Chapter 2
Roles of Cytochrome P450 in Metabolism of Ethanol and Carcinogens

F. Peter Guengerich and Narayan G. Avadhani

Abstract Cytochrome P450 (P450) enzymes are involved in the metabolism of carcinogens, as well as drugs, steroids, vitamins, and other classes of chemicals. P450s also oxidize ethanol, in particular P450 2E1. P450 2E1 oxidizes ethanol to acetaldehyde and then to acetic acid, roles also played by alcohol and aldehyde dehydrogenases. The role of P450 2E1 in cancer is complex in that P450 2E1 is also induced by ethanol, P450 2E1 is involved in the bioactivation and detoxication of a number of chemical carcinogens, and ethanol is an inhibitor of P450 2E1. Contrary to some literature, P450 2E1 expression and induction itself does not cause global oxidative stress *in vivo*, as demonstrated in studies using isoniazid treatment and gene deletion studies with rats and mice. However, a major fraction of P450 2E1 is localized in liver mitochondria instead of the endoplasmic reticulum, and studies with site-directed rat P450 2E1 mutants and natural human P450 2E1 N-terminal variants have shown that P450 2E1 localized in mitochondria is catalytically active and more proficient in producing reactive oxygen species and damage. The role of the mitochondrial oxidative stress in ethanol toxicity is still under investigation, as is the mechanism of altered electron transport to P450s that localize inside mitochondria instead of their typical endoplasmic reticulum environment.

Keywords Ethanol · Oxidation by cytochrome P450, in ethanol oxidation · Mitochondria, and cytochrome P450 · Endoplasmic reticulum, and cytochrome P450 · Cytochrome P450 2E1 · P450 2E1 · Enzyme kinetics · Reactive oxygen species · Mitochondrial toxicity · Chemical carcinogens

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2.1 Introduction to Cytochrome P450 (P450)

The field of P450 research stemmed from work in the areas of the metabolism of steroids, drugs, and carcinogens [18, 29]. The human genome contains 57 P450 (*CYP*) genes, of which almost all are expressed in some tissue [29]. The P450s are classified into families and subfamilies on the basis of sequence similarity [52]. However, sequence similarity may or may not be a guide to function with P450s [23, 29].

Another way to classify P450s is by the types of substrates they use (Table 2.1) [29]. Even this approach has caveats in that some P450s could be placed in multiple categories in Table 2.1 (e.g., P450s 1B1 and 27A1) [29]. Another point to make is that several human P450s have yet to have functions identified, i.e. those in the column labeled "unknown." These are often termed the "orphans [30]. However, even when P450s are classified in the "xenobiotics" category and do happen to be able to oxidize a substrate found in the body (e.g. P450 3A4), it is not clear that this reaction has a significant physiological role [29]. In general, the mouse orthologues of this column of P450s can be deleted without profound physiological effects [26].

Collectively the P450s catalyze about 75% of all reactions involved in the metabolism of drugs [81] (some drugs are not readily metabolized, however, and biologicals are not included in this analysis). In the previous analyses [80, 81], the 3A and 2C Subfamily P450s were those most involved in drug metabolism (i.e., 3A4, 2C9,

Table 2.1 Classification of Human P450s Based on Major Substrate Class [29]

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

[a]X-ray crystal structure(s) reported (for human enzyme)
[b]Rat or rabbit X-ray crystal structure reported

2C19). This pattern still holds for P450s [64]. Further, when one considers oxidation-reduction reactions with all chemicals (for the human enzymes), P450 catalyze 95% of the reactions. Again, the 3A and 2C Subfamily P450s are most prominent [64].

The localization of individual P450s has been reviewed elsewhere [29]. Those P450s involved in critical physiological functions are often found only in a few relevant tissues, e.g. many of the steroidogenic P450s are found only in the adrenals, gonads, etc. Those involved in bile acid processing are generally localized in the liver (e.g. P450 7A1). In general, the xenobiotic-processing P450s are localized principally in the liver, and some are never found outside of the liver, e.g. 1A2, 7A1 [29]. However, some of these are also found at lower levels in many other tissues. Some caution should be used in reviewing the literature, however, in that much of the work is at the mRNA level, not protein.

One of the reasons for the interest in the P450s is their role in the metabolism of toxicants and carcinogens. In general, this process of oxidation (and sometimes reduction) aids in the removal of chemicals from the body and may, along with transporters, be viewed as a mechanism for the body to use a limited set of genes to eliminate natural products that could prove harmful if allowed to accumulate in cells (e.g. alkaloids, terpenes, flavonoids). In general, the oxidation and reduction reactions catalyzed by P450s detoxicate chemicals. However, these same reactions also sometimes create electrophilic species that can react with cellular macromolecules and initiate biological damage, general with DNA or proteins. DNA damage can be misreplicated and fits into the somatic mutation theory of cancer [10]. The relevance of metabolism to chemical carcinogenesis was established largely by the late James and Elizabeth Miller in animal studies [50, 51]. This same concept applies to the toxicity of drugs, with protein damage generally being considered an initial event [24, 37].

Almost all of the information on the roles of human P450s in the metabolism of carcinogens has been published in the past 26 years [72, 73]. One classification indicates that (human) P450s are involved in ~ 2/3 of the reactions in which chemical carcinogens are bioactivated (Fig. 2.1a) [63]. Six human P450s recount for ~ ¾ of these reactions, i.e. P450s 1A1, 1A2, 1B1, 2A6, 2E1, and 3A4 (Fig. 2.1b) [63]. However, P450s are also involved in detoxication of chemical carcinogens [63]. The complexity of these systems can be seen in the classic 1952 Richardson experiment, in which treatment of rats with one carcinogen induced P450s that detoxicate another carcinogen and then had a protective role [65]. We now know that a single P450 catalyze both activation and detoxication of a single carcinogenic chemical, e.g. P450 3A4 oxidizes the hepatocarcinogen aflatoxin B_1 to the 8,9-*exo* epoxide (which reacts efficiently with DNA [36]) and to aflatoxin Q_1 (3α-hydroxylation product), which does not appear to be a carcinogen [77]. This dichotomy appears to be due to the presence of multiple binding modes of the substrate (aflatoxin B_1) in the enzyme active site.

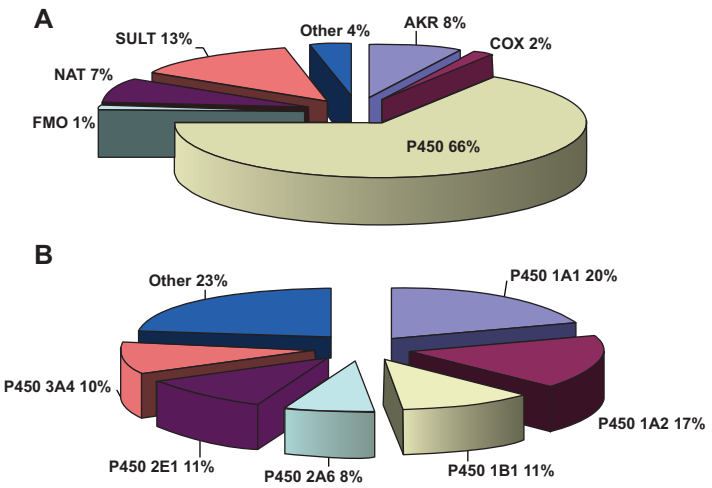

Fig. 2.1 Human enzymes involved in bioactivation of carcinogens [63]. (**a**) fractions of enzyme classes involved in bioactivation; (**b**) individual human P450 contributions to carcinogen activation. *FMO* microsomal flavin-containing monooxygenase, *NAT* N-acetyltransferase, *SULT* sulfotransferase, *AKR* aldo-keto reductase, *COX* cyclooxygenase (prostaglandin synthase)

Table 2.2 Sites of P450 2E1 expression [29]

Liver
Brain
Esophagus
Lung
Nasal mucosa
Pancreas
Small intestine

2.2 P450 2E1

The discovery of P450 2E1 followed largely from work on a microsomal ethanol oxidizing system [47, 57]. P450 2E1 was characterized in rabbit [43] and rat liver [68]. The enzyme is found at highest abundance in liver but also in numerous other tissues of relevance to cancer [25] (Table 2.2).

P450 2E1 is also induced by ethanol [47]. The study of this process has been complicated, in that ethanol also inhibits the enzyme (as expected for a substrate) [82]. Regulation of the CYP2E1 gene is complex and includes both transcriptional and post-transcriptional aspects [25].

