Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
Edited by Costas Ioannides

ISBNs: 0-471-894-66-4 (Hardback); 0-470-84630-5 (Electronic)

2 Cytochrome P450

F. Peter Guengerich

Vanderbilt University School of Medicine, USA

Introduction

Cytochrome P450 (P450) enzymes constitute the major group of enzymes involved in the oxidation of drugs and other xenobiotic chemicals (Wislocki *et al*. 1980; Ortiz de Montellano 1995a; Guengerich 1997a). The functions of the enzymes are a major component of most problems in drug development and chemical toxicity. The P450 enzymes are haemoproteins that generally function in mixed-function oxidase reactions, using the stoichiometry:

$$NADPH + H^+ + O_2 + R \longrightarrow NADP^+ + H_2O + RO \quad (2.1)$$

As discussed later, the identity of the product RO may be obscured by rearrangements, and other variations from the above stoichiometry are possible.

P450s have been known, at least through their actions, for over 50 years. The roots of P450 research are found in studies on the metabolism of steroids (Estabrook *et al*. 1963), drugs (Axelrod 1955; Brodie *et al*. 1958) and carcinogens (Mueller and Miller 1948, 1953). Today these enzymes are studied not only by biochemists but also by inorganic chemists, development biologists, pesticide and plant scientists and physicians. More attention has been probably given to the P450s than to any other single group of enzymes discussed in this book.

Roles of P450s in the metabolism of chemicals

P450s are involved in the metabolism of a wide variety of xenobiotic chemicals. One major role is the clearance of drugs, to which P450s collectively contribute more than any other group of enzymes. Many drugs are rendered more hydrophilic by hydroxylation so that they are excreted from the body, particularly if an oxygen atom is added to be conjugated with a hydrophilic moiety (e.g. glucuronide). The adjustment of rates of metabolism to yield optimal pharmacokinetic profiles ia a major focus in the pharmaceutical industry. P450 oxidation of drugs usually results in their deactivation,

Enzyme Systems that Metabolise Drugs and Other Xenobiotics. Edited by C. Ioannides.

although not always. In some cases drugs are rendered more active by the process of metabolism and the parent drug is considered a 'pro-drug'.

P450s are also involved in the oxidation of 'endogenous' chemicals, those normally found in the body. Vitamins are usually considered in this group because of their essential nature. Deficiencies in the P450s involved in steroid oxidations are often very detrimental. However, the levels of the microsomal P450s, the primary concern of this chapter, can vary considerably in the human population with apparently no problems. For instance, 10- to 40-fold variation in the levels of CYP1A2 and 3A4 are documented, and CYP2D6 is absent in ~7% of Caucasian populations (Guengerich 1995). Although some of the 'xenobiotic-metabolising' P450s can oxidise 'endogenous' substrates (e.g. testosterone, arachidonate), it is not clear that these are critical functions, in the context of the wide variability of levels. Moreover, all the P450-knockout mice developed to date (1A1, 1A2, 1B1, 2E1) show relatively normal phenotypes in the absence of stress from drugs or deleterious chemicals (Lee *et al.* 1996b; Liang *et al.* 1996; Buters *et al.* 1999).

P450s have been of interest in chemical carcinogenesis and toxicology because of their oxidations of protoxicants to reactive electrophilic forms that cause biological damage (Conney 1982; Guengerich 1988a; Guengerich and Shimada 1991). Lists of such reactions have been published (Guengerich and Shimada 1991), and reactive products can covalently modify proteins and DNA (Nelson 1994). Considerable evidence for critical roles of P450 modulation in toxicity and cancer has been developed in experimental animal models (Guengerich 1988a; Nebert 1989), but human epidemiology is not so clear (d'Errico *et al.* 1996). Nevertheless, the role of P450s in influencing the effectiveness of toxicity of drugs in people has now been well established and some of the approaches can also be used with issues of 'environmental' chemicals (Guengerich 1998).

Nomenclature

At the time of the discovery of P450 (Klingenberg 1958) and the demonstration of its role as the terminal oxidase in microsomal mixed-function oxidase systems (Estabrook *et al.* 1963) little evidence existed for the presence of multiple forms. The name 'P450' was developed to indicate a pigment ('P') with a haem Fe^{2+}-carbon monoxide complex having an absorption spectral band at 450 nm (Omura and Sato 1962, 1964). Several lines of investigation led to the view that two or more distinct forms of P450 might exist within a single species (Alvares *et al.* 1967; Hildebrandt *et al.* 1968; Sladek and Mannering 1969). The components of the liver microsomal system—P450, NADPH-P450 reductase, and phospholipid—were separated and then reconstituted in 1968 (Lu and Coon 1968).

In the 1970s and early 1980s a large number of P450s were purified from rat and rabbit liver (Haugen *et al.* 1975; Johnson and Schwab 1984; Ryan *et al.* 1978; Guengerich 1987a) and later human liver (Distlerath and Guengerich 1987; Guengerich 1995). Complex naming systems developed, usually based upon designations of column fractions by investigators. With the growing application of recombinant DNA technology to P450 research in the 1980s, cDNA clones were characterised (Fujii-Kuriyama *et al.* 1982; Kimura *et al.* 1984). A systematic nomenclature system

was developed by Nebert (Nebert *et al*. 1987, 1989; Nelson *et al*. 1993) based upon the coding sequences. Updated versions of the nomenclature can be found on the website <http://drnelson.utmem.edu/CytochromeP450.html>. For comparisons of older nomenclature to the current, see Nelson *et al*. (1993). The nomenclature system follows these basic rules: All P450s are members of the 'superfamily' (some 'functionally' related proteins such as fungal chloroperoxidase and the nitric oxide synthases are not included). The most defining region of the sequence is that surrounding the cysteine that serves as an axial thiolate ligand to the haem ion. Sequences with ⩾40% sequence identity are grouped in the same 'family', specified by a numeral. Sequences with ⩾60% identity fall in the same 'subfamily', denoted with a letter. The last number denotes the individual P450. Families 1–49 are for mammalian and insect genes, 50–99 for plants and fungi, and 100+ for bacteria. There are some exceptions to the rules, including the exact cutoff percentage for classifications and the assignment of families. P450s that seem to be conserved across species (e.g. 1A1, 1A2, 2E1) are given the same name in all species. Other P450s that seem to vary more are given individual names in different species, i.e. 3A4, 3A5, and 3A7 in humans and 3A6 in rabbits. Obviously there may be future problems in fitting all species and new P450s into the existing nomenclature system (see <http://drnelson.utmem.edu/CytochromeP450.html>), but the system has been useful. The terms 'P450' or 'CYP' may be used, but searches with CYP will also identify a yeast transcription factor with the same acronym (Naït-Kaoudjt *et al*. 1997).

Distribution

P450s are rather ubiquitous. In nature they appear almost everywhere from Archebacteria to humans, with the apparent exception of enterobacteria (e.g. *Escherichia coli*). The availability of genomic databases and generally accepted trademark sequences ('cysteine peptide') has made it possible to estimate the number of P450 genes in some organisms. That number currently stands at 53 in humans at the time of this writing (Spring 2000), 70 in *Caenorhabditis elegans*, 90 in Drosophila, and 330 in *Arabidopsis thaliana*. These numbers are probably approaching the final count now with the completion of sequencing of genomes.

This chapter will not deal with prokaryotic P450s except to the extent that they serve as models for the mammalian P450s of interest. The mammalian enzymes discussed in this chapter are found in the endoplasmic reticulum, associated with the auxiliary flavoprotein NADPH-P450 reductase, which delivers electrons from NADPH to the iron atom in the haem of P450. The family 11 mitochondrial P450s function in the hydroxylation of steroids and vitamin D, and accept electrons from the iron-sulphur protein adrenodoxin (Kagawa and Waterman 1995). Family 24 and 27 P450s—total of 6 including the family 11 P450s—are also targeted solely to the mitochondria. It should be pointed out that some microsomal P450s, because of particular processing patterns, are also delivered into the mitochondria (Addya *et al*. 1997; Neve and Ingelman-Sundberg 1999) and the plasma membrane (Loeper *et al*. 1998). In both cases there appears to be functional capability, although the details are not completely clear yet. The fraction of microsomal P450 targeted to the plasma membrane is very low but may be involved in the initiation of auto-immune responses

involving P450s (Loeper *et al*. 1993). The fraction of some P450s localised in mitochondria can be substantial and they may be significant contributors to some reactions (Anandatheerthavarada *et al*. 1997).

The xenobiotic-oxidising P450s are localised principally in the liver, and some are expressed essentially exclusively in the liver (e.g. CYP1A2). Others are expressed in the liver and in a variety of extrahepatic tissues, often with differential mechanisms of regulation (e.g. CYP3A4). Some P450s are expressed in several extrahepatic tissues but almost not at all in the liver (e.g. CYP1A1 and 1B1). The liver is a major site for oxidation of drugs and other xenobiotic chemicals, but P450s in extrahepatic tissues, even if expressed at much lower levels there than in the liver, can have very important roles in at least three situations:

(1) The catalytic specificity for a reaction is so absolute that the substrate will only be oxidised when it encounters this P450 (e.g. some steroid hydroxylations).
(2) Extensive oxidation occurs when the chemical first encounters this P450 because of the route of administration. For instance, there is often a major 'first-pass' effect in the small intestine for drugs ingested following oral administration (esp. CYP3A4 (Kolars *et al*. 1991; Guengerich 1999b)).
(3) Oxidation of a chemical by a P450 yields a reactive product with very limited stability, and only oxidation in the 'target' cells for evoking the toxic response is important in considering bioactivation.

The point should also be emphasised that localisation of P450s within a tissue occurs, and can be important in determining responses to chemicals. In many tissues a number of different cell types exist (e.g. ~40 in the lung) and P450s are usually found in only a few of these (e.g. within the lung, kidney, brain (Dees *et al*. 1982; Ishii-Ohba *et al*. 1984)). In the liver, the P450s are most concentrated in the parenchymal cells (hepatocytes), but different gradations through the regions of the liver (centrilobular, midzonal, periportal) are observed with individual P450s (Baron *et al*. 1981).

Regulation

This area really began with observations in the 1950s that metabolism was inducible in experimental animals (Richardson *et al*. 1952; Conney *et al*. 1956) and humans (Remmer 1957). These phenomena helped lead to the discovery of P450 multiplicity. For many years the study of regulation of P450s was rather descriptive, and until 1980 there was little real evidence that P450 protein concentrations were really increased (immunochemical methods were critical in this work). The field of P450 regulation really developed with the advent of recombinant DNA technology and molecular biology. Although many details are still unknown, what has emerged today is a generalised paradigm of primarily transcriptional regulation, controlled by *trans*-acting factors; although other factors contribute as well.

In the generalised model of transcriptional regulation of P450s, a receptor interacts with a low M_r ligand in the cytosol and somehow changes its conformation, moves to the nucleus, binds to a specific region in the P450 upstream of the start site, and changes the DNA in such a way to make it more accessible to other proteins involved in transcription (Hankinson 1995; Waterman and Guengerich 1997). All the details

have not been elucidated in any case, but several major systems are outlined and fit into the general context of the scheme shown in Figure 2.1.

The 'Ah (aryl hydrocarbon) locus' is involved in the regulation of CYP1A1, 1A2, and 1B1. In this system, the receptor protein binds to a polycyclic hydrocarbon. As a result, the Ah receptor no longer binds to the heat shock protein (hsp)90 but now forms a heterodimer with the ARNT (Ah receptor nuclear transporter) protein and moves to the DNA, binding to XRE (xenobiotic response element) sequences (Hankinson 1995). Other events may be involved, but the result is that transcription of the P450 1A1 or other structural gene is considerably enhanced. Similar systems are involved with PXR (pregnane X receptor) (regulation of *CYP3A* genes) (Kliewer *et al.* 1998), CAR (constitutively active receptor) (regulation of *CYP2B* genes) (Honkakosski *et al.* 1998), and PPAR (peroxisome proliferator activator receptor) (regulation of P450 4A genes and other genes involved in peroxisome proliferation) (Muerhoff *et al.* 1992). PXR, CAR, and PPAR are all in the 'steroid orphan receptor' family and, when bound to their ligands, form heterodimers with RXR (retinoid x receptor)–retinoid complexes. The interactions can be complex in that multiple forms of some of these proteins exist and the properties of the heterodimers are also influenced by the ligands that are bound.

Obviously this is an over-simplification of a complex process. Evidence exists that other proteins interact with the proteins in the heterodimers (e.g. HIF (hypoxia inducible factor) 1α with ARNT, RARs (retinoic acid receptors) with RXR). P450 genes usually contain binding sites for multiple factors and are not controlled only by a single response system. Such elements are usually responsible for the tissue-specific expression of P450s (e.g. HNFs (hepatic nuclear factors) for some P450s in liver).

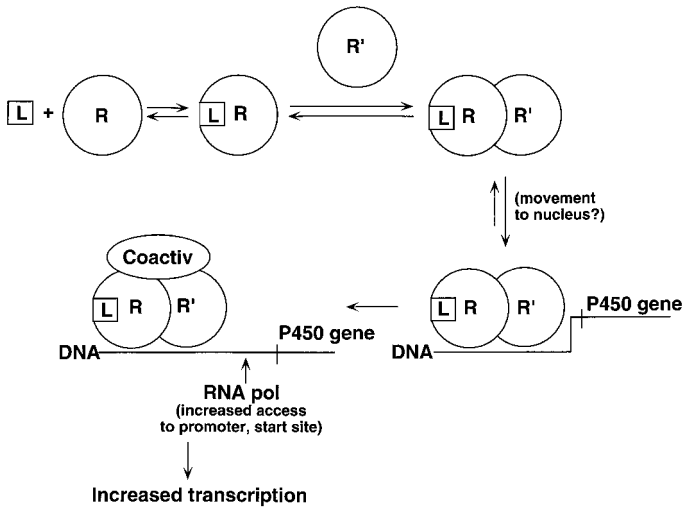


Figure 2.1 Generalised model for regulation of P450 genes by induction. L = ligand, R = receptor, R' = partner protein for heterodimer of R, Coactiv = coactivator, RNA pol = RNA polymerase.

