

1

Diversities and Similarities in P450 Systems: An Introduction

Mary A. Schuler¹ and Stephen G. Sligar²

¹Department of Cell and Developmental Biology, University of Illinois,
Urbana, IL 61801, USA
<maryschu@uiuc.edu>

²Department of Biochemistry, University of Illinois,
Urbana, IL 61801, USA
<s-sligar@uiuc.edu>

1. OXYGENASES: MEDIATORS OF BIOCHEMICAL DIVERSITY	2
2. P450 SUPERFAMILY: DIVERSITY AT THE SEQUENCE LEVEL	3
3. DIVERSITY OF P450 STRUCTURES: FOLDS AND CONFORMATIONS FOR FUNCTIONS	5
4. DIVERSITY IN P450 MECHANISMS	6
4.1. Diversity of Redox Partners	6
4.2. The Heme-Oxygen Catalytic Landscape	9
4.3. The Oxy and Peroxo Iron Intermediates	10
4.4. High-Valent Metal-Oxo Complexes	11
4.5. Uncoupling: Nature's Leakage Pathways	12
4.6. Other Heme-Thiolate Systems: Needs from a Mechanistic Viewpoint	12
5. DIVERSITY IN REGULATION ACROSS THE SUPERFAMILY	13
5.1. Transcriptional Regulation	13
5.2. Post-translational Regulation	15
6. DIVERSITY IN THE EVOLUTION OF COMMON METABOLIC FUNCTIONS	16
6.1. Hormone Biosynthesis	16
6.2. Xenobiotic Catabolism	17
6.3. Fatty Acid Hydroxylases: Bacteria to Mammals to Plants	17

7. SUMMARY AND OUTLOOK	18
ACKNOWLEDGMENTS	19
ABBREVIATIONS	19
REFERENCES	19

1. OXYGENASES: MEDIATORS OF BIOCHEMICAL DIVERSITY

The introduction of oxygen into biochemical processes has had a profound effect on the evolution of life. An appreciation for this traumatic event was presented in a beautiful recent review on the linkages of gene development that occurred at this juncture [1]. Although the first important utilization of atmospheric dioxygen was perhaps through its use as a terminal electron acceptor in metabolic energy conversion, an equally important leap in complexity and diversity was appreciated when, in the mid-1950s, Osamu Hayaishi and Howard Mason discovered the oxygenases [2,3]. This advance changed the simplistic view of how Nature uses atmospheric dioxygen from that of a simple electron acceptor and pointed to the rich metabolic diversity allowed by the incorporation of atmospheric dioxygen into substrate molecules. The discovery, naming and mechanistic understanding of the first ‘oxygenase’ enzymes have provided wonderful opportunities and scientific impetus to understand the great diversity of these systems in the synthesis and catabolism of organic molecules.

Before describing their various levels of diversity, one must consider the prime biochemical similarity that categorizes nearly all of them within the class of ‘oxidases’ that use atmospheric dioxygen as a terminal electron acceptor and, hence, yield an oxidized substrate molecule. In technical terms, the ‘oxygenases’ that exist within this broader oxidase category are classed as ‘mono-’ or ‘di-’, depending on whether one or both atoms of atmospheric dioxygen are incorporated into their respective substrates. In their reaction cycles, the classic stoichiometry of monooxygenases represents a sort of ‘half-way’ point on the pathway for the full four-electron reduction of dioxygen to generate two molecules of water as is typical of the redox counting of cytochrome ‘oxidases’. Positioned midstream in this pathway, monooxygenases require only two electrons and two protons to reductively cleave atmospheric dioxygen, producing only a single water molecule in the process while saving the second atom for the incorporation and formal oxidation of the organic substrate molecule. As a result of their dual functionality, cytochrome P450 monooxygenases (P450s) are often referred to as ‘mixed-function oxidases’ since they possess both ‘oxygenase’ and ‘oxidase’ reactivities. The electron transfer functionalities of P450s also earned them the label of ‘cytochrome’.

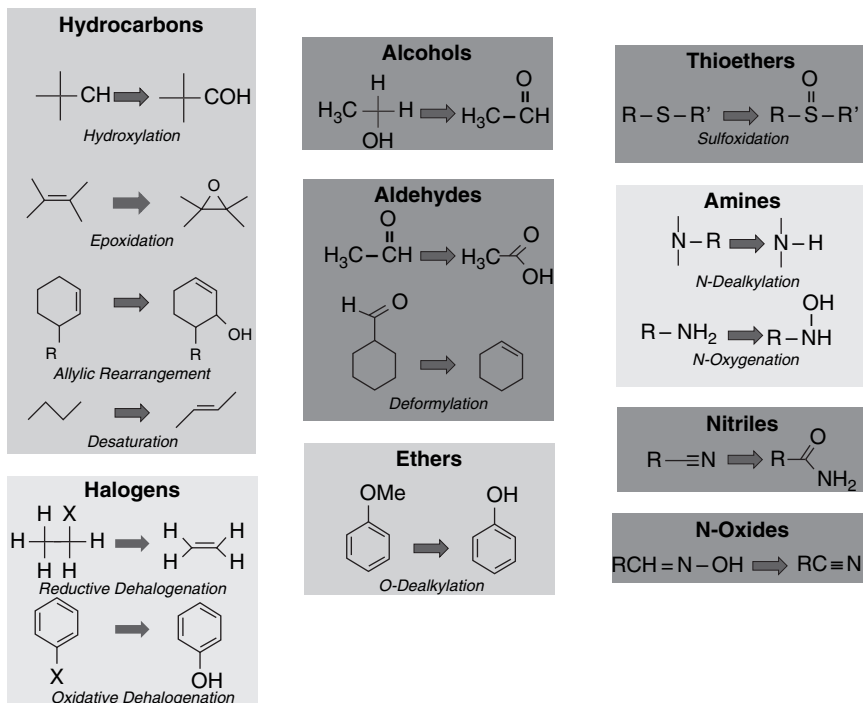


Figure 1. P450 chemistries.

This volume presents the breadth of research efforts focused on cytochrome P450 monooxygenases in their fullest, from structure through function to a deep appreciation of the diversity and complexity of the biotransformations that they catalyze (Figure 1). Historical perspectives on oxygenase discoveries over the past 50 years and mechanistic descriptions of their reaction cycles and metabolic transformations have been the subject of many other recent reviews [4–9]. Here, we focus on exploring the many different levels at which these enzymes (and their respective genes) have diverged in the process of evolution to yield the plethora of enzymes that are now termed ‘P450 monooxygenases’, even though they mediate a multitude of diverse reactions.

2. P450 SUPERFAMILY: DIVERSITY AT THE SEQUENCE LEVEL

Several years ago, realizing how many might be in store for future characterizations, researchers devised a nomenclature system for cytochrome monooxygenases (P450s) that designated sequences based on their degree of primary amino acid

sequence identity [10,11]. In this system, the most highly related monooxygenase proteins were grouped into families whose members shared greater than 40% amino acid identity and designated with numbers (CYP1, CYP2, etc.) following the CYP (Cytochrome P450) designator used for all of these sequences. Families were divided into subfamilies whose members shared greater than 55% amino acid identity and designated with alphabetical characters (A, B, C, etc.). These subfamilies were further subdivided into individual loci designated with an additional set of numbers (CYP1A1, CYP1A2, CYP1A3, etc.). Without definitive genomic information demonstrating the existence of individual P450 loci, P450 sequences sharing more than 97% amino acid identity were designated as allelic variants with additional sets of numbers (v1, v2, etc.) [10,11]. The nearly universal acceptance of this highly structured nomenclature system provided an understandable index of the sequence relationships between the proteins found within a species as well as between the proteins found in the different kingdoms.

Stepping forward to the present, it is now widely appreciated that P450s exist in many bacteria and all archaea, fungi, and higher eukaryotes whose genomic DNA sequences have been completed. The numbers of full-length P450 genes existing in these species vary substantially in bacterial species from one in many species to 18–33 in some streptomycetes and 20–40 in some mycobacteria [12]. The numbers of full-length genes expand further in eukaryotic species, varying from three full-length P450 open reading frames (ORFs) in the *Saccharomyces cerevisiae* (budding yeast) genome to 34 in the *Chlamydomonas reinhardtii* genome, 46 in the *Apis mellifera* (honeybee) genome, 55 in the human genome, 71 in the *Physcomitrella patens* (moss) genome, 83 in the *Drosophila melanogaster* (fruitfly) genome, 100 in the *Anopheles gambiae* (mosquito) genome, 80 in the *Caenorhabditis elegans* (nematode) genome [11,13–16] (<http://drnelson.utm.edu>, <http://p450.antibes.inra.fr>), 246 in the *Arabidopsis thaliana* genome [17–19], (<http://Arabidopsis-P450.biotech.uiuc.edu>, <http://www.biobase.dk/P450/>), and 356 in the *Oryza sativa* (rice) genome [13]. Multiply the number of organisms containing P450s by the number of full-length genes in any of them and there are indeed a large number of sequences within the phylogenetically diverse P450 superfamily. Present counts as of May 2006 include more than 5100 sequences (not counting alleles) in this massive superfamily (<http://drnelson.utm.edu>). In some of the smaller plant genomes containing little repetitive DNA, they are estimated to represent 0.6% of the genome.

Genome-wide comparisons in some of these organisms with high P450 gene copy numbers have indicated that the degree of duplication and divergence in different P450 families and clans (their associated larger family groupings) are not constant. With the CYP51 family involved in sterol biosynthesis representing the only family common to fungi, animals, and plants [12,13,20,21], members of the plant-specific CYP71 family have proliferated to 52 members in *Arabidopsis* and 90 members in rice and members of the insect/animal-specific CYP4 family

have proliferated from 4 members in the honeybee to 11 members in humans and 45 members in the mosquito (<http://drnelson.utmem.edu>; <http://Arabidopsis-P450.biotech.uiuc.edu>; <http://p450.antibes.inra.fr>). With P450 clans designated according to their family members with the lowest numeral, there are clearly some among the ten clans (CYP71) that have expanded substantially in plants compared with those that exist in animals.

