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Yeast Expressed Cytochrome P450 2D6 (CYP2D6) Exposed on the External Face of Plasma Membrane Is Functionally Competent

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ABSTRACT

CYP2D6, a xenobiotic metabolizing cytochrome P450 (P450), was found to be present in significant amount on the outer face of cell plasma membrane in addition to the regular microsomal location. Present work demonstrates that this external P450 is catalytically competent and that activity is supported by NADPH-P450 reductase present on the inner face of plasma membrane. Purified plasma membranes from yeast expressing CYP2D6 sustained NADPH- and cumene hydroperoxide-dependent dextromethorphan demethylation and NADPH-cytochrome c activity confirming previous observations in human hepatocytes. CYP2D6 found on the outside of plasma membrane (by differential immuno-inhibition and acidic shift assays on transformed spheroplasts) was catalytically competent at the cell surface for NADPH-supported activities. Anti-yeast P450-reductase antibodies inhibited neither CYP2D6 nor P450-reductase activities upon incubation with intact spheroplasts. In contrast, both activities were inhibited on isolated plasma membrane fragments. This highly suggested a cytosolic-orientation of the plasma membrane P450-reductase. This finding was confirmed by immunostaining in confocal microscopy. Finally, gene deletion of P450-reductase caused a complete loss of plasma membrane NADPH-supported CYP2D6 activity, which suggests that the reductase participates to some degree in the transmembrane electron transfer chain. This work illustrates that the outside-exposed plasma membrane CYP2D6 is active and may play an important metabolic role.

Data on the occurrence P450s on PM are conflicting. Although several workers evidenced the presence of various P450 isoforms, including CYP2D6, on hepatic or hepatocyte plasma membranes, pulmonary cells, and transfected mammalian cells, others were unable to detect external P450 expression (for a review, see Loeper *et al.*, 1990). PM P450 presence was evidenced by immunocytochemistry, Western blot, spectral, or activity analysis (for a review, see Beaune *et al.*, 1994). P450s were identified as specific targets for autoantibodies in idiopathic autoimmune diseases (for a review, see Lecoeur *et al.*, 1996) or in hepatitis induced by such drugs as tienilic acid (Beaune *et al.*, 1987), dihydralazine (Bourdi *et al.*, 1990), anticonvulsivant (Leeder *et al.*, 1992) and halothane (Eliasson and Kenna, 1996). CYP2D6 is the target of anti-LKM1 autoantibodies (anti-CYP2D6) developed in the type 2 autoimmune hepatitis (Gueguen *et al.*, 1988; Zanger *et al.*, 1988; Kiffel *et al.*, 1989; Manns *et al.*, 1989) and in C viral hepatitis (Parez *et al.*, 1996). Different P450 forms were recognized on the outside face of unpermeabilized hepatocytes by these autoantibodies (Loeper *et al.*, 1990, 1993). B-cell epitopes of some of these autoantibodies were characterized by epitope mapping to be sequential or conformational sites located on the cytosol-exposed part of the P450 (for a review, see Lecoeur *et al.*, 1996). CYP2D6, which exhibits an important genetic polymorphism, is involved in the transformation of a great variety of drugs, most of which act on the central nervous system, and is required for metabolic activation of some of these drugs. Its presence at the surface of human hepatocytes (Loeper *et al.*, 1993) and recognition by anti-CYP2D6 autoantibodies directed against a specific cytosol-exposed epitope were demonstrated (Manns *et al.*, 1991; Yamamoto *et al.*, 1993). This isoform was chosen as a model to investigate catalytic competence of PM P450s.