P450 2E1 substrates include ethanol, a number of drugs (especially anesthetics), *N*-alkylnitrosamines, vinyl monomers, and halogenated hydrocarbons (Table 2.3) [29, 31]. Several of these are of interest in that a number of the nitrosamines, vinyl monomers, and halogenated hydrocarbons are carcinogens [31]. Nitrosamines are

Table 2.3 Pro-carcinogens and suspected carcinogens known to be bioactivated by P450 2E1 [29, 31, 63]

Acrylonitrile
Aniline
Azoxymethane
Benzene
1,3-Butadiene
Carbon tetrachloride
Chloroform
Cisplatin
1,4-Dichlorobenzene
2,2-Dichloro-1,1,1-trifluoroethane
N,*N*-Diethylnitrosamine
N,*N*-Dimethylformamide
1,2-Dimethylhydrazine
N,*N*-Dimethylnitrosamine
Ethanol
Ethyl carbamate (urethane)
Ethylene dibromide
Ethylene dichloride
Furan
4-Ipomeanol
2-Methoxyaniline
Methylene chloride
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
N-Nitrosodi-*n*-propylamine
N-Nitrosodiethanolamine
N-Nitrosoethylbutylamine
N-Nitrosomethylbutylamine
N-Nitrosomethylethylamine
N-Nitrosomethylpropylamine
N-Nitrosomorpholine
N-Nitrosopyrrolidine
Nornitrosonicotine (NNN)
Propylene dichloride
Styrene
Trichloroethylene
Vinyl bromide
Vinyl carbamate
Vinyl chloride
4-Vinyl-1-cyclohexene
Vinylidene chloride

present in tobacco, and the vinyl monomers and halogenated hydrocarbons are used on a large scale in industry. These substrates have the general property of small molecular size, consistent with the crystal structure of P450 2E1 [60, 61]. However, it should be pointed out that even fatty acids can be substrates [61]. Because of the induction and inhibition of P450 2E1 by ethanol, the multiples sites of expression of the enzyme (Table 2.2), and the relevance to humans drinking ethanol and exposed to carcinogens in Table 2.2, the co-carcinogenicity of ethanol has been considered in a number of studies with experimental animals [15, 48]. (Co-carcinogenesis studies involve simultaneous administration of chemicals, as opposed to initiation-promotion regimens, with distinct temporal relationships and mechanisms [58]).

2.3 Ethanol Oxidation by P450 2E1

A general view in the alcohol field has been that alcohol dehydrogenase is the most relevant enzyme at low concentrations of ethanol and that P450 (2E1) is more involved at higher concentrations, i.e. those that would be relevant with high consumption. The situation is also complicated in that catalase can also contribute to ethanol metabolism [14, 76].

Even though ethanol has only two carbons and one oxygen, its oxidation has several complexities (Fig. 2.2). The general view in the field is that there are no receptors as such for ethanol, and essentially all of its biological effects are related to acetaldehyde. In aqueous solution, acetaldehyde exists in a roughly equimolar equilibrium with its hydrate, a "*gem* diol" (Fig. 2.2a) [27, 28].

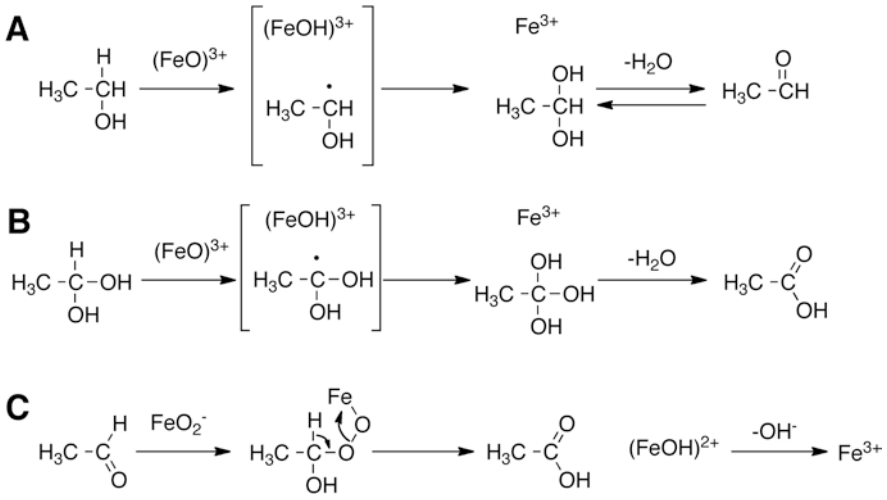

Fig. 2.2 Possible mechanisms of oxidation of ethanol to acetaldehyde and acetic acid [33]. (**a**) oxidation of ethanol to acetaldehyde. (**b**) oxidation of acetaldehyde by a perferryloxo mechanism (Compund I). *C*, oxidation of acetaldehyde by a ferric peroxide mechanism

We were interested in kinetic hydrogen isotope effects (KIEs) for ethanol oxidation because of some reports on KIEs for nitrosamine oxidation by liver microsomes [41, 49]. Work with recombinant human P450 2E1 showed, not unexpectedly, that α-deuterated ethanol showed a 5-fold KIE, expressed in the K_m but not k_{cat} (Fig. 2.3) [11]. This was shown to be the result of "burst" kinetics, i.e. a rate-determining step following product formation [11, 32]. The kinetic analysis is not novel [79], and K_m is a constant that obviously has nothing to do with K_d here (a general axiom in enzymology) [35, 56]. K_m even contains k_{cat} as a variable in this case [32]. That is, for the simplified expression

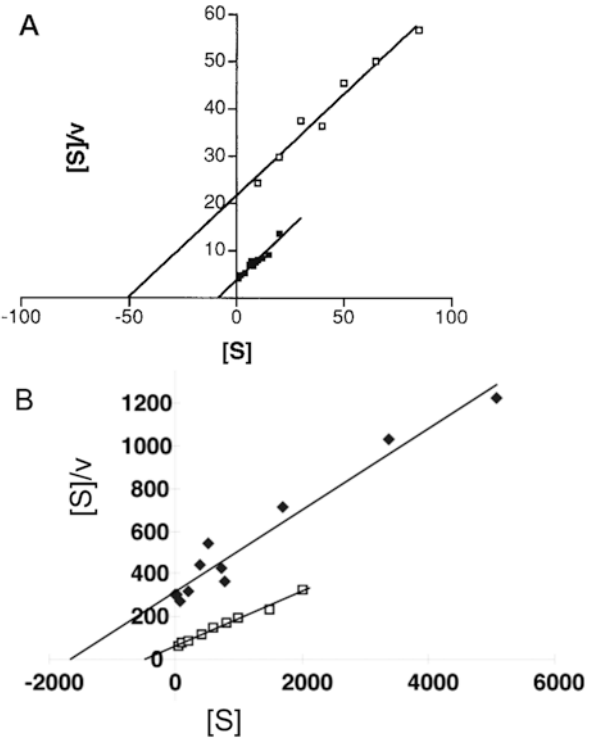

Fig. 2.3 Kinetic deuterium isotope effects with human P450 2E1 [11, 12]. The results are presented in Hanes-Wolff plots ([S]/v vs. [S]). (**a**) ethanol to acetaldehyde. CH_3CH_2OH as substrate (k_{cat}, 2.7 (± 0.2) min^{-1}, K_m 11 (± 2) mM); CH_3CD_2OH as substrate (■, k_{cat} 2.4 (± 0.1) min^{-1}, K_m 53 (± 6) mM). (**b**) acetaldehyde to acetic acid. CH_3CHO as substrate (□, k_{cat} 7.5 (± 0.5) min^{-1}, K_m 0.50 (± 0.2) mM); CH_3CDO as substrate (◆, k_{cat} 5.0 (± 0.4) min^{-1}, K_m 1.5 (± 0.3) mM). This research was originally published in Bell, L. C., and Guengerich, F. P. [11] Oxidation kinetics of ethanol by human cytochrome P450 2E1. Rate-limiting product release accounts for effects of isotopic hydrogen substitution and cytochrome b_5 on steady-state kinetics. *The Journal of Biological Chemistry* **272**, 29,643–29,651 and Bell-Parikh, L. C., and Guengerich, F. P. [12] Kinetics of cytochrome P450 2E1-catalyzed oxidation of ethanol to acetic acid via acetaldehyde. *The Journal of Biological Chemistry* **274**, 23,833–23,840.

$$E \underset{k_2}{\overset{k_1 S}{\rightleftarrows}} ES \overset{k_3}{\rightarrow} EP \overset{k_5}{\rightarrow} E + P$$

where S is the substrate and P the product. Then

$$V_{max} = \frac{k_3 k_5}{k_3 + k_5}[E]_T$$

$$K_m = \frac{k_5 (k_2 + k_3)}{k_1 (k_3 + k_5)}$$

with K_m having units of molarity. k_3 must be considerably larger than k_5, the rate of a step following product formation [44]. The original expressions for V_{max} and K_m are then reduced to

$$V_{max} \cong k_5 [E]_T$$

$$K_m = \frac{k_5 (k_2 + k_3)}{k_1 k_3} \cong \frac{k_5 k_2}{k_1 k_3} + \frac{k_5}{k_1}$$

K_m even contains k_{cat} as a variable in this case [32].

