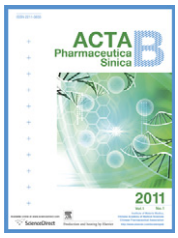
Acta Pharmaceutica Sinica B 2012;2(2):102–106

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

New insights into the role of cytochrome P450 reductase (POR) in microsomal redox biology

Todd D. Porter*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0596, USA

Received 14 January 2012; revised 7 February 2012; accepted 13 February 2012

KEY WORDS

Cytochrome P450;
Squalene monooxygenase;
Heme oxygenase;
Cytochrome b_5;
Microsomes;
7-Dehydrocholesterol reductase

Abstract Cytochrome P450 reductase (POR) is an essential electron transfer protein located on the endoplasmic reticulum of most cell types, and has long been appreciated for its role in cytochrome P450-mediated drug metabolism. Additional roles and electron acceptors for POR have been described, but it is largely with the recent availability of POR-null tissues that these supplemental roles for POR have been able to be explored. These studies have confirmed POR as the principal redox partner for the microsomal P450s responsible for drug and xenobiotic metabolism as well as cholesterol and bile acid synthesis, and for heme oxygenase, which catalyzes the initial step in the breakdown of heme. Surprisingly, these studies have revealed that squalene monooxygenase, an enzyme essential to cholesterol synthesis, has a second unknown redox partner in addition to POR, and that 7-dehydrocholesterol reductase, previously proposed to require POR as an electron donor, functions fully independently of POR. These studies have also helped define the role of cytochrome b_5 in P450 catalysis, and raise the question as to the extent to which POR contributes to b_5-dependent redox pathways.

*Tel.: +11 859 257 1137; fax: +11 859 257 7564.
E-mail address: tporter@uky.edu (Todd D. Porter)

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
doi:10.1016/j.apsb.2012.02.002

1. Early identification of redox partners for cytochrome P450 reductase

The early history of cytochrome P450 reductase (POR) is described in a comprehensive review by Masters and Okita[1]. Cytochrome P450 reductase was initially identified as a TPNH(NADPH)-dependent cytochrome c reductase present in liver extracts, but subsequent studies demonstrated that cytochrome c, a mitochondrial protein, was not this microsomal enzyme's natural redox partner. The redox partner for POR remained unknown for over a decade, until the early 1960s when it was suggested that POR might be involved in drug and steroid oxidations by the newly discovered cytochrome P450 system. The observation that POR activity was inducible by drugs that increased microsomal P450 expression was undertaken. These studies have greatly facilitated by antibody inhibition studies that clearly showed that antibody to POR inhibited microsomal drug oxidations. The reconstitution of cytochrome P450 activity by Lu and Coon in 1968[2] from solubilized components, including a POR-containing fraction, established P450 as the first redox partner for POR.

Antibody inhibition studies were used to identify additional redox partners for POR, and led to the identification of heme oxygenase[3], a microsomal enzyme that catalyzes the first step in heme degradation. Antibody inhibition was also used to show that steroid hydroxylations in the adrenal cortex[4], placenta[5], and testis[6] were dependent on POR, thus expanding the range of P450s that serve as POR partners to those involved in physiological biochemistry as well as drug and xenobiotic metabolism. Squalene monooxygenase (epoxidase) was identified as a redox partner for POR in 1977[7], making it the second non-P450 enzyme, after heme oxygenase, to use POR as an electron source; squalene monooxygenase acts in the cholesterol synthesis pathway. The ability of POR to donate electrons to cytochrome b_5[8] generated considerable interest as to the relevance of this pathway to P450 function, as b_5 was able to donate the second of the two required electrons to cytochrome P450 in isolated and reconstituted preparations. Moreover, cytochrome b_5 is part of the fatty acid desaturation and elongation pathways as well as the post-lanosterol sterol synthesis pathway, placing POR at the interface of drug and xenobiotic metabolism, steroid and cholesterol synthesis, and fatty acid metabolism. The last enzyme to be associated with POR is 7-dehydrocholesterol reductase, which catalyzes the final step in cholesterol synthesis; mutations in the gene for 7-dehydrocholesterol reductase are responsible for Smith-Lemli-Opitz syndrome[9]. Antibody inhibition studies again implicated POR as an electron donor to this enzyme, an idea further supported by reconstitution studies with partially purified enzyme preparations[10]. However, very recent studies from the author's laboratory have demonstrated that POR does not have a role in 7-dehydrocholesterol reductase function[11].

