THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 264, No. 12, Issue of April 25, pp. 6780–6785, 1989
Printed in U.S.A.

Olfactory-specific Cytochrome P-450

cDNA CLONING OF A NOVEL NEUROEPITHELIAL ENZYME POSSIBLY INVOLVED IN CHEMORECEPTION*

(Received for publication, August 8, 1988)

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We isolated cDNA clones for cytochrome P-450 genes expressed in the olfactory neuroepithelium by screening a corresponding rat cDNA library. Sequence analysis and RNA blot hybridization revealed a new cytochrome P-450, designated cytochrome P-450olf1, which is the first reported cytochrome P-450 mRNA uniquely expressed in the chemosensory organ. Cytochrome P-450olf1 shows intermediate level of sequence similarity (38–53% identity) to several liver cytochrome P-450 enzymes, suggesting that it belongs to the cytochrome P-450II family, but defines a new subfamily (cytochrome P-450IIG) within it. Cytochrome P-450II enzymes are known to process diverse organic compounds, including odorants. This, together with the specificity of cytochrome P-450olf1 to the sensory neuroepithelium, may indicate a role for this protein in olfactory reception.

The hepatic microsomes contain many different cytochromes P-450 which bind and oxidize a great variety of exogenous and endogenous compounds (reviewed in Black and Coon, 1987; Adesnik and Atchison, 1986; Nebert and Gonzalez, 1987). Likewise, the olfactory epithelium in the nasal cavity concentrates and actively metabolizes many compounds, including inhaled odorants (Dahl and Hadley, 1983) environmental xenobiotics (Bond, 1983), drugs (Brittebo and Tjalve, 1981, Reed *et al.*, 1986), carcinogens (Brittebo and Ahlman, 1984; Dahl *et al.*, 1982; Bond, 1983), and steroids (Brittebo and Rafter, 1984), suggesting a system similar to the one found in hepatic microsomes.

The cytochrome P-450-dependent metabolism of various substrates is comparable or even higher in olfactory epithelium compared to the liver (Dahl *et al.*, 1982; Reed *et al.*, 1986; Dahl, 1988). Furthermore, cytochrome P-450 enzymes in olfactory epithelium have different kinetic parameters and substrate specificities (Brittebo and Rafter, 1984; Reed *et al.*, 1986) and are more sensitive to cytochrome P-450 inhibitors (Jenner and Dodd, 1988) than those in the liver. Yet, olfactory cytochrome P-450 proteins have not been purified and characterized and the molecular basis for the above mentioned qualitative and quantitative metabolic differences remains unknown. Recently, we reported that a CNBr peptide derived from a major nonglycosylated integral membrane protein unique to bovine olfactory epithelium bears strong sequence similarity to a cytochrome P-450 enzyme (Nef *et al.*, 1988). We therefore decided to apply recombinant DNA technology to characterize olfactory cytochrome P-450 proteins at the molecular level.

Biochemical and genetic studies have permitted the characterization in 10 species of more than 65 different cytochrome P-450 genes, most of them expressed in the liver. Primary structure comparison of cytochrome P-450 proteins in prokaryotes and eukaryotes suggested that an ancestral precursor gene gave rise through multiple duplications to the cytochrome P-450 multi-gene superfamily. Based on the percent identity between aligned cytochrome P-450 amino acid sequences, 14 families and their corresponding subfamilies have been defined (Nebert *et al.*, 1989). The largest family, cytochrome P-450II, includes seven subfamilies (IIA–IIG). In the liver, cytochrome P-450II genes are constitutively expressed (IIA, IIC) or induced by phenobarbital (IIB) or ethanol (IIE). To date, it has not been established which cytochrome P-450 genes are expressed in the olfactory epithelium.

The present report describes the cDNA cloning of a new cytochrome P-450 (cytochrome P-450olf1) unique to the olfactory epithelium. It provides the first molecular biological correlate of a prominent enzymatic activity in this sensory organ, possibly related to its odorant receptor function.

MATERIALS AND METHODS

cDNA Library—A cDNA library was prepared from poly(A)$^+$ RNA derived from olfactory epithelia (including the subepithelium) of 4-week-old Sprague-Dawley rats. A cDNA population larger than 1 kb[1] was inserted into the vector λgt11 and packaged *in vitro*, producing 3 × 10^6 independent phages. The amplified cDNA library had 85% recombinant phages.

Screening Conditions—Typically, 7 × 10^5 phages were screened at a density of 300 phages/cm^2, using *Escherichia coli* strain Y1090. Following incubation at 42 °C for 8 h, phages were transferred to nitrocellulose membranes (Schleicher and Schuell) and processed as described (Benton and Davis, 1977). Hybridization was conducted in 5 × SSPE (SSPE: 0.15 M NaCl, 0.01 M NaH_2PO_4, 0.001 M EDTA), 1 × Denhardt's, 0.1% SDS, and 100 μg/ml sonicated and denatured salmon sperm DNA (Maniatis *et al.*, 1982). Probes were labeled with

* This research was supported in part by Grant NS22063 (to D. L.) from the National Institutes of Health and by grants (to D. L.) from the United States Army Research Office and the Fund for Basic Research administered by the Israel Academy of Sciences and Humanities. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04715.

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[1] The abbreviations used are: kb, kilobase pair(s); SDS, sodium dodecyl sulfate; bp, base pair(s).

[α-^{32}P]dCTP to a specific activity of ~10^8 dpm/μg by nick translation (Rigby *et al.*, 1977). Hybridization (12–18 h) was carried out at 50 °C (low stringency) or 75 °C (high stringency). Filters were then washed at 50 °C for 1 h in 2 × SSC, 0.1% SDS, and for 1 h in 1 × SSC (0.15 M NaCl, 0.015 M Na_3 citrate), 0.1% SDS.

Phage Analysis and Subcloning—Phage recombinants giving a positive signal in plaque hybridization were purified by several cycles of plating at low density. Phage DNA was prepared from phage stocks grown in liquid culture as described (Maniatis *et al.*, 1982). Electrophoretically purified *Eco*RI fragments of the inserts were subcloned either into the vectors pUC8, 9, 18, or 19 (Vieira and Messing, 1982) for restriction mapping or into the M13 vectors mp18/mp19 (Messing and Vieira, 1982) for DNA sequencing.