Some of these elements are involved in basal responses; some contribute to inducibility. With some P450s, GREs (glucocoticoid receptor elements) within the introns can enhance responses.

Although transcriptional systems have received the most attention and can be considered to be the predominant aspect of induction, post-transcriptional regulation is also known. Evidence for mRNA stabilisation is more difficult to obtain (than for enhanced mRNA synthesis), but some systems appear to involve such a mechanism (Zangar and Novak 1998; Dong *et al*. 1988). Mechanisms are less clear than with transcriptional control. With some P450s there is evidence that certain proteins selectively bind the mRNA and stabilise it (Geneste *et al*. 1996).

Another means of regulating P450 levels is post-translational, and there is evidence that such a phenomenon occurs in experimental animals. The sensitivty of P450 to proteolytic degradation can be attenuated by ligand binding in some cases (Steward *et al*. 1985; Eliasson *et al*. 1988) and enhanced by protein alkylation in others (Tephly *et al*. 1986; Correia *et al*. 1992b). Incorporation of haem is a post-translational event. This process is generally considered to occur during translation, i.e. the protein is wrapped around the haem. Haem is ultimately degraded by haem oxygenase after it leaves P450 (Schmid 1973). The question of whether haem equilibrates among P450s is an interesting one, because the general consensus is that the haem site is not very exposed to the protein surface (Guengerich 1978). Some evidence for haem exchange comes from the slightly shorter half-life for P450 haem turnover in rat liver (Sadano and Omura 1983a; Shiraki and Guengerich 1984) and from the immunoprecipitation experiments of Omura (Sadano and Omura 1983b, 1985). The only other postranslational event of possible consequence with P450s is phosphorylation. Evidence for destabilisation of P450s by serine phosphorylation has been presented for rat CYP2B1 and 2E1 (Johansson *et al*. 1991; Bartlomowicz *et al*. 1989). Presumably any post-translational modification is not critical to normal P450 function, because most P450s can be readily expressed in bacterial systems (Waterman *et al*. 1995; Guengerich *et al*. 1996). The degradation of at least some P450s does involve ubiquination (Correia *et al*. 1992a; Tierney *et al*. 1992).

Some other aspects of regulation of P450s should also be mentioned. In rodents some P450s show dramatic gender-specific expression (e.g. CYP2C11 in male rats (Kato and Kamataki 1982; Guengerich *et al*. 1982)). Many studies have been carried out that document the roles of steroid hormone levels (especially at the neonatal stage) and pulsatile growth hormone levels in these responses (Waxman *et al*. 1985; Gustafsson *et al*. 1983). The underlying basis appears to be in factors such as STAT (Ram *et al*. 1996). The relevance of this phenomenon to humans is not clear; in humans the gender effects in drug metabolism are rather small. Another interesting phenomenon is P450 down-regulation. This has been observed with some P450s, with the same chemicals that induce other P450s (e.g. 2C11 and polycyclic hydrocarbons). The mechanism involves lowered mRNA levels but is not well understood beyond this level. P450 levels are also attenuated in rodents or humans treated with interferons or in disease states where levels of interferons or interleukins are elevated (Renton *et al*. 1979). This phenomenon has direct relevance in humans, and individuals with colds (or following flu shots) have generally impaired drug metabolism (Renton and Knickle 1990).

In animals the toxicity of a chemical may be either enhanced or attenuated due to induction of P450s, and the effect may be tissue-specific (Nebert 1989). Exactly how much the toxicity of chemicals is modulated by P450 induction is less clear in humans; differences in lung cancer (among smokers) are associated with variable CYP1A1 inducibility (Kouri *et al*. 1982). A goal of the US National Institute of Environmental Health Sciences Environmental Genome Project is to determine if better associations can be developed between gene responses and disease outcome (Guengerich 1998).

P450 induction has practical significance in drug development. Induction may not be a fatal flaw for a new drug candidate but will raise two important issues. The first is the prospect of drug–drug interactions. Induction of a P450 involved in metabolism of a drug will lower the concentration of that drug in the body. One rather classic example is the induction of CYP3A4 by rifampicin or barbiturates, which leads to more rapid oxidation of the oral contraceptive 2-ethynyloestradiol and its loss of effectiveness (Bolt *et al*. 1977; Guengerich 1988b). Induction of a P450 by a drug which is a substrate leads to a lower AUC (area-under-the-curve) as a function of time. In practice, the clinical consequences of P450 induction are not as common as for inhibition. One of the major concerns about P450 induction by drugs in development is that some general correlations exist between induction of certain P450s in rodents and the potential for tumour promotion (Nims *et al*. 1994). For instance, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (inducer of CYP1A1, 1A2, 1B1), barbiturates (CYP2B) and some hypolipidaemic agents (CYP4A) are all tumour promoters in rodents (Pitot 1995). These phenomena may be largely unimportant for humans (e.g. epileptic patients on lifetime phenobarbital do not develop tumours (Olsen *et al*. 1989)) but are of concern regarding review of rodent bioassays by regulatory agencies.

Structure

The first three-dimensional structure of a P450 was reported by Poulos in 1987 (Poulos *et al*. 1987). At this time the structure of at least eight different P450s is known; six have been published (Poulos *et al*. 1987; Ravichandran *et al*. 1993; Hasemann *et al*. 1994; Cupp-Vickery *et al*. 1996; Park *et al*. 1997; Williams *et al*. 2000). All but one are of soluble enzymes, and all but two are of bacterial origin. One is from a Fusarium mold (Park *et al*. 1997) and one is a slightly modified form of a rabbit enzyme (Williams *et al*. 2000). Because of restrictions on the length of this chapter, these structures will be discussed only briefly and the reader is referred to the original papers (see above) for reference to the actual structures and to discussion of exactly what one can conclude from the results (particularly see Poulos 1991 and Hasemann *et al*. 1995).

All the P450 structures have common elements and look similar at a gross level. The proteins are arranged into a series of helices and folds that are rather similar. The helices are denoted A through L and are well conserved. The I and L helices make contact with the haem. Residues in the B and I helices contact the substrate, and mutations in these regions have had some of the most dramatic effects in site-directed mutagenesis experiments (Kronbach *et al*. 1991; Hasler *et al*. 1994; Halpert and He 1993; Guengerich 1997b). Another region that contacts the substrate is the F helix,

where mutations have also influenced catalytic selectivity (Lindberg and Negishi 1989; Ibeanu *et al*. 1996; Harlow and Halpert 1997). Other structural, homology modelling, and site-directed mutagenesis work indicates that the six 'substrate recognition sequences' (SRS, three already cited in the discussion of the B, I, and F helices, see above) are generally conserved among most P450s (Gotoh 1992).

The most conserved part of the P450 sequence is in the region containing the cysteine that acts as the thiolate ligand to the haem iron. This sequence is used as the 'landmark' to identify P450s in genebanks. Another highly conserved residue is a threonine corresponding to threonine 252 in bacterial P450 101, which has been postulated to have various functions. It may donate a proton to the $Fe^{2+}O_2^-$ complex to facilitate (heterolytic) cleavage of the O–O bond. Arguments against the general need for such an activity include the high pK_a of threonine, the absence of this threonine in some P450s, and the functionality of CYP101 in which a threonine O-methyl ether was substituted using mutagenesis (Kimata *et al*. 1995). The dominant view today is that the threonine and its neighbouring glutamic acid are part of a proton relay system that is useful but not absolutely necessary. In CYP108 (P450eryF), the substrate binds and then mobilises H_2O to serve the same function (Cupp-Vickery *et al*. 1996).

Crystal structures in themselves do not necessarily provide information about function. Indeed, some of the P450s for which structures have been obtained are not well characterised in the sense that in some cases the physiological electron donor has not been identified (Cupp-Vickery *et al*. 1996). CYP101 ($P450_{cam}$) was the first P450 crystallised, and this structure has provided the greatest amount of information, in part because of the wealth of knowledge that exists about protein interactions, substrate specificity and kinetics (Mueller *et al*. 1995). Recently time-resolved crystallographic approaches have been used to obtain the structure of distinct redox forms of P450 101 during the catalytic cycle (Schlichting *et al*. 2000). Although the structure of the latter intermediates is not as clear and the molecular identity is not as certain, the work provides insight into the changes that occur in the course of P450 reaction. Of particular interest is the observation that changes in the structure near the active site occur at every step of the reaction. To some extent then, each of the redox forms may be considered as a slightly different enzyme. In P450 102 there is a long, open substrate channel and only relatively small conformational changes are required (Ravichandran *et al*. 1993; Hasemann *et al*. 1995). However, with several of the other P450s, the substrate is completely enclosed by the protein (e.g. CYP101, 107, 108, 2C5 (Hasemann *et al*. 1995; Williams *et al*. 2000)) and obviously a major rearrangement of the protein is necessary. This rearrangement is not well understood, even in P450 101, and has only been approached from molecular dynamics simulations (Paulsen and Ornstein 1993; Helms and Wade 1998). A key question is how fast the protein motion is and exactly why the substrate is bound. The structural change is also required for the product to leave, and similar questions can be raised. Also, the question arises as whether the product and substrate follow the same paths in entering and leaving.

The availability of a structure of a mammalian P450 is an important development. Rabbit CYP2C5 was subjected to some modification to increase the level of heterologous expression and decrease aggregation (Cosme and Johnson 2000). These ap-

proaches yielded a protein that could be crystallised in the absence of detergents (Williams *et al*. 2000). However, molecular anomalous dispersion phasing techniques and synchotron radiation were required to solve the structure. The structure is of considerable interest in terms of modelling of other mammalian P450s. In this regard, the structure has been compared to that of CYP102, which has already been employed extensively and shows the greatest similarity (Williams *et al*. 2000). The spatial arrangement of the major elements diverges from the other structures, with SRS-4 being the most conserved compared to CYP102. SRS-5 diverges significantly, with 3.3 Å root mean square (rms deviation) and the shape of the base of the active site is significantly different, affecting the orientation and position of substrates as well as selectivity for different substrates. This is not surprising, in that the substrates of CYP102 and 2C5 are fatty acids and steroids, respectively (Williams *et al*. 2000; Fulco 1991; Johnson *et al*. 1983). The spatial organisation of SRS regions 1, 2, 3, and 6 also diverges from CYP102 (4.5–6.0 Å). The high temperature factors of the CYP2C5 B-C loop might be indicative of dynamic fluctuations related to passage of substrates through this area (Williams *et al*. 2000).

The CYP2C5 structure seems to indicate a bound ligand, although no substrate was added. It is possible that this is a reagent utilised in the work (e.g. dithiothreitol). The substrate binding cavity is only slightly larger than that for camphor in CYP 101 (360 $Å^3$) (Williams *et al*. 2000). Progesterone was readily docked into this site. Many P450 modelling efforts have been published, based first on P450 101 and subsequently on CYP102 or aggregate models of the known structures. Undoubtedly structure modelling will now also be based upon CYP2C5. The general features will apply; the usefulness of modelling details of ligand interaction remains to be demonstrated.

Mechanisms of catalysis

A discussion of the chemistry is in order because of relevance to considerations of catalytic selectivity (see below). For earlier and more extensive discussion of the chemistry of catalysis, the reader is directed to several articles (Ortiz de Montellano 1986, 1995b; Dawson 1988; Guengerich and Macdonald 1984, 1990, Guengerich 1996).

General features

The general paradigm for P450 catalysis is shown in Figure 2.2. Briefly, ferric P450 binds substrate in step 1. In the bacterial CYP101 ($P450_{cam}$), this step is associated with a shift from low- to high-spin iron, an increased redox potential ($E_{1/2}$) and faster reduction (Fisher and Sligar 1985; Mueller *et al*. 1995). However, with the microsomal P450s these events may or may not occur and are not coupled (Guengerich 1983; Bäckström *et al*. 1983; Guengerich and Johnson 1997). In step 2, one electron is transferred from the reductase. This is a complex process in that the two flavins (FAD, FMN) are used and a number of redox state combinations are possible. The accepted view is that the step $FADH/FMNH_2 \rightarrow FADH/FMNH$ is associated with step 2 (Vermilion and Coon 1978; Vermilion *et al*. 1981). Step 3 involves binding of O_2 to

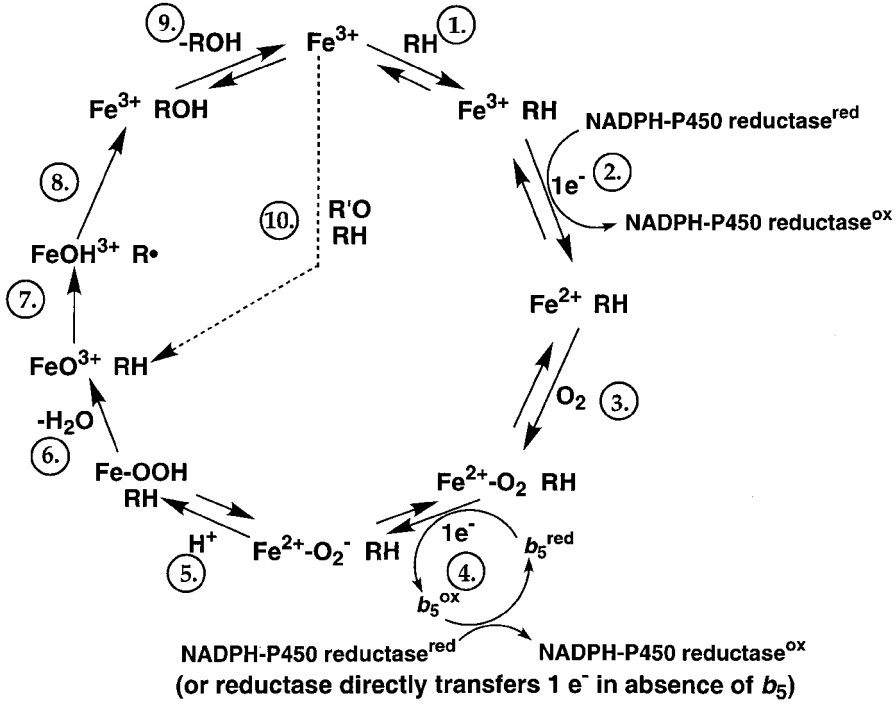


Figure 2.2 Generalised catalytic P450 mechanism.

ferrous P450 (ferric haem does not bind O_2). The 'second' electron is delivered, to this ferrous·O_2 species, in step 4. It is generally thought that the redox potential for this step is much higher (0 mV?) than for step 2 (−300 mV), although estimates are very crude (Guengerich 1983; Pompon and Coon 1984). The reductase step involved in step 4 is $FAD/FMNH_2 \rightarrow FAD/FMNH\cdot$ (which then is recycled in the reductase: $FAD/FMNH\cdot + NADPH + H^+ \rightarrow FADH_2/FMNH\cdot \rightarrow FADH\cdot/FMNH_2$) Vermilion *et al.* 1981). However, there is evidence that in some cases this electron can be provided from ferrous cytochrome b_5 (which has a redox potential of 0 mV (Rivera *et al.* 1998)).