2. Studies with hepatic POR-null mice

The germline deletion of POR in mice results in embryonic lethality by day 14[12], making whole-animal knock-out studies unfeasible. The lethality appears due to the loss of cholesterol synthesis necessary for neuronal development and to the loss of sterol modification of proteins necessary for development[13,14]. To circumvent this problem the selective, tissue-specific deletion of POR expression was undertaken. These studies have greatly facilitated studies on the role of POR in drug metabolism, tissue and organ-specific toxicity of xenobiotics, and organismal development[15,16].

2.1. *Heme oxygenase*

In one of the two seminal studies on hepatic POR-null mice, Gu et al.[15] noted that the inducible form of heme oxygenase, HO-1, was induced 9-fold at the protein level in the livers of these mice, and yet heme oxygenase activity was essentially negligible in this tissue. The Wolf laboratory did not look at heme oxygenase protein or activity in their hepatic POR-null mice, but did note a modest increase (1.4 to 2-fold) in HO-1 mRNA levels in POR-null liver[17]. A microarray study from the Ding laboratory similarly found a 2.3-fold increase in HO-1 mRNA[18]. The expression of heme oxygenase-2, a constitutively expressed form present in normal liver, was not altered at the mRNA or protein level by the loss of POR expression. The lack of heme oxygenase activity in POR-null liver in the face of a significant increase in protein argues strongly that POR is the requisite and only electron transfer partner for this enzyme. Although there was some evidence for oxidative stress in the livers of these animals (evidenced by an increase in glutathione-*S*-transferase gene expression)[18] consistent with the increase in HO-1 expression, no direct evidence of heme toxicity was noted. Biliverdin and bilirubin levels were not measured in the livers or blood of these animals and the question of how heme turnover is handled in a hepatic POR-null liver remains to be determined.

2.2. *Squalene monooxygenase*

Squalene monooxygenase catalyzes the first oxidative step in the synthesis of sterols, yielding 2,3-oxidosqualene which is then cyclized by 2,3-oxidosqualene cyclase to the first sterol in the pathway, lanosterol. Squalene monooxygenase is an FAD-dependent monooxygenase which is unusual in its requirement for an additional electron transfer partner. Early purification studies demonstrated that activity could be reconstituted with POR[7], and it was not until hepatic POR-null microsomes became available that it could be shown that squalene monooxygenase retained partial activity in the absence of POR[19]. The second microsomal reductase, still unidentified, appears able to catalyze approximately 40% of the activity seen in POR-containing liver microsomes. Sterol analysis of the livers of hepatic POR-null mice revealed elevated levels of dihydrolanosterol, a metabolite of lanosterol formed by sterol 24-reductase, a downstream enzyme in cholesterol synthesis. This metabolite also accumulated in hepatocytes isolated from hepatic POR-null mice, demonstrating that squalene is converted to lanosterol *via* squalene monooxygenase and 2,3-oxidosqualene cyclase in the absence of POR. Studies with rat hepatoma cells in which POR expression is suppressed by RNA interference (RNAi) similarly demonstrated the accumulation of lanosterol and dihydrolanosterol[20], confirming that squalene monooxygenase activity was resilient to the loss of POR. Notably, sterol synthesis is blocked at lanosterol demethylase, resulting in the accumulation of lanosterol and dihydrolanosterol; lanosterol demethylase is a cytochrome

P450 (CYP51) that is evidently fully dependent on POR for activity. Squalene monooxygenase protein levels are decreased by approximately 40% in POR-null liver despite a two-fold increase in mRNA levels. While this suggests that lanosterol or its metabolite act post-transcriptionally to decrease squalene monooxygenase expression (as is seen with HMG-CoA reductase[21]), Gill et al.[22], have recently shown that the turnover of squalene monooxygenase is accelerated by cholesterol but not by lanosterol or dihydrolanosterol. In hepatic POR-null cells the inability to convert cholesterol to bile acids (a P450-dependent reaction) may result in the accumulation of sufficient cholesterol to promote the degradation of squalene monooxygenase, although cholesterol levels in these cells are not noticeably elevated (unpublished data).

