CYP26, a Novel Mammalian Cytochrome P450, Is Induced by Retinoic Acid and Defines a New Family*

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A novel member of the cytochrome P450 superfamily, CYP26, which represents a new family of cytochrome P450 enzymes, has been cloned. CYP26 mRNA is up-regulated during the retinoic acid (RA)-induced neural differentiation of mouse embryonic stem cells in vitro and is transiently expressed by embryonic stem cells undergoing predominantly non-neural differentiation. CYP26 transcript is detectable as early as embryonic day 8.5 in mouse embryos, suggesting a function for the gene in early development. CYP26 is expressed in mouse and human liver, as expected for a cytochrome P450, and is also expressed in regions of the brain and the placenta. Acute administration of 100 mg/kg all-trans-RA increases steady-state levels of transcript in the adult liver, but not in the brain. CYP26 is highly homologous to a Zebrafish gene, CYPRA1, which has been proposed to participate in the degradation of RA, but is minimally homologous to other mammalian cytochrome P450 proteins. Thus, we report the cloning of a member of a novel cytochrome P450 family that is expressed in mammalian embryos and in brain and is induced by RA in the liver.

The cytochrome P450 (CYP) superfamily of heme-binding monoxygenases catalyzes a large number of important biological reactions, most notably the nonspecific oxidative conversions of many steroids, lipids, and a variety of xenobiotics and environmental toxins. The CYP superfamily is large, with at least 74 families, and each mammalian species is estimated to have between 60 and 200 distinct superfamily members (1). The mammalian enzymes involved in xenobiotic metabolism are typically expressed in the liver and exhibit broad substrate specificity. Other CYPs participate in a number of specific anabolic reactions, such as the synthesis of several steroid hormones (2). Although generally expressed in the liver, CYPs have also been found in extrahepatic sites such as the kidney, lung, mucosa of the gut, placenta, reproductive organs, embryonic tissues, and the brain (3–8). The functions of CYPs in the brain are not completely understood but may include production of neurosteroids (9).

Retinoic acid (RA), a derivative of vitamin A, has a wide range of biological effects. RA is a potent teratogen (10), and conversely, vitamin A deficiencies lead to severe developmental defects. During development, RA is a suspected morphogen (11). For example, it is thought to be involved in the induction of polarity in developing limb buds in the chick (12), forming anterior-posterior gradients of gene expression in the Xenopus nervous system (13, 14), and is known to influence the expression of Hox genes in the mouse and in other systems (15). RA signaling is mediated through a set of nuclear hormone receptors that bind RA and subsequently alter the expression of target genes (16). Deletions of certain combinations of these receptors lead to developmental defects resembling vitamin A deficiency during pregnancy (17, 18). RA and similar derivatives are detectable in embryonic tissues (19), but neither the production nor the catabolism of retinoids are well understood. Additionally, RA is employed in the treatment of acute lymphocytic leukemia (20). Understanding RA metabolism is, therefore, an important problem.

RA has profound effects on mouse embryonic stem (ES) cells, a cell line resembling the totipotent cells of the inner cell mass of the preimplantation embryo (21, 22). ES cells are known for their totipotency, as evidenced by their use in generating gene-targeted mice. These cells also differentiate into a number of cell types in vitro (23–25). RA induces efficient neural differentiation of ES cells (26) while repressing spontaneous mesodermal differentiation (27). ES cells thus provide an opportunity to study the effects of RA on totipotent cells. We screened for genes which are induced during RA-induced neural differentiation. One such gene, CYP26, defines a novel family of CYPs, and its expression is regulated in vitro and in vivo by RA.

MATERIALS AND METHODS

Cell Culture—D3 mouse ES cells were maintained and differentiated as in Bain et al. (26). Briefly, undifferentiated ES cell stocks were propagated in the presence of leukemia inhibitory factor (LIF). For neural differentiation, cells were cultured as embryoid bodies (EB) in the absence of LIF for 4 days, treated with 500 nM RA for 4 days, and then dispersed and plated onto an adhesive substrate. For differentiation into a mixture of mostly non-neural cell types, RA was omitted. Cultures are described using the following nomenclature: 4 + or → /X+ or →. The number 4 refers to the number of days the ES cells were cultured as EBs, X refers to the number of additional days as EBs, and + or → refers to the presence or absence of RA. For example, 4 +/X+ or → indicates that ES cells were cultured as EBs for 4 days without RA and then 2 days with RA.

