Association of Multiple Developmental Defects and Embryonic Lethality with Loss of Microsomal NADPH-Cytochrome P450 Oxidoreductase*

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Running Title:

Essential Role of CYPOR in Embryonic Development
The microsomal flavoprotein NADPH-cytochrome P450 oxidoreductase (CYPOR)\(^1\) is believed to function as the primary, if not sole, electron donor for the microsomal cytochrome P450 mixed function oxidase system. Development of the mammalian embryo is dependent upon temporally- and spatially- regulated expression of signaling factors, many of which are synthesized and/or degraded via the cytochromes P450 and other pathways involving NADPH-cytochrome P450 oxidoreductase as the electron donor. Expression of CYPOR as early as the 2-cell stage of embryonic development [TIGR Mouse Gene Index – Version5.0, www.tigr.org/tdb/mgi] suggests that CYPOR is essential for normal cellular functions and/or early embryogenesis. Targeted deletion of the translation start site and membrane-binding domain of CYPOR abolished microsomal CYPOR expression and led to production of a truncated, 66 kDa protein localized to the cytoplasm. Although early embryogenesis was not affected, a variety of embryonic defects was observable by day 10.5 of gestation, leading to lethality by day 13.5. Furthermore, a deficiency of heterozygotes was observed in 2-week old mice as well as late gestational age embryos, suggesting that loss of one CYPOR allele produced some embryonic lethality. CYPOR -/- embryos displayed a marked friability, consistent with defects in cell adhesion. Ninety per cent of CYPOR -/- embryos isolated at days 10.5 or 11.5 of gestation could be classified as either Type I, characterized by grossly normal somite formation but having neural tube, cardiac, eye, and limb abnormalities, or Type II, characterized by a generalized retardation of development after approximately day 8.5 of gestation. No CYPOR -/- embryos were observed after day 13.5 of gestation. These studies demonstrate that loss of microsomal CYPOR does not block early embryonic development but is essential for progression past mid-gestation.
INTRODUCTION

NADPH-cytochrome P450 oxidoreductase (CYPOR) is an essential component of the microsomal P450 mixed-function oxidase system (1), mediating electron transfer from NADPH to the cytochromes P450, and a variety of other acceptors, including heme oxygenase (2), fatty acid elongase (3), cytochrome b5 (4) and squalene monoxygenase (5). Electron transfer to the cytochromes P450 as well as membrane anchoring requires the hydrophobic, N-terminal membrane-binding domain of this microsomal flavoprotein (6, 7). No other physiological electron donor to the cytochromes P450 has been identified in vertebrates, although plants contain multiple CYPOR genes (8) and electron transfer from the cytochrome b5/cytochrome b5 system to P450 has been reported in yeast lacking CYPOR (9, 10).

Development of the mammalian embryo requires temporally- and spatially regulated biosynthesis and degradation of signalling factors, many of which, such as retinoic acid, sterols, prostaglandins, and steroids, are dependent upon cytochrome P450 or other CYPOR-dependent pathways. For example, squalene monooxygenase and CYP51 are necessary for biosynthesis of cholesterol (5, 11), while catabolism of retinoic acid proceeds via CYP26A1 (10, 11). The heme oxygenase pathway regulates heme homeostasis (14), and steroidogenesis is dependent upon several of the cytochromes P450 (15). CYPOR is expressed as early as the 2-cell stage of embryonic development (16-18), suggesting that one or more CYPOR-dependent processes are important in early embryonic development.

