Mar. Biotechnol. 6, 453–462, 2005
DOI: 10.1007/s10126-004-4100-3

MARINE BIOTECHNOLOGY

© 2004 Springer Science+Business Media, Inc.

Isolation and Characterization of Ornithine Decarboxylase Gene from Flounder (*Paralichthys olivaceus*)

Jae Hyung Lee,[1] Mi Young Son,[1] Moon-Young Yoon,[2] Jung-Do Choi,[3] and Young Tae Kim[1]

[1]*Department of Microbiology, Pukyong National University, Busan 608-737, Korea*
[2]*Department of Chemistry, Hanyang University, Seoul 133-791, Korea*
[3]*Department of Life Sciences, Chungbuk National University, Cheongju 361-763, Korea*

Abstract: Ornithine decarboxylase (ODC) is a homodimeric enzyme dependent on pyridoxal 5′-phosphate. We identified a complementary DNA clone corresponding to ODC from the brain of adult flounder (*Paralichthys olivaceus*). The flounder ODC cDNA consisted of 2939 bp encoding 272 amino acid residues. The flounder ODC showed 80.3% sequence identity to zebrafish and 70.8% to rat at the amino acid level. Comparison of the structure and nucleotide sequence of the ODC genes revealed that the gene is highly conserved in the flounder, zebrafish, and rat. The presence of ODC mRNA species in brain, kidney, liver, and embryo was confirmed using the reverse transcriptase polymerase chain reaction. The recombinant protein of flounder ODC containing a short histidine tag at the carboxyl terminus was overexpressed in *Escherichia coli* BL21 (DE3) codon plus using an inducible T7 expression system, and was purified by Ni-NTA affinity chromatography.

Key words: ornithine decarboxylase (ODC), pyridoxal 5′-phosphate (PLP), flounder (*Paralichthys olivaceus*).

INTRODUCTION

The polyamines putrescine, spermidine, and spermine are small polycations that are essential for cell growth and function. Intracellular polyamine concentrations are tightly regulated by the enzyme ornithine decarboxylase (ODC), which catalyzes the conversion of ornithine to putrescine. This ODC is the first enzyme in the biosynthesis of polyamines (Tabor and Tabor, 1984; Heby and Persson, 1990; Cohen, 1998).

Received September 30, 2003; accepted December 28, 2003; online publication July 30, 2004.

Corresponding author: Young Tae Kim; e-mail: ytkim@pknu.ac.kr

The eukaryotic ODCs are enzymes dependent on pyridoxal 5′-phosphate (PLP). They are obligate dimers, and each monomer is composed of 2 domains (Osterman et al., 1995b; Kern et al., 1999). The active sites are the N-terminal domain of one monomer and the C-terminal domain of the other (Tobias and Kahana, 1993; Coleman et al., 1994; Osterman et al., 1995a). The N-terminal domain contains residues that interact with PLP (Poulin et al., 1992; Osterman et al., 1995b). ODC, one of the most highly regulated enzymes (Holm et al., 1988; Heby and Persson, 1990), and has a short half-life. The enzyme may be downregulated by polyamines (Pegg, 1986), which exert the most effective repression via antizyme (Coffino, 2001). Antizyme is an ODC-inhibitory protein that is induced by a polyamine-dependent frameshift mechanism (Matsufuji

et al., 1995). It inhibits ODC and triggers its degradation by the 26S proteasome (Hayashi and Murakami, 1995; Hayashi et al., 1996; Coffino, 2001), possibly after a conformational change in the ODC monomer (Coffino, 1998; Almrud et al., 2000; Murakami et al., 2000). The degradation of ODC occurs without ubiquitination (Murakami et al., 1992). ODC activity is regulated at the transcriptional and translational levels, as well as at the level of mRNA and protein stability (Murakami and Hayashi, 1985; Katz and Kahana, 1987; Persson et al., 1988; Grens and Scheffler, 1990; Chen and Chen, 1992).

ODC cDNA genes have been cloned from *Mus musculus* (house mouse; Kahana and Nathans, 1985), *Rattus norvegicus* (Norway rat; van Kranen et al., 1987), *Trypanosoma brucei* (Phillips et al., 1987), *Cricetulus griseus* (Chinese hamster; Grens et al., 1989), *Xenopus laevis* (African clawed frog; Bassez et al., 1990; Cao et al., 2001), *Homo sapiens* (human; Moshier et al., 1990), *Mus pahari* (shrew mouse; Johannes and Berger, 1992), *Gallus gallus* (chicken; Johnson and Bulfield, 1992), *Drosophila melanogaster* (fruit fly; Rom and Kahana, 1993), *Bos taurus* (cow; Yao et al., 1995), and *Danio rerio* (zebrafish; Hascilowicz et al., 2002). However, knowledge of the molecular structure of ODC in marine fishes is extremely limited. In addition, the nature of ODCs in these fish and their roles in the control of the polyamine pathway are still unclear.

The flounder (*Paralichthys olivaceus*) is a commercially important marine aquaculture species in Korea, and it has been used for molecular-level studies of various functional genes (Kim and Kim, 1999; Cho et al., 2001; Lee et al., 2001). In this study we focused on isolating cDNA encoding ODC and the expression of ODC in adult tissues. We report the molecular characteristics and tissue expression of our newly identified ODC cDNA from adult flounder. These data will provide a wider base of knowledge on the primary structure of ODC at the molecular level and its functional diversity.

Materials and Methods

RNA Isolation and Construction of cDNA Library

Mature flounders (*P. olivaceus*) were purchased from a nearby fish market, and 10 brain glands from both sexes were collected. Total RNA was isolated using a Micro-FastTrack 2.0 Kit (Invitrogen). A flounder brain cDNA library was constructed using a λZAP-II cDNA Synthesis Kit (Stratagene), as described in the manufacturer's instructions. The resulting library contained approximately 1×10^5 clones. The library was then amplified up to 3×10^9 clones/ml.

