Identification of a Novel P450 Expressed in Rat Lung: cDNA Cloning and Sequence, Chromosome Mapping, and Induction by 3-Methylcholanthrene†

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ABSTRACT: A novel P450 cDNA was isolated from a rat lung λgt11 library by hybridization with the rat P450 IIB1 cDNA probe. The cDNA-deduced amino acid sequence of this clone was 71% and 73% similar to rat IIA1 and IIA2 P450s; it was, therefore, designated IIA3 as the third member of the rat IIA subfamily. IIA3 demonstrates only 55% amino acid similarity with IIB1. Interestingly, this P450 also shared 85% and 94% amino acid similarities with human IIA3 and a mouse testosterone 15α-hydroxylase P450, respectively, indicating that these P450s are orthologous counterparts to rat IIA3. Chromosome mapping, using mouse–hamster somatic cell hybrids, revealed that the IIA3 gene is localized on mouse chromosome 7. The IIA3 mRNA was detected in rat lung, and its level was induced 3-fold by treatment of rats with 3-methylcholanthrene. No IIA3 mRNA was seen in the liver, kidney, or intestine, even after long exposure of Northern blot filters to X-ray film. In contrast, the orthologous mouse and human IIA3 genes are expressed in liver.

The P450 superfamily[1] consists of eight mammalian, two yeast, and two bacterial gene families [reviewed in Nebert and Gonzalez (1987, 1988)]. The most extensively studied organism in P450 research is the rat. About 15–20 P450s have been isolated and characterized from this animal with respect to both catalytic activities and regulation [reviewed in Guengerich (1987)]. These enzymes are tightly bound to the endoplasmic reticulum lipid bilayer and are extremely difficult to purify. In particular, this applies to P450s present at low levels in untreated rats that are not inducible by classic inducing agents. Most P450s involved in drug and carcinogen metabolism are found in liver, and the cDNAs for virtually every P450 form that has been purified from this organ have been cloned and sequenced.

Although considerable information exists on the biochemistry and molecular biology of liver P450s, relatively little data have accrued on the drug- and carcinogen-metabolizing P450s in extrahepatic tissues. P450s are of particular importance in lung, since this organ is subjected to exposure to environmental contaminants and potential carcinogens, especially those in cigarette smoke. P450s have been isolated from lung (Philpot & Smith, 1984), and some have been cloned (Gasser et al., 1988). However, these and other P450s found in lung are also found in liver (Omiecinski, 1986; Gasser et al., 1988). In general, it is quite difficult to purify P450s from pulmonary tissue, in particular, those forms that are constitutively expressed at low levels. On the basis of sequence similarity between different P450 cDNAs and the complexity of different P450 gene families and subfamilies, we thought it may be possible to screen the libraries from extrahepatic tissues using low-stringency hybridization conditions to isolate cDNA to new previously undetected P450s. To search for P450s expressed in rat lung, we have constructed a cDNA library from untreated rat lung mRNA and screened it with preexisting rat cDNA probes. In the present paper, we describe the isolation and sequence of such a cDNA to a P450 that is specifically expressed in rat lung and inducible by 3-methylcholanthrene.

Materials and Methods

RNA was isolated from untreated 12-week-old female rats by the guanidine thiocyanate method (Chirgwin et al., 1979). A λgt11 library was constructed from 5 μg of poly(A)-selected RNA (Aviv & Leder, 1972) using a method similar to that described by Watson and Jackson (1985). Packaging extract and phosphatase-treated λgt11 arms were obtained from Stratagene Cloning Systems. Approximately 5×10^5 unamplified phage particles were screened with a rat IIB1 full-length cDNA insert. Hybridization was performed at 65 °C in 6× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), 0.5% SDS, 10× Denhardt's medium, and 200 μg/mL denatured and sonicated salmon sperm DNA. After 20 h, the filters were washed with 3× SSC and 0.1% SDS 3 times at 65 °C. Four phage clones were isolated by using these conditions, and an additional two cDNAs were isolated by screening another approximately 5×10^5 unamplified phage from the same library with a fragment obtained from the 5′ end of the IIA3 cDNA that was excised by *Eco*RI digestion (*Eco*RI site is at 268 bp in Figure 1). Their phage cDNA inserts were subcloned into pUC9 and then sequenced by using the shotgun cloning (Deininger, 1983) and dideoxy sequencing (Sanger et al., 1977) methods. Sequence data were analyzed by use of the Beckman Microgenie program. RNA electrophoresis (Lehrach et al., 1977), blotting to nylon filters (Nytran, Schleicher & Schuell), and hybridization and washing (Church & Gilbert, 1984) were performed as described. Filters were exposed to autoradiographic film for various periods of time as indicated in the legend to Figure 4. The levels of hybridization were quantitated by scanning

† The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02852.