Interestingly, the sterol synthesis pathway in Saccharomyces cerevisiae, which yields ergosterol, is not dependent on POR[23] despite the involvement of two P450s (CYP51 and CYP61), as well as squalene monooxygenase. The assumption is that the NADH-cytochrome b_5 pathway supports CYP51 and CYP61 in yeast, but *S. cerevisiae* squalene monooxygenase activity cannot be supported by NADH in microsomes[24], raising the possibility that that this yeast, like mammals, contains a second reductase capable of reducing squalene monooxygenase.

2.3. *Cytochrome b_5*

The earliest studies on the P450 system indicated a role for cytochrome b_5 in this pathway, but establishing the specifics of the interaction was contentious. Cytochrome b_5 is reduced by NADH *via* cytochrome b_5 reductase, and there was good evidence that this NADH-dependent pathway could support modest P450 activity in microsomal preparations[25]. Later reconstitution studies with purified enzymes supported this observation for some, but not all P450 isoforms[26,27], as did recombinant expression studies[28]. Nonetheless, the ability of POR to transfer electronsto b_5[8] complicates the picture, and the ability of cytochrome b_5 to stimulate P450 activity allosterically without electron transfer adds further complexity. The hepatic deletion of POR has provided some insight as to the role the NADH-cytochrome b_5 pathway plays in hepatic drug metabolism *in vivo*[15,16]. Drug metabolism by all microsomal P450s tested was greatly decreased (>90%) in hepatic POR-null microsomes, and in some cases nearly extinguished. It is thus clear from these studies that the NADH-b_5 pathway cannot support P450 activity to any significant extent *in vivo* in the absence of POR, a finding consistent with the inability of this pathway to support most P450s in reconstitution studies. There are two possible exceptions to this observation that microsomal P450 activity is lost in the absence of POR: the finding that approximately 10% of CYP3A-catalyzed testosterone-6β hydroxylase activity[16] and 5% of CYP2E1-catalyzed chlorzoxazone hydroxylase activity[29] remains in POR-null microsomes and hepatocytes. These observations are consistent with reconstitution and recombinant expression studies which have shown that CYP3A4 and CYP2E1 are unique in their ability to accept both electrons from cytochrome b_5 and retain activity in the absence of POR[26–28].

Notably, studies with POR-null microsomes do not address the extent to which cytochrome b_5 can donate the second electron to P450 catalysis (with the first electron coming from POR), an event that is much more facile than donation of the first electron, and is believed to occur with a greater number of P450 isoforms. This question is addressed by the deletion of cytochrome b_5 expression, and indeed, the elimination of cytochrome b_5 decreases the activity of a number of microsomal P450s by a remarkable 50–90%[30,31]. Thus, cytochrome b_5, rather than playing a minor and auxiliary role in drug metabolism, is necessary for maximal activity with a wide range of P450 isoforms. The stimulation afforded by cytochrome b_5 may be either catalytic or allosteric, and likely depends on the P450 isoform; sorting this out may require the transgenic expression of redox-inactive forms of cytochrome b_5 in a b_5-null animal. Perhaps most surprisingly, the germline deletion of cytochrome b_5 was not embryonic-lethal, as was anticipated[32]. Because cytochrome b_5 is the electron donor to two enzymes in the cholesterol synthesis pathway (sterol 4α-methyl oxidase and lathosterol Δ5-desaturase), the loss of cytochrome b_5 should have resulted in embryonic lethality due to the loss of cholesterol synthesis, as seen with germline POR-null animals. The ability of these animals to survive indicates that at least one alternative redox protein must be present in microsomes and is able to substitute for the loss of cytochrome b_5. The possibility that this is POR seems unlikely but cannot be excluded, and a b_5-null/hepatic POR null animal would be particularly interesting in this regard.

The remaining question is to what extent POR donates electrons to cytochrome b_5 *in vivo*. This can best be tested with enzymes that utilize cytochrome b_5 – and not POR – as a redox partner, and would include the microsomal fatty acid desaturases and elongases, and sterol 4α-methyl oxidase and lathosterol Δ5-desaturase in sterol synthesis (noted above). The activity of these enzymes in POR-null liver might be reduced if POR is a significant contributor to the cytochrome b_5 redox steady-state and could be assessed with microsomes prepared from this tissue. An interesting alternative experiment would be to instead knock out cytochrome b_5 reductase expression, thereby removing the NADH pathway for b_5 reduction; remaining activity would presumably be due exclusively to POR-mediated electron transfer to cytochrome b_5. As most of these enzymes demonstrate activity with NADPH in microsomal preparations, it is likely that POR contributes to their activity *in vivo*; the extent of the contribution may depend on relative NADH and NADPH levels in the cell and tissue.