With amino acid sequence identity representing one level of comparison among these many sequences, the organization of genes (as defined by the number and position of intron–exon junctions) within P450 families and subfamilies often supports the evolutionary relationships defined first by comparisons of these protein sequences. As an example of this organizational conservation, many of the 35 members of the *Arabidopsis* CYP71B subfamily have a single intron at the same place in their coding sequence, some have two introns at the same place and just two have no apparent introns (<http://www.p450.kvl.dk>; <http://arabidopsis-p450.biotech.uiuc.edu>). Scattered throughout the genome in clusters of duplicated P450 genes, all but one of the CYP71B loci with two introns are present within one tandem cluster while other clusters contain CYP71B loci with one intron. The same is true of the CYP71A subfamily where each in a cluster of six genes contains a single intron, each in three other sets of genes contain three introns and one more divergent gene contains four introns.

3. DIVERSITY OF P450 STRUCTURES: FOLDS AND CONFORMATIONS FOR FUNCTIONS

Despite these sequence diversities, P450 in many different families and many different organisms share a high degree of structural conservation in their secondary and tertiary folds [22–25] (see Chapter 3 in this volume). If one were to look at ribbon diagrams for the known P450 structures from across the room, all could immediately recognize the protein as belonging to the P450 superfamily. Moving closer, however, subtle variation in the positioning of secondary structure elements and the lengths of interconnecting loop regions contribute to the rich diversity of their catalytic sites and resulting specificities. Their commonality is manifested in a core structure of eleven α -helices (labeled A–K) and β -pleated sheets (labeled 1–4) surrounding the generally hydrophobic catalytic site buried within each protein. Variations in the lengths of the regions making up these core structures and in their intervening loops allow for these elements of secondary structure to create a diversity of three-dimensional active site structures. But, most important in our considerations of P450 catalytic site diversity is the fact that, within this core structure, comparatively small segments of the protein are involved in contacting the substrate and in the catalytic reaction cycle. These more limited regions include the loop between the B- and C-helices positioned over the heme (substrate recognition site 1 or SRS1 as originally

described by Gotoh [26], the I-helix extending over the heme pyrrole ring B (SRS4), the amino-terminus of β -sheet 1–4 (SRS5) and the β -turn at the end of β -sheet 4 (SRS6). While quite variable in their individual sequences, alignments of 45 sequences representing each of the P450 subfamilies existing in *Arabidopsis* have indicated that no significant length variations exist in these internalized SRS regions [27]. Instead, some of the most prominent length variations occur in the previously mentioned α -helices and β -pleated sheets as well as external loop sequences and the loop between the F- and G-helices (between SRS2 and SRS3) that is involved in defining the substrate access and/or interactions with the endoplasmic reticulum membrane.

Alignments of the 20 crystal structures currently available for bacterial, fungal and mammalian P450s (listed in Chapter 3 of this volume [25]) have indicated that the most significant variations in backbone structure occur in three regions that have been designated as the B region (the loop between strand 5 of β -sheet 1, B'-helix, B-helix and B-C loop), the FG region (the C-terminus of the F-helix, the F-G loop and the N-terminus of the G-helix) and the β 4 region (β -sheet 4) [28]. Structural backbone variations as well as side chain variations in these three regions as well as side chain variations in the previously mentioned SRS4 and SRS5 regions are the most likely contributors to diversity in substrate specificity among the P450s.

Many examples of the specificity differences conferred by very small variations in these SRS regions now exist in the naturally occurring differences between two closely related P450 proteins and in synthetically generated variations within a single P450. Examples of naturally occurring variations that lead to variations in substrate specificity are the mouse CYP2A4 and CYP2A5 sequences that mediate testosterone and coumarin hydroxylations, respectively, as the result of a limited number of amino acid variations in the SRS1, SRS2, SRS5, and SRS6 regions [29,30]. Other examples are the spearmint CYP71D15 and peppermint CYP71D18 sequences that differentiate between C6 and C3 limonene hydroxylations, respectively, based on a single amino acid variation in SRS5 (between the K helix and β 1–4 strand) [31]. Examples of synthetically generated mutations that lead to variations in the substrate specificity of vertebrate and plant P450s are covered in several recent reviews [27,32,33].

4. DIVERSITY IN P450 MECHANISMS

4.1. Diversity of Redox Partners

The diversity of the P450 reactions can also be classified by the nature of the redox partners that introduce the two electrons required for the oxygenation of substrate. The systems carrying out this electron transfer reaction have been beautifully reviewed by Peterson and others [34–39]. Two major systems exist

for those P450s that require an external feed from pyridine nucleotide. One of these utilizes a two-component electron transfer complex consisting of simplified FAD dehydrogenase and a small two-iron, two-sulfur redoxin to carry out the coupling of two-electron transfer to the sequential input of two redox equivalents needed by the P450 heme component [40]. The other utilizes a more complex flavoprotein, again with a FAD functioning as a hydride transfer catalyst interfacing with reduced pyridine nucleotide, but in this case, using a FMN prosthetic group cycling through a semiquinone to function as the two-to-one electron transformer.

In some bacterial systems, such as CYP102 (P450BM3) from *Bacillus megaterium*, the diflavin and heme catalytic domains are found linked into a single polypeptide [41]. Regardless of the nature of coupling to pyridine nucleotide, the P450 cycle needs a single electron to reduce the protein so that atmospheric dioxygen can bind and form the ferrous dioxygen complex. This intermediate was characterized first in the microbial P450CAM (CYP101) protein by Peterson, Gunsalus, and colleagues in the early 1970s [42,43] and has more recently been stabilized in the human CYP3A4 protein through incorporation into nanoscale, soluble phospholipid bilayers [44]. Interestingly, recent work from the Ortiz de Montellano laboratory has revealed further diversity in the provision of electron input in the case of CYP119 from the thermophilic *Sulfolobus solfataricus* [45]. Here, in addition to a temperature-stable iron-sulfur protein, a novel 2-oxoacid-ferredoxin oxidoreductase that utilizes pyruvic acid rather than NAD(P)H as the source of reducing equivalents couples to this system. In the future, one may expect to see additional variations in the mechanisms of providing the electrons needed for the classic P450 reaction cycle.

As reviewed in McLean et al. [46], additional diversity exists in some systems that do not use atmospheric dioxygen and two electrons as co-substrates and instead use the reduced dioxygen product, peroxide, as input to provide both the oxygen nucleus and the redox equivalents. One of the more unusual bacterial P450s in this category is *Bacillus subtilis* CYP152A1 that catalyzes hydroxylation of its long-chain fatty acid substrates directly using hydrogen peroxide [47]. Another is *Fusarium oxysporum* CYP55A1 (P450NOR) that catalyzes the hydroxylation of nitric oxide into nitric oxide by reduction of the P450 with NADH [48]. Some of the unusual eukaryotic P450s in this category are those in the plant CYP74A subfamily (allene oxide synthases) in which a hydroperoxide in the substrate is subsequently rearranged to form a reactive allene oxide that is subsequently converted to jasmonic acid [49,50]. Others in this unusual category are the plant CYP74B subfamily proteins (hydroperoxide lyases) and CYP74D subfamily proteins (divinyl ether synthases) that break down 13- and 9-carbon fatty acid hydroperoxides, respectively, into shorter signaling molecules and fungal defense compounds [51–53] and the mammalian CYP5A1 protein (thromboxane synthases) that catalyzes the isomerization of prostaglandin H₂, yielding thromboxane A₂ [54]. Interestingly, nonsteroidal anti-inflammatory

drugs (NSAID) that interfere with the cyclooxygenase activity of prostaglandin endoperoxide H synthase (PGHS) and subsequent production of prostaglandins in mammals also competitively block allene oxide synthases and the subsequent production of jasmonic acid in plants [55].

In organellar compartments, such as animal mitochondria and plant chloroplasts, the electron transfer components are most similar to the bacterial FAD dehydrogenases and two-iron, two-sulfur redoxins. In the inner mitochondrial membranes of mammalian cells, adrenodoxin (Adx) and NADPH-dependent adrenodoxin reductase (AdR) provide electrons to CYP11A and CYP11B proteins, which are involved in cholesterol side chain cleavage and modification, and other P450s localized within the mitochondria [37]. In *Schizosaccharomyces pombe* (fission yeast), an iron sulfur protein (etp1) shares enough structural similarity with mammalian AdR that it can substitute for its activity in heterologous expression systems [56]. The pathogenic bacterium, *Mycobacterium tuberculosis*, also contains an electron transfer component (FprA) that is chemically and structurally related to mammalian AdR [57,58]. In plant chloroplasts, ferredoxin (Fd) and NADPH-dependent ferredoxin reductase (FNR), which are electron transfer components of the photosynthetic electron transfer chain [59], provide electrons to P450s normally localized within this organelle as well as heterologous plant, bacterial and mammalian P450s targeted to this organelle by genetic engineering [60,61]. In several unusual cases, some bacteria utilizing ferredoxin-like proteins have them fused in-frame with P450 coding sequences allowing them to attain optimal electron transfer rates within a single protein. Examples of this include a *Methylococcus capsulatus* CYP51 protein that is fused to a 3Fe-4S ferredoxin and a *Rhodococcus* sp. CYP116B2 that is fused to a dioxygenase reductase and a 2Fe-2S ferredoxin center [62–64].