Screening ODC cDNA and DNA Sequencing

The conserved nucleotide sequences of vertebrate ODCs were determined using the National Center for Biotechnology Information (NCBI) nucleotide and protein sequence database. To prepare a probe to screen for ODC-oligonucleotide degeneracy primers were synthesized at GenoTech (Taejeon, Korea). The main polymerase chain reaction (PCR) program consisted of 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30 seconds. The probe used to screen for ODC was amplified by PCR using upstream (ODC-F; 5′-GTCAACAT(T/C)AT(T/C)GCCAA(G/A)AAGGTC-3′) and downstream (ODC-R; 5′-CC(G/C)AC GGTGTAGGC(G/A)CCCATG-3′) primers labeled with a DIG (digoxigenin) Oligonucleotide 3′-End Labeling Kit (Roche). Approximately 1×10^5 plaques from the cDNA library were screened using this probe. Positive plaques recovered from the first screening were confirmed with a second screening (Kim and Richardson, 1993, 1994; Kim et al., 1997; Cho et al., 1999). Positive plaques were recovered from the second screening, and the phagemid containing the insert was excised according to the manufacturer's instructions (Stratagene). The excised phagemid was sequenced using an ABI PRISM DNA Sequencing Kit and an ABI 377 Genetic Analyzer according to the manufacturer's instructions (Applied Biosystems).

Comparative Sequence Analysis of Flounder ODC

To examine the molecular evolution of ODC, the following vertebrate ODC sequences were imported from the Swiss-Prot databank/GenBank: *H. sapiens* (NP-002530), *B. taurus* (AAA79849), *M. musculus* (NP-038642), *R. norvegicus* (NP-036747), *M. pahari* (AAA39847), *C. griseus* (DCHYOC), *X. laevis* (P27120 for ODC1; Q9I8S4 for ODC2), *D. rerio* (BAB84694), and *P. olivaceus* (AY214169). The nucleotide sequences were analyzed using the program Blast (http://www.ncbi.nlm.nih.gov/BLAST). Multiple sequences were aligned using the program Clustal w (http://www.ebi.ac.uk/clustalw), and sequence identities were calculated using Genedoc (http://www.psc.edu/biomed/genedoc). A phylogenetic tree was constructed by the neighbor-joining (NJ) method with the program Treecon (Van de Peer and De Wachter, 1994) for the amino