To determine whether CYPOR is an essential enzyme in the mouse and the sole electron donor to the cytochromes P450, we have examined the effects of CYPOR gene disruption by removal of the natural translation initiation site and deletion of the membrane-binding domain of
CYPOR. Although homozygous CYPOR -/- embryos produced a truncated, cytoplasmic protein, microsomal CYPOR was absent and a spectrum of embryonic defects leading to mid-gestational lethality was observed, demonstrating that microsomal CYPOR is indeed necessary for normal embryonic development.
METHODS

Isolation of the mouse CYPOR gene. The single-copy (19) mouse CYPOR gene isolated from a Genome Systems 129/SvJ BAC library (http://www.incyte.com) was found to be similar in size (> 40 Kbp) and organization to that of the rat reductase gene (20, 21) (Fig. 1A), with an untranslated first exon approximately 30 kBP upstream of Exon 2, which contains the translation start site. Exon 2 codes for the amino-terminal membrane-binding domain necessary for cytochrome P450-dependent activity (6) and is separated by a 12 Kbp intron from the remaining 14 exons, which are clustered in an 8 Kbp region.

Generation of CYPOR -/- mice. The targeting construct shown in Fig. 1A produces, upon homologous recombination, an allele in which 2.5 Kbp of Intron 1 and all but the last 6 bases of Exon 2 are replaced by the Neo coding sequence, thus deleting the CYPOR translation start site as well as the membrane binding domain. After electroporation into R1 embryonal stem cells (22) and selection for G418 and gancyclovir resistance, colonies were screened for homologous recombination by PCR using the primers Intron 1C (5'-GGAATCAAACTCAGGTCCCTTAAGAT-3') and 313A2 (5'-GTATCAAGGCCTTAAAGAGTTCA-3'), located 5' and 3', respectively, of the homology units (Fig. 1A). These primers produce an 8 Kbp product with the wild-type allele, which is decreased to 7.3 Kbp upon homologous recombination (Fig. 1B). Clones producing both bands were classified as heterozygous for the targeted allele; this was confirmed by EcoRV digestion of the PCR products to give the expected 4.0 and 2.5 Kbp bands, with the 4.0 Kbp band hybridizing, as expected, to an Intron 1 probe. Positive clones were expanded and karyotyped before injection into blastocysts and implantation into pseudopregnant C57BL6 females.
Chimeric males were mated with C57/BL6 females to yield heterozygous F1 progeny, which were then mated with each other to produce CYPOR +/-, +/- and -/- offspring. Mouse tail DNA was isolated using the Qiagen DNeasy kit (www.qiagen.com) and analyzed using two separate PCR reactions (Fig. 1B). The first employed the primers Intron 1C and Common 1 (5’-TCTGAGGGCACCACACGTCTGT-3’), shown in Fig. 1A, which yielded bands of 4.7 Kbp and 4.0 Kb, respectively, for the wild-type and disrupted alleles. A second, confirmatory, reaction, yielding 210 bp and 260 bp fragments, respectively, for the wild-type and disrupted alleles, employed 3 primers: Common 1, which spans the Exon 2/Intron 2 junction and is found in both the wild-type and disrupted alleles, WT1 (5’-GTGTCACCAACATGGGGACTCT-3’), located in the deleted segment of Intron 1 and found only in the wild-type allele, and Neo4 (5’-CTTCCATTTCACGTCCCTGAC-3’), located in the Neo gene of the disrupted allele (Fig. 1A).

Analysis of Embryos. Detection of vaginal plugs in the morning was taken as gestation day 0.5 (E0.5). Embryos were genotyped by removal of either the tail or the hindlimb bud prior to fixation. For histology, embryos were fixed for at least 4 hr in 4% paraformaldehyde in phosphate-buffered saline, dehydrated, and embedded in paraffin. Ten-micron sections were stained with hematoxylin and eosin (23). For scanning electron microscopy, embryos were fixed in Karnovsky’s buffer, postfixed in osmium tetroxide, dehydrated, critical point dried, coated with gold-palladium, and viewed on a Hitachi S-570 scanning electron microscope.

