Fetus-Specific Expression of a Form of Cytochrome P-450 in Human Livers†

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ABSTRACT: The developmentally regulated expression of forms of cytochrome P-450, namely, those encoded by λHFL33 and NF25 or HLp cDNAs, which were isolated from respective fetal and adult human liver cDNA libraries, was investigated. When *Eco*RI fragments of cDNA clones of λHFL33 and NF25 were used as probes, these probes hybridized with RNA from both fetal and adult human livers. However, when oligonucleotides specific to the coding and 3′-noncoding region of λHFL33 (oli-HFL and oli-HFL3′, respectively) were used as probes, these probes gave hybridizable bands with RNA from fetal but not adult livers. On the other hand, an oligonucleotide probe specific to the coding region of NF25 and HLp (oli-NF) gave positive bands with RNA only from adult livers. These results indicate that P-450(HFL33) is expressed specifically in fetal livers and that neither $P\text{-}450_{NF}$ nor HLp is expressed in fetal livers, but one or both are expressed in adult livers.

Cytochrome P-450 is a heme-containing enzyme which catalyzes the oxidation and reduction of a wide variety of endogenous and exogenous substrates such as steroids, drugs, and toxicants. Unlike many experimental animals, human fetal liver possesses cytochrome P-450 (Yaffe et al., 1970), suggesting the pharmacological and toxicological consequences specifically in human fetuses after treatment with such foreign compounds. Recently, we purified a major form of cytochrome P-450 (designated as P-450 HFLa) from human fetal livers (Kitada et al., 1985) and clarified that P-450 HFLa catalyzed the 16α-hydroxylation of dehydroepiandrosterone 3-sulfate as one of the physiological functions (Kitada et al., 1987) and mutagenic activation of aflatoxin B_1 (Kitada et al., 1989a).

More recently we were capable of isolating a cDNA clone (λHFL33, CYP3A6)[1] containing the entire coding region for a form of cytochrome P-450 related to P-450 HFLa from a human fetal liver cDNA library (Komori et al., 1989a). The nucleotide and deduced amino acid sequences of λHFL33 were very similar to but distinct from those of NF25 (phPCN1) (CYP3A4) (Beaune et al., 1986; Gonzalez et al., 1988) and HLp cDNAs (CYP3A3) (Molowa et al., 1986), which were isolated from a human adult liver cDNA library as representative cDNA clones of cytochrome P-450 belonging to the P-450III gene family (Nebert et al., 1987). Northern blot analysis using various cDNA clones as probes showed that human fetal livers expressed limited forms of cytochrome P-450 belonging to this gene family (Komori et al., 1989b; Ohgiya et al., 1989). Thus, this study was undertaken to further clarify the characteristic expression of cytochrome P-450 in human fetal livers. We show here the fetus-specific expression of mRNA for P-450(HFL33)[2] and adult-specific expression of mRNA for $P\text{-}450_{NF}$ (Guengerich et al., 1986) or HLp (Watkins et al., 1985) or both, using specific oligonucleotide probes.

Materials and Methods

Materials. Biochemical reagents were obtained from sources as follows: Klenow fragment of *Escherichia coli* DNA polymerase I, DNA ligation kit, M13 cloning and sequencing kits, DNA labeling kit (Takara Shuzo); nick-translation kit (Nippon Gene); [α-^{32}P]dCTP (3000 Ci/mmol), [α-^{35}S]-dATPαS (650 Ci/mmol), and λgt11 cloning system (Amersham International); nitrocellulose filters (Toyo Roshi). Other chemicals used were of the highest grade available.