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[1] Throughout this paper, we have used the nomenclature recommended by Nebert et al. (1987, 1989).

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rIIA3 CTGGTGGCCTCAGTGGCCTTCCTCAGTGTCCTGGTCTTGATGTCTGTCTGGAAGCAGAGGAAGCTCTCAGGGAAGCTGCCTCCTGGACCCACCCCATTGCCCTTCATCGGG  111
      LeuValAlaSerValAlaPheLeuSerValLeuValLeuMetSerValTrpLysGlnArgLysLeuSerGlyLysLeuProProGlyProThrProLeuProPheIleGly   44

AACTACCTCCAGCTGAACACAGAGAAAATGTACAGCTCTCTCATGAAGATCAGCCAACGTTACGGTCCTGTATTCACCATCCACCTGGGACCTCGCCGAGTTGTGGTGCTGTGCGGA  228
AsnTyrLeuGlnLeuAsnThrGluLysMetTyrSerSerLeuMetLysIleSerGlnArgTyrGlyProValPheThrIleHisLeuGlyProArgArgValValLeuCysGly    83

CAGGAGGCAGTCAAGGAGGCTCTGGTGGACCAAGCTGAGGAATTCAGTGGTCGGGGAGAGCAGGCCACCTTCGACGGGCTTTTCAAAGGCTATGGCGTAGCCTTCAGCAGCGGGGAG  345
GlnGluAlaValLysGluAlaLeuValAspGlnAlaGluGluPheserGlyArgGlyGluGlnAlaThrPheAspGlyLeuPheLysGlyTyrGlyValAlaPheSerSerGlyGlu  122

CGAGCCAAACAGCTAAGGCGCTTCTCCATCGCCACGCTGCGGGACTTCGGCGTGGGCAAGCGTGGCATCGAGGAGCGTATCCAAGAGGAGGCGGGCTTTCTCATCGAGTCATTTCGA  462
ArgAlaLysGlnLeuArgArgPheSerIleAlaThrLeuArgAspPheGlyValGlyLysArgGlyIleGluGluArgIleGlnGluGluAlaGlyPheLeuIleGluSerPheArg  161

AAGACGAACGGTGCCCTCATTGACCCCACCTTCTATCTGAGCCGGACAGTCTCCAATGTCATTAGCTCAATAGTCTTCGGGGACCGCTTCGACTATGAGGACAAAGAGTTCCTGTCA  579
LysThrAsnGlyAlaLeuIleAspProThrPheTyrLeuSerArgThrValSerAsnValIleSerSerIleValPheGlyAspArgPheAspTyrGluAspLysGluPheLeuSer  200

CTGCTTCGAATGATGCTGGGAAGCTTCCAGTTCACAGCTACCTCCACGGGGCAGCTCTATGAGATGTTCTCTTCTGTGATGAAACACCTGCCAGGCCCCCAGCAACAGGCCTTTAAG  696
LeuLeuArgMetMetLeuGlySerPheGlnPheThrAlaThrSerThrGlyGlnLeuTyrGluMetPheSerSerValMetLysHisLeuproGlyProGlnGlnGlnAlaPheLys  239

GAGCTGCAGGGGCTGGAGGACTTCATAACCAAGAAGGTGGAACAGAATCAGCGCACGCTGGATCCCAATTCCCCAAGGGACTTCATCGACTCTTTCCTCATCCGAATGCTGGAGGAA  813
GluLeuGlnGlyLeuGluAspPheIleThrLysLysValGluGlnAsnGlnArgThrLeuAspProAsnSerProArgAspPheIleAspserPheLeuIleArgMetLeuGluGlu  278

AAGAAGAACCCCAATACTGAGTTCTACATGAAGAACTTGGTGCTGACTACCCTAAATCTCTTCTTTGCCGGCACAGAGACCGTCAGCACCACCCTGCGTTACGGCTTTCTGTTGCTC  930
LysLysAsnProAsnThrGluPheTyrMetLysAsnLeuValLeuThrThrLeuAsnLeuPhePheAlaGlyThrGluThrValSerThrThrLeuArgTyrGlyPheLeuLeuLeu  317