Collection of Mouse Tissues and Administration of Retinoic Acid—ICR mice were mated overnight, and on the morning of appearance of vaginal plugs, females were designated 0.5 days pregnant. For RA-administration, mice were injected 100 mg/kg intraperitoneally with 50 mg/ml all-trans-RA (Sigma) in MeSO. Control mice received MeSO alone. 24 h later, the mice were sacrificed by cervical dislocation, and tissues were collected.

Cloning of CYP26—CYP26 was isolated from 4–3+ cDNA that had undergone subtractive hybridization to ES cDNA as in Wang and Brown (28). A 364-nt fragment of CYP26 was cloned corresponding to nt
This fragment was used to probe a cDNA library from P19 embryonal carcinoma cell aggregates treated with RA for three days (29), by standard techniques (30). Two clones were isolated, each with 1.7-kb inserts, including the original CYP26 fragment. Both strands of one phage insert were cycle-sequenced using gene-specific oligos and the AmpliCycle sequencing kit (Perkin-Elmer). To verify that the phage sequence accurately reflected CYP26 mRNA and had not recombined, overlapping RT-PCR reaction products from ES cDNA were sequenced. The conceptual translation was used to search protein data bases using the BLASTP program provided by NCBI. Percent identity to other proteins was determined by manual alignment. Assignment into a novel CYP family was made by Dr. D. Nelson of the P450 Nomenclature Committee (University of Tennessee, Memphis, TN).

RNA Purification—For Northern analysis and ribonuclease protection assays, RNA was collected from ES cultures and mouse tissues by differential precipitation (31). For RT-PCR analysis, RNA was collected by the acid phenol method (32), except in the case of embryonic samples that were collected using RNeasy columns (Qiagen). Human liver RNA was from Dr. Karen O’Malley (Washington University, St. Louis, MO), and F9 cDNA was from Dr. Greg Longmore (Washington University). Human brain RNA was isolated from tissue obtained at autopsy by the Washington University Department of Pathology (2 h after death) from an adult who died of cardiac arrest. The human astrocytoma cell lines used were CCF-STTG1 (ATCC CRL 1718), SW 1088 (ATCC HTB 12), and U-373 MG (ATCC HTB 17). Human brain and astrocytoma RNAs

![Figure 1. Northern analysis of CYP26 expression in embryonic stem cells.](image1)

2 µg of polyA+ RNA were probed with labeled antisense RNA derived from nt 159–523 of CYP26 cDNA (see Fig. 2). ES, undifferentiated embryonic stem cells; 4–3+, ES cells cultured for 4 days as embryoid bodies in the absence of LIF and subsequently treated for 3 days with 500 nM retinoic acid. Size markers are the location of ribosomal bands. The blot was reprobed with a GAPDH antisense riboprobe, and equivalent amounts of RNA were detected in both lanes (not shown).

![Figure 2. Sequence of CYP26 cDNA.](image2)

Sequence analysis of 2 cDNA clones hybridizing to the original CYP fragment (underlined) reveal a 1701 cDNA with a 1491-nucleotide open reading frame. Putative untranslated regions are given in lowercase letters. Stop codons are denoted by asterisks, and a potential polyadenylation signal is double underlined.
Northern Analysis—The original CYP26 fragment was cloned into pBSIIISK(+) and used to generate labeled antisense RNA by in vitro transcription (Boehringer Mannheim). The resulting probe was hybridized overnight to 2 μg of poly(A)+ RNA (prepared using polyAtract, Promega) from ES and 4–3+ cells that had been electrophoresed (30) and transferred to Hybond N+ membranes, all according to the manufacturer recommendations (Amersham). GAPDH probes were generated from the pTRI-GAPDH vector (Ambion Inc.).