In rat and human hepatocytes, CPR was found to be present on PMs and to support NADPH-cytochrome *c* reductase activity (Stasiecki and Oesch, 1980; Loeper *et al.*, 1990; Wu and Cederbaum, 1991; Loeper *et al.*, 1993). In a recent paper, we demonstrated that yeast expression mimicked the PM localization observed in hepatocyte and mammalian cells (Loeper *et al.*, 1998). In this present work, PM CYP2D6 was found functional as in mammalian cells, in NADPH- and cumene hydroperoxide (CumOOH)-supported reactions. And

ABBREVIATIONS: CPR, NADPH-cytochrome P450-reductase; CumOOH, cumene hydroperoxide; FITC, fluorescein isothiocyanate; CYP2D6, cytochrome P450 2D6; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PM, cell plasma membrane; P450, cytochrome P450.

we demonstrated that external PM location allowed NADPH-supported activity or required some hydroperoxide-dependent mechanism for function. CPR was also present in yeast PM but with an opposite orientation compared with CYP2D6. Availability of yeast strains to be disrupted for endogenous CPR or to overexpress it was an additional advantage to test for possible NADPH-dependent activity of CYP2D6 located on the external side of PM This is the first report demonstrating that human CYP2D6 exposed at the outside face of yeast PM is catalytically functional and able to couple with CPR present on the inner face of PM

Materials and Methods

***Saccharomyces cerevisiae* strains and growth conditions.** W303–1B (*MATa, leu2–3112 his3–11 ade2–1 trp1–1 ura3–1 can*^{R} *cyr*$^+$). W(R) was engineered from W303–1B to overexpress yeast CPR gene *YRED* (Pompon *et al.*, 1996). WRΔ derived from W303–1B by disruption of the yeast CPR gene by *TRP1*. Transformation and growth conditions were performed as described previously (Loeper *et al.*, 1998).

Plasmids. pYeDP60 (V60) carries the yeast "2 μ" origin of replication, the *URA3* and *ADE2* selection markers, an expression cassette based on the galactose inducible *GAL10-CYC1* promoter, a multiple cloning site, and the phosphoglycerate kinase gene (*PGK*) terminator. pCYP2D6/V60 (pCYP2D6) was constructed by insertion of human *CYP2D6* open reading frame between the *Bam*HI and *Eco*RI sites of V60 (Gautier *et al.*, 1996).

Subcellular purifications. Transformed yeast cells were exponentially grown in synthetic galactose/tryptophan medium (SLI) to a density of 1×10^7 cells/ml (A_{600} = 1.5). The PM fraction was prepared using electrostatic attachment of spheroplasts on cationic silica microbeads (gift of Dr. Bruce S. Jacobson) as described previously (Loeper *et al.*, 1998). Microsomes were prepared as described (Pompon *et al.*, 1996).

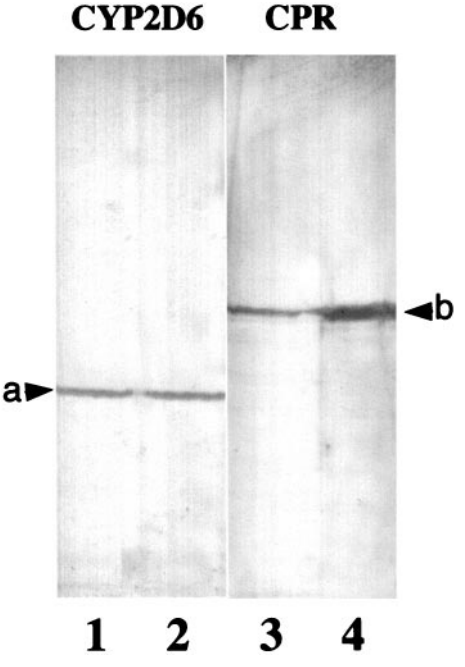

Fig. 1. SDS-PAGE immunoblot analysis of plasma membrane and microsomal fractions of yeast CPR and CYP2D6 expressed in yeast. Polyacrylamide gels (9%) were loaded with 20 μg of PM proteins (*lanes 1, 3*), and 5 μg of microsomal proteins (*lanes 2, 4*). After SDS-PAGE and electrophoretic transfer to nitrocellulose, blots were incubated with anti-CYP2D6 autoantibodies at 1:200 dilution (*lanes 1, 2*) or anti-yeast CPR antibodies at 1:200 dilution (*lanes 3, 4*). a, CYP2D6; b, CPR.