ATGAAGCACCCGGATATTGAGGCTAAGGTCCACGAGGAGATTGACCGGGTGAATGGCAGGAACCGGCAGGCCAAGTATGAGGACCGAATGAAGATGCCCTACACGGAGGCTGTGATC  1047
MetLysHisProAspIleGluAlaLysValHisGluGluIleAspArgValAsnGlyArgAsnArgGlnAlaLysTyrGluAspArgMetLysMetProTyrThrGluAlaValIle  356

CACGAGATCCAGAGATTTGCAGACATGATCCCCATGGGCCTGGCTCGCAGGGTCACCAAGGACACCAAGTTTCGAGAGTTCCTCCTCCCCAAGGGTACTGAAGTATTTCCTATGCTG  1164
HisGluIleGlnArgPheAlaAspMetIleProMetGlyLeuAlaArgArgValThrLysAspThrLysPheArgGluPheLeuLeuProLysGlyThrGluValPheProMetLeu  395

GGCTCTGTACTGAAAGACCCTAAGTTCTTCTCCAACCCCAACGACTTCAACCCAAAGCACTTCCTAGATGACAAGGGACAGTTTAAGAAGAGTGATGCCTTTGTGCCCTTTTCCATT  1281
GlySerValLeuLysAspProLysPhePheSerAsnProAsnAspPheAsnProLysHisPheLeuAspAspLysGlyGlnPheLysLysSerAspAlaPheValProPheSerIle  434

GGAAAACGGTATTGTTTCGGGGAAGGACTGGCAAGGATGGAACTCTTTCTCTTCCTCACAAACATCATGCAGAACTTCTGCTTCAAATCCCCACAGGCACCCCAGGACATCGATGTG  1398
GlyLysArgTyrCysPheGlyGluGlyLeuAlaArgMetGluLeuPheLeuPheLeuThrAsnIleMetGlnAsnPheCysPheLysSerProGlnAlaProGlnAspIleAspVal  473

TCTCCTAGACTTGTGGGCTTTGCCACAATCCCACCAAACTACACTATGAGTTTCTTGTCCCGTTGAGCCAGCATTGCGTGAGAGTAAAGGGAAGAATGGGGGTTGAAACTTAGAAAG  1515
SerProArgLeuValGlyPheAlaThrIleProProAsnTyrThrMetSerPheLeuSerArg *                                                        494

GGTCGGATGTACTGAAGAAACAGAGGAAAAAGAGTAGACCTGCTGAAGATATATATTTCAAAGGTAGAGCCAGAGAAGGGGGAAATATCTACTATGCTGTGAATAGGAATAATAAAAT  1632
AATAAAAGTAGATATTATTTATGGCAn                                                                                            1657
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FIGURE 1: Sequence of the IIA3 cDNA. The nucleotide and deduced amino acid sequences of IIA3 cDNA are displayed. The cDNA is probably lacking seven amino acids from the natural mRNA, and therefore the first amino acid residue in this sequence begins at 8. The conserved "Pro-Pro-Gly-Pro" sequence at the N-terminus and cysteine fragment of the enzyme's active site and the two consecutive poly(A) addition signals are underlined.

the films using a Beckman DU-8 spectrophotometer. The molecular weights of the mRNAs were estimated by using RNA size standards (Bethesda Research Laboratory). Hybridization of Southern blots (Southern, 1975) was done using Hybrisol I (Oncor, Inc.). Filter washing was undertaken at 65 °C using a solution of 20 mM Tris, pH 7.5, and 20 mM NaCl. Chromosome mapping was performed by using the somatic cell hybrid mapping strategy and a panel of well-characterized hybrids (Kozak et al., 1975; Kozak & Rowe, 1979, 1980) in conjunction with Southern blotting.

RESULTS

Isolation and Sequencing of Rat IIA3 cDNA. A rat lung cDNA library was screened by plaque hybridization using the rat IIB1 cDNA as a probe. Surprisingly, we did not isolate from the lung cDNA library any clones that correspond to the IIB1 mRNA. IIB1 protein (Christou et al., 1987) and its mRNA (Omiecinski, 1986) are clearly expressed in this tissue, although it was noted by Omiecinski (1986) that the IIB1 mRNA did not exist in the poly(A)-containing mRNA fraction of rat lung. The reason that we did not detect IIB1 cDNA clones may be related to the lack of a significant length of poly(A) tract on the lung IIB1 mRNAs. Since the poly(A) mRNA tail is required for priming the first strand cDNA synthesis, a particular class of mRNA that lacks poly(A) stretch will not be represented in the cDNA library. This may be the case for IIB1.