DNA Sequencing—Single-stranded DNA of appropriate M13 cDNA subclones was prepared (Davis *et al.*, 1986) and sequenced by the chain termination method (Sanger *et al.*, 1977) using α-^{35}S-dATP and the modified T7 DNA polymerase (Sequenase, US Biochemicals, Cleveland, OH). Labeled fragments were separated on 50 cm long 6% polyacrylamide gels (0.2–0.7 mm thick), run for 2–8 h at 1800 V.

RNA Preparation—Olfactory epithelium, heart, liver, brain, lung, and intestine were dissected from ~4-week-old Sprague-Dawley rats (from the Weizmann Institute animal breeding facility) and stored in liquid nitrogen. Olfactory epithelium (including the subepithelial layer) was obtained by sagittal section of the head following quick decapitation. The pigmented ethmoturbinates were collected, with occasional inclusion of small regions of unpigmented nonsensory epithelium. Bovine olfactory epithelium was similarly obtained at the Jerusalem municipal slaughterhouse. Accurate separation of the sensory and nonsensory areas was achieved by discarding the tissue in the borderline area. Total RNA was prepared by a modification of the method described (Aufray and Rougeon, 1980): 1 g of tissue was homogenized with a polytron (30 s at half-maximum speed) in 5 ml of 5 M guanidinium isothiocyanate, 0.01 M EDTA, 0.05 M Tris-HCl, pH 7.5, 1 M β-mercaptoethanol. The homogenate was added to 25 ml of 4 M lithium chloride, incubated for 12 h at 0 °C, and centrifuged for 45 min at 12,000 rpm in an SW 28 rotor (Beckman). The pellet was resuspended in 3 ml of 10 mM Tris-HCl, pH 7.5, 25 mM EDTA, 0.6% SDS extracted twice with phenol and once with chloroform. Total RNA was ethanol-precipitated at 0 °C for 12 h and resuspended in diethylpyrocarbonate-treated water. Poly(A)$^+$ RNA was prepared by oligo(dT) column chromatography (Maniatis *et al.*, 1982). Poly(A)$^+$ RNAs from 3-month-old bovine olfactory and respiratory epithelia were prepared similarly.

RNA Blots—RNA samples were heat-denatured in sample buffer containing 50% formamide and 6% formaldehyde, and electrophoresed on denaturing 1% agarose gels containing 6% of formaldehyde, as described (Lehrach *et al.*, 1977). Gels were blotted by capillary force onto GeneScreen membranes (Du Pont-New England Nuclear), which were fixed by baking at 80 °C for 2 h. Prehybridization (42 °C, 6 h) and hybridization (42 °C, 18 h) were carried out in the presence of 10% dextran sulfate and 50% deionized formamide. 0.5 μg of the probe was labeled (100–200 μCi/μg) by nick translation. Membranes were washed as indicated in the GeneScreen manual (eliminating the first low stringency wash) at 65 °C for 30 min in 2 × SSC, 0.1% SDS, and at room temperature for 1 h in 0.1 × SSC, 0.1% SDS, followed by autoradiography for 12 to 48 h at −70 °C, using an intensifying screen.

Computer Analysis—Nucleic acid and amino acid sequences were analyzed on a Microvax II computer at the Computer Unit of the biological services at the Weizmann Institute, using the Sequence Analysis Software Package (version 5.0), University of Wisconsin Genetics Computer Group. The Wilbur and Lipman (1983) algorithm was used for the alignment of amino acid sequences.

RESULTS

Isolation of Cytochrome P-450olf1 cDNA—To characterize cytochrome P-450 genes expressed in the olfactory tissue, we screened a rat olfactory cDNA library with a probe derived from rat cytochrome P-450e (subfamily IIB2). A 375-bp *Bgl*II restriction fragment was isolated from a cDNA clone (No. 91) kindly provided by Dr. G. Padmanaban (Ravishankar and Padmanaban, 1985), and subcloned in pUC8. This fragment encodes amino acids 361–486, which are highly conserved among all the cytochrome P-450 gene families (see Fig. 6). High stringency screening revealed positive signals at a frequency of ~1/50,000. Five of the positive phage recombinants were further purified and shown to encode the closely related cytochrome P-450b (subfamily IIB1) by partial DNA sequence analysis (results not shown).

To identify cDNAs for additional forms of cytochrome P-450 we subcloned one of the above cytochrome P-450b clones (No. 6), a 1.9-kb insert encompassing the full protein-coding sequence. We used it to screen the same nitrocellulose filters, but this time at a low stringency. This revealed faint signals at a much higher frequency (~1/500) than that observed at high stringency. Several of these clones were plaque-purified and four of them further characterized. Two clones, No. 30 (1.89 kb) and No. 27 (~2.7 kb) were subjected to DNA sequence analysis as shown in Figs. 1 and 2. Complete identity in coding and noncoding regions over almost 1000 bp indicated that they are derived from the same gene (or from very similar genes). More than 90% of the recombinant phages that hybridized only weakly with the cytochrome P-450b probe showed strong signals with a clone No. 27 probe. This sequence, which appears to be highly represented in the olfactory cDNA library, is termed cytochrome P-450olf1.

Intervening sequences were observed in two clones, No. 27 and No. 15. They define a cytochrome P-450olf1 exon with its flanking intronic sequences (Fig. 3).

Tissue-specific Steady-state Levels of Cytochrome P-450olf1 mRNA—We next asked whether cytochrome P-450olf1 is merely highly represented in the olfactory cDNA library or is also tissue-specific. To compare the level of cytochrome P-450olf1 mRNA in different rat tissues, we first hybridized total RNA blots with a radioactively labeled cytochrome P-450olf1 cDNA insert at high stringency. Cytochrome P-450olf1 mRNA transcripts were easily detected in the olfactory epithelium but not in heart, liver, brain, lung, or intestine (Fig. 4, *A* and *B*). Under the same hybridization conditions, a radioactively labeled cytochrome P-450b probe (clone No. 6) also detected cytochrome P-450b mRNA in the olfactory epithelium (results not shown).

