0013-7227/05/$15.00/0
Printed in U.S.A.

Endocrinology 146(6):2544–2550

doi: 10.1210/en.2005-0096

Minireview: Regulation of Steroidogenesis by Electron Transfer

Walter L. Miller

University of California, San Francisco, San Francisco, California 94143-0978

Cytochrome P450 enzymes catalyze the degradation of drugs and xenobiotics, but also catalyze a wide variety of biosynthetic processes, including most steps in steroidogenesis. The catalytic rate of a P450 enzyme is determined in large part by the rate of electron transfer from its redox partners. Type I P450 enzymes, found in mitochondria, receive electrons from reduced nicotinamide adenine dinucleotide (NADPH) via the intermediacy of two proteins—ferredoxin reductase (a flavoprotein) and ferredoxin (an iron/sulfur protein). Type I P450 enzymes include the cholesterol side-chain cleavage enzyme (P450scc), the two isozymes of 11-hydroxylase (P450c11β and P450c11AS), and several vitamin D-metabolizing enzymes. Disorders of these enzymes, but not of the two redox partners, have been described. Type II P450 enzymes, found in the endoplasmic reticulum, receive electrons from NADPH via P450 oxidoreductase (POR), which contains two flavin moieties. Steroidogenic Type II P450 enzymes include 17α-hydroxylase/17,20 lyase (P450c17), 21-hydroxylase (P450c21), and aromatase (P450aro). All P450 enzymes catalyze multiple reactions, but P450c17 appears to be unique in that the ratio of its activities is regulated at a posttranslational level. Three factors can increase the degree of 17,20 lyase activity relative to the 17α-hydroxylase activity by increasing electron flow from POR: a high molar ratio of POR to P450c17, serine phosphorylation of P450c17, and the presence of cytochrome b_5, acting as an allosteric factor to promote the interaction of POR with P450c17. POR is required for the activity of all 50 human Type II P450 enzymes, and ablation of the *Por* gene in mice causes embryonic lethality. Nevertheless, mutation of the human *POR* gene is compatible with life, causing multiple steroidogenic defects and a skeletal dysplasia called Antley-Bixler syndrome. (*Endocrinology* 146: 2544–2550, 2005)

DISCUSSIONS OF THE regulation of steroidogenesis generally center on the CRH/ACTH/glucocorticoid and renin/angiotensin/mineralocorticoid systems, which are classical examples of endocrine feedback loops regulated by circulating factors acting at a distance from their sites of synthesis. Recent work has expanded our understanding of steroidogenesis to include intracellular factors that directly influence steroidogenic enzymes. Perhaps the best studied of these is the steroidogenic acute regulatory protein, which regulates the acute steroidogenic responses to ACTH and angiotensin II (1, 2) by acting on the outer mitochondrial membrane (3). Steroidogenic acute regulatory protein increases the delivery of substrate (cholesterol) to the cholesterol side-chain cleavage enzyme, P450scc, but does not act on the enzyme itself. Another level of regulation is at the level of the catalytic efficiency of the steroidogenic enzymes themselves, which is regulated by electron transfer. Regulation of electron transfer to enzymes by complex electron transfer chains is a common cellular strategy for regulating many of biochemical processes, such as oxidative phosphorylation. Such biochemical regulation is also central to steroidogenic processes.

First Published Online March 17, 2005

Abbreviations: FAD, Flavin adenine dinucleotide; FMN, flavin mononucleotide; NADPH, reduced nicotinamide adenine dinucleotide; OMb_5, outer mitchondrial membrane cytochrome b_5; POR, P450 oxidoreductase.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Cytochrome P450

Steroidogenic enzymes fall into two broad categories: the cytochrome P450 enzymes and the hydroxysteroid dehydrogenases, which are addressed in another review (4). This review will focus on the P450 enzymes. Cytochrome P450 refers to a large group of enzymes that have about 500 amino acids and a single heme group and have a characteristic absorption peak at 450 nm in their reduced states. There are two biochemical classes of P450 enzymes. Type I enzymes are found in mitochondria (and bacteria), where they receive electrons from reduced nicotinamide adenine dinucleotide (NADPH) via an electron transfer chain consisting of two proteins: a flavoprotein termed ferredoxin reductase (also called adrenodoxin reductase) and an iron-sulfur protein termed ferredoxin (adrenodoxin). Type II enzymes are found in the endoplasmic reticulum, where they receive electrons from NADPH via a single intermediate, termed P450 oxidoreductase, sometimes assisted by cytochrome b_5 (5). The human genome project has identified 57 P450 genes: seven encode type I enzymes, all of which play key roles in sterol biosynthesis, and 50 encode type II enzymes. Of these 50 type II enzymes, 20 participate in the biosynthesis of steroids, sterols, fatty acids, and eicosanoids; 15 principally metabolize xenobiotic agents and drugs; and 15 are orphan enzymes whose functions and activities remain unclear.

Mitochondrial (Type I) P450 Enzymes

The type I enzymes include six enzymes familiar to endocrinologists. P450scc, the cholesterol side chain cleavage enzyme (formal gene name CYP11A1), is the enzymatic rate-limiting step in steroidogenesis (6, 7). P450c11β (CYP11B1) is the classic steroid 11β-hydroxylase that converts 11-deoxy-

cortisol to cortisol and deoxycorticosterone to corticosterone in the adrenal zona fasciculata; its closely related isozyme P450c11AS (CYP11B2) is the aldosterone synthase that catalyzes 11β hydroxylation, 18 hydroxylation, and 18 methyl oxidation in the adrenal zona glomerulosa, thus converting deoxycorticosterone to aldosterone (8, 9). P450c1α (CYP27B1) is the hormonally regulated vitamin D 1α-hydroxylase that activates vitamin D; P450c24 (CYP24) is the vitamin D 24-hydroxylase that initiates inactivation of vitamin D; and P450c27 (CYP27A1) is a hepatic enzyme principally involved in bile acid biosynthesis that is also a minor vitamin D 25-hydroxylase (10–12).

