Proc. Indian natn Sci Acad.(PINSA) **B69** No.6, pp 917-929 (2003)

Involvement of Cytochromes P450 in Development

JOHN B SCHENKMAN[1*], DHARAMAINDER CHOUDHARY[1], INGELA JANSSON[1], MANSOOR SARFARAZI[2] AND IVAYLO STOILOV[2]

Department of Pharmacology[1] and Department of Surgery[2], University of Connecticut Health Center, Farmington, Connecticut 06030, USA

(Received on 30 December 2002; Accepted after revision on 22 March 2003)

Cytochrome P450 is generally thought of as a superfamily of oxidative enzymes that functions in xenobiotic metabolism. The fact is, however, that the many forms of cytochrome P450 are found in almost every phylum and have roles in homeostasis, synthesizing biochemicals used in normal functioning, and in signaling in and between cells and tissues. In this review we point out other functions of cytochrome P450 forms, that of participating in the organization and shaping of the developing organism. Examples are provided in a number of phyla, from fungi to mammals, of cytochrome P450 forms functioning in morphogenesis in the developing organism. A recent, related review focusing on the involvement of cytochrome P450 in plant homeostasis and development has been published (Kim and Tsukaya 2002)

Key Words: Development, Cytochrome 450, Morphogenesis, Embryo/foetus

Introduction

We are constantly under challenge by chemicals in the environment, lipophilic compounds that enter the body via the airways, skin, and alimentary canal, the latter in the food as plant products or contaminating agricultural chemicals used as growth enhancers or pesticides. Chemicals with a high oil/water partition coefficient are poorly excreted, and living organisms have evolved enzyme systems that facilitate renal excretion of such compounds by making them more polar (Schenkman 1999). One such enzyme system involves the cytochrome P450 mono-oxygenase superfamily. The indication that members of these families have a major role in the oxidative metabolism of lipophilic xenobiotics, as well as endogenous lipophilic compounds, such as steroid hormones, was an extension of several earlier observations. In the 1950s it was noted that hepatic endoplasmic reticulum fragments, the microsomes, contained enzymes responsible for the oxidation of drugs and chemicals (Brodie, et al. 1958). The identification of cytochrome P450 as a microsomal hemoprotein (Omura and Sato 1962, Omura and Sato 1964) and its function as a terminal oxidase (Cooper, et al. 1965, Estabrook, et al. 1963) responsible for the oxidations sparked several decades of intense investigation into this superfamily of monooxy-genases.

Individual species have large numbers of cytochrome P450 forms. Humans, for example, have at the latest listing, 57 different cytochromes P450 (http://drnelson.utmem.edu/CytochromeP450.html), contained in 18 families and 33 subfamilies (figure 1). Half of the total number of forms of human cytochrome P450 are found in families 1-3, which are generally thought of as 'xenobiotic metabolizing' enzymes. The enzymes in these three families are responsible for increasing the polarity of exogenous chemicals, taken into the body as drugs or environmental contaminants, as well as endobiotics, chemicals of endogenous origin, such as steroid hormones. Family 1 members are generally considered to be responsible for the metabolism of polycyclic aromatic hydrocarbons and for activation of a large number of procarcinogens (Gonzalez and Gelboin 1994, Shimada, et al. 1996). Members of this family are induced by polycyclic aromatic hydrocarbons and other ligands, like TCDD, of the cytoplasmic aryl hydrocarbon (AH) receptor (Bresnick 1993, Nebert and Jones 1989). Family 2 (figure 1) has the largest number of individual enzyme forms in mammals, and its members are readily, but differentially, induced by a large number of drugs and chemicals, some of which also serve as substrates. For example, members of Subfamily CYP2B are transcriptionally induced by barbiturates, like phenobarbital

*Corresponding Author: *E-mail:* jschenkm@neuron.uchc.edu; *Tel:* (860) 679-3694; *FAX:* (860) 679-2473

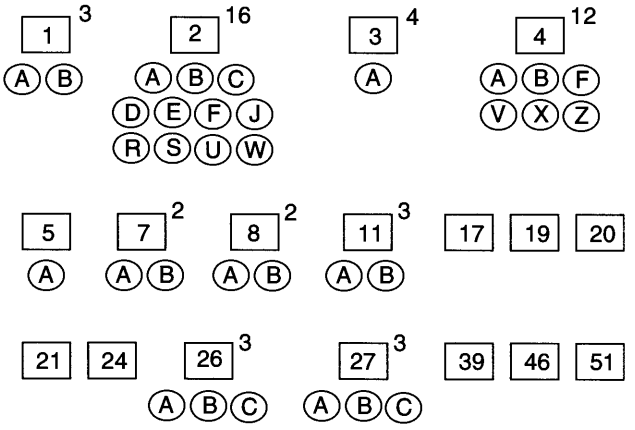

Figure 1. Human cytochrome P450 families and subfamilies. Superscript numbers indicate the number of members in this family.

(Honkakoski & Negishi 1998, Honkakoski et al. 1998), and metabolize a wide variety of drugs, while Subfamily CYP2E members are similarly inducible by a number of small molecules, like ethanol, acetone and pyrazole (Ronis et al. 1991, Song et al. 1987), and metabolize alcohol and a number of nitrosamines. In contrast, CYP2D does not appear to be inducible (Johansson et al. 1993), but metabolizes a number of drugs, including propranolol, codeine and antidepressants. As in rodents, (Funae & Imaoka 1993), human Family 3 members are very active in the oxidative metabolism of steroid hormones, like testosterone, estradiol and progesterone (Guengerich et al. 1993, Waxman et al. 1991). Family 3 has but one subfamily, CYP3A, and members are inducible by steroids, like dexamethasone, (Schuetz et al. 1984) and responsible for steroid 6b-hydroxylations. Being the predominant form of cytochrome P450 in the liver microsomes, CYP3A forms are major contributors in drug metabolism, oxidizing a large number of different drugs (Guengerich 1994). Interestingly, Family 4 is the second largest family of cytochrome P450 in mammals, but unlike Family 2 forms these enzymes do not appear to be important in the metabolism of xenobiotics. In fact, of the fourteen Family 4 human enzymes, only CYP4B1 and CYP4F12 have been reported to be capable of xenobiotic metabolism (Hashizume et al. 2002, Verschoyle et al. 1993). For those other forms tested, only metabolism of lipids e.g., eicosanoids like arachidonate, prostaglandins and leukotrienes have been reported (Bylund et al. 2000, Hardwick 1991, Kikuta et al. 2000, Masters et al. 1993, Sundseth & Waxman 1992). From the very large number of members of this family, and their minimal impact on xenobiotic metabolism, it is possible they play a role in inactivation or generation of bioactive lipids and lipid-derived compounds.

Cytochromes P450 involved in 'housekeeping' functions

Most of the other families of cytochrome P450 in mammals have important roles in maintaining homeostasis in the adult animal, producing components of intermediary metabolism involved in signaling and/or maintenance of the various functions of the different tissues, i.e., what we consider as 'housekeeping' functions (table 1). Biochemicals produced by these enzymes are sterols and steroids, like cholesterol and the corticosteroids and sex steroids, and lipid-derived compounds, like the prostacylins and thromboxanes, as well as the bile acids, and certain vitamins. These families generally have only one or two subfamilies, and rarely more than three members. In contrast to the very broad substrate specificities of the Families 1-3 cytochromes P450, these families have more specific substrate dependence, although some may be able to bind and oxidize a few xenobiotics. Some examples of these specific forms include, CYP51, lanosterol 14a-demethylase, which catalyzes synthesis of cholesterol from lanosterol; orthologous forms of this cytochrome P450 exist in most eukaryotic species, including the unicellular fungi. CYP11A1 cleaves the side chain of cholesterol preparatory for production of a range of steroids. CYP7A1 hydroxylates cholesterol in the 7a-position, sending the sterol into a pathway toward bile acid synthesis in which other forms of cytochrome P450, like CYP8B1 (12a-hydroxylation) participate. CYP27A1 activates Vitamin D3 by 25-hydroxylase activity and will also hydroxylate cholesterol in the 27-methyl group, another step in the formation of bile acids. CYP46 acts to form the oxysterol 24-hydroxycholesterol, and also a substrate for bile acid synthesis (Russell 2000). CYP27B1 is necessary in the 1a-hydroxylation of 25-hydroxy vitamin D3 to the active 1a, 25-dihydroxy vitamin D3. CYP26 is involved in the metabolism of the active vitamin A metabolite, all-trans retinoic acid. Other examples of housekeeping functions include formation of the prostacyclins (CYP8A) thromboxanes (CYP5A1) and aldosterone (CYP11B2).

