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Current Status Review
The cytochrome P450 gene superfamily

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Introduction

The term cytochrome P450 was originally coined by Omura and Sato in 1964 to describe a hepatic microsomal haemoprotein which bound carbon monoxide to give a characteristic absorption spectrum at 450 nm. Since its discovery, cytochrome P450 has been intensively studied by the scientific community because of the realization that it is an important determinant of the toxicity and carcinogenicity of many foreign compounds including drugs. In addition, many of these chemicals, as well as physiological/pathological conditions, modulate the content of cytochrome P450 in both hepatic and extrahepatic tissues and hence modify the toxicity/carcinogenicity of xenobiotics. For a general account of these membrane bound mono-oxygenases see Paine (1981) and Nebert and Gonzalez (1987). More recently it has been discovered that cytochromes P450 play an important role in the metabolism of normal body constituents such as steroid hormones, bile acids, fatty acids, ketones, leukotrienes, prostaglandins and vitamins (Gonzalez 1989, 1990).

The reason for this unusually wide range of substrates began to emerge when Lu *et al.* (1971) purified the hepatic cytochromes P450 induced by administration to rats of phenobarbitone or polycyclic aromatic hydrocarbons and showed them to be different isozymes. Although many other compounds have now been identified as inducers of cytochrome P450, progress in characterizing the forms affected has been slow because of technical difficulties in separating closely related proteins in high enough yield and purity as well as labour intensive procedures required for amino acid sequencing. For example, it was not until 1983 that a microheterogeneity in the phenobarbitone inducible cytochrome P450 was recognized (Yuan *et al.* 1983). These studies demonstrated that the rat hepatic isozyme known as cytochrome P450b was immunochemically indistinguishable from the isozyme known as P450e because these two proteins have a 97% sequence identity. Thus a major breakthrough in the structural analysis of cytochromes P450 came with the application of recombinant DNA technology which resulted in the isolation and sequencing of their individual cDNAs (Fujii-Kuriyama *et al.* 1982). Accordingly, many cDNAs for cytochrome P450 variants have been isolated and sequenced. This wealth of information has provided the basis for a unifying nomenclature for cytochromes P450 based on their divergent evolution and nucleotide deduced amino acid homologies (Nebert *et al.* 1987, 1990).

Nomenclature

This has been established only during the past few years to overcome the difficulty that virtually every laboratory involved in P450 purification developed its own system of nomenclature (Table 1). The purpose of Table 1, or for that matter this overview, is not to list every P450 gene family, subfamily and form but rather to indicate the diversity of previous nomenclature and illustrate how the new nomenclature is improving the understanding of the cytochrome P450 gene

Table 1. Diversity of nomenclature of some mammalian cytochromes P450

Gene designation			Old nomenclature							
			Rat from laboratory of							
			Levin	Guengerich	Waxman	Wolf	Others	Rabbit	Mouse	Human
I	A	1	c	BNF-B	—	MC_{1b}	P448	LM6	P_1450	P_1
I	A	2	d	ISF-G	—	MC_{1a}	P448	LM4	P_3450	P_3
II	A	1	a	UT-F	Form 3	UT_1	—	—	—	—
II	A	3	—	—	—	—	—	—	15αtype 1	Form 1
II	B	1	b	PB-B	PB_4	PB_{3a}	PBRLM5	LM2	—	—
II	B	2	e	PB-D	PB_5	PB_{3b}	PBRLM6	LM2	—	—
II	C	6	k	PB-C	PB-1	PB_{1b}	RLM5a	—	—	—
II	C	11	h	UT-A	2c	PB_{2a}	RLM5,16α	—	P450 16α	—
II	C	12	i	UT-1	2d	—	P450 15β		P450 15β	
II	D	1	—	UT-H	—	—	db1	—	—	db_1
II	E	1	j	—	—	—	RLM6	LM3a	—	j
III	A	1	p	—	—	—	pcn1	LM3	—	P450nf
IV	A	1	—	—	—	—	P452	—	—	—

See Gonzalez (1989) and references contained therein for sources of nomenclature.

superfamily. For more detailed reviews refer to Gonzalez (1989, 1990).

Currently the P450 superfamily comprises 21 gene families, ten of which exist in all mammals, and the total number of functional P450 genes in any given mammalian species range between at least 60 and perhaps 200. To date, each functional gene is known to produce a unique P450 (Gonzalez 1990).