All type I P450 enzymes receive electrons from NADPH via the same electron-transport chain. First, NADPH binds to ferredoxin reductase, a 54-kDa flavoprotein that is loosely associated with the inner mitochondrial membrane and contains a flavin adenine dinucleotide (FAD) moiety (13). The x-ray crystal structure of bovine ferredoxin reductase shows that it is a bilobed protein with an NADPH-binding site in a tightly packed amino-terminal lobe and an FAD-binding domain in the more loosely packed carboxyl-terminal lobe, with the FAD isoalloxazine ring abutting the bound NADPH (14). The cleft containing the FAD is a Rossman fold with numerous basic residues that appear to be important for interacting with acidic residues near the Fe_2S_2 cluster of ferredoxin. Ferredoxin reductase then interacts with and transfers a pair of electrons to ferredoxin, a small (14 kDa) iron/sulfur protein found in the mitochondrial matrix (15) or loosely associated with the inner mitochondrial membrane (16). Ferredoxin accepts electrons by means of an Fe_2S_2 cluster, which resides in an acidic environment containing one Glu and three Asp residues, which are deprotonated, and hence negativley charged (17). This acidic region protrudes from the molecule and interacts with the basic Rossman fold of ferredoxin reductase to accept a pair of electrons and also interacts with the basic, positively charged redox partner binding site of the mitochondrial P450 to donate electrons. Ferredoxin thus forms a l:l complex with ferredoxin reductase, then dissociates, and then subsequently forms a l:l complex with the P450 (Fig. 1), thus functioning as an indiscriminate electron-shuttle system to all type I P450 enzymes (18–20).

Although the same surface of the ferredoxin molecule that interacts with the ferredoxin reductase must also interact with the P450 (21, 22), catalytically active, highly efficient fusion proteins of these three components have been constructed by placing the ferredoxin moiety on a short tether with rotational freedom (23–27). The increased efficiency of fusion proteins suggests that electron transfer is rate limiting in mitochondrial P450 enzymes. In bovine adrenals, in which P450scc and P450c11β are found in approximately equimolar quantities, the ratio of ferredoxin reductase to ferredoxin to total mitochondrial P450 is about 1:3:8 (28). To convert cholesterol to pregnenolone, P450scc must catalyze three sequential reactions, 20-hydroxylation, 22-hydroxylation, and scission of the 20–22 carbon bond, each of which requires a pair of electrons. Thus, three distinct ferredoxin molecules must dock with the P450scc, give up their electrons, and then exit, making way for the next charged (reduced) ferredoxin. Thus, the rate-limiting enzymatic step in steroidogenesis relates to the movement of ferredoxin molecules rather than to actual catalysis by P450scc. As a result of this, the turnover number for P450scc is only about six molecules of cholesterol converted to pregnenolone per molecule of P450scc per minute (29).

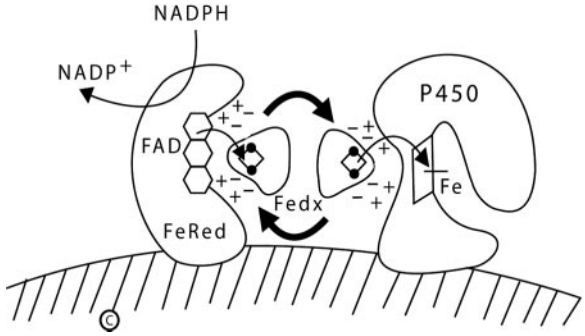

FIG. 1. Diagram of electron transfer by mitochondrial (type I) P450 enzymes. NADPH interacts with ferredoxin reductase (FeRed), which is bound to the inner mitochondrial membrane, and gives up a pair of electrons. The isoalloxazine ring of the FAD moiety of ferredoxin reductase, which lies in a Rossman fold, receives the electrons and in turn passes them to ferredoxin (Fedx). Basic, positively charged residues in ferredoxin reductase, and acidic, negatively charged residues in ferredoxin coordinate the protein interaction, permitting the electrons to be received by the Fe_2S_2 center of ferredoxin, depicted by a ball-and-stick diagram. Ferredoxin then dissociates from ferredoxin reductase and diffuses through the mitochrondrial matrix. The same surface of ferredoxin that received the electrons from ferredoxin reductase then interacts with the redox partner binding site of a type I P450, with electrostatic interactions again coordinating the protein-protein interaction. The electrons from the Fe_2S_2 center of ferredoxin then travel through an ill-described protein conduit in the P450 to reach the heme ring of the P450. The heme iron then mediates catalysis with substrate bound in the P450.

There is only a single gene for ferredoxin reductase (30, 31) located on chromosome 17q24-q25 (32), which is ubiquitously expressed in all tissues, but is most abundantly expressed in steroidogenic tissues (33). It is alternatively spliced into two forms differing by the presence of six residues (30, 31), but these disrupt the FAD binding site so that the longer form is inactive (34). The single functional gene for ferredoxin (35) is located on chromosome 11q22 (32), although there are also several pseudogenes on chromosome 20 (36). The single functional gene encodes several alternately spliced mRNAs that are widely expressed and differ in their 3′ untranslated regions, possibly resulting in different mRNA half-lives (37).

These proteins are not major sites of hormonal regulation. Adrenodoxin mRNA increases sluggishly in response to treatment of steroidogenic cells with cAMP (37, 38), and adrenodoxin reductase mRNA is posttranscriptionally diminished by cAMP, but its transcription does not appear to be regulated by cAMP (33). Similarly, the consequences of mutations in these genes can only be inferred: no mouse knockouts or human disease-causing mutations have been reported. The lack of human disease suggests embryonic lethality. The production of progesterone by placental P450scc is required to suppress maternal uterine contractility, permitting the maintenance of pregnancy, implying that a genetic lesion in any factor required for its synthesis (P450scc, ferredoxin, ferredoxin reductase) will cause spon-

taneous abortion (39). However, three cases of partial or complete absence of P450scc activity have now been described (40–42), suggesting that similar mutations of ferredoxin and ferredoxin might be compatible with life. Mutations in these genes have been sought but not found in at least two cases (43, 44).

Microsomal (Type II) P450 Enzymes

The type II enzymes include hepatic P450 enzymes involved in drug metabolism; several enzymes in the biosynthetic pathways leading to cholesterol, bile acids, and prostaglandins; and three steroidogenic enzymes familiar to endocrinologists. P450c17 (CYP17) catalyzes steroid 17α-hydroxylase and 17,20 lyase activities (45–50) and hence is essential for the synthesis of glucocorticoids (17α-hydroxylase activity) and sex steroids (17,20 lyase activity). P450c21 (CYP21A2) is the single enzyme catalyzing the 21-hydroxylation of both glucocorticoids and mineralocorticoids and is the enzyme that is disordered in the common form of congenital adrenal hyperplasia (51–53). P450aro (CYP19) is the aromatase that converts androgens to estrogens: androstenedione to estrone, testosterone to estradiol, and 16α hydroxytestosterone to estriol (54, 55).

