



## Polymorphism of P450 in *Drosophila melanogaster*

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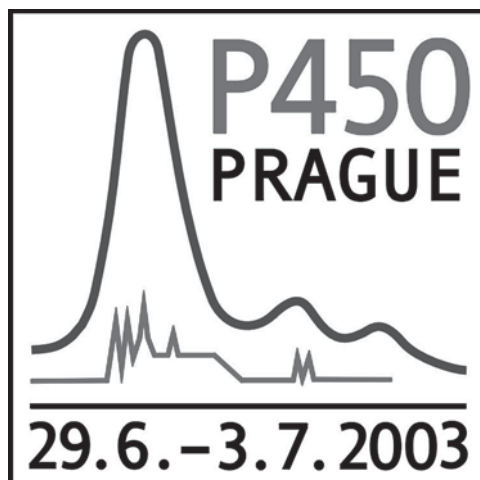
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in the microsomal fractions of minipig and human origin as well as in the respective reconstituted systems. Inhibition studies with diethyldithiocarbamate complement the study.

Both the chlorzoxazone 6-hydroxylating activity and the p-nitrophenol hydroxylating one has been determined using the methods described in literature<sup>4,5</sup>. The protein itself has been isolated according to procedure published earlier<sup>2,3</sup>. Enzyme kinetics has been analyzed using a LSW Data Analysis software (www.lsw.com). The results obtained show conclusively that the minipig cytochrome P450 2E1 is able to catalyze both reactions exhibiting the activity well comparable to this of the human enzyme. Diethyldithiocarbamate, a specific CYP2E1 inhibitor, has been also shown to be functional in minipig reconstituted system inhibiting its activity to 10% and less. Hence, the minipig enzyme shows the characteristics typical of a CYP2E1 enzyme.

#### Acknowledgment

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### TP27 TWO NOVEL HUMAN P450 ENZYMES, CYP4Z1 AND CYP4X1

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The CYP4 family was originally discovered as the lauric acid omega-hydroxylase, which was induced in rat liver by peroxisome proliferators<sup>1,2</sup>. The cloning of members of the CYP4B and CYP4F families has revealed that there are additionally fatty acid substrates for the CYP4 family<sup>2-5</sup>. The CYP4 family has a potentially important role in metabolism of endogenous fatty compounds.

We prepared an extensive alignment of members of the CYP4 family using the program SAGA; this alignment included both vertebrate and invertebrate P450 proteins. The alignment showed unexpectedly high sequence similarity

in the region of the C-helix for members of the CYP4 family. In order to test the significance of this finding, we used the sequence of CYP4A1 in the conserved region to search DNA databases. The conserved sequence detected exclusively members of the CYP4 family, and two additional partial EST sequences.

Subsequent cloning of the two novel gene sequences was achieved by RACE-PCR, and yielded full-length sequences for CYP4Z1 and CYP4X1. These data are consistent with subsequent human genomic sequences. The sequence of CYP4Z1 and CYP4X1 predict functional cytochromes P450. Antisera were raised against recombinant fragments of CYP4X1 and CYP4Z1. These antisera are characterised. The tissue-specific distribution of CYP4Z1 and CYP4X1 is discussed.

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### TP28 POLYMORPHISM OF P450 IN DROSOPHILA MELANOGASTER

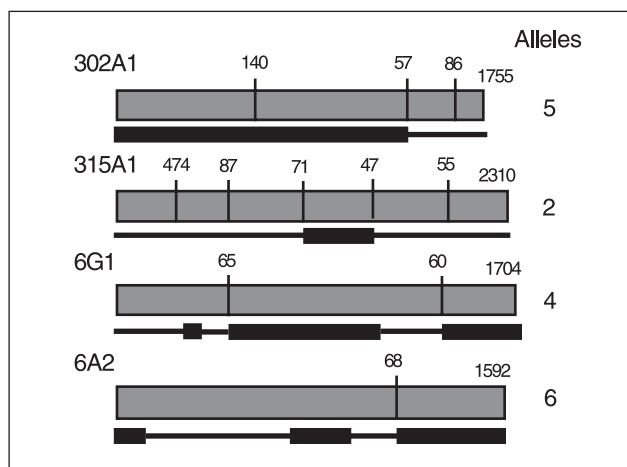
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Because of its impact on drug metabolism, cytochrome P450s polymorphism is under the scope in humans<sup>1</sup>. In insects, they are involved in numerous instances of insecticide resistance and adaptation to chemical stresses. Available large collections of strains, easyness of wild populations collections and genome knowledge make *Drosophila melanogaster* a suitable species for a global study of P450s polymorphism in insects. In this first study, we analyzed in 7 strains the polymorphism of Cyp6a2 and Cyp6g1 (xenobiotics metabolism and insecticide resistance<sup>2,3</sup>), Cyp302a1 and Cyp315a1 (steroid synthesis, dib and sad genes<sup>4,5</sup>) and of Cyp6w1 and Cyp6u1, two P450s physically close to Cyp6a2<sup>6</sup>.