Developmental functions of cytochrome P450

Over the last few years evidence has begun to appear suggestive of a role for cytochrome P450 in development of the organism (Stoilov 2001, Stoilov, et al. 2001). In 1997, a study of primary congenital glaucoma, a disease affecting newborns, linked the disease to mutations in the gene of a form of

Table 1. *Cytochrome P450 Housekeeping Genes*

CYP	Common name	Substrates	Function
CYP5A1	Thromboxane synthase	PGH_2	Forms thromboxane A_2
CYP7A1	Cholesterol 7α-hydroxylase	Cholesterol	Bile acid synthesis
CYP7B1	Oxysterol 7α-hydroxylase	25OH-Cholesterol & 27OH-Cholesterol	Neurosteroid synthesis
CYP8A1	Prostacyclin synthase	PGH_2	Prostaglandin synthesis
CYP8B1	Sterol 12α-hydroxylase	7αOH 4-cholesten-3-one	Bile acid synthesis
CYP11A1	P450scc	Cholesterol	Pregnenolone
CYP11B1	11β-hydroxylase	Deoxycorticosterone 11-deoxycortisol	Corticosteroid synthesis
CYP11B2	Aldosterone synthase	Corticosterone 18OH-Corticosterone	Corticosteroid synthesis
CYP17	17α-hydroxylase	Pregnenolone Progesterone 17αOH-Pregnenolone 17αOH-Progesterone	Steroid synthesis
CYP19	Aromatase	Androstenedione Testosterone	Estrogen synthesis
CYP20	–	–	–
CYP21	Sterol 21-hydroxylase	Progesterone 17OH-Progesterone	Corticosteroid synthesis
CYP24	1α,25-Dihydroxyvitamin D_3 -24-hydroxylase	1α,25-Dihydroxyvitamin D_3	Vitamin D degradation
CYP26	Retinoic acid hydroxylase	Retinoic acid	Retinoate degradation
CYP27A1	Sterol 27-hydroxylase	Cholesterol Vitamin D_3	Bile acid synthesis Vitamin D3 25-hydrox.
CYP27B1	25OH-vitamin D_3-1-α-hydroxylase	25OH-vitamin D_3	Vitamin D_3 activation
CYP39	Oxysterol 7α-hydroxylase	24OH-Cholesterol	Bile acid synthesis
CYP46	Cholesterol 24-hydroxylase	Cholesterol	Bile acid synthesis
CYP51	Lanosterol 14α-demethylase	Lanosterol	Cholesterol synthesis

cytochrome P450, CYP1B1 (Stoilov et al. 1998, Stoilov et al. 1997). Confirmation soon followed in reports by other groups (Bejjani et al. 1998, Bejjani et al. 2000, Mashima et al. 2001, Panicker et al. 2002, Plasilova et al. 1998, Plasilova et al. 1999). This gene has orthologous forms in different species of mammals and is the only member of its subfamily. An examination of species in which CYP1B1 has been detected, indicates that Subfamily 1B members are found in mammals, as well as in the earlier evolving bony fishes, such as zebrafish and fugu (http://drnelson.utmem.edu/CytochromeP450.html), indicating an early evolutionary appearance. The disease phenotype presents as elevated intraocular pressure and abnormal development of the trabecular meshwork in the eye and also with defects in the Canal of Schlemm (WuDunn 2002). These studies provide direct evidence that a form of cytochrome P450 is involved in normal eye development in the mammalian organism. An earlier examination of a Cyp1b1-null mouse strain revealed no gross phenotypical defects in the homozygous null animals (Buters et al. 1999). However, more recent histological examination showed anomalies in the trabecular meshwork and Canal of Schlemm in the homozygous Cyp1b1-null mouse (Libby et al. 2003). Studies on Cyp1b1 in the mouse eye revealed an absence of mRNA transcripts around the trabecular meshwork at all in utero stages, but presence in the retinal neuroepithelium at developmental age E15 (Bejjani et al. 2002). The morphogenic pathway and nature of the morphogenic agent affected by CYP1B1 in eye development is, at present, unknown.

Xenobiotic-metabolizing P450 Family Members in the Conceptus

One of the earliest reports of the presence of cytochrome P450 in the conceptus, showed the presence of the hemoprotein in human fetal liver as early as 14 weeks (Yaffe et al. 1970). These investigators also reported the presence of NADPH-cytochrome P450 reductase in this tissue. Early attempts to identify forms of cytochrome P450, which were produced in the embryo, indicated the presence of CYP3A subfamily members. A form of cytochrome P450 not found in the adult liver, CYP3A7, was purified from human fetal liver (Kitada et al. 1985). Attempts to see CYP1A1, and CYP1A2 mRNA in poly(A)-rich RNA extracts of 21-31 day rabbit fetus were negative, while CYP3A6 only appeared just prior to birth (Pineau et al. 1991). In contrast, a number of studies have shown the presence of the Family 1 cytochrome P450 in the conceptus (table 2). Interestingly, Cyp1a1- and Cyp1a2-null mice showed normal development (Dalton et al. 2000, Liang et al. 1996). Transcripts for Cyp51 have been found in the pre-implantation developing mouse blastocyst, but transcripts for other 'housekeeping' forms of cytochrome P450, those involved in steroid metabolism, Cyp17, Cyp11a1, Cyp19 and Cyp27, were not detected at that developmental stage (Stromstedt et al. 1996). To date, a number of forms of cytochrome P450 have been reported to be constitutively present in developing mammals in utero, the majority being identified from mRNA transcripts. These include another member of the CYP1 Family, CYP1B1, as well as a dozen from Family 2, and half that number from Family 3A. Most of the latter have been found in late prenatal developmental stages of the fetus (table 2). The functional role played by the presence of these forms has been suggested to involve xenobiotic metabolism and to be associated with toxicities produced by reactive intermediates (Miller et al. 1996).

Cytochrome P450 is a terminal oxidase that requires for its function the enzyme NADPH-cytochrome P450 reductase. In support of a developmental role for forms of cytochrome P450 in the conceptus, mRNA for NADPH-cytochrome P450 reductase was detected in the 4-day, preimplantation mouse blastocyst (Stromstedt et al. 1996). NADPH-cytochrome P450 reductase was shown to have a specific spatial orientation in the developing mouse embryo (Keeney and Waterman 1999). Further, lethality was associated with knockout of the NADPH-cytochrome P450 reductase gene (Shen et al. 2002). These studies suggest a role for cytochrome P450 in the development of the embryo. However, the reductase can also participate in lipid metabolism (Schenkman & Jansson 2003), a defect in which might also be lethal to the developing embryo.