P450 oxidoreductase

All type II P450 enzymes receive electrons from NADPH through the intermediacy of P450 oxidoreductase (POR), sometimes with the assistance of cytochrome b_5. POR is an 82-kDa, membrane-associated protein first isolated in 1969 (56); the cDNA was cloned in 1989 (57), but the gene was not characterized and sequenced until the human genome project showed it consists of 15 exons spanning 32 kb on chromosome 7q11.2 (GenBank sequences GI: 4508114, GI: 11181841, and GI: 24307876). Like ferredoxin reductase, POR contains a molecule of FAD that accepts a pair of electrons from NADPH, but unlike ferredoxin reductase, POR also contains a molecule of flavin mononucleotide (FMN), which can accept the electrons from the FAD moiety and donate them one at a time directly to the P450 enzyme so that POR is a self-contained electron transfer system that does not need another protein such as the ferredoxin used by type I P450 enzymes. The first electron is always transferred more rapidly than the second (58); in some type II P450 systems, cytochrome b_5 can substitute for POR and donate the second but not the first electron, but the presence of POR is mandatory (59, 60).

The structure and function of POR are well understood, in large measure from the x-ray crystal structure of a soluble, amino-terminally deleted form of rat POR (61). The FAD and FMN moieties are contained in distinct domains separated by a flexible hinge region. It appears that binding of NADPH and receipt of electrons by the FAD moiety elicits flexion of the hinge, aligning the isoalloxazine rings of the FAD and FMN moieties so that electrons can pass from FAD to FMN. On doing so, the hinge flexes once more, permitting the FMN domain to become associated with the redox partner binding site of the cytochrome P450 (Fig 2). The surface charge of the FMN domain of POR is negative, produced by acidic residues (61–63), whereas the redox partner binding sites of

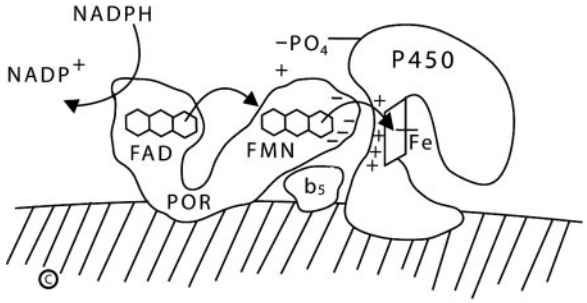

FIG. 2. Diagram of electron transfer by microsomal (type II) P450 enzymes. NADPH interacts with POR, bound to the endoplasmic reticulum, and gives up a pair of electrons, which are received by the FAD moiety. Electron receipt elicits a conformational change, permitting the isoalloxazine rings of the FAD and FMN moieties to come close together so that the electrons pass from the FAD to the FMN. After another conformational change that returns the protein to its original orientation, the FMN domain of POR interacts with the redox partner binding site of the P450. Electrons from the FMN domain of POR reach the heme group to achieve catalysis, as described for type I P450 enzymes. The interaction of POR and the P450 is coordinated by negatively charged acidic residues on the surface of the FMN domain of POR and positively charged basic residues in the redox partner binding site of the P450. In the case of human P450c17, this interaction was facilitated by the allosteric action of cytochrome b_5 and the serine phosphorylation of P450c17.

microsomal P450 enzymes have a positive surface charge produced by basic (Lys and Arg) residues (64–69). The redox partner binding site of the P450 is on the opposite side of the plane of the P450 heme group from the substrate-binding site; hence, electrons from the FMN moiety of the POR must travel about 18 Å to reach the heme iron of the P450 (70). It appears likely that there are multiple pathways for this electron flow in various P450 enzymes.

The availability of electrons from POR is limiting in most microsomal P450 reactions. In both the liver and steroidogenic tissues, the microsomal P450 component is found in a great molar excess to POR (71), possibly as high as 20:1; this has a profound influence on steroidogenesis. P450c17 catalyzes both the 17α-hydroxylation required to produce 17 hydroxy 21-carbon precursors to cortisol (17-hydroxyprenenolone and 17-hydroxyprogesterone) and the 17,20 lyase activity needed to produce 19-carbon precursors of sex steroids. In posing the question of why most adrenal steroidogenesis stops at C21 steroids, Yanagibashi and Hall (72) found that the ratio of POR to P450c17 was 3- to 4-fold higher in testicular microsomes than adrenal microsomes, and that addition of exogenous POR increased the 17,20 lyase reaction far more than the 17α-hydroxylase reaction, although the hydroxylase to lyase ratio never fell below 2.0. This key finding has been confirmed for human P450c17 (73) and forms the basis for the view that the onset of adrenal androgen synthesis (adrenarche) is regulated by events that govern electron flow from POR to P450c17 (74, 75).

Cytochrome b_5

Because a single POR molecule interacts with the redox partner binding sites of five distinct microsomal P450 enzymes, it seems logical to infer that different P450 enzymes will have different affinities for POR. In this situation it is easy to conceptualize how another factor, in this case cyto-

chrome b_5, can act allosterically to optimize the positioning of the POR and P450 with respect to one another and thus foster catalysis indirectly. Similarly, one would predict that the allosteric effect would be greater for some P450 enzymes than for others, depending on the surface geometry and charge distribution in the redox partner binding site of the P450. Modeling and mutagenesis studies with hepatic P450 2B4 indicate that cytochrome b_5 and POR interact with overlapping portions of the negatively charged redox partner binding site of the P450 (76). By optimizing the interaction of POR and the P450, one would expect to see an increased reaction velocity, but one would not expect to see significant changes in substrate binding or product dissociation because these parameters reflect events on the far side of the plane of the heme group, away from the redox partner binding site. Substantial experimental data support this allosteric mechanism for the action of cytochrome b_5 with selected hepatic drug-metabolizing P450 enzymes (77–79).

Recent work has highlighted the central role of cytochrome b_5 and other factors regulating electron flux from POR to P450c17 in the intracellular regulation of human androgen synthesis. Early studies suggested that cytochrome b_5 increased the 17,20 lyase activity of P450c17, but it was thought that its mechanism of action was to function as an alternative donor for the second electron in the P450 catalytic cycle (80, 81), as can happen with some hepatic P450 enzymes (59, 60). However, work with a humanized yeast system that expresses human P450c17 and human (rather than yeast), POR has now established that cytochrome b_5 exerts no action on the 17α-hydroxylase reaction of human P450c17. Instead, cytochrome b_5 profoundly stimulates the 17,20 lyase reaction through an allosteric mechanism, rather than as an electron donor (82, 83). Thus, cytochrome b_5 promotes the association of P450c17 with POR to increase the efficiency of electron donation from POR.