Several weakly hybridizing plaques were identified and their phages purified by repeated plaque purification. These plaques were only identified when filters were washed with high salt concentrations. Washing the filters at 65 °C with 0.1× SSC abolished the signals. The cDNA clones containing the longest inserts were processed further by subcloning into pUC9 and sequencing. The complete sequence of the longest cDNA obtained, designated IIA3, is displayed in Figure 1. IIA3 cDNA contains 1657 bp and an open reading frame that begins at the first nucleotide of the sequence. Interestingly, two consecutive poly(A) addition signals are found in the 3′ untranslated region of the mRNA. A conserved "Pro-Pro-Gly-Pro" and cysteine-containing fragment, the latter of which is known to be in the P450 active-site heme pocket, are found in the open reading frame (Figure 1). Alignment of this reading frame with several other P450 amino acid sequences revealed similarity with members of the IIA subfamily. The IIA3 open reading frame shared 71% and 73% cDNA-deduced amino acid similarities with rat IIA1 (Nagata et al., 1987) and rat IIA2 (Matsunaga et al., 1988), respectively. The IIA3 showed only 55% amino acid similarity with IIB1. The IIA3 sequence also displayed 85% and 94% cDNA-deduced amino acid sequence similarity to a full-length cDNA related to human P450(1) (Phillips et al., 1985a)[2] and mouse testosterone 15α-hydroxylase (Squires & Negishi, 1988), respectively. On the basis of amino acid alignment with mouse 15α, it appears that the rat IIA3 is missing seven amino acids at its amino terminus. Attempts to isolate the full coding region of this P450 have failed. This may be due to the fact that the IIA3 mRNA is present at very low levels in the lung or that the 5′ leader sequence of this mRNA is short.

Southern Blotting and Chromosome Mapping of the IIA3 Gene. To determine the complexity of the IIA3 gene, Southern blot analysis was carried out using rat liver DNA and the IIA3 cDNA probe. Digestion of rat DNA with several restriction enzymes revealed simple patterns indicative of a single gene sharing high nucleotide similarity with the IIA3 probe (Figure 2). Hybridization and washing stringency conditions used in this experiment selected for sequences sharing greater than 90% similarity. The size of the hybridizing fragments, de-

[2] This cDNA was isolated in our laboratory (S. Yamano and F. J. Gonzalez, unpublished data) and contains an amino acid coding sequence that displays 46 amino acid differences from the partial amino acid sequence deduced from the P450(1) cDNA sequence published by Phillips et al. (1985a). IIA3 displays 75% amino acid sequence similarity to P450(1).

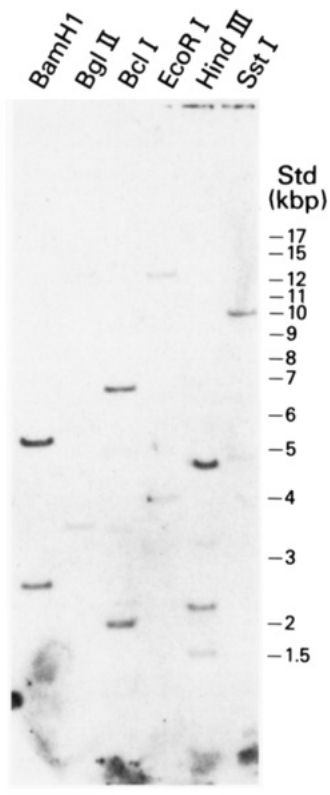

FIGURE 2: Southern blot analysis of the IIA3 gene. Rat liver DNA was digested with the restriction enzymes indicated and electrophoresed on a 0.4% agarose gel, treated by depurination and denaturation, and transferred to Biotrace nylon filters (Gelman Science, Inc.). The filter was hybridized and washed, and autoradiography was conducted with the aid of a Dupont Lighting Plus intensifying screen for 2 days at −80 °C. The molecular weight standards consist of the 1-kb ladder and high molecular weight standard kits supplied by Bethesda Research Laboratories.

pending on the enzyme used for digestion, ranged from 1.5 to 15 kbp (Figure 2).