The system of nomenclature is based on the alignment of amino acid sequences and so any P450 that has less than 36% sequence identity to another is placed in a separate gene family and these are designated by Roman numerals. For example, and shown in Fig. 1, the gene families I to IV code for the hepatic microsomal P450s involved in drug metabolism whereas families XI, XVII, XIX and XXI designate adrenal P450s involved in steroid biosynthesis. Furthermore, the LI and CI families code for a yeast lanosterol demethylase and a bacterial P450 found in *Pseudonomas putida* respectively. Several other P450s have been identified or characterized but as their cDNAs or genes have not been cloned and sequenced it is unknown whether they fall into existing gene families or comprise new families. Accordingly, gaps have been left in the nomenclature to accommodate new families. This has been facilitated because the Roman numerals chosen are also intended to aid workers in the field. For example, the enzymes for the 17α- and 21-hydroxylations of steroids have been assigned to the XVII and XXI families respectively while the P450 involved in 11β-hydroxylation of steroids is encoded by a gene in the XI family. Finally, the 'aromatase system' involved in the syn-

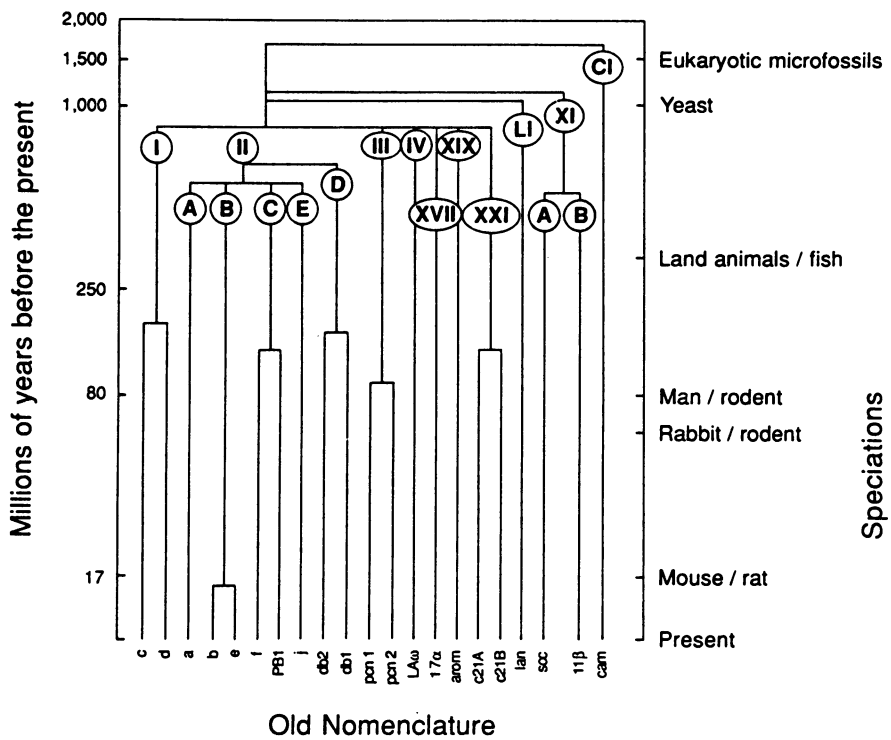

Fig. 1. Evolutionary aspects of the P450 gene superfamily. Modified from Nebert and Gonzalez (1987). Although only 10 gene families are illustrated here there are more than 100 total sequences presently available that enlarges the superfamily to 21 families.

thesis of oestrogens from androgens has recently been shown to represent a unique P450 gene family designated XIX because aromatization of the steroid A-ring includes the loss of carbon-19.

As depicted in Fig. 1, each P450 family diverged from one another about 600–900 million years ago so that, as previously mentioned, any protein in one gene family is less than 36% similar to proteins in any of the other gene families. A P450 with a sequence homology of 59% or more to another P450 is placed into the same gene subfamily. Subfamilies are indicated by use of sequential capital letters e.g. IIA, IIB, IIC, IID and IIE (Fig. 1) and individual genes and their protein products within a subfamily are denoted by sequential Arabic numerals. For example, only two genes, IA1 and IA2, reside in the polycyclic aromatic hydrocarbon inducible P450I family of rat, rabbit, mouse and man (Table 1). It should be noted here that the nomenclature makes a distinction between genes and their protein products. Thus, while individual proteins may be designated P450IIB1, the corresponding gene is named with the root '*CYP*' to denote *cy*tochrome *P*450 followed by the family and subfamily in italicised letters and numerals. Thus the gene *CYP2B1* encodes cytochrome P450IIB1.

