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Review

Function of human cytochrome P450s: Characterization of the orphans

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Abstract

The human genome has now been established to contain 57 cytochrome P450 genes. The proteins can be grouped into categories of types of substrates, including sterols, fatty acids, eicosanoids, and fat-soluble vitamins. Some P450s have also been demonstrated to have significant roles in the metabolism of drugs and chemicals. In addition to these, at least 13 can be considered to still be without apparent function with endogenous or xenobiotic substrates. The current list includes P450s 2A7, 2S1, 2U1, 2W1, 3A43, 4A22, 4F11, 4F22, 4V2, 4X1, 4Z1, 20A1, and 27C1. Limited information is available about the sites of mRNA expression of some of these orphans. Some strategies for characterization are discussed.

Keywords: Cytochrome P450; Xenobiotic metabolism; Orphans; Carcinogens

One of the most important groups of oxygenases is the cytochrome P450 (P450) enzymes [1]. These enzymes usually function as monooxygenases and are found throughout nature, being present in most Eubacter and Archaebacter species [2]. Plants have particularly high numbers of P450 (or *CYP*) genes; humans have 57 P450 genes [3].

The P450s can be classified on the basis of their substrates, as done in previous treatments from this laboratory [4,5] and in update form in Table 1. A major issue is the classification of an enzyme as being present for a particular biological function(s), involving substrates already present in the body (or vitamins), or being a useful addition to help protect the organisms against the stress of exogenous chemicals. These considerations include not only the P450s but all enzymes that can be involved in ''xenobiotic'' metabolism, as discussed 25 years ago by Jakoby [6]. We define a ''xenobiotic'' as a non-essential chemical that enters the body from the environment. The term includes many secondary natural products (alkaloids, terpenes, etc.), drugs, carcinogens, and various synthetic chemicals.

The classification of a P450 to column 2 of Table 1, ''xenobiotic metabolism,'' is not done on the basis of the rates of catalysis (many drugs can be oxidized by some P450s with rates and efficiencies that exceed those of critical P450s oxidizing true physiological substrates). We apply three general criteria for inclusion in this group: (i) The concentration of the P450 can vary widely among individuals, without an apparent effect on health. (ii) For the identified substrates and products, none of the transformations are known to be critical to health. Thus, P450 3A4 is an efficient testosterone 6β-hydroxylase but we are not aware of any evidence that fluctuations in the levels of the hepatic activity are critical. The enzyme also catalyzes 17β-estradiol hydroxylation ($k_{cat} \sim 4\ min^{-1}$) [7]; the extent of variation among people is considerable [8], although no clear health impact is known. Likewise, the level of P450 2D6 can vary $>10^4$-fold among apparently normal people [4], and P450 1A2 levels vary 40-fold with limited impact aside from the ability to consume coffee [9]. Admittedly, these views are based upon present knowledge, and future research could document some beneficial role for one or more of these P450s beyond protection against certain xenobiotics, e.g., a somewhat extended lifetime, decreased incidence of neurological deficit in old age, etc. (iii) Studies with transgenic animals lacking in what appears to be the ortholog do not demonstrate a clear biological effect of the deficiency [10]. In this regard, it should be noted that

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Table 1
Classification of human P450s based on major substrate class [4,5]

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins	Unknown
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17	2C18				4F22
19	2C19				4V2
21A2	2D6				4X1
27A1	2E1				4Z1
39	2F1				20A1
46	3A4				27C1
51	3A5				
	3A7				

ablation of NADPH-P450 reductase in mouse liver in conditional knock-out animals, which effectively eliminates the function of all hepatic P450s, appears to have only a very limited physiological effect beyond the accumulation of cholesterol [11,12].

Even with these boundaries and definitions, the classification is somewhat arbitrary, e.g., P450s 1B1 and 27A1 could be grouped in either of two categories [4], although P450 1B1 apparently has a role in the eye [13]. Many P450s can use fatty acids as substrates, but the four cited in the list in Table 1 are cases where more evidence of a major role has been presented. Under the heading of eicosanoids, P450s 5A1 and 8A1 are thromboxane and prostacyclin syntheses, respectively, and the roles for these "rearrangement" P450s are now well established. The three 4F subfamily P450s under the eicosanoid heading have hydroxylation activity at the ω-ends of prostaglandins. With many of the P450s in these classes, particularly sterols and vitamins, a genetic insufficiency can be debilitating or fatal [14].

Classifying a P450 under the heading of xenobiotics is also somewhat arbitrary, for more than one reason. Some of these P450s do also oxidize steroids and fatty acids (e.g., P450 3A4 hydroxylates testosterone [15,16] and P450 2C enzymes hydroxylate fatty acids [17]). However, the evidence for critical roles of these activities of these enzymes is lacking. Another issue is whether the rate of oxidation by one of these enzymes is enough to be significant in the in vivo metabolism of any particular drug, carcinogen, or any other xenobiotic. We give P450 3A5 the benefit of doubt in this regard; P450 2F1 may be less clear. Although the identification of a P450 as a "xenobiotic metabolizer" may seem somewhat artificial, we emphasize that the characterization of roles of individual P450s in issues of drug bioavailability, interactions, and toxicity has been considerable, and this area can be considered one of the most practical successes of studying P450s and even oxygenases in general. The evidence for roles of P450 variations as a factor in cancer etiology is less compelling but interesting leads have support in the epidemiology [18,19].