Analysis of Subcellular Fractions. For preparation of embryonic S1 and microsomal fractions, whole embryos were homogenized in 50 mM Tris, pH 7.7, 0.15 M KCl, 1 mM EDTA, containing 100 µM PMSF and 10 µg/ml aprotinin, and centrifuged at 14,000 x g for 30 minutes. This low-speed supernatant fraction, designated S1, was centrifuged at 100,000 x g for 60 min
and the microsomal pellet resuspended in 50 mM Tris, pH 7.7, 0.1 mM dithiothreitol, 20% glycerol. Protein was determined using the Pierce BCA Protein Assay Kit (http://www.piercenet.com). Western blotting was carried out using a polyclonal anti-rat CYPOR antibody, with ECL detection using the BM Chemiluminescence Western blotting kit from Roche (http://biochem.roche.com). EROD and cytochrome c reductase activities were measured as described (24, 25). Cytochrome c reductase assays contained 23-50 (liver) or 10-30 (embryonic) µg microsomal protein. EROD assays contained 100-200 (liver) or 50-100 (embryonic) µg microsomal protein. Limits of detection were ≤0.001 µmol/min/mg protein for cytochrome c and ≤0.02 pmol/min/mg protein for EROD activity.
RESULTS

Homologous recombination between the targeting construct and the mouse CYPOR gene deleted 2.5 Kbp of the 3’-end of Intron 1 and all but the last 6 bases of Exon 2 (Fig. 1A), thus removing both the translation start site and the membrane-binding domain necessary for electron transfer to cytochrome P450 (20). Two separate clones, designated G1 and H11, yielded chimeras that subsequently transmitted the disrupted allele to their progeny. Mice analyzed in this study were obtained from five founder males, four derived from the G1 clone and one from H11, and were maintained on a mixed 129SvJ/C57/BL6 background. No differences were noted among the progeny of the five founders.

Heterozygote crosses produced an average litter size of 6 ± 2, with a normal male: female ratio (0.52) and no apparent perinatal mortality. Genotyping of 2-week old offspring of heterozygote crosses yielded no homozygous -/- offspring (Table 1). Heterozygotes were obtained at a frequency of 56% (Table 1), significantly less (P< 0.05) than the 67% expected for a 1:2 wild-type: heterozygote ratio, suggesting that loss of one CYPOR allele reduced survival. Genotyping of E8.5 to E10.5 embryos from heterozygote crosses produced the expected 1:2:1 Mendelian ratios; however, the frequency of homozygotes declined thereafter and no homozygous embryos were observed after E13.5. Genotyping of late gestational age embryos also revealed a deficiency of heterozygous embryos, suggesting an increased mortality of +/- embryos.

Examination of the gross morphology of the embryos revealed a spectrum of abnormalities in the CYPOR -/- embryos (Fig. 2). While E8.5 and E9.5 -/- embryos were generally indistinguishable from +/- and +/- embryos, older CYPOR -/- embryos were
noticeably more friable than their littermates. At E10.5 and E11.5, 90% of CYPOR -/- embryos (compared to 22% of +/- and 12% of +/+ embryos) displayed abnormalities visible by light microscopy. These CYPOR -/- embryos could be divided into two classes, with Type I embryos being relatively well-developed but displaying neural tube, cardiac, eye and limb bud abnormalities (Fig. 2B), and Type II embryos exhibiting a generalized retardation of development (Fig. 2C).

At E10.5, somite formation was grossly normal in CYPOR -/- Type I embryos (Fig. 2B). Fore and hind limb buds were present and well-formed; however, neural tube abnormalities were evident. Fig. 2A displays normal neural, tail, and limb development in an E10.5 CYPOR wild-type embryo. In contrast, its CYPOR -/- littermate (Fig. 2B) exhibited an exencephalic phenotype, with failure of neural fold elevation, subsequent failure of dorsal fusion, and eversion of the neural tube in the hindbrain and midbrain region giving a “ruffled” appearance to the head. Other gross abnormalities include a fluid-filled dilated pericardial cavity (Fig. 2B and C), alteration in the size and positioning of the branchial arches relative to the fronto-nasal region (Fig. 2A vs. 2B), as well as truncation of the tail (Fig. 2B). Petechial hemorrhaging was noted in the CYPOR -/- embryos and was particularly prominent at the edges of the hindbrain neural folds (Fig. 2B). Failure of the caudal neuropore to close was also observed (not shown).

