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Structural diversity of cytochrome P450 enzyme system

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Cytochrome P450 enzyme system consists of P450 and its NAD(P)H-linked reductase or reducing system, and catalyses monooxygenation reactions. The most prevalent type in eukaryotic organisms is 'microsomes type', which consists of membrane-bound P450 and NADPH-P450 reductase. The second type is 'mitochondria type', in which P450 is bound to the inner membrane while the reducing system consisting of an NADPH-linked flavoprotein and a ferredoxin-type iron–sulphur protein is soluble in the matrix space. The third type is 'bacteria type', in which both P450 and the reducing system are soluble in the cytoplasm. In addition to these three types, several forms of P450-reductase fusion proteins have been found in prokaryotic organisms. On the other hand, some P450s catalyse the re-arrangement of the oxygen atoms in the substrate molecules that does not require the supply of reducing equivalents for the reaction. A peculiar P450, P450nor, receives electrons directly from NADH for the reduction of nitric oxide.

Keywords: Bacterial P450/cytochrome P450/microsomal P450/mitochondrial P450/P450-reductase fusion proteins.

Monooxygenase reactions catalysed by cytochrome P450 require the supply of electrons from NADPH or NADH, and P450 is always found associated with its NAD(P)H-linked reductase or reducing system in the cell. Several different types of P450 enzyme systems including P450-reductase fusion proteins have been reported. The structural diversity of the P450 enzyme system may be related with the highly diversified physiological functions of P450 in eukaryotic and prokaryotic organisms.

P450 is present in both endoplasmic reticulum (microsomes) and mitochondria in animal cells, whereas it is found only in endoplasmic reticulum in plant and fungus cells. The components of microsomal P450 system are all membrane-bound, whereas the mitochondrial P450 system consists of membrane-bound P450 and soluble reducing system. Both P450 and its reducing system are soluble in the cells of prokaryotic organisms. Various P450-reductase fusion proteins have been found in bacteria.

The substrates of P450-catalysed monooxygenase reactions are mostly hydrophobic organic compounds. The membrane-bound nature of microsomal and mitochondrial P450s reflects the hydrophobic properties of the substrates, which tend to be concentrated to the membranes in the cells. The soluble nature of the P450s of prokaryotic organisms might be due to the absence of intracellular membrane systems to anchor P450 in the cells.

The structural diversity of P450 enzyme system is an interesting case where the physiological diversification of an enzyme has been accompanied with the alteration of the partner enzyme. P450 gene superfamily, which codes for a large number of different P450s, has apparently evolved from a single ancestral gene and highly diversified along with the evolution of living organisms. Since P450 requires the reductase for its function, the molecular evolution of P450 must have been restricted by the necessity for the interaction with the partner enzyme. This review summarizes the present knowledge on the structural diversity of P450 enzyme system and discusses its relation with the functional diversification of P450 during the evolution of eukaryotic and prokaryotic organisms.

Discovery of various cytochrome P450 enzyme systems

P450 was first characterized in the microsomal fraction of animal liver in 1962 (*1*), and the enzymatic activity of adrenal cortex microsomal P450 as the monooxygenase was elucidated in the next year (*2*). Role of microsomal NADPH-cytochrome *c* reductase in P450-catalysed NADPH-dependent monooxygenation activity was suggested by a reconstitution experiment in 1968 (*3*). Later studies confirmed the essential role of NADPH-cytochrome *c* reductase in microsomal P450-catalysed monooxygenation reactions, and the reductase is now called NADPH-cytochrome P450 reductase. Both P450 and the reductase are tightly bound to the microsomal membrane.

Presence of P450 in the mitochondria isolated from adrenal cortex was discovered in 1964 (*4*). The NADPH-dependent steroid hydroxylase activity of the adrenal cortex mitochondria was found to be composed of membrane-bound P450 and the soluble NADPH-P450 reductase system consisting of adrenodoxin and NADPH-adrenodoxin reductase in 1966 (*5*). Mitochondrial P450 enzyme system is found in animals, whereas fungi and plants do not seem to contain P450 in mitochondria. However, a few papers reported the presence of P450 in the choloroplasts of plants (*6*).

A bacterial P450 was first found in *Rhizobium* bacteroids in 1967 (*7*). In contrast to the microsomal and mitochondrial P450s reported before, the *Rhizobium*

P450 was soluble. However, the function of the P450 in *Rhizobium* bacteroids was not elucidated. In the next year, two more bacterial P450s were reported. One was found in *Pseudomonas putida* (*8*) and the other in *Coryebacterium* sp., (*9*). The *Pseudomonas* P450, P450cam (CYP101), catalysed NADH-dependent oxidation of camphor, and required a soluble NADH-P450 reductase system consisting of putidaredoxin and NADH-putidaredoxin reductase for the reaction (*8*). *Corynebacterium* P450 catalysed NADH-dependent oxidation of *n*-octane to 1-octanol. More soluble P450s have been found in various prokaryotic organisms in later years.

The first self-sufficient P450-reductase fusion enzyme was found in *Bacillus megaterium* in 1987 and named P450 BM-3 (CYP102A1) (*10*). Similar P450-reductase fusion proteins have been found in other bacterial species and also in several fungi in the following years. Recent analyses of the genomic sequences of various prokaryotic organisms have revealed the presence of several novel P450-reductase fusion proteins in bacteria.

P450s can catalyse monooxygenation reactions in the absence of molecular oxygen when provided with organic or inorganic peroxides. This type of the monooxygenase activity of P450s, which does not need the supply of reducing equivalents by the reductase, was found in 1975 (*11*, *12*). Some P450s catalyse the rearrangement of oxygen atoms in the substrate molecules (*13*), which is also independent of the reductase.

An interesting exceptional case is P450nor (CYP55A1) discovered in the fungus *Fusarium oxysporum* in 1993 (*14*). P450nor catalyses the reduction of nitric oxide, and its haeme prosthetic group receives electrons directly from NADH (*14*).

Microsomal cytochrome P450 enzyme system

The microsomal P450 enzyme system is widespread among all eukaryotic organisms, and catalyses NADPH-dependent oxygenation of various endogenous and exogenous substrates. It consists of P450 and NADPH-P450 reductase. Both P450 and the reductase are tightly bound to the cytoplasmic surface of endoplasmic reticulum in the cells. They are synthesized by the membrane-bound ribosomes in the cell, and anchor to the membrane by their amino-terminal hydrophobic signal-anchor sequences (*15*, *16*). Membrane binding is essential for their interaction. To reconstitute the monooxygenase activity from purified P450 and NADPH-P450 reductase, their incorporation into phospholipids liposomes or detergent micelles is necessary.

Requirement of NADPH-P450 reductase for microsomal P450-catalysed oxygenation reactions was confirmed by *in vitro* reconstitution experiments using purified components, and also *in vivo* by conditional deletion of hepatic NADPH-P450 reductase. Liver-specific deletion of NADPH-P450 reductase in mice ablated the P450-dependent monooxygenase activities in the liver, although the amount of microsomal P450 was rather elevated (*17*, *18*). Since both P450 and NADPH-P450 reductase are bound to the membrane of endoplasmic reticulum, the transfer of electrons from the reductase to P450 depends on their interaction on the membrane. One possibility is the random collision of membrane-bound P450 and reductase molecules due to their free lateral movement on the membrane (*19*). Formation of molecular clustering of P450 and the reductase on the membrane is another possible mechanism for their interaction, but the molar ratio of the reductase to P450 in liver microsomes is far larger than unity; 1 mol of reductase to 10–20 mol of P450. Moreover, NADPH-P450 reductase supplies electrons not only to P450 but also to some other microsomal membrane-bound enzymes, haeme oxygenase, squalene oxygenase, etc. Evidence has been presented for the formation of molecular clustering of P450 and NADPH-P450 reductase on the liposomal and microsomal membranes (*20*, *21*), but the aggregates must be in a dynamic equilibrium between the free-moving components (*22*).