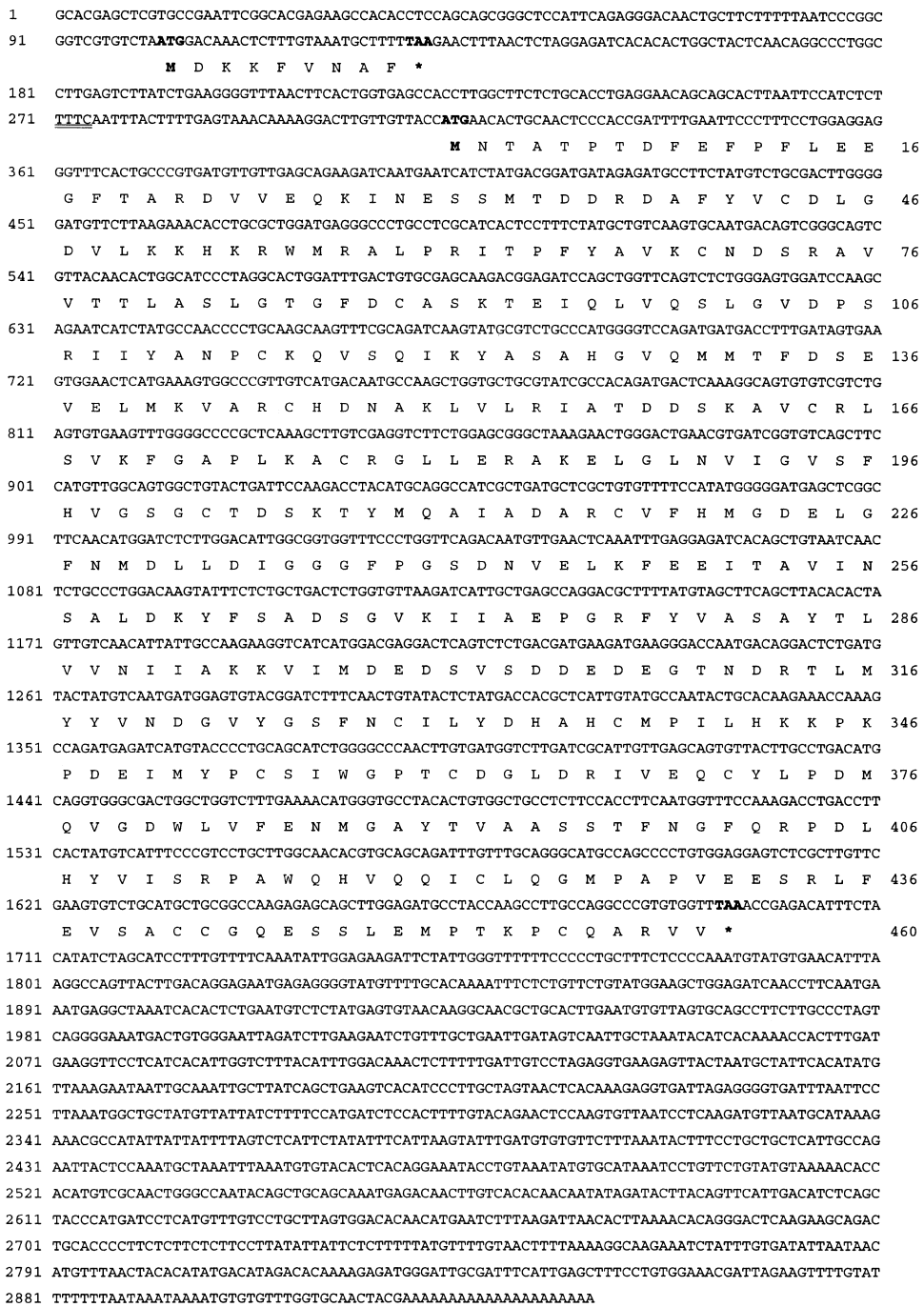


Figure 1. Nucleotide and deduced amino acid sequence of cDNA encoding flounder ODC. The nucleotide sequence is numbered to the left and the amino acid to the right. The bold type indicates the initiator codon. The asterisk and bold type indicate the stop codon. The potential internal ribosome entry site sequence is double-underlined. The polyadenylation signal (AATAAA) is underlined. The nucleotide sequence has accession number AY214169 in the GenBank database.

acid sequences of ODCs from *H. sapiens*, *B. taurus*, *R. norvegicus*, *M. musculus*, *C. griseus*, *X. laevis* (ODC1 and ODC2), *D. rerio*, and *P. olivaceus*.

Reverse Transcriptase Polymerase Chain Reaction

In order to perform RT-PCR, total RNA was isolated from brain, kidney, muscle, liver, and embryo from mature flounders ($n = 10$; size, 45 ± 10 cm; body weight, 900 ± 300 g; 3 years old). The Titan one-tube RT-PCR system (Roche) was used. Master Mix 1 contained 0.2 mM dNTPs, 5 mM dithiothreitol, 50 pmol upstream (ODC-RF: 5′-CCACCGATTTTGAATTCCC-3′) and downstream (ODC-RR: 5′-CTCAAATTTGAGGAGATCACAG-3′) primers, template RNA, and 5 U of RNase inhibitor. Master Mix 2 consisted of 5× RT-PCR buffer and enzyme mix. Mixes 1 and 2 were added to a 0.2-ml thin-walled PCR tube on ice. Then the sample was placed in a thermocycler GeneAmp PCR system (Applied Biosystems) and incu-

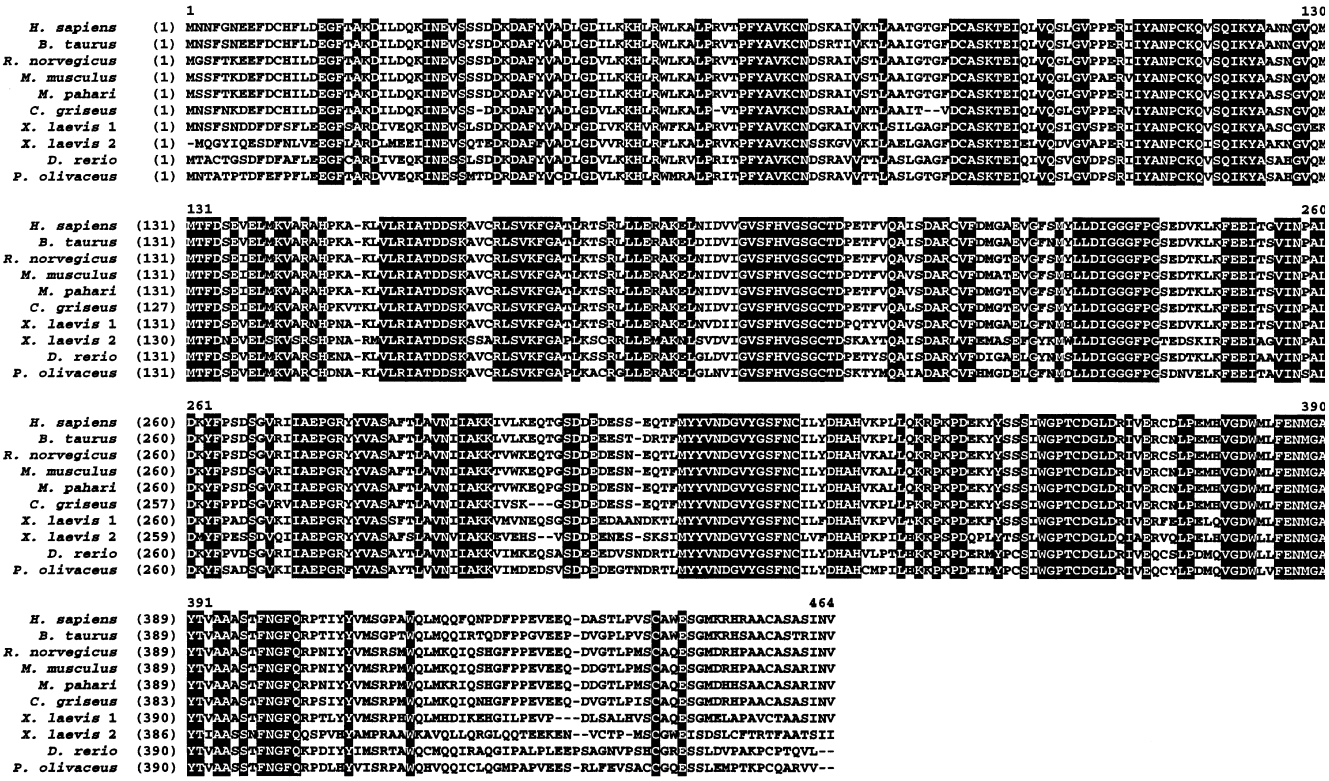


Figure 2. Multiple alignment of the predicted flounder ODC amino acid sequence with known ODC protein sequences taken from GenBank. Accession numbers are given in the text. Residues identical in all sequences are highlighted .

bated for 1 hour at 50°C for reverse transcription followed by thermocycling. The temperature profile consisted of an initial 94°C for 5 minutes, 30 cycles at 94°C for 40 seconds, 54°C for 30 seconds, and 72°C for 2 minutes, and a final 7-minute extension at 72°C. Twenty microliters of the reaction products was separated by electrophoresis through 1.5% agarose gels and stained with ethidium bromide.