Screening of the Adult Human Liver cDNA Library. For the preparation of a probe and for the examination of the specificity of synthetic oligonucleotide probes, a cDNA clone possessing nucleotide sequences identical with NF25 was obtained. The screening of the commercially available human adult liver cDNA library in λgt11 (Clontech Laboratory Inc.; titer, 2×10^9/mL; average insert size, 0.82 kb) was performed by plaque hybridization using the middle *Eco*RI fragment of λHFL33 as a probe. Phage DNAs of positive clones were isolated by standard protocols (Maniatis et al., 1982), and their inserts were subcloned into pUC18 (Boehringer Mannheim). Plasmid DNA was isolated by the alkaline lysis method (Maniatis et al., 1982). The nucleotide sequences of cDNA inserts were determined by the dideoxy termination method (Sanger et al., 1980).

Isolation of RNA. Human fetal livers were excised from fetuses, stillborn or prematurely delivered by therapeutic

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[1] Nomenclature for the human P-450III gene family used were as follows: CYP3A3, HLp; CYP3A4, NF25 (hPCN1); CYP3A6, P-450-(HFL33). The nomenclature CYP3A6 for P-450(HFL33) has been recently proposed by Dr. D. W. Nebert.

[2] We indicate the name of cytochrome P-450 encoded by λHFL33 as P-450(HFL33).

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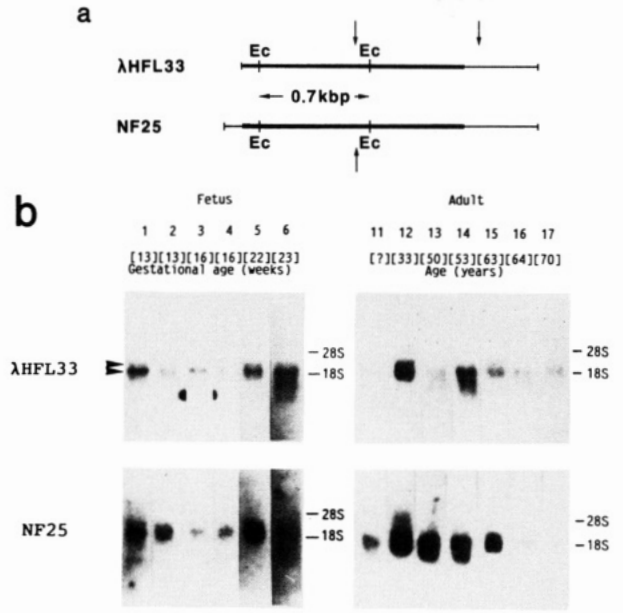

FIGURE 1: Northern blot analysis of RNA from human livers with cDNA fragments as probes. (a) The 0.7-kb *Eco*RI fragments of λHFL33 and NF25, which were ^{32}P-labeled by nick-translation and used as probes, are shown. Thick and thin lines indicated the coding and noncoding regions, respectively. Vertical arrows indicate the positions of oligonucleotide probes. (b) Total RNA (5 μg) from each sample was used. The conditions of hybridization and after-washing were described previously (Imai et al., 1988). Numbers in brackets represent gestational age (weeks) or age (years) of fetuses and adults, respectively. The age of the sample on lane 11 was unknown. Arrowheads indicate two different species of mRNA (near 19 S and 23 S).

abortion. Specimens of human adult livers were obtained by hepatic surgery of cancer. Total RNA was isolated from six fetal and seven adult livers by the guanidine thiocyanate method (Raymond & Shore, 1979).

Synthesis of Oligonucleotide Probes. Oligonucleotides, oli-HFL and oli-NF, were synthesized (Applied Biosystems) as specific probes (19-mer), which were in the coding region of λHFL33 (nucleotides 643–661) (Komori et al., 1989a,b) and NF25 (nucleotides 709–727) (Beaune et al., 1986), respectively. A 17-mer oligonucleotide (oli-HFL33′) was also synthesized as a specific probe for the 3′-noncoding region of λHFL33 (nucleotides 1586–1602).