Following the $Fe^{2+}\cdot O_2$ complex (between steps 3 and 4), only limited spectral characterisation has been obtained and some of the evidence is based on biomimetic models (Ortiz de Montellano 1995b). Step 5 involves the protonation of the formal $Fe^{2+}O_2$-complex, which appears to be mediated by a Thr/Asp/H_2O 'relay' system in CYP101 and some other P450s for which three-dimensional structures are available (Martinis *et al.* 1989; Cupp-Vickery *et al.* 1996). This relay system is is also believed to facilitate the heterolytic cleavage of the O–O band to generate a formal FeO^{3+} complex in step 6. This complex can formally be written $Fe^{V} = O$ but the prevailing view is that a better electronic representation is as $Fe^{4+}O$/porphyrin radical, analogous to peroxidase Compound I (Dawson 1988). Step 7 involves abstraction of a hydrogen atom ($C{-}H \rightarrow C\cdot + FeOH$), or possibly a non-bonded or π electron,

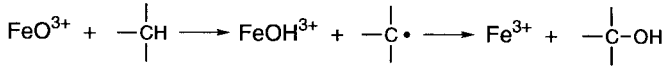
followed by rapid 'oxygen rebound' in step 8 to yield the product. The product is released in step 9, to regenerate the starting ferric, unliganded protein.

Types of oxidations

So many P450 substrates exist that can only be considered in major groups, on a mechanistic basis. Some minor paths are not presented, unless they have a major bearing on general mechanisms, but the reader is referred to discussions presented elsewhere (Ortiz de Montellano 1995b; Guengerich and Macdonald 1990).

CARBON HYDROXYLATION

This process results in the formation of alcohols and is common in the metabolism of sterols, alkanes, etc. The most generally accepted mechanism involves hydrogen abstraction followed by oxygen rebound:

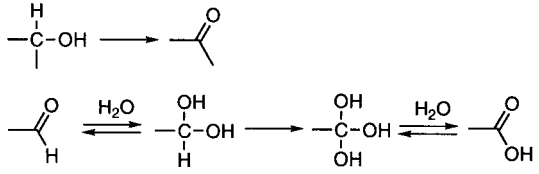


(2.2)

Major evidence for this pathway comes from the high kinetic hydrogen effects and the scrambling of putative methylene and allylic radicals (Ortiz de Montellano 1995b; Groves and Subramanian 1984; Ortiz de Montellano 1986).

DEHYDROGENATION

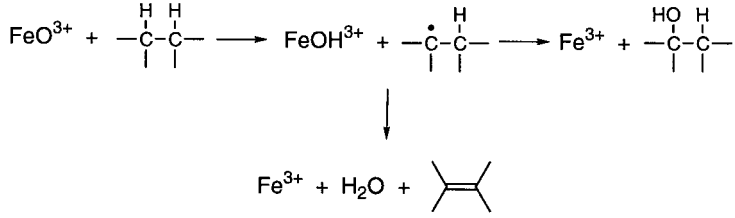
Several reactions are included here:



(2.3)

The 2-electron oxidation of an alcohol can be readily explained by the carbon hydroxylation mechanism. The oxidation of an aldehyde to a carboxylic acid can also be explained in this way, although an alternate mechanism is possible (see below).

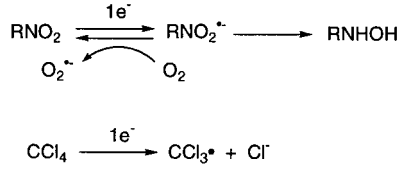
Desaturation of alkanes is not uncommon. It usually accompanies carbon hydroxylation and is postulated to result from bifurcation of the putative radical intermediate:



(2.4)

1e⁻ REDUCTION

When the oxygen tension is low, ferrous P450 may transfer an electron to a substrate before binding O_2 (Wislocki *et al.* 1980). Some reductions catalysed by P450s include those of nitroaromatics and CCl_4:

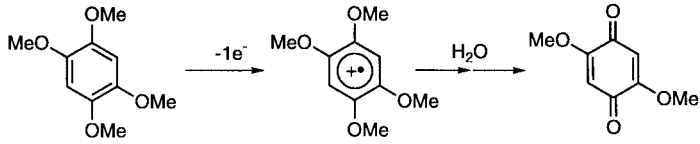


(2.5)

Other reductions by P450 (e.g. epoxides) are not as well characterised (Wislocki *et al.* 1980; Yamazoe *et al.* 1978).

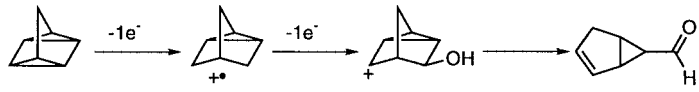
1e⁻ OXIDATION

This process has been postulated in a number of reactions where low redox potentials are involved (see below). The concept has relevance in the discussion of 1e⁻ oxidation of polycyclic aromatic hydrocarbons (Cavalieri and Rogan 1995), for which direct evidence is not available. Recently this laboratory has presented evidence for the 1e⁻ oxidation of 1,2,4,5-tetramethoxybenzene ($E_{1/2}$ 0.94 versus SCE), which can be observed in the steady state (Sato and Guengerich 2000):



(2.6)

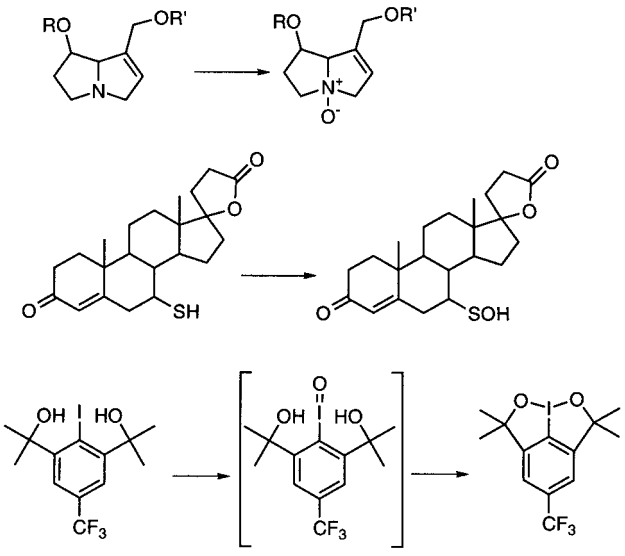
The rearrangement of strained cycloalkanes (e.g. quadricylane) with low potentials has also been rationalised in the context of 1e⁻ oxidation (Stearns and Ortiz de Montellano 1985):



(2.7)

HETEROATOM OXYGENATION

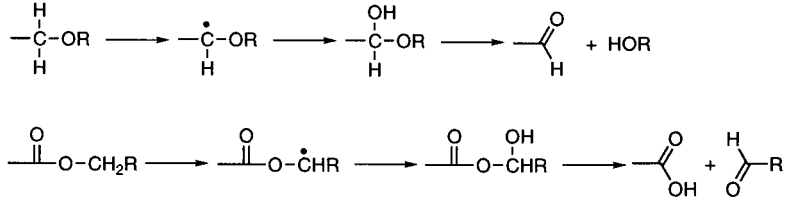
This reaction is very common for the enzyme flavin-containing monooxygenase but it is also catalysed by P450s, even in situations where α-hydrogen atoms are available. The reaction is commonly seen with amines and sulphides and has also been implicated in a reaction with an iodide (Watanabe *et al.* 1982; Seto and Guengerich 1993; Guengerich 1989).



(2.8)

HETEROATOM DEALKYLATION

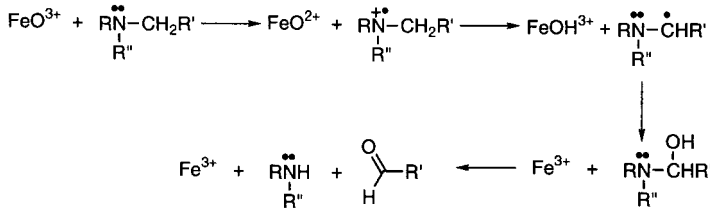
The cleavage of a substrate at a heteroatom is a common P450 reaction. In general, the cleavage of ethers and carboxylic acid esters and amides (Guengerich 1987; Guengerich *et al.* 1988a; Peng *et al.* 1995) is generally thought to involve the carbon hydroxylation mechanism presented above.



(2.9)

In situations where $E_{1/2}$ is low ($\sim$1 V versus SCE (Guengerich and Macdonald 1984; Guengerich and Macdonald 1990, 1993)), the mechanism is postulated to involve initial $1e^-$ oxidation (if spatially accessible) followed by base-catalysed

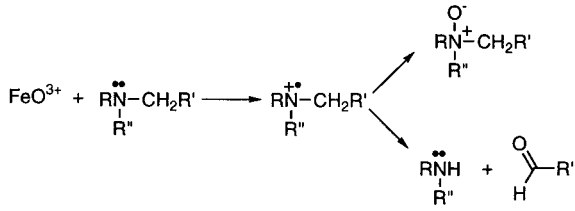
rearrangement involving the FeO_2^{2+} entity (Okazaki and Guengerich 1993; Ortiz de Montellano 1987):



(2.10)

Similar mechanisms can be involved for the dealkylation of sulphides (Guengerich and Macdonald 1984) and alkyl phenylethers with low $E_{1/2}$ (Yun *et al.* 2000).

$1e^-$ oxidation may be a common starting point for amine/sulphide oxygenation and dealkylation, because the two processes are usually associated with each other (Guengerich and Macdonald 1984):



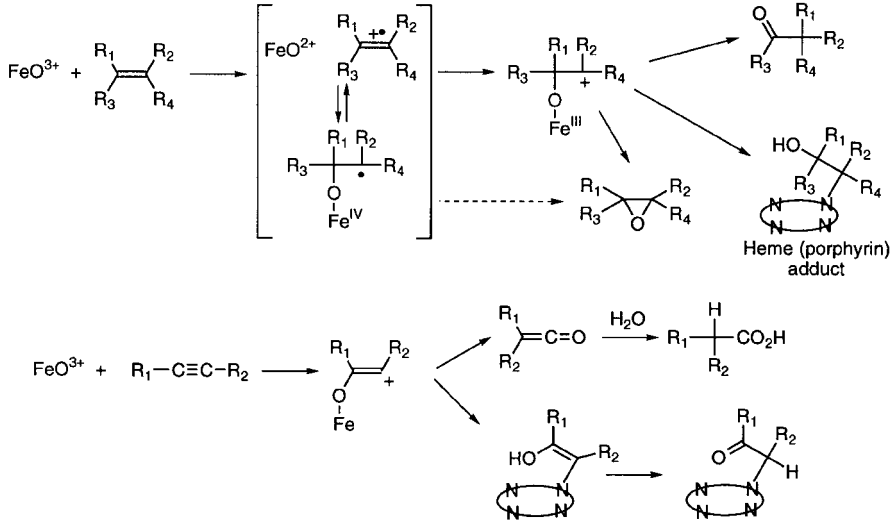
(2.11)

However, the lack of a linear free energy relationship (Seto and Guengerich 1993) and certain molecular orbital calculations (Hammons *et al.* 1985) suggest that N-oxygenation may not be so simple and might possibly involve a further electron transfer (Hammons *et al.* 1985):

(2.12)

OXIDATION OF OLEFINS AND ACETYLENES

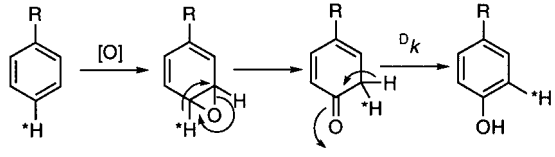
A very common product of olefin oxidation is an epoxide (Daly *et al.* 1972). However, the oxidation of olefins is probably best understood in the context of a stepwise mechanism with ionic intermediates, which may or may not follow radical intermediates that could undergo collapse first:



(2.13)

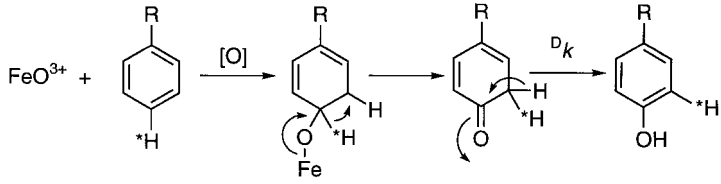
OXIDATION OF AROMATIC RINGS

Hydroxylation of aromatic rings is a reaction observed not only with P450s but also with numerous other oxygenases, including non-haem iron, copper, and flavin proteins (Walsh 1979; Guengerich 1990). Studies with the non-haem iron phenylalanine hydroxylase showed that a hydrogen label in the *para* position of the substrate could undergo a 1,2-shift, which was rationalised in the context of a hydrogen isotope effect and an epoxide intermediate:



(2.14)

This migration, commonly termed the 'NIH Shift' (Daly *et al.* 1972; Guroff *et al.* 1967), is consistent with the formation of an epoxide intermediate but does not in itself constitute proof of an epoxide. Epoxides are often intermediates in aromatic oxidations, or demonstrated by their isolation (or dihydrodiols in the presence of epoxide hydrolase) (Daly *et al.* 1972). A more generalised mechanism of aromatic oxidation includes the existence of Fe–O-substrate intermediates, with the 1,2-anion shifts well-rationalised (Guengerich and Macdonald 1990; Ortiz de Montellano 1995b).