In the cytosolic compartments of vertebrate, insect and plant cells, membrane-bound P450s found in the endoplasmic reticulum utilize NADPH-dependent P450 reductases and, sometimes, NADH-dependent cytochrome b_5 reductase/cytochrome b_5 complexes. Compared with the diversity of P450s in these organisms, their electron transfer partners are few in number and reasonably well conserved. In the organisms where complete genomic sequences are available, P450 reductase is encoded by one gene in the human, *C. elegans* (nematode), *A. gambiae* (mosquito), and *D. melanogaster* genomes and three genes in the *A. mellifera* (honeybee) genome (Genbank accessions). For reasons that are as yet unclear, multiple NADPH-dependent P450 reductase genes exist in most higher plant genomes with two identified in the sequenced *A. thaliana* and *Oryza sativa* (rice) genomes and two and three identified in *Helianthus tuberosus* (artichoke) and *Populus* sp. (poplar) cDNA collections, respectively [65,66]. Sequence comparisons among these indicate that the *Musca domestica* (housefly) P450 reductase most commonly used for heterologous expression in insect cell systems is 84% identical to *D. melanogaster* P450 reductase, 76% identical to *A. gambiae* P450 reductase and 66% identical to *Bombyx mori*

(silkworm) P450 reductase and 55–56% identical to vertebrate P450 reductases. In comparison, the *Arabidopsis* P450 reductases are 61% identical to one another, 65–70% identical to the artichoke P450 reductases, 55–66% identical to the rice P450 reductases and 40–41% to vertebrate and fruitfly P450 reductases; the rice P450 reductases share approximately the same degree of relatedness to each other (63% identical) as the *Arabidopsis* P450 reductases share to one another. Cytochrome b_5 and NADH-dependent cytochrome b_5 reductase genes in these organisms have similarly low copy numbers in the vertebrate and insect genomes, higher copy numbers in plant genomes and high degrees of conservation among all.

Relevant to some of the post-translational regulatory mechanisms moderating P450 activities discussed in post-translational regulation (Section 5.2.), several of these electron transfer partners are subject to phosphorylation events that can modulate their activities.

4.2. The Heme-Oxygen Catalytic Landscape

Many very recent review articles have appeared which document the development of the current understanding of the P450 monooxygenase catalytic mechanism [7,67,68]. The most typical discussion of the reaction cycles of the cytochrome P450s utilizes a cyclic reaction path that begins with the protein substrate-free and the heme iron in the ferric state with the five d-electrons in a low spin ($S = 1/2$) configuration. This low-spin state is formed due to a large ligand field contributed by the axial thiolate ‘fifth’ ligand contributed by a cysteine residue and an axially coordinated ‘sixth’ water molecule.

The overall goal of any mechanistic understanding is the description of the intermediate states of heme, oxygen and substrate as well as the electrons/protons needed to link these states into a reaction cycle. As described earlier, the diversity of catalytic specificities in P450s exists because of the variations in particular sets of active site residues that have allowed these enzymes to evolve with varying degrees of plasticity. In some cases, such as the enzymes involved in the regiospecific hormone oxygenations occurring in humans, insects and plants, the catalytic site provides for a great deal of specificity and selectivity. In other cases, such as the human hepatic and insect midgut enzymes involved in xenobiotic detoxifications, the catalytic sites appear to have selectivities for broad classes of compounds, but are rather promiscuous with respect to the organic structures within particular classes. In both cases, however, it now appears that a complementarity between substrate and the active site geometry, as well as any linked induced conformational changes permitted, can result in displacement of the axial water with a resultant weakening of the ligand field and conversion of the protein to the high spin ($S = 5/2$) electronic configuration. This complementarity of substrate and active site displacing the water heme ligand has a substantial functional significance. Since the next step in the reaction cycle

involves a ferric–ferrous reduction of the heme iron and the ferrous iron must be in the five coordinated high-spin state for subsequent dioxygen binding, a change in coordination of the ferric iron to the high-spin electronic configuration will facilitate electron transfer through a change in the redox potential of the metal center [69,70].

4.3. The Oxy and Peroxo Iron Intermediates

In the P450s studied to date, the oxy-ferrous state is only quasi-stable, and decays to the ferric resting state with the release of superoxide very rapidly. In the membrane-bound systems where substrate turnover is slower, this autoxidation reaction is thought to be responsible for the ultimate production of cytotoxic reactive oxygen species (ROS). A second electron input from the redox donor would then yield a two-electron reduced dioxygen heme center, a formal ferric-peroxo or ferric-hydroperoxo state. Although postulated for many decades based on simple electron counting, this state was only recently observed directly via low-temperature cryoradiolytic reduction in a series of seminal papers by Hoffman and colleagues [71–73]. The ferric-peroxo state represents a critical branch point in the diversity of P450 reactions as indicated in Figure 2. The now classic P450 reaction cycle involves specific protonation of the peroxo anion to form the hydroperoxo and a subsequent proton delivery to result in a heterolytic scission of the O–O bond of heme-bound dioxygen. As envisioned by Groves

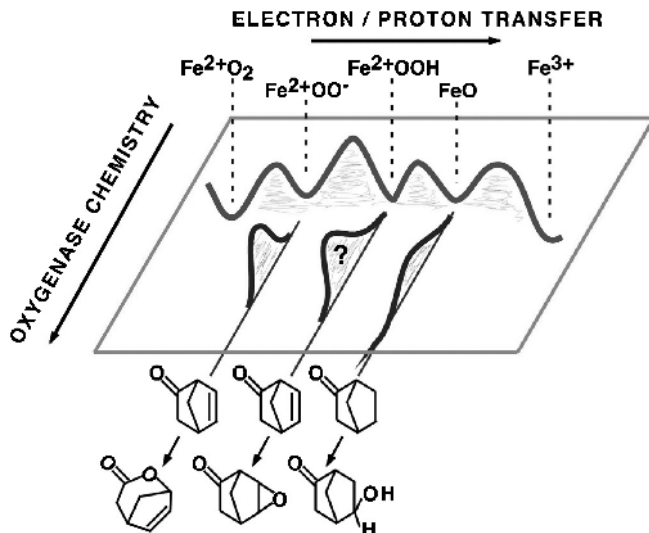


Figure 2. Schematic of the cytochrome P450 reactivity landscape.

(see Section 4.4. below) nearly three decades ago (and further reported in this volume), substrate oxygenation occurs through a step-wise hydrogen abstraction and radical recombination event in the enzyme active site.

However, in principal, each of the intermediates shown in Figure 2 could be active in substrate metabolism. The ferrous oxygenated state could potentially operate as a nucleophile since early Mössbauer spectroscopy from the Debrunner laboratory demonstrated that the iron was more ferric-like and the well-known ‘alpha-effect’ of the proximal oxygen lone pairs should push electron density out toward the distal oxygen atom [74]. Despite many attempts to show that a P450 ferrous-oxy state could catalyze ester hydrolysis or hemiacetal formation, no such reactivity has been demonstrated. However, a recent publication suggests that the simple one-electron reduced dioxygen complex can indeed serve as a catalyst in CYP2E1-mediated deboronation of bortezomib, a potent inhibitor of the 26S proteasome [75]. The input of a second reducing equivalent would form the peroxy anion, an even more potent nucleophile. Indeed, a peroxy heme adduct has been suggested to serve as the catalytic intermediate in the final step in the aromatization of the A-ring in estrogen biosynthesis [76] as well as in the production of nitric oxide by nitric oxide synthase [77].

4.4. High-Valent Metal-Oxo Complexes

Following formation of a hydroperoxy intermediate in the P450 reaction cycle, a second proton delivery will result in cleavage of the oxygen–oxygen bond and the formation of a higher-valent metal-oxo complex that is at the redox level of the peroxidase ‘Compound I’. While a ‘Compound I’ state has been characterized structurally and spectroscopically in several of the peroxidases, it has yet to be observed in the dioxygen-dependent reaction cycle of P450. However, by focusing on the substrate and the resultant stereochemistry of carbon center functionalization, Groves and co-workers made the seminal discovery of a step-wise hydrogen abstraction mechanism [78,79]. Coon and colleagues first demonstrated that, under unfavorable substrate oxygenation conditions, a reaction cycle intermediate could be reduced by two additional redox equivalents to form a second water molecule [80], and the Sligar laboratory used isotope effects to show that commitment to oxygenase catalysis and water production shared a common intermediate [81]. These indirect tools have provided strong evidence for the existence of a higher-valent metal-oxo state in P450 reaction mechanisms.

The extremely hot ‘Fe=O’ oxidant could easily oxidize an unactivated alkane as well as perform easier heteroatom, alkene, or allylic oxygenations. The natural question that emerges when one contemplates the great diversity of substrates and metabolic profiles is whether product distribution and substrate specificity are controlled by protein constraints, inherent substrate site reactivities and/or specific isozyme requirements. White and colleagues have examined

these parameters in the context of the bacterial CYP101 protein [82], but in more complex P450 active site architectures, there remains the possibility that complex homotropic and heterotropic cooperativity can alter the observed metabolic profiles [33]. This diversity of substrate recognition is of paramount importance to human health in that, in many cases, observed drug–drug interactions and determination of effective therapeutic dose are dictated by P450 turnover rates. The application of *in vitro* studies for the prediction of drug–drug interactions *in vivo* has recently been reviewed [83] and more detailed analyses, including the possible time-dependent interaction between multiple P450s has been presented for CYP2D6 [84] and CYP3A4 [85].