There are two human genes for cytochrome b_5. The gene on chromosome 18q23 has six exons that undergo alternative splicing: exons 1, 2, 3, and 4 encode the 98AA soluble form of cytochrome b_5 found principally in erythropoietic tissues, whereas exons 1, 2, 3, 5, and 6 encode the widely expressed 134AA form bound to the endoplasmic reticulum (84, 85). A second gene on chromosome 16q22.1 consists of five exons that encode OMb_5, a 146AA form of cytochrome b_5 that is bound to the mitochondrial outer membrane (86). Because some domains of OMb_5 share 70% amino acid sequence identity with microsomal cytochrome b_5, it is likely that antisera raised against one will cross-react with the other. Rat OMb_5 can facilitate 17,20 lyase activity *in vitro* but exerts an even greater effect on 17α-hydroxylase activity (87). Because the principal form of cytochrome b_5 found in the adrenal is the 134AA microsomal form (88) and because cytochrome b_5 has no apparent effect on human 17α-hydroxylase activity, it appears that the 134AA microsomal form is largely responsible for the observed effects on 17,20 lyase activity.

Whereas most information about the activity and presumed role of cytochrome b_5 in human androgen synthesis derives from biochemical studies *in vitro*, physiologic support for this role is beginning to emerge. Immunocytochemical analysis of human (89–91) and rhesus monkey (92, 93) adrenals show that cytochrome b_5 is overwhelmingly more abundant in the zona reticularis that in the other zones and that its degree of expression increases in parallel with the increased secretion of 19-carbon steroids during adrenarche, *i.e.* in parallel with increased 17,20 lyase activity. A proposed role for cytochrome b_5 in the 17,20 lyase activity of P450c17 would suggest that mutations in the gene for cytochrome b_5 might present clinically as isolated 17,20 lyase deficiency. Only a single case of cytochrome b_5 deficiency has been reported (94), having a splice-site mutation between exons 1 and 2 (95). Because a major physiologic role of cytochrome b_5 is in the reduction of methemoglobin, the principal clinical manifestation in this patient was methemoglobinemia, which is most commonly caused by disordered cytochrome b_5 reductase. Unlike individuals with cytochrome b_5 reductase disorders, the patient with mutant cytochrome b_5 also had ambiguous genitalia in a 46,XY male, but unfortunately, androgen synthesis was not assessed in this patient. Hence, it is possible that cytochrome b_5 deficiency will disrupt androgen synthesis, but this is not established.

Phosphorylation of P450c17

In addition to high molar ratios of POR to P450c17 and the allosteric action of cytochrome b_5, a third mechanism that increases 17,20 lyase activity is the serine/threonine phosphorylation of P450c17 (96). Very few P450 enzymes undergo posttranslational modification. P450aro (aromatase) can be glycosylated, but this does not appear to affect its catalytic ability (97). To date, P450c17 is one of the few cytochrome P450 enzymes that is known to undergo phosphorylation and the only case in which a posttranslational modification has been shown to exert a major influence on catalysis. Serine/threonine phosphorylation of P450c17 confers 17,20 lyase activity on the enzyme, and dephosphorylation by treating human adrenal microsomes with alkaline phosphatase ablates 17,20 lyase activity without affecting 17α-hydroxylase activity (96). The responsible kinase appears to be responsive to cAMP but remains unidentified. A kinase-enriched cytoplasmic fraction of human adrenal NCI-H295A cells can phosphorylate dephospho-P450c17 expressed in eukaryotic cells or in bacteria and can confer 17,20 lyase activity to the P450c17 (98). Treatment with inhibitors of various protein phosphatases, RNA interference studies, and protein transfection studies indicate that the phosphorylation of P450c17 is counterbalanced by protein phosphatase 2A, which, in turn, is negatively regulated by phosphoprotein SET (98). Because serine phosphorylation of the β-chain of the insulin receptor will produce insulin resistance (99, 100), it appears likely that serine phosphorylation is the mechanistic link between the insulin resistance and hyperandrogenism that characterize some forms of the polycystic ovary syndrome (75, 96, 101). Serine phosphorylation of P450c17 apparently increases 17,20 lyase activity by increasing the association of P450c17 with POR and increasing the efficiency of electron transfer. Strong evidence for this model comes from the recent observation that serine phosphorylation of P450c17 and addition of cytochrome b_5 can each saturate the 17,20 lyase activity of P450c17, *i.e.* the effects are neither additive nor cooperative (88). Thus, three mechanisms, the abundance of POR,

the presence of cytochrome b_5, and the serine phosphorylation of P450c17, all regulate 17,20 lyase activity, and hence androgen production, by modulating the flow of electrons from NADPH to P450c17.

P450 oxidoreductase deficiency

Because POR is required for the activity of all 50 human type II (microsomal) P450 enzymes, one might presume that ablation of POR would have dire consequences. In fact, mice lacking only the membrane-anchoring amino-terminal domain of POR (but retaining residues 107–677, which can reduce cytochrome c *in vitro*) die by embryonic d 13.5 (102), and mice lacking the entire POR gene suffer embryonic lethality by d 9.5 (103). This lethality is apparently a consequence of disordered extrahepatic P450 enzymes because liver-specific POR knockout mice have normal development and normal reproductive capacity, despite severely impaired drug metabolism (104). Thus, it was most surprising when Flück *et al.* (105) reported POR missense mutations in both a phenotypically normal adult woman with primary amenorrhea and three children with disordered steroidogenesis and a severe skeletal malformation disorder called Antley-Bixler syndrome. These patients have steroidal findings suggesting partial combined deficiencies of 17α-hydroxylase and 21-hydroxylase and occasionally evidence of fetoplacental aromatase deficiency as well (106). Several other groups have also reported similar cases (107–109).

Although this steroidal profile was first reported in 1985 (110) and it was suggested that the disorder might be in POR (111, 112), POR was not investigated until the human genome project made the gene sequence available. All patients studied to date have had a missense (amino acid replacement) mutation on at least one allele; hence, it is not clear whether total ablation of POR is compatible with human life. With the completion of a large international series, a total of 21 POR missense mutations have been identified, providing excellent scanning mutagenesis of POR and the opportunity to correlate clinical, biochemical, and genetic findings (113). All affected individuals have disordered 17,20 lyase activity; the defects in 17α-hydroxylase, 21-hydroxylase, and aromatase activities are more variable. This is consistent with the observations that the 17,20 lyase activity of P450c17 is sensitive to mutations in its redox partner binding site that do not affect 17α-hydroxylase activity and the observations that the 17,20 lyase activity of P450c17 requires the assistance of either serine phosphorylation or the allosteric action of cytochrome b_5. Not surprisingly, the biochemical assay of POR activity that most closely correlates with the clinical findings is the degree to which a mutant form of POR is able to support the 17,20 lyase activity of P450c17 *in vitro*, in the presence of saturating amounts of cytochrome b_5 (105, 106, 113). The potential effects of such POR mutations or POR polymorphisms on drug metabolism by hepatic P450 enzymes has not yet been investigated but may become an important area of pharamacogenomics.