(2.15)

As pointed out earlier, evidence has been for $1e^-$ oxidation of low $E_{1/2}$ aromatic systems but no clear evidence for the coupling of stepwise electron transfer and epoxidation/hydroxylation has been obtained (Anzenbacher *et al.* 1996).

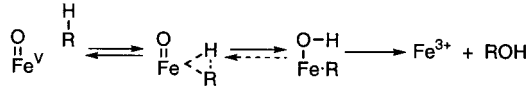
Nature of reactive oxidant

Early work in the P450 field was dominated by a search for 'mobile' oxidants, e.g. O_2^-, 'oxene' (Ullrich *et al.* 1982; Strobel and Coon 1971; Ullrich 1972). However, the general view today is that catalysis is the result of interaction of substrates with Fe–oxygen complexes. However, several possibilities are still viable.

The dominant view is that of a FeO^{3+} entity analogous to peroxidase Compound I (McMurry and Groves 1986; Ortiz de Montellano 1995b; Guengerich 1991). Most of the mechanisms of oxidation are reasonably rationalised with such an intermediate (see above). Moreover, most of the P450 reactions can be reproduced in systems in which the reductase and NADPH replaced by the 'oxygen surrogate' iodosylbenzene (step 10 in Figure 2.2), which is only capable of forming a mono-oxygen Fe complex. Reactions supported by this oxygen surrogate are usually faster than the 'normal' P450 reactions but linearity suffers from the harshness of the iodosylbenzene in destroying P450 haem (Lichtenberger *et al.* 1976; Gustafsson *et al.* 1979). If P450 and peroxidases have a similar set of reaction intermediates, why doesn't horseradish peroxidase catalyse all P450 oxidations? Apparently the steric restriction of the haem FeO entity causes electron transfer through the porphyrin edge but precludes base catalysis and oxygen transfer (Ortiz de Montellano 1987; Okazaki and Guengerich 1993).

An alternate view to the hydrogen atom abstraction pathway (Groves *et al.* 1978; McMurry and Groves 1986; Ortiz de Montellano 1995a) has been proposed by Newcomb (Newcomb *et al.* 1995a, 2000). The issue is the limited rearrangement of strained alkane 'radical clocks'. The lack of rearrangement has been interpreted as evidence that a discrete carbon-centred radical would have a lifetime too short to qualify as a bonafide intermediate (Newcomb *et al.* 1995b; 2000). An alternative to the hydrogen atom abstraction mechanism (which actually should be considered a caged system) is a rather concerted mechanism. However, such a mechanism does not explain the degree of rearrangement seen in some reactions (Newcomb *et al.* 2000; White *et al.* 1986) and may not be considered general. Another issue regarding the use of radical clocks for more than qualitative studies is their reliability inside enzymes (Jin and Lipscomb 1999; Frey 1997).

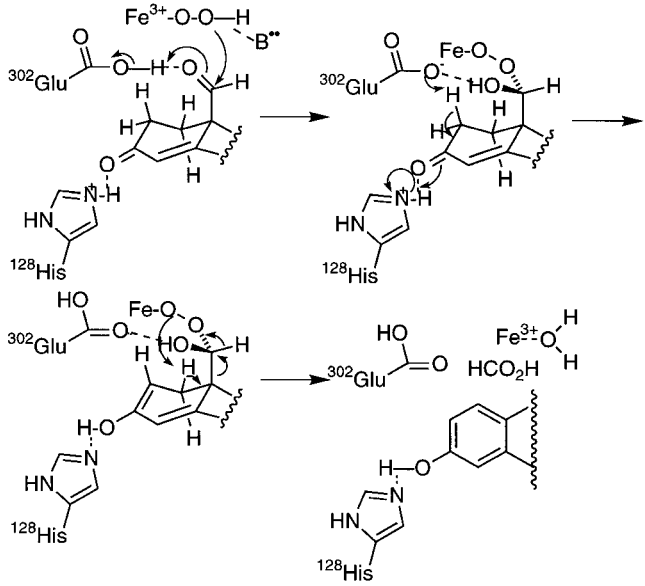
Collman has raised the hypothesis of 'agostic' mechanisms, involving Fe–C bonds:



(2.16)

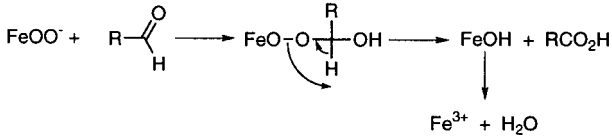
This proposal is based largely on bioorganic models (Collman *et al.* 1998); evidence in support of this has not been presented with P450 enzyme systems.

One 'alternative' mechanism (to the FeO^{3+} odd-electron abstraction/oxygen rebound system) that does have reasonable support is the iron-peroxide mechanism, originally proposed by Akhtar *et al.* (1982) to explain the third reaction in the sterol demethylation sequences.



(2.17)

Further studies have provided evidence that this nucleophilic mechanism can be implicated in the oxidation of simple aldehydes and formation of epoxides (Vaz *et al.* 1998):



(2.18)

A mechanism involving FeOOH has also been proposed for the formation of alcohols (Newcomb *et al.* 2000).

Rate-limiting steps

The issue of rate-limiting steps in P450 reactions has been considered since the early days of research in the field (Diehl *et al.* 1970; White and Coon 1980). Early work was

focussed on rates of reduction of ferric P450 (Diehl *et al*. 1970) and on intermolecular kinetic isotope effects, which were generally very small (Ortiz de Montellano 1986). Unfortunately, knowledge in this area is still very limited and restricted to certain P450/substrate pairs. Many investigators attach great significance to the steady-state kinetic parameters $k_{cat}(V_{max})$ and K_m with no interpretation of their meaning. For a more general consideration of this issues see Northrop (1998). Possibilities regarding each step in Figure 7.2 will be considered briefly.

Step 1 is generally considered to be fast, and the binding itself probably nearly diffusion-limited ($k_{on} \sim 10^8\ M^{-1}\ s^{-1}$), as judged by rates of change in the Soret spectrum (White and Coon 1980). It is possible (and probable; Poulos *et al*. 1995; Schlichting *et al*. 2000) that a conformational change occurs upon binding, but no rate has been measured.

Step 2, the reduction of ferric P450, has been studied extensively (White and Coon 1980; Peterson and Prough 1986). With some P450s the rate does appear to limit the overall reaction, especially in liver microsomes, where the concentration of reductase is far less than that of P450. The extent of stimulation of reduction rates by the P450 substrate can vary considerably (Guengerich and Johnson 1997). However, many P450-catalysed oxidations are very slow ($\sim$1 min^{-1}) and not really limited by the rate of reduction. Thus, rate-limiting reduction is probably limited to the faster substrate oxidations.

Step 3, the binding of O_2 to ferrous iron, is probably fast. The rate of binding has been measured to be $7.7 \times 10^5\ M^{-1}\ s^{-1}$ for CYP101 (Griffin and Peterson 1972); thus at 200 μM O_2 the rate would be $9 \times 10^3\ min^{-1}$.

Step 4, the transfer of the second electron, is considered to be the rate-limiting step in the oxidation of bacterial CYP101 (Mueller *et al*. 1995). This is probably a rate-limiting step in some reactions catalysed by microsomal P450s, in that cytochrome b_5 can stimulate some reactions by supplying the electron at this step (Peterson and Prough 1986). However, it should be noted that some cytochrome b_5 enhancements cannot be explained by electron transfer (Yamazaki *et al*. 1996; Aucus *et al*. 1998).

Steps 5 and 6 may be rate-limiting, because disruption of the threonine/glutamic acid/H_2O relay system can attenuate substrate oxidation and increase abortive O_2 reduction (Imai *et al*. 1989; Martinis *et al*. 1989; Kimata *et al*. 1995).

Step 7 involves C–H bond breaking in many cases and is subject to study using isotopic hydrogen substitution. Intramolecular isotope studies provide an estimate of the intrinsic isotope effect but do not in themselves yield information as to whether C–H bond breakage is a rate-limiting step (Ortiz de Montellano 1986). Non-competitive intermolecular hydrogen isotope effect studies can. In many cases with P450 reactions, no significant isotope effects could be measured (Ortiz de Montellano 1986; Ullrich 1969). However, in several cases these isotope effects are large (>10) and clearly implicate rate-limiting C–H cleavage (Guengerich *et al*. 1988; Yun *et al*. 2000).

Step 9, product release, has generally been considered to be rapid. However, in our own work on the interpretation of a kinetic deuterium isotope effect on K_m but not k_{cat} for the oxidation of ethanol to acetaldehyde and acetic acid (by CYP2E1), the pre-steady-state burst kinetics clearly implicated a rate-limiting step *following* product formation (Bell and Guengerich 1997; Bell-Parikh and Guengerich 1999). The best

explanation, based on simulated fitting of all data for these reactions, is a scheme in which actual substrate oxidation is very fast (500–1000 min^{-1}), as clearly shown experimentally by the burst rates, and the k_{cat} approximates a putative conformational relaxation step that occurs following product formation (Bell-Parikh and Guengerich 1999). This paradigm may explain why several CYP2E1 reactions have similar rates (Guengerich *et al*. 1991). However, it should be pointed out that other CYP2E1 reactions can have slower rates, show different isotope effects, and not show burst kinetics because they are limited by other steps (Bell and Guengerich 1997; Bell-Parikh and Guengerich 1999).

These considerations should be kept in mind as one considers static models of P450 substrate interactions and their meaning. In many cases, the rates of P450 reactions may be much slower than the rate of 'abortive' P450 turnover, so the 'fit' within the active site/transition state may effectively govern reaction selectivity. However, the significance of steady-state kinetic parameters is usually not clear. In some cases the meaning of k_{cat} may be developed through the appropriate experiments. The meaning of K_m in complex systems such as P450-catalysed reactions is usually unknown, as demonstrated in our own work on K_m isotope effects with CYP2E1 (Bell and Guengerich 1997; Bell-Parikh and Guengerich 1999). Investigators should not equate K_m with K_d, the substrate dissociation constant, unless they have specific evidence to justify such a conclusion. This is simply a recapitulation of the advice of basic kinetic texts (Fersht 1999; Northrop 1998).

Catalytic specificity

BASIC CONSIDERATIONS

Catalytic selectivity has been one of the most practical issues involving P450 enzymes, particularly in the context of reactions with new substrates such as drugs, pesticides, etc. What really determines catalytic specificity is will a compound be a substrate and, if so, at which site it will be oxidized and how fast? These are questions that build on two points already discussed, structures and catalytic mechanisms.

One view of catalytic selectivity is that the protein structure is the only determinant and serves only to position substrates near the chemically reactive FeO centre. Reactivity and rates of oxidation are functions of the distance of each substrate atom to the FeO entity. Substrates that do not fit tightly into the active site can 'rattle', and regioselectivity of oxidation is largely a function of the ease of chemical oxidation at each substrate atom. There is a certain amount of credibility to this view. For instance, with relatively small substrates it has been possible to use series of chemicals to develop linear free energy relationships (Burka *et al*. 1985; Macdonald *et al*. 1989). With the growing popularity of protein models, many docking efforts have been made on the basis of the ability of substrates to fit into models with the lowest energy barriers (Jones *et al*. 1996; de Groot and Vermeulen 1997; Lewis and Lake 1995).

No one would exclude the influence of protein structure in selectivity considerations, but some mechanistic differences are also operative. For instance, if electron abstraction is involved in a mechanism, it should be feasible over a longer distance than 'direct' oxygenation or hydrogen abstraction. The possibility that either $Fe^{2+}OOH$

(or $Fe^{2+}O_2^-$) or FeO^{3+} can be the oxidant will also influence selectivity (Sahali-Sahly *et al*. 1996). Rate-limiting steps influence rates and therefore selectivity. For instance, for some P450 2E1 reactions we showed that the rate-limiting step follows product formation but that the K_m for the reaction is isotopically sensitive (because the bond-breaking step is attenuated) (Bell and Guengerich 1997; Bell-Parikh and Guengerich 1999). In other reactions the rate of P450 reduction may be the issue (Guengerich and Johnson 1997).

Thus, the balance of the individual rate-limiting steps is a part of the catalytic selectivity. Having said this, it should be emphasised that, to a large extent, the rate-limiting steps are also a function of the steric fit of the substrate into the protein. For instance, a poor fit may not facilitate efficient reduction for some P450 substrates (Guengerich and Johnson 1997). With all these complexities, predicting substrate and regioselectivity has been very challenging and few if any good prospective examples are available. Predicting rates has been nearly impossible except within sets of small molecules where linear free energy relationships are operative (Burka *et al*. 1985; Macdonald *et al*. 1989).