Diversity of cytochromes P450

Based on the amino acid sequences of cytochromes P450 complex evolutionary trees have been constructed (e.g. Gonzalez 1990). Nevertheless, the simple tree shown in Fig. 1 indicates that according to this type of evolutionary analysis the ancestral P450 gene is probably more than 1500 million years old. The first point of divergence coincides with the separation of prokaryotes (CI family) and eukaryotes about 1400 million years ago (MYA) while a second divergence occurring around 1360 MYA separated cytochromes P450 in two different cellular organelles, namely the mitochondria and endoplasmic reticulum. Thereafter, the families diverged from one another between 600 and 900 MYA. Thus, many P450 genes diverged prior to the formation of discrete eukaryotic chromosomes resulting in the dispersal of loci over several mouse and human chromosomes (Table 2). Nevertheless, proteins within the same subfamily are approximately 70% or more similar to each other and so appear to have diverged within the past 150 million years given an average unit evolutionary period (the time in millions of years to effect a 1% change in amino acid sequence) for P450s of 4. Accordingly, the human IIA and IIB subfamilies, the human IIC and IIE subfamilies as well as the mouse IIA, IIB and IIE subfamilies are linked to the same chromosomes (Table 2). However, an interesting feature of P450 gene evolution is the occurrence of multiple genes that, in many cases, are present in some species but not others. These are presumed to have arisen through the process of gene duplication and fixation of duplicated genes through natural selection. From a practical point of view, the occurrence of multiple genes presents a problem in identifying orthologous P450 genes. From all existing data, orthologous gene products exhibit a 71–80% similarity in amino acid sequence and, for example, although human P450 form 1 is more similar (60%) to rat P450a than to any other protein it is, therefore, unlikely to be the orthologue of rat P450a (Table 1). Accordingly 'human P450 form 1' has been assigned like rat P450a to the IIA subfamily but is believed to be another member of this subfamily. By the same reasoning, it is unlikely that IIC1 to IIC5 found in rabbits are orthologues of human IIC7 to IIC10 or for that matter orthologues of rodent IIC6, IIC11 and IIC12 (Table 1). Thus the P450IIC subfamily appears to be the most highly diverged of all subfamilies. The reason for this may be related to the evolution of distinct interspecies, dietary habits and consequent changes in the toxins contained within their foodstuffs. Thus 'plant–animal warfare' is believed to be the driving force for the 'recent burst' in new P450 genes,

Table 2. Comparison of some P450 genes and their products

Subfamily	Chromosome		No. of genes in human	Specific gene	Size of gene (kb)	No. of exons	Size mRNA (kb)	Protein MW SDS-PAGE
	Mouse	Human						
IA	9	15	2	1A1	6.1	7	2.9	55K
IIA	7	19	2	2A1	12.8	9	2&3	47.8K
IIB	7	19	3	2B1	23.0	9	2.1	52.5K
IIC	19	10	3	2C11	35.0	9	1.8	52.8K
IID	15	22	3	2D2	4.0	9	2.0	52K
IIE	7	10	1	2E1	10.4	9	1.8	51K
IIIA	6	7	3	3A1	?	>10	2.0	51K
IVA	4	1	?	4A1	?	12/13	2.2	52K
XIA	?	15	1	11A1	20.0	9	1.8	51.7K
XVIIA	?	10	1	17A1	6.6	8	2.2	59K
XXIA	17	6	1/2	21A1	3.5	10	2.2	49K

These data, obtained from the references contained in Nebert *et al.* (1990) indicate the importance of splicing events (see section on molecular mechanisms of regulation) in producing mRNAs and hence protein products of similar sizes. Gene, mRNA and protein sizes for subfamilies IA through to IVA are for rat while those for subfamilies XIA and XVIIA are for human and XXIA are for cow.

?, Unknown.

particularly in the *CYP2* family (Nebert & Gonzalez 1987). Although humans have more recently been exposed to a tremendous number of man-made chemicals it is obvious, given an average unit evolutionary period for P450s of 4, that our P450s cannot evolve rapidly enough to meet this challenge. This may be an important factor underlying the increase in chemically induced diseases, particularly cancer.

Regulation, tissue specific expression and catalytic activities of P450 families

P450 I gene family

There are two P450I genes (IA1 and IA2) in every mammalian species so far examined. The enzyme activity known as aryl hydrocarbon (benz[*a*]pyrene) hydroxylase (AHH) is associated predominantly with P450IA1 while P450IA2 exhibits a high level of catalytic activity towards arylamines such as the potent hepatocarcinogen 2-acetylaminofluorene and the analgesic phenacetin. IA2 is constitutively expressed in liver with little expression in extra-hepatic tissues while IA1 is detectable in liver and extra-hepatic tissues only after treatment with inducers. However, both genes probably lie in tandem on mouse chromosome 9 and human chromosome 15 (Table 2) and are highly inducible by environmental pollutants such as polycyclic aromatic hydrocarbons (PAH) contained in cigarette smoke and tetrachlorodibenzodioxin (TCDD).

The discovery of an allelic variant of IA1 among inbred mouse strains has resulted in much more being known about the regulation of this gene than any other P450 gene. AHH activity is readily induced by *aromatic hydrocarbons* in the C57BL/6 mouse strain but not in DBA/2 mice and so the gene controlling the induction processes was named the *Ah* locus. A defect in a cytosolic *Ah* receptor, resulting in the receptor having a decreased affinity for inducers, has been shown to be responsible for the refractory nature of DBA/2 mice to PAH induction (Poland *et al.* 1976). In responsive mice the PAH inducer binds to the *Ah* receptor and the complex translocates to the nucleus and activates IA1 gene transcription leading to an increased abundance of its mRNA and protein product. Transcription of the IA1 gene is believed to be governed by a complex interaction of trans-acting factors within DNA sequence elements lying within 1 kilobase upstream of the RNA polymerase II start site. These elements include a TCDD inducible enhancer but also include an element that augments 'constitutive expression' and a separate control element involved in a negative autoregulatory loop (Nebert & Jones 1989). The Ah locus also regulates the expression of detoxification enzymes such as UDP glucuronyl transferases and glutathione transferases. Other enzymes and enzyme activities known to be increased by TCDD or PAH, which may therefore be under the control of the Ah receptor, include aldehyde dehydrogenase, phosphatidyl choline biosynthesis, arachidonic acid metabolism, lipid deacylation, phospholipase A2, γ-glutamyl transpepsidase and protein kinase C (Nebert & Gonzalez 1987).