Participation of cytochrome *b5* in microsomal P450-catalysed reactions was known since early studies on this enzyme system. Although microsomal P450 depends on NADPH-P450 reductase for the supply of electrons from NADPH, addition of NADH was found to stimulate the *in vitro* NADPH-dependent oxidation of substrates by liver microsomes (*23*), and participation of cytochrome *b5* in the supply of electrons from NADH to P450 was suggested in 1971 (*24*). It was proposed that the reduction of the ferric haeme of P450 depends on NADPH-P450 reductase, while the electron for the activation of the oxygen molecule bound to the ferrous haeme of P450 can be supplied by cytochrome *b5* (*25*). However, apo-cytochrome *b5* was also effective in stimulating the NADPH-dependent oxidation of substrates in reconstituted systems (*26*, *27*). The molecular mechanism of the stimulation of microsomal P450-catalysed reactions by cytochrome *b5* is still controversial (*28–30*), although cytochrome *b5* may be regarded to be a component of the microsomal P450 enzyme system.

Presence of cytochrome *b5* in the reconstituted systems not only stimulates the NADPH-dependent monooxygenase activities of various microsomal P450s, but also affects the regioselectivity of the monooxygenase reactions catalysed by some P450s. In the case of P450c17 (CYP17A), which catalyses both 17α-hydroxylation and 17,20-lyase reaction of progesterone, the addition of cytochrome *b5* to the reconstituted systems stimulates the lyase reaction more than 17α-hydroxylation reaction (*27*, *31–33*).

In addition to cytochrome *b5*, another cytochrome *b5*-like hemoprotein was found in animal liver. Since the novel hemoprotein was localized on the outer membrane of the mitochondria, it was named OM-b in 1980 (*34*). OM-b is spectrally almost identical with cytochrome *b5*, but the amino-acid sequence of rat liver OM-b was only 58% identical with rat cytochrome *b5* (*35*). It was later found that OM-b is expressed in various animal tissues, and is present in the endoplasmic reticulum in certain tissues including adrenal cortex and testis (*36*). Involvement of OM-b in

NADPH-dependent androgen synthesis by P450c17 in rat testicular Leydig cells was reported (*36*). Participation of 'type 2 *b5*', possibly OM-b, in the 17,20-lyase activity of human P450c17 was also reported (*37*).

Microsomal P450s are retained to the endoplasmic reticulum by some retention mechanisms (*38–40*). However, several papers reported the presence of microsome-type P450s on the plasma membrane of animal cells (*41*, *42*). Under certain physiological conditions, a small fraction of microsome-type P450s seem to leave the endoplasmic reticulum to move to the cell surface possibly via the secretory pathway (*43*, *44*). However, some papers denied the presence of P450 on the cell surface (*45*). The expression of microsome-type P450s on the plasma membrane is still controversial.

Multiple molecular species of P450 co-exist on the membrane of endoplasmic reticulum in some animal tissues like liver and kidney, and the expression level of each of them is separately regulated by external and internal stimuli. Some P450s specialized in the metabolism of foreign compounds like P450 1A1 in the liver is rapidly and remarkably induced by particular xenobiotic chemicals, whereas NADPH-P450 reductase and cytochrome *b5*, other components of the microsomal P450 enzyme system, are not much affected. Apparently, microsmal P450 enzyme system is a highly flexible system that can respond to the need for new metabolic activity by changing only one component, a particular form of P450, of the enzyme system. The fluid nature of the microsomal membrane, to which the components of the enzyme system are incorporated, allows this dynamic and economical response of the enzyme system to new physiological needs.

Mitochondrial cytochrome P450 enzyme system

Several molecular species of P450 are localized in the mitochondria of animal tissue cells. They are a minor group in the total P450 family members of animals compared with the microsomal P450s. In the case of human, seven P450s are found in mitochondria, whereas the total number of P450 genes in the human genome is 57. The mitochondrial P450s form a unique branch in the phylogenic tree of animal P450s (*46*). They are generally specialized in the metabolism of endogenous steroid substrates, and each of them catalyses a site-selective reaction of specific substrates. This is in contrast with their microsomal counterparts that show rather broad substrate specificities and metabolize even xenobiotic compounds. P450 has not been found in the mitochondria of plants and fungi.

Mitochondrial P450s are bound to the matrix-side surface of the inner membrane (*47*), and their reductase system consisting of adrenodoxin and NADPH-adrenodoxin reductase are soluble in the matrix (*5*), Adrenodoxin was first isolated from adrenal cortex mitochondria in 1965 (*48*), It is a ferredoxin-type iron–sulphur protein containing one [2Fe–2S] iron–sulphur cluster in the molecule. NADPH-adrenodoxin reductase is a FAD-containing flavoprotein. All of these components of mitochondrial P450 enzyme system are coded to nuclear genes and their mRNAs are translated on cytoplasmic ribosomes to produce the precursor peptides that have cleavable mitochondria-targeting signal sequence at the amino terminus (*46*). The precursors are imported into mitochondria and proteolytically processed to mature peptides in the matrix space, and the mature P450 molecules are incorporated into the inner membrane. The molecular sizes of mitochondrial P450 precursor peptides with the cleavable mitochondria-targeting sequence, about 500 amino-acid residues, are almost the same with microsomal P450s that have uncleavable signal-anchor sequence at the amino terminus.

The binding of mitochondrial P450s to the inner membrane seems to be rather weak compared with their microsomal counterparts that are anchored to the membrane of endoplasmic reticulum by the hydrophobic *trans*-membrane signal anchor sequence. Some hydrophobic amino-acid residues in the F–G loop region of P450 molecules seem to be responsible for the binding of mitochondrial P450s to the inner membrane (*49*, *50*), although the precise mechanism of the binding is not yet elucidated.

Although adrenodoxin and NADPH-adrenodoxin reductase are recovered in the supernatant when isolated mitochondria are disrupted by sonication, adrenodoxin seems to be associated with the membrane-bound P450s in the mitochondria. Electronmicroscopic observation of adrenocortical cells showed the association of adrenodoxin and NADPH-adrenodoxin reductase with the inner membrane of mitochondria (*51*, *52*). Adrenodoxin has high affinity for P450s, and formation of molecular aggregates of adrenocortical P450s and the reductase components *in vitro* was reported (*53*, *54*). It is likely that P450s and their reductase components are associated in the mitochondria to facilitate the electron transfer.