PRACTICAL ASPECTS

The difficulty of predicting catalytic specificity with potential substrates has been discussed above. However, when a reaction is established to occur, it is relatively straightforward to determine which P450 is involved. General approaches have been presented elsewhere (Guengerich and Shimada 1991).

In vitro experiments can be done with microsomes (usually liver) or (less often) with isolated hepatocytes. With rat liver microsomes, considerable information can be obtained from comparisons of gender effects and treatments of the rats with various inducers. For instance, very high inducibility with phenobarbital would first suggest CYP2B1 or 2B2; a male-specific activity that is attenuated in rats treated with polycyclic hydrocarbons would suggest CYP2C11, etc. (Guengerich 1987a). Another means of assessing selectivity is to simply compare isolated enzymes for their parameters. Today most preparations are from heterologous expression and not derived from tissues; some are commercially available. The enzymes can be utilised in purified form (along with the reductase, phospholipids, and cytochrome b_5, when appropriate) or within the context of cruder preparations derived from expression systems, e.g. membranes (Guengerich *et al*. 1996; Gonzalez *et al*. 1991). Another approach is to use crude animal or human systems (e.g. microsomes) as the source of enzymes and then determine the effects of selective inhibitors of individual P450s (Correia 1995; Halpert and Guengerich 1997). In principle, the fraction of inhibition is indicative of the extent to which that P450 contributes. The same approach can be applied with inhibitory antibodies (Thomas *et al*. 1977; Guengerich 1987a; Krausz *et al*. 1997). Finally, if one P450 is dominant in catalysing a particular reaction, then a high correlation should be seen in different human liver samples between the activity and that form of P450 (Beaune *et al*. 1986). To a first approximation, the correlation coefficient r^2 indicates the fraction of the relationship (activity versus parameter of a particular P450) than can be accounted for by that P450.

In vivo experiments can also be done to establish roles of individual P450s in

reactions, both in experimental animals and in humans. Induction experiments can be done and the effects on *in vivo* parameters measured. Several inhibitors can be used safely in humans (e.g. quinidine for CYP2D6, ketoconazole for CYP3A4). Genetic systems can also be of value. With humans, genotypic 'poor metabolisers' (with regard to a particular P450) are expected to also slow oxidation of a drug if that P450 is dominant in metabolism. With animals, several P450-knockout mouse lines are now available (Medinsky *et al*. 1997; Buters *et al*. 1999) (the applicability to humans must be evaluated, however).

Progress has been made in using *in vitro* information (from human liver microsomes and hepatocytes) to make *in vivo* human predications. All approaches have some deficiencies, particularly when issues such as transport are problems. Nevertheless, the general approach has promise (Iwatsubo *et al*. 1997).

Inhibition

Inhibition of P450s is a very practical issue today in the pharmaceutical industry. Before discussing the practical considerations, the basic principles will be reviewed briefly. More extensive treatments of enzyme inhibition in general (Kuby 1991; Segel 1975) and P450 inhibition (Ortiz de Montellano and Correia 1983; Ortiz de Montellano and Reich 1986; Correia 1995; Halpert and Guengerich 1997) have been published and the reader is referred to these articles.

Competitive inhibition is relatively straightforward and, in its simplest form, explained by direct competition of two ligands in the substrate binding site, yielding an increase in the apparent K_m but no change in k_{cat}. However, for discussion of the potential complexity see Segel (1975).

Mechanism-based inactivation utilises the oxidation of a substrate to an inhibitory form. Such oxidation is time-dependent and requires the usual cofactors (NADPH, O_2). In the strictest sense, mechanism-based inactivation implies the formation of an intermediate in the reaction that can either (1) react with the enzyme to destroy it or (2) go on to yield a stable product. The ratio of these two reactions (2/1) is termed the partition coefficient (which can be viewed as the average number of times the enzyme turns over before inactivation). This behaviour has been seen with many drugs and is even a strategy in the design of some drugs intended to inactivate targets, e.g. the CYP19-catalysed oxidation of androgens to oestrogens in hormone-dependent cancers (Vanden Bossche *et al*. 1994). Three major types of inactivation are seen. The first involves destruction of the P450 haem, by alkylation of a pyrrole nitrogen (Ortiz de Montellano *et al*. 1979). Following this process, the iron is lost and the porphyrin derivative is removed from the P450 protein. The second process involves the interaction of a reaction intermediate with the P450 protein. A number of products have been characterised (Roberts *et al*. 1997). The third type of P450 inactivation involves the degradation of haem and crosslinking of the fragments to the protein, which occurs with oxidants and certain chemicals (Osawa and Pohl 1989).

Some other types of inhibition often presented in introductory biochemistry texts are non-competitive and uncompetitive inhibition. In principle, the former involves a decreased k_{cat} and change in K_m. The latter involves interaction of an inhibitor with only the substrate-bound form of the enzyme. Although these cases are often

presented in graph example, they are seldom observed in practice. Another type of inhibition is 'slow-binding tight inhibition', in which a time-dependent development of inhibition occurs without transformation of the compound. For instance, a conformational change is thought to be involved in such a process when prostaglandin synthase is inhibited by non-steroidal anti-inflammatory drugs (Kalgutkar and Marnett 1994). Such a process has not been documented for any P450s (Shimada *et al*. 1998). Another type of inhibition occurs when a reaction product is a reactive electrophile and binds to the P450 protein. Distinguishing such reactions from true mechanism-based inactivation may be difficult (Silverman 1995), and often this type of inhibition is grouped under the same heading.

In the course of our own work, we found that some peptides were very effective in blocking the binding of substrates to CYP3A4 but were more than an order of magnitude less efficient in blocking catalytic activity (Hosea *et al*. 2000). The basis appears to be the selective binding of the peptides (which were shown not to be substrates for oxidation) to the ferric P450 (compared to ferrous P450). This situation is of conceptual interest in that different redox states of P450 are considered as distinct enzymes in the analysis of the catalytic cycle (Hosea *et al*. 2000; Segel 1975).

A complex issue that will be mentioned briefly is that of P450 stimulation by chemicals. The phenomenon is not new (Cinti 1978) but remains poorly understood (Halpert and Guengerich 1997). Two modes are seen: (1) heterotropic cooperativity, in which an added chemical other than the substrate can enhance oxidation of the substrate (whether or not the added chemical is also a substrate), and (2) homotropic cooperativity, where plots of reaction velocity versus substrate are not hyperbolic. Several models have been proposed, including those with a large site capable of binding $\geqslant$2 substrates (Johnson *et al*. 1988; Huang *et al*. 1981; Hosea *et al*. 2000; Shou *et al*. 1999). These models have not been distinguished by biophysical experiments, and several possibilities must still be considered possible to account for all the observations. The cooperativity is probably not simply a biochemical curiosity. Some sigmoidal kinetic behaviour has been observed in kinetic assays with hepatocyte suspensions (Witherow and Houston 1998), and heterotropic stimulation of animal P450s has been demonstrated *in vivo* (Lasker *et al*. 1982; Lee *et al*. 1996a).

As mentioned earlier, P450 inhibition is a practical issue and has been implicated in adverse drug–drug interactions (Kivistö *et al*. 1994; Guengerich 1999a). Most pharmaceutical companies do assays early in the screening/development phase. Screens for competitive inhibition can be readily done in relatively high-throughput modes.

Several issues can be considered. One of the major decisions to make is how much information to acquire. More data may be useful in reaching decision points but more time and resources are needed. Assays for mechanism-based inactivation are much more laborious than for simple competitive inhibition. One problem, even with competitive inhibition, is the generality of results obtained with one particular reaction, and this problem is probably greatest with CYP3A4. For instance, some of the different fluorescence-based high-throughput reactions show varying effects (stimulation or inhibition) when the same compound is added (Miller *et al*. 1999). A potentially valuable new fluorescence assay was developed by Chauret *et al*. (1999) and appears to be congruent with the prototype testosterone 6β-hydroxylation. Another concern is how much organic solvent to use to dissolve the substrate. Many

drugs are notoriously insoluble in H_2O and solvents are needed. In this laboratory we have generally operated with ⩽1% (v/v), but even this concentration can be an issue (Yoo *et al*. 1987; Chauret *et al*. 1998).

A major issue is exactly how much *in vitro* inhibition is serious *in vivo* (Iwatsubo *et al*. 1997). Calculations can be made by simply doing substitutions in the standard Michaelis–Menten formulae, letting [S] = plasma concentration of drug and [I] = plasma concentration of inhibitor, although protein binding and transport phenomena can influence the extrapolations. Mechanism-based inactivation has been more difficult to model because of its very nature. As the databases of drugs with both *in vivo* history and valid inactivation parameters ($k_{inactivation}$, K_i) grow, better prediction of *in vivo* problems should be possible.

References

Addya S, Anandatheerthavarada HK, Biswas G, Bhagwat SV, Mullick J and Avadhani NG (1997) Targeting of NH_2-terminal-processed microsomal protein to mitochondria: a novel pathway for the biogenesis of hepatic mitochrondrial P450MT2. *Journal of Cell Biology*, **139**, 589–599.

Akhtar M, Calder MR, Corina DL and Wright JN (1982) Mechanistic studies on C-19 demethylation in oestrogen biosynthesis. *Biochemical Journal*, **201**, 569–580.

Alvares AP, Schilling G, Levin W and Kuntzman R (1967) Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. *Biochemical and Biophysical Research Communications*, **29**, 521–526.

Anandatheerthavarada HK, Addya S, Dwivedi RS, Biswas G, Mullick J and Avadhani NG (1997) Localization of multiple forms of inducible cytochromes P450 in rat liver mitochrondria: immunological characteristics and patterns of xenobiotic substrate metabolism. *Archives of Biochemistry and Biophysics*, **339**, 136–150.

Anzenbacher P, Niwa T, Tolbert LM, Sirimanne SS and Guengerich FP (1996) Oxidation of 9-alkyl anthracenes by cytochrome P450 2B1, horseradish peroxidase, and iron tetraphenylporphin/iodosylbenzene systems. Anaerobic and aerobic mechanisms. *Biochemistry*, **35**, 2512–2520.

Aucus RJ, Lee TC and Miller WL (1998) Cytochrome b_5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *The Journal of Biological Chemistry*, **273**, 3158–3165.

Axelrod J (1955) The enzymatic deamination of amphetamine. *The Journal of Biological Chemistry*, **214**, 753–763.

Bäckström D, Ingelman-Sundberg M and Ehrenberg A (1983) Oxidation-reduction potential of soluble and membrane-bound rabbit liver microsomal cytochrome P-450 LM2. *Acta Chemica Scandinavica*, **37**, 891–894.

Baron J, Redick JA and Guengerich FP (1981) An immunohistochemical study on the localizations and distributions of phenobarbital- and 3-methylcholanthrene-inducible cytochromes P-450 within the livers of untreated rats. *The Journal of Biological Chemistry*, **256**, 5931–5937.

Bartlomowicz B, Friedberg T, Utesch D, Molitor E, Platt K and Oesch F (1989) Regio- and stereoselective regulation of monooxygenase activities by isoenzyme-selective phosphorylation of cytochrome P450. *Biochemical and Biophysical Research Communications*, **160**, 46–52.

Beaune P, Kremers PG, Kaminsky LS, de Graeve J and Guengerich FP (1986) Comparison of monooxygenase activities and cytochrome P-450 isozyme concentrations in human liver microsomes. *Drug Metabolism and Disposition*, **14**, 437–442.

Bell LC and Guengerich FP (1997) Oxidation kinetics of ethanol by human cytochrome P450 2E1. Rate-limiting product release accounts for effects of isotopic hydrogen substitution and cytochrome b_5 on steady-state kinetics. *The Journal of Biological Chemistry*, **272**, 29643–29651.

Bell-Parikh LC and Guengerich FP (1999) Kinetics of cytochrome P450 2E1-catalyzed oxidation of ethanol to acetic acid via acetaldehyde. *The Journal of Biological Chemistry*, **274**, 23833–23840.

Bolt HM, Bolt M and Kappus H (1977) Interaction of rifampicin treatment with pharmacokinetics and metabolism of ethinyloestradiol in man. *Acta Endocrinologica*, **85**, 189–197.

Brodie BB, Gillette JR and LaDu BN (1958) Enzymatic metabolism of drugs and other foreign compounds. *Annual Review of Biochemistry*, **27**, 427–454.

Burka LT, Guengerich FP, Willard RJ and Macdonald TL (1985) Mechanism of cytochrome P-450 catalysis. Mechanism of N-dealkylation and amine oxide deoxygenation. *Journal of the American Chemical Society*, **107**, 2549–2551.

Buters JTM, Sakai S, Richter T, Pineau T, Alexander DL, Savas U, Doehmer J, Ward JM, Jefcoate CR and Gonzalez FJ (1999) Cytochrome P450 CYP1B1 determines susceptibility to 7,12-dimethylbenz[*a*]anthracene-induced lymphomas. *Proceedings of the National Academy of Sciences, USA*, **96**, 1977–1982.

Cavalieri EL and Rogan EG (1995) Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica*, **25**, 677–688.

Chauret N, Gauthier A and Nicoll-Griffith DA (1998) Effect of common organic solvents on in vitro cytochrome P450-mediated metabolic activities in human liver microsomes. *Drug Metabolism and Disposition*, **26**, 1–4.

Chauret N, Tremblay N, Lackman RL, Gauthier J-Y, Silva JM, Marois J, Yergey JA and Nicoll-Griffith DA (1999) Description of a 96-well plate assay to measure cytochrome P4503A inhibition in human liver microsomes using a selective fluorescent probe. *Analytical Biochemistry*, **276**, 215–226.

Cinti DL (1978) Agents activating the liver microsomal mixed function oxidase system. *Pharmacology and Therapeutics*, **2**, 727–749.