Primer Extension Assays—Primer extension assays were performed (30) using 106 cpm of end-labeled oligo 5'-AAAGCAGCAGCGGACACGAGGACGGCACC-3', 30 μg of total RNA, and Superscript II RT (Life Technologies, Inc.).

Ribonuclease Protection Assays—Ribonuclease protection assays (RPA) were performed as described (33). 25 μg of total RNA was hybridized to labeled antisense CYP26 or GAPDH RNA generated as above, digested with ribonuclease mixture (Amersham), and resolved on 6% acrylamide/urea gels.

RT-PCR—Reverse transcription-PCR was performed as described (34). All cDNAs were positive for GAPDH expression at 30 cycles (CLONTECH). The upstream oligo for CYP26 detection was 5'-TCTCGCACAACGGAGAAGGTAATT-3'; the downstream oligo was 5'-ATGTTGGTATGGCTAGTTAAGT-3'. 5 pmol of each oligo was used to amplify 1 μl of cDNA for 35 cycles under the parameters 94 °C at 30 s, 60 °C at 30 s, and 72 °C at 1 min. Reaction products were evaluated on 2% agarose gels containing ethidium bromide. For cDNA samples from the embryo prior to E12.5, RNA was not quantitated prior to cDNA synthesis. RNA from two embryos or one deciduum was used in each cDNA synthesis reaction; 1% of cDNA was used per reaction. For human samples, the positive control was detection of the transferrin receptor (CLONTECH); the upstream oligo was 5'-CCTGCTGCTGCTTTCCTGGCTGCGA-3', the downstream oligo was 5'-GACCGA-CACCCAGCGGTGCTTCCCGA-3', and samples were subjected to 33 rounds of amplification.

RESULTS

Identification of CYP26 cDNA—CYP26 was isolated as a 364-nt cDNA fragment that detected a transcript up-regulated during the RA-induced neuronal differentiation of mouse ES cells. The subtractive PCR method of Wang and Brown (28) was used to identify genes expressed at higher levels in 4–3+ cells than in the undifferentiated stem cells. At this stage of differentiation, 3 days after the administration of RA, the cells express early neural regulatory genes but do not express many markers of terminal differentiation (26, 27). RPA showed that the full-length mRNA, less the poly(A) tail, had been cloned. Primer extension analysis of total RNA using a 36-nt primer with its 3' end corresponding to nt 44 generated an approximately 80-nt product from RNA from 4–3+ cells, liver, and from RA-treated mouse, but not from kidney, spleen, or yeast tRNA (Fig. 3). Thus, the primer was extended approximately 44 nt in the 5' direction, suggesting that the 5'-end of the cDNA corresponds closely to the 5'-end of the native transcript.

CYP26 Encodes a 1.9-kb mRNA Regulated during ES Cell Neural Differentiation—The CYP26 fragment was used to probe a Northern blot of ES and 4–3+ RNA. 2 μg of poly(A)+ RNA were fractionated and probed with an antisense RNA transcribed from the CYP26 fragment. The riboprobe detects a 1.9-kb species found in 4–3+ poly(A)+ RNA but not in ES poly(A)+ RNA (Fig. 1). Only one band, also 1.9 kb, was detected in liver RNA (not shown), suggesting that the probe does not detect other CYP family members. The same blot was probed with GAPDH to confirm the loading and integrity of the RNA (not shown). This data confirms the regulation in CYP26 and estimates the size of the mRNA at 1.9 kb.