We also showed that (human) P450 2E1 can oxidize acetaldehyde to acetic acid [12], in support of reports by others with the rat enzyme [45, 75] (Figs. 2.2, 2.3b, c). This oxidation also displays burst kinetics [12] and a KIE in the K_m parameter. Pulse-chase experiments showed that the conversion of radiolabeled ethanol into acetic acid was not attenuated by the addition of unlabeled acetaldehyde. However, the results cannot be explained by a high affinity of P450 2E1 for acetaldehyde. A scheme involving a slow conformational step after the formation of acetaldehyde (and acetic acid) has been proposed [12]. Some similar results were found in the oxidation of *N*,*N*-dimethyl- and *N*,*N*-diethyl nitrosamine by P450 2E1 [17].

2.4 Source of Oxidative Stress Due to P450 2E1 in Tissues

A role for P450 2E1 in generation of reactive oxygen species (ROS) is often assumed in much of the literature in the field. Much of this view is based on early studies with liver microsomes [22, 62] and studies in cell culture [16]. The ROS field is complicated in that the vast majority of the work is *in vitro* and many of the methods of detecting ROS are not validated, e.g. see [40]. In the field, a "gold standard" is F_2-isoprostane production, which can be measured both *in vitro* and *in vivo* [38, 39].

Treatment of rats with typical P450 enzyme inducers did not produce enhanced levels of liver isoprostanes, except in the case of barbiturate-type induction [20]. In particular, treatment of rats or mice with isoniazid, an established inducer of P450

2E1 [68], did not increase isoprostane levels [20, 21]. Further, *Cyp2e1*$^{-/-}$ mice had very similar levels of liver, kidney, brain, and urinary isoprostanes [21]. Thus, P450 2E1 does not appear to increase global levels of ROS. Increased levels of ROS related to treatment of animals with ethanol are not linked only to induction of P450 2E1. Further, although the utilization of reducing equivalents (NADPH) by purified P450 2E1 is low, it is not inferior to several other (human) P450s we have worked with [74, 84].

As has been suggested in the literature [42, 83], ROS production in the presence of ethanol is probably not due to P450 2E1 induction per se but possibly to the generation (and reaction) of $CH_3CHO\cdot$ radicals.

The ROS increases following treatment of rats and mice with barbiturates have been attributed to induction of nicotinamide *N*-methyl transferase and depletion of pyridine nucleotides to support ROS destruction [21].

2.5 Roles of Mitochondrial P450s

Of the 57 human P450s (Table 2.1), seven are *bona fide* mitochondrial enzymes (11A1, 11B1, 11B2, 24A1, 27A1, 27B1, 27C1). These P450s have distinct roles in the metabolism of sterols and fat-soluble vitamins and they utilize electrons supplied by NADPH-adrenodoxin reductase (ADR) and the iron-sulfur protein adrenodoxin (Adx) [29].

The other 50 P450 proteins, when expressed, are targeted to the endoplasmic reticulum (ER). However, fractions of these P450s can, in some cases, be found in mitochondria. This was first shown in rats, where P450 2B1 could be found in mitochondria and catalyze the oxidation of aflatoxin B_1 [53]. Further studies established this phenomenon, i.e. partial localization in mitochondria and catalytic activity there [55, 71]. Studies from several groups showed that mitochondrial Adx (plus ADR) and bacterial ferredoxin (plus NADPH-ferredoxin reductase) could efficiently donate electrons to mitochondria-localized P450 1A1, 2E1, 2C8, 2B1, 2D6, 3A4, and 17A1 [5, 6, 9, 19, 59, 70], demonstrating that mitochondria-localized P450s are catalytically active. It was also shown that the N-terminal acidic domain of Adx interacted with mitochondria-localized P450 1A1 through charge-charge interaction [1]. These Adx-interacting domains are conserved in other P450 Family 1 members and also in P450s 2B1, 2B4, 2D6, 2E1, 3A4, 17A1, and 21A2 [5, 6, 9, 46, 59, 70].

One of the important factors in signaling proteins for endoplasmic reticulum (ER) localization is signal recognition particle (SRP) [69]. SRP interacts with hydrophobic residues in the N-terminal regions of proteins declined for ER localization. We reasoned that the introduction of charged residues in the N-terminal region of rat P450 2E1 might lead to decreased SRP recognition and increased mitochondrial import [7]. This hypothesis was tested, and positive results were obtained, particularly with the Mt^{++} derivative (Fig. 2.4). Mitochondria from cells

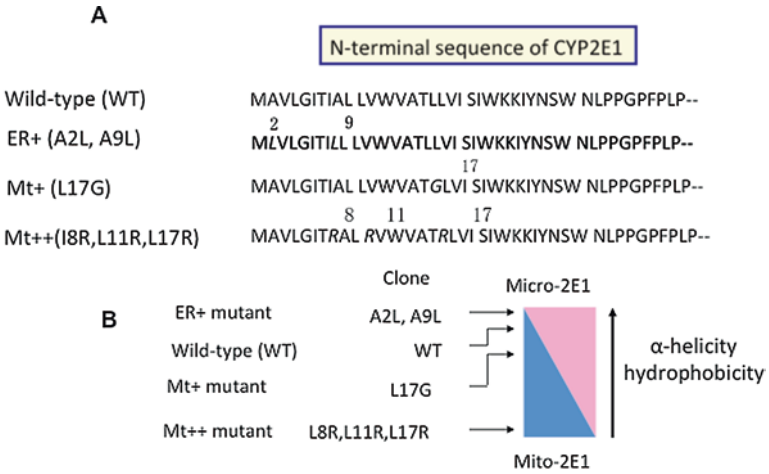

Fig. 2.4 A mutational approach for altering the bimodal targeting efficiency of rat P450 2E1 [7]. (**a**) the WOLFPSORT program was utilized to alter the SRP binding and mitochondria-targeting efficiencies of the N-terminal signal regions. (**b**) predicted targeting efficiencies of WT and mutant P450 2E1 proteins. This research was originally published in Bansal, S., Liu, C. P., Sepuri, N. B., Anandatheerthavarada, H. K., Selvaraj, V., Hoek, J., Milne, G. L., Guengerich, F. P., and Avadhani, N. G. [7] Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol-mediated oxidative stress. *The Journal of Biological Chemistry* **285**, 24,609–24,619.

transfected and expressing the Mt^{++} variant also showed increased mitochondrial ROS production, as judged by isoprostane measurements (Fig. 2.5). When the variants were expressed in yeast, the cell showed similar phenotypes when the yeast were grown in glucose medium regardless of the N-terminal sequence (Fig. 2.6). However, with the non-fermentable carbon source lactic acid in the media, the cells transfected with the Mt^{++} variant, localized to the mitochondria, showed selective toxicity. The phenotype was petite, indicating a loss of mitochondrial function.

Consistent with the data obtained with COS and yeast cells expressing the Mt^{++} variant of P450 Δ2E1, hepatic mitochondria from rats fed ethanol showed a time-dependent increase in F_2-isoprostane production, while hepatic microsomal membranes from these rats showed no significant increase [7]. The increased mitochondrial ROS production was accompanied by attenuated mitochondrial DNA contents, as well as cytochrome *c* oxidase and other electron transport complex activities, suggesting that mitochondrial P450 2E1 promotes alcohol-mediated mitochondrial dysfunction [1, 2, 13, 66, 70]. A recent study with livers of alcohol-fed mice suggested that mitochondrial P450 2E1 contributes significantly to the metabolic profiles of the liver tissue with several P450 2E1 substrates, including 4-nitrophenol, aniline, and styrene [34].