Overexpression of ODC Gene in *E. coli*

In order to express the ODC gene, the cloned ODC cDNA was subcloned into pET101/D-Topo expression vector (Invitrogen), which allows expression of a recombinant protein with a C-terminal fusion His tag. To this end, the ODC gene was amplified by PCR, using primers 5′-CCGCGGATGAACACTGC-3′ and 5′-CTCGAGAACCACACGGG-3′, and inserted into pET101/D-Topo. The resulting plasmid pET101/D-Topo-ODC was transformed into *E. coli* strain BL21 (DE3) codon plus. The cells harboring the ODC gene were cultured in LB medium (containing 50 μg/ml ampicillin) and induced by adding isopropyl-β-thiogalactopyranoside (IPTG) to a final concentration of 1 mM at a cell density corresponding to OD_{600} of 0.5. Expressed proteins were analyzed by 12% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). Western blot analysis was conducted by standard procedures (Gershoni and Palade, 1983). Proteins were electrophoretically transferred from a polyacrylamide gel to a nitrocellulose membrane, probed with goat antiserum against 6-His tag, and incubated with alkaline phosphatase coupled to goat antibody raised against goat IgG. The nitrocellulose membrane was developed using the BCIP/NBT.

Purifying ODC and Assaying Its Activity

The pET101/D Topo-ODC plasmid has ODC-His-tagged DNA sequences. The ODC-His fusion protein was eluted using a His Trap Kit (Amersham Pharmacia Biotech). The cultured cells were harvested by centrifugation. The cell pellets were resuspended with resuspension buffer (20 mM Tris-HCl, pH 8.0), sonicated with an ultrasonicator, and harvested again by centrifugation. The cell pellets were resuspended by adding isolation buffer (2 M urea, 20 mM Tris-HCl, 0.5 M NaCl, 2% Triton X-100, pH 8.0) and

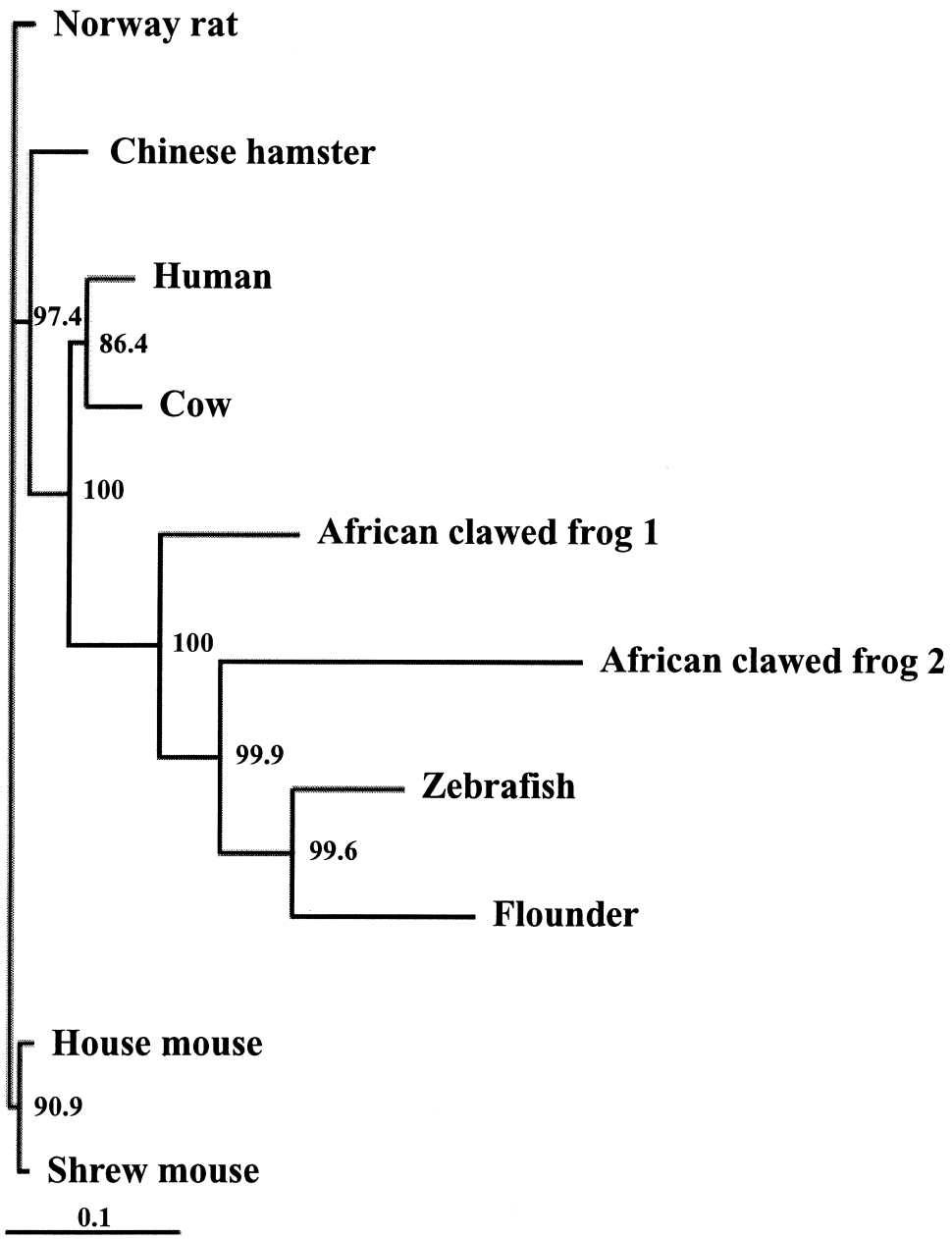


Figure 3. Molecular phylogenetic tree of ODC based on NJ method. The values shown on each internal branch are the percentage of support determined from a bootstrap analysis with 1000 replications.

sonicated. Finally, the cell pellets were resuspended in binding buffer (20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0) and filtered with a 0.45-μm membrane filter. The prepared sample was eluted by affinity purification using a HiTrap Chelating HP column. The ODC enzymatic activity was assayed using the method described by Wang and Bachrach (2000).