Examples of a more severe, generalized retardation of development, the Type II phenotype, are presented in Fig. 2C. Although the majority of E10.5 Type II -/- embryos were turned, they were, in comparison with wild-type littermates, poorly-developed and small (Fig. 2C). These embryos displayed an observable heartbeat in spite of obvious pericardial edema. Fore- and hindlimb formation, as well as cranial development, were delayed. Finally, -/- embryos with an apparently normal yolk sac, but no recognizable embryonic structures were also
isolated (Fig. 2C).

Scanning electron microscopy of the Type I CYPOR -/- embryos provided a detailed view of the neural tube defects (Fig. 3). By E10.5, the neural tube was completely closed in the wild-type embryo (Fig. 3A). Fig. 3B displays the predominant pattern of neural tube abnormalities in E10.5 -/- embryos. The rostral portion of the forebrain and the posterior neural tube (spinal cord) were closed; however, the region extending from the caudal portion of the forebrain to the rostral end of the hindbrain remained elevated and open. The midbrain neuroepithelium was elevated, with the neural folds assuming a concave position, but not fused. In the hindbrain, the neural folds remained in a convex position in spite of continued proliferation of the neuroepithelium. Alternate patterns were also observed; Fig. 3C shows a Type I embryo where the cranial portion of the neural tube was closed, but the hindbrain neural folds failed to elevate and fuse. Malformation of the branchial arches and eye abnormalities were apparent in these embryos, as well as a rough surface, consistent with the observed increased friability, and suggesting defects in cell adhesion properties and/or membrane structure (Fig. 3, B and C). No Type II embryos remained intact through the fixation process.

Histological examination of the homozygous null embryos also demonstrates the convex position and lack of elevation of the neural folds in the Type I CYPOR -/- embryo (compare Fig. 4A and 4B). As was observed in the intact embryo, red blood cells were present in the region of the neural folds (Fig. 4, B and C). Numerous cells containing pyknotic nuclei were also detected in the CYPOR -/- embryo adjacent to the fronto-nasal midline (Fig. 4D), consistent with apoptosis. Apoptosis was also noted in the apical epidermal ridges of the limb bud (not shown), although the fore- and hindlimb buds were well-formed overall. Significantly, no evidence of necrosis was observed in the CYPOR -/- embryos.
Concomitant with enlargement of the pericardial cavity (Fig. 2B), Type I CYPOR −/− embryos also displayed histological evidence of defective heart development. Fig. 5 displays histological sections from the cardiac region of E11.5 wild-type (A and C) and −/− (B and D) embryos. Although development of the atrioventricular cushion (Fig. 5, A vs. B) and positioning of the atrium and ventricle (Fig. 5, C vs. D) were normal in the CYPOR −/− embryo, development of the outflow tract and ventricle was impaired. Within the outflow tract, the wild-type embryo showed an increase in mesenchyme destined to differentiate to form the spiral septum, whereas, little evidence of mesenchyme proliferation was noted in the null embryo (Fig. 5, A vs. D). In the ventricle, the CYPOR −/− embryo exhibited a marked reduction in the development of trabeculae (Fig. 5, C vs. D, arrowheads). Alterations were also noted in the myocardial wall, with the CYPOR−/- embryo in Panel D displaying a thin myocardium compared to its wild-type littermate (Fig. 5C), while a thickened myocardial wall was observed in other −/- embryos (not shown).

Western blot analysis of both S1 and microsome fractions from CYPOR +/+ embryos demonstrated, as expected, a single immunoreactive 78 kDa protein, which was absent in E10.5 CYPOR −/− Type I embryos, consistent with deletion of the wild-type translation start site (Fig. 6). However, the −/− embryos contained a new immunoreactive protein with an apparent molecular weight of 66 kDa, found in the S1 fraction but not in microsomes. While both proteins were found in the S1 fraction of CYPOR +/- embryos, only the 78 kDa protein was found in heterozygote microsomes.