Considerable interest in the Ah locus has been generated by reports that enzymes in the IA family could play an important role in chemically induced human cancer. In this respect it is noteworthy that IA1 is induced in humans by cigarette smoke and that an increased risk of bronchogenic carcinoma appears to be associated with a phenotype of high P450IA1 inducibility. Nevertheless, although restriction fragment length polymorphisms (RFLPs) have been detected with a human IA1 cDNA no correlation between these RFLP patterns and lung cancer incidence has been found to date (Gonzalez 1990).

P450II gene family

Initial studies on genes in the P450II family were carried out in phenobarbitone-treated animals. However, it now appears that the

phenobarbitone inducible genes comprise only a small proportion of this family.

IIA subfamily. Rat liver P450IIA1 is highly specific for testosterone 7α-hydroxylase activity. In contrast, IIA2 exhibits a high level of testosterone 15α-hydroxylase. In the rat, both IIA1 and IIA2 are liver specific, but they are regulated very differently. IIA1 production is increased in young male and female rats but this gene is suppressed in males at the onset of puberty. In contrast, IIA2 is never expressed in females and the gene is activated only when males reach puberty (Matsunaga *et al.* 1988). This sex-specific expression is controlled in part by the pattern of pituitary hormone secretion (Waxman *et al.* 1985, 1989). Finally, IIA1, but not IIA2, is induced by PAH administration. In contrast to the rat, mouse IIA genes are expressed in both liver and kidney (Squires & Negishi 1988).

A third gene in the rat IIA subfamily, designated IIA3, has recently been isolated and shown to be expressed only in the lung (Gonzalez 1989). P450IIA3 has a nucleotide deduced amino acid sequence which is 85% similar to a human P450IIA gene family member which is expressed in liver (Gonzalez 1990). In view of this varied tissue expression it seems that the P450IIA gene family members have evolved quite differently in rodents and humans. (See section on interspecies differences.)

IIB subfamily. The cDNAs for IIB1 and IIB2 were among the first to be isolated and completely sequenced. The respective proteins exhibit a 97% amino acid similarity (viz. only 14 substitutions out of 491 amino acids) and although the enzymes have similar substrate specificities, purified IIB1 has catalytic activities towards most substrates examined which conservatively are an order of magnitude greater than IIB2. A third cDNA in the rat IIB subfamily, designated IIB3, has also been isolated and shown to have a 77% sequence identity to IIB1 and IIB2. However, all three genes are differentially regulated in that IIB1 is absent in liver until phenobarbitone (PB) treatment while IIB2 is constitutively expressed in liver and PB inducible, while IIB3 is constitutively expressed and not inducible by PB. Furthermore, IIB1 is constitutively expressed and not inducible by PB in lung and testis while IIB2 is absent from these tissues regardless of treatment (Gonzalez 1989, 1990). These observations suggest that the genes encoding the rat IIB subfamily contain tissue specific regulatory and inducer control elements. However, the mechanism by which they are regulated is poorly understood. Thus although phenobarbitone treatment is known to cause a rapid transcriptional activation of IIB1 and IIB2 genes, a receptor for phenobarbitone has not yet been found (cf. induction of P450IA1 by PAH).

IIC subfamily. The subfamily is generally considered to represent a class of constitutively expressed genes which are noted for their sex-specific and developmentally regulated expression in rats, although a few are also induced by phenobarbitone.

Rabbit cDNAs corresponding to IIC1 to IIC5 gene products were the first identified. These genes diverged long after the rabbit–rodent and rabbit–human speciations (Fig. 1) and therefore neither the rodent nor the human is expected to have orthologues of the rabbit genes.

Several rat IIC cDNAs have been characterized and the mechanism by which these genes are regulated during development has been extensively investigated. Hepatic IIC6 and IIC7 genes are transcriptionally activated just before male and female rats reach puberty. Accordingly, serum testosterone levels probably do not play a role in the regulation of these genes (Waxman *et al.* 1989). In contrast, IIC11 and IIC12 genes are controlled by testosterone, while IIC12 is partially dependent on oestradiol. The effect of sex hormone exposure, especially during the neonatal period, appears to be related to the subsequent pattern of growth hormone secretions which is pulsatile in male and

constant in female rats. Hypophysectomy of male rats results in the loss of IIC11 mRNA which is partially restored upon intermittent injection of growth hormone. If hypophysectomized male rats are given continuous infusion of growth hormone then IIC11 mRNA remains suppressed but the female specific IIC12 mRNA is increased (Zaphiropoulos *et al.* 1988). It is also noteworthy that other P450s, including IIB1/2, IIA2 and IIIA2, are regulated by pituitary hormone levels (Gonzalez 1989). However, similar sex differences in P450 mediated metabolism have not been observed in humans even though a polymorphism in the human IIC subfamily results in poor metabolism of mephenytoin, hexobarbital and tolbutamide. (See section on interspecies differences.)