Since the elucidation of the constitution of the P450 enzyme system of adrenal cortex mitochondria in 1967 (*5*), requirement for adrenodoxin and NADPH-adrenodoxin reductase in the NADPH-dependent monooxygenase activity had long been regarded to be an important signature of mitochondrial P450s. However, it was shown later that some mitochondrial P450s that were genetically engineered to target to endoplasmic reticulum accepted electrons from microsomal NADPH-P450 reductase when expressed in yeast cells (*55*). It was also shown that some microsomal P450s could accept electrons from adrenodoxin in reconstituted systems (*56*, *57*). Apparently mitochondrial P450s have the ability to interact with microsomal NADPH-P450 reductase, and microsomal P450s can receive electrons from adrenodoxin although less efficiently than from their natural partners.

The subcellular distribution of mitochondrial and microsomal P450s are usually strictly regulated in the cells, but presence of some microsome-type P450s in mitochondria has often been reported (*58–60*). It seems that a small portion of microsome-type P450s are missorted in the cells and imported into

mitochondria under certain physiological conditions. The imported microsome-type P450s seem to be enzymatically active in mitochondria (*58*, *61*), but the physiological significance of the presence of microsome-type P450s in mitochondria is not yet clearly understood.

An exceptional cytochrome P450, P450nor (CYP55)

An eukaryotic P450 with exceptional properties, P450nor, was found in the fungus *Fusarium oxysporum* in 1993, and named from its NADH-dependent nitric oxide reductase activity (*13*). It is a soluble P450, and is distributed between cytoplasm and mitochondria in the fungal cells, although both forms are coded to the same single gene (*62*). It receives electrons for nitric oxide reduction directly from NADH without participation of any NADH-linked reductase or reducing system. The direct transfer of electrons from NADH to the haeme of P450nor was confirmed by kinetic experiments and also by X-ray crystallographic analysis of the molecular structure of P450nor (*63*, *64*). P450nor-like soluble P450s have later been found in the yeast *Trichosporon cutaneum* (*65*, *66*) and in the fungi *Cylindrocarpon tonkinense* (*67*) and *Histoplasma capsulatum* (*68*). They show significant amino-acid sequence homology with *Fusarium* P450nor and also catalyse NADH-dependent nitric oxide reduction.

Bacterial cytochrome P450 enzyme system

After successful characterization of the soluble camphor-oxidizing P450 enzyme system of *Pseudomonas putida* in 1968 (*8*), similar soluble P450 enzyme systems have been found in several other bacterial species and characterized (*69–73*). They are soluble enzyme systems consisting of P450, ferredoxin and NADH–ferredoxin reductase. The three components constitution of the bacterial P450 system is seemingly similar to the mitochondrial P450 system in animals, but the bacterial P450 systems generally prefer NADH as the electron donor for the monooxygenation reactions whereas the animal mitochondrial system is NADPH-dependent. Recently, a novel P450 enzyme system was found in *Citrobacter braakii*. When grown on cineole as the sole source of carbon and energy, *C. braakii* expresses P450cin (CYP176) (*74*), which receives electrons for cineole hydroxylation from cindoxin, an FMN-containing flavoprotein, not from ferredoxin (*75*).

It was initially thought that the bacterial P450s are specialized in the metabolism of some particular foreign substrates, camphor in the case of *Pseudomonas putida*, to utilize them as the energy and carbon sources, and the P450s are encoded to plasmids. Since some bacterial species including the model gram-negative bacterium *Escherichia coli* were found to be devoid of P450, distribution of P450 in bacteria was regarded to be limited. However, more recent studies have revealed the presence of many P450s encoded to chromosomal genes in various bacterial species; 20 P450 genes in *Mycobacterium tuberculosis* (*76*), nine P450 genes in *Bacillus subtilis* (*77*), 18 P450 genes in *Streptomyces versicolor* (*78*), etc. Apparently, P450 is more widely distributed among bacteria than was previously thought.

P450 has also been found in some archaea (*79*, *80*). Archean P450s are also soluble in the cytoplasm, and generally heat resistant (*81*). The P450 enzyme system of the archaeon *Sulfolobus sulfataricus* utilizes pyruvate as the electron donor. It is a three components system consisting of P450 (CYP119), ferredoxin and 2-oxoacid-ferredoxin oxidoreductase (*82*). This is an exceptional case in the P450 enzyme systems that usually utilize NADPH or NADH as the electron donor.

Elucidation of the physiological functions of these P450s discovered from the analysis of bacterial genomes has proved to be difficult. Out of the twenty P450 genes of *M. tuberculosis*, one was found to code a P450 similar to eukaryotic CYP51A (*82*) that catalyses the 14α-demethylation of lanosterol in animals and the 14α-demethylation of obtusifoliol in plants. The *M. tuberculosis* '*Cyp51*-like gene' was expressed in *E. coli*, and was found to catalyse the 14α-demethylation of lanosterol and obtusifoliol (*83*). However, sterols have not been found in the lipid components of *M. tuberculosis* cells. The physiological function of the *Cyp51*-like gene is still unknown. *Streptomyces versicolor* also has a *Cyp51*-like gene, but the gene was not essential for the viability of the bacteria, and sterol was not detected in the bacterial cells (*84*). The P450 of *Rhizobium* was the first bacterial P450 reported in 1967 (*7*), and the genes-coding three P450s (*Cyp112*, *Cyp114* and *Cyp117*) were identified in 1998 (*85*), but the enzymatic activities and physiological functions of the P450s are still unknown. Gene disruption experiments suggested that the P450s are not needed for the symbiosis and the formation of root nodules (*86*).

Bacterial P450s are soluble in the cytoplasm, and their molecular sizes, about 400 amino-acid residues long, are significantly smaller than microsomal or mitochondrial membrane-bound P450s. However, comparison of the amino-acid sequences of bacterial and eukaryotic P450s has confirmed that all of them have originated and diversified from a single common ancestor. The soluble nature of bacterial P450s is due to the absence of the amino-terminal portion of eukaryotic P450s where the membrane-anchoring sequence resides. Bacterial cells do not have extensive intracellular membrane systems like the endoplasmic reticulum in eukaryotic cells to anchor P450s. The lack of intracellular membrane systems in prokaryotic cells may be correlated with the soluble nature of bacterial P450s.

Cytochrome P450-reductase fusion enzymes

Several P450-reductase fusion proteins have been found in bacteria. The first one was P450 BM-3 (CYP102A1) found in *Bacillus megaterium* in 1983 (*9*). It was a fusion protein of P450 and NADPH-dependent P450 reductase containing FAD and FMN (*9*) and catalysed ω-1-, ω-2- and ω-3-hydroxylations of long chain fatty acids and alcohols. The reductase domain was fused to the carboxy

terminus of the P450 domain. Interestingly, the amino-acid sequence of the P450 domain showed significant homology with animal P450s, and the reductase domain was also significantly homologous with mammalian NADPH-P450 reductase (*87*, *88*), suggesting their eukaryotic origin. In the following years, P450 BM-3-type fusion proteins have been found in some other bacteria (*89*) and also in the fungus *Fusarium oxysporum* (*90*). The *Fusarium* counterpart of P450 BM-3, P450foxy (CYP505), is membrane-bound, whereas the bacterial fusion proteins are soluble. A similar membrane-bound P450 BM-3-type fusion protein has been found in the fungus *Fusarium verticillioides* (*91*).