In the examination of human 17-23 week embryonic cDNA libraries of different tissues (figure 2), selective and differential expression of CYP1B1 transcripts was observed , suggestive of a role for this enzyme in the human embryo. CYP1B1 transcripts were seen in lung, skeletal muscle, and heart, and levels were especially prominent in spleen (Lane 8), thymus (Lane 9) and kidney (Lane 5). Signals were absent in cDNA libraries of brain and liver. While a number of forms of cytochrome P450 have been reported to be expressed in the human embryo and fetus, to date, only a few of forms of cytochrome P450 have been detected in the mouse conceptus (table 2). However, an examination of mouse embryo cDNA libraries at different stages of development revealed a temporal component to the developmental appearance of a number of different cytochrome P450 forms (Choudhary et al. 2003). This is exemplified by the data shown in (figure 3) for Cyp1b1, where the signal for Cyp1b1 did not appear until post-conception day 11 (E11). In mouse 93 forms of cytochrome P450 have been identified. These are contained in 18 families and 33 subfamilies (figure 4). Recent studies from our laboratory, using gene-specific RT-PCR methodology, indicate the appearance of at least 27 different cytochromes P450 at different stages of embryonic development (Choudhary et al. 2003). Since only 40 gene-specific primers were utilized in that study, it is clear that the number of forms appearing will be considerably higher, when the complete complement of forms is examined.

Forms of Cytochrome P450 Involved in Morphogenesis

The role of a gene product in development is more readily identified when a mutation in the gene results in a mutational phenotype. The effects of cytochrome P450 gene mutations illustrating a role in development in several phyla are indicated below and in (figure 5). A recent essay reviewing concepts on morphogens and pattern formation (Neumann and Cohen 1997) provides criteria for a compound to meet in order to be considered a morphogen: a) the agent must have a direct effect on target cells and b) the influence of the agent must be concentration dependent. A known morphogen,

Table 2. *Cytochromes P450 in the Conceptus*

CYPs	Species (age)	Reference
CYP1A1	Mouse (E7)	(Kimura et al. 1987)
	Rat (late prenatal)	(Rich and Boobis 1997)
	Rabbit (late prenatal)	(Rich et al. 1993)
	Human (E45-60)	(Omiecinski et al. 1990, Yang et al. 1995)
	Human (11-13 wks)	(Shimada et al. 1996)
CYP1A2	Rat (late prenatal)	(Rich et al. 1993)
	Rabbit (late prenatal)	(Rich and Boobis 1997)
CYP1B1	Mouse (E11-17)	This study
	Human (12-19 wks)	(Hakkola et al. 1997)
	Human (17-23 wks)	(Jansson et al. 2001)0
CYP2A6	Human (91-125d)	(Gu et al. 2000)
CYP2A13	Human (91-125d)	(Gu et al. 2000)
CYP2B6	Human (91-125d)	(Gu et al. 2000)
CYP2B15	Rat (?)	(Keeney et al. 1998)
CYP2B19	Mouse (E15.5-postnatal)	(Keeney et al. 1998)
CYP2C8	Human (11-17 wks)	(Hakkola et al. 1994)
CYP2C9	Human (16-40 wks)	(Hines and McCarver 2002)
CYP2C18	Human (16-40 wks)	(Hines and McCarver 2002)
CYP2D6	Human (17-40 wks)	(Treluyer et al. 1991)
	Human (11-17wks)	(Hakkola et al. 1994)
CYP2E1	Human (7-16 wks)	(Brzezinski et al. 1999)
	Human (16-24 wks)	(Carpenter et al. 1996)
CYP2J2	Human (91-125d)	(Gu et al. 2000)
CYP2J5	Mouse (E18)	(Ma et al. 1999)
CYP3A1	Rat (late prenatal)	(Simmons and Kasper 1989)
CYP3A4	Human (9-12 wks)	(Hakkola et al. 2001)
CYP3A5	Human (9-12 wks)	(Hakkola et al. 2001)
CYP3A6	Rabbit (E30)	(Pineau et al. 1991)
CYP3A7	Human (55-81d)	(Kitada 1991 Yang et al. 1994)
CYP3A16	Mouse (E17)	(Itoh et al. 1994)
CYP7B	Mouse (12.5-18.5)	(Bean et al. 2001)
CYP11A	Mouse (13.5-17.5)	(Keeney et al. 1995)[a]
CYP17A	Mouse (E11-15)	(Keeney et al. 1995)
	Rat (E10.5-19.5)	(Compagnone et al. 1995)
CYP19	Equine (E12-15)	(Walters et al. 2000)
CYP21	Mouse (E14-18)	(Raschella et al. 1989)
CYP26A1	Mouse (E6-17)	(Fujii et al. 1997)
	Human (E57-110)	(Trofimova-Griffin et al. 2000)
	Human (20-26wks)	(Trofimova-Griffin and Juchau 1998)
CYP26B1	Mouse (E8-postnatal)	(Abu-Abed et al. 2002, MacLean et al. 2001)
	Human (E57-224)	(Trofimova-Griffin and Juchau 2002)
CYP51	Mouse (E4)	Stromstedt et al. 1996

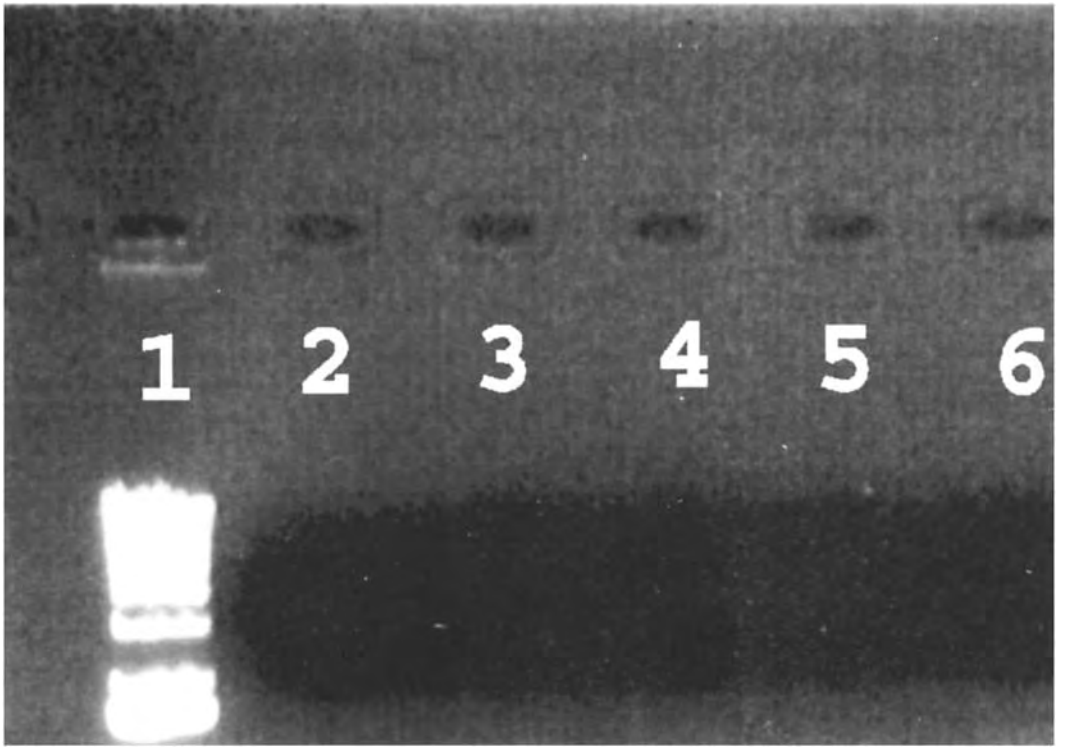

Figure 2. Profile of expression of CYP1B1 transcripts in human fetal tissue cDNA libraries. Lane 2: Fetal Brain. Lane 3: Fetal Lung. Lane 4: Fetal Liver. Lane 5: Fetal Kidney. Lane 6: Fetal Heart. Lane 7: Fetal Skeletal Muscle. Lane 8: Fetal Spleen. Lane 9: Fetal Thymus. Lanes 1 and 10: 1 Kb Plus DNA Ladder (GibcoBRL). PCR of each cDNA library was carried out for 27 cycles of amplification to allow comparison of relative amounts of transcript in the libraries. 10 ml of each amplification reaction was applied to the 1% agarose gel and after electrophoresis was visualized by fluorescence of ethidium bromide. G3PDH band (1Kb) is seen in lanes 2-9. The number of amplification cycles was optimized in a preliminary experiment to ensure that the PCR reaction had not reached its plateau in order to be able to compare amounts of signal from the different tissues. [after Jansson et al. (2001) *Pharmacogenetics* **11** 1-9, with permission from the publishers (Williams & Wilkins)].