2.4. *7-Dehydrocholesterol reductase*

7-dehydrocholesterol reductase (DHCR7) catalyzes the last step in cholesterol synthesis. The highly hydrophobic 55 kDa protein is proposed to contain 6–9 membrane-embedded segments that bear close similarity to the sterol sensing domains of HMG-CoA reductase and sterol cleavage activating protein (SCAP)[33]. The enzyme has been expressed in yeast and is active in microsomes in the presence of NADPH[33,34]; the enzyme has not been purified from native or yeast sources and has not been expressed in a prokaryotic organism, perhaps due to its highly hydrophobic structure. As a result, the possibility that accessory microsomal proteins are necessary for activity, including NADPH-dependent electron transfer proteins such as POR, has not been firmly established.

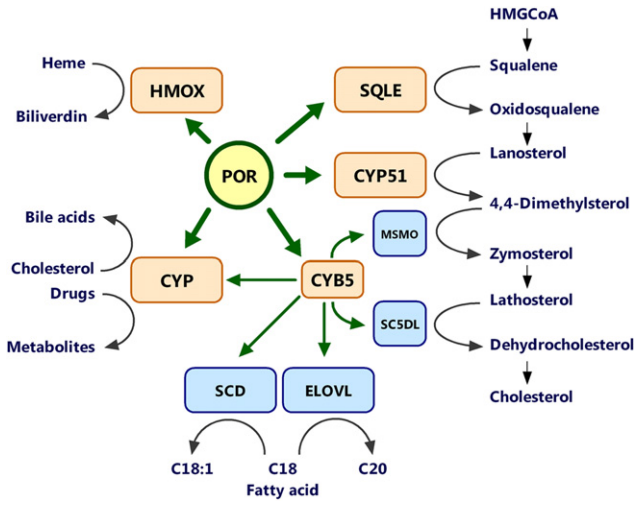


Figure 1 POR redox partners and pathways. Arrows indicate electron flow. HMOX, heme oxygenase (2 genes); SQLE, squalene monooxygenase; CYP, cytochromes P450 (51 microsomal forms); CYB5, cytochrome b_5 (2 genes); MSMO, methylsterol monooxygenase; SC5DL, sterol-C5-desaturase; SCD, stearoyl-CoA desaturase (2 genes); ELOVL, fatty acid elongase (7 genes).

The absence of a canonical cofactor binding domain raises the possibility that a redox partner is required for activity and indeed, Nishino and Ishibashi[10] reported evidence that DHCR7 required POR for activity, based on antibody inhibition studies and partial purification and reconstitution studies. We[11] tested the requirement for POR by using microsomes from hepatic POR-null mice and were surprised to find that DHCR7 activity was greater in these preparations than in wild-type microsomes, and correlated with the two-fold higher levels of DHCR7 protein. Addition of POR to POR-null microsomes did not augment DHCR7 activity. Antibody to POR was not inhibitory to DHCR7 in our hands, and antibodies to each protein did not cross-react. We concluded that POR was not a required electron donor to 7-dehydrocholesterol reductase, and did not augment DHCR7 activity. Final proof will require, at a minimum, expression of a functional enzyme in a host system that lacks POR, or demonstration of activity with the purified protein.

3. Conclusions and perspectives

Cytochrome P450 reductase is a ubiquitous microsomal electron transfer protein most often associated with drug and xenobiotic metabolism, but its role in cellular biochemistry is much larger than that (summarized in Fig. 1). POR is essential to steroid hormone synthesis, cholesterol homeostasis through bile acid synthesis, heme catabolism, and perhaps most importantly, cholesterol synthesis. Indeed, germline deletion of POR leads to fetal lethality due to loss of cholesterol synthesis. POR likely contributes to other redox pathways through its ability to reduce cytochrome b_5, allowing b_5-dependent biochemical pathways to draw on both NADH and NADPH pools for reducing equivalents. These pathways include fatty acid metabolism (desaturases, elongases) and downstream steps in cholesterol synthesis. Recent studies with POR-null and cytochrome b_5-null tissues have revealed the presence of additional microsomal redox enzymes that may substitute for or augment POR and b_5-dependent pathways, and have elucidated the unexpected importance of cytochrome b_5 to P450 biochemistry. Future studies are likely to be directed at identifying these additional microsomal electron transfer proteins and their role in microsomal redox biology.