4.5. Uncoupling: Nature's Leakage Pathways

The complexity of the enzymatic cycle and the presence of several reactive intermediates along the reaction coordinate gives rise to another intrinsic feature of the P450 mechanism, which is the ‘uncoupling’ or leaking of reducing equivalents into nonproductive pathways capable of producing cytotoxic reactive oxygen species such as superoxide and peroxide. The overall efficiency of converting the consumption of electrons from pyridine nucleotide oxidation to the critical active intermediate(s) necessary for substrate metabolism depends on the kinetic partitioning between the commitment to catalysis of a particular P450 intermediate versus the dissociation of the corresponding iron-superoxide or iron-peroxide complex. Through a number of studies, this efficiency or uncoupling ratio has been shown to be sensitive to the hydration of the distal site of the heme and accessibility to solvent. The influence of the protein structure and dynamics on these leakage pathways is reviewed by Jung [86] (see Chapter 7 of this volume). Other factors which strongly modulate uncoupling include the substrate structure and mobility within the active center of the protein and can also be systematically probed using point mutations of the distal amino acids [87–90].

4.6. Other Heme-Thiolate Systems: Needs from a Mechanistic Viewpoint

There are several other classes of heme proteins which contain thiolate as a proximal heme axial ligand such as the nitric oxide synthases (NOS), chloroperoxidase, cystathionine β -synthase, and the sensor proteins CooA and eIF2a kinase. A brief overview of the properties of these has recently been presented by Omura [91]. An important difference between the enzymes and sensor proteins listed above is in the fact that the former retain their thiolate proximal ligand in the reduced ferrous state and show the definitive Soret band at 442–450 nm when saturated with carbon monoxide (CO) as opposed to the ‘sensor’ proteins wherein the thiolate is displaced by CO or nitric oxide (NO) in the reduced

state. It is beyond the scope of this introductory chapter to provide a discussion of these other heme-thiolate systems although a complete understanding of the bioinorganic mechanisms of the P450 oxygenases benefits from discoveries in these related enzymes.

5. DIVERSITY IN REGULATION ACROSS THE SUPERFAMILY

5.1. Transcriptional Regulation

Layered on the previously mentioned structural diversity in catalytic site residues is transcriptional diversity in the range of tissues and stimuli capable of expressing individual P450 genes (except in organisms containing a single constitutively expressed P450). Because of this transcriptional diversity, even P450 loci coding for highly similar proteins have potential for mediating different physiological functions with individual P450s being expressed in one tissue and not the next. In mammalian systems, examples of this transcriptional diversity exist in the vertebrate CYP1A1 and CYP1A2 genes where CYP1A1 is not expressed at any detectable constitutive level, but highly induced by arylhydrocarbons in many tissues and CYP1A2 is constitutively expressed in liver and further induced by arylhydrocarbons only in liver [92–94]. Other examples exist in the numerous members of the human CYP2 family that are expressed in adults at distinctly higher levels in liver (CYP2E1), lung (CYP2S1), thymus (CYP2U1), etc. Displaying distinct developmental variations, these same loci are expressed in fetal tissues at varying levels that are sometimes higher and sometimes lower than observed in adult tissues [95].

In the insect world, examples of differentially regulated sets of related P450 transcripts are fewer in number, primarily because many of these have been cloned only in recent times. Those that display distinct developmental and tissue-specific expression patterns are the *D. melanogaster* mitochondrial CYP302A1, CYP314A1, and CYP315A1 proteins (also designated as the Halloween genes *dib*, *shd* and *sad*) which mediate the 22-, 20- and 2-hydroxylations, respectively, on the ecdysteroid nucleus [96]. Another microsomal CYP306A1 protein (*D. melanogaster phm*) mediates the 25-hydroxylation on the ecdysteroid nucleus [97,98]. Consistent with their role in early ecdysone synthesis, *dib*, *sad* and *phm* are expressed early in larval development in the prothoracic gland cells of the larval ring gland. And, *shd* that has a function in later ecdysone modifications, is expressed later in larval development and in multiple tissues. Another *D. melanogaster* CYP307A1 (designated as *spo*) in this pathway has an expression pattern suggesting that it has a role in early embryogenesis outside of prothoracic glands, but its exact function has not yet been defined [99].

The larger complements of P450 genes in plant genomes as well as the large-scale cDNA/EST and microarray projects being carried out in

Arabidopsis and *Oryza* (<http://www.arabidopsis.org/>; <http://rgp.dna.affrc.go.jp/>; <http://www.tigr.org/tdb/e2k1/osa1/>) have provided many more examples of differentially regulated transcripts within individual P450 subfamilies. Examples of this exist in the 5-member CYP86A subfamily that contains the functionally characterized CYP86A1, CYP86A2, CYP86A4, CYP86A7, and CYP86A8 involved in fatty acid hydroxylations [100–102], the 37-member CYP71B subfamily that contains the genetically characterized CYP71B15 in camalexin synthesis [103] and 36 uncharacterized members and the 17-member CYP71A subfamily whose functions are completely uncharacterized. Transcript profiling by microarray analyses as well as RT-PCR analyses have indicated that each of the members within these subfamilies is independently regulated, with some being expressed exclusively in one or another tissue and others being constitutively expressed in all tissues, albeit to different levels [104]. Enumerations of the full-length cDNAs existing for each of the 246 full-length cDNAs in *Arabidopsis* [104] make this point eminently clear with several loci in these particular subfamilies represented by 5–7 full-length cDNAs, others represented by 3–4 full-length cDNAs and yet others not represented by any cDNAs or ESTs.

Apart from tissue and developmental cues triggering transcription, individual P450 loci in animals and plants are capable of responding to varying sets of chemical inducers encountered in their dietary sources or through exposure to environmental toxins. Among the best characterized of these transcriptional activating molecules are polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene (3-MC), benzo[α]pyrene, β -naphthoflavone and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) and the drug phenobarbital (PB). In mammalian systems, the signal transduction cascades and the range of genes activated by these two groups of compounds differ: arylhydrocarbons induce expression of genes in the CYP1A subfamily whereas phenobarbital and its related compounds induce expression of genes in the CYP2A, CYP2B, CYP2C, and CYP2D subfamilies [105,106]. In addition to these extensively studied xenobiotic inducers, many of the compounds encountered in plant food sources or used to preserve food materials are capable of inducing mRNAs for vertebrate and insect phase I (P450) and phase II (glutathione S-transferase) detoxicative activities. Among these, furanocoumarins represent a much-studied group of plant defense compounds that are capable of inducing CYP1A1, CYP1A2, and CYP2B1 transcripts in rats [107,108] and CYP6B and CYP9A subfamily transcripts in insects [109–115]. Other larger groups of compounds that are capable of activating vertebrate antioxidant response cascades include antioxidants (e.g., the common food preservative *tert*-butylhydroquinone) [116], natural α,β -unsaturated aldehydes (e.g., *trans*-2-hexenal) [117] and simple phenolic compounds (e.g., caffeic acid) [118].

In addition to these examples of transcriptional induction of individual genes, a small number of P450 loci are subject to transcriptional suppression. One of the most prominent examples of this phenomenon is the vertebrate male-specific

CYP2C11 that is down-regulated in response to arylhydrocarbons, such as 3-MC and TCDD without changing the half-life of its transcript [119,120]. Other examples of P450 suppression are reviewed in Lee and Riddick [119].

5.2. Post-translational Regulation

Studies on a variety of vertebrate P450s have reported that some are post-translationally modified by phosphorylation (CYP2B1, CYP2B4, CYP2E1, CYP11A1, CYP17A1), glycosylation (CYP11A1, CYP19A1), nitration (CYP4A subfamily), and ubiquitination (CYP3A4, CYP2B1) [122–124]. Evidence indicates that, in the cases of CYP2B1, CYP2B4 and CYP2E1, phosphorylation of a serine located on the P450 surface in the C-helix is effected by cAMP-dependent protein kinase A (PKA). Modification at these sites, which occur at analogous positions in these three proteins, provides for the rapid post-translational repression of these particular enzymes and significant reduction in the synthesis of their downstream components. In the case of CYP2E1, phosphorylation has been shown to act as a switch inactivating the microsomal enzyme immediately with kinetics much more rapid than if its activity was regulated by transcriptional repression or protein degradation [123]. Phosphorylation at this site has also been shown to decrease the proportion of CYP2E1 targeted to the endoplasmic reticulum membrane, allowing some to be targeted into the mitochondria using this protein's cryptic organellar targeting sequence [125].

In the case of mitochondrial CYP11A1, phosphorylation of serines and threonines is effected by cAMP-dependent protein kinase C (PKC) resulting in the activation of this particular enzyme's activity [126]. And, in the case of CYP17A1, phosphorylation of serines and threonines by PKA results in the activation of this enzyme's activity [127]. In other cases, such as CYP3A4, CYP2B1, and CYP2E1, ubiquitination targets these proteins to degradation pathways [128,129] through mechanisms that are distinct from the classical ubiquitin-dependent degradation pathways [130].

According to our present understanding of this post-translational regulatory mechanism, low-level ubiquitination of the CYP3A proteins appears to be linked to extended activation of their expression and followed by formation of unusual high-molecular-weight CYP3A4-ubiquitin aggregates in microsomal membranes free of cytosolic components. The fact that these aggregates are subsequently degraded by the cytosolic components and blocked from forming in the presence of substrates suggests a link between ubiquitination and substrate stabilization of this particular group of vertebrate P450s [130,131].

The effects of some other post-translational modifications are less well characterized. In the case of CYP19A1, glycosylation has been shown to occur on a residue within the N-terminal signal sequence that is capable of directing insertion of this P450 across the ER membrane in the types of insect cells used for heterologous expression [132], but the significance of this modification is not

yet clear. These examples serve to illustrate the sorts of modifications affecting individual P450 activities and are not meant to be comprehensive. Further information on the range of vertebrate P450s affected by these modifications are available in Aguiar et al. [124]. Little is yet known about the extent of post-translational modification occurring on insect and plant P450s.