Cloning of Full-length CYP26 cDNA—A cDNA library from RA-induced, naturally-differentiating P19 embryonal carcinoma (EC) cells was probed with the CYP26 fragment. Two clones hybridizing to the 364-nt CYP26 probe were isolated and contained inserts of 1.7 kilobase pairs. Sequence analysis revealed that CYP26 cDNA is 1701 nt long and contains an open reading frame (ORF) of 1491 nt, beginning with the first ATG at nt 18 (Fig. 2). This ATG is surrounded by a consensus Kozak initiation sequence (35), and the ORF is predicted to be translated based on mammalian codon usage using the Microgene DNA analysis program. The predicted translation product is a 56.1-kDa polypeptide consisting of 497 amino acids. The ORF ends with 2 stop codons, and 23 nt from the end, there is the canonical polyadenylation signal, AATAAA. The presence of a complete ORF and the size of the cDNA (1.7 kb) compared with the species detected by Northern analysis (1.9 kb) suggested that the full-length mRNA, less the poly(A) tail, had been cloned. Primer extension analysis of total RNA using a 36-nt primer with its 3' end corresponding to nt 44 generated an approximately 80-nt product from RNA from 4–3+ cells, liver, and from RA-treated mouse, but not from kidney, spleen, or yeast tRNA (Fig. 3). Thus, the primer was extended approximately 44 nt in the 5' direction, suggesting that the 5'-end of the cDNA corresponds closely to the 5'-end of the native transcript.

CYP26 Defines a Novel CYP Family—The first sequence comparisons with other known CYP members revealed that CYP26, while sharing general homology to other CYP members, did not share high overall homology to any one member. The closest homolog is the conceptual translation of a gene from the cyanobacterium Synchocystis. Excluding the membrane anchoring region, which the bacterial protein lacks, there is 34% identity. CYP26 has some homology to three plant CYPs, tomato CYP homolog from Solanum lycopersicum (22% identity), CYP90 from Arabidopsis thaliana (23% identity), and...
Dwarf 3 from Zea mays (22% identity). The closest mammalian homolog is rat lanosterol a-14-demethylase, with 16% identity. Examination of phylogenetic and functional relationships between CYP families has established that greater than 40% identity typically constitutes membership within a family (1). Thus, CYP26 appears to belong to a novel family of cytochrome p450 genes that is most closely related to a putative bacterial protein and three plant CYPs. This conclusion was supported by the P450 Nomenclature Committee (1), which assigned CYP26 to a novel family based on amino acid sequence homology to superfamily members. After this first analysis and taxonomic assignment, a novel CYP was reported (37), which appears to be the Zebrafish homolog of CYP26. Thus, the CYP26 family is present in this non-mammalian species as well.

CYP26 Expression Is Induced by RA in Differentiating ES Cells—The expression of CYP26 in differentiating ES cells was examined by RPA. Undifferentiated ES cells grown in the presence of LIF do not express detectable amounts of CYP26 (Fig. 5). When EB are treated with RA to induce neural differentiation, the abundance of CYP26 increases to detectable levels within 2 days (42 days). This expression level is maintained in the ES cultures throughout the 4-day RA treatment and 5 days post-induction (5 dpi), when EBs have been dispersed and overt neural differentiation has occurred. When EBs are treated with RA, the cells differentiate into a variety of cell types, with neural cells being relatively rare (21, 25, 26). Under these conditions, CYP26 expression increases to detectable levels by 4 – 2 – , but this expression is lower than in the RA-treated samples. Expression at the 4 – 2 – stage is approximately equal to 4 – 4 + cells, but unlike the RA-treated cells, expression levels decline as the cells mature (5 dpi – ). Interestingly, CYP26 is induced in EBs that have been treated with RA for 4 days with no initial RA-free culture period (41 days). ES cells treated in this fashion do not efficiently neurally differentiate.2 42 cultures, which are cultured the same as 41 cultures, except without RA, do not detectably up-regulate CYP26. Thus, neural differentiation is not necessary for CYP26 induction. Two other in vitro models of embryonic differentiation were assayed for CYP26 regulation by RT-PCR (Fig. 6). P19 embryonal carcinoma cells express CYP26 transcript before and after differentiation, while F9 teratocarcinoma cells appear to up-regulate the transcript during differentiation. However, the up-regulation of CYP26 in F9 was not verified by a quantitative analysis. These results show that CYP26 is expressed by three models of early embryonic cells.