To determine the chromosome location of the IIA3 gene in mouse, hamster–mouse somatic cell hybrids were analyzed. The hybrid cell lines were formed by fusion of E36 hamster cells with mouse cells. Individual cell lines containing a background of hamster chromosomes and one or more mouse chromosomes are then typed by karyotypic histological analysis, assays that distinguish mouse and hamster genes or gene products, and direct Southern blot analysis of mouse genes. Preliminary experiments were performed to determine the appropriate restriction enzyme that can be used to distinguish between mouse and hamster sequences that could be clearly resolved from hamster fragments on 20-cm-long 0.4% agarose gels. A typical result of a Southern blot of hybrid DNAs showing the presence or absence of mouse fragments in different mouse–hamster hybrids is seen in Figure 3. Mouse fragments of 16.0, 13.5, 8.5, and 7.5 kbp were detected, and the presence or absence of these fragments was segregated with the presence or absence of chromosome 7 (Table I). This is indicated by the lack of discordancy for chromosome 7; i.e., no cell lines were found that lacked chromosome 7 and contained the IIA3 hybridizing fragments or contained the fragments and lacked chromosome 7.

Expression of the IIA3 Gene. To determine if the IIA3 gene is expressed in liver and other tissues, Northern blot analysis was performed. First, RNAs isolated from liver, lung, small intestine, and kidney of 6-week-old untreated, phenobarbital-treated, and 3-methylcholanthrene-treated rats were analyzed by Northern blots using the IA1 and IIB1 probes.

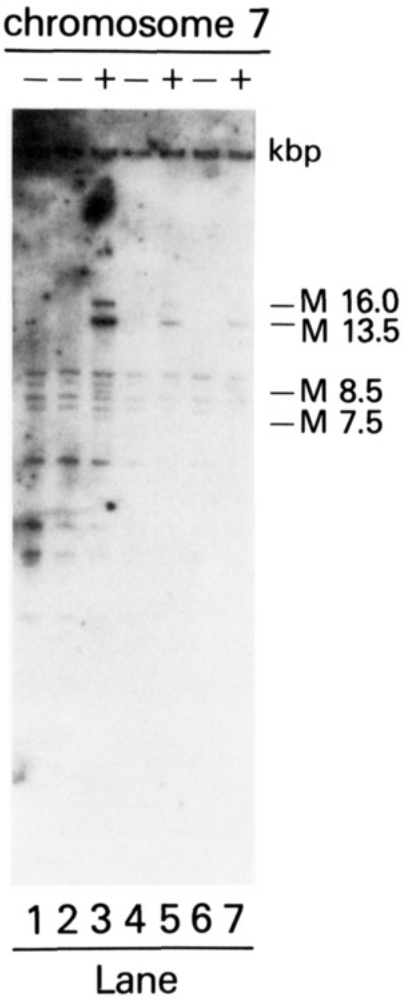

FIGURE 3: Mapping of the IIA3 gene using mouse–hamster somatic cell hybrids. Hybrid DNAs from a few select cell lines were digested with *Eco*RI and treated as described in the Figure 2 legend. Only a small subset of the DNAs used to generate the data in Table I are displayed. The sizes of the mouse-specific fragments (M) were calculated by using molecular weight standards.

The IA1 mRNA was induced by 3-methylcholanthrene in kidney, lung, and small intestine (data not shown), as demonstrated in previous studies (Kimura et al., 1986). The IIB1 and/or IIB2 mRNAs were induced in liver and small intestine by phenobarbital and constitutively expressed in lung (Figure 4). These results, which are consistent with previously published reports in which oligonucleotide probes were used to quantify levels of IIB1 and IIB2 in liver and lung (Omiecinski, 1986) and intestine (Traber et al., 1988), establish that the RNA isolated from the extrahepatic tissues in this experiment was intact and the rats were fully induced. Surprisingly, and in contrast to the presence of the IA1 and IIB mRNAs in liver of inducer-treated rats, the 1.8-kb IIA3 mRNA was only expressed in lung, and its level was induced about 3-fold by 3-methylcholanthrene administration (Figure 4). Phenobarbital treatment actually decreased the level of IIA3 mRNA in lung (Figure 4). No IIA3 mRNA was detected in liver, kidney, or small intestine, even after exposure of the Northern blot filter to X-ray film for 1 month.

DISCUSSION

The approach of using cDNA probe cross-hybridization to identify previously unknown P450s has been used before to isolate two P450s that had never been purified. One of them is the male-specific PCN2; characterization of this cDNA led to the clarification of several observations regarding the reg-

Table I: Correlation between Mouse Chromosome 7 and the P450 IIA3 Gene among 21 Mouse X Hamster Somatic Cell Hybrids[a]

mouse chromosome	no. of hybrids with IIA3 vs chromosome retention				
	+/+	−/−	+/−	−/+	% discordancy
1	7	1	8	4	60
2	11	1	5	3	38
3	3	2	6	3	64
4	6	4	9	1	50
5	1	4	14	0	74
6	8	3	8	2	48
7	16	5	0	0	0
8	4	3	9	2	61
9	6	5	10	0	48
10	2	5	14	0	67
11	0	5	10	0	67
12	6	1	3	4	50
13	10	3	5	2	35
14	5	5	11	0	52
15	10	0	0	5	33
16	4	2	10	1	65
17	11	0	5	5	48
18	6	2	8	3	58
19	7	3	8	1	47
20	7	1	9	4	62