Conclusion

Whereas endocrinology has traditionally emphasized regulation by circulating hormonal factors, regulation by intracellular factors has assumed equal importance. Many biochemical pathways including steroidogenic pathways are delicately regulated by electron-donation and redox state. The elucidation of the structures of these redox partner proteins, their biochemical activities, and their genetic deficiency states are opening a major new area of endocrine investigation.

Acknowledgments

The author thanks all the members of the Miller laboratory for their contributions to this work.

Received January 24, 2005. Accepted March 1, 2005.

Address all correspondence and requests for reprints to: Professor Walter L. Miller, Department of Pediatrics, Building MR-4, Room 209, University of California, San Francisco, San Francisco, California 94143-0978.

This work was supported by National Institutes of Health Grants DK37922 and HD41958.

References

1. **Stocco DM, Clark BJ** 1996 Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 17:221–244
2. **Bose HS, Sugawara T, Strauss III JF, Miller WL** 1996 The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med 335: 1870–1878
3. **Bose HS, Lingappa WR, Miller WL** 2002 Rapid regulation of steroidogenesis by mitochondrial protein import. Nature 417:87–91
4. **Agarwal AK, Auchus RJ** 2005 Redox state and hydroxysteroid dehydrogenase directionality. Endocrinology 146:2531–2538
5. **Hildebrandt A, Estabrook RW** 1971 Evidence for participation of cytochrome b_5 in hepatic microsomal mixed-function oxidation reactions. Arch Biochem Biophys 143:66–79
6. **Chung B, Mattson KJ, Voutilainen R, Mohandras TK, Miller WL** 1986 Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning assignment of the gene to chromosome 15, and expression in the placenta. Proc Natl Acad Sci USA 83:8962–8966
7. **Miller WL** 1988 Molecular biology of steroid hormone synthesis. Endocr Rev 9:295–318
8. **White PC, Curnow KM, Pascoe L** 1994 Disorders of steroid 11β-hydroxylase isozymes. Endocr Rev 15:421–438
9. **Fardella CE, Miller WL** 1996 Molecular biology of mineralocorticoid metabolism. Annu Rev Nutrition 16:443–470
10. **Fu GK, Lin D, Zhang MYH, Bikle DD, Shackleton CHL, Miller WL, Portale AA** 1997 Cloning of 25-hydroxyvitamin D lα-hydroxylase and mutations causing vitamin D-dependent rickets type I. Mol Endocrinol 11:1961–1970
11. **Miller WL, Portale AA** 2000 Vitamin D lα-hydroxylase. Trends Endocrinol Metab 11:315–319
12. **Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW** 2004 Genetic evidence that the human CYP 2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 101:7711–7715
13. **Kimura T, Suzuki K** 1967 Components of the electron transport system in adrenal steroid hydroxylase. J Biol Chem 242:485–491
14. **Ziegler GA, Vonrhein C, Hanukoglu I, Schulz GE** 1999 The structure of adrenodoxin reductase of mitochondrial P450 systems: electron transfer for steroid biosynthesis. J Mol Biol 289:981–990
15. **Gnanaiah W, Omdahl JL** 1986 Isolation and characterization of pig kidney mitochondrial ferredoxin: NADP+ oxidoreductase. J Biol Chem 261:12649–12654
16. **Hanukoglu I, Suh BS, Himmelhoch S, Amsterdam A** 1990 Induction and mitochondrial localization of cytochrome P450scc system enzymes in normal and transformed ovarian granulosa cells. J Cell Biol 111:1373–1381
17. **Muller A, Muller JJ, Muller YA, Uhlmann H, Bernhardt R, Heinemann U** 1998 New aspects of electron transfer revealed by the crystal structure of a truncated bovine adrenodoxin, Adx(4–108). Structure 6:269–280
18. **Lambeth JD, Seybert D, Kamin H** 1979 Ionic effects on adrenal steroidogenic electron transport: the role of adrenodoxin as an electron shuttle. J Biol Chem 254:725–7204
19. **Hanukoglu I, Spitsberg V, Bumpus JA, Dus KM, Jefcoate CR** 1981 Adrenal mitochondrial cytochrome P450scc: cholesterol and adrenodoxin interactions at equilibrium and during turnover. J Biol Chem 256:4321–4328
20. **Coghlan VM, Vickery LE** 1989 Expression of human ferredoxin and assembly of the [2Fe-2S] center in *Escherichia coli*. Proc Natl Acad Sci USA 85:835–839
21. **Coghlan VM, Vickery LE** l991 Site-specific mutations in human ferredoxin that affect binding to ferredoxin reductase and cytochrome P450scc. J Biol Chem 266:18606–18612
22. **Coghlan VM, Vickery LE** 1992 Electrostatic interactions stabilizing ferre-