For each gene, primers were designed to amplify by PCR fragments no longer than 320 bp in the coding sequence. We first analysed these fragments by SSCP. Results showed that Cyp6a2, Cyp6g1 and Cyp302a1 (dib) were polymorphic with 6, 4 and 5 alleles respectively contrary to Cyp315a1 (sad) which had only 2 alleles. Work is in





**Fig. 1.** The 4 genes were represented with the position and the size of the introns. The polymorphic regions are indicated under the gene with black bars and narrow bars respectively.

progress with Cyp6u1 and Cyp6w1. There was a region polymorphic in all the genes which code for the G, H and I helices but polymorphism was not distributed among the genes uniformly. Although polymorphism was expected for Cyp6a2 and to a lesser extend for Cyp6g1, we were surprised to classify Cyp302a1 in the polymorphic P450s category. Indeed, this gene is involved in steroid hormone synthesis and thus should be submitted to a high selection pressure. Nevertheless, we are sequencing these alleles to identify which polymorphism event is translated and thus putatively effective on protein function.

The amount of polymorphism of each gene, its repartition among the gene and its putative effects on the encoded protein activity will be discussed taking account of the function of the gene and P450 structure/activity relationships.

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## TP29 BACTERIAL MONOOXYGENASES IN BIOTRANSFORMATION AND BIODEGRADATION OF ALIPHATIC AND AROMATIC COMPOUNDS

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Bacteria are able to degrade or transform many natural or xenobiotic compounds occurring in the environment using variety of oxidative reactions. An important group of enzymes are the monooxygenases including P-450 enzymes. The best known and characterized bacterial P-450 was the P-450cam isolated from *Pseudomonas*<sup>1</sup>.

Study of dependent of the levels of cytochromes  $b_5$  and P450 from structure of substrate was by purpose of this work. Two series of the compounds were used for these investigations. Aliphatic and alicyclic compounds represent the first, halogenated aromatic compounds represent the second series. *Pseudomonas fluorescens* B-22 was used for the transformation aliphatic and alicyclic compounds, *Rhodococcus opacus* B-2243 for the degradation of halogenated aromatic compounds. The levels of cytochromes  $b_5$  and P450 was determined spectrophotometrically by the method Omura and Sato<sup>2</sup>. The concentrations of cyt P450 were calculated from reduced carbon monoxide difference spectrum, using an extinction coefficient  $92,8 \text{ mM}^{-1} \text{ cm}^{-1}$  in according to the paper<sup>3</sup>. The results of these studies are represented in Table 1 and Table 2.

The change of the levels of cytochromes  $b_5$  and P450 in *Pseudomonas fluorescens* B-22 (Table 1) probably is caused by distinction of chemical reactions on cyt P450. Hexan is capable to hydroxylate with formation of alcohols; hexen-1 may be hydroxylated, but preferable it epoxydates; cyclohexen and nonen-4 epoxydate, what was confirmed in further study with GC.

**Table 1.** Content of cytochromes  $b_5$  and P450 in *Pseudomonas fluorescens* B-22

Substrate	Contents of cytochrome $b_5$ , nmol/mg of protein		Contents of cytochrome P450, nmol/mg of protein	
	Exponential growth phase	Stationary growth phase	Exponential growth phase	Stationary growth phase
Glucose	0,04	0,02	0,02	-
Hexan	0,12	0,05	0,10	0,08
Hexen-1	0,18	0,03	0,21	0,03
Cyclohexen	0,04	0,04	0,23	0,02
Nonen-4	0,05	0,01	0,18	0,02