Collman JP, Chien AS, Eberspacher TA and Brauman J,I. (1998) An agostic alternative to the P-450 rebound mechanism. *Journal of the American Chemical Society*, **120**, 425–426.

Conney AH (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes memorial lecture. *Cancer Research*, **42**, 4875–4917.

Conney AH, Miller EC and Miller JA (1956) The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. *Cancer Research*, **16**, 450–459.

Correia MA (1995) Rat and human liver cytochromes P450. Substrate and inhibitor specificities and functional markers. In *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd edition, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 607–630.

Correia MA, Davoll SH, Wrighton SA and Thomas PE (1992a) Degradation of rat liver cytochromes P450 3A after their inactivation by 3,5-dicarbethoxy-2,6-dimethyl-4-ethyl-1,4-dihydropyridine: characterization of the proteolytic system. *Archives of Biochemistry and Biophysics*, **297**, 228–238.

Correia MA, Yao K, Wrighton SA, Waxman DJ and Rettie AE (1992b) Differential apoprotein loss of rat liver cytochromes P450 after their inactivation by 3,5-dicarbethoxy-2,6-dimethyl-4-ethyl-1,4-dihydropyridine: a case for distinct proteolytic mechanisms? *Archives of Biochemistry and Biophysics*, **294**, 493–503.

Cosme J and Johnson EF (2000) Engineering microsomal cytochrome P450 2C5 to be a soluble, monomeric enzyme. Mutations that alter aggregation, phospholipid dependence of catalysis, and membrane binding. *The Journal of Biological Chemistry*, **275**, 2545–2553.

Cupp-Vickery JR, Han O, Hutchinson CR and Poulos TL (1996) Substrate-assisted catalysis in cytochrome P450eryF. *Nature Structural Biology*, **3**, 632–637.

Cupp-Vickery J, Anderson R and Hatziris Z (2000) Crystal structures of ligand complexes of P450eryF exhibiting homotropic cooperativity. *Proceedings of the National Academy of Sciences, USA*, **97**, 3050–3055.

d'Errico A, Taioli E, Chen X and Vineis P (1996) Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers*, **1**, 149–173.

Daly JW, Jerina DM and Witkop B (1972) Arene oxides and the NIH shift: the metabolism, toxicity and carcinogenicity of aromatic compounds. *Experientia*, **28**, 1129–1264.

Dawson JH (1988) Probing structure–function relations in heme-containing oxygenases and peroxidases. *Science*, **240**, 433–439.

de Groot MJ and Vermeulen NPE (1997) Modeling the active sites of cytochrome P450s and glutathione *S*-transferases, two of the most important biotransformation enzymes. *Drug Metabolism Reviews*, **29**, 747–799.

Dees JH, Masters BSS, Muller-Eberhard U and Johnson EF (1982) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and phenobarbital on the occurrence and distribution of four cytochrome P-450 isozymes in rabbit kidney, lung, and liver. *Cancer Research*, **42**, 1423–1432.

Diehl H, Schädelin J and Ullrich V (1970) Studies on the kinetics of cytochrome P-450 reduction in rat liver microsomes. *Hoppe–Seyler's Zeischrift fur Physioligische Chemie*, **351**, 1359–1371.

Distlerath LM and Guengerich FP (1987) Enzymology of human liver cytochromes P-450. In *Mammalian Cytochromes P-450, Volume 1*, Guengerich FP (ed.), CRC Press, Boca Raton, FL, pp. 133–198.

Dong Z, Hong J, Ma Q, Li D, Bullock J, Gonzalez FJ, Park SS, Gelboin HV and Yang CS (1988) Mechanism of induction of cytochrome P-450ac (P-450j) in chemically induced and spontaneously diabetic rats. *Archives of Biochemistry and Biophysics*, **263**, 29–35.

Eliasson E, Johansson I and Ingelman-Sundberg M (1988) Ligand-dependent maintenance of ethanol-inducible cytochrome P-450 in primary rat hepatocyte cell cultures. *Biochemical and Biophysical Research Communications*, **150**, 436–443.

Estabrook RW, Cooper DY and Rosenthal O (1963) The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex. *Biochemische Zeitschrift*, **338**, 741–755.

Fersht A (1999) *Structure and Mechanism in Protein Science*, Freeman, New York, p. 110.

Fisher MT and Sligar SG (1985) Control of heme protein redox potential and reduction rate: linear free energy relation between potential and ferric spin state equilibrium. *Journal of the American Chemical Society*, **107**, 5018–5019.

Frey PA (1997) Radicals in enzymatic reactions. *Current Opinion in Chemical Biology*, **1**, 347–356.

Fujii-Kuriyama Y, Mizukami Y, Kawajiri K, Sogawa K and Muramatsu M (1982) Primary structure of a cytochrome P-450: coding nucleotide sequence of phenobarbital-inducible cytochrome P-450 cDNA from rat liver. *Proceedings of the National Academy of Sciences, USA*, **79**, 2793–2797.

Fulco AJ (1991) $P450_{BM\text{-}3}$ and other inducible bacterial P450 cytochromes: biochemistry and regulation. *Annual Review of Pharmacology and Toxicology*, **31**, 177–203.

Geneste O, Raffalli F and Lang MA (1996) Identification and characterization of a 44 kDa protein that binds specifically to the 3′-untranslated region of CYP2a5 mRNA: inducibility, subcellular distribution and possible role in mRNA stabilization. *Biochemical Journal*, **313**, 1029–1037.

Gonzalez FJ, Crespi CL and Gelboin HV (1991) cDNA-expressed human cytochrome P450s: a new age of molecular toxicology and human risk assessment. *Mutation Research*, **247**, 113–127.

Gotoh O (1992) Substrate recognition sites in cytochrome P450 family 2 (CYP2) proteins inferred from comparative analysis of amino acid and coding nucleotide sequences. *The Journal of Biological Chemistry*, **267**, 83–90.

Griffin BW and Peterson JA (1972) Camphor binding by *Pseudomonas putida* cytochrome P-450. Kinetics and thermodynamics of the reaction. *Biochemistry*, **11**, 4740–4746.

Groves JT and Subramanian DV (1984) Hydroxylation by cytochrome P-450 and metalloporphyrin models. Evidence for allylic rearrangement. *Journal of the American Chemical Society*, **106**, 2177–2181.

Groves JT, McClusky GA, White RE and Coon MJ (1978) Aliphatic hydroxylation by highly purified liver microsomal cytochrome P-450: Evidence for a carbon radical intermediate. *Biochemical and Biophysical Research Communications*, **81**, 154–160.

Guengerich FP (1978) Destruction of heme and hemoproteins mediated by liver microsomal reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase. *Biochemistry*, **17**, 3633–3639.

Guengerich FP (1983) Oxidation-reduction properties of rat liver cytochrome P-450 and

NADPH-cytochrome P-450 reductase related to catalysis in reconstituted systems. *Biochemistry*, **22**, 2811–2820.
Guengerich FP (1987a) Enzymology of rat liver cytochromes P-450. In *Mammalian Cytochromes P-450, Volume 1*, Guengerich FP (ed.) CRC Press, Boca Raton, FL, pp. 1–54.
Guengerich FP (1987b) Oxidative cleavage of carboxylic esters by cytochrome P-450. *The Journal of Biological Chemistry*, **262**, 8459–8462.
Guengerich FP (1988a) Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Research*, **48**, 2946–2954.
Guengerich FP (1988b) Oxidation of 17α-ethynylestradiol by human liver cytochrome P-450. *Molecular Pharmacology*, **33**, 500–508.
Guengerich FP (1989) Oxidation of halogenated compounds by cytochrome P-450, peroxidases, and model metalloporphyrins. *The Journal of Biological Chemistry*, **264**, 17198–17205.
Guengerich FP (1990) Enzymatic oxidation of xenobiotic chemicals. *Critical Reviews in Biochemistry and Molecular Biology*, **25**, 97–153.
Guengerich FP (1991) Reactions and significance of cytochrome P-450 enzymes. *The Journal of Biological Chemistry*, **266**, 10019–10022.
Guengerich FP (1995) Human cytochrome P450 enzymes. In *Cytochrome P450*, 2nd edition, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 473–535.
Guengerich FP (1996) The chemistry of cytochrome P450 reactions. In *Cytochromes P450: Metabolic and Toxicological Aspects*, Ioannides C (ed.), CRC Press, Boca Raton, FL, pp. 55–74.
Guengerich FP (1997a) Cytochrome P450 enzymes. In *Biotransformation, Volume 3, Comprehensive Toxicology*, Guengerich FP (ed.), Elsevier Science, Oxford, pp. 37–68.
Guengerich FP (1997b) Comparisons of catalytic selectivity of cytochrome P450 subfamily members from different species. *Chemico-Biological Interactions*, **106**, 161–182.
Guengerich FP (1998) The environmental genome project: functional analysis of polymorphisms. *Environmental Health Perspectives*, **106**, 365–368.
Guengerich FP (1999a) Inhibition of drug metabolizing enzymes: molecular and biochemical aspects. In *Handbook of Drug Metabolism*, Woolf TF (ed.), Marcel Dekker, New York, pp. 203–227.
Guengerich FP (1999b) Human cytochrome P-450 3A4: regulation and role in drug metabolism. *Annual Review of Pharmacology and Toxicology*, **39**, 1–17.
Guengerich FP and Johnson WW (1997) Kinetics of ferric cytochrome P450 reduction by NADPH-cytochrome P450 reductase. Rapid reduction in absence of substrate and variation among cytochrome P450 systems. *Biochemistry*, **36**, 14741–14750.
Guengerich FP and Macdonald TL (1984) Chemical mechanisms of catalysis by cytochromes P-450: a unified view. *Accounts of Chemical Research*, **17**, 9–16.
Guengerich FP and Macdonald TL (1990) Mechanisms of cytochrome P-450 catalysis. *FASEB Journal*, **4**, 2453–2459.
Guengerich FP and Macdonald TL (1993) Sequential electron transfer reactions catalyzed by cytochrome P-450 enzymes. In *Advances in Electron Transfer Chemistry, Volume 3,* Mariano PS (ed.), JAI Press, Greenwich, CT, pp. 191–241.
Guengerich FP and Shimada T (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chemical Research in Toxicology*, **4**, 391–407.
Guengerich FP, Dannan GA, Wright ST, Martin MV and Kaminsky LS (1982) Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. *Biochemistry*, **21**, 6019–6030.
Guengerich FP, Peterson LA and Böcker RH (1988) Cytochrome P-450-catalyzed hydroxylation and carboxylic acid ester cleavage of Hantzsch pyridine esters. *The Journal of Biological Chemistry*, **263**, 8176–8183.
Guengerich FP, Kim D-H and Iwasaki M (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chemical Research in Toxicology*, **4**, 168–179.
Guengerich FP, Gillam EMJ and Shimada T (1996) New applications of bacterial systems to problems in toxicology. *CRC Critical Reviews in Toxicology*, **26**, 551–583.

Guroff G, Daly JW, Jerina DM, Renson J, Witkop B and Udenfriend S (1967) Hydroxylation-induced migration: the NIH shift. *Science*, **157**, 1524–1530.
Gustafsson JÅ, Rondahl L and Bergman J (1979) Iodosylbenzene derivatives as oxygen donors in cytochrome P-450 catalyzed steroid hydroxylations. *Biochemistry*, **18**, 865–870.
Gustafsson J-Å, Mode A, Norstedt G and Skett P (1983) Sex steroid induced changes in hepatic enzymes. *Annual Review of Physiology*, **45**, 51–60.
Halpert JR and Guengerich FP (1997) Enzyme inhibition and stimulation. In *Biotransformation, Volume 3, Comprehensive Toxicology*, Guengerich FP (ed.), Elsevier Science, Oxford, pp. 21–35.
Halpert JR and He Y (1993) Engineering of cytochrome P450 2B1 specificity: conversion of an androgen 16β-hydroxylase to a 15α-hydroxylase. *The Journal of Biological Chemistry*, **268**, 4453–4457.
Hammons GJ, Guengerich FP, Weis CC, Beland FA and Kadlubar FF (1985) Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. *Cancer Research*, **45**, 3578–3585.
Hankinson O (1995) The aryl hydrocarbon receptor complex. *Annual Review of Pharmacology and Toxicology*, **35**, 307.
Harlow GR and Halpert JR (1997) Alanine-scanning mutagenesis of a putative substrate recognition site in human cytochrome P450 3A4: role of residues 210 and 211 in flavonoid activation and substrate specificity. *The Journal of Biological Chemistry*, **272**, 5396–5402.
Harlow GR and Halpert JR (1998) Analysis of human cytochrome P450 3A4 cooperativity: construction and characterization of a site-directed mutant that displays hyperbolic steroid hydroxylation kinetics. *Proceedings of the National Academy of Sciences, USA*, **95**, 6636–6641.
Hasemann CA, Ravichandran KG, Peterson JA and Deisenhofer J (1994) Crystal structure and refinement of cytochrome $P450_{terp}$ at 2.3 Å resolution. *Journal of Molecular Biology*, **236**, 1169–1185.
Hasemann CA, Kurumbail RG, Boddupalli SS, Peterson JA and Deisenhofer J (1995) Structure and function of cytochromes P450: a comparative analysis of three crystal structures. *Structure*, **2**, 41–62.
Hasler JA, Harlow GR, Szlarz GD, John GH, Kedzie KM, Burnett VL, He YA, Kaminsky LS and Halpert JR (1994) Site-directed mutagenesis of putative substrate recognition sites in cytochrome P450 2B11: importance of amino acid residues 114, 290, and 363 for substrate specificity. *Molecular Pharmacology*, **46**, 338–345.
Haugen DA, van der Hoeven TA and Coon MJ (1975) Purified liver microsomal cytochrome P-450: separation and characterization of multiple forms. *The Journal of Biological Chemistry*, **250**, 3567–3570.
Helms V and Wade RC (1998) Hydration energy landscape of the active cavity in cytochrome $P450_{cam}$. *Proteins*, **32**, 381–396.
Hildebrandt A, Remmer H and Estabrook RW (1968) Cytochrome P-450 of liver microsomes: one pigment or many. *Biochemical and Biophysical Research Communications*, **30**, 607–612.
Honkakosski P, Zelko I, Sueyoshi T, Negishi M (1998) The nuclear orphan receptor CAR-RXR heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. *Molecular and Cellular Biology*, **18**, 1–7.
Hosea NA, Miller GP and Guengerich FP (2000) Elucidation of distinct binding sites for cytochrome P450 3A4. *Biochemistry*, **39**, 5929–5939.
Huang MT, Chang RL, Fortner JG and Conney AH (1981) Studies on the mechanism of activation of microsomal benzo[*a*]pyrene hydroxylation by flavonoids. *The Journal of Biological Chemistry*, **256**, 6829–6836.
Ibeanu GC, Ghanayem BI, Linko P, Li L, Pedersen LG and Goldstein JA (1996) Identification of residues 99, 220, and 221 of human cytochrome P450 2C19 as key determinants of omeprazole hydroxylase activity. *The Journal of Biological Chemistry*, **271**, 12496–12501.
Imai M, Shimada H, Watanabe Y, Matsushima-Hibiya Y, Makino R, Koga H, Horiuchi T and Ishimura Y (1989) Uncoupling of the cytochrome P-450cam monooxygenase reaction by a

single mutation, threonine-252 to alanine or valine: a possible role of the hydroxy amino acid in oxygen activation. *Proceedings of the National Academy of Sciences, USA*, **86**, 7823–7827.