CYP26 Is Expressed in the Early Mouse Embryo—The above results from in vitro models of early differentiation suggested that CYP26 is expressed in the early embryo. To test this possibility, expression in embryos was assayed by RT-PCR. In this qualitative assay (Fig. 6), detectable levels of CYP26 are found in early embryos. Low but reproducible signals are seen in the E5.5 and E6.5 decidua, which include embryos, but are

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FIG. 4. Alignment of CYP26 with closely related CYPs. The closest relatives to CYP26 were determined using the BLASTP program provided by NCBI, and the amino acid sequences of the top three were manually aligned to CYP26 in such a way as to maximize similarity while minimizing gaps. Abbreviations are: bacterial, predicted protein from the cyanobacterium Synchocystis (GenBank accession number D64003); tomato CYP homolog, CYP homolog from Solanum lycopersicum, (GenBank U54770); CYP90, A. thaliana CYP member CYP90 (GenBank S55379). Identical residues are cross-hatched, and dashes represent gaps for alignment purposes. Regions of high conservation in the CYP superfamily are indicated by bracketing above the CYP26 sequence. Anchor region, predicted membrane-spanning helix; proline, proline-rich domain; oxygen binding, site of O2 binding; steroid binding, predicted steroid binding region; and heme binding, consensus sequence for the heme-binding pocket.
A RA-responsive CYP Expressed in Liver, Brain, and Embryos

Fig. 5. Ribonuclease protection assay for CYP26 expression. Labeled antisense RNA from a 364-nt fragment of CYP26 cDNA (nt 159–523, Fig. 2) was hybridized to 25 μg of total RNA and digested with ribonucleases. The protected products were resolved on a sequencing gel and exposed to film for 20 h. Samples are shown in lanes identified as: ES, undifferentiated embryonic stem cells; 4 − , ES cells cultured as EB for 4 days without RA; 4 − 2 +, ES cells cultured as EB for 4 days and treated with RA for 2 days; 4 − 4 +, ES cells cultured as EB for 4 days and treated with RA for 4 days; 5 dpi +, ES cells 5 days post-RA induction in which overt neural differentiation has occurred; 4 − 2 −, ES cells cultured as EB for a total of 6 days; 4 − 4 −, ES cells cultured as EB for 8 days; 5 dpi −, ES cells that had differentiated into a mixture of cell types 5 days after the 4 − 4 − treatment; and 4 +, EB treated with RA for 4 days. E12.5 body and head are samples collected from embryonic tissue from timed-pregnant females; E16, embryonic day 16 brain; P2, post-natal day 2 brain. RNA samples were also assayed for GAPDH transcript levels as a control for RNA concentration and integrity, and all samples were equal (not shown).

Fig. 6. Reverse-transcription PCR assay for CYP26 expression. First-strand cDNA derived from 50 ng of total RNA were subjected to 35 cycles of PCR using gene-specific oligos as described under “Materials and Methods.” Embryonic samples are 1% of the cDNA derived from two embryos or one embryonic decidua. Reaction products were resolved on 2% agarose gels containing ethidium bromide. Size markers in bp are given on the left. The positive reaction product is the 562-nt band seen strongly in the 4 − 3 + positive control reaction. Samples are shown in lanes identified as follows. Water, negative control; mouse DNA, 500 ng mouse genomic DNA as a control for genomic amplification; 4 − 3 +, ES cells cultured as embryoid bodies for 4 days and treated with RA for 3 days. Embryonic samples are described by days post-coitum. Samples followed by the abbreviation “dec.” indicate that the collected tissue was both embryonic and decidua. Samples not followed by “dec.” were entirely embryonic. P19, undifferentiated P19 embryonal carcinoma cells; P19 + RA, P19 cells treated with RA for 4 days to induce neural differentiation; F9, undifferentiated F9 teratocarcinoma cells; F9 + RA, F9 cells treated with RA for 7 days to induce differentiation of visceral endoderm-like cells. All others are adult organs. S. cord, spinal cord; S. intestine, small intestine; and Sk. muscle, skeletal muscle. All cDNA samples were positive for GAPDH at 30 cycles of amplification (not shown).