Results and Discussion

Fish are the largest and most diverse group of vertebrates. Their evolutionary position relative to other vertebrates and their ability to adapt to a wide variety of environments make them ideal for studying both organism and molecular evolution. ODC is a key enzyme in the biosynthesis of polyamines because it regulates intracellular concentrations of polyamines, which are essential for cell growth and function. Several ODC genes have been studied in fish, and some have been analyzed at the molecular level.

In order to identify the ODC gene from flounder, degenerate oligonucleotide primers encoding parts of the conserved domains were prepared and used to produce a probe utilizing a flounder brain cDNA library, constructed with a λZAP-II cDNA Synthesis Kit. The ODC sequence, conserved in humans and several mammals, was deter-

mined using the NCBI nucleotide and protein sequence database and utilized as a screening probe. Using primers ODC-F and ODC-R, a 300-bp PCR product was obtained. Approximately 1×10^5 plaques were screened; several positive clones were obtained, and their nucleotide sequences were analyzed.

Figure 1 shows the nucleotide sequence of the complete cDNA encoding the flounder ODC gene (GenBank accession number AY214169) and its deduced amino acid sequence. The sequence contains a 312-bp 5′-untranslated region (UTR) followed by a 1380-bp coding region, which corresponds to a 460-amino-acid protein, and a 1247-bp 3′-UTR. As shown in Figure 1, the flounder ODC cDNA clone contains an in-frame termination codon (TAA) at bases 1693 to 1695. A putative polyadenylation signal (AATAAA) can be found 26 bp upstream from the poly(A) tail. Both the 5′- and 3′-UTRs of the mammalian ODC are reported to play critical roles in translational regulation of the ODC gene (Shantz and Pegg, 1999; Pyronnet et al., 2000), although the details of this regulation are not fully understood. The 5′-UTR is considered responsible for hampering ODC mRNA expression (Shantz and Pegg, 1999; Pyronnet et al., 2000), while the 3-UTR interferes with this translation-suppressing effect and participates in the 5′-UTR-independent hypotonic ODC induction. As Figure 1 shows, the 5′-UTR of the flounder ODC gene contains nucleotides predicted to form a short upstream open reading frame (MDKKFVNAF, spanning nucleotides 102 to 129), and a potential internal ribosome entry sit. This sequence might lead to different mechanisms of translational regulation in flounder ODC.

Figure 2 shows an alignment of the amino acid sequences of ODC in flounder and other species. The ODCs were compared using the BLAST protein database (NCBI). The deduced flounder amino acid sequence was about 80.3% and 70.6% identical with zebrafish ODC and human ODC, respectively. The highest level of similarity was in residues responsible for active site formation and stabilization of ODC, which are almost identical in the species dimers (Murakami et al., 1992; Kern et al., 1999). Many structural elements are involved in regulating ODC translation and degrading the enzyme (antizyme-binding element of ODC protein and its C-terminal end) (Li and Coffino, 1993; Shantz and Pegg, 1999; Murakami et al., 2000). The flounder ODC contains residues that are important for ODC dimer formation (D134, K169, K294, Y324, Y332, D365, G388, and F398), the enzyme active site (K69, D88, R154, K169, H197, G235, G236, G237, R277, Y324, D333, C361, D362, Y390, and N399), the putative antizyme-binding region (residues 117 to 140), and 2 degradation elements (corresponding to residues 376 to 424 and 422 to 461 of the human and mouse ODC sequences) in the C-terminus (Kern et al., 1999; Almrud et al., 2000; Hascilowicz et al., 2002). The flounder ODC also contains N-linked glycosylation sites (N-X-S/T) (residues 29 to 31; 71 to 73).

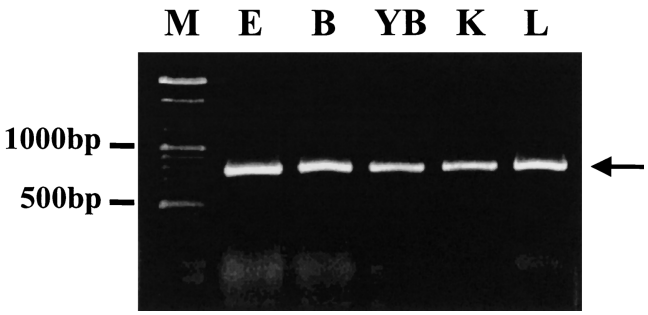
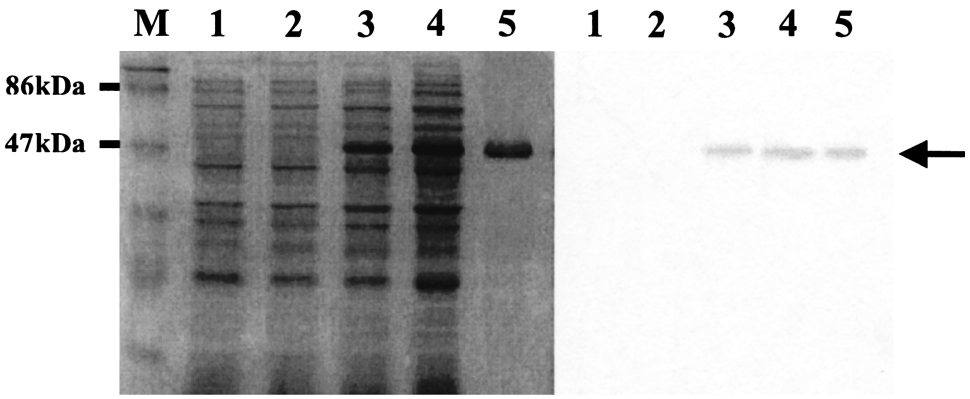