With some of these modifications impacting the activities of the P450 monooxygenases on which they occur, it is worth noting that the extent and types of post-translational modification vary substantially among the heterologous expression systems currently being used for production and analysis of P450 activities.

6. DIVERSITY IN THE EVOLUTION OF COMMON METABOLIC FUNCTIONS

Despite their obvious differences in physical appearance, many organisms maintain a set of common metabolic processes that include the synthesis of hormones, modification of fatty acids, catabolism of xenobiotics, signaling molecules, etc., mediated in different ways by highly divergent P450s. Several recent reviews detail the range of P450-mediated reactions currently known in bacteria and fungi [12], insects [96,133,134], plants [18,19,104,135], and humans [33]. The broad range of P450s mediating common functions are exemplified in the following three categories of reactions.

6.1. Hormone Biosynthesis

Leading to production of sterols that serve as essential structural components of membranes and as precursors for steroid hormones, CYP51 sequences exist in all P450-containing organisms [12,13,20,136]. Testosterone and estradiol, which represent well-characterized examples of the sex-specific steroid hormones occurring in vertebrates, are derived from their cholesterol precursor via CYP11A, CYP17A, and CYP19A subfamily members that are partitioned between the mitochondria and endoplasmic reticulum [33]. Subsequent modifications are mediated by CYP2A, CYP2B, CYP2C, and CYP3A subfamily members located in the endoplasmic reticulum. Brassinosteroids, which represent the plant equivalents of these vertebrate steroid hormones, are synthesized by multiple P450-mediated modifications on the phytosterol skeleton derived from cholesterol [137–139]. Several members in the plant CYP85A, CYP90A, CYP90B, CYP90C, and CYP90D subfamilies mediate these modifications in a biochemical grid that interconnects various intermediates in the synthesis of castasterone and brassinolide, the biologically active forms [140–146]. Ecdysone, which is the insect steroid hormone, is synthesized by an array of P450s in

the CYP302A, CYP306A, CYP307A, CYP314A and CYP315A subfamilies that were previously mentioned in Section 5.1.

Interestingly, some of these P450-mediated modifications as well as other independent P450-mediated modifications on these steroidal hormones lead to their inactivation. Here, examples include the CYP3A-mediated 6 β -, 16 β -, and 11 β -hydroxylations of testosterone in vertebrates [33,147], the CYP734A1-mediated 26-hydroxylation of brassinolide in plants as well as the CYP72C-mediated hydroxylation of brassinolide (at an undefined position) [148–151]. P450s also mediate the inactivation of ecdysone in insects via a 26-hydroxylation [152].

6.2. Xenobiotic Catabolism

With many P450s known to mediate xenobiotic catabolism in vertebrates, an appreciation of diversity and inter-organismal comparisons are best focused by addressing P450s involved in catabolism of plant toxins encountered to varying degrees by the vertebrates and insects that ingest them. Inactivations of furanocoumarins, which were also previously mentioned in transcriptional regulation (Section 5.1), are mediated by the CYP3A4 proteins in humans and closely related enzymes in other vertebrates [33] and CYP6B subfamily proteins as well as the CYP321A1 protein in insects [153–157]. Vertebrate and insect P450s involved in detoxification of this class of compounds attack the double bond of the furan ring as well as methoxy and other substituents on the furanocoumarin core structure [158–160]. Owing to the dimensions of their catalytic sites, P450s in other families and subfamilies are often targets for inactivation by furanocoumarins with, for example, human CYP2A6 being inhibited by binding of this compound in its narrowly constrained catalytic site [161,162].

6.3. Fatty Acid Hydroxylases: Bacteria to Mammals to Plants

Fatty acid hydroxylations are mediated by a wide range of P450s in vastly different subfamilies in different organisms. In the broad set of activities catalyzed by fatty acid hydroxylases, those that catalyze the hydroxylation of the terminal methyl group on aliphatic fatty acid chains are designated as ‘ ω -hydroxylases’ while those that catalyze hydroxylations on internal carbons are designated as ‘in-chain hydroxylases’. Heterologous expressions of these enzymes have indicated they differ in their preferences for fatty acid chain length and degree of saturation and substitution on these chains.

In bacteria, one of the first enzymes characterized was the previously mentioned *B. megaterium* CYP102A1 (P450BM3) that hydroxylates C12 to C18 fatty acids. The more recently *B. subtilis* CYP102A2 and CYP102A3 proteins have been shown to have similar preferences for long-chain fatty acids [163].

In the blue-green alga *Anabaena variabilis*, the CYP110 protein mediates hydroxylation of long-chain saturated and unsaturated fatty acids [164]. In the alkane-assimilating yeast *Candida maltosa*, CYP52A subfamily proteins mediate hydroxylations of C12 to C16 fatty acids with varying preferences and efficiencies [165]. In animals, several of the many CYP4A subfamily members have been shown to hydroxylate fatty acids [166,167]. In comparison with the fatty acid hydroxylases existing in bacteria and plants, several of these mammalian fatty acid hydroxylases are unusual in that they covalently ligate the heme to the backbone of the I-helix at a glutamic acid positioned four residues prior to the (D/E)T in the course of their hydroxylation reactions [168]. In insects, only one protein, *D. melanogaster* CYP6A8, has been shown to mediate any sort of fatty acid hydroxylation [169]. In plants, members of many P450 subfamilies have been shown to catalyze ω -hydroxylations, in-chain hydroxylations and epoxidations of medium- and long-chain fatty acids [170]. The range of plant-specific P450 families involved include the CYP81B, CYP86A, and CYP94A families as well as the most recently identified CYP76A and CYP709C families [100–102,171–178]. The breadth of P450 families involved in fatty acid transformations in these organisms exists in striking contrast with the widely conserved CYP51 family involved in core sterol synthesis.

7. SUMMARY AND OUTLOOK

Looking across the tree of life, it is amazing that one finds cytochrome P450s represented in all life forms. Interestingly, common to these various life forms is a bipartite functionality. One major function of these monooxygenases is in xenobiotic metabolism and detoxification. Here, one finds the use of these monooxygenases in reactions ranging from the metabolism of pharmaceuticals in humans [33,179] to the removal of toxic substances in insects and plants. A second major role in all life forms finds the P450s functioning in the biosynthesis of hormones and signaling molecules, again providing critical functions in mammals, insects and plants.

It is clear that, despite their many differences, their similarities and existing technologies available for transferring genes from organism to the next are now allowing researchers with clear insight into the machinations of these enzymes to engineer P450s for many biotechnological applications. As summarized by Bernhardt [180], recombinant P450s have already been used for improving production of pharmaceuticals in bacterial systems and enhancing synthetic processes in plants with many remaining to be tested. Looking ahead in this ever-expanding field of research is exciting with work on P450 diversity providing the tools for many future biotechnological applications.

ACKNOWLEDGMENTS

The authors thank Dr Ilia Denisov and Mr Sanjeeva Rupasinghe for scientific contributions and Ms Anu Murphy and Ms Kara Sandfort for compiling references. Research on insect P450s is supported by National Institutes of Health R01 GM071826 (MAS), on plant P450s is supported by National Science Foundation grant NSF2010 MCB 0115068 (MAS), and on bacterial and mammalian P450s is supported by National Institutes of Health R37 GM31756 and R37 GM33775 (SGS).

ABBREVIATIONS

Adr	adrenodoxin reductase
Adx	adrenodoxin
FAD	flavin adenine dinucleotide
Fd	ferredoxin
FMN	flavin mononucleotide
FNR	NADPH-dependent ferredoxin reductase
3-MC	3-methylcholanthrene
NAD(P)H	nicotinamide adenine dinucleotide (phosphate), reduced
NOS	nitric oxide synthase
NSAID	nonsteroidal anti-inflammatory drug
ORF	open reading frame
PB	phenobarbital
PGHS	prostaglandin endoperoxide H synthase
PKA	cAMP-dependent protein kinase A
PKC	cAMP-dependent protein kinase C
ROS	reactive oxygen species
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin

REFERENCES

1. J. Raymond and D. Segre, *Science*, *311*, 1764–1767 (2006).
2. O. Hayaishi, M. Katagiri, and S. Rothberg, *J. Am. Chem. Soc.*, *77*, 5450–5451 (1955).
3. H. S. Mason, L. Fowlks, and E. Peterson, *J. Am. Chem. Soc.*, *77*, 2851–2914 (1955).
4. M. Sono, M. P. Roach, E. D. Coulter, and J. H. Dawson, *Chem. Rev.*, *96*, 2841 (1996).
5. F. P. Guengerich, *Chem. Res. Toxicol.*, *14*, 611–650 (2001).
6. F. P. Guengerich, *Curr. Drug Metab.*, *2*, 93–115 (2001).
7. I. G. Denisov, T. M. Makris, S. G. Sligar, and I. Schlichting, *Chem. Rev.*, *105*, 2253–2277 (2005).