Ishii-Ohba H, Guengerich FP and Baron J (1984) Localization of epoxide-metabolizing enzymes in rat testis. *Biochimica et Biophysica Acta*, **802**, 326–334.

Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, Ishizaki T, Green CE, Tyson CA and Sugiyama Y (1997) Prediction of *in vivo* drug metabolism in the human liver from *in vitro* metabolism data. *Pharmacology and Therapeutics*, **73**, 147–171.

Jin Y and Lipscomb JD (1999) Probing the mechanism of C-H activation: oxidation of methylcubane by soluble methane monooxygenase from *Methylosinus trichosporium* OB3b. *Biochemistry*, **38**, 6178–6186.

Johansson I, Eliasson E and Ingelman-Sundberg M (1991) Hormone controlled phosphorylation and degradation of CYP2B1 and CYP2E1 in isolated rat hepatocytes. *Biochemical and Biophysical Research Communications*, **174**, 37–42.

Johnson EF, Schwab GE and Dieter HH (1983) Allosteric regulation of the 16α-hydroxylation of progesterone as catalyzed by rabbit microsomal cytochrome P-450 3b. *The Journal of Biological Chemistry*, **258**, 2785–2788.

Johnson EF and Schwab GE (1984) Constitutive forms of rabbit-liver microsomal cytochrome P-450: enzymatic diversity, polymorphism and allosteric regulation. *Xenobiotica*, **14**, 3–18.

Johnson EF, Schwab GE and Vickery LE (1988) Positive effectors of the binding of an active site-directed amino steroid to rabbit cytochrome P-450 3c. *The Journal of Biological Chemistry*, **263**, 17672–17677.

Jones BC, Hawksworth G, Horne VA, Newlands A, Morsman J, Tute MS and Smith DA (1996) Putative active site template model for cytochrome P4502C9 (tolbutamide hydroxylase). *Drug Metabolism and Disposition*, **24**, 260–266.

Kagawa N and Waterman MR (1995) Regulation of steroidogenic and related P450s. In *Cytochrome* P450, 2nd edition, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 419–442.

Kalgutkar AS and Marnett LJ (1994) Rapid inactivation of prostaglandin endoperoxide synthases by *N*-(carboxyalkyl)maleimides. *Biochemistry*, **33**, 8625–8628.

Kato R and Kamataki T (1982) Cytochrome P-450 as a determinant of sex difference of drug metabolism in the rat. *Xenobiotica*, **12**, 787–800.

Kimata Y, Shimada H, Hirose T and Ishimura Y (1995) Role of Thr-252 in cytochrome $P450_{CAM}$: a study with unnatural amino acid mutagenesis. *Biochemical and Biophysical Research Communications*, **208**, 96–102.

Kimura S, Gonzalez FJ and Nebert DW (1984) Mouse cytochrome P3-450: complete cDNA and amino acid sequence. *Nucleic Acids Research*, **12**, 2917–2927.

Kivistö KT, Neuvonen PJ and Klotz U (1994) Inhibition of terfenadine metabolism: pharmacokinetic and pharmacodynamic consequences. *Clinical Pharmacokinetics*, **27**, 1–5.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Wilson TM, Zetterström RH, Perlmann T and Lehmann JM (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell*, **92**, 73–82.

Lee CA, Lillibridge JH, Nelson SD and Slattery JT (1996a) Effects of caffeine and theophylline on acetaminophen pharmacokinetics: P450 inhibition and activation. *Journal of Pharmacology and Experimental Therapeutics*, **277**, 287–291.

Lee SST, Buters JTM, Pineau T, Fernandez-Salguero P and Gonzalez FJ (1996b) Role of CYP2E1 in the hepatotoxicity of acetaminophen. *The Journal of Biological Chemistry*, **271**, 12063–12067.

Lewis DFV and Lake BG (1995) Molecular modelling of members of the P4502A subfamily: application to studies of enzyme specificity. *Xenobiotica*, **25**, 585–598.

Liang HCL, Li H, McKinnon RA, Duffy JJ, Potter SS, Puga A and Nebert DW (1996) *Cyp1a2*(-l-) null mutant mice develop normally but show deficient drug metabolism. *Proceedings of the National Academy of Sciences, USA*, **93**, 1671–1676.

Lichtenberger F, Nastainczyk W and Ullrich V (1976) Cytochrome P-450 as an oxene transferase. *Biochemical and Biophysical Research Communications*, **70**, 939–946.

Lindberg RLP and Negishi M (1989) Alteration of mouse cytochrome $P450_{coh}$ substrate specificity by mutation of a single amino-acid residue. *Nature*, **339**, 632–634.

Loeper J, Descatoire V, Maurice M, Beaune P, Belghiti J, Houssin D, Ballet F, Feldman G, Guengerich FP and Pessayre D (1993) Cytochromes P-450 in human hepatocyte plasma membrane: recognition by several autoantibodies. *Gastroenterology*, **104**, 203–216.

Loeper J, Le Berre A and Pompon D (1998) Topology inversion of CYP2D6 in the endoplasmic reticulum is not required for plasma membrane transport. *Molecular Pharmacology*, **53**, 408–414.

Lu AYH and Coon MJ (1968) Role of hemoprotein P-450 in fatty acid ω-hydroxylation in a soluble enzyme system from liver microsomes. *The Journal of Biological Chemistry*, **243**, 1331–1332.

Macdonald TL, Gutheim WG, Martin RB and Guengerich FP (1989) Oxidation of substituted *N*,*N*-dimethylanilines by cytochrome P-450: estimation of the effective oxidation-reduction potential of cytochrome P-450. *Biochemistry*, **28**, 2071–2077.

Martinis SA, Atkins WM, Stayton PS and Sligar SG (1989) A conserved residue of cytochrome P-450 is involved in heme-oxygen stability and activation. *Journal of the American Chemical Society*, **111**, 9252–9253.

McMurry TJ and Groves JT (1986) Metalloporphyrin models for cytochrome P-450. In *Cytochrome P-450*, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 1–28.

Medinsky MA, Asgharian B, Farris GM, Gonzalez FJ, Schlosser PM, Seaton MJ and Valentine JL (1997) Benzene risk assessment: transgenic animals and human variability. *Chemical Industry Institute of Toxicology Activities*, **17**, 1–5.

Miller VP, Crespi CL, Ackermann JM, Stresser DM and Busby WF, Jr. (1999) Novel high throughput fluorescent cytochrome P450 assays. *Proceedings, 7th European Meeting, International Society for the Study of Xenobiotics, Budapest, 22–26 August*, **14**, 63.

Mueller EJ, Loida PJ and Sligar SG (1995) Twenty-five years of $P450_{cam}$ research: mechanistic insights into oxygenase catalysis. In *Cytochrome P450*, 2nd edition, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 83–124.

Mueller GC and Miller JA (1948) The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. *The Journal of Biological Chemistry*, **176**, 535–544.

Mueller GC and Miller JA (1953) The metabolism of methylated aminoazo dyes. II. Oxidative demethylation by rat liver homogenates. *The Journal of Biological Chemistry*, **202**, 579–587.

Muerhoff AS, Griffin KJ and Johnson EF (1992) The peroxisome proliferator-activated receptor mediates the induction of CYP4A6, a cytochrome P450 fatty acid ω-hydroxylase, by clofibric acid. *The Journal of Biological Chemistry*, **267**, 19051–19053.

Naït-Kaoudjt R, Williams R, Guiard B and Gervais M (1997) Some DNA targets of the yeast CYP1 transcriptional activator are functionly asymmetric: evidence of two half-sites with different affinities. *European Journal of Biochemistry*, **244**, 301–309.

Nebert DW (1989) The *Ah* locus: genetic differences in toxicity, cancer, mutation, and birth defects. *CRC Critical Reviews in Toxicology*, **20**, 153–174.

Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR (1987) The P450 gene superfamily: recommended nomenclature. *DNA*, **6**, 1–11.

Nebert DW, Nelson DR, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips I, Sato R and Waterman M (1989) The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA*, **8**, 1–13.

Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K and Nebert DW (1993) The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA and Cell Biology*, **12**, 1–51.

Nelson SD (1994) Covalent binding to proteins. In *Methods in Toxicology, Volume 1B,* Academic Press, San Diego, pp. 340–348.

Neve EPA and Ingelman-Sundberg M (1999) A soluble NH_2-terminally truncated catalytically active form of cytochrome P450 2E1 targeted to liver mitochrondria. *FEBS Letters*, **460**, 309–314.

Newcomb M, Le Tadic-Biadatti MH, Chestney DL, Roberts ES and Hollenberg PF (1995a) A

nonsynchronous concerted mechanism for cytochrome P-450 catalyzed hydroxylation. *Journal of the American Chemical Society*, **117**, 12085–12091.

Newcomb M, Letadic MH, Putt DA and Hollenberg PF (1995b) An incredibly fast apparent oxygen rebound rate constant for hydrocarbon hydroxylation by cytochrome P-450 enzymes. *Journal of the American Chemical Society*, **117**, 3312–3313.

Newcomb M, Shen R, Choi SY, Toy PH, Hollenberg PF, Vaz ADN and Coon MJ (2000) Cytochrome P450-catalyzed hydroxylation of mechanistic probes that distinguish between radicals and cations. Evidence for cationic but not for radical intermediates. *Journal of the American Chemical Society*, **122**, 2677–2686.

Nims RW, McClain RM, Manchand PS, Belica PS, Thomas PE, Mellini DW, Utermahlen WE Jr and Lubet RA (1994) Comparative pharmacodynamics of hepatic cytochrome P450 2B induction by 5,5-diphenyl- and 5,5-diethyl-substituted barbiturates and hydantoins in the male F344/NCr rat. *Journal of Pharmacology and Experimental Therapeutics*, **270**, 348–355.

Northrop DB (1998) On the meaning of K_m and V/K in enzyme kinetics. *Journal of Chemical Education*, **75**, 1153–1157.

Okazaki O and Guengerich FP (1993) Evidence for specific base catalysis in *N*-dealkylation reactions catalyzed by cytochrome P450 and chloroperoxidase: differences in rates of deprotonation of aminium radicals as an explanation for high kinetic hydrogen isotope effects observed with peroxidases. *The Journal of Biological Chemistry*, **268**, 1546–1552.

Olsen JH, Boice JD Jr Jensen JPA and Fraumeni JF Jr (1989) Cancer among epileptic patients exposed to anticonvulsant drugs. *Journal of the National Cancer Institute*, **81**, 803–808.

Omura T and Sato R (1962) A new cytochrome in liver microsomes. *The Journal of Biological Chemistry*, **237**, 1375–1376.

Omura T and Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *The Journal of Biological Chemistry*, **239**, 2370–2378.

Ortiz de Montellano PR (1986) Oxygen activation and transfer. In *Cytochrome P-450*, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 217–271.

Ortiz de Montellano PR (1987) Control of the catalytic activity of prosthetic heme by the structure of hemoproteins. *Accounts of Chemical Research*, **20**, 289–294.

Ortiz de Montellano PR (1995a) *Cytochrome P450: Structure, Mechanism, and Biochemistry*, Plenum Press, New York, Ed. 2.

Ortiz de Montellano PR (1995b) Oxygen activation and reactivity. In *Cytochrome P450*, 2nd edition, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 245–303.

Ortiz de Montellano PR and Correia MA (1983) Suicidal destruction of cytochrome P-450 during oxidative drug metabolism. *Annual Review of Pharmacology and Toxicology*, **23**, 481–503.

Ortiz de Montellano PR and Reich NO (1986) Inhibition of cytochrome P-450 enzymes. In *Cytochrome P-450*, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 273–314.

Ortiz de Montellano PR, Kunze KL, Yost GS and Mico BA (1979) Self-catalyzed destruction of cytochrome P-450: covalent binding of ethynyl sterols to prosthetic heme. *Proceedings of the National Academy of Sciences, USA*, **76**, 746–749.

Osawa Y and Pohl LR (1989) Covalent bonding of the prosthetic heme to protein: a potential mechanism for the suicide inactivation or activation of hemoproteins. *Chemical Research in Toxicology*, **2**, 131–141.