General issues with P450 orphans

The human P450s listed under "unknown" can be termed "orphans" in the sense of the use of the word for describing receptor proteins without known ligands [20]. The classification presented in Table 1 is somewhat arbitrary, in that some evidence is available about reactions catalyzed by these P450s but it is either only quantitative or the reactions are considered too slow to make a major contribution.

A significant amount of mRNA expression information is available for many, but not all, of the orphan P450s (Table 2). Only P450s 4F22, 20A1, and 27C1 are lacking any data. However, the point should be made that in several cases the information presented to date from different sources is not very consistent. Protein expression data have only been presented in the case of P450s 2A7 [43] and 4A22 [35,36], at least at the time of preparation of this article. In some cases, heterologous expression of the P450s has been achieved.

Approaches to defining functions of orphans

In traditional biochemistry (Fig. 1), the starting point is usually an existing in vivo reaction. If one could develop an assay, the enzyme could at least theoretically be purified.

Table 2
Human P450 orphans

P450	Reported expression site	Possible substrate	Heterologous expression	References
2A7	(Liver)			[21,22]
2S1	Trachea, lung, stomach small intestine, spleen, skin, colon	Retinoic acid	*Escherichia coli*, CHO cells, baculovirus	[23–28]
2U1	Thymus, heart, brain (cerebellum), spleen, prostate	Arachidonic acid	Baculovirus	[28–30]
2W1	Prostate, pancreas, placenta, lung, colon,small intestine		—	[28]
3A43	Liver, testis		*E. coli*	[31–34]
4A22	(Kidney? Liver?)		—	[35,36]
4F11	Liver, kidney, heart, skeletal muscle, brain	Erythromycin	*Saccharomyces cerevisiae*	[37]
4F22			—	
4V2	Retina, cornea, lymphocytes		—	[38]
4X1	Brain, trachea, aorta, pancreas, lung, kidney, prostate		—	[28,39,40]
4Z1	Breast, breast carcinoma		—	[40,41]
20A1			—	[42] (mouse)
27C1			—	

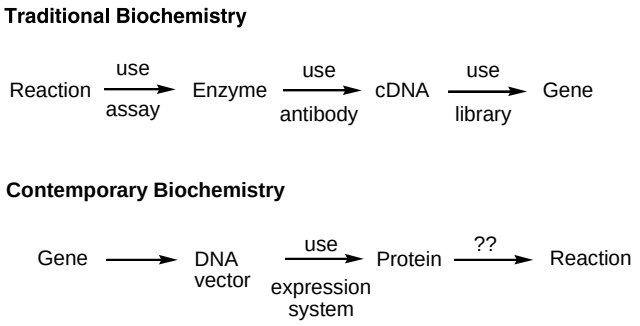

Fig. 1. Strategies used in "traditional" and "contemporary" biochemistry.

Later (after ~1980), the cDNA and gene could be cloned. However, today the human genome is in hand and the genomes of many other organisms are rapidly becoming available. The problem is that the functions of the majority of gene products are unknown, and new strategies and paradigms are needed to address the problem.

One approach is simple trial and error. However, the choices of substrates are considerable and, frankly, we do not even know about all of the potential substrates in a cell. One way to raise the probability of success is to use homology to other P450s with known substrates. This approach has been used, even if not systematically, and some information has come from it. In principle, obtaining the three-dimensional structures of P450s might provide answers about the geometry of potential substrates. However, this approach has not been very successful because the structures of P450s with bound ligands are very different than the open, unbound forms.

Theoretically, an orphan P450 can be heterologously expressed and used as an affinity reagent to isolate ligands/substrates from crude extracts of the tissues in which the P450s are expressed. This approach has had limited application with the steroid receptor superfamily orphans. In principle, this approach should work, although the k_{off} rate of a ligand would have to be very low (and thus the affinity very high). Another issue is that high affinity is not necessarily a requisite of a substrate, e.g., many of the enzymes in primary metabolic pathways have relatively high K_m values, to afford better regulatory control [44].

We have proposed new methods to identify substrates of orphan P450s, based upon some considerations of the many P450s in Streptomyces, which are mostly orphans. All are heavily based upon the use of HPLC–mass spectrometry (MS). Much of the strategy relies on the fact that most (but not all) [45] P450 transformations involve the addition of the 16 a.m.u. of oxygen. Several possibilities can be envisaged with the use of $^{16}O_2$ and $^{18}O_2$, and we are developing systems to allow searching for new products in crude tissue extracts incubated with purified enzymes.