retinoic acid (RA), the active metabolite of vitamin A, has been studied for over 60 years. Deficiency of vitamin A results in a wide range of malformations, while excess of RA during pregnancy also leads to developmental defects. Retinoic acid has a role in development in patterning and formation of the anterior-posterior axis {see review (Maden 1999)}. Retinoic acid exerts its effects by serving as a ligand for nuclear retinoic acid receptors (RAR) and retinoate X receptor (RXR). The level of retinoic acid in tissues is regulated by a balance in the rate of synthesis and oxidative degradation by retinoic acid oxidase, Cyp26, which catalyzes removal of the retinoic acid (Fujii et al. 1997, Hollemann et al. 1998, Swindell et al. 1999). In order for RA to exert its effects properly there has to be an uneven distribution of the RA, and this is provided by a differential expression of CYP26. In fact, the presence of RA in regions where Cyp26 would normally prevent its elevation resulted in severe developmental defects in the Cyp26-null mouse, including caudal truncation, resulting in embryonic and fetal lethality (Sakai et al. 2001). In this case, and unlike the effect of destructive CYP1B1 mutation, the identity of the morphogenic agent and the role of the cytochrome P450 is known. A number of other forms of cytochrome P450 have been shown to

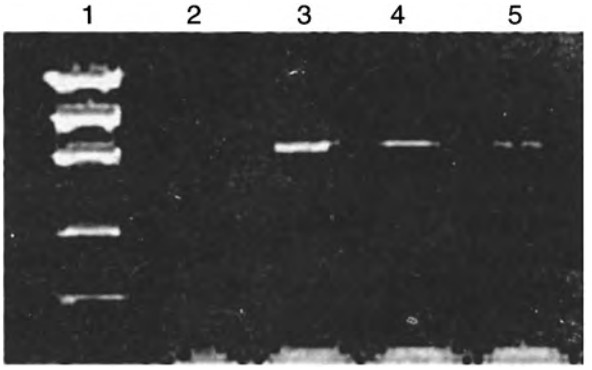

Figure 3. Expression of Cyp1b1 transcripts in mouse fetal tissue cDNA libraries (Clontech) at E7, E11, E15 and E17 (tracks 2-5 respectively). Conditions were as in Figure 2. Track 1 contains a low DNA mass ladder (Invitrogen).

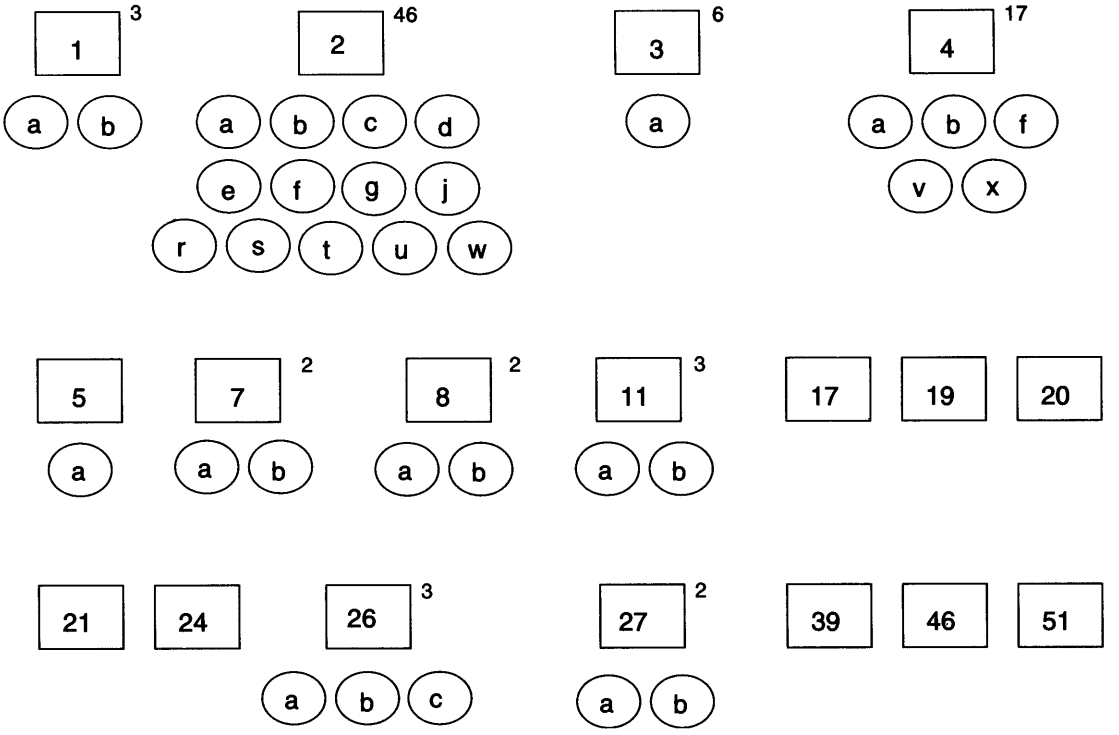

Figure 4. Mouse cytochrome P450 families and subfamilies. Superscript numbers indicate the number of members in this family.

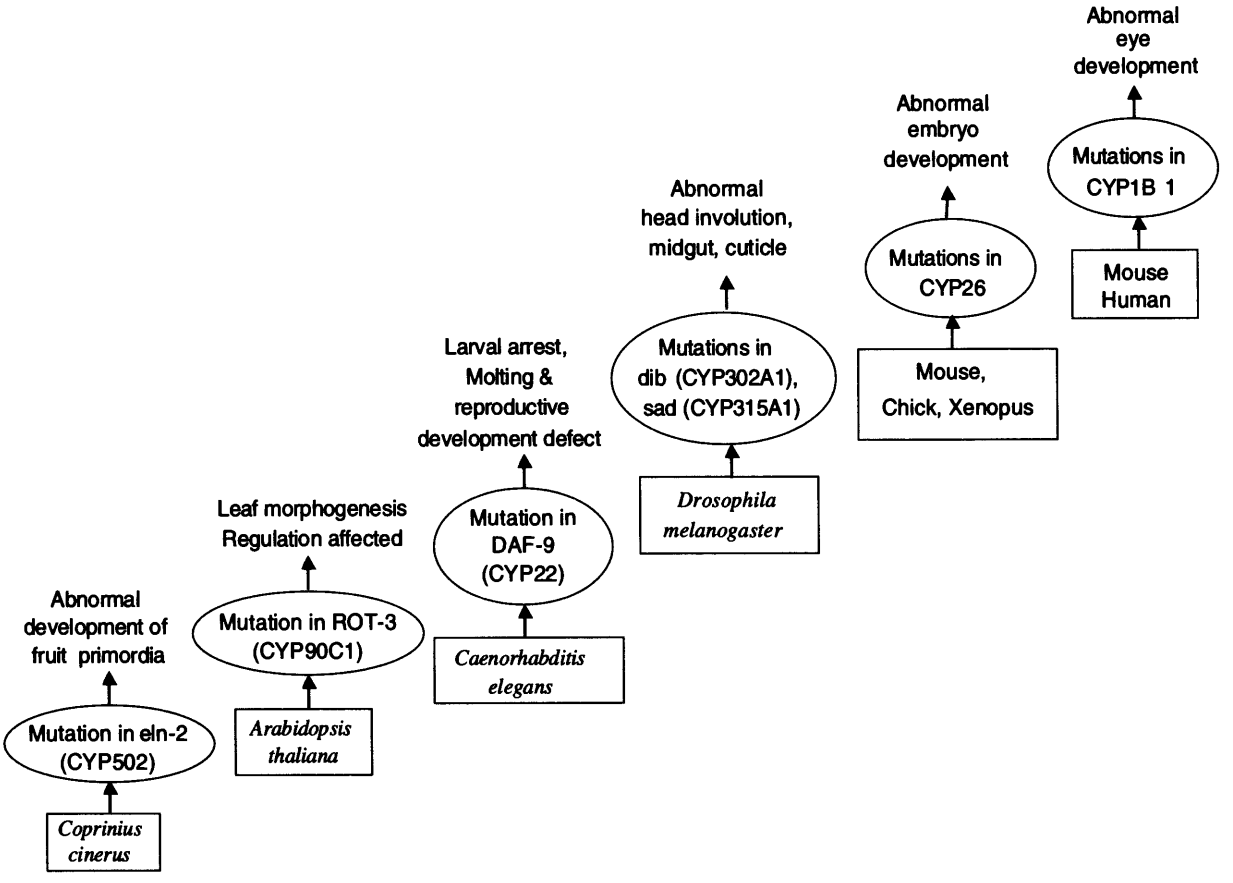

Figure 5. Developmentally active forms of cytochrome P450 in different phyla.