IID subfamily. A human polymorphism in debrisoquine-4-hydroxylase has been described in which the 'extensive metaboliser' (EM phenotype) is able to break down this anti-hypertensive agent 10 to 200 times faster than the 'poor metaboliser' (PM phenotype). The metabolism of more than 20 clinically used drugs appears to be related to this same polymorphism. Subsequent studies showed that this polymorphism results from the absence of the debrisoquine hydroxylase protein resulting from a mutation in *CYP2D1* (now known as *2D6*) (Meyer *et al.* 1990). This knowledge provides the basis for a screening test to identify this polymorphism which affects about 10% of the Caucasian population and has been associated with altered susceptibility to lung and bladder cancer (Gough *et al.* 1990).

IIE subfamily. A unique form of P450 purified from rabbits (Koop & Coon 1984), rats (Ryan *et al.* 1985) and man (Wrighton *et al.* 1987) has been shown to metabolize ethanol, ketone bodies, and the hepatocarcinogen dimethylnitrosamine. A cDNA, designated IIE1, encoding this protein has also been isolated from the three species and its use demonstrated that the mechanism by which IIE1 is regulated is quite distinct from other P450s. Administration of ethanol, acetone, pyrazole or methyl pyrazole to rats causes a four to nine-fold increase in the synthesis of IIE1 without a concomitant increase in IIE1 mRNA, suggesting that these inducers stabilize the IIE1 apoprotein against degradation. In contrast, IIE1 mRNA levels are markedly increased when rats are starved or made diabetic, but this increase results from post-transcriptional mRNA stabilization. The increase due to diabetes can be reversed by administration of insulin which, together with its catalytic activity towards acetone and other ketone bodies, may suggest that IIE1 is involved in the pathway of gluconeogenesis during the fasting state (Gonzalez 1989, 1990).

IIG subfamily. A number of studies on the xenobiotic metabolizing activity of olfactory mucosa have been performed because of the observation that parenteral administration of certain chemicals to rats and mice selectively damages their nasal turbinates (e.g. see Brandt *et al.* 1990). The mechanism underlying this selectivity appears to result from the preferential expression of the P450IIG subfamily in olfactory mucosa (Nef *et al.* 1990). The normal function of this subfamily may be in olfactory recognition, either by inactivating or activating odour molecules, although it should be remembered that the nasal cavity is also exposed to a wide range of airborne pollutants.

P450III gene family

Two P450s (IIIA1 and IIIA2) induced by phenobarbitone have been purified from rat liver and shown to have catalytic activities for testosterone 6β-hydroxylation, ethylmorphine and erythromycin demethylation, as well as the metabolism of the macrolide antibiotic triacetyloleandomycin (TAO) and S-mephenytoin. Antibodies to IIIA1 inhibit metabolism of the immunosuppressive agent cyclosporin in human liver (Kronbach *et al.* 1988) as well as the metabolism of nifedi-

pine, a calcium channel blocking drug. However, rat and human P450 III genes appear to be regulated quite differently. Firstly, in rats, both IIIA1 and IIIA2 are induced by phenobarbitone administration while IIIA1, and not IIIA2, is induced by treatment with the synthetic steroid pregnenolone 16α-carbonitrile (PCN). IIIA2 is specific to the adult male rat while human liver samples from adult males and females were all found to contain a P450 immunochemically related to IIIA2. Another interesting contrast between rats and humans is the finding that the IIIA family appears to be expressed in human foetal liver whereas in rats, expression is activated shortly after birth (Gonzalez 1989, 1990). For obvious reasons the regulation of the P450III gene family has been most extensively investigated in rats, where two genes exist, while at least three genes exist in humans. In rats, IIIA1 is not constitutively expressed in liver but is transcriptionally activated by administration of glucocorticoids or PCN. Conversely, IIIA2 mRNA is constitutively expressed in adult male rat liver but is not increased after treatment with PCN, even though both IIIA1 and IIIA2 are elevated by PB administration. Interpretation of these observations is complicated by the knowledge that post-transcriptional events also play a role in the regulation of IIIA1 and IIIA2 (Watkins *et al.* 1986). In conclusion, the hepatic P450III family is important both quantitatively, as in humans it can comprise 50% of the total hepatic P450 content (C. Palmer, personal communication), as well as in the range of clinically used drugs metabolized, but understanding the mechanisms underlying its regulation is perhaps proving more difficult than for any other P450 gene family.