Cytochrome c reductase activity in liver microsomes isolated from adult heterozygous mice was approximately 50% that found in wild-type microsomes (Table 2), while EROD activity was decreased to 77% of wild-type. Cytochrome c reductase activity in microsomes
prepared from either wild-type or heterozygous embryos (E11.5) was approximately 10% that of adult liver microsomes and was undetectable in microsomes isolated from Type I CYPOR -/- embryos. EROD activity was also undetectable in embryonic CYPOR -/- microsomes (Table 2).
DISCUSSION

Absence of microsomal CYPOR leads to embryonic lethality, with a variety of embryonic defects observable by day 10.5 of gestation. CYPOR -/- embryos displayed a marked friability and apparent generalized defects in cell adhesion. The remaining defects could be divided into two independent classes: Type I, displaying neural tube, cardiac, eye and limb abnormalities, and Type II, exhibiting a generalized retardation of development after approximately E8.5. These results confirm the critical role of CYPOR in embryonic development and are consistent with the report that RNA-mediated interference of CYPOR expression in the nematode Caenorhabditis elegans produces embryonic lethality and undefined structural abnormalities (26). Although yeast are able to survive in the absence of CYPOR, with cytochrome b5 serving as an alternate electron donor to the cytochromes P450 (9, 10), it is clear that the functions of CYPOR are essential in higher organisms.

Although the authentic CYPOR translation initiation site has been deleted in the disrupted allele, the presence of a 66 kDa protein in heterozygous and Type I homozygous embryos indicates that translation must have initiated at an alternate start site. Analysis by 5’-RACE of mRNA from CYPOR +/- embryos (not shown) reveals, in addition to the wild-type transcript, an alternative transcript containing Exon 1 spliced to Exon 3. Exon 3 contains two potential translation initiation sites, with initiation at the ATG at position 107 of the wild-type protein producing a protein of 65 kDa, remarkably similar to the size observed. This protein would retain binding sites for the isoalloxazine, but not the phosphate, of FMN, as well as the FAD and NADPH domains (27). The absence of the membrane-binding domain, essential for formation of a productive catalytic complex with cytochrome P450 (6), and its cytoplasmic
localization argues against the ability of the 66 kDa protein to support microsomal P450 activities.

Although it is not known which CYPOR-dependent activities are essential for embryonic development, detection of CYPOR transcripts in two-cell mouse embryos suggests an essential role in the early stages of embryogenesis (18). The question of whether the survival of CYPOR -/- embryos to as late as E13.5 is the result of residual activity of the cytoplasmic protein or some other compensatory activity remains. Possible compensatory mechanisms include both the presence of an alternate electron donor which substitutes for the electron transfer functions of CYPOR, such as adrenodoxin/adrenodoxin reductase (28) or cytochrome b₅ (9, 10), or alternate sources of metabolites produced by CYPOR-dependent enzymes, for example, maternal sources of cholesterol (29). Regardless of the source, these pathways are insufficient to support normal embryonic development beyond mid-gestation.

CYPOR is a key participant in a variety of metabolic pathways as a consequence of its interactions with the cytochromes P450, heme oxygenase, squalene monooxygenase, and cytochrome b₅. The heterogeneity of the observed CYPOR -/- phenotypes may be a reflection of these multiple biochemical pathways involving CYPOR as well as multiple compensatory mechanisms. Further studies are in progress to characterize these phenotypes and their transmission patterns. CYPOR, alone or via interactions with other proteins, mediates the synthesis and/or degradation of several cellular regulatory factors, with two significant examples being retinoic acid and cholesterol.