In order to further examine the specificity of cytochrome P-450olf1 expression, we decided to compare cytochrome P-450olf1 mRNA levels in olfactory epithelium with those of the adjacent nasal respiratory epithelium. This could not be readily done in the rat, because of technical problems in accurate tissue separation. Instead, we carried out such comparison in bovine tissue, where the two epithelial areas are clearly demarcated. Although cytochrome P-450olf1 cDNA probe (clone No. 27) was found to cross-hybridize with an mRNA in the bovine olfactory epithelium, the closely related respiratory epithelium mRNA did not show cross-reactivity with this probe (Fig. 5).

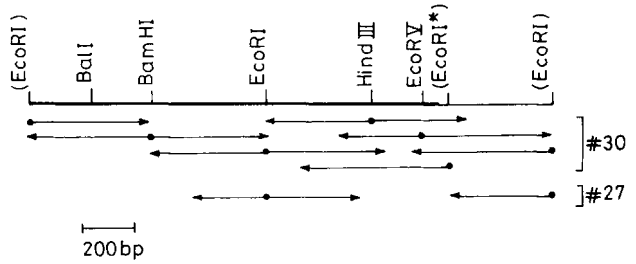

FIG. 1. **Restriction map of cytochrome P-450olf1 cDNA.** *Eco*RI restriction sites in *parentheses* indicate the location of the *Eco*RI linker added during the cDNA library construction. Also shown in *parentheses* is an *Eco*RI* site (CAATTG, position 1520) which has been used in sequencing clone No. 30. *Arrows* describe the sequencing strategy. The continuity through the internal *Eco*RI site was established by the absence of additional *Eco*RI fragments by Southern analysis.

FIG. 2. **Nucleotide and deduced protein sequences of the rat cytochrome P-450olf1.** A functional polyadenylation signal (AATAAA) is found ~350 bp downstream from the stop codon; a poly(A) sequence begins 21 bp downstream from this signal. Sequence analysis has established that clone No. 30 begins 2 bp before the amino acid position number 2. Clone No. 30 does not show an initiator methionine. However, since its amino terminus already extends beyond that of cytochromes P-450a, b, f, and j (Fig. 6), only a few amino-terminal residues are probably missing. The open reading frame of 493 amino acid residues defines a polypeptide with a calculated minimal molecular mass of 56,576 daltons. There is a single potential site for *N*-linked glycosylation at Asn-206 and a potential phosphorylation site for cAMP-dependent protein kinase at Ser-131.

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                    10                  20                  30                  40
  A L G G A F S I F M T L C L S C L L I L I A W K R T S R G G K L P P G P T P I
  GGCACTGGGAGGGGCCTTCAGCATCTTTATGACGCTCTGCTTGTCTTGCCTGCTCATCCTGATAGCTTGGAAGCGAACCAGCAGGGGAGGAAAGCTGCCTCCGGCCCCACACCAATA  120
                    50                  60                  70                  80
P F L G N L L Q V R I D A T F Q S F L K L Q K K Y G S V F T V Y F G P R P V V I
CCATTTTTGGGGAACCTGTTACAAGTACGCATTGATGCCACATTTCAGTCCTTTCTGAAGCTCCAGAAAAAGTATGGCTCTGTGTTCACCGTATACTTTGGCCCAAGGCCAGTGGTTATC  240
                    90                  100                 110                 120
L C G H E A V K E A L V D Q A D D F S G R G E M P T L E K N F Q G Y G L A L S N
TTATGTGGACATGAGGCAGTGAAGGAGGCCCTGGTGGACCAGGCAGATGACTTCAGTGGTCGTGGTGAAATGCCTACACTAGAGAAGAACTTTCAAGGTTATGCTTTAGCTCTGTCCAAT  360
                    130                 140                 150                 160
G E R W K I L R R F S L T V L R N F G M G K R S I E E R I Q E E A G Y L L E E L
GGAGAACGATGGAAGATTCTTCGGCGTTTTCCCTGACTGTCGTTCGAAACTTTGGAATGGGGAAGCGAAGCATTGAGGAGCGGATCCAGGAGGAGCCCGGCTACCTACTGGAGGAACTC  480
                    170                 180                 190                 200
H K V K G A F I D P T F Y L S R T V S N V I C S V V F G K R F D Y E D Q R F R S
CATAAGGTCAAGGGAGCTCCCATAGATCCCACCTTCTACCTGAGTCCGCACTGTGTCCAATGTCATCTGTTCCGTCGTCTTTGGAAAACGTTTCGACTATGAGGACCAGCGTTTCCGGAGC  600
                    210                 220                 230                 240
L M K M I N E S F V E M S M P W A Q L Y D M Y W G V I Q Y F P G R H N R L Y N L
CTCATGAAGATGATCAATGAGAGCTTTGTCGAGATGAGCATGCCCTGGGCACAGTTATATGATATGTACTGGGGAGTCATACAGTATTTTCCAGGAAGACACAACCGCCTCTACAACTTG  720
                    250                 260                 270                 280
I E E L K D F I A S R V K I N E A S F D P S N P R D F I D C F L I K M Y Q D K S
ATAGAAGAGCTTAAGGACTTCATTGCTTCCAGGGTCAAGATCAATGAAGCATCCTTTGACCCCTCAAATCCTAGGGACTTCATCGACTGCTTTCTTATTAAGATGTACCAGCATAAAAGT  840
                    290                 300                 310                 320
D P H S E F N L K N L V L T T L N L F F A G T E T V S S T L R Y G F L L L M K Y
GACCCACACTCAGAATTCAACCTCAAGAACTTGGTTCTCACCACCCTCAACCTCTTCTTTGCTGGCACGGAAACTGTGAGTTCCACACTACGATATGGATTCTTGTTGCTAATGAAGTAC  960
                    330                 340                 350                 360
P E V E A K I H E E I N Q V I G T H R T P R V D D R A K M P Y T D A V I H E I Q
CCCGAGGTGGAGGCCAAGATCCATGAGGAGATTAACCAGGTGATTGCAACACACCGTACCCCAAGAGTGGATGACCGGGCCAAGATGCCCTACACTGATGCTGTCATCCATGAGATACAG 1080
                    370                 380                 390                 400
R L T D I V P L G V P H N V I R D T H F R G Y F L P K G T D V Y P L I G S V L K
AGATTGACAGATATTGTGCCTCTGGGTGTTCCCCATAATGTCATACGGCACACTCATTTCCGAGGCTACTTCCTACCCAAGGGTACAGATGTGTATCCCCTCATTGGCTCAGTCCTCAAA 1200
                    410                 420                 430                 440
D P K Y F R Y P E A F Y P Q H F L D E Q G R F K K N D A F V A F S S G K R I C V
GATCCCAAATACTTCCGCTACCCAGAAGCTTTTTATCCTCAGCACTTCCTGGATCAACAAGGGCGCTTCAAGAAGAATGATGCCTTTGTGGCCTTTTCCTCTGGAAAGCGCATCTGTGTC 1320
                    450                 460                 470                 480
G E A L A R M E L F L Y F T S I L Q R F S L R S L V P P A D I D I A H K I S G F
GGGGAGGCCCTGGCCCGTATGGAGCTCTTCCTCTACTTCACCTCCATCCTTCAGAGGTTCTCCTTACGTTCTCTGGTGCCTCCTGCTGATATCGACATCGCTCACAAAATCTCAGGCTTT 1440
                    490
G N I P P T Y E L C F M A R
GGCAACATTCCCCCAACATATGAGCTCTGCTTCATGGCCCCGCTGAGTGCCCACTTCGAGACCAACCAAGAGTCAATTGAGAGAGGAGCTCTGCTGGAGTTCACGCACACCCGATCTCCCT 1560