Another viable strategy involves the use of collision-induced dissociation (CID) of candidate product peaks, such as those identified with the $^{18}O_2$ approach discussed above. The concept is to find other peaks (the substrate) that have CID spectra similar to those of the candidate product. Software of this type is already available for use with post-translationally modified peptides [46].

Another approach, shown in Fig. 2, is challenging but may have the greatest potential. Subtractive HPLC–MS is involved, where a control ion profile is subtracted from the profile obtained with the complete in vitro P450 system at each m/z value. The traces should show some peaks and troughs, which should be related to each other by 16 a.m.u., as shown (Fig. 2). With appropriate software, an automated search can be done to find candidates.

Current status of orphans

Limited information about the sites of mRNA expression of several of the orphan P450s has been published,

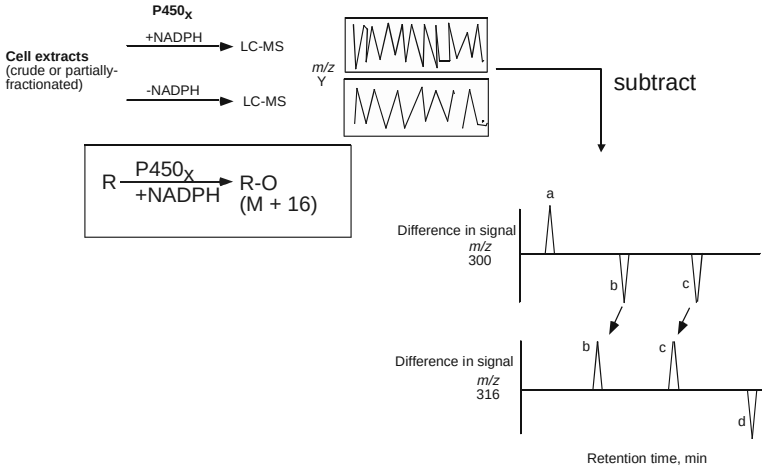

Fig. 2. A proposed method of identifying candidate reactions for an orphan P450. A purified P450(x) is reconstituted with reductase, phospholipid, an NADPH-generating system, and a crude (or partially purified) cell extract, which is the source of the substrate. The products are analyzed by HPLC–MS. A parallel incubation devoid of NADPH (or the P450) is also run and analyzed. One ion trace is subtracted from the other, at each m/z value, to yield sets of difference chromatograms, which should be linked to each other by 16 a.m.u. differences for oxygenations. The candidate substrates and products are further separated and analyzed.

although some of it is inconsistent in the literature (Table 2).

Whether P450 2A7 is expressed or not is unclear. In an earlier work, Gonzalez's laboratory expressed the protein in a vaccinia system but no heme was incorporated [43]. Crossover of the P450 2A6 and 2A7 genes has been reported to yield proteins [21,22].

P450 2S1 mRNA expression has been detected in several tissues (Table 2). The gene is induced by polycyclic hydrocarbons [23]. The protein has been heterologously expressed and some activity towards retinoic acid has been reported [26]. A limited set of carcinogens was examined and none of them was found to be activated by P450 2S1 [27].

P450 2U1 has been reported to show preferential expression in thymus and the brain cerebellum [30]. Some activity towards arachidonic acid has been reported but not quantified [30].

P450 3A43 is listed here, although the protein has been expressed and considered along with the other three P450 3A subfamily members (3A4, 3A5, and 3A7). The expression levels and catalytic activities are very low relative to the other three proteins [31,34].

P450 4F11 mRNA is expressed in a number of tissues (Table 2). Low activity towards some endobiotic and xenobiotic substrates has been demonstrated with a recombinant protein expressed in a yeast system [37].

P450 4V2 is expressed in the eye [38] but to our knowledge no substrates are known. A crystalline corneoretinal dystrophy has been linked to mutations in this gene.

P450 4X1 and 4Z1 appear to share some of the regulatory properties of the 4A subfamily [40], and P450 4Z1 was found to be preferentially expressed in carcinoma of the breast [41].

Presently no other information about the expression or possible activities of the other enzymes has been reported (2W1, 4A22, 4F22, 20A1, and 27C1).

Conclusions

We have grouped 13 of the 57 human P450s as orphans. This is a subjective classification and is based on the limited knowledge about the them at the present time, with no conclusion about importance. Some of these proteins have been reported to show low rates of activity towards arachidonic acid and retinoic acid, but interpretations about biological relevance must be considered premature. The limited activity of P450s 3A43 and 4F11 towards some model xenobiotics is also low [31,34,37,47] and assignments as xenobiotic-metabolizing enzymes are also very tentative.

The tissue localizations of many of these P450s invite speculation about function. Exactly what the roles of these P450s will be is still difficult to predict. However, development of better approaches to defining functions of orphan proteins is a need that goes beyond the P450 problem.

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