P450IV gene family

Structurally diverse xenobiotics, including the oxyisobutyrate class of hypolipidaemic drugs (e.g. clofibrate), industrial plasticisers such as di-2(ethylhexyl)phthalate, and chlorinated phenoxy acid herbicides, induce a rat hepatic form of cytochrome P450 designated P450IVA1. P450IVA1 is characterized by a narrow substrate specificity for the oxidation of the terminal (ω) carbon of fatty acids such as lauric and arachidonic acids. Purification of this cytochrome from clofibrate induced rat liver microsomes and subsequent immunochemical studies demonstrated that this cytochrome exhibits a complete lack of cross-reactivity with P450s induced by PAH or phenobarbitone (Tamburini *et al.* 1984). A full-length cDNA for P450IVA1 has been characterized and the nucleotide deduced amino acid sequence is less than 33% similar to that encoded by genes comprising the other P450 gene families (Gonzalez 1989, 1990) suggesting that the IVA1 gene diverged early in the evolution of the P450 gene superfamily (Fig. 1).

Clofibrate administration to rats results in a rapid transcriptional activation of genes encoding both P450IVA1 and peroxisome proliferation and recent evidence suggests that these effects may be receptor mediated (Issemann & Green 1990). Interest in both phenomena results from the knowledge that induction of hepatic IVA1 is temporally related to hepatic peroxisome proliferation, hepatomegaly and ultimately hepatocellular carcinoma in rodents (Lock *et al.* 1989).

Other fatty acid hydroxylases have been demonstrated in rat kidney (IVA2), lung (IVB1), colon and placenta (Gonzalez 1990). Accordingly, it is believed that the P450IV family may carry out physiologically important hydroxylation reactions related to prostaglandin and leukotriene metabolism.

P450XI, XVII, XIX and XXI gene families

These four mammalian gene families are involved in steroid biosynthesis and their deficiency results in several forms of congenital adrenal hyperplasia in humans (Nebert & Gonzalez 1987).

The P450XI gene family codes for two mitochondrial P450s, namely steroid 11β-

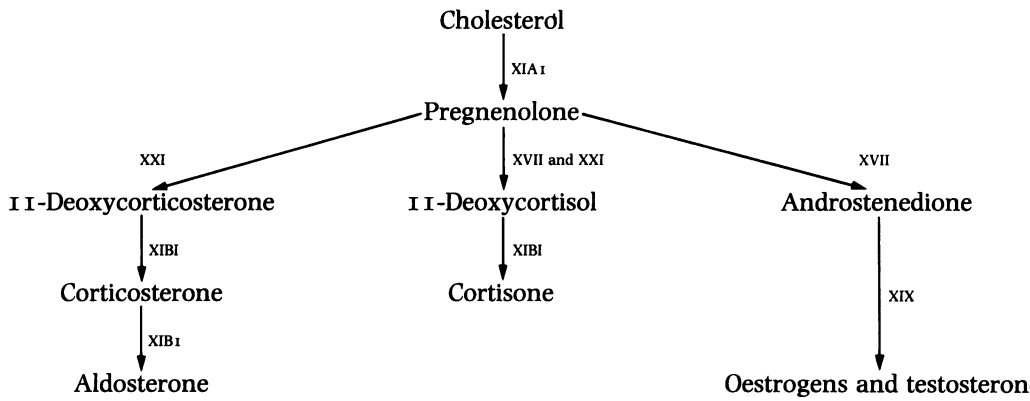

Fig. 2. Simplified scheme demonstrating involvement of P450XI, XVII, XIX and XXI gene families in steroid biosynthesis

hydroxylase (XIB1) and cholesterol side-chain cleavage enzyme (XIA1). Conversion of cholesterol to pregnenolone via cleavage of an isocaproic group from the cholesterol side chain is the first rate-limiting step in the synthesis of steroid hormones and involves three consecutive mono-oxygenation steps, all of which are catalysed by P450 XIA1 (Fig. 2). XIA1 has been demonstrated in adrenal cortex, ovary, testes and placenta. XIB1 is expressed only in adrenal cortex and mediates both the 11β-hydroxylation of 11-deoxycortisol to cortisol as well as the synthesis of aldosterone (the key mineralocorticoid required for salt retention) from 11-deoxycorticosterone.

The intermediate steps leading from the conversion of pregnenolone to 11-deoxycortisol and 11-deoxycorticosterone occur in the endoplasmic reticulum and are mediated by P450 XVII and P450XXI (Fig. 2). P450XVII is found in all steroidogenic tissues and, as previously mentioned, is a critical determinant of the synthesis of mineralocorticoids and glucocorticoids. Furthermore, as P450XVII also functions as a 17, 20-lyase its activity also distinguishes between the synthesis of glucocorticoids and the C19 precursors of androgens. Aromatization of androgens into oestrogens is catalysed by P450XIX. The P450XXI family catalyses the hydroxylation of progesterone and 17-hydroxyprogesterone at carbon 21 to yield 11-deoxycorticosterone and 11-deoxycortisol respectively. A deficiency of this 21-hydroxylase, which occurs in about 1 in every 7000 persons, results in adrenal hyperplasia and hence is a common inborn error of metabolism (Nebert & Gonzalez 1987).