Studies with rat P450 2E1 [3, 67] showed that the N-terminal 160 amino acid region of the protein contained signals for targeting to both the ER and mitochondria. The positively charges residues of immediately upstream of the transmembrane

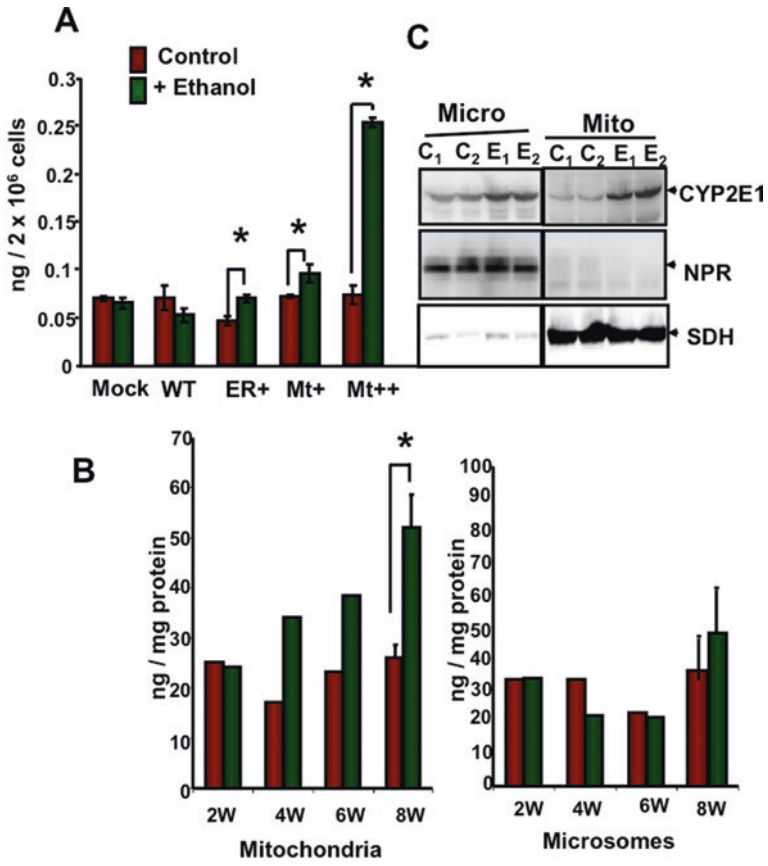

Fig. 2.5 Ethanol-induced F_2-isoprostanes in P450 2E1-expressing cells and in liver fractions from ethanol-treated rats [7]. (**a**) F_2-isoprostanes were assayed using gas chromatography-mass spectrometry. Asterisks represent significant increase in F_2-isoprostanes in ER^+, Mt+, and Mt^{++} cells after ethanol treatment ($p < 0.05$). Values represent the means ± SD of three assays. *B*, F_2-isopreostanes were measured in mitochondria and microsomes isolated from the livers of rats fed with alcohol for 2–8 weeks (W) and pair-fed controls. In each case 100 μg of protein was used. The means ± SD in the 8-week-fed rats were based on assays carried out in three rats each in control and fed groups. Asterisks represent significant difference ($p < 0.05$) from pair-fed controls. The values presented in boxes below the graph indicate the ratios of P450 (CYP) 2E1 contents between pair-fed controls and alcohol-fed rat livers. This research was originally published in Bansal, S., Liu, C. P., Sepuri, N. B., Anandatheerthavarada, H. K., Selvaraj, V., Hoek, J., Milne, G. L., Guengerich, F. P., and Avadhani, N. G. [7] Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol-mediated oxidative stress. *The Journal of Biological Chemistry* **285**, 24,609–24,619. © The American Society for Biochemistry and Molecular Biology

domain of the protein functioned as a cryptic mitochondria targeting signal. Furthermore protein kinase A-mediated phosphorylation at Ser-129 enhanced mitochondrial translocation of the protein through enhanced affinity for binding to cytosolic HSP70 chaperone protein—which has been implicated in presenting

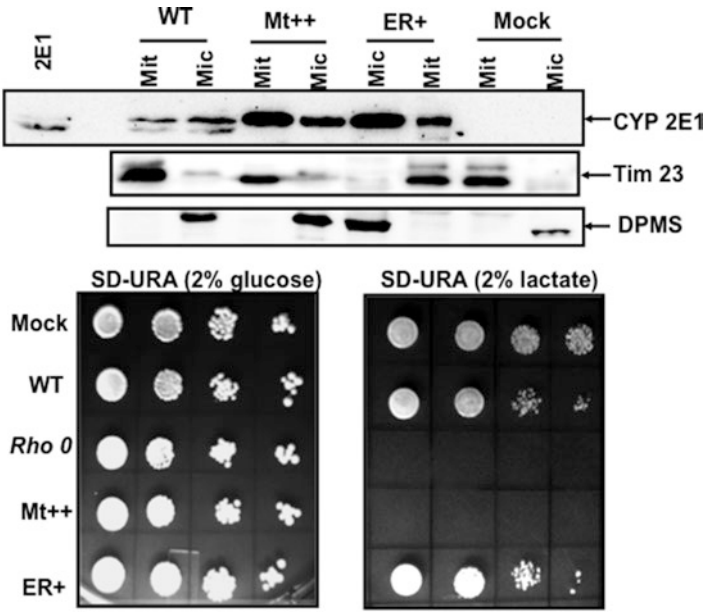

Fig. 2.6 Mitochondrial P450 2E1-induced respiratory deficiency in yeast cells [7]. (**a**) mitochondrial and microsomal CYP2E1 contents in yeast cells stably expressing WT and mutant rat P450 (CYP) 2E1 cDNA constructs. The mitochondrial and microsomal proteins (50 μg each) were analyzed using immunoblotting with anti-P450 2E1. Two identically run (parallel) blots were probed with antibody to the mitochondria-specific marker Tim23 and the microsome-specific marker dolicholphosphate mannose synthase (DPMS). (**b**), yeast cells expressing ER^+, WT, and Mt^{++} rat P450 2E1 were grown in yeast extract/peptone/dextrose medium supplemented with appropriate amino acids. Cells (~ 2.0 OD_{600} units) were pelleted and resuspended in 1 ml of sterile water. The culture was serially diluted 10 times, and 10 μl of each dilution was spotted onto plates containing 2% glucose (w/v) (left panel) and 2% lactate (w/v) (right panel), which were incubated at 30 °C for 4 days. This research was originally published in Bansal, S., Liu, C. P., Sepuri, N. B., Anandatheerthavarada, H. K., Selvaraj, V., Hoek, J., Milne, G. L., Guengerich, F. P., and Avadhani, N. G. [7] Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol-mediated oxidative stress. *The Journal of Biological Chemistry* **285**, 24,609–24,619.

mitochondrial passenger proteins to mitochondrial outer membrane translocators. Subsequent work [7] showed that the hydrophobicity of the N-terminal targeting domains of P450 Family 2 proteins determines their affinity for SRP binding and thus the level of ER vs. mitochondrial targeting [7]. P450 2E1—with a more hydrophobic N-terminus—was mostly targeted to the ER, and proteins with higher hydrophilicity were increasingly targeted to mitochondria.