References

1. Masters BS, Okita RT. The history, properties, and function of NADPH-cytochrome P-450 reductase. *Pharmacol Ther* 1980;**9**: 227–44.
2. Lu AY, Coon MJ. Role of hemoprotein P-450 in fatty acid omega-hydroxylation in a soluble enzyme system from liver microsomes. *J Biol Chem* 1968;**243**:1331–2.
3. Schacter BA, Nelson EB, Masters BSS, Marver HS. Immunochemical evidence for an association of heme oxygenase with microsomal electron-transport system. *J Biol Chem* 1972;**247**: 3601–7.
4. Masters BSS, Taylor WE, Isaacson EL, Baron J, Harkins JB, Nelson EB, et al. Studies on function of adrenodoxin and tpnh-cytochrome c reductase in mitochondria and microsomes of adrenal-cortex, utilizing immunochemical techniques. *Ann NY Acad Sci* 1973;**212**:76–88.
5. Thompson EA, Siiteri PK. Involvement of human placental microsomal cytochrome-P-450 in aromatization. *J Biol Chem* 1974;**249**: 5373–8.
6. Betz G, Roper M, Tsai P. Steroid 17,20-lyase from testis microsomes – participation of nadph cytochrome-c reductase. *Arch Biochem Biophys* 1974;**163**:318–23.
7. Ono T, Ozasa S, Hasegawa F, Imai Y. Involvement of nadph-cytochrome c-reductase in rat-liver squalene epoxidase system. *Biochim Biophys Acta* 1977;**486**:401–7.
8. Enoch HG, Strittmatter P. Cytochrome b_5 reduction by NADPH-cytochrome P-450 reductase. *J Biol Chem* 1979;**254**:8976–81.
9. Porter FD. Smith-Lemli-Opitz syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet* 2008;**16**:535–41.
10. Nishino H, Ishibashi T. Evidence for requirement of NADPH-cytochrome P450 oxidoreductase in the microsomal NADPH-sterol Delta7-reductase system. *Arch Biochem Biophys* 2000;**374**: 293–8.
11. Zou L, Li L, Porter TD. 7-Dehydrocholesterol reductase activity is independent of cytochrome P450 reductase. *J Steroid Biochem Mol Biol* 2011;**127**:435–8.
12. Shen AL, O'Leary KA, Kasper CB. Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase. *J Biol Chem* 2002;**277**:6536–41.
13. Otto DM, Henderson CJ, Carrie D, Davey M, Gundersen TE, Blomhoff R, et al. Identification of novel roles of the cytochrome P-450 system in early embryogenesis: effects on vasculogenesis and retinoic Acid homeostasis. *Mol Cell Biol* 2003;**23**:6103–16.
14. Schmidt K, Hughes C, Chudek JA, Goodyear SR, Aspden RM, Talbot R, et al. Cholesterol metabolism: the main pathway acting downstream of cytochrome P450 oxidoreductase in skeletal development of the limb. *Mol Cell Biol* 2009;**29**:2716–29.
15. Gu J, Weng Y, Zhang QY, Cui H, Behr M, Wu L, et al. Liver-specific deletion of the NADPH-cytochrome P450 reductase gene: impact on plasma cholesterol homeostasis and the function and regulation of microsomal cytochrome P450 and heme oxygenase. *J Biol Chem* 2003;**278**:25895–901.
16. Henderson CJ, Otto DM, Carrie D, Magnuson MA, McLaren AW, Rosewell I, et al. Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *J Biol Chem* 2003;**278**:13480–6.