Teratogenicity has been associated with retinoic acid excess as well as deficiency, with major targets being the craniofacial region, heart, skeleton, limbs, eye, and central nervous system (30). Several catabolic pathways, including those mediated by CYP26A1, catalyze
spatially- and temporally-regulated breakdown of retinoic acid (12). Although CYP26A1 knockouts are lethal between E9.5 and birth and display some phenotypes in common with the CYPOR knockout, including exencephaly and arrested development, the predominant phenotypes, caudal truncations and vertebrate transformations, differ from those observed here (31, 32).

Cholesterol, in addition to being an essential component of eucaryotic cell membranes, has been recently shown to be necessary for activity of the sonic hedgehog protein, a mediator of patterning in the vertebrate embryo (33). CYPOR is required at two points in the biosynthesis of cholesterol: squalene monoxygenase catalyzes formation of squalene-2, 3-epoxide (5) while CYP51 catalyzes lanosterol demethylation (11). The structural defects and increased friability observed in the CYPOR -/- embryos are consistent with defects in cholesterol biosynthesis and are reminiscent of those observed in a human genetic disorder of cholesterol biosynthesis, desmosterolosis (29). Defective neural tube closure and developmental delay have been observed in a knockout of another enzyme involved in cholesterol biosynthesis, squalene synthase; however, lethality in these mice occurred somewhat earlier (E9.5) (34).

In view of the redundancy observed in many cellular functions, it is surprising that there is not an efficient alternative electron donor to the cytochromes P450. Although evolutionarily ancient and present in multiple copies in plants (8), CYPOR has been shown to exist as a single copy gene in the mouse (19). This situation may be different in primates, including humans, where CYPOR has been localized to a region of Chromosome 7 that is duplicated (35); this may provide a means to insure against the catastrophic consequences of CYPOR deletion.
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FOOTNOTES

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Abbreviations used are: CYPOR, NADPH-cytochrome P450 oxidoreductase; PCR, polymerase chain reaction; PMSF, phenylmethyl sulfonyl fluoride; ECL, enhanced chemiluminescence; EROD, ethoxyresorufin O-demethylase.
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FIGURE LEGENDS

Figure 1. Targeted deletion of murine CYPOR. **A**. Restriction maps of the murine wild-type CYPOR gene, targeting construct, and CYPOR null allele. Exons are shown as solid boxes and the double-headed arrow indicates the deleted region. Arrowheads indicate the locations of the PCR primers used in screening. X, Xba1; R, EcoR1; H, Hind3; B, BamH1; O, Xho1; G, Bgl2; S, Sal1; N, Not1. **B**. PCR analysis of genotypes. Lines indicate the products obtained from the wild-type and null alleles. Fragment sizes are indicated at the left and genotypes at the top.

Figure 2. Multiple phenotypes of CYPOR -/- embryos. * indicates the boundaries of the open neural tube. The arrow indicates bleeding along the neural folds. ▶ indicates the enlarged pericardial cavity. fb, forebrain; mb, midbrain; hb, hindbrain; ba, branchial arches; h, heart; t, tail; hl, hindlimb bud; fl, forelimb bud. **A**. CYPOR +/+ embryo. **B**. Type I CYPOR -/- embryo. **C**. Type II CYPOR -/- embryos.

Figure 3. Scanning electron microscopy of E10.5 CYPOR +/- and -/- embryos. * indicates the boundaries of the open neural tube. ▶ indicates exencephaly. fb, forebrain; mb, midbrain; hb, hindbrain; e, eye; ba, branchial arches; fn, fronto-nasal region. **A**. CYPOR +/+ embryo. **B** and **C**. Failure of neural tube closure in CYPOR -/- embryos.

Figure 4. Neural tube abnormalities of CYPOR -/- embryos. Hematoxylin and eosin-stained coronal sections of E11.5 +/- and -/- embryos. se, surface ectoderm; ne, neuroepithelium; e, eye. **A**. CYPOR +/+ embryo, magnification 2.5X. **B**. CYPOR -/- embryo, magnification 2.5X. * indicates the neural tube region infiltrated by blood cells which is magnified in panel C. ▶ indicates the fronto-nasal region containing apoptotic cells which is magnified in panel D. **C**. 20X magnification of the region indicated by the * in Panel B. **D**. 100X magnification of the
region indicated by ▶ in Panel B, with arrowheads indicating cells with pyknotic nuclei.