Analysis of human liver samples from our collection showed varying degrees of mitochondrial localization, > 90% in some cases [8] (Fig. 2.7). DNA sequence analysis showed the presence of nucleotides coding for positively charged residues in the N-terminal region of P450 2E1 in these human samples. Liver samples with high mitochondrial P450 2E1 contents showed reduced cytochrome *c* oxidase

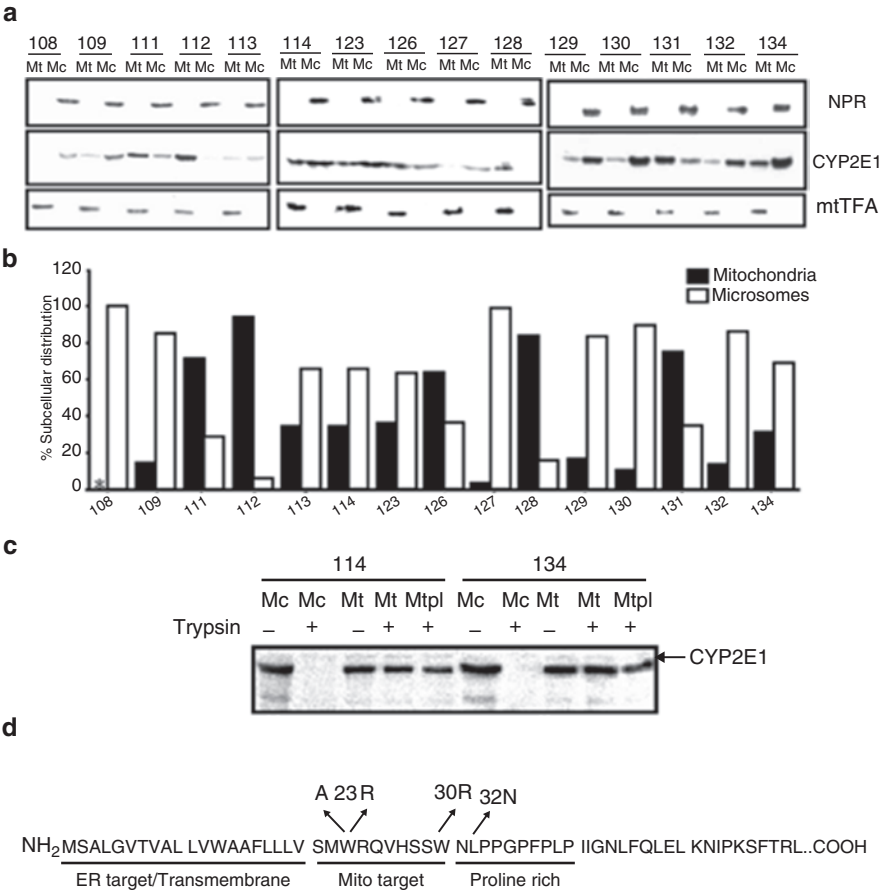

Fig. 2.7 Interindividual variations in P450 2E1 content of human liver samples [8]. **(a)** immunoblotting analysis of mitoplast (Mt) and microsomal (Mc) fractions isolated from human liver samples (50 μg of protein each) using polyclonal antibodies to human P450 (CYP) 2E1 (1:1000, v/v) and the mitochondrial marker protein mtTFA (1:3000, v/v) and a monoclonal antibody to microsomal NADPH-P450 reductase (1:1500, v/v). (**b**) densitometric analysis was performed to determine the distribution of P450 2E1 in human mitochondria and microsomes. (**c**) immunoblot analysis (with anti-human P450 2E1) of human liver mitochondrial and microsomal proteins from liver samples (HL, 'human liver') HL114 and HL134, subjected to limited trypsin digestion (150 μg/mg protein, 20 min on ice). **(d)** N-terminal amino acid sequence of human P450 2E1 protein indicating the ER targeting domain, mitochondrial targeting domain, and proline-rich domain. Variations within the putative signal sequence region are shown by the arrows. This research was originally published in Bansal, S., Anandatheerthavarada, H. K., Prabu, G. K., Milne, G. L., Martin, M. V., Guengerich, F. P., and Avadhani, N. G. [8] Human cytochrome P450 2E1 mutations that alter mitochondrial targeting efficiency and susceptibility to ethanol-induced toxicity in cellular models. *The Journal of Biological Chemistry* **288**, 12,627–12,644. © The American Society for Biochemistry and Molecular Biology

activity and markedly depleted cytochrome *c* oxidase I, IVi1, and Vb subunits [8]. Consisted with observations with the rat P450 2E1, the N-terminal 160 amino acids of human P450 2E1 were sufficient to direct the organelle localization of P450s [8]. Accordingly, this region could be fused to a reporter protein (dihydrofolate reductase, DHFR) to monitor membrane localization. Sequence analysis of the N-terminal coding regions cDNAs from human liver samples showed W23R and W30R substitutions and, as with rat P450 2E1 [7], the N-terminal sequence variants with the more basic residue substitutions (i.e., W23R/W30R) showed higher mitochondrial translocation and more mitochondrial ROS production (Fig. 2.8). In other assays, mitochondria from HepG2 cells transfected with the mitochondria-directed variants showed increased uncoupled respiratory rates and partial mitochondrial DNA depletion (Fig. 2.9). Thus, although the direct role of P450 2E1 in ROS production at the whole organ level remains suspect, the mitochondria-targeted P450 2E1 appears to augment alcohol toxicity by inducing mitochondrial respiratory dysfunction.

2.6 Future Questions

Collectively, the studies with mitochondria show a selective response of P450 2E1 localized there, in terms of ROS production. The mitochondrial P450 2E1 showed a similar catalytic activity in *N*,*N*-dimethylnitrosamine oxidation (as the microsomal P450 2E1) [7]. The results, taken collectively, argue for a selective role of mitochondrial P450 2E1 in ethanol toxicity. Exactly how this relates to ethanol-linked cancer is not clear. P450 2E1 may be generating more mitochondrial DNA adducts, and there is evidence for a higher level of DNA adducts in mitochondria with some carcinogens [4, 54, 78]. What is not clear is how genetic mutations in the mitochondrial DNA would yield tumors, in the context of current theories regarding roles of mutations in oncogenes and tumor suppressor genes.

Another basic question is how Adx interacts with mitochondrial P450 2E1 to deliver electrons. Several other human P450s have now been found to have significant fractions localized in mitochondria (i.e., 1B1, 2C8, 2D6) [5, 6, 9, 29, 70] and this is a similar question in these cases. One possibility is that electron transfer to the P450 with Adx occurs through a different domain, or with altered efficiency as with the normal redox partner NADPH-P450 reductase (an ER protein) and may determine the relative activities. Additionally, the mitochondria localized P450s 1A1, 2B1, and 2E1 show significant changes in their α-helical and β-sheet contents, suggesting altered folding. Many of these questions remain to be addressed in a more detailed manner.