Figure 4. Pattern of ODC expression detected by RT-PCR. Lane M, molecular marker; lane E, total RNA template for RT-PCR isolated from flounder embryo; lane B, brain; lane YB, young brain; lane K, kidney; and lane L, liver.

A molecular phylogenetic tree was constructed to analyze the evolutionary relationships of the ODC proteins (Figure 3). It shows the evolutionary divergence of the ODC genes of zebrafish, flounder, frog, mouse, rat, Chinese hamster, cow, and human. The flounder ODC protein had 80.3% sequence identity with the zebrafish ODC and 70.6% with the human ODC.

The tissue distribution of the flounder ODC gene was investigated using RT-PCR with total RNA isolated from flounder tissues as template. As shown in Figure 4, the resulting DNA banding patterns provided evidence of ODC expression in tissues from the embryo, brain, young brain, kidney, and liver. The flounder ODC mRNA has a wide tissue distribution.

To express the flounder ODC gene in a prokaryotic system, the ODC cDNA was subcloned into pET101/D-Topo expression vector. The resulting pET101/D-Topo-ODC plasmid was transformed into *E. coli* BL 21(DE3) codon plus. The expression patterns of the ODC proteins were analyzed using SDS-PAGE (Figure 5, A). The cloned ODC gene was strongly expressed with IPTG induction. The optimal induction time was approximately 3 hours after IPTG induction. As shown in Figure 5(A), the molecular weight of the ODC-His fusion protein is approximately 53 kDa, while the predicted ODC protein is



(A) (B)

Figure 5. Analysis of expressed proteins using SDS-PAGE and Western blotting. **A:** The expressed proteins were analyzed using 12% SDS-PAGE. Lane M, standard protein molecular weight markers; lane 1, proteins (10 μg) from uninduced cell extracts (control); lane 2, proteins (10 μg) from induced cell extracts 0 hours after IPTG induction; lane 3, proteins (20 μg) from induced cell extracts 1 hour after IPTG induction; lane 4, proteins (30 μg) from induced cell extracts 3 hours after IPTG induction; lane 5, purified ODC protein (10 μg). **B:** Western blot analysis of expressed proteins. Proteins were electrophoretically transferred from an SDS-PAGE gel to a nitrocellulose membrane, probed with goat antiserum against 6-His tag, and incubated with alkaline phosphatase coupled to goat antibody against goat IgG. The nitrocellulose membrane developed using the BCIP/NBT. Lane 1, uninduced cell extracts as control; lanes 2, 3, and 4, induced cell extracts 0, 1, and 3 hours after IPTG induction, respectively; lane 5, purified ODC protein.

approximately 50 kDa. The pET101/D-TOPO expression vector has a C-terminal fusion tag (3 kDa). The expressed protein was purified by affinity chromatography and determined to have a molecular weight of 53 kDa, which was confirmed by Western blotting (Figure 5, B).

To determine whether the cloned cDNA encodes a functional protein, an ODC clone was expressed in *E. coli*. The enzymatic activity of the recombinant flounder ODC was determined using a chemiluminescence-based method (Wang and Bachrach, 2000). Cell lysates after IPTG induction were incubated with ornithine for 1 hour, and the ODC activity was measured (Figure 6). There was a linear correlation between the amount of compound and the intensity of chemiluminescence, indicating that the expressed protein was enzymatically active. When a standard putrescine solution was subjected to the chemiluminescence-based assay, a standard linear curve was obtained. The ODC activity expressed in terms of putrescine formation corresponded well with the amount of cell lysate used. This suggests that the expressed ODC has enzymatic activity and the fusion of 6-His residues at the C-terminus of ODC does not interfere with the catalytic function of the ODC protein.

Our study provides phylogenic information on ODC that is essential for understanding the molecular evolution

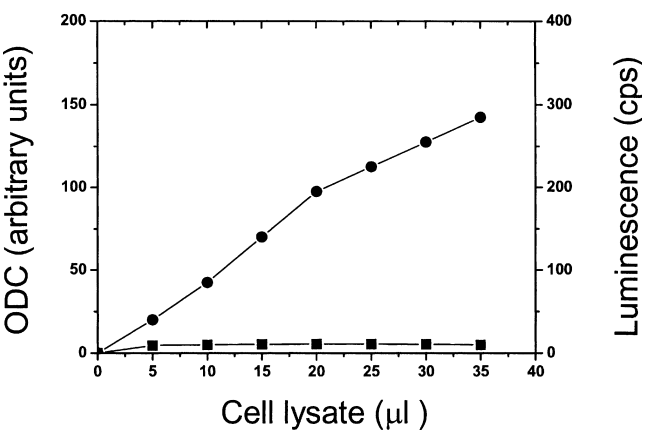


Figure 6. Enzymatic activity of flounder ODC. Cell extracts from IPTG induction (3 hours) were incubated with ornithine for 1 hour, and the enzymatic activity of ODC (●) was measured using a chemiluminescence-based method (Wang and Bachrach, 2000). A control experiment (■) for measuring the catalytic activity was performed with cell extracts of nontransformed *E. coli* strain BL21 (DE3) codon plus.

of this gene in vertebrates. A comparative analysis of the structure, expression, and function of the ODC gene may be necessary in order to elucidate the mechanism responsible for controlling the biosynthetic polyamine pathway and the intracellular polyamine concentrations. Fish are the

most primitive vertebrates, and genetic information obtained from fish can reveal the origin and diversion of genes with similar functions in other organisms. Observations and genetic manipulations of the flounder ODC make this species a useful model for studying the mechanism of polyamine participation in the ODC regulation system during development.