One of the remarkable properties of P450 BM-3 is its high activity in fatty-acid hydroxylation. The turnover number of the P450 BM-3-catalysed hydroxylation reaction is 4,000–5,000 nmol of fatty acid hydroxylated per nanomole of P450 per minute (*92*), which is two orders of magnitude higher than the P450s of eukaryotic organisms. The efficient transfer of electrons from the flavins of the reductase domain to the haeme of the P450 domain of the molecule has been shown to be responsible for this rapid catalysis of the reaction. P450 BM-3 is active only in the homodimeric form, and the electrons are transferred intermolecularly from the FAD of the reductase domain to the FMN in the reductase domain of the partner molecule of the dimer. The electron transfer from FMN to the haeme of P450 is intramolecular. (*93*).

Other types of P450-reductase fusion proteins have been found in some bacterial species. *Rhodococcus sp. NCIMB9784* contained a soluble NADH-dependent P450 enzyme system. It was a fusion protein of P450 (P450 RhF) and an NADH-linked reductase domain that contained FMN and [2Fe–2S] iron–sulphur center (*94*).

Incited by the remarkable high activity of the fusion enzyme P450 BM-3, many artificial P450-reductase fusion proteins have been constructed by genetic engineering. P450 1A1–NADPH–P450 reductase fusion protein (*95*), P450scc-adrenodoxin-adrenodoxin reductase fusion protein (*96*), P450cam-putidaredoxin-putidaredoxin reductase fusion protein (*97*), etc. They were heterologously expressed in microbial systems, and most of them showed significantly higher catalytic activities compared with the original P450s.

Cytochrome P450-catalysed reactions that do not require the supply of electrons

P450-catalysed oxygenation reactions can be supported by inorganic or organic peroxides. Neither molecular oxygen nor the supply of electrons from NAD(P)H to P450 is necessary. The peroxide-dependent oxygenation activity of P450 was first shown in 1975 for the *O*-dealkylation of several drugs by liver microsomes in the presence of cumene hydroperoxide (*11*), and for the hydroxylation of steroids by adrenal cortex microsomes supported by sodium periodate (*12*). The molecular mechanism of the peroxide-dependent reactions catalysed by P450s was studied in the following years, and proved to involve the formation of hydroperoxo intermediate via a short cut pathway in the P450-catalysed reaction cycle of substrate oxygenation. Hydrogen peroxide can also support the P450-catalysed oxygenation reactions, and some P450s specifically require hydrogen peroxide for their catalytic activity. Two bacterial P450s, CYP152A1 of *B. subtilis* and CYP152B1 of *Sphingomonas paucimobilis*, catalyse hydrogen peroxide-dependent α- and β-hydroxylation of fatty acids (*98*). It was proposed to call them peroxygenase rather than monooxygenase (*98*).

Some P450s catalyse the re-arrangement of the oxygen atoms in the substrate molecules. Synthesis of prostacyclin from prostaglandin H2 is a microsomal P450 (CYP8A1)-catalysed reaction (*13*). Thromboxan A2 is also synthesized from prostaglandin H2 by the catalysis of a microsmal P450 (CYP5A) (*99*). These reactions are rearrangements of the substrate molecules, and do not require the supply of electrons to P450.

Plant allene oxide synthases are P450s (CYP74), which catalyse the conversion of fatty-acid hydroperoxides to allene epoxides without the supply of reducing equivalents (*99*, *100*). The primary structures of allene oxide synthases showed the presence of a chloroplast transit peptide at the amino-terminus (*100*, *101*).

Evolution and diversification of cytochrome P450 enzyme system

Comparison of the amino-acid sequences of eukaryotic and prokaryotic P450s has led to the conclusion that the P450 gene superfamily has evolved and diversified from a single ancestral gene. Recent analyses of the genomes of various organisms have enabled the construction of phylogenic trees of eukaryotic P450s. The data generally support the notion that CYP51, which catalyses the 14α-demethylation of sterol precursors, is the oldest form of P450 in eukaryotic organisms (*102*). Since sterols are essential constituents of the cell membrane of animals, fungi and plants, the origin of P450 is at least as old as the emergence of eukaryotic organisms in the history of life, and the microsomal P450 system is possibly the oldest form of the P450 enzyme systems in the eukaryotes. Presence of various intracellular membrane systems including endoplasmic reticulum, where the microsomal P450 system resides, is a characteristic feature of eukaryotic cells.

Several P450s are present in the mitochondria of animal tissues. They form a unique branch in the phylogenic tree of animal P450s, and catalyse the oxygenation reactions of cholesterol, steroids and vitamin D (*46*). Since P450 has not been found in the mitochondria of plants and fungi, P450 was possibly introduced into mitochondria after animal lineage diverged from plants and then from fungi during the evolution of eukaryotic organisms. Translocation of a P450 from endoplasmic reticulum to mitochondria due to a mutation (or mutations) of the organelle-targeting signal at the amino terminus is a likely story. The P450

imported into mitochondria found a suitable electron donor, adrenodoxin, in the organelle to enable the P450 function in the new intracellular compartment, and then diversified to several mitochondrial P450s during the evolution of animals. Adrenodoxin and adrenodoxin reductase are also present in yeast (*103*, *104*), and have been shown to function in the synthesis of iron–sulphur cluster in mitochondria (*105*), which is an essential prosthetic group of several enzymes including some of the components of the mitochondrial respiratory chain. It is likely that ancient eukaryotic organisms acquired the genes-encoding adrenodoxin and its reductase from the prokaryotic symbiont that became mitochondria.

The evolutional relationship between prokaryotic and eukaryotic P450s is not clear. Whether or not the most ancestral P450 gene, from which all of the present P450 genes were derived, was present in some prokaryotic organism before the emergence of eukaryotes is still an open question. High mutation rates of bacterial genes and possible horizontal gene transfer from eukaryotic organisms to prokaryotes make this problem difficult to analyse. P450 BM-3 in bacteria is possibly eukaryotic origin (*86*, *87*). In the case of CYP51, one paper (*102*) suggested its prokaryotic origin, whereas another paper proposed that *Mycobacterium tuberuculosis* obtained its *Cyp51*–like gene from some eukaryotic organism, possibly from a plant, by horizontal gene transfer (*106*).

P450 genes have diversified by gene duplications and mutations to many genes coding different molecular species of P450, whereas the partner of the microsomal P450 enzyme system NADPH-P450 reductase is coded to a single gene in animals (*107*). Plants and fungi seem to have two genes-encoding NADPH-P450 reductase (*108*, *109*). The reductase is apparently a fusion enzyme produced by the fusion of an FAD-containing flavoprotein and an FMN-containing flavoprotein (*110*). The reductase supplies electrons not only to P450s but also to some microsomal enzymes, haeme oxygenase, squalene epoxidase, etc., and is essential for the embryonic development of animals since the disruption of the gene coding NADPH-P450 reductase is embryonic lethal in mice (*107*). The evolutional origin of NADPH-P450 reductase, the partner of microsomal P450s, is also an interesting problem to be clarified.