be capable of retinoic acid degradation, such as CYP3A7, CYP3A5, CYP2C18, CYP2C8, and CYP3A4 (Marill et al. 2000). However, for these forms to function in development, they would need a spatial and temporal expression pattern paralleling that of retinoic acid synthesis, as indicated above. A number of other examples of cytochrome P450 influencing development have appeared in recent years (figure 5). As with CYP1B1, the developmental influence of the cytochrome P450 in these examples was determined from examination of the basis of mutation phenotypes:

CYP502. The mushroom Coprinus cinereus develops a fruit body by differentiation of its tissues, and an expansion of differentiated cells causing elongation of its stipe. A dominant mutation (eln2-1) in the elongationless2 (eln2) gene results in 'dumpy primordia' with mature fruit bodies on short stipes (Maraguchi & Kamada 2000). The gene was shown to code for a form of cytochrome P450, CYP502, and the mutation, a truncating one causing loss of 18 residues from the carboxy-terminal end, might be producing a deleterious effect on the cellular morphogenesis. Since the mutation is a dominant one, the possibility exists that the mutated protein produces an abnormal metabolite or amount of metabolite that prevents the normal morphogenic response of the stipe cells. As with CYP1B1, the nature of the critical metabolite influenced by the wild-type enzyme is unknown at this time.

CYP90C1. In Arabidopsis thaliana, the homozygous rot3 mutant had shortened leaves, a defect in the polar elongation of the leaves. Molecular cloning of the ROT3 gene revealed it to encode a form of cytochrome P450, named CYP90C1 (Kim et al. 1998). Structurally, the deduced sequence had domains that were homologous to substrate recognition domains of steroid hydroxylase forms of cytochrome P450, and it was suggested that it plays a role in leaf polarization by generating a steroid, perhaps a brassinosteroid known to influence plant development (Kim et al. 1999).

Daf-9. Caenorhabiditis elegans has the ability to go into a developmentally dormant or dauer larval stage in which it may exist for several months under unfavorable environmental conditions. Entrance into this stage is affected by a nuclear hormone receptor, DAF-12, that responds to sensory transduction pathway signals and controls dauer and non-dauer morphogenesis of the larval development. The natural ligand for DAF-12 is at present unknown. However, a mutation in a C. elegans gene, daf-9, was found to cause the entrance into a dauer larval state, indicating it was upstream in the pathway, perhaps producing some DAF-12 ligand. Daf-9 was found to encode a cytochrome P450 protein, similar to forms of cytochrome P450 with phylogenetic similarity to CYP2C3, a rabbit progesterone hydroxylase, human CYP17, and CYP21 and to *Drosophila* CYP18. The similarity suggested the possibility that the daf-9 gene product is a steroid hydroxylase responsible for production of a steroid receptor ligand (Jia et al. 2002), the nature of which is, as yet, unknown. The CYP designation for daf-9 has been assigned as CYP22 on the Nelson's Web Page (http://drnelson. utmem. edu/C.elegans.RNAi.html), where it is indicated that inactivation of another C. elegans cytochrome P450 form, CYP13A8, has been found to result in embryonic lethality.

CYP 302A1 and CYP315A1. In Drosophila a mutation in the disembodied (dib) gene was shown to result in poorly differentiated embryonic cuticle. Mutations were also shown to prevent dorsal closure, midgut morphogenesis and headinvolution. The deduced sequence of dib revealed that it coded for a form of cytochrome P450, which was named CYP302A1 (Chavez et al. 2000). Ecdysteroids are necessary for cuticle formation. Since dib mutations cause cuticle defects, it was suggested that this form of cytochrome P450 might be involved in ecdysteroid biosynthesis or breakdown. Examination of the ecdysteroid titers in the mutants revealed severely reduced levels of ecdysone and 20-hydroxyecdysone, suggesting CYP302A1 is involved in the biosynthesis of the ecdysteroid (Chavez et al. 2000). CYP302A1 has subsequently been shown to catalyze 22-hydroxylation of 2,22-dideoxyecdysone (ketotriol) to 2-deoxyecdysone and 22-deoxyecdysone to ecdysone (Warren et al. 2002). Another gene, sad, has also been found to code for a form of cytochrome P450, CYP315A1. Sad catalyzes the hydroxylation of ketotriol to 22-deoxyecdysone, and 2-deoxyecdysone to ecdysone (Warren et al. 2002). Thus, sad and dib appear to function together in the pathway of 20-hydroxyecdysone biosynthesis. Ecdysteroids bind to nuclear receptors (EcR) which heterodimerize with the Drosophila retinoate X receptor (RXR) for transcriptional regulation and might explain the strong developmental impairment in dib mutations. A form of cytochrome P450, CYP6H1, has been isolated from the insect Locusta migratoria, that may function as ecdysone 20-hydroxylase in the locust (Winter et al. 1999), but has only about 20% sequence identity with CYP302A.

Future Developments

Other developmental forms of cytochrome P450 also exist, such as the DIF-3 hydroxylase in the slime mold, Dictyostelium discoideum, which facilitates removal of the differentiation-inducing factor DIF-1 (Kay et al. 1999, Morandini et al. 1995). The function of this form of cytochrome P450 is similar to that of CYP26. Obviously, the very large number of cytochrome P450 families in the many species will eventually be shown to contain many more developmentally active forms of cytochrome P450 and should be the subject of a more inclusive review. The use of newer tools, such as microarray and proteomics will help in clearly delineating the complete complement of constitutive gene expression profiles at each time-point in specific developing tissues. The knowledge gathered from such data will be useful not only in understanding the role of CYPs in normal physiological processes, but also under clinical conditions, and understanding the pharmacokinetics of administered drugs during pregnancy.

Conclusions

Data accumulated to date point to a role for cytochrome P450 forms in the development of living organisms in the different phyla. In some manner this very versatile enzyme has interdigitated itself into the developmental process aiding in the morphogenesis of different tissues in the different species (figure 5). In some instances, the ability of cytochrome P450 to perform such functions appears to be by modifying lipophilic chemicals of endogenous origin, such as steroids, or developmentally important compounds such as vitamins. Perhaps the very broad substrate specificity of members of this superfamily is what makes it such a useful tool. However, such a versatile tool has proven it can also be a sword of Damocles, ready to doom developing organisms by its very presence, during exposure to certain chemicals. The ability to metabolize such a large range of chemicals means that it is only a matter of time before a metabolite of some chemical is produced which can inadvertently imitate a developmentally active chemical, thereby triggering teratologies or exerting neoplastic transformation. Many examples of such effects are known today. The future, however, is a bright one. As more is learned about the forms of cytochrome P450 present in the embryo and fetus it should be possible to generate lists of drugs and chemical agents that should be contraindicated or to be avoided during pregnancy.

Acknowledgements

Supported in part by grant 2 R01 ES03154 from the NIH and American Health Assistance Foundation National Glaucoma Research Grant G2001-021.