8. T. M. Makris, I. Denisov, I. Schlichting, and S. G. Sligar, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (P. R. Ortiz de Montellano, ed.), 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005, pp. 149–182.
9. T. M. Makris, K. von Koenig, I. Schlichting, and S. G. Sligar, *J. Inorg. Biochem.*, **100**, 507–518 (2006).
10. D. R. Nelson, T. Kamataki, D. J. Waxman, F. P. Guengerich, R. W. Estabrook, R. Feyereisen, F. J. Gonzalez, M. J. Coon, I. C. Gunsalus, O. Gotoh, K. Okuda, and D. W. Nebert, *DNA Cell Biol.*, **12**, 1–51 (1993).
11. D. R. Nelson, L. Koymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon, R. W. Estabrook, I. C. Gunsalus, and D. W. Nebert, *Pharmacogenetics*, **6**, 1–41 (1996).
12. S. L. Kelly, D. E. Kelly, C. J. Jackson, A. G. S. Warrilow, and D. C. Lamb, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (P. R. Ortiz de Montellano, ed.), 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005, pp. 585–617.
13. D. R. Nelson, M. A. Schuler, S. M. Paquette, D. Werck-Reichhart, and S. Bak, *Plant Physiol.*, **135**, 756–772 (2004).
14. D. R. Nelson, *Phytochem. Rev.*, in press (2006).
15. N. Tijet, C. Helvig, and R. Feyereisen, *Gene*, **262**, 189–198 (2001).
16. C. Claudianos, H. Ranson, R. M. Johnson, S. Biswas, M. A. Schuler, M. R. Berenbaum, R. Feyereisen, and J. G. Oakeshott, *Insect. Mol. Biol.*, **15**, 615–636 (2006).
17. S. M. Paquette, S. Bak, and R. Feyereisen, *DNA Cell Biol.*, **19**, 307–317 (2000).
18. D. Werck-Reichhart, S. Bak, and S. Paquette, in *The Arabidopsis Book* (C. R. Somerville and E. M. Meyerowitz, eds), American Society of Plant Biologists, Rockville, MD, 2002, doi/10.1199/tab.0028, <http://www.aspb.org/publications/arabidopsis>
19. M. A. Schuler and D. Werck-Reichhart, *Annu. Rev. Plant Biol.*, **54**, 629–667 (2003).
20. N. Debeljak, M. Fink, and D. Rozman, *Arch. Biochem. Biophys.*, **409**, 159–171 (2003).
21. M. R. Waterman and G. I. Lapesheva, *Biochem. Biophys. Res. Commun.*, **338**, 418–422 (2005).
22. S. E. Graham and J. A. Peterson, *Arch. Biochem. Biophys.*, **369**, 24–29 (1999).
23. E. F. Johnson and C. D. Stout, *Biochem. Biophys. Res. Commun.*, **338**, 331–336 (2005).
24. T. L. Poulos and E. F. Johnson, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (P. R. Ortiz de Montellano, ed.), 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005, pp. 87–111.
25. T. L. Poulos and Y. T. Meharena, in *The Ubiquitous Roles of P450 Proteins*, Vol. 3 of *Metal Ions in Life Sciences* (A. Sigel, H. Sigel, and R. K. O. Sigel, eds), John Wiley & Sons, Ltd, Chichester, UK, 2007, pp. 57–96.
26. O. Gotoh, *J. Biol. Chem.*, **267**, 83–90 (1992).
27. S. Rupasinghe and M. A. Schuler, *Phytochem. Rev.*, in press (2006).
28. J. Baudry, S. Rupasinghe, and M. A. Schuler, *Protein Eng. Design Selec.*, **19**, 345–353 (2006).

29. R. L. P. Lindberg and M. Negishi, *Nature*, *339*, 632–634 (1989).
30. M. Negishi, M. Iwasaki, R. O. Juvonen, T. Sueyoshi, T. A. Darden, and L. G. Pedersen, *Mutat. Res.*, *350*, 43–50 (1996).
31. M. Schalk and R. Croteau, *Proc. Natl. Acad. Sci. USA*, *97*, 11948–11953 (2000).
32. T. L. Domanski and J. R. Halpert, *Curr. Drug Metab.*, *2*, 117–137 (2001).
33. F. P. Guengerich, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (P. R. Ortiz de Montellano, ed.), 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005, pp. 377–530.
34. M. J. Hintz and J. A. Peterson, *J. Biol. Chem.*, *256*, 6721–6728 (1981).
35. P. W. Roome and J. A. Peterson, *Arch. Biochem. Biophys.*, *266*, 32–40 (1988).
36. P. W. Roome and J. A. Peterson, *Arch. Biochem. Biophys.*, *266*, 41–50 (1988).
37. A. V. Grinberg, F. Hannemann, B. Schiffler, J. Muller, U. Heinemann, and R. Bernhardt, *Proteins*, *40*, 590–612 (2000).
38. I. F. Sevrioukova, H. Li, and T. L. Poulos, *J. Mol. Biol.*, *336*, 889–902 (2004).
39. M. J. I. Paine, N. S. Scrutton, A. W. Munro, A. Gutierrez, G. C. K. Roberts, and C. R. Wolf, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (P. R. Ortiz de Montellano, ed.), 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005, pp. 115–148.
40. M. B. Murataliev, R. Feyereisen, and F. A. Walker, *Biochim. Biophys. Acta*, *1698*, 1–26 (2004).
41. L. O. Narhi and A. J. Fulco, *J. Biol. Chem.*, *261*, 7160–7169 (1986).
42. J. A. Peterson, Y. Ishimura, and B. W. Griffin, *Arch. Biochem. Biophys.*, *149*, 197–208 (1972).
43. J. D. Lipscomb, S. G. Sligar, M. J. Namtvedt, and I. C. Gunsalus, *J. Biol. Chem.*, *251*, 1116–1124 (1976).
44. I. G. Denisov, Y. V. Grinkova, B. J. Baas, and S. G. Sligar, *J. Biol. Chem.*, *281*, 23313–23318 (2006).
45. A. V. Puchkaev and P. R. Ortiz de Montellano, *Arch. Biochem. Biophys.*, *434*, 169–177 (2005).
46. K. J. McLean, M. Sabri, K. R. Marshall, R. J. Lawson, D. G. Lewis, D. Clift, P. R. Balding, A. J. Dunford, A. J. Warman, J. P. McVey, A. M. Quinn, M. J. Sutcliffe, N. S. Scrutton, and A. W. Munro, *Biochem. Soc. Trans.*, *33*, 796–801 (2005).
47. I. Matsunaga, A. Ueda, N. Fujiwara, T. Sumimoto, and K. Ichihara, *Lipids*, *34*, 841–846 (1999).
48. Y. Shiro, M. Fujii, T. Iizuka, S. Adachi, K. Tsukamoto, K. Nakahara, and H. Shoun, *J. Biol. Chem.*, *270*, 1617–1623 (1995).
49. W. C. Song, C. D. Funk, and A. R. Brash, *Proc. Natl. Acad. Sci. USA*, *90*, 8519–8523 (1993).
50. N. Tijet and A. R. Brash, *Prostaglandins Other Lipid Mediat.*, *68–69*, 423–431 (2002).
51. K. Matsui, M. Shibutani, T. Hase, and T. Kajiwara, *FEBS Lett.*, *394*, 21–24 (1996).
52. A. Itoh and G. A. Howe, *J. Biol. Chem.*, *276*, 3620–3627 (2001).
53. A. N. Grechkin, *Prostaglandins Other Lipid Mediat.*, *68–69*, 457–470 (2002).
54. L. H. Wang, A. L. Tsai, and P. Y. Hsu, *J. Biol. Chem.*, *276*, 14737–14743 (2001).
55. Z. Pan, B. Camara, H. W. Gardner, and R. A. Backhaus, *J. Biol. Chem.*, *273*, 18139–18145 (1998).

56. M. Bureik, B. Schiffler, Y. Hiraoka, F. Vogel, and R. Bernhardt, *Biochemistry*, *41*, 2311–2321 (2002).
57. R. T. Bossi, A. Aliverti, D. Raimondi, F. Fischer, G. Zanetti, D. Ferrari, N. Tahallah, C. S. Maier, A. J. Heck, M. Rizzi, and A. Mattevi, *Biochemistry*, *41*, 8807–8818 (2002).
58. K. J. McLean, N. S. Scrutton, and A. W. Munro, *Biochem. J.*, *372*, 317–327 (2003).
59. D. Ohta and M. Mizutani, *Front. Biosci.*, *9*, 1587–1597 (2004).
60. D. P. O’Keefe, J. M. Tepperman, C. Dean, K. J. Leto, D. L. Erbes, and J. T. Odell, *Plant Physiol.*, *105*, 473–482 (1994).
61. T. Lacour and H. Ohkawa, *Biochim. Biophys. Acta.*, *1433*, 87–102 (1999).
62. C. J. Jackson, D. C. Lamb, T. H. Marczyllo, A. G. Warrilow, N. J. Manning, D. J. Lowe, D. E. Kelly, and S. L. Kelly, *J. Biol. Chem.*, *277*, 46959–46965 (2002).
63. G. A. Roberts, G. Grogan, A. Greter, S. L. Flitsch, and N. J. Turner, *J. Bacteriol.*, *184*, 3898–3908 (2002).
64. D. J. Hunter, G. A. Roberts, T. W. Ost, J. H. White, S. Muller, N. J. Turner, S. L. Flitsch, and S. K. Chapman, *FEBS Lett.*, *579*, 2215–2220 (2005).
65. M. Mizutani and D. Ohta, *Plant Physiol.*, *116*, 357–367 (1998).
66. D. K. Ro, J. Ehltling, and C. J. Douglas, *Plant Physiol.*, *130*, 1837–1851 (2002).
67. P. R. Ortiz de Montellano (ed.), *Cytochrome P450, Structure, Mechanism, and Biochemistry*, 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005.
68. S. G. Sligar, T. M. Makris, and I. G. Denisov, *Biochem. Biophys. Res. Commun.*, *338*, 346–354 (2005).
69. S. G. Sligar, *Biochemistry*, *15*, 5399–5406 (1976).
70. M. Fisher and S. G. Sligar, *J. Am. Chem. Soc.*, *107*, 5018–5019 (1985).
71. R. Davydov, T. M. Makris, V. Kofman, D. E. Werst, S. G. Sligar, and B. M. Hoffman, *J. Am. Chem. Soc.*, *123*, 1403–1415 (2001).
72. R. Davydov, V. Kofman, H. Fujii, T. Yoshida, M. Ikeda-Saito, and B. M. Hoffman, *J. Am. Chem. Soc.*, *124*, 1798–1808 (2002).
73. T. M. Makris, R. Davydov, I. G. Denisov, B. M. Hoffman, and S. G. Sligar, *Drug Metab. Rev.*, *34*, 691–708 (2002).
74. M. Sharrock, P. G. Debrunner, C. Schulz, J. D. Lipscomb, V. Marshall, and I. C. Gunsalus, *Biochim. Biophys. Acta*, *420*, 8–26 (1976).
75. J. Labutti, I. Parsons, R. Huang, G. Miwa, L.-S. Gan, and J. S. Daniels, *Chem. Res. Toxicol.*, *19*, 539–546 (2006).
76. M. Akhtar, M. Calder, D. Corina, and J. Wright, *Biochem. J.*, *201*, 569–580 (1982).
77. H.-G. Korth, R. Sustmann, C. Thater, A. R. Butler, and K. U. Ingold, *J. Biol. Chem.*, *269*, 17776–17779 (1994).
78. J. T. Groves, G. A. McClusky, R. E. White, and M. J. Coon, *Biochem. Biophys. Res. Commun.*, *81*, 154–160 (1978).
79. J. T. Groves and C. C.-Y. Wang, *Curr. Opin. Chem. Biol.*, *4*, 687–695 (2000).
80. L. D. Gorsky, D. R. Koop, and M. J. Coon, *J. Biol. Chem.*, *259*, 6812–6817 (1984).
81. W. M. Atkins and S. G. Sligar, *Biochemistry*, *27*, 1610–1616 (1988).
82. R. E. White, M. B. McCarthy, K. D. Egeberg, and S. G. Sligar, *Arch. Biochem. Biophys.*, *228*, 493–502 (1984).
83. R. S. Obach, R. L. Walsky, K. Venkatakrisnan, J. B. Houston, and L. M. Tremaine, *Clin. Pharmacol. Ther.*, *78*, 582–592 (2005).