CCAACCCTCCCTACTCATCTAAGAGAAGTCGCCTCTCATTGCTGTGCTGAGACCACGGGTCAGGAGAGGTGTCTGCAAAAACATGGCTGGATGGTATCAAGCAGGGTTCTAGCCACAGTCT 1680

CGGTTTCGGAGAGCCCTGAATTTCTCAACCTTGGTCTCGGTGGGCAGCAAACTTACAGATGCTGTGGGTTTACATAATCTATTGACTTTGCCCTGATATTGACCAGAATGTACTCCGCAA 1800

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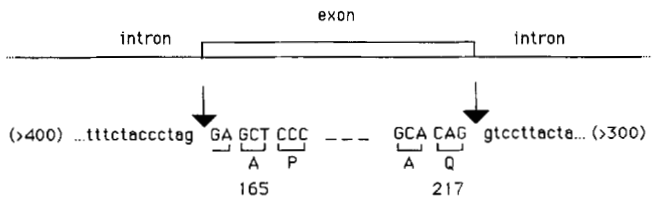

FIG. 3. **Schematic structure of cytochrome P-450olf1 exon.** The exon structure was deduced from cDNA clone No. 15 (0.7 kb). This clone includes two regions that are absent in cytochrome P-450olf1, most probably intronic sequences, flanking the region shown in *capital letters*, which is identical to nucleotides 494–654 of cytochrome P-450olf1. The 5′ boundary of this exon is confirmed by the sequence of clone No. 27, which shows a normally spliced cDNA sequence from the poly(A) stretch to nucleotide 494, but includes the same intervening sequence upstream. *Lowercase nucleotide symbols* indicate acceptor and donor sites of intervening sequences (*cf.* Lerner *et al.*, 1980). *Numbers* in *parentheses* indicate the minimal length in base pairs of the corresponding introns. The borders of the exon shown, located at the numbered amino acid positions, are exactly homologous (Fig. 6) to those of exon 4 in other cytochrome P-450II family members (*cf.* Adesnik and Atchison, 1986).

DISCUSSION

Structural Features of Cytochrome P-450olf1—We have deduced the primary structure of cytochrome P-450olf1 encoded by cDNA clones isolated from a rat olfactory epithelial cDNA library. Cytochrome P-450olf1 shows considerable sequence identity to other cytochrome P-450 proteins of family II (Fig. 6). Segments of high sequence similarity are found all along the protein sequence except for positions 2–30, 195–229, and 234–258, where high variability is seen among all the cytochrome P-450 proteins analyzed. Of the 181 consensus residues of the cytochrome P-450II family, 162 (90%) are also found in cytochrome P-450olf1 (marked by *asterisks* in Fig. 6).

Cytochrome P-450olf1 protein is similar to other cytochrome P-450 species in additional ways: 1) olf1 hydrophobicity profile shows a striking similarity to that obtained by averaging the profiles of representative members of the rat cytochrome P-450II family (Fig. 7). Furthermore, the profile indicates that the first 28 amino acids of cytochrome P-450olf1 form a very hydrophobic region. This is in accordance with the profile of the other cytochrome P-450II proteins (Fig. 7), and with the observation that an amino-terminal hydrophobic region is present in all the microsomal cytochrome P-450 enzymes and serves as an insertion and stop transfer signal (Szczesna-Skorupa *et al.*, 1988; Sakaguchi *et al.*, 1987). This region has been proposed to anchor the protein in the membrane (Nelson and Strobel, 1988; De Lemos-Chiarandini *et al.*, 1987). 2) The highly conserved region (residue 430–450), probably involved in the formation of the heme binding pocket (Nebert and Gonzalez, 1987), contains at position 438 the cysteine bound to the fifth coordination site of the iron atom (Poulos *et al.*, 1986). These observations indicate that cytochrome P-450olf1 is a functional cytochrome P-450 molecule.

A single potential *N*-glycosylation site (Asn-205) is present, but is probably fortuitous, as it is absent from all the other cytochrome P-450 proteins. Furthermore, the region surrounding this Asn residue has not been proposed to be in the endoplasmic reticulum lumen in the membrane topology models for cytochrome P-450 (Nelson and Strobel, 1988; De Lemos-Chiarandini *et al.*, 1987, see also Black and Coon, 1987) and hence is not likely to be glycosylated.