**Figure 5. Cardiac abnormalities in CYPOR -/- embryos.** Magnification 10X. Sagittal sections of E11.5 +/+ (A and C) or -/- (B and D) embryos were stained with hemtoxylin and eosin. ▶ indicates trabeculae and the arrow (▷) indicates the myocardial wall. AV, atrio-ventricular cushion; O, outflow tract; V, ventricle; A, atrium.

**Figure 6. Immunoblot analysis of mouse embryo CYPOR.** Standard lanes contained purified full-length CYPOR (WT) or CYPOR lacking its membrane-binding domain (Δ56). S1 indicates the S1 fraction and M the microsomal fraction. Molecular weights are indicated at the left and genotypes at the top. Sample lanes contained microsomal protein (1 µg) or an amount of S1 fraction normalized to the volume in which the microsomes were resuspended.
Table 1.
Observed Genotypes from CYPOR +/- Crosses

| Gestational Age | Genotype (%)<sup>a</sup> | Total<sup>b</sup> |
|-----------------|--------------------------|------------------|
|                 | +/- | +/- | +/- | +/- | Resorbed | Total |
| E8.5            | 18  | 52  | 27  | 3   |          | 33    |
| E9.5            | 31  | 38  | 31  | 0   |          | 16    |
| E10.5           | 20  | 48  | 21  | 11  |          | 101   |
| E11.5           | 34  | 44  | 14  | 8   |          | 78    |
| E12.5           | 30  | 55  | 5   | 10  |          | 40    |
| E13.5           | 17  | 54  | 4   | 25  |          | 24    |
| E14.5-18.5      | 27  | 36  | 0   | 36  |          | 52    |
| 2 weeks postnatal (G1<sup>c</sup>) | 44  | 56  | 0   | -   |          | 551   |
| 2 weeks postnatal (H11<sup>d</sup>) | 45  | 55  | 0   | -   |          | 67    |

<sup>a</sup>Embryos derived from clone G1 or pups derived from clones G1 or H11 were genotyped as described in METHODS and expressed as % of the total. Resorbed embryos were not genotyped and are expressed as % of total embryos.

<sup>b</sup>Total number of pups or embryos, including resorbed embryos.

<sup>c</sup>Pups derived from clone G1.

<sup>d</sup>Pups derived from clone H11.
Table 2

Adult and Embryonic Microsomal CYPOR-Dependent Catalytic Activities

| Specific Activity | Adult       | Embryo      |
|-------------------|-------------|-------------|
|                   | +/+         | +/-         | +/+         | +/-      | -/-       |
| **Cytochrome c**  |             |             |             |          |           |
| (μmol/min/mg protein) | 0.34 ± 0.02 (4) | 0.17 ± 0.03 (4) | 0.02 ± 0.008 (3) | 0.02 ± 0.008 (3) | ND (2)    |
| **EROD**          |             |             |             |          |           |
| (pmol/min/mg protein) | 22.2 ± 2.0 (4) | 16.9 ± 2.5 (4) | 0.12 ± 0.06 (3) | 0.12 ± 0.02 (3) | ND (2)    |

Microsomes were prepared from livers of 4-month old mice or from E11.5 embryos isolated from a single dam. Genotypes are as indicated. Values are expressed as mean ± SD (number of animals/embryos). ND, not detectable.
Fig. 1

A

CYPOR Gene

Targeting Construct

Disrupted Allele

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Fig. 2

A  +/-

B  -/-

C  -/-

1 mm
Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase
Anna L. Shen, Kathleen A. O'Leary and Charles B. Kasper

J. Biol. Chem. published online December 12, 2001

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