Enzymatic assays. NADPH-dependent dextromethorphan demethylation was performed using subcellular fractions (0.1 mg of microsomal protein in 0.2 ml or 0.1 mg of PM protein bound on the silica beads in 0.4 ml) in 50 mM Tris/HCI, pH 7.4, 1 mM EDTA buffer, 50 mM dextromethorphan (Roche, Switzerland), and 0.15 mM NADPH. After 15 min. incubation at 28° the reaction was extracted with an equal volume of ethylacetate. The upper phase was collected, air-dried and used for high performance liquid chromatography analysis. CumOOH-dependent dextromethorphan demethylation was determined as described previously (Loeper *et al.*, 1998). Dextrorphan fluorescence was detected using excitation at 270 nm and emission at 312 nm. CPR activity measurements were performed as described (Pompon *et al.*, 1996) except that some PM or microsomal samples were treated with detergent (0.5% sodium cholate, 0.5% triton X100, w/v) for 5 min on ice before the assay. Protein concentrations were determined using the Pierce bicinchoninic acid assay with bovine serum albumin as standard. Ergosterol content was measured by gas chromatography after PM-beads hexane extraction as described previously (Duport *et al.*, 1998).

Immunoblotting analysis. A female New Zealand rabbit was immunized with 300 μg of purified yeast CPR and complete Freund's adjuvant. A final immunization with 100 μg of protein was performed 10 days before testing the antibodies. IgG fractions from sera were prepared by sodium sulfate precipitation and dialyzed against 0.15 M NaCl. For control experiments, preimmune serum was collected. Microsomal proteins and PM were subjected to SDS-PAGE on 9% polyacrylamide gels. Proteins were transferred onto nitrocellulose membrane and immunodetected with either anti-CYP2D6 antibodies as described elsewhere (Loeper *et al.*, 1998b) or with anti-CPR antibodies diluted at 1:200.

Immunocytochemistry. For all experiments, anti-CYP2D6 and anti-CPR and secondary antibodies were adsorbed overnight at 4° against whole cells and fixed spheroplasts from control yeast (with an empty plasmid). Transformed cells were converted to spheroplasts, fixed with 4% paraformaldehyde and incubated with either anti-CYP2D6, anti-CPR, antibodies, control human IgG or preimmune sera (diluted 1:200). FITC conjugated secondary antibodies (Pasteur production, Marnes-la-Coquette, France) were used for immunofluorescence and confocal scanning microscopy. Control spheroplasts (with an empty plasmid) were treated under the same conditions. Labeled cells were analyzed on a MRC-1024 confocal scanning laser (Bio-Rad, Richmond, CA). Excitation was at 488 nm with a krypton-argon gas laser.

Results

Subcellular location of CPR and human CYP2D6 in transformed yeast. *S. cerevisiae* strain W(R) that overproduced yeast CPR was transformed with a galactose-inducible expression vector (pCYP2D6/V60) for CYP2D6. Western blot analysis shown that CYP2D6 was expressed in yeast microsomes (Fig. 1, *lane 2*). As demonstrated previously, presence of CYP2D6 in the yeast PM was also evidenced (Fig. 1, *lane 1*) by the purification method developed by Bruce S. Jacobson (Schmidt *et al.*, 1983). This method allowed preparation of PM practically devoid of microsomal contamination (Loeper *et al.*, 1998a).