References

Abu-Abed S, MacLean G, Fraulob V, Chambon P, Petkovich M and Dolle P 2002 Differential expression of the retinoic acid-metabolizing enzymes CYP26A1 and CYP26B1 during murine organogenesis; *Mech. Dev.* **110** 173-7.

Bean R, Seckl J R, Lathe R and Martin C 2001 Ontogeny of the neurosteroid enzyme CYP7b in the mouse.; *Mol. Cell Endocrinol.* **174** 137-144.

Bejjani B A, Lewis R A, Tomey K F, Anderson K L, Dueker D K, Jabak M, Astle W F, Otterud B, Leppert M and Lupski J R 1998 Mutations in CYP1B1, the gene for cytochrome P450 1B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia.; *Am. J. Hum. Genet.* **62** 325-333.

Bejjani B A, Stockton D W, Lewis R A, Tomey K F, Dueker D K, Jabak M, Astle W F and Lupski J R 2000 Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus.; *Hum. Mol. Genet.* **9** 367-374.

Bejjani B A, Xu L, Armstrong D, Lupski J R and Reneker L W 2002 Expression patterns of cytochrome P4501B1 (Cyp1b1) in FVN/N mouse eyes.; *Exp. Eye Res.* **75** 249-257.

Bresnick E 1993 Induction of cytochromes P450 1 and P450 2 by xenobiotics.; in Cytochrome P450 (edn) pp 503-524 eds J Schenkman and H Gleim (Berlin: Springer-Verlag).

Brodie B B, Gillette J R and La Du B N 1958 Enzymatic metabolism of drugs and other foreign compounds; *Ann. Rev. Biochem.* **27** 427-454.

Brzezinski M R, Boutelet-Bochan H, Person R E, Fantel A G and Juchau M R 1999 Catalytic activity and quantitation of cytochrome P-450 2E1 in prenatal human brain; *J. Pharmacol. Exp. Ther.* **289** 1648-53.

Buters J T M, Doehmer J and Gonzalez F J 1999 Cytochrome P450-null mice; *Drug Metab. Rev.* **31** 437-447.

Bylund J, Hidestrand M, Ingelman-Sundberg M and Oliw E H 2000 Identification of CYP4F8 in human seminal vesicles as a prominent 19-hydroxylase of prostaglandin endoperoxides; *J. Biol. Chem.* **275** 21844-9.

Carpenter S P, Lasker J M and Raucy J L 1996 Expression, induction, and catalytic activity of the ethanol-inducible cytochrome P450 (CYP2E1) in human fetal liver and hepatocytes; *Mol. Pharmacol.* **49** 260-268.

Chavez V M, Marques G, Delbecque J P, Kobayashi K, Hollingsworth M, Burr J, Natzle J and O'Connor M B 2000 The Drosophila disembodied gene controls late embryonic morphogenesis and codes for a cytochrome P450 enzyme that regulates embryonic ecdysone levels; *Development* **127** 4115-4126.

Choudhary D, Jansson I, Schenkman J B, Sarfarazi M and Stoilov I 2003 Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues; *Arch. Biochem. Biophys.* **(In Press)**

Compagnone N A, Bulfone A, Rubenstein J L and Mellon S H 1995 Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system; *Endocrinol.* **136** 5212-23.

Cooper D Y, Levin S, Narasimhulu S, Rosenthal O and Estabrook R W 1965 Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems; *Science* **147** 400-402.

Dalton T P, Dieter M Z, Matlib R S, Childs N L, Shertzer H G, Genter M B and Nebert D W 2000 Targeted knockout of Cyp1a1 gene does not alter hepatic constitutive expression of other genes in the mouse [Ah] battery; *Biochem. Biophys. Res. Commun.* **267** 184-9.

Estabrook R W, Cooper D Y and Rosenthal O 1963 The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex; *Biochem. Z.* **338** 741-755.

Fujii H, Sato T, Kaneko S, Gotoh O, Fujii-Kuriyama Y, Osawa K, Kato S and Hamada H 1997 Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos; *EMBO J.* **16** 4163-4173.

Funae Y and Imaoka S 1993 Cytochrome P450 in rodents.; in Cytochrome P450 edn) pp 221-238 eds J Schenkman and H Greim (Berlin: Springer-Verlag).

Gonzalez F J and Gelboin H V 1994 Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins; *Drug Metab. Rev.* **26** 165-183.

Gu J, Su T, Chen Y, Zhang Q-Y and Ding X 2000 Expression of biotransformation enzymes in human fetal olfactory mucosa: Potential roles in developmental toxicity; *Tox. Applied Pharmacol.* **165** 158-162.

Guengerich F P 1994 Catalytic selectivity of human cytochrome P450 enzymes: relevance to drug metabolism and toxicity; *Toxicol. Lett.* **70** 133-138.

Guengerich F P, Gillam E M J, Ohmori S, Sandhu P, Brian W R, Sari M A and Iwasaki M 1993 Expression of human cytochrome-P450 enzymes in yeast and bacteria and relevance to studies on catalytic specificity; *Toxicology* **82** 21-37.

Hakkola J, Pasanen M, Pelkonen O, Hukkanen J, Evisalmi S, Anttila S, Rane A, Mantyla M, Purkunen R, Saarikoski S, Tooming M and Raunio H 1997 Expression of CYP1B1 in human and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells; *Carcinogenesis* **18** 391-397.

Hakkola J, Pasanen M, Purkunen R, Saarikoski S, Pelkonen O, Maenpaa J, Rane A and Raunio H 1994 Expression of xenobiotic-metabolizing cytochrome P450 forms in human adult and fetal liver; *Biochem. Pharmacol.* **48** 59-64.

———, Raunio H, Purkunen R, Saarikoski S, Vahakangas K, Pelkonen O, Edwards R J, Boobis A R and Pasanen M 2001 Cytochrome P450 3A expression in the human fetal liver: evidence that CYP3A5 is expressed in only a limited number of fetal livers; *Biol. Neonate* **80** 193-201.

Hardwick J P 1991 CYP4A subfamily - functional analysis by immunohistochemistry and in situ hybridization.; in Cytochrome P450 edn) pp 273-283.

Hashizume T, Imaoka S, Mise M, Terauchi Y, Fujii T, Miyazaki H, Kamataki T and Funae Y 2002 Involvement of CYP2J2 and CYP4F12 in the metabolism of ebastine in human intestinal microsomes; *J. Pharmacol. Exp. Ther.* **300** 298-304.

Hines R N and McCarver D G 2002 The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes; *J. Pharmacol. Exp. Ther.* **300** 355-60.

Hollemann T, Chen Y, Grunz H and Pieler T 1998 Regionalized metabolic activity establishes boundaries of retinoic acid signalling; *EMBO J.* **17** 7361-7372.

Honkakoski P and Negishi M 1998 Regulatory DNA elements of phenobarbital-responsive cytochrome P450 CYP2B genes; *J. Biochem. Molecular Toxicology* **12** 3-9.

———, Zelko I, Sueyoshi T and Negishi M 1998 The nuclear orphan receptor CAR-RXR heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene.; *Mol. Cell. Biol.* **18** 1-7.

Itoh S, Satoh M, Abe Y, Hashimoto H, Yanagimoto T and Kamataki T 1994 A novel form of mouse cytochrome P450 3A (Cyp3a-16). Its cDNA cloning and expression in fetal liver; *Eur J Biochem* **226** 877-82.

Jansson I, Stoilov I, Sarfarazi M and Schenkman J B 2001 Effect of two mutations of human CYP1B1, G61E and R469W, on stability and endogenous steroid substrate metabolism; Pharmacogenetics **11** 1-9.

Jia K, Albert P S and Riddle D L 2002 Daf-9, a cytochrome P450 regulating C. elegans larval development and adult longevity; *Development* **129** 221-231.