84. K. Ito, D. Hallifax, R. S. Obach, and J. B. Houston, *Drug Metab. Dispos.*, **33**, 837–844 (2005).
85. A. Galetin, K. Ito, D. Hallifax, and J. B. Houston, *J. Pharmacol. Exp. Ther.*, **314**, 180–190 (2005).
86. C. Jung, in *The Ubiquitous Roles of P450 Proteins*, Vol. 3 of *Metal Ions in Life Sciences* (A. Sigel, H. Sigel, and R. K. O. Sigel, eds), John Wiley & Sons, Ltd, Chichester, UK, 2007, pp. 187–234.
87. P. J. Loida and S. G. Sligar, *Protein Eng.*, **6**, 207–212 (1993).
88. P. J. Loida and S. G. Sligar, *Biochemistry*, **32**, 11530–11538 (1993).
89. M. Budde, M. Morr, R. D. Schmid, and V. B. Urlacher, *Chembiochem.*, **7**, 789–794 (2006).
90. J. P. Clark, C. S. Miles, C. G. Mowat, M. D. Walkinshaw, G. A. Reid, S. N. Daff, and S. K. Chapman, *J. Inorg. Biochem.*, **100**, 1075–1090 (2006).
91. T. Omura, *Biochem. Biophys. Res. Commun.*, **338**, 404–409 (2005).
92. F. J. Gonzalez and Y. H. Lee, *FASEB J.*, **10**, 1112–1117 (1996).
93. J. P. Whitlock, Jr., *Annu. Rev. Pharmacol. Toxicol.*, **39**, 103–125 (1999).
94. Y. Fujii-Kuriyama and J. Mimura, *Biochem. Biophys. Res. Commun.*, **338**, 311–317 (2005).
95. D. Choudhary, I. Jansson, I. Stoilov, M. Sarfarazi, and J. B. Schenkman, *Arch. Biochem. Biophys.*, **436**, 50–61 (2005).
96. L. I. Gilbert, *Mol. Cell. Endocrinol.*, **215**, 1–10 (2004).
97. R. Niwa, T. Matsuda, T. Yoshiyama, T. Namiki, K. Mita, Y. Fujimoto, and H. Kataoka, *J. Biol. Chem.*, **279**, 35942–35949 (2004).
98. J. T. Warren, A. Petryk, G. Marques, J. P. Parvy, T. Shinoda, K. Itoyama, J. Kobayashi, M. Jarcho, Y. Li, M. B. O'Connor, C. Dauphin-Villemant, and L. I. Gilbert, *Insect Biochem. Mol. Biol.*, **34**, 991–1010 (2004).
99. T. Namiki, R. Niwa, T. Sakudoh, K. Shirai, H. Takeuchi, and H. Kataoka, *Biochem. Biophys. Res. Commun.*, **337**, 367–374 (2005).
100. I. Benveniste, N. Tijet, F. Adas, G. Philipps, J.-P. Salaün, and F. Durst, *Biochem. Biophys. Res. Commun.*, **243**, 688–693 (1998).
101. K. Wellesen, F. Durst, F. Pinot, I. Benveniste, K. Nettesheim, E. Wisman, S. Steiner-Lange, H. Saedler, and A. Yephremov, *Proc. Natl. Acad. Sci. USA*, **98**, 9694–9699 (2001).
102. H. Duan and M. A. Schuler, *Plant Physiol.*, **137**, 1067–1081 (2005).
103. N. Zhou, T. L. Tootle, and J. Glazebrook, *Plant Cell*, **11**, 2419–2428 (1999).
104. M. A. Schuler, H. Duan, M. Bilgin and S. Ali, *Phytochem. Reviews*, in press (2006).
105. D. J. Waxman, *Arch. Biochem. Biophys.*, **369**, 11–23 (1999).
106. T. Sueyoshi and M. Negishi, *Annu. Rev. Pharmacol. Toxicol.*, **41**, 123–143 (2001).
107. J. H. Gwang, *Cancer Lett.*, **109**, 115–120 (1996).
108. A. Baumgart, M. Schmidt, H. J. Schmitz, and D. Schrenk, *Biochem. Pharmacol.*, **69**, 657–667 (2005).
109. H. Prapaipong, M. R. Berenbaum, and M. A. Schuler, *Nucleic Acids Res.*, **22**, 3210–3217 (1994).
110. C.-F. Hung, H. Prapaipong, M. R. Berenbaum, and M. A. Schuler, *Insect Biochem. Mol. Biol.*, **25**, 89–99 (1995).
111. C.-F. Hung, T. L. Harrison, M. R. Berenbaum, and M. A. Schuler, *Insect Mol. Biol.*, **4**, 149–160 (1995).

112. J. L. Stevens, M. J. Snyder, J. F. Koener, and R. Feyereisen, *Insect Biochem. Mol. Biol.*, **30**, 559–568 (2000).
113. W. Li, M. R. Berenbaum, and M. A. Schuler, *Insect Biochem. Mol. Biol.*, **31**, 999–1011 (2001).
114. R. A. Petersen, A. R. Zangerl, M. R. Berenbaum, and M. A. Schuler, *Insect Biochem. Mol. Biol.*, **31**, 679–690 (2001).
115. X. Li, M. R. Berenbaum, and M. A. Schuler, *Insect Biochem. Mol. Biol.*, **11**, 343–351 (2002).
116. F. Shahidi, *Nahrung.*, **44**, 158–163 (2000).
117. R. B. Tjalkens, S. W. Luckey, D. J. Kroll, and D. R. Petersen, *Arch. Biochem. Biophys.*, **359**, 42–50 (1998).
118. A. K. Jaiswal, R. Venugopal, J. Mucha, A. M. Carothers, and D. Grunberger, *Cancer Res.*, **57**, 440–446 (1997).
119. C. Lee and D. S. Riddick, *Biochem. Pharmacol.*, **59**, 1417–1423 (2000).
120. A. Bhatena, C. Lee, and D. S. Riddick, *Drug Metab. Dispos.*, **30**, 1385–1392 (2002).
121. D. S. Riddick, C. Lee, A. Bhatena, Y. E. Timsit, P. Y. Cheng, E. T. Morgan, R. A. Prough, S. L. Ripp, K. K. Miller, A. Jahan, and J. Y. Chiang, *Drug Metab. Dispos.*, **32**, 367–375 (2004).
122. B. Oesch-Bartlomowicz and F. Oesch, *Biol. Chem.*, **383**, 1587–1592 (2002).
123. B. Oesch-Bartlomowicz and F. Oesch, *Arch. Biochem. Biophys.*, **409**, 228–234 (2003).
124. M. Aguiar, R. Masse, and B. F. Gibbs, *Drug Metab. Rev.*, **37**, 379–404 (2005).
125. M. A. Robin, H. K. Anandatheerthavarada, G. Biswas, N. B. Sepuri, D. M. Gordon, D. Pain, and N. G. Avadhani, *J. Biol. Chem.*, **277**, 40583–40593 (2002).
126. I. Vilgrain, G. Defaye, and E. M. Chambaz, *Biochem. Biophys. Res. Commun.*, **125**, 554–561 (1984).
127. L. H. Zhang, H. Rodriguez, S. Ohno, and W. L. Miller, *Proc. Natl. Acad. Sci. USA*, **92**, 10619–10623 (1995).
128. K. K. Korsmeyer, S. Davoll, M. E. Figueiredo-Pereira, and M. A. Correia, *Arch. Biochem. Biophys.*, **365**, 31–44 (1999).
129. M. A. Correia, S. Sadeghi, and E. Mundo-Paredes, *Annu. Rev. Pharmacol. Toxicol.*, **45**, 439–464 (2005).
130. R. C. Zangar, A. L. Kimzey, J. R. Okita, D. S. Wunschel, R. J. Edwards, H. Kim, and R. T. Okita, *Mol. Pharmacol.*, **61**, 892–904 (2002).
131. A. L. Kimzey, K. K. Weitz, F. P. Guengerich, and R. C. Zangar, *Biochemistry*, **42**, 12691–12699 (2003).
132. O. Shimozawa, M. Sakaguchi, H. Ogawa, N. Harada, K. Mihara, and T. Omura, *J. Biol. Chem.*, **268**, 21399–21402 (1993).
133. J. G. Scott and Z. Wen, *Pest Manag. Sci.*, **57**, 958–967 (2001).
134. R. Feyereisen, in *Comprehensive Molecular Insect Science*, Vol. 4, (L. I. Gilbert, K. Latrou, and S. S. Gill, eds), Elsevier, Oxford, 2005, pp. 1–77.
135. K. A. Nielsen and B. L. Moller, in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (P. R. Ortiz de Montellano, ed.), 3rd edn, Kluwer Academic/Plenum Publishers, New York, 2005, pp. 553–583.
136. R. Bernhardt and M. R. Waterman, in *The Ubiquitous Roles of P450 Proteins*, Vol. 3 of *Metal Ions in Life Sciences* (A. Sigel, H. Sigel, and R. K. O. Sigel, eds), John Wiley & Sons, Ltd, Chichester, UK, 2007, pp. 361–396.