The potential site for Ser/Thr phosphorylation by cAMP-dependent protein kinase (RR*X*S or RR*X*T) found at position 130 of cytochrome P-450olf1 is more likely to be functional, as it occurs in all the cytochrome P-450olf1 sequences of Fig. 6, as well as in many other cytochrome P-450 proteins (Nelson and Strobel, 1988).

A New Cytochrome P-450II Subfamily—Alignment of cytochrome P-450olf1 with other rat microsomal cytochrome P-450 sequences revealed 38–53% identity with the members of family II and less than 30% identity with members of other families (Table I), suggesting that it belongs to family II. Since cytochrome P-450olf1 does not show identity greater than 53% with any other known cytochrome P-450 sequence, it represents the first member of a new cytochrome P-450

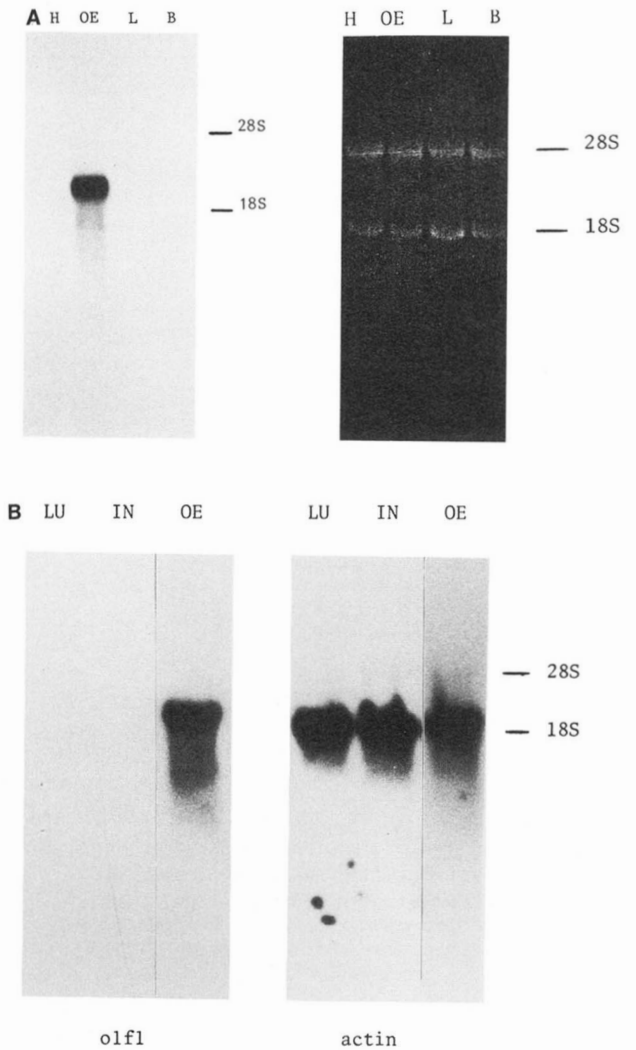

FIG. 4. **RNA blot analysis of rat cytochrome P-450olf1 transcripts.** *A*, *left*, 20 μg total RNA from rat heart (*H*), olfactory epithelium (*OE*), liver (*L*), and brain (*B*) were blotted and hybridized to the radioactively labeled cytochrome P-450olf1 probe (clone No. 27). Similar results were obtained with a clone No. 30 probe. The estimated size of cytochrome P-450olf1 mRNA is 2.0–2.2 kb. *Right*, ethidium bromide staining of 18 S and 28 S rRNA provides a measure of the quantity of undegraded RNA in the correspondingly marked lanes. *B*, *left*, a similar comparison of cytochrome P-450olf1 hybridization of a clone No. 30 probe to total RNA from rat lung (*LU*), intestine (*IN*), and olfactory epithelium (*OE*). *Right*, blotting of the same lanes with a β-actin probe (Hanukoglu *et al.*, 1983) to provide a measure of the amount of RNA loaded.

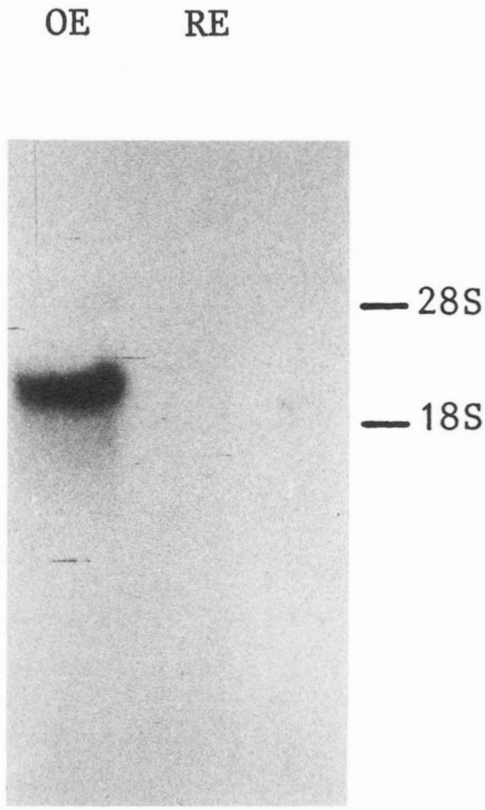

FIG. 5. **RNA blot analysis of bovine cytochrome P-450olf1 related transcripts.** 10 μg polyadenylated RNA from cow olfactory (*OE*) and respiratory (*RE*) epithelia were hybridized as described in Fig. 4. The size of cow cytochrome P-450olf1 transcripts is in the same range as the rat cytochrome P-450olf1 mRNA (~2.1 kb). Both lanes had similar amounts of undegraded RNA, as estimated by ethidium bromide staining, as in Fig. 4*A* (not shown).

subfamily, which is designated cytochrome P-450IIG in accordance with the most recent update of the cytochrome P-450 nomenclature (Nebert *et al.*, 1989). The assignment of cytochrome P-450olf1 to family II is strengthened by the finding that the splicing positions delimiting one exon of cytochrome P-450olf1 (Fig. 3) are identical to those of exon 4 present in genes of subfamilies IIB, IIC, IID, IIE, but different from genes of families I and XXI (*cf.* Adesnik and Atchison, 1986; Nebert and Gonzalez, 1987).