[a] A total of 21 hybrid cell lines were analyzed for the presence or absence of sequences hybridizing to the rat IIA3 cDNA probe. The hybrid cell DNA was digested with *Eco*RI, electrophoresed on a 0.4% agarose gel, and blotted onto a nylon membrane. Four mouse fragments were identified of approximately 16.0, 13.5, 8.5, and 7.5 kbp (Figure 3). Fourteen hybrids were karyotyped, and all others were typed for the presence or absence of specific marker loci. +/+, containing the IIA3 gene and indicated chromosome; −/−, lacking the IIA3 gene and indicated chromosome; +/−, containing the IIA3 gene but lacking the indicated chromosome; −/+, lacking the IIA3 gene but containing the indicated chromosome. Discordancy indicates the presence of the chromosome when the gene is absent or the absence of the chromosome despite the presence of the gene. The sum of these numbers divided by total hybrids examined × 100 represents percent discordancy.

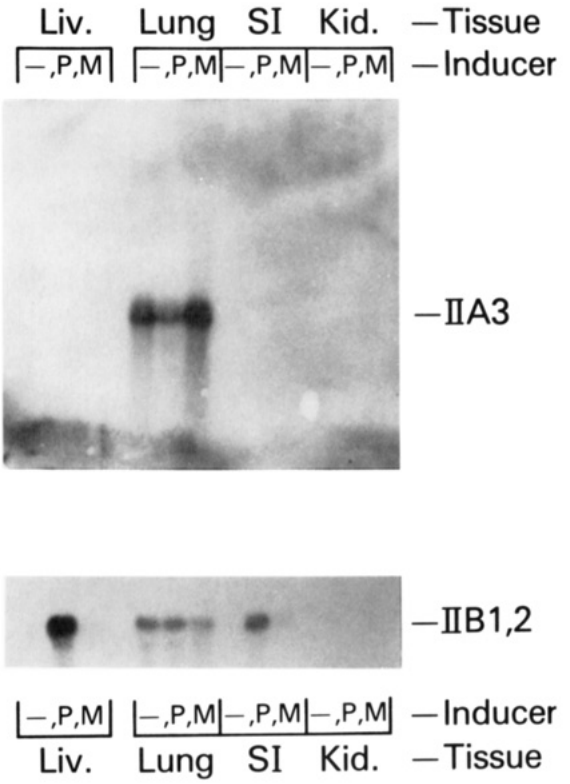

FIGURE 4: Northern blot analysis of RNAs from untreated (−), phenobarbital-treated (P), and 3-methylcholanthrene-treated rats (M). Rats were treated with 3-methylcholanthrene or phenobarbital for 24 h prior to killing. Total RNA (10 μg) from each tissue liver (Liv), lung, small intestine (SI), and kidney (Kid) was electrophoresed in 1% agarose–2.2 M formaldehyde gels and transferred to Nytran membrane. The filter was hybridized, washed, and exposed to film for 48 h (top panel) and 6 h (lower panel).

ulation of the steroid-inducible PCN1 (Gonzalez et al., 1986). This enzyme, designated IIIA2, has since been purified (Halpert, 1988). Recently, another cDNA, designated IIB3, constitutively expressed in rat liver, was isolated and sequenced (Labbé et al., 1988). In contrast to the IIB1 and IIB2 genes, the IIB3 gene is not induced by treatment of rats with phenobarbital. This P450 has not yet been purified from rats.

In the present paper, we have used cross-hybridization with a IIB1 P450 cDNA probe to extract a previously unknown P450 cDNA from rat lung. We believe that this cDNA codes for a P450 because significant sequence similarities to other P450s are found in this newly identified cDNA-deduced amino acid sequence; most notably, the heme binding cysteine-containing region near the carboxy terminus of the enzyme and the "Pro-Pro-Gly-Pro" segment near the amino terminus are highly conserved among many P450s (Black & Coon, 1986).