CPR was also detected on Western blot, both in microsomal and PM fractions using anti-CPR antibodies (Fig. 1, *lanes 3* & *4*). The level of PM CPR was approximately 10-fold lower than the microsomal one. When fixed (unpermeabilized) spheroplasts were incubated with anti-CPR antibodies or preimmune sera and analyzed by confocal scanning microscopy, no labeling of the cell surface was observed (Fig. 2C). In contrast, presence of CYP2D6 at the surface of the cells, already described using immunoelectron microscopy and

flow-cytometry analysis (Loeper *et al.*, 1998), was clearly visualized by confocal analysis (Fig. 2, A and B). No signal was observed with control human IgG or control cells. On permeabilized cells, similar staining of the endoplasmic reticulum was obtained with anti-CYP2D6 and anti-CPR antibodies (Fig. 2D). These data demonstrate that CPR is also present at the PM but only CYP2D6 is exposed on the outside of cells.

CYP2D6 and CPR activities at the yeast plasma membrane. P450 activities were evaluated for NADPH- and hydroperoxide-supported dextromethorphan demethylase activities. Only the NADPH-supported activity, which involved electron transfers, required CPR, whereas P450 was self-sufficient for CumOOH-dependent reactions. In purified PM from CYP2D6 expressing cells, these specific activities were about half of the values found in microsomal fractions (Table 1). This result, along with a 4-fold lower content based on Western blotting (Fig. 1, *lanes 1* and *2*) suggests that CYP2D6 antigen corresponds to catalytically competent protein.

NADPH-cytochrome *c* reductase activities of isolated and detergent solubilized PM fractions were found to be 10% and 20% of the microsomal fractions, respectively (Table 1). The CPR activity measured in the absence of detergent pretreatment was consistent with previous value reported for purified yeast PM (Zinser *et al.*, 1991). Also this activity well correlated Western blot signals in PM and in microsomes (Fig. 1, lanes *3* and *4*). Experiments using purified PM or microsomes from WRΔ strain (disrupted for the yeast *CPR* gene) expressing CYP2D6, revealed the absence of residual NADPH-supported cytochrome c reductase or CYP2D6 activities. In contrast, CumOOH-dependent activities in WRΔ remained identical to those in W(R) strain (Table 1). Taken together, these experiments suggest that CPR is required to support the NADPH-dependent activities of both microsomal and PM located CYP2D6.

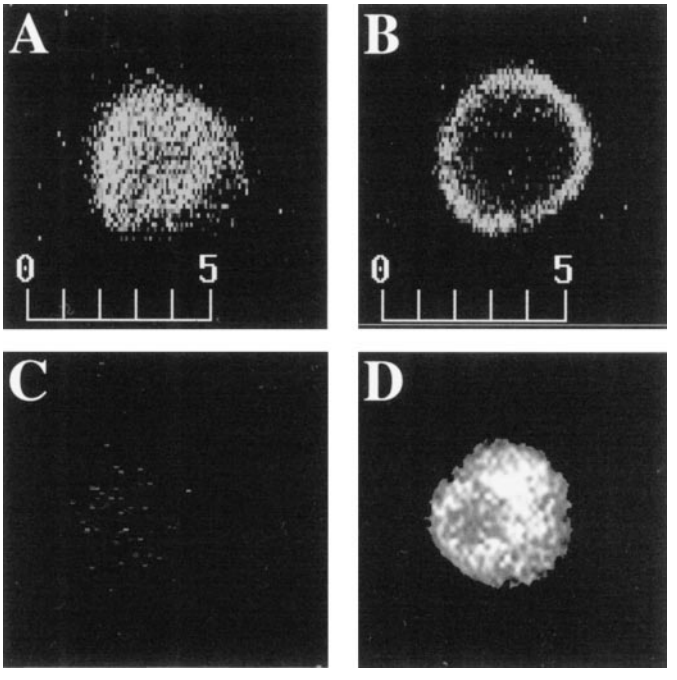

Fig. 2. Plasma membrane localization of CYP2D6 expressed in yeast and yeast CPR by confocal analysis. Fixed unpermeabilized spheroplasts expressing CYP2D6 and yeast CPR were incubated with anti-CYP2D6 or anti-CPR antibodies and with FITC-conjugated antibodies. Top (A) and middle-cut (B) of cell expressing CYP2D6 that exhibits a clear immunostaining only at the surface of the spheroplast. C, No staining was observed at the cell surface with anti-CPR antibodies. D, On permeabilized cell, staining around the nucleus and thorough the cytoplasm was given by anti-CPR antibodies. *Bars*, 5 μm.