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References

1. Omura, T., and Sato, R. (1962) A new cytochrome in liver microsomes. *J. Biol. Chem.* **237**, PC1375–PC1376
2. Estabrook, R.W., Cooper, D.Y., and Rosenthal, O. (1963) The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex. *Biochem. Z.* **338**, 741–755
3. Lu, A.Y.H., and Coon, M.J. (1968) Role of hemoprotein P-450 in fatty acid ω-hydroxylation in a soluble enzyme system from liver microsomes. *J. Biol. Chem.* **243**, 1331–1332
4. Harding, B.W., Wong, S.H., and Nelson, D.H. (1964) Carbon monoxide-combining substances in rat adrenal. *Biochim. Biophys. Acta.* **92**, 415–417
5. Omura, T., Sanders, E., Estabrook, R.W., Cooper, D.Y., and Rosenthal, O. (1966) Isolation from adrenal cortex of a nonheme iron protein and a flavoprotein functional as a reduced triphosphopyridine nucleotide-cytochrome P-450 reductase. *Arch. Biochem. Biophys.* **117**, 660–673
6. Watson, C.J.W., Froelich, J.E., Josefsson, C.A., Chapple, C., Durst, F., Benveniste, I., and Coolbaugh, R.C. (2001) Localization of CYP86B1 in the outer envelope of chloroplasts. *Plant Cell Physiol.* **42**, 873–878
7. Appleby, C.A. (1967) A soluble haemoprotein P-450 from nitrogen-fixing Rhizobium bacteroids. *Biochim. Biophys. Acta* **147**, 399–402
8. Katagiri, M., Ganguli, B.N., and Gunsalus, I.C. (1968) A soluble cytochrome P-450 functinonal in methylene hydroxylation. *J. Biol. Chem.* **243**, 3543–3546
9. Cardini, G., and Jurtshuk, P. (1968) Cytochrome P-450 involvement in the oxidation of n-octane by cell-free extracts of *Corynebacterium sp. Strain 7E1C*. *J. Biol. Chem.* **243**, 6070–6072
10. Narhi, L.O., and Fulco, A.J. (1987) Identification and characterization of two functional domains in cytochrome P-450 BM-3, a catalytically self-sufficient monooxygenase induced by bartiturates in *B. metgatelium*. *J. Biol. Chem.* **262**, 6683–6690
11. Rahintula, A.D., and O'Brien, P.J. (1975) Hydroperoxide dependent O-dealkylation reactions catalyzed by liver microsomal cytochrome P-450. *Biochem. Biophys. Res. Commun.* **62**, 268–275
12. Hrycay, E.G., Gustafsson, J., Ingelman-Sundberg, M., and Ernster, L. (1975) Sodium periodate, sodium chlorite, organic hydroperoxide, and H2O2 as hydroxylating agents in steroid hydroxylation reactions catalyzed by partially purified cytochrome P-450. *Biochem. Biophys. Res. Commun.* **66**, 209–216
13. DeWitt, D.J., and Smith, W.L. (1983) Purification of prostacyclin synthase from bovine aorta by immunoaffinity chromatography. Evidence that the enzyme is hemoprotein. *J. Biol. Chem.* **258**, 3285–3293
14. Nakahara, K., Tanimoto, T., Hatano, K., Usuda, K., and Shoun, H. (1993) Cytochrome P-450 55A1 (P-450 dNIR) acts as nitric oxide reductase employing NADH as the direct electron donor. *J. Biol. Chem.* **268**, 8350–8355
15. Sakaguchi, M., Mihara, K., and Sato, R. (1987) A short amino-terminal segment of microsomal cytochrome P-450 functions both as an insertion signal and as a stop-transfer sequence. *EMBO J.* **6**, 2425–2431
16. Kida, Y., Ohgiya, S., Mihara, K., and Sakaguchi, M. (1998) Membrane topology of NADPH-cytochrome P450 reductase on the endoplasmic reticulum. *Arch. Biochem. Biophys.* **351**, 175–179
17. Henderson, C.J., Otto, D.M., Carie, D., Magnuson, M.A., Laren, A.W., Rosewell, I., and Wolf, C.R. (2003) Inactivation of hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *J. Biol. Chem.* **278**, 13480–13486
18. Gu, J., Weng, Y., Zhang, Q.Y., Cui, H., Behr, M., Wu, L., Yang, W., and Zhang, L. (2003) Liver-specific deletion of the NADPH-cytochrome P450 reductase gene. Impact on plasma cholesterol homeostasis and the function and regulation of microsomal cytochrome P450 and heme oxygenase. *J. Biol. Chem.* **278**, 25895–25910