Cytochrome P-450b in the Olfactory Epithelium—Cytochrome P-450b transcripts are hardly detectable in total liver RNA prepared from untreated rat (Affolter *et al.*, 1986; Omiecinski *et al.*, 1985). However, the hepatic gene is actively transcribed after phenobarbital injection. The fact that we have isolated many cytochrome P-450b cDNA clones and easily detected cytochrome P-450b mRNA in total olfactory RNA from untreated rats indicates either that cytochrome P-450b gene expression is constitutively higher in the olfactory tissue or that some environmental chemicals have served as inducing agents.

Interestingly, we have not isolated cytochrome P-450e cDNA clones. Cytochrome P-450e is also phenobarbital-inducible and shares 97% amino acid identity with cytochrome P-450b (Suwa *et al.*, 1985). Northern analyses have recently demonstrated that cytochrome P-450e transcripts are also absent in the lung and testes of untreated animals, whereas cytochrome P-450b is constitutively expressed in these tissues (Omiecinski, 1986). At present we do not know whether the mechanism of cytochrome P-450b/e gene regulation in olfactory epithelium is similar to that in other tissues.

Possible Function of Olfactory Cytochromes P-450—Our RNA blot analysis (Figs. 4 and 5) shows that cytochrome P-450olf1 transcripts are olfactory-specific and conserved in at least two mammalian species. Cytochromes P-450olf1 and cytochrome P-450b are good candidates for catalyzing the modification and detoxification of inhaled xenobiotics (*cf.* Dahl *et al.*, 1982; Brittebo and Ahlman, 1984; Dahl, 1988). The cytochrome P-450II family, which includes both cytochrome P-450olf1 and cytochrome P-450b, is the largest characterized monooxygenase family, and its members are known to catalyze the conversion of numerous hydrophobic compounds. Among these are several known odorants, such as anisole and coumarin derivatives (Dahl *et al.*, 1982; Jenner and Dodd, 1988). Thus, it is likely that the cytochrome P-450 species specifically expressed in olfactory epithelium catalyze the conversion of odorants, either as a means of generating a

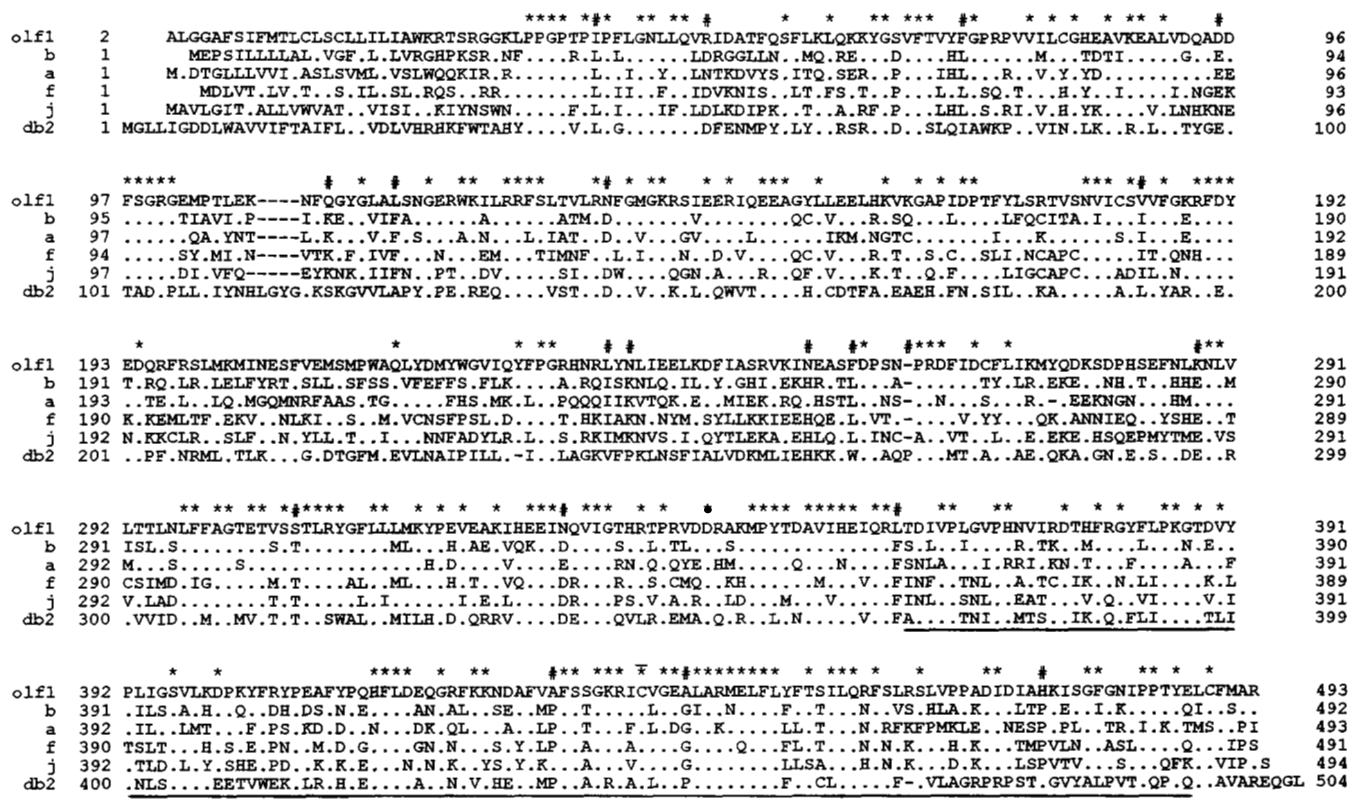

FIG. 6. **Alignment of cytochrome P-450olf1 with representative rat cytochrome P-450II protein sequences.** *b*, cytochrome P-450b, subfamily IIB; *a*, cytochrome P-450a, subfamily IIA; *f*, cytochrome P-450f, subfamily IIC; *j*, cytochrome P-450j, subfamily IIE; *db2*, cytochrome P-450db2, subfamily IID (sequence information is from Nelson and Strobel, 1988). A *dot* shows identity to the amino acid in cytochrome P-450olf1. Positions at which four or all five of the non cytochrome P-450olf1 sequences are identical are marked on top: *, agreement of cytochrome P-450olf1 with this consensus; #, deviation of olf1 from the consensus. The functional Cys-438 is marked with a *bar* above the *asterisk*. The position of the cytochrome P-450e probe used for library screening is marked by a *line* (positions 362–487). The percent identity between cytochrome P-450olf1 and the other proteins is shown in Table I.