doxin electron transfer complexes. Disruption by "conservative" mutations. J Biol Chem 267:8932–8935
23. **Harikrishna JA, Black SM, Szklarz GD, Miller WL** 1993 Construction and function of fusion enzymes of the human cytochrome P450scc system. DNA Cell Biol 12:371–379
24. **Black SM, Harikrishna JA, Szklarz GD, Miller WL** 1994 The mitochondrial environment is required for cytochrome P450scc function. Proc Natl Acad Sci USA 91:7247–7251
25. **Dilworth FJ, Black SM, Guo YD, Miller WL, Jones G** 1996 construction of a P450c27 fusion enzyme—a useful tool for analysis of vitamin D_3-25-hydroxylase activity. Biochem J 320:267–271
26. **Sibbesen O, DeVoss JJ, Ortiz de Montellano PR** 1996 Putidaredoxin reductase-putidaredoxin-cytochrome P450cam triple fusion protein. J Biol Chem 271:22462–22469
27. **Cao P, Bülow H, Dumas B, Bernhardt R** 2000 Construction and characterization of a catalytic fusion protein system: P450–11β-adrenodoxin reductase-adrenodoxin. Biochim Biophys Acta 1476:253–264
28. **Hanukoglu I, Hanukoglu Z** 1986 Stoichiometry of mitochondrial cytochromes P450, adrenodoxin, and adrenodoxin reductase in adrenal cortex and corpus luteum. Eur J Biochem 157:27–31
29. **Kuwada M, Kitajima R, Suzuki H, Horie S** 1991 Purification and properties of cytochrome P-450 (SCC) pig testis mitochondria. Biochem Biophys Res Commun 176:1501–1508
30. **Solish SV, Picado-Leonard J, Morel Y, Kuhn RW, Mohandas TK, Hanukoglu I, Miller WL** 1988 Human adrenodoxin reductase: two mRNAs encoded by a single gene of chromosome 17 cen→q25 are expressed in steroidogenic tissues. Proc Natl Acad Sci USA 85:7104–7108
31. **Lin, D, Shi Y, Miller WL** 1990 Cloning and sequence of the human adrenodoxin reductase gene. Proc Natl Acad Sci USA 87:8516–8520
32. **Sparkes RS, Klisak I, Miller WL** 1991 Regional mapping of genes encoding human steroidogenic enzymes: P450scc to 15q23–q24, adrenodoxin to 11q22, adrenodoxin reductase to 17q24–q25, and P450c17 to 10q24–q25. DNA Cell Biol 10:359–365
33. **Brentano ST, Black SM, Lin D, Miller WL** 1992 cAMP post-transcriptionally diminishes the abundance of adrenodoxin reductase mRNA. Proc Natl Acad Sci USA 89:4099–4103
34. **Brandt ME, Vickery LE** 1992 Expression and characterization of human mitochondrial ferredoxin reductase in *Escherichia coli.* Arch Biochem Biophys 294:735–740
35. **Chang CY, Wu DA, Lai CC, Miller WL, Chung B** 1988 Cloning and structure of the human adrenodoxin gene. DNA 7:609–615
36. **Morel Y, Picado-Leonard J, Wu DA, Chang C, Mohandas TK, Chung B, Miller WL** 1988 Assignment of the functional gene for adrenodoxin to chromosome 11q13→qter and of two adrenodoxin pseudogenes to chromosome 20 cen→q13.1. Am J Hum Genet 43:52–59
37. **Picado-Leonard J, Voutilainen R, Kao L, Chung B, Strauss III JF, Miller WL** 1988 Human adrenodoxin: cloning of three cDNAs and cycloheximide enhancement in JEG-3 cells. J Biol Chem 263:3240–3244
38. **Voutilainen R, Picado-Leonard J, BiBlasio AM, Miller WL** 1988 Hormonal and developmental regulation of human adrenodoxin mRNA in steroidogenic tissues. J Clin Endocrinol Metab 66:383–388
39. **Miller WL** 1988 Why nobody has P450scc (20,22 desmolase) deficiency. J Clin Endocrinol Metab 83:1399–1400 (Letter to Editor)
40. **Tajima T, Fujieda AK, Konda N, Nakae J, Miller WL** 2001 Heterozygous mutation in the cholesterol side chain cleavage enzyme (P450scc) gene in a patient with 45, XY sex reversal and adrenal insufficiency. J Clin Endocrinol Metab 86:3820–3825
41. **Katsumata N, Ohtake M, Hojo T, Ogawa E, Hara T, Sato N, Tanaka T** 2002 Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. J Clin Endocrinol Metab 87:3808–3813
42. **Hiort O, Holterhaus PM, Werner R, Marschke C, Hoppe U, Partsch J, Riepe FG, Achermann JC, Struve D** 2005 Homozygous disruption of P450scc (CYP11A1) is associated with prematurity, complete 46, XY sex reversal and severe adrenal failure. J Clin Endocrinol Metab 90:538–541
43. **Lin D, Gitelman SE, Saenger P, Miller WL** 1991 Normal genes for the cholesterol side chain cleavage enzyme, P450scc, in congenital lipoid adrenal hyperplasiz. J Clin Invest 88:1955–1962
44. **Gassner HL, Toppai J, Quinteiro-Gonzalez S, Miller WL** 2004 Near-miss apparent SIDS from adrenal crisis. J Pediatr 145:178–183
45. **Nakajin S, Hall PF** 1981 Microsomal cytochrome P450 from neonatal pig testis. Purification and properties of a C_{21} steroid side-chain cleavage system (17α-hydroxylase-C17–20 lyase) J Biol Chem 256:3871–3876
46. **Nakajin S, Hall PF, Onoda M** 1981 Testicular microsomal cytochrome P450 for C_{21} steroid side chain cleavage J Biol Chem 256:6134–6169
47. **Nakajin S, Shively JE, Yuan P, Hall PF** 1981 Microsomal cytochrome P450 from neonatal pig testis. Two enzymatic activities (17α-hydroxylase and C17,20-lyase) associated with one protein. Biochemistry 20:4037–4042
48. **Nakajin S, Shinoda M, Haniu M, Shively JE, Hall PF** 1984 C_{21} steroid side-chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17α hydroxylase/C17,20 lyase cytochrome P450. J Biol Chem 259:3971–3976