19. Richter, C., Winterhalter, K.H., and Cherry, R.J. (1979) Rotational diffusion of cytochrome P-450 in Rat Liver Microsomes. *FEBS Lett*. **102**, 151–154
20. Gut, J., Richter, C., Cherry, R.J., Winterhalter, K.H., and Kawato, S. (1982) Rotation of cytochrome P-450. II. Specific interactions of cytochrome P-450 reductase in phospholipid vesicles. *J. Biol. Chem*. **257**, 7030–7036
21. Kuznetsov, V.Y., Ivanov, Y.D., and Archakov, A.I. (2004) Atomic force microscopy revelation of molecular complexes in the multiprotein cytochrome P450 2B4 containing system. *Proteomics*. **4**, 2390–2396
22. Miwa, G.T., and Lu, A.Y.H. (1984) The association of cytochrome P-450 and NADPH-cytochrome P-450 reductase in phospholipids membranes. *Arch. Biochem. Biophys*. **234**, 161–166
23. Cohen, B.S., and Estabrook, R.W. (1971) Microsomal electron transport reactions. II. The use of reduced triphosphopyridine nucleotide and/or reduced diphosphopyridine nucleotide for the oxidative N-demethylation of aminopyrine and other drug substrates. *Arch. Biochem. Biophys*. **143**, 46–53
24. Hildebrandt, A., and Estabrook, R.W. (1971) Evidence for the participation of cytochrome *b5* in hepatic microsomal mixed-function oxidation reactions. *Arch. Biochem. Biophys*. **143**, 66–79
25. Noshiro, M., Ullrich, V., and Omura, T. (1981) Cytochrome *b5* as electron donor for oxy-cytochrome P-450. *Eur. J. Biochem*. **116**, 521–526
26. Yamazaki, H., Johnson, W.W., Ueng, Y.F., Shimada, T., and Guengerich, F.P. (1996) Lack of electron transfer from cytochrome *b5* in stimulation of catalytic activities of cytochrome P450 3A4. *J. Biol. Chem*. **271**, 27438–27444
27. Auchus, R.J., Lee, T.C., and Miller, W.L. (1998) Cytochrome *b5* augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J. Bio. Chem*. **273**, 3158–3165
28. Guryev, O.L., Gilep, A.A., Usanov, S.A., and Estabrook, R.W. (2001) Interaction of apo- cytochrome *b5* with cytochrome P450 3A4 and P450 17A Relevance of heme transfer reactions. *Biochemistry*. **40**, 5018–5031
29. Yamazaki, H., Shimada, T., Martin, M.V., and Guengerich, F.P. (2001) Stimulation of cytochrome P450 reactions by apo- cytochrome *b5*: Evidence against transfer of heme from cytohrome P450 3A4 to apocytochrome *b5* or heme oxygenase. *J. Biol. Chem*. **276**, 30885–30891
30. Reed, J.R., and Hollenberg, P.F. (2003) Examining the mechanism of stimulation of cytochrome P450 by cytochrome *b5*: The effect of cytochrome *b5* on the interaction between cytochrome P450 2B4 and P450 reductase. *J. Inorg. Biochem*. **97**, 265–275
31. Onoda, M., and Hall, P.F. (1982) Cytochrome *b5* stimulates purified testicular microsomal cytochrome P-450 (C-21 side-chain cleavage). *Biochem. Biophys. Res. Commun*. **108**, 454–460
32. Kominami, S., Ogawa, N., Morimune, R., Huang, D.Y., and Takemori, S. (1992) The role of cytochrome *b5* in adrenal microsomal steroidogenesis. *J. Steroid Biochem. Mol. Biol*. **42**, 57–64
33. Katagiri, M., Kagawa, N., and Waterman, M.R. (1995) The role of cytochrome *b5* in the biosynthesis of androgens by human P450c17. *Arch. Biochem. Biophys*. **317**, 343–347
34. Ito, A. (1980) Cytochrome *b5*-like hemoprotein of outer mitochondrial membrane, OM cytochrome. *b. J. Biochem*. **87**, 63–71
35. Lederer, F., Ghrir, R., Guiard, B., Cortial, S., and Ito, A. (1983) Two homologous cytochrome *b5* in a single cell. *Eur. J. Biochem*. **132**, 95–102
36. Ogishima, T., Kinoshita, J., Mitani, F., Suematsu, M., and Ito, A. (2003) Identification of outer mitochondrial cytochrome *b5* as a modulator for androgen synthesis in Leydig cells. *J. Biol. Chem*. **278**, 21204–21211
37. Soucy, P., and Luu-The, V. (2002) Assessment of the ability of type 2 cytochrome *b5* to modulate 17, 20-lyase activity of human P450c17. *J. Steroid Biochem. Mol. Biol*. **80**, 71–75
38. Murakami, K., Mihara, K., and Omura, T. (1994) The transmembrane region of microsomal cytochrome P450 identified as the endoplasmic reticulum retention signal. *J. Biochem*. **116**, 164–175
39. Szeczesna-Skorupa, E., Ahn, K., Chen, C.D., Doray, B., and Kemper, B. (1995) The cytoplasmic and N-terminal transmembrane domains of cytochrome P450 contain independent signals for retention in the endoplasmic reticulum. *J. Biol. Chem*. **270**, 24327–24333
40. Szczesna-Skorpa, E., and Kemper, B. (2000) Endoplasmic retention determinants in the transmembrane and linker domains of cytochrome P450 2C1. *J. Biol. Chem*. **275**, 19409–19415
41. Amarneh, B.A., and Simpson, E.R. (1996) Detection of aromatase cytochrome P450, 17α-hydroxylase cytochrome P450 and NADPH-P450 reductase on the surface of cells in which they are expressed. *Mol. Cell. Endocrinol*. **119**, 69–74
42. Neve, E.P.A., and Ingelman-Sundberg, M. (2000) Molecular basis for the transport of cytochrome 2E1 to the plasma membrane. *J. Biol. Chem*. **275**, 17130–17135
43. Robin, M.A., Maratrat, M., Loeper, J., Durand-Schneider, A.M., Tinet, M., Ballet, F., Beaune, P., Feldman, G., and Passayre, D. (1995) Cytochrome P450 2B follows a vesicular route to the plasma membrane in cultured rat hepatocytes. *Gastroenterol*. **108**, 1110–1123
44. Robin, M.A., Maratrat, M., LeRoy, M., LeBretton, F.P., Bonierbale, E., Dansette, P., Ballet, F., Mansuy, D., and Passayre, D. (1996) Antigenic targets in Tienillic acid hepatitis: Both cytochrome P450 2C11 and 2C11-tienillic acid adducts are transported to the plasma membrane of rat hepatocytes and recognized by human sera. *J. Clin. Invest*. **98**, 1471–1480
45. Yamamoto, A.M., Mura, C., De Lemos-Chiarandini, C., Krishnamoorthy, R., and Alvarez, F. (1993) Cytochrome IID6 recognized by LKM1 antibody is not exposed on the surface of hepatocytes. *Clin. Exp. Immunol*. **92**, 381–390
46. Omura, T. (2006) Mitochondrial P450s. *Chemico-Biol. Interaction* **163**, 86–93
47. Churchill, P.F., and Kimura, T. (1979) Topological studies of cytochrome P-450scc andnP-45011β in bovine adrenocortical inner mitochondrial membranes. Effects of controlled tryptic digestion. *J. Biol. Chem*. **254**, 10443–10448
48. Suzuki, k., and Kimura, T. (1965) An iron protein as a component of steroid 11β-hydroxylase complex. *Biochem. Biophys. Res. Commun*. **19**, 340–345
49. Murtazina, D., Puchkaev, A.V., Schein, C.H., Oezguen, N., Braun, W., Nanavati, A., and Pikuleva, I.A. (2002) Membrane-protein interactions contribute to efficient 27-hydroxylation of cholesterol by mitochondrial cytochrome P450 27A1. *J. Biol. Chem*. **277**, 37582–37589
50. Headlam, M., Wilce, M.C.J., and Tuckey, R.C. (2003) The F-G loop region of cytochrome P450scc (CYP11A1) interact with the phospholipids membrane. *Biochim. Biophys. Acta*. **1617**, 96–108