The amino acid sequence similarity cutoff point used to assign P450 gene subfamilies was previously set at 65% (Nebert et al., 1987). Therefore, any sequence greater than 65% similar to another sequence is assigned to the same gene subfamily. The IIA3 was assigned to the IIA subfamily on the basis of its 71% and 73% cDNA-deduced amino acid sequence similarities to the previously characterized IIA1 and IIA2. In spite of these similarities, under usual hybridization stringencies, the IIA3 probe does not hybridize with the IIA1 and IIA2 mRNAs. This is readily apparent, since the sizes of these two mRNAs are 3.0 and 2.6 kb and both are expressed in liver and are not expressed in lung (Matsunaga et al., 1988). The IIA3 mRNA, in contrast, is 1.8 kb and is only expressed in lung. On the basis of the average calculated unit evolutionary period of P450s, estimated from comparing orthologous P450 sequences among different species (Nebert & Gonzalez, 1987; Nelson & Strobel, 1987), the approximate time the IIA3 gene was formed during evolution can be determined. The IIA3 gene and the ancestor to the IIA1 and IIA2 genes were formed about 120 million years ago, and the IIA1 and IIA2 genes were formed about 40 million years ago. Since the man–rodent radiation occurred at around 75 million years ago, we predict that two IIA genes might exist in man.

The IIA gene was mapped to mouse chromosome 7. This result is in line with previous studies mapping the human P450(1) gene to human chromosome 19 (Phillips et al., 1985b), since a linkage conservation group of genes from mouse 7 is found on the long arm of human 19. It is also of interest that the IIB (Simmons & Kasper, 1983), IIC (Meehan et al., 1988), and IIE (Umeno et al., 1988a) subfamilies are also located on mouse chromosome 7. The fact that the IIE subfamily (Umeno et al., 1988b) and IIC subfamily (Okino et al., 1987) are located on human chromosome 10, while the IIA subfamily (Phillips et al., 1985b) and IIB subfamily (Miles et al., 1988; Santisteban et al., 1988) are on human chromosome 19, implies that these four subfamilies are not tightly linked on mouse chromosome 7. The IID subfamily is located on mouse chromosome 15 (Gonzalez et al., 1987) and human chromosome 22 (Gonzalez et al., 1988). The separation of the IID subfamily from the other four subfamilies follows from the fact that the IID gene(s) diverged much earlier in evolution than the IIA, IIB, IIC, and IIE genes (Nebert & Gonzalez, 1988).

To our knowledge, IIA3 is only the second example of a P450 constitutively expressed in rat lung and absent from liver.

Others have reported that the orthologous counterpart to rabbit P450 isozyme 5 is present in the lungs of rats, mice, guinea pig, and monkey but is absent in the livers of these species (Vanderslice et al., 1987). In contrast, rabbits and hamsters express this enzyme in both tissue. These data suggest an interesting paradox involving tissue-specific regulation of isozyme 5 in different species. Interestingly, the mouse IIA3 gene is expressed in a tissue-specific manner that is distinct from that of its rat counterpart (see below).

Another form of rabbit P450 has been extensively studied that is expressed in lung. This enzyme is a prostaglandin ω-hydroxylase that is induced in pregnant rabbit lung by progesterone (Williams et al., 1984; Yamamoto et al., 1984). However, this P450 mRNA is present, to some extent, in the liver and is constitutively expressed at a higher level in kidney (Matsubara et al., 1987). The progesterone-induced P450 is presumed to play a role in prostaglandin metabolism and perhaps maintainance of the pregnant state. However, the function of IIA3 and isozyme 5 in lung is still unknown. The fact that IIA3 mRNA is inducible by 3-methylcholanthrene in lung suggests that it may be primarily a xenobiotic metabolizing enzyme.

Of interest was the recent elucidation of the structure of a mouse testosterone 15α-hydroxylase (Squires & Negishi, 1988). Two cDNAs, designated type I and type II, were sequenced, and these display about 94% deduced amino acid similarity with rat IIA3. This high level of similarity indicates that these mouse cDNAs are orthologues of rat IIA3 (Nebert et al., 1989). Despite this similarity, both mouse genes are expressed in liver and kidney in contrast to IIA3. The apparent human orthologue of IIA3, P450(1) (Phillips et al., 1985a), is also found in liver. These comparisons suggest marked species differences in expression of IIA3 genes.

Even within the IIA subfamily, differences exist in the inducibility and time-specific expressiosn of IIA1, IIA2, and IIA3. Both IIA3 and IIA1 (Nagata et al., 1987) are induced by 3-methylcholanthrene, suggesting that a DNA control element governing inducibility may be conserved between both genes. Despite a similar mode of regulation by 3-methylcholanthrene, IIA1 is liver specific and IIA3 is lung specific, suggesting that these genes possess distinct tissue-specific regulatory domains.