Northern and Southern Blot Analysis. The 5′ ends of synthetic oligonucleotides were ^{32}P-labeled by T4 polynucleotide kinase (Takara Shuzo). Total RNA from each sample was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde and transferred to a nitrocellulose filter. Northern blot hybridization using cDNA fragments as probes was carried out as described previously (Imai et al., 1988). In the case of Southern blot hybridization, subcloned plasmids containing the middle *Eco*RI fragments of λHFL33 and NF25 were digested with *Eco*RI, and the digests were electrophoresed on a 1% agarose gel and then transferred to a nitrocellulose filter. ^{32}P-Labeled oligonucleotide probes were hybridized with the filters in a solution containing 0.75 M NaCl, 0.075 M sodium citrate, 0.02% Ficoll 400, 0.02% poly(vinylpyrrolidone), 0.02% bovine serum albumin, 10 mM EDTA, 0.1% SDS, and 0.25 μg/mL heat-denatured salmon sperm DNA at a temperature 8 °C below the T_m. After hybridization, filters were washed with a solution containing 0.75 M NaCl, 0.075 M sodium citrate, and 0.1% SDS first at room temperature and then at the same temperature of hybridization. Autoradiography was carried out for 16–24 h at −80 °C with an intensifying screen. The nitrocellulose filters were stained with methylene blue after autoradiography as described (Maniatis et al., 1982).

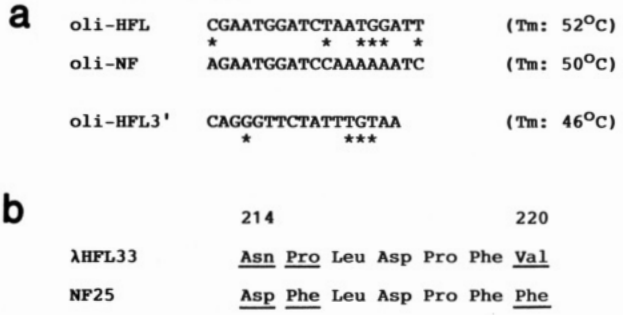

FIGURE 2: Nucleotide sequence of oli-HFL, oli-NF, and oli-HFL3′. The nucleotide (a) and the deduced amino acid sequences (b) of λHFL33 and NF25 or phPCN1, which were selected for the synthesis of specific oligonucleotide probes, are shown. Nucleotides different between λHFL33 and NF25 (or phPCN1) are indicated by asterisks.

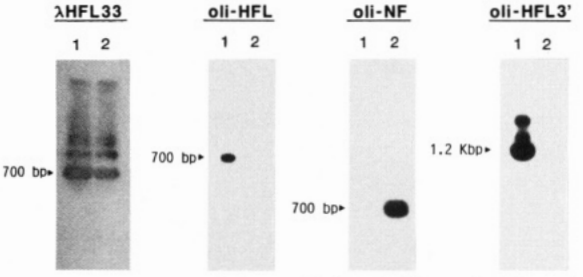

FIGURE 3: Examination of the specificity of oligonucleotide probes for respective cDNAs. Southern plot hybridization was carried out using the indicated cDNA fragment or synthetic oligonucleotide probes. Subcloned plasmid DNAs containing the *Eco*RI fragments of λHFL33 (lane 1) and NF25 (lane 2) were digested with *Eco*RI. The positions of the middle *Eco*RI fragment (0.7 kb) and 3′ side *Eco*RI fragment (1.2 kb) of λHFL33 and NF25 were indicated by arrowheads. Upper bands were derived from undigested ones.

Results and Discussion

A cDNA clone (approximately 1.9 kb) corresponding to NF25 could be isolated from adult human liver cDNA library in λgt11 by using the middle *Eco*RI fragment of λHFL33 as a probe. This clone contained at least two *Eco*RI fragments (0.7 and 1.2 kb). The nucleotide sequence of this 0.7-kb *Eco*RI fragment, which was used as a probe for following Northern blot analysis, was identical with that of the middle *Eco*RI fragment of NF25.