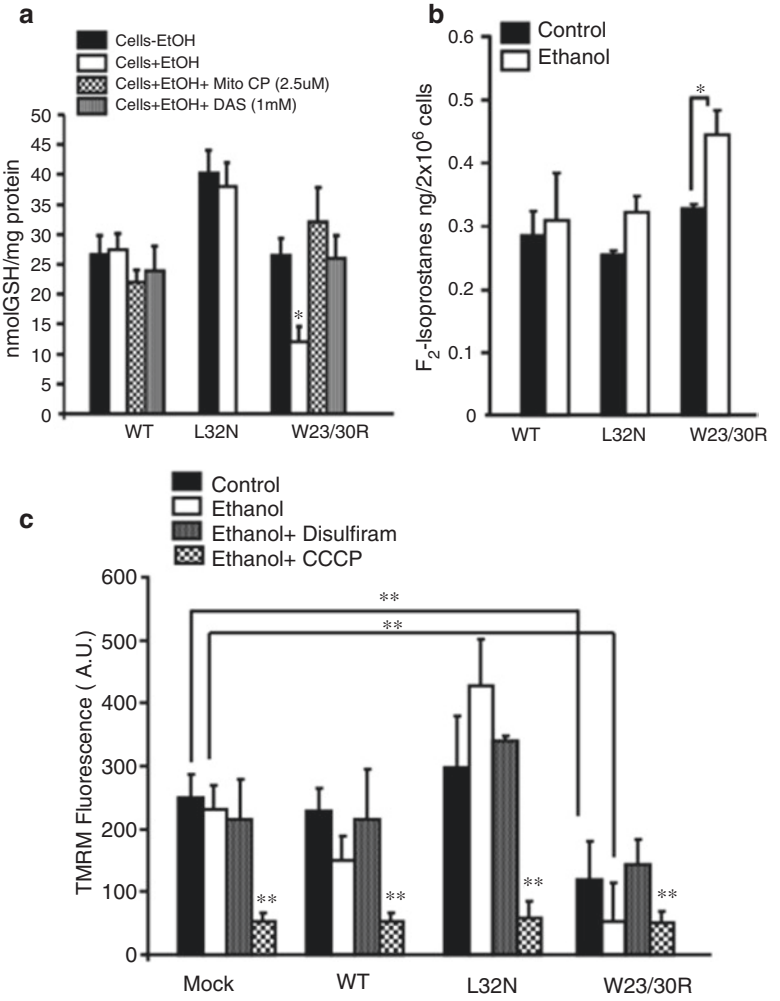

Fig. 2.8 Effects of ethanol on cellular toxicity in cells expressing WT and mutant human P450 2E1 constructs [8] (**a**) cellular GSH levels (nmol/mg protein). (**b**) ethanol-induced F_2-isoprostanes in human P450 2E1-expressing cells, assayed using gas chromatography-mass spectrometry (expressed as ng/2 × 10^6 total cells). (**c**) mitochondrial membrane potential ($\Delta\Psi_m$) measured spectrophotometrically in stable cells using a fluorescent dye, tetramethylrhodamine methyl ester (TMRM). Briefly, cells were incubated with and without ethanol and also treated with disulfiram (25 μM) overnight. Cells were washed and loaded with 150 nM tetramethylrhodamine methyl ester in an assay involving a Chameleon microplate reader (excitation wavelength 535 nm, emission wavelength 590 nm). For a control, 10 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was used in each series. *, p <0.05; **, p <0.001. Values represent means ± SE from three independent measurements. A.U., arbitrary units. This research was originally published in Bansal, S., Anandatheerthavarada, H. K., Prabu, G. K., Milne, G. L., Martin, M. V., Guengerich, F. P., and Avadhani, N. G. [8] Human cytochrome P450 2E1 mutations that alter mitochondrial targeting efficiency and susceptibility to ethanol-induced toxicity in cellular models. *The Journal of Biological Chemistry* **288**, 12,627–12,644. © The American Society for Biochemistry and Molecular Biology

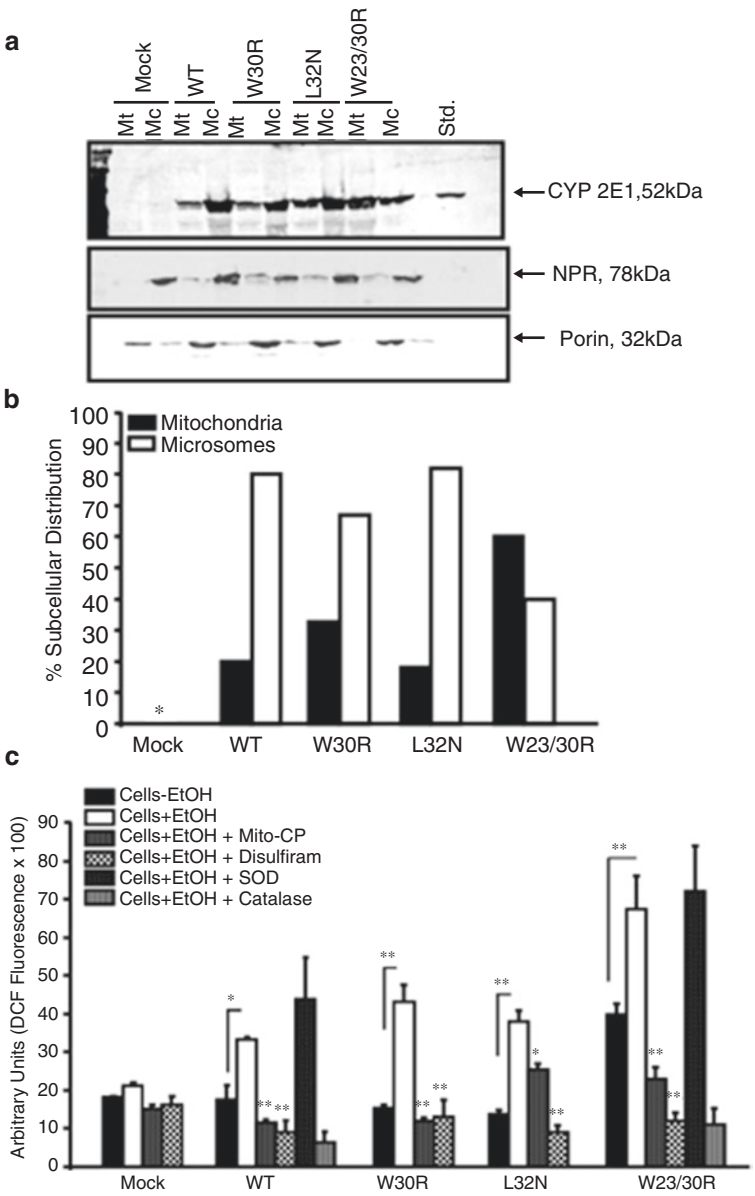

Fig. 2.9 Subcellular distribution and ethanol-mediated ROS in HepG2 cells stably expressing WT or mutant human P450 2E1. [8] (**a**) mitochondria and microsomes from HepG2 cells stably expressing human P450 2E1 cDNAs. Mitochondrial and microsomal fractions (50 μg of protein) were subjected to immunoblotting analysis (anti- human P450 2E1). The blot was also co-developed with anti-NADPH-P450 reductase (a microsomal marker) and porin (a mitochondrial marker) to assess relative cross-contamination. (**b**) percentage subcellular distribution was calculated based on band intensity. (**c**) ROS levels in whole cells grown with or without ethanol (300 mM) were estimated using the dye dichlorofluorescin (DCF) as the substrate.

References

1. Anandatheerthavarada HK, Addya S, Mullick J, Avadhani NG (1998) Interaction of adrenodoxin with P4501A1 and its truncated form $P450_{MT2}$ through different domains: differential modulation of enzyme activities. Biochemistry 37:1150–1160
2. Anandatheerthavarada HK, Amuthan G, Biswas G, Robin MA, Murali R, Waterman MR, Avadhani NG (2001) Evolutionarily divergent electron donor proteins interact with $P450_{MT2}$ through the same helical domain but different contact points. EMBO J 20:2394–2403
3. Avadhani NG, Sangar MC, Bansal S, Bajpai P (2011) Bimodal targeting of cytochrome P450s to endoplasmic reticulum and mitochondria: the concept of chimeric signals. FEBS J 278:4218–4229
4. Backer JM, Weinstein IB (1980) Mitochondrial DNA is a major cellular target for a dihydrodiol-epoxide derivative of benzo[*a*]pyrene. Science 209:297–299
5. Bajpai P, Sangar MC, Singh S, Tang W, Bansal S, Chowdhury G, Cheng Q, Fang JK, Martin MV, Guengerich FP, Avadhani NG (2013) Metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by mitochondrion-targeted cytochrome P450 2D6: implications in Parkinson disease. J Biol Chem 288:4436–4451
6. Bajpai P, Srinivasan S, Ghosh J, Nagy LD, Wei S, Guengerich FP, Avadhani NG (2014) Targeting of splice variants of human cytochrome P450 2C8 (CYP2C8) to mitochondria and their role in arachidonic acid metabolism and respiratory dysfunction. J Biol Chem 289:29614–29630
7. Bansal S, Liu CP, Sepuri NB, Anandatheerthavarada HK, Selvaraj V, Hoek J, Milne GL, Guengerich FP, Avadhani NG (2010) Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol-mediated oxidative stress. J Biol Chem 285:24609–24619
8. Bansal S, Anandatheerthavarada HK, Prabu GK, Milne GL, Martin MV, Guengerich FP, Avadhani NG (2013) Human cytochrome P450 2E1 mutations that alter mitochondrial targeting efficiency and susceptibility to ethanol-induced toxicity in cellular models. J Biol Chem 288:12627–12644
9. Bansal S, Leu AN, Gonzalez FJ, Guengerich FP, Chowdhury AR, Anandatheerthavarada HK, Avadhani NG (2014) Mitochondrial targeting of cytochrome P450 (CYP) 1B1 and its role in polycyclic aromatic hydrocarbon-induced mitochondrial dysfunction. J Biol Chem 289:9936–9951
10. Bauer KH (1928) Mutationstheorie der Geschwulstenstehung. Springer, Berlin
11. Bell LC, Guengerich FP (1997) Oxidation kinetics of ethanol by human cytochrome P450 2E1. Rate-limiting product release accounts for effects of isotopic hydrogen substitution and cytochrome b_5 on steady-state kinetics. J Biol Chem 272:29643–29651
12. Bell-Parikh LC, Guengerich FP (1999) Kinetics of cytochrome P450 2E1-catalyzed oxidation of ethanol to acetic acid via acetaldehyde. J Biol Chem 274:23833–23840