more active stimulus or as a means of turning off the olfactory signal so that new stimuli can be received (*cf.* Dahl *et al.*, 1982; Brittebo and Ahlman, 1984; Margolis, 1987; Dahl, 1988; Lancet *et al.*, 1988). In this, olfactory cytochrome P-450 may complement the proposed function of odorant binding proteins (Snyder *et al.*, 1988).

It has been reported that olfactory epithelium contains high and tissue-specific cytochrome P-450-dependent enzymatic activities, *e.g.* 7-ethoxycoumarin deethylase and hexobarbitone oxidase (Reed *et al.*, 1986). We propose that cytochrome P-450olf1 protein may be related to such activities, as it is also highly and specifically expressed in the olfactory epithelium. Additional cytochrome P-450 species are probably present in the olfactory epithelium, to accommodate the broad spectrum of odorants. Cytochrome P-450olf1 cDNA could serve as a useful probe to identify such enzymes.

Tissue Expression of Cytochrome P-450olf1—A central finding of the present report is that cytochrome P-450olf1 is expressed in olfactory epithelium, but not in several control tissues. This comparison relates only to uninduced levels, and possible induction by various chemicals in olfactory epithelium and other tissues is currently under study. The absence of cytochrome P-450olf1 in the anatomically adjacent respiratory epithelium is particularly notable, since both nasal epithelia are exposed to a very similar chemical environment during inhalation. A possible explanation is that odorant receptors, which appear to be coupled to stimulatory GTP-binding protein and adenylate cyclase (*cf.* Lancet, 1986; Lancet and Pace, 1987; Snyder *et al.*, 1988), may bind and concentrate odorants in olfactory, but not in respiratory epithelium. This would necessitate a tissue-specific odorant modification and clearance mechanism.

At present, the cellular localization of cytochrome P-450olf1 within the olfactory epithelium or subepithelium is unknown. Previously, cytochrome P-450 enzymes were shown to be present in the epithelial supporting cells and in the subepithelial Bowman's glands (Voigt *et al.*, 1985; Morgan, 1988). Morgan (1988) has proposed on the basis of cytochrome P-450 reductase distribution in the olfactory epithelium that the sensory neurons may have their own subtypes of the enzyme. The presence of cytochrome P-450olf1 in any of these cellular sites remains to be investigated.

An interesting subject for future studies would be the control of cytochrome P-450olf1 expression in olfactory epithelium. Previous studies have shown that cytochrome P-450-dependent enzymatic activities and cytochrome P-450-related epitopes are not readily induced in this tissue by classical modulators such as phenobarbital and 3-methylcholanthrene (Bond, 1983; Foster *et al.*, 1986). It is possible that olfactory cytochrome P-450 enzymes are strictly constitutive. Alternatively, they may be induced by as yet unknown chemicals, including odorants. Such induction experiments could also help establish the substrate specificity of cytochrome P-450olf1 and shed light on its possible function.

Acknowledgments—We are grateful to Dr. G. Padmanaban (Indian Institute of Science, Bangalore, India) for the generous gift of cDNA

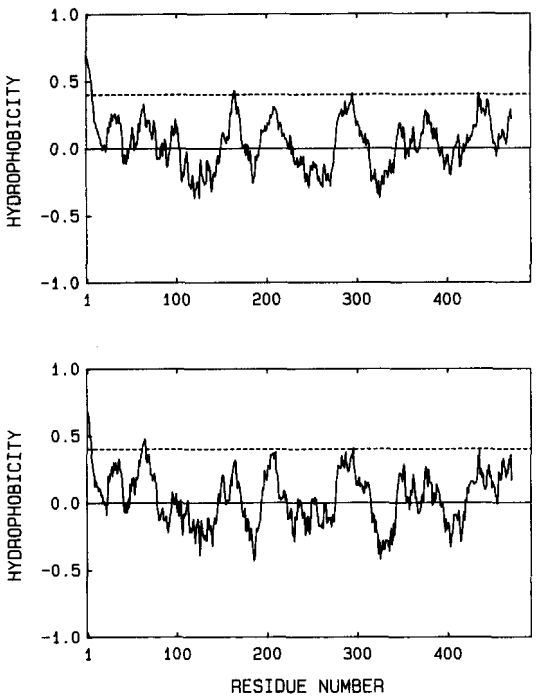

FIG. 7. **Hydrophobicity profile.** Comparison of the hydrophobicity profile of cytochrome P-450olf1 (*bottom*) with an average profile (*top*) of the five rat cytochrome P-450II sequences of Fig. 6. The normalized scale of Eisenberg *et al.* (1984) was used with a 21-residue "window." The *dotted line* indicates a minimal cutoff hydrophobicity value of +0.42 proposed by Eisenberg *et al.* (1984) for predicting membrane spanning segments. The four extra residues of cytochrome P-450db2 at positions 113–117 (Fig. 6) are not included in the analysis.

TABLE I

Percent identity between the amino acid sequence of protein olf1 (subfamily IIG) and rat cytochrome P-450 proteins derived from four gene families (I, II, III, IV)

Protein sequence references from Nebert *et al.* (1989).

Trivial name	Family	Subfamily	%
c	I	A	30
a	II	A	51
b	II	B	53
f	II	C	49
db2	II	D	38
j	II	E	49
p	III	A	22
LAω	IV	A	21

clone 91 and to Dr. M. Ballivet (University of Geneva, Geneva, Switzerland) for the kind support in performing some of the DNA sequence determination.