In conclusion, a comparison of the amino acid sequences of vertebrate ODCs indicated that the flounder ODC is highly conserved with those of other species. Flounder ODC mRNA was detected in embryo, brain, young brain, kidney, and liver. The recombinant flounder ODC expressed in *E. coli* was enzymatically active. This suggests that the flounder ODC protein has functions during vertebrate development similar to those in other species. Therefore, our results contribute to a deeper understanding of the mechanisms involved in the polyamine-mediated ODC regulation system.

Acknowledgments

This work was supported by a Korea Research Foundation Grant (KRF-2002-070-C0064). We are grateful to Dr. Thomas T. Chen (University of Connecticut) for critical comments on the manuscript.The GenBank accession number for the ODC nucleotide sequence reported here is AY214169.

References

Almrud, J.J., Oliveira, M.A., Kern, A.D., Grishin, N.V., Philips, M.A., and Hackert, M.L. (2000). Crystal structure of human ornithine decarboxylase at 2.1 Å resolution: structual insights to antizyme binding. *J Mol Biol* 295:7–16.

Bassez, T., Paris, J., Omilli, R, Dorel, C., and Osborne, H.B. (1990). Post-transcriptional regulation of ornithine decarboxylase in *Xenopus laevis* oocytes. *Development* 110:955–962.

Cao, Y., Zhao, H., Hollemann, T., Chen, Y., and Grunz, H. (2001). Tissue-specific expression of an ornithine decarboxylase paralogue, XODC2, in *Xenopus laevis*. *Mech Dev* 102:243–246.

Chen, Z.P., and Chen, K.Y. (1992). Mechanism of regulation of ornithine decarboxylase gene expression by asparagines in a variant mouse neuroblastoma cell line. *J Biol Chem* 267:6946–6951.

Cho, J.J., Lee, J.H., Kim, S-K., Choi, T-J., and Kim, Y.T. (1999). cDNA encoding *nm23*/NDP kinase gene from Korean tiger shark *Scyliorhinus torazame*. *Mar Biotechnol* 1:131–136.

Cho, J.J., Sung, B.K., Lee, J.H., Chung, J-K., Choi, T-J., and Kim, Y.T. (2001). cDNA for an immune response gene encoding low molecular weight polypeptide from flounder, *Paralichthys olivaceus*. *Mol Cells* 11:226–230.

Coffino, P. (1998). Marine Biotechnology. In: *Ubiquitin and the Biology of the Cell*, Peters, J.M., Harris, J.D., and Finley, D. (eds.). New York, N.Y.: Plenum Press, pp 411–427.

Coffino, P. (2001). Regulation of cellular polyamines by antizyme. *Nat Rev Mol Cell Biol* 2:188–194.

Cohen, S.S. (1998). A Guide to the Polyamines. New York, N.Y.: Oxford Univ Press, pp 231–259.

Coleman, C.S., Stanley, B.A., Viswanath, R., and Pegg, A.E. (1994). Rapid exchange of subunits of mammalian ornithine decarboxylase. *J Biol Chem* 269:3155–3158.

Gershoni, J.M., and Palade, G.E. (1983). Protein blotting: principles and application. *Anal Biochem* 131:1–15.

Grens, A., and Scheffler, I.E. (1990). The 5′- and 3′-untranslated regions of ornithine decarboxylase mRNA affect the translational efficiency. *J Biol Chem* 265:11810–11816.

Grens, A., Steglich, C., Pilz, R., and Scheffler, I.E. (1989). Nucleotide sequence of the Chinese hamster ornithine decarboxylase gene. *Nucleic Acids Res* 17:10497.

Hascilowicz, T., Murai, N., Matsufuji, S., and Murakami, Y. (2002). Regulation of ornithine decarboxylase by antizymes and antizyme inhibitor in zebrafish (*Danio rerio*). *Biochim Biophys Acta* 1578:21–28.

Hayashi, S., and Murakami, Y. (1995). Rapid and regulated degradation of ornithine decarboxylase. *Biochem J* 306:1–10.

Hayashi, S., Murakmi, Y, and Matsufuji, S. (1996). Ornithine decarboxylase antizyme: a novel type of regulatory protein. *Trends Biochem Sci* 21:27–30.

Heby, O., and Persson, L. (1990). Molecular genetics of polyamin synthesis in eukaryotic cells. *Trends Biochem Sci* 15:153–158.

Holm, I., Persson, L., Heby, O., and Seiler, N. (1988). Feedback regulation of polyamine synthesis in Ehrlich ascites tumor cells: analysis using nonmetabolizable derivatives of putrescine and spermine. *Biochim Biophys Acta* 972:239–248.

Johannes, G., and Berger, F.G. (1992). Alterations in mRNA translation as a mechanism for the modification of enzyme synthesis during evolution: the ornithine decarboxylase model. *J Biol Chem* 267:10108–10115.

Johnson, R., and Bulfield, G. (1992). Molecular cloning and sequence analysis of a chicken ornithine decarboxylase cDNA. *Anim Genet* 23:403–409.

Kahana, C., and Nathans, D. (1985). Nucleotide sequence of murine ornithine decarboxylase mRNA. *Proc Natl Acad Sci U S A* 82:1673–1677.