CYP2D6 located on the external face of the plasma membrane is catalytically competent. To establish the functionality of P450 exposed on the outside of the PM, the following experiment was performed. Intact spheroplasts from cells expressing CYP2D6 were incubated with anti-CYP2D6 autoantibodies or control antibodies. After spheroplast washing, PM were prepared. To quantify CYP2D6 activity, CPR activity was used as an internal standard; protein content could not be estimated because of immunoglobulin interferences. Incubation with anti-CYP2D6 antibodies decreased by 95% the NADPH-dependent CYP2D6 activity in PM fractions isolated from spheroplasts compared with incubation with control sera (Table 2). In contrast, homogenates prepared from spheroplasts incubated with anti-CYP2D6 or control sera showed unchanged CYP2D6 activities. This indicated that antibodies did not penetrate intact spheroplasts and demonstrated that CYP2D6 activity was specifically immuno-inhibited at the PM external face.

To further confirm external CYP2D6 activity, spheroplasts were submitted to a short duration pH-shift (from 6.3 to 3) with the aim of deactivating the externally located enzyme. In such conditions, the internal cell pH is easily maintained by yeast proton-pumping PM ATPases (de Kerchove *et al.*, 1995). In treated cells, the PM CYP2D6 activity was reduced by 70% compared with normal PM (Table 3), whereas in the same experiment, microsomes prepared after cell lysis did not show a significant alteration of the activity (less than 10%). This data confirms that PM CYP2D6 activity is associated with the outside oriented enzyme.

CPR bound to the inner plasma membrane face supports the activity of CYP2D6 located on the outer face. Spheroplast incubation with anti-CYP2D6 antibodies before cell lysis and preparation of PM fraction led to the selective immuno-inhibition of the externally oriented PM CYP2D6 (see above and Table 2). In contrast, CPR-supported CYP2D6 and NADPH-cytochrome *c* reductase activities were not immuno-inhibited after spheroplast incubation with anti-CPR antibodies. This indicated that no functional CPR was present on the outer face of PM. When PM were isolated and incubated with anti-CPR antibodies to allow inhibition of CPR on both faces of PM, NADPH-cytochrome *c* reductase activity was lost. Interestingly, this loss was correlated with a 90% decrease in CYP2D6 activity (Table 4). No inhibition was found on either activities with preimmune or control sera. As reported previously, because immunoglobulin treatment precluded PM protein quantification, activity determinations were standardized based on ergosterol (a major PM component) content. These experiments demonstrate that CPR, in contrast to CYP2D6, is not exposed on the outer surface of the cells, but is cytosolically oriented at the PM. In addition, functional coupling between internal CPR and external CYP2D6 on PM is evidenced.

An artifact could avoid such conclusion: PM fusion formed by inverted orientations on silica beads during membrane purification. To rule out formation of a possible fusion between inverted orientations of CPR and CYP2D6, NADPH-dependent CYP2D6 activity was measured on PM isolated

TABLE 1

Dextromethorphan demethylase and cytochrome *c* reductase activities in subcellular fractions from yeast expressing CYP2D6

Activities were measured as described under Materials and Methods. W(R) strain overexpressed the yeast CPR when WRΔ strain does not express at all this enzyme. Values represent the mean ± standard deviation of six independent determinations.