The bovine XIA, XIB, XVII and XXI genes have been shown to be transcriptionally activated in cultured adrenocortical cells by ACTH which acts via cyclic AMP. It has been postulated that cAMP does not stimulate transcription directly but rather that cAMP activates a gene encoding a hypothetical *s*teroid *h*ydroxylase *i*nducing *p*rotein (SHIP) (Nebert & Gonzalez 1987). Two other important biosynthetic reactions are catalysed by cytochromes P450.

Bile acid synthesis. The first step in the biosynthesis of bile acids in mammalian liver is the 7α-hydroxylation of cholesterol catalysed by the P450VII gene family (Li *et al.* 1990).

Metabolism of vitamins. (a) Retinoic acid: vitamin A (retinol) must be converted in the target tissue to retinoic acid before it can cause epithelial differentiation. Retinoic acid cannot be stored and is rapidly metabolized to 4-hydroxyretinoic acid. The involvement of P450 in the 4-hydroxylation of retinoic

acid is indicated by the requirement for NADPH and oxygen and by inhibition of the reaction by carbon monoxide and SKF525A (Van den Bossche 1988). In addition, epidermal microsomes have been known, for many years, to metabolize testosterone and drugs (Bickers *et al.* 1982). Thus, multiple forms of P450 are probably present in skin and although absorption through skin represents an important route of human exposure to xenobiotics, its P450 gene families remain uncharacterized (Mukhtar & Khan 1989). (b) Vitamin D: cholecalciferol is hydroxylated in the liver to produce 25-hydroxy vitamin D3. This P450 is immunochemically different from the liver P450s purified from phenobarbitone and PAH treated rats (Hayashi *et al.* 1984). In order to achieve full biological activity, 25-hydroxy vitamin D3 must be converted by a kidney P450 to 1α,25-dihydroxy vitamin D3. This hydroxylation step also occurs in osteocytes and melanomas. The biological activity of 1,25-dihydroxy vitamin D3 is terminated by P450 mediated hydroxylation at carbon 24 (Crivello 1985).

Molecular mechanisms of P450 gene regulation

In view of the large number of isozymes in the P450 gene superfamily, as well as their wide range of functions, it is not too surprising that different mechanisms of regulation exist. These can be confusing if considered from the substrate, inducer or gene classification points of view. However, Goldfarb (1990) has eloquently demonstrated that cellular P450 contents are in fact regulated no differently from other mammalian gene products. Thus, in summary, the various levels of control are the rate of gene activation and transcription, pre-mRNA splicing, mRNA stability, post-translational modification, and the rate of degradation of individual proteins.

Gene activation involving the unwinding of condensed, inactive, chromatin is brought about by gene specific demethylation events and these have clearly been demonstrated in the regulation of rat hepatic P450IIE1 (Gonzalez 1989). DNA can be maintained in the activated state by binding specific proteins known as 'transcription factors'. Therefore, while all the cells of an organism may have the same genetic potential to synthesize cytochromes P450, their tissue specific expression can be accounted for by the existence of tissue specific transcription factors. Thus liver specific transcription factors such as HNF1 (Cereghini *et al.* 1990) or DBP (Mueller *et al.* 1990) may underlie the expression of certain hepatic P450s. Accordingly, only in some tissues will the combination of interactions of a receptor–inducer complex and activating proteins result in P450 gene expression, for example, the involvement of the Ah locus in P450IA1 expression (Nebert & Jones 1989), induction of IIIA1 and possibly IVA1 by steroid-receptor binding mechanisms (Shuetz & Guzelian 1984; Issemann & Green 1990), or XIB1 induction by binding of the hypothetical cAMP activated 'SHIP'. Such interactions permit RNA polymerases to transcribe the gene and synthesize a pre-mRNA. In addition to such complex interactions controlling the rates of transcription, another point of control is the efficiency of removing the non-coding regions (introns) from the pre-mRNA and the subsequent splicing of its coding regions (exons) to form a mature mRNA which can then be translocated to the cytoplasm where it can be translated by ribosomes and assembled into an intact P450. Thus RNA splicing is a determinant of cellular P450 levels and aberrant splicing seems to underlie the observed polymorphisms in the human IIC and IID subfamilies (Goldfarb 1990; Gough *et al.* 1990). In the expression of other, non-P450, genes 'true' alternative splicing (that is, the differential processing of the same pre-mRNA transcript) is a major strategy by which cells produce different mRNAs and hence enzymes with different catalytic function but, to date, there is no evidence for this mechanism operating in the P450 gene superfamily. Thus each functional P450 gene appears to produce a

single mRNA and hence a single protein (Gonzalez 1989, 1990).