49. **Zuber MX, Simpson ER, Waterman MR** 1986 Expression of bovine 17α-hydroxylase cytochrome P450 cDNA in non-steroidogenic (COS-1) cells. Science 234:1258–1261
50. **Chung BC, Picado-Leonard J, Haniu M, Bienkowski M, Hall PF, Shively JE, Miller WL** 1987 Cytochrome P450c17 (steroid 17α-hydroxylase/17,20 lyase). Cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. Proc Natl Acad Sci USA 84:407–411
51. **Miller WL, Morel Y** 1989 Molecular genetics of 21-hydroxylase deficiency. Annu Rev Genet 23:371–393
52. **White PC, Speiser PW** 2000 Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21:245–291
53. **Forest MG** 2004 Recent advances in the diagnosis and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Hum Reprod Update 10:469–485
54. **Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshlelwood MM, Graham-Lorence S, Amarneh B, Ito YJ, Fisher CR, Michael MD, Mendelson CR, Bulun SE** 1994 Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev 15:342–355
55. **Grumbach MM, Auchus RJ** 1999 Estrogen: consequences and implications of human mutations in synthesis and action. J Clin Endocrinol Metab 84: 4677–4694
56. **Lu AY, Junk KW, Coon MJ** 1969 Resolution of the cytochrome P450-containing ω-hydroxylation system of liver microsomes into three components. J Biol Chem 244:3714–3721
57. **Yamano LS, Aoyama T, McBride OW, Hardwick JP, Gelboin HV, Gonzalez FJ** 1989 NADPH-P450 oxidoreductase. Complementary DNA cloning, sequence, vaccinia virus-mediated expression, and localization of the CYPOR gene to chromosome 7. Mol Pharmacol 35:83–88
58. **Oparian DD, Coon MJ** 1982 Oxidation-reduction states of FMN and FAD in NADPH-cytochrome P450 reductase during reduction by NADPH. J Biol Chem 257:8935–8944
59. **Tamburini PP, Gibson GG** 1983 Thermodynamic studies of the protein-protein interactions between cytochrome P450 and cytochrome b_5. J Biol Chem 258:3444–3452
60. **Guengerich FP, Johnson WW** 1997 Kinetics of ferric cytochrome P450 reduction by NADPH-cytochrome P450 reductase. Rapid reduction in the absence of substrate and variations among cytochrome P450 systems. Biochemistry 36:14741–14750
61. **Wang M, Roberts DL, Paschke R, Shea TM, Masters BSS, Kim JJ** 1997 three-dimensional structure of NADPH-cytochrome P450 reductase: prototype for FMN- and FAD-containing enzymes. Proc Natl Acad Sci USA 94: 8411–8416
62. **Shen AL, Kasper CB** 1995 Role of acidic residues in the interaction of NADPH-cytochrome P450 oxidoreductase with cytochrome P450 and cytochrome c. J Biol Chem 270:27475–27480
63. **Estabrook RW, Shet MS, Fisher CW, Jenkins CM, Waterman MR** 1996 The interaction of NADPH-P450 reductase with P450: an electrochemical study of the role of the flavin mononucleotide-binding domain. Arch Biochem Biophys 333:308–315
64. **Hasemann CA, Kirumbail RG, Boddupalli SS, Peterson JA, Deisenhofer J** 1995 Structure and function of cytochromes P450: a comparative analysis of three crystal structures. Structure 3:41–62
65. **Fisher CW, Shet MS, Estabrook RW** 1996 Construction of plasmids and expression in *Escherichia coli* of enzymatically active fusion proteins containing the heme-domain of a P450 linked to NADPH-P450 reductase. Methods Enzymol 272:15–25
66. **Geller DH, Auchus RJ, Medonca BB, Miller WL** 1997 The genetic and functional basis of isolated 17,20-lyase deficiency. Nat Genet 17:201–205
67. **Auchus RJ, Miller WL** 1999 Molecular modeling of human P450c17 (17α-hydroxylase/17,20-lyase: insights into reaction mechanisms and effects of mutations. Mol Endocrinol 13:1169–1182
68. **Kondo S, Sakaki T, Ohkawa H, Inouye K** 1999 Electrostatic interaction between cytochrome P450 and NADPH-P450 reductase: comparison of mixed and fused systems consisting of rat cytochrome P450 1A1 and yeast NADPH-P450 reductase. Biochem Biophys Res Commun 257:273–278
69. **Davydov DR, Kariakin AA, Petushkova NA, Peterson JA** 2000 Association of cytochromes P450 with their reductases: opposite sign of the electrostatic interaction in P450BM-3 as compared with the microsomal 2B4 system. Biochemistry 39:6489–6497
70. **Sevrioukova IF, Li H, Zhang H, Peterson JA, Poulos TL** 1999 Structure of a cytochrome P450-redox partner electron-transfer complex. Proc Natl Acad Sci USA 96:1863–1868
71. **Estabrook RW, Franklin MR, Cohen B, Shizamatsu A, Hildebrandt AG** 1971 Influence of hepatic microsomal mixed function oxidation reactions on cellular metabolic control. Metabolism 20:187–199
72. **Yanagibashi K, Hall PF** 1986 Role of electron transport in the regulation of the lyase activity of C_{21} side chain cleavage P450 from porcine adrenal and testicular microsomes. J Biol Chem 261:8429–8433
73. **Lin D, Black SM, Nagahama Y, Miller WL** 1993 Steroid 17α-hydroxylase and 17,20-lyase activities of P450c17: contributions of serine 106 and P450 reductase. Endocrinology 132:2498–2506