A RA-responsive CYP Expressed in Liver, Brain, and Embryos

CYP26 Expression in the Adult Mouse—CYP26 expression in the adult mouse was assayed by both RPA and RT-PCR. As shown in Figs. 5 and 6, the liver and brain were the only adult tissues of those examined that were positive. Sensitive RT-PCR assays were negative for many tissues at 35 cycles of amplification, including heart, lung, spleen, pancreas, stomach, small intestine, kidney, skeletal muscle, and testes. Low signals were occasionally detected in the spinal cord. Using the less sensitive RPA, CYP26 expression was found in the liver (Fig. 5), and longer exposures showed expression in the liver and brain but not in kidney, spleen, or heart. Thus, CYP26 appears to be expressed both in liver and brain in the adult mouse.

CYP26 Is Induced by RA in the Liver—The expression of CYP26 in RA-treated ES cells raised the possibility that this gene is RA-responsive in vivo. To test this hypothesis, adult mice were treated with RA, and the expression of CYP26 RNA was analyzed. 100 mg/kg RA in Me6SO, or Me6SO alone, were injected intraperitoneally, and the animals were sacrificed 24 h later. Brain and liver RNA samples were collected and assayed for CYP26 transcript levels using RPA. As shown in Fig. 7, RA induced the expression of CYP26 severalfold in adult liver (control liver signal is more faint than in Fig. 5 due to a shorter exposure time). This induction occurred in animals of both sexes in 5 of 5 animals tested. Longer exposures showed that RA treatment had no effect on expression levels in brain. Thus, CYP26 RNA is induced by RA in the adult mouse liver.

CYP26 Is Present in Humans—To determine if a human homolog of CYP26 exists, RT-PCR was performed on human liver cDNA. Two PCR products, covering 40% of the ORF, were generated from human liver and sequenced. Of the 598 nt covered, 90.6% were identical. 195 of the 200 amino acids predicted from the nt sequence were identical, and the 5 substitutions were conservative (data not shown). Furthermore, human CYP26 is represented in the human expressed sequence tag data base. Three are present in a female post-natal day 73 brain cDNA library (GenBank accession numbers R51129, R51021, and R21282), and two are from a human placental library (H97372 and H87920). Thus, a human homolog of CYP26 exists.

CYP26 Expression in Human Liver, Brain, and Placenta—To assay for CYP26 expression in human tissues, a human-specific RT-PCR assay was developed and used to analyze transcript presence in a variety of human brain regions, glial cell lines, and placenta. As shown in Fig. 8, the RT-PCR assay amplifies a 281-nt region of CYP26 cDNA from human liver but predominantly uterine tissue. Expression was detected in E8.5, E9.5, and E12.5 samples, which are exclusively from embryonic tissue. However, RPA did not detect CYP26 expression in E12.5 body or head (Fig. 5), even after a 5 day exposure, suggesting a low level of expression. As shown in Fig. 6, a variety of adult tissue cDNAs were negative for CYP26 expression, serving as a biological negative control. Thus, CYP26 appears to be expressed early in embryogenesis.
not from water or genomic DNA. After 33 cycles of amplification, expression was found in one of three astrocytoma lines tested, olfactory bulb, temporal cortex, and hippocampus, with other regions giving lower signal, including the parietal cortex, the medulla/pons region, and the putamen. Two of the three astrocytoma lines tested were negative at 33 cycles, as was frontal cortex, caudate, cerebellum, thalamus, and spinal cord. Expression was also detected in placental tissues. Thus, CYP26 is expressed in the human brain with some regional specificity and is expressed in the placenta.