51. Mitani, F., Ishimura, Y., Izumi, S., and Watanabe, K. (1979) Immunohistochemical localization of adrenodoxin and adrenodoxin reductase in bovine adrenal cortex. *Acta Endocrinol.* **90**, 317–327
52. Hatano, O., Sagara, Y., Omura, T., and Takakusu, A. (1989) Immunohistochemical localization of adrenodoxin in bovine adrenal cortex by protein A-gold technique. *Histochem.* **91**, 89–97
53. Lambeth, J.D., Geren, L.M., and Millett, F. (1984) Adrenodoxin interaction with adrenodoxin reductase and cytochrome P450scc. Cross-linking of protein complexes and effects of adrenodoxin modification by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. *J. Biol. Chem.* **259**, 10025–10029
54. Hara, T., and Kimura, T. (1989) Active complex between adrenodoxin reductase and adrenodoxin in the cytochrome P450scc reduction reaction. *J. Biochem.* **105**, 601–605
55. Sakaki, T., Kominami, S., Hayashi, K., Akiyoshi-Shibata, M., and Yabusaki, Y. (1996) Molecular engineering study on electron transfer from NADPH-P450 reductase to rat mitochondrial P450c27 in yeast microsomes. *J. Biol. Chem.* **271**, 26209–26213
56. Lehnerer, M., Schulze, J., Bernhardt, R., and Hlavica, P. (1999) Some properties of mitochondrial adrenodoxin associated with its non-mitochondrial electron donor functional toward rabbit liver microsomal cytochrome P450 2B4. (1999). *Biochem. Biophys. Res. Coomun.* **254**, 83–87
57. Pechurskaya, T.A., Harastai, I.N., Grabovec, I.P., Gilep, A.A., and Usanov, S.A. (2007) Adrenodoxin supports reactions catalyzed by microsomal steroidogenic cytochrome P450s. *Biochem. Biophys. Res. Commun.* **353**, 598–604
58. Honkakoski, P., Kojo, A., Raunio, M., Pasanen, R., Juvonen, R., and Lang, M.A. (1988) Hepatic mitochondrial coumarin 7-hydroxylase; comparison with the microsomal enzyme. *Arch. Biochem. Biophys.* **267**, 558–567
59. Niranjan, B.G., Raza, H., Shayig, R.M., Jefcoate, C.R., and Avadhani, N.G. (1988) Hepatic mitochondrial cytochrome P450 system. Identification and characterization of a precursor form of mitochondrial cytochrome P450 induced by 3-methylcholanthrene. *J. Biol. Chem.* **263**, 575–580
60. Genter, M.B., Clay, C.D., Dalton, T.P., Dong, H., Nebert, D.W., and Schertzer, H.G. (2006) Comparison of mouse hepatic mitochondrial versus microsomal cytochromes P450 following TCDD treatment. *Biochem. Biophys. Res. Commun.* **342**, 1375–1381
61. Anandatheerthavarada, H.K., Vijayasarathy, C., Bhagwat, S.V., Biswas, G., Mullick, J., and Avadhani, N.G. (1999) Physiological role of the N-terminal processed P450 1A1 targeted to mitochondria in erythromycin metabolism and reversal of erythromycin-mediated inhibition of mitochondrial protein synthesis. *J. Biol. Chem.* **274**, 6617–6625
62. Nakahara, K., and Shoun, H. (1996) N-terminal processing and amino acid sequence of two isoforms of nitric oxide reductase cytochrome P450nor from Fusarium oxysporum. *J. Biochem.* **120**, 1082–1087
63. Umemura, M., Su, F., Takaya, N., Shiro, Y., and Shoun, H. (2004) D88A mutant of cytochrome P450nor provides kinetic evidence for direct complex formation with electrondonor NADH. (2004) *Eur. J. Biochem.* **271**, 2887–2894
64. Oshima, R., Fushinobu, S., Su, F., Zhang, L., Takaya, N., and Shoun, H. (2004) Structural evidence for direct hydride transfer from NADH to cytochrome P450nor. *J. Mol. Biol.* **342**, 207–217
65. Yang, Y., Zhang, D., and Cerniglia, C.E. (1997) Purification and characterization of a cytosolic cytochrome P450 from the yeast *Trichosporon cutaneum*. *FEMS Microbiol. Lett.* **154**, 347–353
66. Stuendl, U.M., Schmidt, I., Scheller, U., Schmid, R., Schunk, W.H., and Schauer, F. (1998) Purification and characterization of cytosolic cytochrome P450 forms from yeasts belonging to the genus *Trichosporon*. *Arch. Biochem. Biophys.* **357**, 131–136
67. Toritsuka, N., Shoun, H., Singh, U.P., Park, S.Y., Iizuka, T., and Shiro, Y. (1997) Functional and structural comparison of nitric oxide reductases from denitrifying fungi *Cylindrocarpon tonkinense* and *Fusarium oxysporum*. *Biochem. Biophys. Acta.* **1338**, 93–99
68. Nittler, M.P., Hocking-Murray, D., Foo, C.K., and Sil, A. (2005) Identification of *Histoplasma capsulatum* transcripts induced in response to reactive nitrogen species. *Mol. Biol. Cell.* **16**, 4792–4813
69. Dardas, A., Gal, D., Barrelle, M., Sauret-Ignazi, G., Sterjiades, R., and Pelmont, J. (1985) The demethylation of guaiacol by a new bacterial cytochrome P-450. *Arch. Biochem. Biophys.* **236**, 585–592
70. Mueller, R., Asperger, O., and Kleber, H.P. (1989) Purification of cytochrome P-450 from n-hexadecane-grown *Acinetobacter calcoaceticus*. *Biomed. Biochim. Acta.* **48**, 243–254
71. Trower, M.K., Sariaslani, F.S., and O'Keefe, D.P. (1989) Purification and characterization of a soybean flour-induced cytochrome P-450 from *Streptomyces griseus*. *J. Bact.* **171**, 1781–1787
72. Ullah, A.J.H., Murray, R.I., Bhattachuaryya, P.K., Wagner, G.C., and Gunsalus, I.C. (1990) Protein components of a cytochrome P-450 linalool 8-methyl hydroxylase. *J. Biol. Chem.* **265**, 1345–1351
73. Peterson, J.A., Lu, J.Y., Griesselsoder, J., Graham-Lorence, S., Carmona, C., Witney, F., and Lorence, M.C. (1992) Cytochrome P-450terp. Isolation and purification of the protein and cloning and sequenceing of its operon. *J. Biol. Chem.* **267**, 14193–14203
74. Hawkes, D.B., Adams, G.W., Burlingame, A.L., Ortiz de Montellano, P.R., and DeVoss, J.J. (2002) Cytochrome P450cin (CYP176A), isolation, expression, and characterization. *J. Biol. Chem.* **277**, 27725–27732
75. Kimmich, N., Das, A., Sevrioukova, I., Macharenna, Y., Sligar, S.G., and Poulos, T.L. (2007) Electron transfer between cytochrome P450cin and its FMN-containing redox partner, cindoxin. *J. Biol. Chem.* **282**, 27006–27011
76. McLean, K.J., Clift, D., Lewis, D.G., Sabri, M., Balding, P.R., Sutcliffe, M.J., Leys, D., and Munro, A.W. (2006) The preponderance of P450s in the *Mycobacterium tuberculosis* genome. *Trends Microbiol.* **14**, 220–228
77. Lawson, R.J., Leys, D., Sutcliffe, M.J., Kemp, C.A., Cheesman, M.R., Smith, S.J., Clarkson, J., Smith, W.E., Haq, I., Perkinas, J.B., and Munro, A.W. (2004) Thermodynamic and biophysical characterization of cytochrome P450Biol from *Bacillus subtilis*. *Biochemistry.* **43**, 12410–12426
78. Lamb, D.C., Skaug, T., Song, H.L., Jackson, C.J., Produst, L.M., Waterman, M.R., Kell, D.B., Kelly, D.E., and Kelly, S.L. (2002) The cytochrome P450 complement (CYPone) of *Streptomyces versicolor* A3. *J. Biol. Chem.* **277**, 24000–24005
79. Wright, R.L., Harris, K., Solow, B., White, R.H., and Kennelly, P.J. (1996) Cloning of a potential cytochrome