REFERENCES

Adesnik, M., and Atchison, M. (1986) *CRC Crit. Rev. Biochem.* **19,** 247–305

Affolter, M., Labbe, D., Jean, A., Raymond, M., Noel, D. Labelle, Y., Parent-Vaugeois, C., Lambert, M., Bojanowski, R., and Anderson, A. (1986) *DNA* **5,** 209–218

Aufray, C., and Rougeon, F. (1980) *Eur. J. Biochem.* **107,** 303–314

Benton, W., and Davis, R. (1977) *Science* **196,** 180–182

Black, S., and Coon, M. (1987) *Adv. Enzymol.* **60,** 35–85

Bond, J. A. (1983) *Cancer Res.* **43,** 4805–4811

Brittebo, E. B., and Tjälve, H. (1981) *Carcinogenesis* **2,** 959–963

Brittebo, E., and Ahlman, M. (1984) *Chem. Biol. Interact.* **50,** 233–245

Brittebo, E., and Rafter, J. (1984) *J. Steroid Biochem.* **20,** 1147–1151

Dahl, A. R. (1988) in *Molecular Neurobiology of the Olfactory System* (Margolis, F. L., and Getchell, T. V., eds) pp. 51–70, Plenum Publishing Corp., New York

Dahl, A., and Hadley, W. (1983) *Toxicol. Pharmacol.* **67,** 200–205

Dahl, A., Hadley, W., Hahn, F., Benson, J., and McClellan, R. (1982) *Science* **216,** 57–59

Davis, L., Dibner, M., and Battey, J. (1986) *Basic Methods in Molecular Biology*, Elsevier Science Publishing Co., Inc., New York

De Lemos-Chiarandini, C., Frey, A. B., Sabatini, D. D., and Kreibich, G. (1987) *J. Cell Biol.* **104,** 209–219

Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. (1984) *J. Mol. Biol.* **179,** 125–142

Foster, G. R., Elcombe, C. R., Boobis, A. R., Davies, D. S., Sesardic, D., McQuade, J., Robson, R. T., Hayward, C., and Lock, E. A. (1986) *Biochem. Pharmacol.* **35,** 4543–4554

Hanukoglu, I., Tanese, N., and Fuchs, E. (1983) *J. Mol. Biol.* **163,** 673–678

Jenner, J., and Dodd, G. (1988) *Biochem. Pharmacol.* **37,** 558–559

Lancet, D. (1986) *Annu. Rev. Neurosci.* **9,** 329–355

Lancet, D., and Pace, U. (1987) *Trends Biochem. Sci.* **12,** 63–66

Lancet, D., Lazard, D., Heldman, J., Khen, M., and Nef, P. (1988) *Cold Spring Harbor Symp. Quantit. Biol.* **53,** 343–348

Lehrach, H., Diamond, D., Wozney, J. M., and Boedtker, H. (1977) *Biochemistry* **16,** 4743–4751

Lerner, M. A., Boyle, S. A., Mount, S. M., Wonin, S. L., and Steitz, S. A. (1980) *Nature* **283,** 220–223

Maniatis, T., Fritsch, E., and Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Margolis, F. L. (1987) in *Sensory Transduction, Discussion in Neuroscience* (Hudspeth, A. J., MacLeish, P. R., Margolis, F. L., and Wiesel, T. N., eds) Vol. 4, No. 3, pp. 47–52, FESN, Geneva, Switzerland

Messing, J., and Vieira, J. (1982) *Gene (Amst.)* **19,** 269–276

Morgan, J. I. (1988) in *Molecular Neurobiology of the Olfactory System* (Margolis, F. L., and Getchell, T. V., eds) pp. 269–296, Plenum Publishing Corp., New York

Nebert, D., and Gonzalez, F. (1987) *Annu. Rev. Biochem.* **56,** 945–993

Nebert, D., Nelson, D. R., Adesnik, M., Coon, M., Estabrook, R., Gonzalez, F., Guengerich, F., Gunsalus, I., Johnson, E., Kemper, B., Levin, W., Phillips, I., Sato, R., and Waterman, M. (1989) *DNA* **8,** 1–13

Nef, P., Lazard, D., Heldman, J., Khen, M., and Lancet, D. (1988) in *The Molecular Biology of Signal Transduction, Abstracts of the LIII Cold Spring Harbor Symposium on Quantitative Biology*, p. 27, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Nelson, D. R., and Strobel, H. W. (1988) *J. Biol. Chem.* **263,** 6038–6050

Omiecinski, C. (1986) *Nucleic Acids Res.* **12,** 1349–1359

Omiecinski, C. J., Walz, F. G., Jr., and Vlasuk, G. P. (1985) *J. Biol. Chem.* **260,** 3247–3250

Poulos, T. L., Finzel, B. C., and Howard, A. J. (1986) *Biochemistry* **25,** 5314–5322

Ravishankar, H., and Padmanaban, G. (1985) *J. Biol. Chem.* **260,** 1588–1592

Reed, C., Lock, E., and Matteis, F. (1986) *Biochem. J.* **240,** 585–592

Rigby, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251

Sakaguchi, M., Mihara, K., and Sato, R. (1987) *EMBO J.* **6,** 2425–2431

Sanger, F., Nicklen, S., and Coulson, A. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5463–5467

Snyder, S. H., Sklar, P. B., and Pevsner, J. (1988) *J. Biol. Chem.* **263,** 13971–13974

Suwa, Y., Mizukami, Y., Sogawa, K., and Fujii-Kuriyama, Y. (1985) *J. Biol. Chem.* **260,** 7980–7984

Szczesna-Skorupa, E., Browne, N., Mead, D., and Kemper, B. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 738–742

Vieira, J., and Messing, J. (1982) *Gene (Amst.)* **19,** 259–268

Voigt, J. M., Guengerich, F. P., and Baron, J. (1985) *Cancer Lett.* **27,** 241–247

Wilbur, W., and Lipman, D. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 726–730