Katz, A., and Kahana, C. (1987). Transcriptional activation of mammalian ornithine decarboxylase during stimulated growth. *Mol Cell Biol* 7:2641–2643.

Kern, A.D., Oliveira, M.A., Coffino, P., and Hackert, M.L. (1999). Structure of mammalian ornithine decarboxylase at 1.6 Å resolution: stereochemical implications of PLP-dependent amino acid decarboxylases. *Structure Fold Des* 7:567–581.

Kim, D.S., and Kim, Y.T (1999). Identification of an embryonic growth factor IGF-II from the central nervous system of the teleost, flounder, and its expressions in adult tissues. *J Microbiol Biotechnol* 9:113–118.

Kim, Y.T., and Richardson, C.C. (1993). Bacteriophage T7 gene 2.5 protein: an essential protein for DNA replication. *Proc Natl Acad Sci U S A* 90:10173–10177.

Kim, Y.T., and Richardson, C.C. (1994). Acidic carboxyl-terminal domain of gene 2.5 protein of bacteriophage T7 is essential for protein-protein interactions. *J Biol Chem* 269:5270–5278.

Kim, Y.T., Song, Y.H., and Churchich, J.E. (1997). Recombinant brain 4-aminobutyrate aminotransferases: overexpression, purification, and identification of Lys-330 at the active site. *Biochim Biophys Acta* 1337:248–256.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.

Lee, E-Y., Park, H-H., Kim, Y.T., and Choi, T-J. (2001). Cloning and sequence analysis of the interleukin-8 gene from flounder (*Paralichthys olivaceus*). *Gene* 274:237–243.

Li, X., and Coffino, P. (1993). Degradation of ornithine decarboxylase: exposure of the C-terminal target by a polyamine-inducible inhibitory protein. *Mol Cell Biol* 13:2377–2383.

Moshier, J.A., Gilbert, J.D., Skunca, M., Dosescu, J., Almodovar, K.M., and Luk, G.D. (1990). Isolation and expression of a human ornithine decarboxylase gene. *J Biol Chem* 265:4884–4892.

Matsufuji, S., Matsufji, T., Miyazaki, Y., Murakami, Y., Atkins, J.F., Gesteland, R.F., and Hayashi, S. (1995). Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. *Cell* 80:51–60.

Murakami, Y., and Hayashi, S. (1985). Role of antizyme in degradation of ornithine decarboxylase in HTC cells. *Biochem J* 226:893–896.

Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., Tanaka, K., and Ichihara, A. (1992). Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. *Nature* 360:597–599.

Murakami, Y., Matsufuji, S., Hayashi, S., Tanahashi, N., and Tanaka, K. (2000). Degradation of ornithine decarboxylase by the 26S proteasome. *Biochem Biophys Res Commun* 267:1–6.

Osterman, A.L., Kinch, L.N., Grishin, N.V., and Phillips, M.A. (1995a). Acidic residues important for substrate binding and cofactor reactivity in eukaryotic ornithine decarboxylase identified by alanine scanning mutagenesis. *J Biol Chem* 270:11797–11802.

Osterman, A.L., Lueder, D.V., Quick, M., Myers, D., Canagarajah, B.J., and Phillips, M.A. (1995b). Domain organization and a protease-sensitive loop in eukaryotic ornithine decarboxylase. *Biochemistry* 34:13431–13436.

Pegg, A.E. (1986). Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem J* 234:249–262.

Persson, L., Holm, I., and Heby, O. (1988). Regulation of ornithine decarboxylase mRNA translation by polyamines: studies using a cell-free system and a cell line with an amplified ornithine decarboxylase gene. *J Biol Chem* 263:3528–3533.

Phillips, M.A., Coffino, P., and Wang, C.C. (1987). Cloning and sequencing of the ornithine decarboxylase gene from *Trypanosoma brucei*: implications for enzyme turnover and selective difluoromethylornithine inhibition. *J Biol Chem* 262:8721–8727.

Poulin, R., Lu, L., Ackerman, B., Bey, P., and Pegg, A.E. (1992). Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethyl ornithine: characterization of sequence at the inhibitor and coenzyme binding sites. *J Biol Chem* 267:150–158.

Pyronnet, S., Pradayrol, L., and Sonenberg, N. (2000). A cell cycle-dependent internal ribosome entry site. *Mol Cell* 5:607–616.

Rom, E., and Kahana, C. (1993). Isolation and characterization of the *Drosophila* ornithine decarboxylase locus: evidence for the presence of two transcribed ODC genes in the *Drosophila* genome. *DNA Cell Biol* 12:499–508.

Shantz, L.M., and Pegg, A.E. (1999). Translational regulation of ornithine decarboxylase and other enzymes of the polyamine pathway. *Int J Biochem Cell Biol* 31:107–122.

Tabor, C.W., and Tabor, H. (1984). Polyamines. *Annu Rev Biochem* 53:749–790.

Tobias, K.E., and Kahana, C. (1993). Intersubunit location of the active site of mammalian ornithine decarboxylase as determined by hybridization of site-directed mutants. *Biochemistry* 32:5842–5847.

Van de Peer, Y., and De Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* 10:569–570.

van Kranen, H.J., van de Zande, L., van Kreijl, C.F., Bisschop, A., and Wieringa, B. (1987). Cloning and nucleotide sequence of rat ornithine decarboxylase cDNA. *Gene* 60:145–155.

Wang, Y., and Bachrach, U. (2000). A luminescence-based test for determining ornithine decarboxylase activity. *Anal Biochem* 287:299–302.

Yao, J., Zadworny, D., Kuhnlein, U., and Hayes, J.F. (1995). Molecular cloning of a bovine ornithine decarboxylase cDNA and its use in the detection of restriction fragment length polymorphisms in Holsteins. *Genome* 38:325–331.