The expression of mRNA related to λHFL33 and NF25 was examined by Northern blot analysis using the middle *Eco*RI fragments of λHFL33 and NF25 as probes (Figure 1a). As shown in Figure 1b, a major transcript (near 19 S) and a minor one (near 23 S) were detectable in both human fetal and adult livers with either probe in a similar manner, suggesting that the middle *Eco*RI fragments of cDNA clones λHFL33 and NF25 are not specific to determine mRNAs coding for each cytochrome P-450. Although the cDNA fragment of λHFL33 gave a weak band in fetus 2 and adult 13, these signals were confirmed by the autoradiogram of longer exposure (data not shown). In accordance with the results of Northern blot analysis, we observed a cross-reactivity between antibodies to P-450 HFLa and P-450-HM1 (Komori et al., 1988) which is believed to be identical with P-450$_{NF}$ and encoded by the clone NF25 (data not shown). The evidence that fetal and adult forms of cytochrome P-450 are different from each other has been provided recently by another laboratory. Wrighton and Vandenbranden purified HLp2 (Wrighton & Vandenbranden, 1989), which seems to be identical with P-450 HFLa, from human fetal livers and

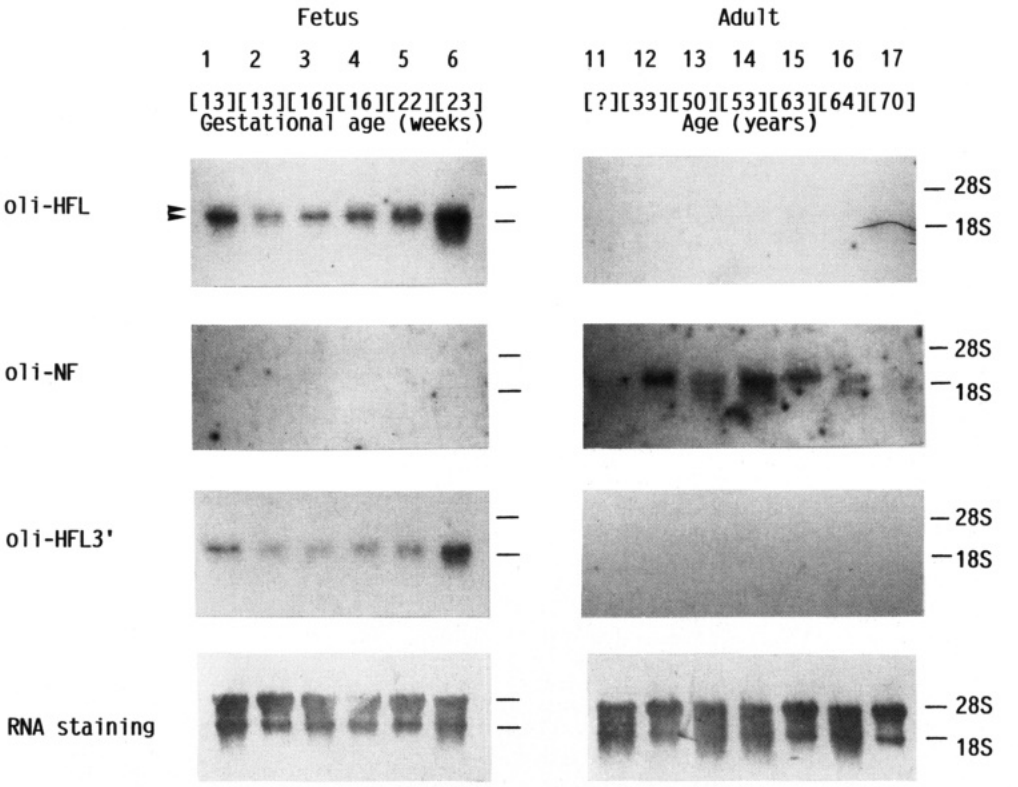

FIGURE 4: Northern blot analysis of RNA from human livers with specific oligonucleotide probes. Total RNA (20 μg) from each sample was used. Hybridization was carried out as described under Materials and Methods. Numbers in brackets represent gestational age (weeks) or age (years) of fetuses and adults, respectively. The age of the sample on lane 11 was unknown.

showed that HLp2 and HLp were different from each other in their peptide maps.