DISCUSSION

We have identified a novel member of the cytochrome P450 superfamily, CYP26, which is expressed in the early mouse embryo, brain, and liver. CYP26 was isolated as a cDNA fragment from a subtractive hybridization scheme designed to identify genes up-regulated during RA-induced differentiation of mouse ES cells in vitro. Northern analysis shows that CYP26 is an mRNA of 1.9 kb, induced during ES neural differentiation. Full-length cDNA contains an ORF predicted to encode a 497-amino acid protein with homology to members of the cytochrome P450 family. The putative protein of 56.1 kDa contains a membrane anchoring domain, a proline-rich region, an oxygen-binding domain, and a heme-binding region, all characteristic of CYPs. While there was strong homology in highly conserved regions to other CYP members, there were no existing CYP members with extensive overall homology, suggesting that CYP26 defines a novel family. This analysis was confirmed by the P450 Nomenclature Committee (1). Subsequently, a novel CYP in Zebrafish has been identified, CYPRA1, and appears to be the homolog of CYP26 (37). Thus, CYP26 and CYPRA1 represent a new family of cytochrome P450 enzymes.

The expression of CYP26 suggests that this enzyme may respond to RA or be involved in RA metabolism in vivo. First, CYP26 is expressed in ES cells, an in vitro model of early embryonic differentiation. In this system, transcript abundance increases with differentiation, an effect enhanced by RA administration. Additionally, administration of all-trans-RA leads to a substantial increase in the levels of CYP26 transcript in the mouse liver within 24 h. This induction did not occur in the brain, suggesting a tissue-specific response. The induction of CYP26 transcript abundance after RA treatment suggests a number of possibilities. One, many CYP enzymes display broad substrate selectivity, as their function is to catalyze xenobiotics of a general class. Some of these enzymes are known to be induced by their substrate at the level of transcription, as in the cases of polycyclic aromatic compounds, phenobarbital, and ethanol (2). It is suspected that all-trans-RA is converted to 4-OH-RA by an unidentified cytochrome in the liver (36). Perhaps CYP26 is induced by retinoids and participates in this or other related activities. A second possibility is that CYP26 is specifically up-regulated after a RA dose to increase synthesis or degradation of specific responder molecules. It has recently been shown that CYPRA1 promotes the degradation of all-trans-RA into 4-OH-RA and 4-oxo-RA when transfected into COS-1 cells, supporting the first possibility (37). It will be interesting to determine if this activity is a result of direct catalysis and if CYP26 performs a similar function in mammals.

The presence of CYP26 transcript in ES, P19, and F9 cells suggested an embryonic expression of CYP26. RT-PCR data, as shown in Fig. 6, confirm that CYP26 is expressed by embryos as early as E8.5 and is detectable in the E5.5 deciduum although expression by uterine tissues in those samples is not excluded. Embryonic expression levels are likely to be low, since no CYP26 expression was detected in 5 day exposures of E12.5 body or head RNA samples using RPA, but is readily detectable by RT-PCR from the same RNA sample.

RA, a derivative of vitamin A, is well known as a teratogen (10). There is accumulating evidence that RA functions normally in development as well, possibly acting as a morphogen (15). Perhaps CYP26 functions in some aspect of retinoid signaling or metabolism in the embryo. Interestingly, two of the plant homologs of CYP26 are involved in development. CYP90
from *Arabidopsis* is essential for normal development and participates in the synthesis of the steroid hormone brassinolide (38). Dwarf3 from *Zea mays* is required for proper growth and development in that species (39). These relationships, along with the embryonic expression and potential RA catabolizing activity, warrant investigation of CYP26 in mammalian embryogenesis. CYP26 is somewhat unusual among the CYP superfamily in that it is expressed in the brain. RT-PCR data from regions of the human brain suggest that part of the reason that the expression levels are low is that expression is regionally restricted in the CNS. Some CYP family members participate in the synthesis of neurosteroids, which are known to affect brain function in a variety of ways (40). It will be interesting to identify the precise locations of CYP26 expression and determine if CYP26 has an important role in brain physiology.

In summary, the expression of CYP26, which represents a novel cytochrome P450 enzyme family, implicates the putative enzyme in the synthesis of physiological systems, such as development, brain function, and retinoid metabolism in mammals. Given that RA is a known teratogen, a possible morphogen, and a chemotherapeutic agent for some forms of leukemia (20), the understanding of enzymes acting downstream of RA or on RA directly will be of considerable interest.

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