	Dextromethorphan conversion per mg of proteins		NADPH-cytochrome *c* reductase activity	
	NADPH-dependent activity	CumOOH-dependent activity	Plus detergent[a]	Minus detergent[a]
	%		*μmol/min/mg of proteins*	
CYP2D6 Mic W(R)	11 ± 1	9 ± 1	5.0 ± 0.3	2.6 ± 0.3
CYP2D6 PM W(R)	5 ± 1	4 ± 1	1.2 ± 0.05	0.23 ± 0.01
CYP2D6 PM WRΔ	0.1 ± 0.1	4 ± 1	0.02 ± 0.01	0.02 ± 0.01

[a] 0.5% of triton X100 and 0.5% sodium cholate (w/v) were added for 5 min on ice before the assay.

TABLE 2

Immunoinhibition analysis of catalytic competence of inside and outside exposed PM CYP2D6

Spheroplasts were incubated for 1 hr on ice with anti-CYP2D6 or control antibodies (diluted 1:50 in 1.2 M sorbitol and PBS). After three washes, the incubated spheroplasts were attached on the silica microbeads for PM preparations. Before disruption of bead-bound spheroplasts, an aliquot was taken to prepare an homogenate to monitor the intracellular P450 activity. Values are the mean ± standard deviation (two replicates on three independent cultures).

Type of membranes	Control antibodies	Anti-2D6 antibodies	Dextromethorphan conversion per unit of CPR[a]
PM			11 ± 2
PM	External[b]		12 ± 1
PM		External[b]	0.6 ± 0.1
PM	External[b] + internal[c]		13 ± 2
PM		External[b] + internal[c]	0 ± 0
PM	External[b]	Internal[c]	0.0 ± 0.1
PM	Internal[c]	External[b]	1.0 ± 0.2
Homogenate	External[b]		7 ± 1
Homogenate		External[b]	7 ± 1

[a] Arbitrary unit.
[b] Spheroplasts were first incubated with antibodies and washed before disruption.
[c] Isolated plasma membranes were incubated with antibodies before the activity assays.

from mixtures of spheroplasts prepared from W(R) strain (containing the CPR but no CYP2D6) and from WRΔ strain transformed by CYP2D6 (containing CYP2D6 but no CPR). Although the CumOOH-dependent P450 activity was proportional to the fraction CYP2D6/WRΔ spheroplasts, no NADPH-supported activity was observed whatever the mixture (Table 5). We conclude that in our experimental conditions, no membrane fusion occurs. Consequently, observed coupling between CPR and CYP2D6 in PM membranes during coexpression is not an artifact. Interestingly these data suggest the existence of an unidentified transmembrane electron transfer system.

TABLE 3

Acidic inactivation of outside exposed PM CYP2D6

Freshly prepared spheroplasts were incubated for 2 min on ice, in ammonium acetate buffer, pH 3, (sample) or 6.3 (control). pH was shifted to 6.3 and material was divided into two parts. One part was attached on the silica microbeads for PM preparations. The other part was used to prepare microsomes. Values are the mean ± standard deviation (two duplicates on three independent cultures).

Type of membranes	Dextromethorphan conversion		CPR activity[b]
	Per mg of protein	Per unit of CPR[a]	
	%		*μmol/min/mg of proteins*
PM	5 ± 1	11 ± 2	1.2 ± 0.1
Acidic PM	1.6 ± 0.5	4 ± 2	1.2 ± 0.2
Microsomes	11 ± 1	6 ± 2	5.0 ± 0.3
Acidic microsomes	9.8 ± 0.9	7 ± 2	4.5 ± 0.5

[a] Arbitrary unit.
[b] CPR activity was evaluated as NADPH-cytochrome *c* reductase activity.