Fig. 2.9 (continued) Cells were treated with the mitochondria-targeted antioxidant Mito-CP (2.5 μM)and the P450 2E1 inhibitor disulfiram (25 μM) (cell-permeable superoxide dismutase and catalase were used as controls). Values represent means ± SD (error bars) from three independent experiments. *, $p < 0.05$; **, $p < 0.001$. This research was originally published in Bansal, S., Anandatheerthavarada, H. K., Prabu, G. K., Milne, G. L., Martin, M. V., Guengerich, F. P., and Avadhani, N. G. [8] Human cytochrome P450 2E1 mutations that alter mitochondrial targeting efficiency and susceptibility to ethanol-induced toxicity in cellular models. *The Journal of Biological Chemistry* **288**, 12,627–12,644. © the American Society for Biochemistry and Molecular Biology

13. Bhagwat SV, Biswas G, Anandatheerthavarada HK, Addya S, Pandak W, Avadhani NG (1999) Dual targeting property of the N-terminal signal sequence of P4501A1. Targeting of heterologous proteins to endoplasmic reticulum and mitochondria. J Biol Chem 274:24014–24022
14. Bradford BU, Seed CB, Handler JA, Forman DT, Thurman RG (1993) Evidence that catalase is a major pathway of ethanol oxidation *in vivo*: dose-response studies in deer mice using methanol as a selective substrate. Arch Biochem Biophys 303:172–176
15. Castonguay A, Rivenson A, Trushin N, Reinhardt J, Spathopoulos S, Weiss CJ, Reiss B, Hecht SS (1984) Effects of chronic ethanol consumption on the metabolism and carcinogenicity of *N´*-nitrosonornicotine in F344 rats. Cancer Res 44:2285–2290
16. Cederbaum AI (2006) Cytochrome P450 2E1-dependent oxidant stress and upregulation of anti-oxidant defense in liver cells. J Gastroenterol Hepatol 21(Suppl 3):S22–S25
17. Chowdhury G, Calcutt MW, Nagy LD, Guengerich FP (2012) Oxidation of methyl and ethyl nitrosamines by cytochrome P450 2E1 and 2B1. Biochemistry 51:9995–10007
18. Ortiz de Montellano, PR (ed) (n.d.) Cytochrome P450: Structure, Mechanism, and Biochemistry, 4th edn. Springer, New York
19. Dong MS, Yamazaki H, Guo Z, Guengerich FP (1996) Recombinant human cytochrome P450 1A2 and an N-terminal-truncated form: construction, purification, aggregation properties, and interactions with flavodoxin, ferredoxin, and NADPH-cytochrome P450 reductase. Arch Biochem Biophys 327:11–19
20. Dostalek M, Brooks JD, Hardy KD, Milne GL, Moore MM, Sharma S, Morrow JD, Guengerich FP (2007) In vivo oxidative damage in rats is associated with barbiturate response but not other cytochrome P450 inducers. Mol Pharmacol 72:1419–1424
21. Dostalek M, Hardy KD, Milne GL, Morrow JD, Chen C, Gonzalez FJ, Gu J, Ding X, Johnson DA, Johnson JA, Martin MV, Guengerich FP (2008) Development of oxidative stress by cytochrome P450 induction in rodents is selective for barbiturates and related to loss of pyridine nucleotide-dependent protective systems. J Biol Chem 283:17147–17157
22. Ekström G, Ingelman-Sundberg M (1989) Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). Biochem Pharmacol 38:1313–1319
23. Enright JM, Toomey MB, Sato SY, Temple SE, Allen JR, Fujiwara R, Kramlinger VM, Nagy LD, Johnson KM, Xiao Y, How MJ, Johnson SL, Roberts NW, Kefalov VJ, Guengerich FP, Corbo JC (2015) Cyp27c1 red-shifts the spectral sensitivity of photoreceptors by converting vitamin A_1 into A_2. Curr Biol 25:3048–3057
24. Evans DC, Watt AP, Nicoll-Griffith DA, Baillie TA (2004) Drug-protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. Chem Res Toxicol 17:3–16
25. Gonzalez FJ (2007) The 2006 Bernard B Brodie Award Lecture. Cyp2e1. *Drug Metab Dispos* 35:1–8
26. Gonzalez FJ, Kimura S (2003) Study of P450 function using gene knockout and transgenic mice. Arch Biochem Biophys 409:153–158
27. Greenzaid P, Luz Z, Samuel D (1967a) A nuclear magnetic resonance study of the reversible hydration of aliphatic aldehydes and ketones. I. oxygen-17 and protonspectra and equilibrium constants. J Am Chem Soc 89:749–755
28. Greenzaid P, Luz Z, Samuel D (1967b) A nuclear magnetic resonance study of the reversible hydration of aliphatic aldehydes and ketones. II. The acid-catalyzed oxygen exchange of acetaldehyde. J Am Chem Soc 89:756–759
29. Guengerich FP (2015) Human cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed) *Cytochrome P450: structure, mechanism, and biochemistry*, 4th edn. Springer, New York, pp 523–785
30. Guengerich FP, Cheng Q (2011) Orphans in the human cytochrome P450 superfamily: approaches to discovering functions and relevance in pharmacology. Pharmacol Rev 63:684–699
31. Guengerich FP, Kim DH, Iwasaki M (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 4:168–179

32. Guengerich FP, Bell LC, Okazaki O (1995) Interpretations of cytochrome P450 mechanisms from kinetic studies. Biochimie 77:573–580
33. Guengerich FP, Sohl CD, Chowdhury G (2011) Multi-step oxidations catalyzed by cytochrome P450 enzymes: Processive vs. distributive kinetics and the issue of carbonyl oxidation in chemical mechanisms. Arch Biochem Biophys 507:126–134
34. Hartman JH, Martin HC, Caro AA, Pearce AR, Miller GP (2015) Subcellular localization of rat CYP2E1 impacts metabolic efficiency toward common substrates. Toxicology 338:47–58
35. Johnson KA (2003) Introduction to kinetic analysis of enzyme systems. In: Johnson KA (ed) *Kinetic analysis of macromolecules. A practical approach*. Oxford University Press, Oxford, pp 1–18
36. Johnson WW, Guengerich FP (1997) Reaction of aflatoxin B_1 *exo*-8,9-epoxide with DNA: Kinetic analysis of covalent binding and DNA-induced hydrolysis. Proc Natl Acad Sci U S A 94:6121–6125
37. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB (1973) Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. J Pharmacol Exp Ther 187:195–202
38. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, Fitzgerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts, L. J., 2nd, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP, Barrett JC (2005a) Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 38:698–710
39. Kadiiska MB, Gladen BC, Baird DD, Graham LB, Parker CE, Ames BN, Basu S, Fitzgerald GA, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts, L. J., 2nd, Rokach J, Shigenaga MK, Sun J, Walter PB, Tomer KB, Barrett JC, Mason RP (2005b) Biomarkers of oxidative stress study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl_4 poisoning. Free Radic Biol Med 38:711–718
40. Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB, Mann GE, Moore K, Roberts, L. J., 2nd, Ischiropoulos H (2012) Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radic Biol Med 52:1–6
41. Keefer LK, Lijinsky W, Garcia H (1973) Deuterium isotope effect on the carcinogenicity of dimethylnitrosamine in rat liver. J Natl Cancer Inst 51:299–302
42. Kono H, Bradford BU, Yin M, Sulik KK, Koop DR, Peters JM, Gonzalez FJ, McDonald T, Dikalova A, Kadiiska MB, Mason RP, Thurman RG (1999) CYP2E1 is not involved in early alcohol-induced liver injury. Am J Phys 277:G1259–G1267
43. Koop DR, Morgan ET, Tarr GE, Coon MJ (1982) Purification and characterization of a unique isozyme of cytochrome P-450 from liver microsomes of ethanol-treated rabbits. J Biol Chem 257:8472–8480
44. Kuby SA (1991) A study of enzymes, I, Enzyme catalysis, Kinetics, and substrate binding. CRC Press, Boca Raton
45. Kunitoh S, Imaoka S, Hiroi T, Yabusaki Y, Monna T, Funae Y (1997) Acetaldehyde as well as ethanol is metabolized by human CYP2E1. J Pharmacol Exp Ther 280:527–532
46. Lehnerer M, Schulze J, Bernhardt R, Hlavica P (1999) Some properties of mitochondrial adrenodoxin associated with its nonconventional electron donor function toward rabbit liver microsomal cytochrome P450 2B4. Biochem Biophys Res Commun 254:83–87
47. Lieber CS, DeCarli LM (1970) Hepatic microsomal ethanol oxidizing system: In vitro chracteristics and adaptive properties in vivo. J Biol Chem 245:2505–2512
48. McCoy GD, Hecht SS, Katayama S, Wynder EL (1981) Differential effect of chronic ethanol consumption on the carcinogenicity of *N*-nitrosopyrrolidine and *N′*-nitrosonornicotine in male Syrian golden hamsters. Cancer Res 41:2849–2854