137. G. J. Bishop and C. Koncz, *Plant Cell*, *14*, S97–S110 (2002).
138. S. Fujioka and T. Yokota, *Annu. Rev. Plant Biol.*, *54*, 137–164 (2003).
139. S. Choe, *Plant Physiol.*, *126*, 539–548 (2006).
140. M. Szekeres, K. Németh, Z. Koncz-Kálmán, J. Mathur, A. Kauschmann, T. Altmann, G. P. Rédei, F. Nagy, J. Schell and C. Koncz, *Cell*, *85*, 171–182 (1996).
141. S. Choe, B. P. Dilkes, S. Fujioka, S. Takatsuto, A. Sakurai, and K. A. Feldmann, *Plant Cell*, *10*, 231–243 (1998).
142. G. J. Bishop, T. Nomura, T. Yokota, K. Harrison, T. Noguchi, S. Fujioka, S. Takatsuto, J. D. Jones, and Y. Kamiya, *Proc. Natl. Acad. Sci. USA*, *96*, 1761–1766 (1999).
143. Y. Shimada, S. Fujioka, N. Miyauchi, M. Kushiro, S. Takatsuto, T. Nomura, T. Yokota, Y. Kamiya, G. J. Bishop, and S. Yoshida, *Plant Physiol.*, *126*, 770–779 (2001).
144. Y. Shimada, H. Goda, A. Nakamura, S. Takatsuto, S. Fujioka, and S. Yoshida, *Plant Physiol.*, *131*, 287–297 (2003).
145. T.-W. Kim, J.-Y. Hwang, Y.-S. Kim, S.-H. Joo, S. C. Chang, J. S. Lee, S. Takatsuto, and S. K. Kim, *Plant Cell*, *17*, 2397–2412 (2005).
146. T. Nomura, T. Kushiro, T. Yokota, Y. Kamiya, G. J. Bishop, and S. Yamaguchi, *J. Biol. Chem.*, *280*, 17873–17879 (2005).
147. M. H. Choi, P. L. Skipper, J. S. Wishnok, and S. R. Tannenbaum, *Drug Metab. Dispos.*, *33*, 714–718 (2005).
148. M. M. Neff, S. M. Nguyen, E. J. Malancharuvil, S. Fujioka, T. Noguchi, H. Seto, M. Tsubuki, T. Honda, S. Takatsuto, S. Yoshida, and J. Chory, *Proc. Natl. Acad. Sci. USA*, *96*, 15316–15323 (1999).
149. E. M. Turk, S. Fujioka, H. Seto, Y. Shimada, S. Takatsuto, S. Yoshida, M. A. Denzel, Q. I. Torres, and M. M. Neff, *Plant Physiol.*, *133*, 1643–1653 (2003).
150. M. Nakamura, T. Satoh, S. Tanaka, N. Mochizuki, T. Yokota, and A. Nagatani, *J. Exp. Bot.*, *56*, 833–840 (2005).
151. N. Takahashi, M. Nakazawa, K. Shibata, T. Yokota, A. Ishikawa, K. Suzuki, M. Kawashima, T. Ichikawa, H. Shimada, and M. Matsui, *Plant J.*, *42*, 13–22 (2005).
152. D. R. Williams, M. J. Fisher, and H. H. Rees, *Arch. Biochem. Biophys.*, *376*, 389–398 (2000).
153. C.-F. Hung, M. R. Berenbaum, and M. A. Schuler, *Insect Biochem. Mol. Biol.*, *27*, 377–385 (1997).
154. J.-S. Chen, M. R. Berenbaum, and M. A. Schuler, *Insect Mol. Biol.*, *11*, 175–186 (2002).
155. Z. Wen, L. Pan, M. R. Berenbaum, and M. A. Schuler, *Insect Biochem. Mol. Biol.*, *33*, 937–947 (2003).
156. X. Li, J. Baudry, M. R. Berenbaum, and M. A. Schuler, *Proc. Natl. Acad. Sci. USA*, *101*, 2939–2944 (2004).
157. M. Sasabe, Z. Wen, M. R. Berenbaum, and M. A. Schuler, *Gene*, *338*, 163–175 (2004).
158. G. W. Ivie, in *Light-Activated Pesticides* (J. R. Heitz and K. R. Downum, eds), Vol. 339 of *American Chemical Society Symposium Series*, Washington, 1987, pp. 216–230.

159. J. K. Nitao, M. Berhow, S. M. Duval, D. Weisleder, S. F. Vaughn, A. Zangerl, and M. R. Berenbaum, *J. Chem. Ecol.*, *29*, 671–682 (2003).
160. W. Mao, S. Rupasinghe, A. Zangerl, M. A. Schuler, and M. R. Berenbaum, *Insect Mol. Biol.*, *15*, 169–179 (2006).
161. J. Maenpaa, R. Juvonen, H. Raunio, A. Rautio, and O. Pelkonen, *Biochem. Pharmacol.*, *48*, 1363–1369 (1994).
162. J. K. Yano, M. H. Hsu, K. J. Griffin, C. D. Stout, and E. F. Johnson, *Nature Struct. & Mol. Biol.*, *12*, 822–823 (2005).
163. M. C. Gustafsson, O. Roitel, K. R. Marshall, M. A. Noble, S. K. Chapman, A. Pessegueiro, A. J. Fulco, M. R. Cheesman, C. von Wachenfeldt and A. W. Munro, *Biochemistry*, *3*, 5474–5487 (2004).
164. S. Torres, C. R. Fjetland and P. J. Lammers, *BMC Microbiol.*, *5*, 16–27 (2005).
165. U. Scheller, T. Zimmer, E. Kargel, and W. H. Schnuck, *Arch. Biochem. Biophys.*, *328*, 245–254 (1996).
166. A. E. Simpson, *Gen. Pharmacol.*, *28*, 351–359 (1997).
167. R. T. Okita and J. R. Okita, *Curr. Drug. Metab.*, *2*, 265–281 (2001).
168. K. R. Henne, K. L. Kunze, Y.-M. Zheng, P. Christmas, R. J. Soberman, and A. E. Rettie, *Biochemistry*, *40*, 12925–12931 (2001).
169. C. Helvig, N. Tijet, R. Feyereisen, F. A. Walker, and L. L. Restifo, *Biochem. Biophys. Res. Commun.*, *325*, 1495–1502 (2004).
170. J.-P. Salaün and C. Helvig, *Drug Interact.*, *12*, 261–283 (1995).
171. F. Cabello-Hurtado, Y. Bataud, J.-P. Salaün, F. Durst, F. Pinot, and D. Werck-Reichart, *J. Biol. Chem.*, *273*, 7260–7267 (1998).
172. N. Tijet, C. Helvig, F. Pinot, R. Le Bouquin, A. Lesot, F. Durst, J. P. Salaun, and I. Benveniste, *Biochem. J.*, *332*, 583–589 (1998).
173. R. LeBouquin, F. Pinot, I. Benveniste, J. P. Salaün, and F. Durst, *Biochem. Biophys. Res. Commun.*, *261*, 156–162 (1999).
174. R. LeBouquin, M. Skrabs, R. Kahn, I. Benveniste, J. P. Salaün, L. Schreiber, F. Durst, and F. Pinot, *Eur. J. Biochem.*, *268*, 3083–3090 (2001).
175. R. A. Kahn, R. LeBouquin, F. Pinot, I. Benveniste and F. Durst, *Arch. Biochem. Biophys.*, *391*, 180–187 (2001).
176. F. Xiao, S. M. Goodwin, Y. Xiao, Z. Sun, D. Baker, X. Tang, M. A. Jenks, and J. M. Zhou, *EMBO J.*, *23*, 2903–2913 (2004).
177. S. Kandel, M. Morant, I. Benveniste, E. Blee, D. Werck-Reichhart, and F. Pinot, *J. Biol. Chem.*, *280*, 35881–35889 (2005).
178. K. Tamaki, H. Imaishi, H. Ohkawa, K. Oono, and M. Sugimoto, *Biosci. Biotechnol. Biochem.*, *69*, 406–409 (2005).
179. P. B. Danielson, *Curr. Drug Metab.*, *3*, 561–597 (2002).
180. R. Bernhardt, *J. Biotechnol.*, *124*, 128–145 (2006).