P450 from the archaeon Sulfolobus solfataricus. *FEBS Lett*. **384**, 235–239
80. Ho, W.W., Li, H., Nishida, C.R., Ortiz de Montellano, P.R., and Poulos, T.L. (2008) Crystal structure and properties of CYP231A2 from the thermoacidophilic archaeon *Picrophilus torridus*. *Biochemistry*. **47**, 2071–2079
81. McLean, M.A., Maves, S.A., Weiss, K.E., Krepich, S., and Sligar, S.C. (1998) Characterization of a cytochrome P450 from the acidothermophilic archae *Sulfolobus sulfataricus*. *Biochem. Biophys. Res. Commun*. **252**, 166–172
82. Puchkaev, A.V., and Ortiz de Montellano, P.R. (2005) The *Sulfolobus sulfataricus* electron donor partners of thermophilic CYP119: An unusual non-NAD(P)H-dependent cytochrome P450 system. *Arch. Biochem. Biophys*. **434**, 169–177
83. Bellamine, A., Mangla, A.T., Nes, W.D., and Waterman, M.R. (1999) Characterization and catalytic properties of the sterol 14α-demethylase from *Mycobacterium tuberculosis*. *Proc. Natl Acad. Sci. USA* **96**, 8937–8942
84. Lamb, D.C., Fowler, K., Kieser, T., Manning, N., Podust, L.M., Waterman, M.R., Kelly, D., and Kelly, S.L. (2002) Sterol 14a-demethylase activity in *Streptomyces coelicolor* A3 (2) is associated with an unusual member of the CYP51 gene family. *Biochem. J*. **364**, 555–562
85. Tully, R.E., van Berkum, P., Lovins, K.W., and Keister, D.L. (1998) Identification and sequencing of a cytochrome P450 gene cluster from *Bradyrhizobium japonicum*. *Biochim. Biophys. Acta*. **1398**, 243–255
86. Tully, R.E., and Keister, D.L. (1993) Cloning and mutagenesis of a cytochrome P-450 locus from *Bradyrhizobium japonicum* that is expressed anaerobically and symbiotically. *App. Environ. Microbiol*. **59**, 4136–4142
87. Narhi, L.O., Kim, B.H., Stevenson, P.M., and Fulco, A.J. (1983) Partial characterization of a barbitrate-induced cytochrome P-450-dependent fatty acid monooxygenase from *Bacillus magaterium*. *Biochem. Biophys. Res. Commun*. **116**, 851–858
88. Ruettinger, R.T., Wen, L.P., and Fulco, A.J. (1989) Coding nucleotide, 5′-regulatory, and deduced amino acid sequences of P-450 BM-3, a single peptide cytochrome P-450:NADPH-P-450 reductase from *Bacillus magaterium*. *J. Biol. Chem*. **264**, 10987–10995
89. De Mot, R., and Parret, A.H.A. (2002) A novel class of self-sufficient cytochrome P450\monooxygenases in prokaryotes. *Trends Microbiol*. **10**, 502–508
90. Kitazume, T., Takaya, N., Nakayama, N., and Shoun, H. (2000) *Fusarium oxysporum* fatty acid subterminal hydroxylase (CYP505) is a membrane-bound eukaryotic counterpart of *Bacillus megaterium* cytochrome P450BM3. *J. Biol. Chem*. **275**, 39734–39740
91. Seo, J.A., Proctor, R.H., and Plattner, R.D. (2001) Characterization of four clustered and coregulated genes associated with fumonisin biosynthesis in *Fusarium vercillioides*. *Fungal Genet. Boil*. **34**, 155–165
92. Nahri, L.O., and Fulco, A.J. (1986) Characterization of a catalytically self-sufficient 119,000 dalton cytochrome P-450 monooxygenase induced by barbitrates in *Bacillus megaterium*. *J. Biol. Chem*. **261**, 7160–7169
93. Kitazume, T., Haines, D.C., Estabrook, R.W., Chen, B., and Peterson, J.A. (2007) Obligatory intermolecular electron-transfer from FAD to FMN in dimeric P450 BM-3. *Biochemistry*. **46**, 11892–11901
94. Roberts, G.A., Celik, A., Hunter, D.J.B., Ost, T.W.B., White, J.H., Chapmen, S.K., Turner, N.J., and Flitsch, S.L. (2003) A self-sufficient cytochrome P450 with a primary structural organization that includes a flavin domain and a [2Fe − 2S] redox center. *J. Biol. Chem*. **278**, 48914–48920
95. Sakaki, T., Kominami, S., Takemori, S., Ohkawa, H., Ariyoshi-Shibata, M., and Yabusaki, Y. (1994) Kinetic studies on a genetically engineered fused enzyme between rat cytochrome P450 1A1 and yeast NADPH-P450 reductase. *Biochemistry*. **33**, 4933–4939
96. Harikrishna, J.A., Black, S.M., Szklarz, G.D., and Miller, W.L. (1993) Construction and function of fusion enzymes of the human cytochrome P450scc system. *DNA Cell Biol*. **12**, 371–379
97. Sibbesen, O., De Voss, J.J., and Ortiz de Montellano, P.R. (1996) Putidaredoxin reductase-putidaredoxin-P450cam triple fusion protein. Construction of a self-sufficient *Escherichia coli* catalytic system. *J. Biol. Chem*. **271**, 22462–22469
98. Matsunaga, I., Yamada, A., Lee, D-S., Obayashi, E., Fujiwara, N., Kobayashi, K., Ogura, H., and Shiro, Y. (2002) Enzymatic reaction of hydrogen peroxide-dependent peroxygenase cytochrome P450: Kinetic deuterium isotope effects and analyses by resonance Raman spectroscopy. *Biochemistry*. **41**, 1886–1892
99. Haurand, M., and Ullrich, V. (1985) Isolation and characterization of thromboxane synthase from human platelet as a cytochrome P-450 enzyme. *J. Biol. Chem*. **260**, 15059–15067
100. Song, W.C., Funk, C.D., and Brash, A.R. (1993) Molecular cloning of an allene oxide synthase: a cytochrome P450 specialized for the metabolism of fatty acid hydroperoxides. *Proc. Natl Acad. Sci. USA* **90**, 8519–8523
101. Laudert, D., Pfannschmidt, U., Lottspeich, F., Hallaender-Czytko, H., and Weiler, E.W. (1996) Cloning, molecular and functional characterization of *Arabidopsis thaliana* allene oxide synthase CYP74, the first enzyme of the octadecfanoid pathway to jasmonates. *Plant Mol. Biol*. **31**, 323–335
102. Yoshida, Y., Noshiro, M., Aoyama, Y., Kawamoto, T., Horiuchi, T., and Gotoh, O. (1997) Structural and evolutionary studies on sterol-14-demethylase P450 (CYP51), the most conserved P450 monooxygenase: II. Evolutionay analysis of protein and gene structures. *J. Biochem*. **122**, 1122–1128
103. Barros, M.H., and Nobrega, F.G. (1999) *YAH1* of *Saccharomyces cerevisiae*: a new essential gene that codes for a protein homologous to human adrenodoxin. *Gene*. **233**, 197–203
104. Lacour, T., and Dumas, B. (1996) A gene encoding a yeast equivalent of mammalian NADPH-adrenodoxin oxidoreductases. *Gene*. **174**, 289–292
105. Lange, H., Kaut, A., Kispal, G., and Lill, R. (2000) A mitochondrial ferredoxin is essential for biogenesis of cellular iron-sulfur proteins. *Proc. Natl Acad. Sci. USA*. **97**, 1050–1055
106. Rezen, T., Debeljak, N., Kordis, D., and Rozman, D. (2004) New aspects on lenosterol 14a-demethylase and cytochrome P450 evolution: Lanosterol/cycloarterol diversification and lateral transfer. *J. Mol. Evol*. **59**, 51–58
107. Shen, A.L., O7Leary, K.A., and Kasper, C.B. (2002) Association of multiple developmental defects and embryonic lethality with loss of microsomal

NADPH-cytochrome P450 oxidoreductase. *J. Biol. Chem*. **277**, 6536–6541
108. Urban, P., Mignotte, C., Kazmaier, M., Delorme, F., and Pompon, D. (1997) Cloning, yeast expression, and characterization of the coupling of two distantly related *Arabidopsis thaliana* NADPH-cytochrome P450 reductase with P450 CYP73A5. *J. Biol. Chem*. **272**, 19176–19186
109. Lah, L., Krasevec, N., Trontej, P., and Komel, R. (2008) High diversity and complex evolution of fungal cytochrome P450 reductase: cytochrome P450 system. *Fungal Genetics Biol*. **45**, 446–458
110. Porter, T.D., and Kasper, C.B. (1986) NADPH-cytochrome P-450 oxidoreductase: Flavin mononucleotide and flavin adenine dinucleotide domains evolved from different flavoproteins. *Biochemistry*. **25**, 1682–1687