To determine mRNAs related to λHFL33 and NF25 separately, we synthesized oligonucleotide probes expected to be specifically hybridizable with λHFL33 or NF25. Figure 2a shows the nucleotide sequences designed to use in the following experiments. The positions of nucleotides synthesized were as indicated by vertical arrows in Figure 1a. Oli-HFL and oli-NF are distinct from each other; there are six base changes in this region between λHFL33 and NF25. These base changes lead to three changes of the amino acids (Figure 2b). The nucleotide sequence of HLp cDNAs (Molowa et al., 1986) was identical with that of NF25 in this region. Oli-HFL3′ contains four different nucleotides from phPCN1, which is believed to be identical with NF25 (Figure 2a). Two more base changes exist in this region between λHFL33 and HLp cDNA.

First, we examined the specificities of the probes to hybridize with corresponding cDNAs by Southern blot analysis (Figure 3). When the middle *Eco*RI fragment of λHFL33 was used as the probe, it hybridized with both cDNA fragments of λHFL33 and NF25. On the other hand, oli-HFL or oli-HFL3′ and oli-NF hybridized only with the cDNA fragment of λHFL33 and NF25, respectively.

The expression of P-450(HFL33) and P-450_{NF} was investigated by Northern blot analysis using these specific oligonucleotide probes. When oli-HFL was used as a probe, hybridizable bands were detectable only in fetal liver RNA (Figure 4). On the other hand, when oli-NF was used as a probe, mRNAs for P-450_{NF} and HLp were found to express only in adult livers. Furthermore, the results using oli-HFL3′ as a probe highly supported the idea that mRNA for P-450-(HFL33) was specifically expressed in fetal livers (Figure 4). As shown at the bottom of the figure, equal amounts of RNA were applied to each lane in the electrophoresis. This is the

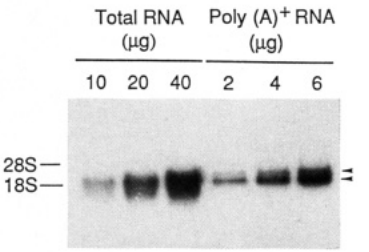

FIGURE 5: Detection of two species of mRNA for P-450(HFL33) using oli-HFL as a probe. The indicated amounts of total and poly(A+) RNA were used. Two species of mRNA (near 19 S and 23 S) were indicated by arrowheads.

first example of the fetus-specific expression of cytochrome P-450 in human livers. However, the mechanism and the biological significance of such developmentally regulated expression of P450III gene in human livers are still unclear.

When oli-HFL was used as a probe, a minor band near 23 S besides a major band around 19 S was detected in all fetal livers as shown in Figure 4, though their autoradiograms were not clear in the case of oli-NF and oli-HFL3′. This minor band was detected more clearly when poly(A+) RNA was used instead of total RNA, as shown in Figure 5. This probably suggests that there would be at least two mRNA species for P-450(HFL33), which were different in the length of their 3′-noncoding region, as in the case of mRNAs reported for P-450_{NF} (Bork et al., 1989).

Our immunohistochemical studies using anti-P-450 HFLa antibodies have shown that P-450 HFLa was detectable in various tissues of gynecologic malignancies (Okajima et al., 1988) and hepatocellular carcinomas (Kitada et al., 1989b). This finding may indicate that P-450 HFLa is one of the onco-feto-placental antigens. Thus, the possibility that P-450 HFLa might serve as a tumor marker is currently under investigation in our laboratory.

Registry No. Cytochrome P-450, 9035-51-2.

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