Once in the cytoplasm, the mRNA can be degraded by ribonucleases and so the susceptibility of individual P450 mRNAs to degradation will determine their translatability and hence apoprotein levels. Interestingly, inhibition of mRNA degradation accounts for the elevated hepatic content of P450IIE1 in the diabetic or fasting state as well as the induction of the P450IIIA subfamily by TAO. In addition to such post-transcriptional effects, cytochromes P450 have also been shown to be controlled at post-translational levels. Obviously the availability of the haem prosthetic group is an important factor (Padmanaban *et al.* 1989) but more specific mechanisms of post-translational control have been demonstrated to be the protection of IIE1 by ethanol, acetone or pyrazole and the protection of IIIA apoproteins by TAO against degradation. The role of phosphorylation by cAMP dependent protein kinases in the regulation of cytochrome P450 activity is less clear but appears to lead to inactivation of several forms, namely IIB1, IIC2, IIE1 and CI (Goldfarb 1990).

Interspecies differences in drug and carcinogen metabolism

In is now well established that cytochromes P450 play a pivotal role in the toxicity and carcinogenicity of many xenobiotics but the evolutionary scheme depicted in Fig. 1 shows that different mammalian species have varying numbers of P450 genes particularly within the IIC subfamily (Table 1). In addition, orthologues of the rat hepatic IIB subfamily are unlikely to exist in mouse, rabbit or human. Similarly, the rabbit CYP2E subfamily has two members which arose via gene duplication after the rabbit–rodent divergence, meaning that only one of these genes exists in rat, mouse and human (Gonzalez 1989). Furthermore, a particular mouse CYP2D enzyme has testosterone 16α-hydroxylase activity while the rat CYP2D enzymes do not. Also, human and rat CYP2D enzymes metabolize debrisoquine while the mouse CYP2D enzymes do not (Puga & Nebert 1990). Clearly, these observations have important implications for the extrapolation of toxicological data obtained in rodents and lagomorphs to man, and in many instances will explain the significant species differences in xenobiotic metabolism and in susceptibility to chemical toxins and carcinogens. It is therefore of utmost importance to identify where similarities and differences in P450 mediated xenobiotic metabolism exists between species. Catalytic activity alone is not much help in this respect because Lindberg and Negishi (1989) have shown that a single amino acid difference in a peptide of about 500 residues is critical in changing catalytic activity from a coumarin 7-hydroxylase to a testosterone 15α-hydroxylase. Thus where orthologues exist it is not always possible to predict their substrate specificities. It is therefore vital to improve our understanding of the function and regulation of P450s in order to predict more accurately the human response to drugs and other environmental chemicals.

Conclusion and future perspectives

Recombinant DNA technology combined with protein chemistry has made a significant impact on our understanding of the P450 gene superfamily and has created new opportunities to determine its members' precise role in human disease. Just two of the many outstanding and rather complex questions are:

(1) In order to better understand the strengths and weaknesses of animal models for the prediction of human disease, what are the similarities between rodent and human P450s?

In view of the difficulty in obtaining sufficient material to purify P450s from human tissues, this aspect is likely to progress by transfecting cDNAs for human P450s into yeast or mammalian cell lines in order to synthesize catalyti-

cally active human P450s (e.g. see Aoyama *et al.* 1990a,b). These techniques, coupled with site directed mutagenesis and the preparation of chimeric enzymes, will undoubtedly improve our understanding of structure–function relationships in the catalytic cycle of P450s (e.g. see Lindberg & Negishi 1989; Kronbach *et al.* 1989).

(2) Is the observed variability in the expression of P450s due to genetic, environmental or hormonal factors and is there a linkage between P450 polymorphisms and human disease where xenobiotics are a contributing factor?

There appear to be relationships between human polymorphisms in P450IA, P450IIC, IID and IIE subfamilies and susceptibility to malignancies of the liver, gastrointestinal tract and lung (Nebert & Gonzalez 1987; Gonzalez 1989, 1990). Accordingly, it is anticipated that there will soon be more clinical studies attempting to correlate RFLP patterns of P450 genes with individual risk to cancer. Similar studies may be important in predicting those individuals with a peculiar sensitivity to drugs. For example, identification of the human orthologues of the P450III gene family may identify those individuals sensitive to macrolide antibiotics in much the same way that current knowledge of polymorphisms in the IID subfamily could identify the potential for iatrogenic disease resulting from the prescription of hypotensive agents. Future studies will undoubtedly be coupled to the knowledge that environmental chemicals can modulate the toxic response. For example, more than 200 pharmacological and chemically unrelated compounds are known to induce cytochromes P450 in experimental animals and so an understanding of the precise molecular mechanisms involved will not only reduce the number of experiments that have to be performed on living animals in order to identify these effects but also help predict iatrogenic disease resulting from multiple prescription and/or genetic polymorphisms. Furthermore, it is necessary to understand the way in which pituitary, gonadal, adrenal, thyroid and pancreatic hormones are also able to control P450 mediated drug metabolism. The use of cells in culture, particularly hepatocytes, should help answer whether such hormonal effects are indirect, but a problem to be overcome here is that cell culture systems rapidly lose cytochrome P450 content and also do not faithfully reflect the induction process (e.g. see Paine 1990; Shean & Paine 1990; Padgham *et al.* 1990).

In conclusion, the study of the molecular biology of cytochromes P450 has provided many important avenues to explore, and therefore offers the fascination that many surprises are still in store.

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