Discussion

Native CYP2D6 is present in two subcellular locations: the microsomal membrane and the external face of PM. Similarly, in hepatocytes, clara cells, and transfected mammalian cells, the presence of surface-exposed P450s was established by immunolabeling (for review, see Loeper *et al.*, 1990; Eliasson and Kenna, 1996). The possibility of NADPH-dependent activities of PM P450s and CPR were advanced in rat and human liver tissue or cells, (Jarasch *et al.*, 1979; Stasiecki and Oesch, 1980; Loeper *et al.*, 1990; Wu and Cederbaum, 1992; Loeper *et al.*, 1993;). In the present study, PM CYP2D6 expressed in yeast sustained dextromethorphan demethylation in both NADPH- and CumOOH-supported reactions. Peroxides derived from lipid peroxidation could participate in P450 functions under physiological conditions (Nordblom *et al.*, 1976). Observation that PM specific activities reached half the value in microsomes and suitable controls excluded microsomal contamination. However, a major advance was the demonstration of outside-oriented CYP2D6 functionality at the PM. NADPH-supported competence of CYP2D6 present on the cell surface was evidenced by CYP2D6 immuno-inhibition and acidic treatment of the spheroplast surface before spheroblast disruption for PM isolation. In these conditions, PM outside-exposed CYP2D6 activity was decreased by 95% and 70%, respectively.

CPR was found to be exposed on the cytosolic side of PM by Western blot analysis, confocal scanning microscopy, NADPH-dependent cytochrome *c* reductase, and CYP2D6 activities. Interestingly, these data suggest the existence of a transmembrane electron transfer system to sustain func-

TABLE 4

Determination of the CPR orientation on plasma membrane

To determine CPR orientation by immuno-inhibition, spheroplasts were incubated for 1 hr on ice with anti-CPR or preimmune antibodies (diluted 1:50 in 1.2 M sorbitol, PBS), that led to the selective immunoinhibition of the externally oriented enzymes. After three washes, incubated spheroplasts were mixed with silica microbeads and treated to prepare PM. As controls, these PM were incubated a second time with anti-CPR or preimmune antibodies to inhibit the inside oriented enzymes that were not inhibited during the first incubation. NADPH-supported CYP2D6 and detergent solubilized CPR activities were measured as described under Materials and Methods. The values shown represent the mean ± standard deviation of results on four independent cultures.

Preimmune antibodies	Anti-CPR antibodies	Dextromethorphan conversion per unit of CPR[a]	CPR activity
			nmol / min / μg of ergosterol
		11 ± 2	118 ± 15
External[b]		11 ± 1	115 ± 10
	External[b]	10 ± 1	111 ± 12
External[b] + internal[c]		10 ± 2	113 ± 11
External[b]	Internal[c]	1.0 ± 0.2	11 ± 1
	External[b] + internal[c]	0.8 ± 0.2	0
Internal[c]	External[b]	10 ± 1	95 ± 9

[a] Arbitrary unit.
[b] Spheroplasts were incubated with sera and washed before disruption.
[c] Isolated PM were incubated with sera before the activity assays.

TABLE 5

Absence of fusion between CPR and CYP2D6 PM during silica bead membrane purification

Dextromethorphan demethylase activities were measured in PM samples prepared from mixtures in variable amounts of spheroplasts from strain expressing CYP2D6 in WRΔ (no CPR) and strain W(R) overexpressing only CPR. Absence of fusion was deduced from the lack of reconstitution of a coupled CPR-P450 system. Values are the mean ± standard deviation of three independent determinations.

Plasma membranes		Dextromethorphan conversion	
CYP2D6/WRΔ	W(R)	NADPH-dependent activity	CumOOH-dependent activity
%		*% per mg of protein*	
100	0	0.1 ± 0.1	5 ± 1
75	25	0.05 ± 0.05	3.3 ± 0.5
50	50	0.05 ± 0.05	2.7 ± 0.5
25	75	<0.05	1.6 ± 0.5
0	100	<0.05	<0.05

tional coupling between the PM CPR and P450. A similar hypothesis was raised for iron uptake by *S. cerevisiae*, in which the reduction of iron (III) at the cell surface via a *trans*-PM electron transfer system implied two proteins encoded by *FRE1* and *FRE2* genes (Klausner and Dancis, 1994). This multi-heme complex (similar to human neutrophil cytochrome *b*558) involved CPR to transfer electrons across PM from NADPH to external iron (Lesuisse *et al.*, 1996, 1997). Interestingly, in the CPR-free yeast strain (WRΔ), NADPH-dependent P450 activity was no longer detectable on isolated PM, an indication that the reductase is an obligatory component. These findings imply that a relationship may exist between an electron carrier (a FRE1p-, FRE2p-like system), the NADPH-dependent CPR (oriented on the cytosolic side) and activity of CYP2D6 oriented on the external side of the PM (Fig. 3).