Added in Proof

A genomic clone containing the rat IIA3 gene has been isolated and sequenced (T. Veno and F. J. Gonazlez, unpublished data). The sequence missing from our cDNA beginning with the initiator Met is as follows: ATGCTGGCCTCAGGACTCCTT coding for the amino acids MetLeuAlaSerGlyLeuLeu.

Registry No. Cytochrome P450, 9035-51-2; 3-methylcholanthrene, 56-49-5.

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Expression of $G_{o\alpha}$ mRNA and Protein in Bovine Tissues†

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ABSTRACT: $G_{o\alpha}$ is a 39-kDa guanine nucleotide-binding protein (G protein) similar in structure and function to $G_{s\alpha}$ and $G_{i\alpha}$ of the adenylate cyclase complex and to transducin ($G_{t\alpha}$) of the retinal photon receptor system. Although expression of $G_{o\alpha}$ protein has been reported to be tissue-specific, other workers have found $G_{o\alpha}$ mRNA in all rat tissues examined. In order to clarify this contradiction, studies to verify the distribution of $G_{o\alpha}$ mRNA and protein in bovine and rat tissues were performed. Tissues were screened for the presence of $G_{o\alpha}$ mRNA by use of a series of restriction fragments of a bovine retinal cDNA clone, λGO9, and oligonucleotide probes complementary to sequences specific among G_α subunits for the 5′ untranslated and coding regions of $G_{o\alpha}$. These probes hybridized predominantly with mRNA of 4.0 and 3.0 kb in bovine brain and retina. A 2.0-kb mRNA in retina also hybridized strongly with the cDNA but weakly with the oligonucleotide probes. In bovine lung, two mRNAs of 1.6 and 1.8 kb hybridized with the cDNA while only the 1.6-kb species hybridized with the coding-region oligonucleotide. In bovine heart, only a 4.0-kb mRNA was detected and in amounts much less than those in the other tissues. A similar distribution of $G_{o\alpha}$ mRNAs was seen in rat tissues. In bovine tissues, $G_{o\alpha}$ protein was identified with rabbit polyclonal antibodies directed against purified bovine brain $G_{o\alpha}$. An immunoreactive 39-kDa membrane protein was found principally in retina and brain, and in a lesser amount in heart. Thus, in the rat and bovine tissues examined, $G_{o\alpha}$ or $G_{o\alpha}$-like mRNAs, as well as $G_{o\alpha}$ protein, are expressed in a tissue-specific manner.

Guanine nucleotide-binding proteins (G proteins)[1] function as signal transducers from cell surface receptors to inner membrane effector enzymes in a number of regulatory systems (Stryer, 1986; Gilman, 1987). The G proteins include G_s and G_i, the stimulatory and inhibitory proteins of the adenylate cyclase system, the transducins, and G_o (Stryer, 1986; Gilman, 1987; Sternweis & Robishaw, 1984; Florio & Sternweis, 1985). Transducin activates a retinal cyclic GMP phosphodiesterase in response to photoexcitation of rhodopsin (Stryer, 1986). G_o, initially identified in brain, interacts in vitro with rhodopsin as well as muscarinic and other receptors; however, its physiological role is presently unclear (Sternweis & Robishaw, 1984; Florio & Sternweis, 1985; Kurose et al., 1986; Tsai et al., 1987; Ueda et al., 1988).

These G proteins share functional, structural, and immunological similarities (Stryer, 1986; Gilman, 1987; Moss & Vaughan, 1988). They are composed of three subunits: α, β, and γ (Stryer, 1986; Gilman, 1987; Hildebrandt et al., 1984). The β subunits appear to be very similar in all four proteins (Manning & Gilman, 1983). The γ subunits of the transducins are different from those of G_s, G_i, and G_o (Hildebrandt et al., 1985). The α subunits are unique for each G protein and presumably convey specificity to the interactions with cell surface receptors and effector enzymes (Northup et al., 1982, 1983; Brandt et al., 1983; Kanaho et al., 1984;

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[1] Abbreviations: G proteins, guanine nucleotide-binding regulatory proteins; G_s and G_i, stimulatory and inhibitory G proteins of adenylate cyclase; G_o, G protein purified from brain; $G_{s\alpha}$, α subunit of G_s; $G_{i\alpha}$, α subunit of G_i; $G_{o\alpha}$, α subunit of G_o; SDS, sodium dodecyl sulfate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; GTPase, guanosinetriphosphatase.