Johansson I, Lundqvist E, Bertilsson L, Dahl M L, Sjoqvist F and Ingelmansundberg M 1993 Inherited amplification of an active gene in the cytochrome-P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine; *Proc. Natl. Acad. Sci. USA* **90** 11825-11829.

Kay R R, Flatman P and Thompson C R 1999 DIF signalling and cell fate; *Semin Cell Dev. Biol.* **10** 577-85.

Keeney D S, Ikeda Y, Waterman M R and Parker K L 1995 Cholesterol side-chain cleavage cytochrome P450 gene expression in the primitive gut of the mouse embryo does not require steroidogenic factor 1; *Mol. Endocrinol.* **9** 1091-1098.

Keeney D S, Jenkins C M and Waterman M R 1995 Developmentally regulated expression of adrenal 17 alpha-hydroxylase cytochrome P450 in the mouse embryo; *Endocrinol.* **136** 4872-9.

Keeney D S, Skinner C, Traverse J B, Capdevila J H, Nanney L B, King L E, Jr. and Waterman M R 1998 Differentiating keratinocytes express a novel cytochrome P450 enzyme, CYP2B19, having arachidonate monooxygenase activity; *J. Biol. Chem.* **273** 32071-32079.

Keeney D S and Waterman M R 1999 Two novel sites of expression of NADPH cytochrome P-450 reductase during murine embryogenesis: limb mesenchyme and developing olfactory neuroepithelia; *Dev. Dynam.* **216** 511-517.

Kikuta Y, Kasyu H, Kusunose E and Kusunose M 2000 Expression and catalytic activity of mouse leukotriene B4 omega-hydroxylase, CYP4F14; *Arch. Biochem. Biophys.* **383** 225-32.

Kim G T and Tsukaya H 2002 Regulation of the biosynthesis of plant hormones by cytochrome P450s; *J. Plant Res.* **115** 169-77.

Kim G T, Tsukaya H, Saito Y and Uchimiya H 1999 Changes in the shapes of leaves and flowers upon overexpression of cytochrome P450 in Arabidopsis; *Proc. Natl. Acad. Sci.* USA **96** 9433-7.

Kim G-T, Tsukaya H and Uchimiya H 1998 The ROTUNDIFOLIA3 gene of Arabidopsis thaliana encodes a new member of the cytochrome P-450 family that is required for the regulated polar elongation of leaf cells; *Genes & Dev.* **12** 2381-2391.

Kimura S, Donovan J C and Nebert D W 1987 Expression of the mouse P(1)450 gene during differentiation without foreign chemical stimulation; *J. Exp. Pathol.* **3** 61-74.

Kitada M 1991 Cytochrome-P-450 in human fetal liver - properties of P-450 HFLa, a form of cytochrome-P-450 in human fetal livers; *Yakugaku Zasshi-Journal of the Pharmaceutical Society of Japan* **111** 158-169.

Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kato R and Kanakubo Y 1985 Purification and properties of cytochrome P-450 from homogenates of human fetal livers; *Arch. Biochem. Biophys.* **241** 275-280.

Liang H-C, Li H, McKinnon R A, Duffy J J, Potter S S, Puga A and Nebert D W 1996 Cyp1a2(-/-) null mutant mice develop normally but show deficient drug metabolism; *Proc. Natl. Acad. Sci. USA* **93** 1671-1676.

Libby R T, Smith R S, Savinova O V, Zabaleta A, Martin J E, Gonzalez F J and John S W 2003 Modification of ocular defects in mouse developmental glaucoma models by tyrosinase; *Science* **299** 1578-81.

Ma J, Qu W, Scarborough P E, Tomer K B, Moomaw C R, Maronpot R, Davis L S, Breyer M D and Zeldin D C 1999 Molecular cloning, enzymatic characterization, developmental expression, and cellular localization of a mouse cytochrome P450 highly expressed in kidney; *J. Biol. Chem.* **274** 17777-88.

MacLean G, Abu-Abed S, Dolle P, Tahayato A, Chambon P and Petkovich M 2001 Cloning of a novel retinoic-acid metabolizing cytochrome P450, Cyp26B1, and comparative expression analysis with Cyp26A1 during early murine development; *Mechisms of Development* **107** 195-201.

Maden M 1999 Heads or tails? Retinoic acid will decide; *BioEssays* **21** 809-812.

Maraguchi H and Kamada T 2000 A mutation in the eln2 gene encoding a cytochrome 450 of Coprinus cinereus affects mushroom morphogenesis; *Fungal Genet. Biol.* **29** 49-59.

Marill J, Cresteil T, Lanotte M and Chabot G G 2000 Identification of human cytochrome P450s involved in the formation of all-trans-retinoic acid principal metabolites; *Mol. Pharmacol.* **58** 134 1-1348.

Mashima Y, Suzuki Y, Sergeev Y, Ohtake Y, Tanino T, Kimura I, Miyata H, Aihara M, Tanihara H, Inatani M, Azuma N, Iwata T and Araie M 2001 Novel cytochrome P4501B1 (CYP1B1) gene mutations in Japanese patients with primary congenital glaucoma; Invest. Ophthalmol.Vis. Sci. **42** 2211-2216.

Masters B S S, Clark J E, Roman L J, Nishimoto M, Mccabe T J, Demontellano P R O, Plopper C G, Gebremedhin D, Ma Y H, Harder D R and Roman R J 1993 Functional aspects of eicosanoid hydroxylation by lung and kidney cytochromes-P450. Expression of cDNAs in mammalian cells and E-coli; *Journal of Lipid Mediators* **6** 353-360.

Miller M S, Juchau M R, Guengerich F P, Nebert D W and Raucy J L 1996 Drug metabolic enzymes in developmental toxicology; *Fund. App. Toxicol.* **34** 165-175.

Morandini P, Offer J, Traynor D, Nayler O, Neuhaus D, Taylor G W and Kay R R 1995 The proximal pathway of metabolism of the chlorinated signal molecule differentiation-inducing factor-1 (DIF-1) in the cellular slime mould Dictyostelium; *Biochem. J.* **306 (Pt 3)** 735-43.

Nebert D W and Jones J E 1989 Regulation of the mammalian cytochrome p1-450 (CYP1A1) gene; *Int. J. Biochem.* **21** 243-252.

Neumann C and Cohen S 1997 Morphogens and pattern formation; *BioEssays* **19** 721-729.

Omiecinski C J, Hassett C and Costa P 1990 Developmental expression and in situ localization of the phenobarbital-inducible rat hepatic mRNAs for cytochromes CYP2B1, CYP2B2, CYP2C6, CYP3A1; *Mol. Pharmacol.* **38** 462-470.

Omura T and Sato R 1962 A new cytochrome in liver microsomes; *J. Biol. Chem.* **237** 1375-1376.

Omura T and Sato R 1964 The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature; *J. Biol. Chem.* **239** 2370-2378.

Panicker S G, Reddy A B, Mandal A K, Ahmed N, Nagarajaram H A, Hasnain S E and Balasubramanian D 2002 Identification of novel mutations causing familial primary congenital glaucoma in Indian pedigrees; *Invest Ophthalmol Vis Sci* **43** 1358-66.

Pineau T, Daujat M, Pichard L, Girard F, Angevain J, Bonfils C and Maurel P 1991 Developmental expression of rabbit cytochrome-P450 CYP1A1, CYP1A2 and CYP3A6 genes. Effect of weaning and rifampicin; *Eur. J. Biochem.* **197** 145-153.

Plasilova M, Ferakova E, Kadasi L, Polakova H, Gerinec A, Ott J and Ferak V 1998 Linkage of autosomal recessive primary congenital glaucoma to the GLC3A locus in Roms (Gypsies) from Slovakia; *Human Heredity* **48** 30-33.

Plasilova M, Stoilov I, Sarfarazi M, Kadasi L, Ferakova E and Ferak V 1999 Identification of a single ancestral CYP1B1 mutation in Slovak Gypsies (Roms) affected with primary congenital glaucoma; *J. Med. Genet.* **36** 290-294.