17. Wang XJ, Chamberlain M, Vassieva O, Henderson CJ, Wolf CR. Relationship between hepatic phenotype and changes in gene expression in cytochrome P450 reductase (POR) null mice. *Biochem J* 2005;**388**(3)857–67.
18. Weng Y, DiRusso CC, Reilly AA, Black PN, Ding X. Hepatic gene expression changes in mouse models with liver-specific deletion or global suppression of the NADPH-cytochrome P450 reductase gene. Mechanistic implications for the regulation of microsomal cytochrome P450 and the fatty liver phenotype. *J Biol Chem* 2005;**280**:31686–98.
19. Li L, Porter TD. Hepatic cytochrome P450 reductase-null mice reveal a second microsomal reductase for squalene monooxygenase. *Arch Biochem Biophys* 2007;**461**:76–84.
20. Porter TD, Banerjee S, Stolarczyk EI, Zou L. Suppression of cytochrome P450 reductase (POR) expression in hepatoma cells replicates the hepatic lipidosis observed in hepatic POR-null mice. *Drug Metab Dispos* 2011;**39**:966–73.
21. Song BL, Javitt NB, DeBose-Boyd RA. Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. *Cell Metab* 2005;**1**:179–89.
22. Gill S, Stevenson J, Kristiana I, Brown AJ. Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. *Cell Metab* 2011;**13**:260–73.
23. Sutter TR, Loper JC. Disruption of the Saccharomyces cerevisiae gene for NADPH-cytochrome P450 reductase causes increased sensitivity to ketoconazole. *Biochem Biophys Res Commun* 1989;**160**: 1257–66.
24. Satoh T, Horie M, Watanabe H, Tsuchiya Y, Kamei T. Enzymatic properties of squalene epoxidase from Saccharomyces cerevisiae. *Biol Pharm Bull* 1993;**16**:349–52.
25. Porter TD. The roles of cytochrome b5 in cytochrome P450 reactions. *J Biochem Mol Toxicolo* 2002;**16**:311–6.
26. Yamazaki H, Nakano M, Imai Y, Ueng YF, Guengerich FP, Shimada T. Roles of cytochrome b_5 in the oxidation of testosterone and nifedipine by recombinant cytochrome P450 3A4 and by human liver microsomes. *Arch Biochem Biophys* 1996;**325**:174–82.
27. Yamazaki H, Nakano M, Gillam EM, Bell LC, Guengerich FP, Shimada T. Requirements for cytochrome b_5 in the oxidation of 7-ethoxycoumarin, chlorzoxazone, aniline, and *N*-nitrosodimethylamine by recombinant cytochrome P450 2E1 and by human liver microsomes. *Biochem Pharmacol* 1996;**52**:301–9.
28. Mokashi V, Li L, Porter TD. Cytochrome b_5 reductase and cytochrome b_5 support the CYP2E1-mediated activation of nitrosamines in a recombinant Ames test. *Arch Biochem Biophys* 2003;**412**:147–52.
29. Li L, Porter TD. Chlorzoxazone hydroxylation in microsomes and hepatocytes from cytochrome P450 oxidoreductase-null mice. *J Biochem Mol Toxicol* 2009;**23**:357–63.
30. Finn RD, McLaughlin LA, Ronseaux S, Rosewell I, Houston JB, Henderson CJ, et al. Defining the *in vivo* role for cytochrome b_5 in cytochrome P450 function through the conditional hepatic deletion of microsomal cytochrome b_5. *J Biol Chem* 2008;**283**: 31385–93.
31. McLaughlin LA, Ronseaux S, Finn RD, Henderson CJ, Roland Wolf C. Deletion of microsomal cytochrome b_5 profoundly affects hepatic and extrahepatic drug metabolism. *Mol Pharmacol* 2010;**78**:269–78.
32. Finn RD, McLaughlin LA, Hughes C, Song C, Henderson CJ, Roland Wolf C. Cytochrome b_5 null mouse: a new model for studying inherited skin disorders and the role of unsaturated fatty acids in normal homeostasis. *Transgenic Res* 2011;**20**:491–502.
33. Bae SH, Lee JN, Fitzky BU, Seong J, Paik YK. Cholesterol biosynthesis from lanosterol. Molecular cloning, tissue distribution, expression, chromosomal localization, and regulation of rat 7-dehydrocholesterol reductase, a Smith-Lemli-Opitz syndrome-related protein. *J Biol Chem* 1999;**274**:14624–31.
34. Moebius FF, Fitzky BU, Lee JN, Paik YK, Glossmann H. Molecular cloning and expression of the human delta7-sterol reductase. *Proc Nat Acad Sci U.S.A.* 1998;**95**:1899–902.