Quantitatively, PM P450s represent only a minor part (about 10%) of total human hepatocyte P450s (Loeper *et al.*, 1993). However, P450 presentation on the outer face of PM was found to play a critical role in autoimmunity neoantigen formation (Loeper *et al.*, 1990, 1993). Therefore, a further role for external PM P450 might be considered in central nervous system where P450s were found in the blood-brain barrier microvessels (Ghersi-Egea *et al.*, 1994). Surface-exposed enzyme could contribute to the release of activated metabolites into the circulation and thus induce cytotoxicity toward host tissues such as bone marrow and kidney (Wei *et al.*, 1994). In addition, CYP2D family which metabolizes dextromethorphan was present in the synaptic PM fraction of neural tissue (Fonne-Pfister *et al.*, 1987). Furthermore, neuronal dopamine transporter and CYP2D1 bind similar substrates and inhibitors (Tyndale *et al.*, 1991), which suggests that synaptic CYP2D could participate in xenobiotic, neurotransmitter or neurotoxin metabolisms (Fonne-Pfister *et al.*, 1987; Suzuki *et al.*, 1992). Demonstration of the catalytic competence of PM-located P450s opens a new field of investigation with respect to their potential physiological roles.

Although liver PM P450s are the targets of different autoantibodies in drug-induced hepatitis, little is known of the ability of PM P450s to produce adducts *in situ*. Such a potential role was investigated in the case of PM protein alkylation after administration of isaxonine, a drug that induces immunoallergic hepatitis after its P450-dependent biotransformation into reactive metabolites. *In vitro*, no alkylation

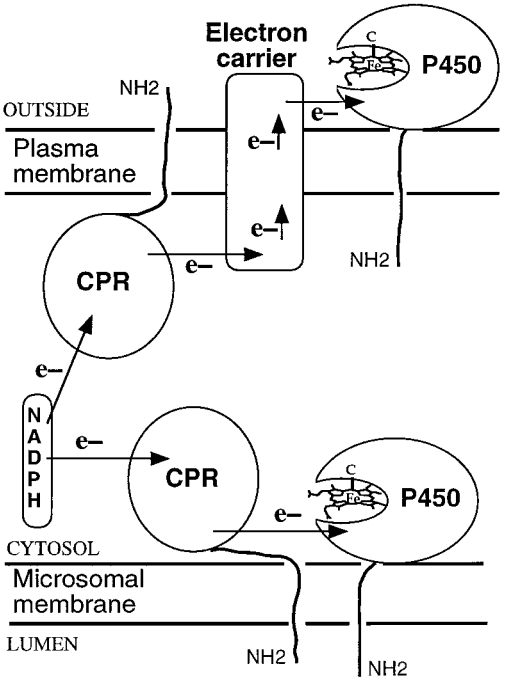

Fig. 3. Proposed mechanism involving the presence of a plasma membrane electron carrier for NADPH-supported activity of outside PM P450 with CPR oriented on the opposite side of PM

was observed in isolated PM but interpretation was limited by the low sensitivity of the assay (Loeper *et al.*, 1989). Furthermore, formation of alkylated CYP2C11 in the presence of tienilic acid was evidenced at the surface of rat hepatocytes (Robin *et al.*, 1996). Adduct formation with CYP2C11 at the endoplasmic reticulum followed by transport of the alkylated P450 toward PM was suggested. Nevertheless, alternate mechanism involving direct formation of P450 adducts on PM merits consideration.

Acknowledgments

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