Raschella G, Smets G, Claeys A, Verdood P, Romeo A and Hooghe-Peters E L 1989 Transcriptional pattern of 21-hydroxylase gene (P-450C21) during embryonic development, before, and after birth in mice as determined by in situ hybridization; *J. Histochem. Cytochem.* **37** 751-756.

Rich K J and Boobis A R 1997 Expression and inducibility of P450 enzymes during liver ontogeny; *Microscopy Research and Technique* **39** 424-435.

Rich K J, Foster J R, Edwards R J, Davies D S and Boobis A R 1993 Ontogenetic development of the distribution of constitutive and 3-methylcholanthrene-induced CYP1A1 and CYP1A2 in rabbit liver; *J. Histochem. Cytochem.* **41** 915-925.

Ronis M J J, Johansson I, Hultenby K, Lagercrantz J, Glaumann H and Ingelmansundberg M 1991 Acetone-regulated synthesis and degradation of cytochrome-P4502E2 and cytochrome-P4502B1 in rat liver; *Eur. J. Biochem.* **198** 383-389.

Russell D W 2000 Oxysterol biosynthetic enzymes; *Biochim. Biophys. Acta* **1529** 126-135.

Sakai Y, Meno C, Fujii H, Nishino J, Shiratori H, Saijoh Y, Rossant J and Hamada H 2001 The retinoic acid-inactivating enzyme CYP26 is essential for establishing an uneven distribution of retinoic acid along the anterio-posterior axis within the mouse embryo; *Genes & Dev.* **15** 213-225.

Schenkman J B 1999 The fate of xenobiotics in the body: enzymes of metabolism.; in Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes. edn) pp 1-20 eds E Arinc et al. (New York: Plenum Publishers).

Schenkman J B and Jansson I 2003 The many roles of cytochrome b_5.; *Pharmacol. Ther.* **97** 139-152.

Schuetz E, Wrighton S A, Barwick J L and Guzelian P S 1984 Induction of cytochrome P-450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16a-carbonitrile regulate de novo synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver *in vivo*.; *J. Biol. Chem.* **259** 1999-2006.

Shen A L, O'Leary K A and Kasper C B 2002 Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase; *J. Biol. Chem.* **277** 6536-6541.

Shimada T, Hayes C L, Yamazaki H, Amin S, Hecht S S and Guengerich F P 1996 Activation of chemically diverse procarcinogens by human cytochrome P450 1B1; *Cancer Res.* **56** 2979-2984.

Shimada T, Yamazaki H, Mimura M, Wakamiya N, Ueng Y-F, Guengerich F P and Inui Y 1996 Characterization of microsomal cytochrome P450 enzymes involved in the oxidation of xenobiotic chemicals in human fetal livers and adult lungs; *Drug Metab. Dispos.* **24** 515-522.

Simmons D L and Kasper C B 1989 Quantitation of mRNAs specific for the mixed-function oxidase system in rat liver and extrahepatic tissues during development; *Arch. Biochem. Biophys.* **271** 10-20.

Song B J, Matsunaga T, Hardwick J P, Park S S, Veech R L, Yang C S, Gelboin H V and Gonzalez F J 1987 Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat; *Mol. Endocrinol.* **1** 542-547.

Stoilov I 2001 Cytochrome P450s: coupling, development and environment; *Trends Genet.* **17** 629-632.

Stoilov I, Akarsu A N, Alozie I, Child A, Barsoum-Homsy M, Turacli M E, Or M, Lewis R A, Ozdemir N, Brice G, Aktan S G, Chevrette L, Coca-Prados M and Sarfarazi M 1998 Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1; *Am. J. Hum. Genet.* **62** 573-584.

Stoilov I, Akarsu A N and Sarfarazi M 1997 Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (buphthalmos) in families linked to the GLC3A locus on chromosome ep21; *Hum. Mol. Genet.* **6** 641-647.

Stoilov I, Jansson I, Sarfarazi M and Schenkman J B 2001 Roles of cytochrome P450 in development; *Drug Metab. Drug Interac.* **18** 1-23.

Stromstedt M, Keeney D S, Waterman M R, Paria B C, Conley A J and Dey S K 1996 Preimplantation mouse blastocysts fail to express CYP genes required for estrogen biosythesis; *Mol. Reproduc. Develop.* **43** 428-436.

Sundseth S S and Waxman D J 1992 Sex-dependent expression and clofibrate inducibility of cytochrome-P450 4A fatty acid mega-hydroxylases. Male specificity of liver and kidney Cyp4A2 messenger RNA and tissue-specific regulation by growth hormone and testosterone; *J. Biol. Chem.* **267** 3915-3921.

Swindell E C, Thaller C, Sockanathan S, Petkovich M, Jessell T M and Eichele G 1999 Complementary domains of retinoic acid production and degradation in the early chick embryo; *Dev. Biol.* **216** 282-296.

Treluyer J M, Jacqzaigrain E, Alvarez F and Cresteil T 1991 Expression of CYP2D6 in developing human liver; *Eur. J. Biochem.* **202** 583-588.

Trofimova-Griffin M E, Brzezinski M R and Juchau M R 2000 Patterns of CYP26 expression in human prenatal cephalic and hepatic tissues indicate an important role during early brain development; *Developmental Brain Research* **120** 7-16.

Trofimova-Griffin M E and Juchau M R 1998 Expression of cytochrome P450RAI (CYP26) in human fetal hepatic and cephalic tissues; *Biochem. Biophys. Res. Commun.* **252** 487-491.

Trofimova-Griffin M E and Juchau M R 2002 Developmental expression of cytochrome CYP26B1 (P450RAI-2) in human cephalic tissues; *Brain Res. Dev. Brain. Res.* **136** 175-178.

Verschoyle R D, Philpot R M, Wolf C R and Dinsdale D 1993 Cyp4B1 activates 4-Ipomeanol in rat lung; *Tox. Applied Pharmacol.* **123** 193-198.

Walters K W, Corbin C J, Anderson G B, Roser J F and Conley A J 2000 Tissue-specific localization of cytochrome P450 aromatase in the equine embryo by in situ hybridization and immunocytochemistry; *Biology of Reproduction* **62** 1141-5.

Warren J T, Petryk A, Marqués G, Jarcho M, Parvy J-P, Dauphin-Villemant C, O'Conner M B and Gilbert L I 2002 Molecular and biochemical characterization of two P450 enzymes in the ecdysteroidogenic pathway of Drosophila melanogaster; *Proc. Natl. Acad. Sci. USA* **99** 11043-11048.

Waxman D J, Lapenson D P, Aoyama T, Gelboin H V, Gonzalez F J and Korzekwa K 1991 Steroid hormone hydroxylase specificities of eleven cDNA-expressed human cytochrome-P450s; *Arch. Biochem. Biophys.* **290** 160-166.

Winter J, Bilbe G, Richener H, Sehringer B and Kayser H 1999 Cloning of a cDNA encoding a novel cytochrome P450 from the insect Locusta migratoria: CYP6H1, a putative ecdysone 20-hydroxylase; *Biochem. Biophys. Res. Commun.* **259** 305-310.

WuDunn D 2002 Genetic basis of glaucoma; *Curr. Opinion in Ophthalmology* **13** 55-60.

Yaffe S, Rane A, Sjöqvist F, Boréus L and Orrenius S 1970 The presence of a monooxygenase system in human fetal liver microsomes; *Life Sci.* **9** 1189-1200.

Yang H Y, Lee Q P, Rettie A E and Juchau M R 1994 Functional cytochrome P4503A isoforms in human embryonic tissues: expression during organogenesis; *Mol. Pharmacol.* **46** 922-928.

Yang H-Y L, Namkung M J and Juchau M R 1995 Expression of functional cytochrome P4501A1 in human embryonic hepatic tissues during organogenesis; *Biochem. Pharmacol.* **49** 717-726.