74. **Miller WL, Auchus RJ, Geller DH** 1997 The regulation of 17,20 lyase activity. Steroids 62:133–142
75. **Auchus RJ, Geller DH, Lee TC, Miller WL** 1998 The regulation of human P450c17 activity: relationship to premature adrenarche, insulin resistance and the polycystic ovary syndrome. Trends Endocrinol Metab 9:47–50
76. **Bridges A, Gruenke L, Chang YT, Vakser IA, Loew G, Waskell L** 1998 Identification of the binding site on cytochrome P450 2B4 for cytochrome b_5 and cytochrome P450 reductase. J Biol Chem 273:17036–17049
77. **Yamazaki H, Johnson WW, Ueng YF, Shimada T, Guengerich FP** 1996 Lack of electron transfer from cytochrome b_5 in stimulation of catalytic activities of cytochrome P450 3A4. Characterization of a reconstituted cytochrome P450 3A4/NADPH-cytochrome P450 reductase system and studies of apo-cytochrome b_5. J Biol Chem 271:27438–27444
78. **Yamazaki H, Gillam EM, Dong MS, Johnson WW, Guengerich FP, Shimada T** 1997 Reconstitution of recombinant cytochrome P450 2C10 (2C9) and comparison with cytochrome P450 3A4 and other forms. Effects of cytochrome P450–P450 and cytochrome P450-b_5 interactions. Arch Biochem Biophys 342: 329–337
79. **Loughran PA, Roman LJ, Miller RT, Masters BSS** 2001 The kinetic and spectral characterization of the *E. coli*-expressed mammalian CYP4A7: cytochrome b_5 effects vary with substrates. Arch Biochem Biophys 385:311–321
80. **Onoda M, Hall PF** 1982 Cytochrome b_5 stimulates purified testicular microsomal cytochrome P450 (C_{21} side-chain cleavage). Biochem Biophys Res Commun 108:454–460
81. **Kominami S, Ogawa N, Morimune R, Huang DY, Takemori S** 1992 The role of cytochrome b_5 in adrenal microsomal steroidogenesis. J Steroid Biochem Mol Biol 42:57–64
82. **Auchus RJ, Lee TC, Miller WL** 1998 Cytochrome b_5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. J Biol Chem 273:3158–3165
83. **Geller DH, Auchus RJ, Miller WL** 1999 P450c17 mutations R347H and R358Q selectively disrupt 17,20-lyase activity by disrupting interactions with P450 oxidoreductase and cytochrome b_5. Mol Endocrinol 13:167–175
84. **Giordano SJ, Steggles AW** 1991 The human liver and reticulocyte cytochrome b_5 mRNAs are products from a single gene. Biochem Biophys Res Commun 178:38–44
85. **Giordano SJ, Yoo M, Ward DC, Bhatt M, Overhauser J, Steggles AW** 1993 The human cytochrome b_5 gene and two of its pseudogenes are located on chromosomes 18q23, 14q31–32.1 and 20p11.2, respectively. Hum Genet 92: 615–618
86. **Kuroda R, Ikenoue T, Honsho M, Tsujimoto S, Mitoma JY, Ito A** 1998 Charged amino acids at the carboxyl-terminal portions determine the intracellular locations of two forms of cytochrome b_5. J Biol Chem 273:31097–31102
87. **Ogishima T, Kinoshita J, Mitani F, Suematsu M, Ito A** 2003 Identification of outer mitochondrial membrane b_5 as a modulator for androgen synthesis in Leydig cells. J Biol Chem 278:21204–21211
88. **Pandey AV, Miller WL** 2005 Regulation of 17,20 lyase activity by cytochrome b_5 and by serine phosphorylation of P450c17. J Biol Chem 280:13265–13271
89. **Yanase T, Sasano H, Yubisui T, Sakai Y, Takayanagi R, Nawata H** 1998 Immunohistochemical study of cytochrome b_5 in human adrenal gland and in adrenocortical adenomas from patients with Cushings's syndrome. Endocr J 45:89–95
90. **Suzuki T, Sasano H, Takeyama J, Kaneko C, Freije WA, Carr BR, Rainey WE** 2000 Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: immunohistochemical studies. Clin Endocrinol (Oxf) 53:739–747
91. **Dharia S, Slane A, Jian M, Conner M, Conley AJ, Parker CR** 2004 Colocalization of P450c17 and cytochrome b_5 in androgen-synthesizing tissues of the human. Biol Reprod 71:83–88
92. **Mapes S, Corbin CJ, Tarantal A, Conley A** 1999 The primate adrenal zona retucularis is defined by expression of cytochrome b_5, 17α-hydroxylase/17,20 lyase cytochrome P450 (P450c17) and NADPH-cytochrome P450 reductase (reductase) but not 3β-hydroxysteroid dehydrogenase/Δ5–4 isomerase (3β-HSD). J Clin Endocrinol Metab 84:3382–3385
93. **Mapes S, Tarantal AF, Parker CR, Moran FM, Bahr JM, Pyter L, Conley AJ** 2002 Adrenocortical cytochrome b_5 expression during fetal development in the rhesus macaque. Endocrinology 143:1451–1458
94. **Hegesh E, Hegesh J, Kaftory A** 1986 Congenital methemoglobinemia with deficiency of cytochrome b_5. N Engl J Med 3124:757–761
95. **Giordano SJ, Kaftory A, Steggles AW** 1994 A splicing mutation in the cytochrome b_5 gene from a patient with congenital methemoglobinemia and pseudohermaphroditism. Hum Genet 93:568–570
96. **Zhang LH, Rodriguez H, Ohno S, Miller WL** 1995 Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. Proc Natl Acad Sci USA 92:10619–10623
97. **Shimozawa O, Sakaguchi M, Ogawa H, Harada N, Mihara K, Omura T** 1993 Core glycosylation of cytochrome P450(arom). Evidence for localization of N terminus of microsomal cytochrome P450 in the lumen. J Biol Chem 268: 21399–21402
98. **Pandey AV, Mellon SH, Miller WL** 2003 Protein phosphatase 2A and phosphoprotein SET regulate androgen production by P450c17. J Biol Chem 278: 2837–2844
99. **Takeyama S, White MF, Kahn CR** 1988 Phorbol ester-induced serine phosphorylation of the insulin receptor decreases its tyrosine kinase activity. J Biol Chem 263:3440–3447
100. **Chin JE, Dickens M, Tavare JM, Roth RA** 1993 Overexpression of protein kinase C isozymes α, βI, γ, and ϵ in cells overexpressing the insulin receptor. J Biol Chem 268:6338–6347
101. **Miller WL** 1999 The molecular basis of premature adrenarche: an hypothesis. Acta Paediatrica 88(Suppl 433):60–66
102. **Shen AL, O'Leary KA, Kasper CB** 2002 Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase. J Biol Chem 277:6536–6541
103. **Otto DM, Henderson CJ, Carrie D, Davey M, Gundersen TE, Blomhoff R, Adams RH, Tickle C, Wolf CR** 2003 Identification of novel roles of the cytochrome P450 system in early embryogenesis: effects on vasculogenesis and retinoic acid homeostasis. Mol Cell Biol 21:6103–6116
104. **Henderson CJ, Otto DM, Carrie D, Magnuson MA, McLaren AW, Rosewell I, Wolf CR** 2003 Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. J Biol Chem 278: 13480–13486
105. **Flück CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, Jabs EW, Mendonca BB, Fujieda K, Miller WL** 2004 Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. Nat Genet 36:228–230
106. **Miller WL** 2004 P450 oxidoreductase deficiency: a new disorder of steroidogenesis with multiple clinical manifestations. Trends Endocrinol Metab 15:311–315
107. **Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, Chalder SM, Borucka-Mankiewicz M, Hauffa BP, Malunowicz EM, Stewart PM, Shackleton CH** 2004 Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 363: 2128–2135
108. **Adachi M, Tachibana K, Asakura Y, Yamamoto T, Hanaki K, Oka A** 2004 Compound heterozygous mutations of cytochrome P450 oxidoreductase gene (POR) in two patients with Antley-Bixler syndrome. Am J Med Genet 128A:333–339
109. **Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, Okuyama T, Nakai H, Soneda S, Tachibana K, Matsuo N, Sato S, Homma K, Nishimura G, Hasegawa T, Ogata T** 2005 POR (P450 oxidoreductase) mutations and Antely-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. J Clin Endocrinol Metab 90:414–426
110. **Peterson RE, Imperato-McGinley J, Gautier T, Shackleton C** 1985 Male pseudohermaphroditism due to multiple defects in steroid-biosynthetic mixed-function oxidases. A new variant of congenital adrenal hyperplasia. N Engl J Med 313:1182–1191
111. **Miller WL** 1986 Congential adrenal hyperplasia. N Engl J Med 314:1321–1322
112. **Augarten A, Pariente C, Gazit E, Chayen R, Goldfarb H, Sack J** 1992 Ambiguous genitalia due to partial activity of cytochromes P450c17 and P450c21. J Steroid Biochem Mol Biol 41:37–41
113. **Huang N, Pandey AV, Agrawal V, Reardon W, Lapunzina PD, Mowat D, Jabs EW, vanVliet G, Sack J, Flück CE, Miller WL** 2005 Diversity and function of mutations in P450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. Am J Hum Genet, 76:729–749

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**