Park S, Shimizu H, Adachi S, Shiro Y, Iizuka T, Nakagawa A, Tanaka I, Shoun H and Hori H (1997) Crystallization, preliminary diffraction and electron paramagnetic resonance studies of a single crystal of cytochrome P450nor. *FEBS Letters*, **412**, 346–350.

Paulsen MD and Ornstein RL (1993) Substrate mobility in thiocamphor-bound cytochrome $P450_{cam}$: an explanation of the conflict between the observed product profile and the X-ray structure. *Protein Engineering*, **6**, 359–365.

Peng HM, Raner GM, Vaz ADN and Coon MJ (1995) Oxidative cleavage of esters and amides to carbonyl products by cytochrome P450. *Archives of Biochemistry and Biophysics*, **318**, 333–339.

Peterson JA and Prough RA (1986) Cytochrome P-450 reductase and cytochrome b_5 in cytochrome P-450 catalysis. In *Cytochrome P-450*, Ortiz de Montellano PR (ed.), Plenum Press, New York, pp. 89–117.

Pitot HC (1995) The role of receptors in multistage carcinogenesis. *Mutation Research*, **333**, 3–14.

Pompon D and Coon MJ (1984) On the mechanism of action of cytochrome P-450: oxidation and reduction of the ferrous dioxygen complex of liver microsomal cytochrome P-450 by cytochrome b_5. *The Journal of Biological Chemistry*, **259**, 15377–15385.

Poulos TL (1991) Modeling of mammalian P450s on the basis of $P450_{cam}$ X-ray structure. *Methods in Enzymology*, **206**, 11–30.

Poulos TL, Finzel BC and Howard AJ (1987) High-resolution crystal structure of cytochrome $P450_{cam}$. *Journal of Molecular Biology*, **195**, 687–700.

Poulos TL, Finzel BC and Howard AJ (1995) Crystal structure of substrate-free *Pseudomonas putida* cytochrome P-450. *Biochemistry*, **25**, 5314–5322.

Ram PA, Park SH, Choi HK and Waxman DJ (1996) Growth hormone activation of Stat 1, Stat 3, and Stat 5 in rat liver: differential kinetics of hormone desensitization and growth hormone stimulation of both tyrosine phosphorylation and serine/threonine phosphorylation. *The Journal of Biological Chemistry*, **271**, 5929–5940.

Ravichandran KG, Boddupalli SS, Hasemann CA, Peterson JA and Deisenhofer J (1993) Crystal structure of hemoprotein domain of P450 BM-3, a prototype for microsomal P450's. *Science*, **261**, 731–736.

Remmer H (1957) The acceleration of evipan oxidation and the demethylation of methylaminopyrine by barbiturates. *Naunyn–Schmiedeberg's Archive für experimentiel Pathologie und Pharmakologie*, **237**, 296–307.

Renton KW and Knickle LC (1990) Regulation of hepatic cytochrome P-450 during infectious disease. *Canadian Journal of Physiology and Pharmacology*, **68**, 777–781.

Renton KW, Keyler DE and Mannering GJ (1979) Suppression of the inductive effects of phenobarbital and 3-methylcholanthrene on ascorbic acid synthesis and hepatic cytochrome P-450-linked monooxygenase systems by the interferon inducers, poly rI-rC and tilorone. *Biochemical and Biophysical Research Communications*, **88**, 1017–1023.

Richardson HL, Stier AR and Borsos-Nachtnebel E (1952) Liver tumor inhibition and adrenal histologic responses in rats to which 3′-methyl-4-dimethylaminoazobenzene and 20-methylcholanthrene were simultaneously administered. *Cancer Research*, **12**, 356–361.

Rivera M, Seetharaman R, Girdhar D, Wirtz M, Zhang XJ, Wang XQ and White S (1998) The reduction potential of cytochrome b_5 is modulated by its exposed heme edge. *Biochemistry*, **37**, 1485–1494.

Roberts ES, Hopkins NE, Foroozesh M, Alworth WL, Halpert JR and Hollenberg PF (1997) Inactivation of cytochrome P450s 2B1, 2B4, 2B6, and 2B11 by arylalkynes. *Drug Metabolism and Disposition*, **25**, 1242–1248.

Ryan D, Lu AYH and Levin W (1978) Purification of cytochrome P-450 and P-448 from rat liver microsomes. *Methods in Enzymology*, **52**, 117–123.

Sadano H and Omura T (1983a) Turnover of two drug-inducible forms of microsomal cytochrome P-450 in rat liver. *Journal of Biochemistry*, **93**, 1375–1383.

Sadano H and Omura T (1983b) Reversible transfer of heme between different molecular species of microsome-bound cytochrome P-450 in rat liver. *Biochemical and Biophysical Research Communications*, **116**, 1013–1019.

Sadano H and Omura T (1985) Incorporation of heme to microsomal cytochrome P-450 in the absence of protein biosynthesis. *Journal of Biochemistry*, **98**, 1321–1331.

Sahali-Sahly Y, Balani SK, Lin JH and Baillie TA (1996) In vitro studies on the metabolic activation of the furanopyridine L-754,394, a highly potent and selective mechanism-base inhibitor of cytochrome P450 3A4. *Chemical Research in Toxicology*, **9**, 1007–1012.

Sato H and Guengerich FP (2000) Oxidation of 1,2,4,5-tetramethoxybenzene to a radical cation by cytochrome P450. *Journal of the American Chemical Society*, submitted.

Schlichting I, Berendzen J, Chu K, Stock AM, Maves SA, Benson DE, Sweet BM, Ringe D, Petsko GA and Sligar SG (2000) The catalytic pathway of cytochrome $P450_{cam}$ at atomic resolution. *Science*, **287**, 1615–1622.

Schmid R (1973) Synthesis and degradation of microsomal hemoproteins. *Drug Metabolism and Disposition*, **1**, 256–258.

Segel IH (1975) *Enzyme Kinetics*, Wiley, New York.

Seto Y and Guengerich FP (1993) Partitioning between *N*-dealkylation and *N*-oxygenation in the oxidation of *N,N*-dialkylarylamines catalyzed by cytochrome P450 2B1. *The Journal of Biological Chemistry*, **268**, 9986–9997.

Shimada T, Yamazaki H, Foroozesch M, Hopkins NE, Alworth WL and Guengerich FP (1998) Selectivity of polycyclic inhibitors for human cytochromes P450 1A1, 1A2, and 1B1. *Chemical Research in Toxicology*, **11**, 1048–1056.

Shiraki H and Guengerich FP (1984) Turnover of membrane proteins: kinetics of induction and degradation of seven forms of rat liver microsomal cytochrome P-450, NADPH-cytochrome P-450 reductase, and epoxide hydrolase. *Archives of Biochemistry and Biophysics*, **235**, 86–96.

Shou M, Mei Q,ettore MW, Jr., Dai R, Baillie TA and Rushmore TH (1999) Sigmoidal kinetic model for two co-operative substrate-binding sites in a cytochrome P450 3A4 active site: an example of the metabolism of diazepam and its derivatives. *Biochemical Journal*, **340**, 845–853.

Silverman RB (1995) Mechanism-based enzyme inactivators. *Methods in Enzymology*, **249**, 240–283.

Sladek NE and Mannering GJ (1969) Induction of drug metabolism. II. Qualitative differences in the microsomal N-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital. *Molecular Pharmacology*, **5**, 186–199.

Stearns RA and Ortiz de Montellano PR (1985) Cytochrome P-450 catalyzed oxidation of quadricyclane. Evidence for a radical cation intermediate. *Journal of the American Chemical Society*, **107**, 4081–4082.

Steward AR, Wrighton SA, Pasco DS, Fagan JB, Li D and Guzelian PS (1985) Synthesis and degradation of 3-methylcholanthrene-inducible cytochromes P-450 and their mRNAs in primary monolayer cultures of adult rat hepatocytes. *Archives of Biochemistry and Biophysics*, **241**, 494–508.

Strobel HW and Coon MJ (1971) Effect of superoxide generation and dismutation on hydroxylation reactions catalyzed by liver microsomal cytochrome P-450. *The Journal of Biological Chemistry*, **246**, 7826–7829.

Tephly TR, Black KA, Green MD, Coffman BL, Dannan GA and Guengerich FP (1986) Effect of the suicide substrate 3,5-diethoxycarbonyl-2,6-dimethyl-4-ethyl-1,4-dihydropyridine on the metabolism of xenobiotics and on cytochrome P-450 apoproteins. *Molecular Pharmacology*, **29**, 81–87.

Thomas PE, Lu AYH, West SB, Ryan D, Miwa GT and Levin W (1977) Accessibility of cytochrome P450 in microsomal membranes: inhibition of metabolism by antibodies to cytochrome P450. *Molecular Pharmacology*, **13**, 819–831.

Tierney DJ, Haas AL and Koop DR (1992) Degradation of cytochrome P450 2E1: selective loss after labilization of the enzyme. *Archives of Biochemistry and Biophysics*, **293**, 9–16.

Ullrich V (1969) On the hydroxylation of cyclohexane in rat liver microsomes. *Hoppe–Seyler's Zeitschrift für Physiologische Chemie*, **350**, 357–365.

Ullrich V (1972) Enzymatic hydroxylations with molecular oxygen. *Angewandte Chemie—International Edition in English*, **11**, 701–712.

Ullrich V, Castle L and Haurand M (1982) Cytochrome P-450 as an oxene transferase. In *Oxygenases and Oxygen Metabolism*, Academic Press, New York, pp. 497–509.

Vanden Bossche H, Moereels H and Koymans LMH (1994) Aromatase inhibitors—mechanisms for non-steroidal inhibitors. *Breast Cancer Research and Treatment*, **30**, 43–55.

Vaz ADN, McGinnity DF and Coon MJ (1998) Epoxidation of olefins by cytochrome P450: evidence from site-specific mutagenesis by hydroperoxo-iron as an electrophilic oxidant. *Proceedings of the National Academy of Sciences USA*, **95**, 3555–3560.

Vermilion JL and Coon MJ (1978) Identification of the high and low potential flavins of liver microsomal NADPH-cytochrome P-450 reductase. *The Journal of Biological Chemistry*, **253**, 8812–8819.

Vermilion JL, Ballou DP, Massey V and Coon MJ (1981) Separate roles for FMN and FAD in catalysis by liver microsomal NADPH-cytochrome P-450 reductase. *The Journal of Biological Chemistry*, **256**, 266–277.

Walsh C (1979) *Enzymatic Reaction Mechanisms*, W. H. Freeman, San Francisco, pp. 34–35.

Watanabe Y, Oae S and Iyanagi T (1982) Mechanisms of enzymatic *S*-oxygenation of thioanisole derivatives and *O*-demethylation of anisole derivatives promoted by both microsomes and a reconstituted system with purified cytochrome P-450. *Bulletin of the Chemical Society of Japan*, **55**, 188–195.

Waterman MR and Guengerich FP (1997) Enzyme regulation. In *Biotransformation, Volume 3, Comprehensive Toxicology*, Guengerich FP (ed.), Elsevier Science, Oxford, pp. 7–14.

Waterman MR, Jenkins CM and Pikuleva I (1995) Genetically engineered bacterial cells and applications. *Toxicology Letters*, **82/83**, 807–813.

Waxman DJ, Dannan GA and Guengerich FP (1985) Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry*, **24**, 4409–4417.

White RE and Coon MJ (1980) Oxygen activation by cytochrome P-450. *Annual Review of Biochemistry*, **49**, 315–356.

White RE, Miller JP, Favreau LV and Bhattacharyya A (1986) Stereochemical dynamics of aliphatic hydroxylation by cytochrome P-450. *Journal of the American Chemical Society*, **108**, 6024–6031.

Williams PA, Cosme J, Sridhar V, Johnson EF and MeRee DE (2000) Mammalian microsomal cytochrome P450 monooxygenase: structural adaptations for membrane binding and functional diversity. *Molecular Cell*, **5**, 121–131.

Wislocki PG, Miwa GT and Lu AYH (1980) Reactions catalyzed by the cytochrome P-450 system. In *Enzymatic Basis of Detoxication, Volume 1,* Jakoby WB (ed.), Academic Press, New York, pp. 135–182.

Witherow LE and Houston JB (1998) Sigmoidal kinetics of CYP3A4 substrates: an approach for scaling dextromethorphan metabolism in rat microsomes and isolated hepatocytes to predict *in vivo* clearance. *Proceedings of the International Society for the Study of Xenobiotics*, **12**, 26.

Yamazaki H, Johnson WW, Ueng Y-F, Shimada T and Guengerich FP (1996) Lack of electron transfer from cytochrome b_5 in stimulation of catalytic activities of cytochrome P450 3A4: characterization of a reconstituted cytochrome P450 3A4/NADPH-cytochrome P450 reductase system and studies with apo-cytochrome b_5. *The Journal of Biological Chemistry*, **271**, 27438–27444.

Yamazoe Y, Sugiura M, Kamataki T and Kato R (1978) Reconstitution of benzo[*a*]pyrene 4,5-oxide reductase activity by purified cytochrome P-450. *FEBS Letters*, **88**, 337–340.

Yoo JSH, Cheung RJ, Patten CJ, Wade D and Yang CS (1987) Nature of *N*-nitrosodimethylamine demethylase and its inhibitors. *Cancer Research*, **47**, 3378–3383.

Yun C-H, Miller GP and Guengerich FP (2000) Rate-determining steps in phenacetin oxidations by human cytochrome P450 1A2 and selected mutants. *Biochemistry*, submitted.

Zangar RC and Novak RF (1998) Posttranslational elevation of cytochrome P450 3A levels and activity by dimethyl sulfoxide. *Archives of Biochemistry and Biophysics*, **353**, 1–9.