49. Mico BA, Swagzdis JE, Hu HS-W, Keefer LK, Oldfield NF, Garland WA (1985) Low-dose *in vivo* pharmacokinetic and deuterium isotope effect studies of *N*-nitrosodimethylamine in rats. Cancer Res 45:6280–6285
50. Miller JA (1970) Carcinogenesis by chemicals: an overview. G.H.A. Clowes Memorial Lecture. Cancer Res 30:559–576
51. Mueller GC, Miller JA (1948) The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. J Biol Chem 176:535–544
52. Nebert DW, Nelson DR, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B et al (1989) The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. DNA 8:1–13
53. Niranjan BG, Avadhani NG (1980) Activation of aflatoxin B_1 by a mono-oxygenase system localized in rat liver mitochondria. J Biol Chem 255:6575–6578
54. Niranjan BG, Bhat NK, Avadhani NG (1982) Preferential attack of mitochondrial DNA by aflatoxin B1 during hepatocarcinogenesis. Science 215:73–75
55. Niranjan BG, Wilson NM, Jefcoate CR, Avadhani NG (1984) Hepatic mitochondrial cytochrome P-450 system: distinctive features of cytochrome P-450 involved in the activation of aflatoxin B1 and benzo[a]pyrene. J Biol Chem 259:12495–12501
56. Northrop DB (1998) On the meaning of K_m and *V/K* in enzyme kinetics. J Chem Educ 75:1153–1157
57. Orme-Johnson WH, Ziegler DM (1965) Alcohol mixed function oxidase activity of mammalian liver micromes. Biochem Biophys Res Commun 21:78–82
58. Paolini M, Sapigni E, Hrelia P, Scotti M, Morotti M, Cantelli-Forti G (1991) Wide spectrum detection of precarcinogens in short-term bioassays by simultaneous superinduction of multiple forms of cytochrome P450 isoenzymes. *Carcinogenesis* 12:759–766
59. Pechurskaya TA, Harnastai IN, Grabovec IP, Gilep AA, Usanov SA (2007) Adrenodoxin supports reactions catalyzed by microsomal steroidogenic cytochrome P450s. *Biochem Biophys Res Commun* 353:598–604
60. Porubsky PR, Meneely KM, Scott EE (2008) Structures of human cytochrome P-450 2E1. Insights into the binding of inhibitors and both small molecular weight and fatty acid substrates. *J Biol Chem* 283(48):33698–33707
61. Porubsky PR, Battaile KP, Scott EE (2010) Human cytochrome P450 2E1 structures with fatty acid analogs reveal a previously unobserved binding mode. *J Biol Chem* 285(29):22282–22290
62. Rashba-Step J, Cederbaum AI (1994) Generation of reactive oxygen intermediates by human liver microsomes in the presence of NADPH or NADH. *Mol Pharmacol* 45:150–157
63. Rendic S, Guengerich FP (2012) Contributions of human enzymes in carcinogen metabolism. *Chem Res Toxicol* 25:1316–1383
64. Rendic S, Guengerich FP (2015) Survey of human oxidoreductases and cytochrome P450 enzymes involved in the metabolism of xenobiotic and natural chemicals. *Chem Res Toxicol* 28:38–42
65. Richardson HL, Stier AR, Borsos-Nachtnebel E (1952) Liver tumor inhibition and adrenal histologic responses in rats to which 3′-methyl-4-dimethylaminoazobenzene and 20-methylcholanthrene were simultaneously administered. *Cancer Res* 12:356–361
66. Robin MA, Anandatheerthavarada HK, Fang JK, Cudic M, Otvos L, Avadhani NG (2001) Mitochondrial targeted cytochrome P450 2E1 (P450 MT5) contains an intact N-terminus and requires mitochondrial specific electron transfer proteins for activity. *J Biol Chem* 276:24680–24689
67. Robin MA, Anandatheerthavarada HK, Biswas G, Sepuri NB, Gordon DM, Pain D, Avadhani NG (2002) Bimodal targeting of microsomal CYP2E1 to mitochondria through activation of an N-terminal chimeric signal by cAMP-mediated phosphorylation. *J Biol Chem* 277:40583–40593
68. Ryan DE, Ramanathan L, Iida S, Thomas PE, Haniu M, Shively JE, Lieber CS, Levin W (1985) Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. *J Biol Chem* 260:6385–6393

69. Sakaguchi M, Mihara K, Sato R (1984) Signal recognition particle is required for co-translational insertion of cytochrome P-450 into microsomal membranes. *Proc Natl Acad Sci U S A* 81:3361–3364
70. Sangar MC, Anandatheerthavarada HK, Tang W, Prabu SK, Martin MV, Dostalek M, Guengerich FP, Avadhani NG (2009) Human liver mitochondrial cytochrome P450 2D6individual variations and implications in drug metabolism. *FEBS J* 276:3440–3453
71. Shayiq, R. M., and Avadhani, N. G. (1989) A phenobarbital inducible hepatic mitochondrial cytochrome P-450 immunochemically related to microsomal P-450_b.
72. Shimada T, Guengerich FP (1989) Evidence for cytochrome P-450_{NF}, the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. *Proc Natl Acad Sci U S A* 86:462–465
73. Shimada T, Iwasaki M, Martin MV, Guengerich FP (1989) Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by umu gene response in *Salmonella typhimurium* TA 1535/pSK1002. *Cancer Res* 49:3218–3228
74. Shinkyo R, Guengerich FP (2011) Cytochrome P450 7A1 cholesterol 7alpha-hydroxylation: individual reaction steps in the catalytic cycle and rate-limiting ferric iron reduction. *J Biol Chem* 286:4632–4643
75. Terelius Y, Norsten-Höög C, Cronholm T, Ingelman-Sundberg M (1991) Acetaldehyde as a substrate for ethanol-inducible cytochrome P450 (CYP2E1). *Biochem Biophys Res Commun* 179:689–694
76. Thurman RG, Ley HG, Scholz R (1972) Hepatic microsomal ethanol oxidation. Hydrogen peroxide formation and the role of catalase. *Eur J Biochem/FEBS* 25:420–430
77. Ueng Y-F, Shimada T, Yamazaki H, Guengerich FP (1995) Oxidation of aflatoxin B_1 by bacterial recombinant human cytochrome P450 enzymes. *Chem Res Toxicol* 8:218–225
78. Van Houten B, Woshner V, Santos JH (2006) Role of mitochondrial DNA in toxic responses to oxidative stress. *DNA Repair* 5:145–152
79. Walsh C (1979) *Enzymatic reaction mechanisms*. W. H. Freeman Co, San Francisco, pp 67–71
80. Wienkers LC, Heath TG (2005) Predicting in vivo drug interactions from in vitro drug discovery data. *Nat Rev Drug Discov* 4:825–833
81. Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, Ball SE (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUC_i/AUC) ratios. *Drug Metab Dispos* 32:1201–1208
82. Yang CS, Ishizaki H, Lee M, Wade D, Fadel A (1991) Deuterium isotope effect in the interaction of *N*-nitrosodimethylamine, ethanol, and related compounds with cytochrome P-450IIE1. *Chem Res Toxicol* 4:408–413
83. Yin M, Gabele E, Wheeler MD, Connor H, Bradford BU, Dikalova A, Rusyn I, Mason R, Thurman RG (2001) Alcohol-induced free radicals in mice: direct toxicants or signaling molecules? *Hepatology* 34:935–942
84. Yun CH, Kim KH, Calcutt MW, Guengerich FP (2005) Kinetic analysis of oxidation of coumarins by human cytochrome P450 2A6. *J Biol Chem* 280:12279–12291
85. American Cancer Society (ACS) (2017) American Cancer Society, Atlanta, GA. https://www.cancer.org/content/dam/cancerorg/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf