10 Steroidogenic Enzymes in Leydig Cells

Anita H. Payne, PhD

SUMMARY

This chapter describes the enzymes expressed in Leydig cells that are required for the biosynthesis of testosterone from cholesterol, as well as the two enzymes, steroid 5α-reductase and P450arom, that metabolize testosterone to dihydrotestosterone and estradiol, respectively. The emphasis is on human and mouse enzymes.

Key Words: CYP11A; CYP17A; CYP19A, 3β-HSD; 17β-HSD; Leydig cell; P450arom; P450c17; P450scc; steroidogenesis; steroid-α-reductase.

INTRODUCTION

The Leydig cell is the only cell in the male that expresses all of the enzymes essential for the conversion of cholesterol to testosterone, the major male sex hormone secreted by the testis. Testosterone can be metabolized further in the Leydig cell by S5A (5α-reductase) to yield dihydrotestosterone *(1)*, or by the enzyme, CYP19A1 (P450arom) to yield estradiol *(2,3)*. Testosterone or its metabolite, dihydrotestosterone, is essential for male sexual differentiation, expression of male secondary sex characteristics *(4)*, and maintenance of spermatogenesis *(5)*. The biosynthesis of testosterone is dependent on both acute and chronic stimulation of Leydig cells by the pituitary hormone luteinizing hormone (LH). LH binds to specific high affinity receptors on the surface of Leydig cells activating adenylate cyclase, resulting in increased production of cyclic AMP. The acute stimulation results in the rapid transport of cholesterol from the outer to the inner mitochondrial membrane, the site of the first enzyme in the pathway of cholesterol to testosterone. This process is mediated by the steroidogenic acute regulatory protein (StAR) *(6), see* Chapter 9. Chronic stimulation of Leydig cells by LH or cAMP is required for optimal expression of the enzymes required for the biosynthesis of testosterone from cholesterol. This chapter describes the enzymes involved in the biosynthesis of testosterone from cholesterol as well as the two enzymes, steroid 5α-reductase and P450arom that metabolize testosterone to dihydrotestosterone and estradiol, respectively (Fig. 1). The enzymes can be divided into two major classes of proteins: the cytochrome P450 heme-containing proteins CYP11A1 (P450scc), CYP17A1 (P450c17), and P450arom, and the hydroxysteroid dehydrogenases 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-hydroxysteroid dehydrogenase (17β-HSD). 5α-reductase belongs to the steroid 5α-reductase family (Table 1C; ref. *7*).

The initial step in the biosynthesis of testosterone from cholesterol is the conversion of the C27 cholesterol to the C21 steroid, pregnenolone. This reaction is catalyzed by the cytochrome P450 enzyme, cholesterol side-chain cleavage (P450scc), located in the inner mitochondrial membrane. Pregnenolone diffuses across the mitochondrial membrane and is further metabolized by enzymes associated with the smooth endoplasmic reticulum. These include the cytochrome P450 17α-hydroxylase C17-C20 lyase, P450c17, which catalyzes the conversion of the C21 steroids pregnenolone or progesterone to the C19 steroids dehydroepiandrosterone or androstenedione, respectively, and 3β-hydroxysteroid dehydrogenase which catalyzes the conversion of the Δ5 steroids, pregnenolone, or dehydroepiandrosterone to the Δ4 steroids, progesterone, or androstenedione, respectively, and 17β-hydroxysteroid dehydrogenase, which catalyzes the final step in the biosynthesis of testosterone (Fig. 1).

CYTOCHROME P450s

The cytochrome P450 enzymes are members of a superfamily of heme-containing proteins *(8)*. They

From: *Contemporary Endocrinology: The Leydig Cell in Health and Disease*
Edited by: A. H. Payne and M. P. Hardy © Humana Press Inc., Totowa, NJ

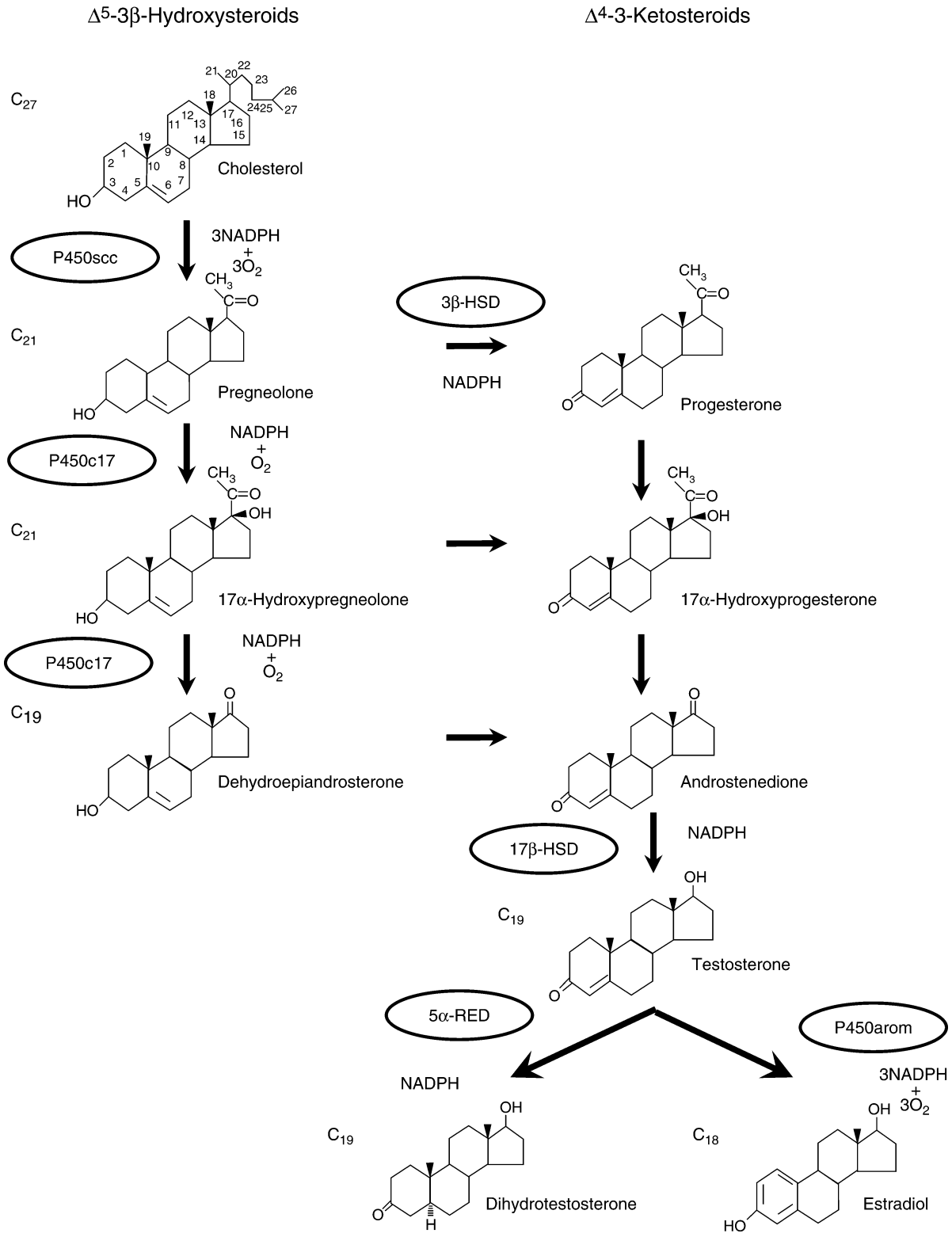

Fig. 1. Steroid biosynthetic pathways in Leydig cells.

derive their name from the characteristic which, when complexed in vitro with exogenous carbon monooxide, absorb light maximally at 450 nm. They function as monooxygenases utilizing reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the donor for the reduction of molecular oxygen. The general reaction is: $RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$. In this reaction, the oxygen is activated by P450, and one oxygen atom is introduced into the substrate RH as a hydroxyl group, and the other oxygen is reduced to H_2O. The electrons from NADPH are transferred to the substrate by two distinct electron transfer systems. The mitochondrial transfer involves the transfer of high potential electrons to a flavoprotein, adrenodoxin reductase (ferredoxin reductase), and then sequentially to adrenodoxin (ferredoxin), a nonheme iron–sulfur protein, then P450, and finally, to the substrate. The microsomal electron transfer system involves only one protein, cytochrome P450 oxidoreductase, a protein that contains two flavins. The electrons are transferred from NADPH, to flavinadenine dinucleotide, followed sequentially by transfer to flavinmononucleotide and then the substrate *(7)*.

P450scc

Reaction Catalyzed

P450scc catalyzes the conversion of cholesterol to pregnenolone, the first and rate-limiting enzymatic step in the biosynthesis of testosterone (Fig. 1). P450scc catalyzes three sequential oxidation reactions of cholesterol. Each reaction requires one molecule of oxygen and one molecule of NADPH, and the mitochondrial electron transfer system *(7)*. The first reaction is the hydroxylation at C22, followed by hydroxylation at C20 to yield 20,22R-hydroxycholesterol that is cleaved between C22 and C20 to yield the C21 steroid pregnenolone, and isocapraldehyde *(9,10)* Isocapraldehyde is further metabolized to isocaproic acid *(11)*.

Investigations utilizing the purified protein as well as studies on recombinant proteins from P450scc cDNAs have provided conclusive evidence that a single protein catalyzes all three reactions at a single active site *(12,13)*. The pair of electrons required for each of the reactions is transferred from NADPH to a flavoprotein, ferredoxin reductase, and then sequentially to a nonheme iron–sulfur protein, ferredoxin, to P450scc, and finally, to the substrate *(14)*. The P450scc enzyme is typical of all mitochondrial cytochrome P450 enzymes that share the same electron transfer proteins *(15)*. It has been shown that the P450scc enzyme only functions in the mitochondrion. This requirement appears to be for the mitochondrial environment rather than the specific mitochondrial electron transfer system *(16)*. A model of the interactions between P450scc and the electron transport proteins has been proposed based on the expression of mutants. The results of these studies indicate that the acidic residues, Asp 76, and Asp 79, of ferredoxin interact with the basic residues of ferredoxin reductase and P450scc *(17)*.

Molecular Structure

P450scc is the product of a single gene. The cDNA was first isolated in 1984 from bovine adrenal cortex mRNA *(18)*. Subsequently, P450scc cDNA has been cloned from human *(19)*, rat *(20)*, mouse *(21)*, and numerous other species. The deduced amino acid (aa) sequence displays high homology among species, equal to or more than 71%. The open reading frame of human cDNA encodes a peptide consisting of 521 aa *(19,22)*. The 39 aa at the amino-terminus includes the N-terminal leader sequence essential for the translocation of the protein to the inner mitochondrial membrane. The removal of this leader sequence yields a protein of 482 aa *(16,19)*. The aa sequence contains a heme-binding region common to the P450 superfamily located close to the carboxyl terminus containing a single cysteine residue *(18)*, and a specific 20 aa region of high homology among species located at the amino-terminus which is proposed to be the P450scc-specific substrate binding region *(23)*.

The structure of the cholesterol-side chain cleavage gene designated as *CYP11A1* has been determined in human *(22)* and rat *(24)*. The gene is at least 20 kb in length, and consists of nine exons containing an unusual exon/intron junctional sequence that begins with GC found in the sixth intron of both the human gene *(22)* and the rat gene *(24)*. The human gene is located on chromosome 15q23-q24 *(19)*, and the mouse gene is found on chromosome 9 at 31 cM *(25)*.

P450c17

Reaction Catalyzed

P450c17 catalyzes two mixed function oxidase reactions, 17α-hydroxylation, and C17–C20 cleavage. Each reaction requires one molecule of NADPH, and one molecule of oxygen, and the microsomal electron transfer protein, cytochrome P450 oxidoreductase *(7)*. The two reactions catalyzed by P450c17 are the 17α-hydroxylation of either the Δ5-C21 steroid, pregnenolone, or the Δ4-C21 steroid, progesterone, followed by cleavage of the C17-20 bond to yield either the C19 steroids, dehydroepiandrosterone (DHEA), or androstenedione, respectively (Fig. 1). In this two

Table 1
Leyding Cell Steroidogenic Enzymes

Gene		*Chromosomal location*		*Protein name (molecular mass)*	*Subcellular location*	*Synonyms*	*Protein Gene ID (accession no)*	
Human	*Mouse*	*Human*	*Mouse*				*Human*	*Mouse*
A. Cytochrome P450								
CYP11A1	*Cyp11a1*	15q23-q24	9 (31 cM)	CYP11A1 (56 kDa)	Mitochondria *(146)*	P450scc, cholesterol side chain cleavage, cholesterol desmolase, cytochrome P450 cholesterol side chain cleavage, cholesterol 20-22 desmolase	1583 (P05108)	13070 (Q9QZ82)
CYP17A1	*Cyp17a1*	10q24.3	19 (46 cM)	CYP17A1 (57 kDA)	Microsomal *(146)*	P450c17, 17α- hydroxylase/ 17,20 lyase, P450 17α-hydroxylase/ c17-20 lyase	281739 (P05093)	13074 (P27786)
CYP19A1	*Cyp19a1*	15q21.1	9 (31 cM)	CYP19A1 (58 kDa)	Microsomal *(57)*	Aromatase, P450arom, cytochrome P450arom, estrogen syntase	1588 (P11511)	13075 (P28649)
B. Hydroxysteroid dehydrogenases								
HSD3B1	*Hsd3b6*	1p13.1	3 (F2.2)	3β-HSDI human 3β-HSD VI mouse (42 kDa)	Microsomal	3β-HSD type-I (type-VI), 3β-HSD/Δ 4–Δ5 isomerase, 3β-hydroxyΔ5-steroid dehydrogenase, 3β-hydroxy-5-ene steroid dehydrogenase	3283 (P14060)	15497 (O35469)
HSD3B2	*Hsd3b1*	1p13.1	3 (49.8 cM)	3β-HSDII human 3β-HSDI mouse (42 kDa)	Mitochondria and microsomal *(146)*	*See* previous entry	3284 (P26439)	15492 (P24815)
HSD17B3	Hsd17b3	9q22	13	17β-HSD3 (34.5 kDa)	Microsomal (147)	17β-HSD 3, testicular 17β-HSD	3293 (P37058)	15487 (P70385)
C. Steroid 5α-reductases								

SRDA1	*Srda1*	5p15	13 (39 cM)	SRDA1 (29.5 kDa) human (24.9 kDa) mouse	Microsomal *(148)*	3-oxo-5α-reductase, 5α-reductase	6715 (P18405)	24950
SRDA 2	*Srda1*	2p23	17 (E2)	SRDA2 (28.4 kDa) human (24.9 kDa) mouse	Microsomal *(148)*	*See* previous entry	6716 (P31213)	94224 (Q99N99)

Swiss-Prot http://us.espasy.org/cgi-bin/sprot-search.ac.

step reaction, 17α-hydroxypregnenolone or 17α-hydroxyprogesterone is synthesized as an intermediate. Initially, it was believed that each reaction was catalyzed by a distinct enzyme, but studies by Hall and colleagues *(26,27)* demonstrated that a single purified protein catalyzed both 17α-hydroxylation and C17–C20 cleavage (lyase) activity. Subsequent cloning of a bovine P450c17 cDNA and expression of this cDNA confirmed that both reactions were catalyzed by the same protein *(28)*. Although, the P450c17 enzyme from various species catalyzes both the hydroxylation and the lyase reaction, there are marked species-dependent differences in the utilization of either 17α-hydroxypregnenolone (Δ5) or 17α-hydroxyprogesterone (Δ4) as substrate for the lyase reaction. The human and bovine enzymes use 17α-hydroxypregnenolone as the preferential substrate yielding DHEA as the product, whereas the rodent enzyme utilizes 17α-hydroxyprogesterone as the substrate yielding androstenedione as the product *(29)*. Auchus and colleagues provided conclusive evidence that human testes synthesize testosterone predominantly by the Δ5 pathway *(30)*. These species-dependent differences in substrate preference for the C17–C20 lyase activity are not related to differences in the aa sequence of the bovine and human enzyme, compared with the rodent enzyme. However, the differences are a property of the human and bovine enzyme required for high molar concentration of cytochrome P450 reductase *(31,32)*, serine/threonine phosphorylation of P450c17 *(33,34)*, and the accessory protein cytochrome b_5 in promoting lyase activity of 17α-hydroxypregnenolone, but not of 17α-hydroxyprogesterone *(29,35)*.

Additional activities to that of the classic 17α-hydroxylation/C17–C20 cleavage have been observed. Swart et al. *(36)* reported that human P450c17 also expresses 16α-hydroxylase activity at the same site as 17α-hydroxylase activity. More recent study by Liu et al. *(37)* using a P450c17 deficient MA10 mouse tumor Leydig cell culture indicated that P450c17 expresses a secondary activity, squalene monooxygenase (epoxidase) activity, suggesting that this enzyme may also be involved in cholesterol biosynthesis as proposed several years earlier by Lieberman and Warne *(38)*.

Molecular Structure

Genomic Southern blotting and/or cloning has established that in mouse *(39)*, rat *(40)*, and human *(41,42)* as well as in other species there is a single gene designated as *CYP17A1* in human and *Cyp17a1* in mouse. The *CYP17A1* gene is approx 6 kb in length and contains eight exons with the location of intron–exon boundaries conserved among species. The 5′ upstream region of the human, bovine, porcine, rat, and mouse gene share a high homology over the first 550 bp including the same nonconsensus TATA box *(41–46)*. The human *CYP17A1* gene has been mapped to chromosome 10q24.3 *(47,48)* and the mouse *Cyp17a1* gene to chromosome 19 at 46 cM *(39)*. The human P450c17 protein contains 508 aa *(49)* compared with 507 aa in the mouse *(39)* and rat *(40)* proteins. The molecular mass of the P450c17 protein is approx 57 kDa. Comparison of the mouse aa sequence to rat and human sequences indicates that they are 83 and 66% identical, respectively. The P450c17 protein of different species contains regions of high homology common to members of the P450 gene family *(39)*. These are the putative binding regions for mouse aa 434–454 *(39)*, human aa 435–455 *(50)*, and the ozols tridecapeptide sequence (343–372 aa) *(51)* that may play a role in substrate specificity *(52)*. In addition, there is a region that is specifically conserved among different species of P450c17 (296–319) that may function in catalysis *(53)*. Arginine346 in the rat enzyme *(54,55)* and arginine347 in the human enzyme were found to be critical for catalyzing lyase activity.

P450arom

Reaction Catalyzed

P450arom catalyzes the conversion of the C19 androgens, androstenedione, and testosterone, to the C18 estrogens, estrone, and estradiol, respectively. The reaction requires three molecules of oxygen and three molecules of NADPH using the microsomal electron transfer system. The first two oxygen molecules are required for the oxidation of the C19 methyl group by standard hydroxylation reactions, whereas the third oxygen molecule is required for a reaction proposed to be a peroxidative attack on the C19 methyl group combined with elimination of the 1β hydrogen to yield a phenolic A ring and formic acid *(7)*.

Molecular Structure

P450arom (*CYP19A1*) is the product of a single gene in human *(56,57)*, mouse *(58)*, and rat *(59)*. The human gene has been mapped to 15q21.1 *(60)*, and the mouse gene is located on chromosome 9 at 31 cM *(25)*. The human gene contains 10 exons, nine of which includes the coding region spanning approx 30 kb *(56)*. Upstream of exon II are several alternative exon 1s that are spliced into the 5′ untranslated region, which determines the tissue-specific expression of the protein *(56)*. The proximal promoter II determines testicular and ovarian expression of P450arom, and the transcript originates immediately upstream of the

translational start site, approx 26 bp downstream of the putative TATA sequence *(57,61,62)*. Although, the transcripts have different termini in the different tissues, the coding region of the expressed protein is identical.

The deduced aa sequence of human P450arom in comparison to the rat and mouse protein exhibits 81% homology *(56)*. Both the human and mouse protein consist of 503 aa with a molecular mass of 58 kDa *(56)*. The P450arom proteins from different species contain the same structural features described for the other cytochrome P450 enzymes: the heme-binding region containing a conserved cysteine residue that serves as the fifth coordinating ligand of the heme iron, and the substrate binding site in the amino-terminal I-helix region.

HYDROXYSTEROID DEHYDROGENASES

The hydroxysteroid dehydrogenases, 3β-HSD and 17β-HSD, involved in the biosynthesis of testosterone from cholesterol, belong to the same phylogenetic protein family, namely the short-chain alcohol dehydrogenase reductase superfamily. In general, they are involved in the reduction and oxidation of steroid hormones, requiring $NAD^+/NADP^+$ as acceptors and their reduced forms as donors of reducing equivalents. One of the major differences between the P450 enzymes and the hydroxysteroid dehydrogenases is that each of the P450 enzymes is a product of a single gene, whereas there are several isoforms for 3β-HSDs and several isozymes of the 17β-HSDs, each a product of a distinct gene. The number of isoforms or isozymes varies in different species, in tissue distribution, catalytic activity (whether they function predominantly as dehydrogenases or reductases), substrate and cofactor specificity, and subcellular distribution. The 3β-HSD isoform expressed in Leydig cells is 3β-HSD II in human *(63)*, 3β-HSD I *(64)* and VI *(65)* in mouse, and 3β-HSD I in rat *(66)* The 17β-HSD3 is the 17β-HSD isoform exclusively expressed in both human *(67)* and rodent adult Leydig cells *(68,69)*.

3β-Hydroxysteroid Dehydrogenase

Reaction Catalyzed

The 3β-HSD isoforms expressed in Leydig cells catalyze the conversion of the Δ5-3β-hydroxysteroids, pregnenolone, 17α-hydroxypregnenolone, and DHEA, to the Δ4-3-ketosteroids, progesterone, 17α-hydroxyprogesterone, and androstenedione, respectively. Two sequential reactions are involved in the conversion of the Δ5-3β-hydroxysteroid to a Δ4-3-ketosteroid. The first reaction is the dehydrogenation of the 3β-equatorial hydroxysteroid, requiring the coenzyme NAD^+, yielding the Δ5-3-keto intermediate, and reduced NADH. The reduced NADH, activates the isomerization of the Δ5-3-keto steroid to yield the Δ4-3-ketosteroid *(7,70,71)*. This reaction is catalyzed by a single dimeric protein without the release of the intermediate or coenzyme *(71)*. Four isoforms in rat have been identified *(7,72)*. Each of these isoforms is the product of a distinct gene. Human *HSD3B* genes are found on chromosome 1p31.1 *(73)* and the *Hsd3b* mouse genes are located in a cluster on mouse chromosome 3 close to the centromeric region that shows conservation of gene order and physical distance with the centromeric region of human chromosome 1 *(74,75)*. All of the *HSD3B* genes consist of four exons, with the start site of translation found in exon 2 *(75)*. The two human genes are approx 7.8 kb, and their nucleotide sequences are highly homologous including their intronic sequences and the 1250 bp sequence of the 5′ flanking region that exhibits 81.9% identity *(72)*. The size of the mouse genes varies as result of differences in the size of their introns *(75,76)*. Intron 1 of the mouse *Hsd3b6* gene was found to be 3.1 kb *(76)* compared with 126, 125, and 132 bp found in mouse *Hsd3b1* and human *HSD3B1* and *HSD3B2 (75)*. The open reading frames of mouse I and mouse VI 3β-HSD, the isoforms expressed in Leydig cells, encode a protein including the initiator methionine of 373 aa *(65)*, whereas human II 3β-HSD encodes a protein of 372 aa *(72)*. The aa sequences among the different isoforms and between mouse and human isoforms show a high degree of identity. Mouse 3β-HSD I is 84% identical to mouse VI, and 71% identical to human II *(65,72)*. Although the aa sequence predicts a molecular mass of 42 kDa for all of the 3β-HSD proteins, when subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the mobilities of mouse 3β-HSD I and VI are distinct, exhibiting an apparent molecular mass of 42 and 44 kDa, respectively *(65)*. The cofactor binding site is located in the amino-terminal sequence. Investigations using homology modeling of human 3β-HSD I demonstrated that Asp36 is responsible for the NAD(H) binding site *(71)*. In earlier studies investigating the difference in the aa sequence of mouse 3β-HSD I, which requires NAD^+ as a cofactor, and mouse 3β-HSD IV and V, which require $NADP^+$ as cofactor, it was found that Asp36 was essential for NAD^+-mediated dehydrogenation/isomerization, and replacement of Asp36 with phenylalanine at position 36 changed the cofactor specificity to $NADP^+$. *(77,78)* The dehydrogenase activity has been localized to the Y154-P-H-S-K158 domain and the isomerase site to Tyr269 and Lys273 of the human 3β-HSD protein *(79)*.

17-Hydroxysteroid

Till date, 11 distinct 17β-HSDs have been identified. Unlike the 3β-HSDs described earlier, there is little homology among the different 17β-HSD isozymes *(7)*. But unlike the 3β-HSDs, the orthologs among the different species are assigned the same number. Thus, the isozyme expressed in Leydig cells is 17β-HSD 3 in human, rat, and mouse.

Reaction Catalyzed

The 17β-HSD catalyzes the last step in the biosynthesis of testosterone. It converts androstenedione, a weak androgen, to testosterone, a potent androgen. 17β-HSD 3 prefers NADPH as a cofactor, and its primary activity is reductive.

Molecular Structure

The human gene *HSD17B3* maps to chromosome 9q22 *(67)* and the mouse gene *Hsd17b3* is located on chromosome 13 *(68)*. The human gene is 60 kb in length and contains 11 exons *(67)*. It encodes a protein of 310 aa with a molecular mass of 34.5 kDa and no apparent membrane spanning region *(67)*. The mouse gene encodes a protein of 305 aa, five fewer than the human with a molecular mass of 33.7 kDa *(68)*. The mouse protein is missing four of the aa at the amino-terminus and Val245 of the human sequence; the aa identity between the mouse and the human protein is 72.5%, and similarity is 94.8% *(68)*. Site-directed mutational analysis of human 17β-HSD 3 demonstrated that arginine at position 80 was critical for binding of the cofactor NADPH *(80)*.

STEROID 5α-REDUCTASE

Reaction Catalyzed

Steroid 5α-reductase (3-oxo-5α-steroid reductase) catalyzes the irreversible conversion of a 3-keto Δ4–Δ5 structure to the corresponding 5α-reduced metabolite, e.g., testosterone to 5α-dihydrotestosterone (Fig. 1) or progesterone to 5α-dihydroprogesterone. NADPH is the donor of the electrons. Conversion of testosterone to dihydrotestosterone is predominant in androgen target tissues, although, it is also present in the testis. In several species, including rat *(81,82)* and mouse *(83,84)*, there is a peak of 5α-reductase activity in Leydig cells during pubertal development. The major androgen produced in mouse Leydig cells during pubertal development is 5α-androstanediol reaching a maximal production between 25 and 30 d postnatal *(83)*.

Molecular Structure

Two isoforms of steroid 5α-reductase, each a product of a distinct gene, have been identified in human *(85)*, rat *(85)*, and mouse *(86)*. Both the human, and most likely the mouse and rat genes, contain five exons with the positions of the introns being essentially identical in the two isoforms *(85)*. The two genes, human *SDR5A1* and *SDR5A2*, are located on distinct chromosomes. *SDR5A1* maps to the distal arm of chromosome 5p15 *(87)*, whereas *SDR5A2* is located on chromosome 2p23 *(88)*, the mouse *Sdr5a1* maps to chromosome 13 39.0 cM and mouse *Sdr5a2* to 17E2.

The steroid 5α-reductases are hydrophobic intrinsic membrane-bound proteins: the human 5α isozyme type-1 consists of 259 aa whereas the type-2 consists of 254 aa with a molecular mass of 29.5 kDa and 28.4 kDa, respectively (Table 1C; *89*). The respective mouse 5α-reductases 1 and 2 consist of 217 and 254 aa, and a molecular mass of 24.9 kDA and 28.6 kDa, respectively (Table 1C; ref. *90*). The enzymes show aberrant electrophoretic mobilities in sodium dodecyl sulfate polyacrylamide gels. The human isozymes migrate with molecular weights of 21–27 kDa instead of the predicted 28 and 29 kDa *(85)*.

REGULATION OF EXPRESSION OF STEROIDOGENIC ENZYMES IN LEYDIG CELLS

P450 Enzymes

A major nuclear factor that is essential for cell-specific expression for P450 steroidogenic enzymes was identified by two laboratories in 1992. This nuclear DNA-binding protein, referred to as SF-1 by Lala et al. *(91)* or Ad4BP by Morohashi et al. *(92)* belongs to the orphan nuclear receptor family and binds to variants of an AGGTCA sequence motif found in the proximal promoter of all P450 steroidogenic enzymes *(93,94)*. Although SF-1 is essential for cell-specific gonadal expression, other factors are necessary for determining maximal as well as cell-specific expression of these enzymes.

Chronic stimulation of Leydig cells by the pituitary hormone LH is required for the maintenance of optimal expression of the enzymes. LH, acting via G protein-coupled receptors, activates adenylate cyclase thereby, increasing cAMP, which in turn, leads to increased synthesis of P450 steroidogenic enzymes. The regulation of LH stimulation via cAMP is not mediated by the cAMP response element (CRE/CRE-binding protein [CREB]) system with the exception of *CYP19A1*. It has been reported that cAMP acts via CRE/CREB in the rat *Cyp19a1* promoter expressed in rat granulosa cells and in R2C Leydig cells *(95)* and in the PII human *CYP19A1* promoter expressed in human granulosa cells

(96). cAMP-responsive sequences found in the promoters of *CYP11A1* and *CYP17A1* differ among these *CYP* genes and among the same genes of different species *(45,97)*. Although, hormone-stimulated increases in cAMP enhance the expression of all of steroidogenic P450 enzymes, additional factors are involved in maintaining maximal expression. Recent studies have provided evidence for a role of GATA-4 and GATA-6 as phosphorylated intermediates in cAMP-stimulated expression of P450scc, P450c17, P450arom, and 3β-HSD *(98–100)*.

In vitro studies using isolated Leydig cells in culture have contributed to our understanding of the regulation of steroidogenic enzymes. Early studies reported that treatment of immature porcine Leydig cells in culture with hCG increased the *de novo* synthesis of P450scc and adrenodoxin *(101)*. Similar studies using rat Leydig cells in culture demonstrated that treatment with hCG or cAMP increased the synthesis of P450scc and adrenodoxin *(102)*.

Studies by this author and colleagues using primary culture of mouse Leydig cells demonstrated that LH or cAMP are essential for the expression of P450c17 enzyme activity *(103,104)*, *de novo* synthesis of P450c17 protein *(105,106)*, and the expression of P450c17 mRNA *(107)*. The synthesis of P450c17 ceases in the absence of cAMP *(105)*. In a subsequent study, Youngblood and Payne identified the cAMP-responsive region between -346 and -245 bp upstream of the start site of transcription of the *Cyp17a1* promoter *(45)*. The essential role of cAMP for the expression of P450c17 in other species has been reviewed by Waterman and Keeney *(108)*. In contrast to the absolute requirement for cAMP for the expression of P450c17 in mouse Leydig cells, expression of P450scc and 3β-HSD are not dependent on cAMP *(105)*. Although LH, hCG, or cAMP may not be critical for expression of P450scc in Leydig cells in culture, treatment of mouse MA-10 Leydig tumor cells with cAMP *(106,109)* or forskolin *(110)* increases the amount of P450scc protein and mRNA.

In the studies on the requirement of cAMP for the expression of P450c17, evidence was obtained that testosterone produced during LH or cAMP stimulation repressed cAMP induction of P450c17 activity *(104)*, *de novo* synthesis *(106)*, and the amount of mRNA *(107)*. This negative effect of testosterone could be mimicked by the androgen agonist mibolerone *(107)* and prevented by the androgen antagonist hydroxyflutamide *(106)* indicating that androgen-mediated repression of P450c17 expression was mediated by the androgen receptor. A subsequent study demonstrated that androgen-mediated repression involved the binding of the androgen receptor to sequences within the cAMP-responsive region of the *Cyp17a1* promoter *(111)*.

Glucocorticoids have been implicated in the regulation of testicular steroidogenesis. Increased production of glucocorticoids in pathological conditions of the adrenal cortex, such as Cushing's syndrome, can be associated with decreased circulating testosterone and reproductive dysfunction *(112)*. Studies by Hales and Payne *(109)* and Payne and Sha *(107)* demonstrated that the glucocorticoids, cortisol, corticosterone, or the synthetic glucocorticoid, dexamethsone, repress both basal and cAMP-induced synthesis of P450scc protein and mRNA. The glucocorticoid-mediated decrease in P450scc synthesis was prevented by the antiglucocorticoid, RU486, suggesting that glucocorticoid repression of P450 synthesis is mediated by the glucocorticoid receptor found in Leydig cells *(113)*.

For regulation of P450c19 in Leydig cells, *see* Chapter 19.

Hydroxysteroid Dehydrogenases

3β-HSD

Gonadal expression of human 3β-HSD II and mouse 3β-HSD I is dependent on SF-1 as described for the gonadal-specific expression of the P450 steroidogenic enzymes *(114,115)*. A study on the mouse *Hsd3b1* promoter identified three potential SF-1 consensus binding sites in the proximal promoter of the gene *(75)*. In a subsequent study, it was shown that SF-1, also, was required for the expression of mouse 3β-HSD I protein *(76)*.

Studies involving the regulation of 3β-HSD mRNA in mouse Leydig cells in culture, demonstrated high constitutive expression of 3β-HSD *(107)*. In a subsequent study, it was found that mouse Leydig cells express two distinct isoforms of 3β-HSD, 3β-HSD I, and VI *(65)*. Investigations using gonadotropin-deficient mice to study the role of LH/hCG in regulating the expression of 3β-HSD I and VI mRNA in the adult Leydig cell lineage revealed that the expression of 3β-HSD I is independent of LH stimulation *(116)*. In contrast, the expression of 3β-HSD VI mRNA is highly dependent on LH/hCG stimulation. GATA factors appear to be important in the expression of human 3β-HSD II in steroidogenic cells *(100)*. In a recent study, Martin et al. identified a proximal element in the *HSD3B2* promoter that interacts with GATA 4 and 6 which physically interact with SF-1 or LHR-1 to determine cell-specific and maximal expression of *HSD3B2* in Leydig cells *(117)*. In addition, Martin and Tremblay *(118)* identified a response element located at -130 bp specific for another orphan nuclear receptor, Nur 77, which was found to be important for both basal- and

hormone-induced human *HSD3B2* promoter activity Nur 77 expression is induced in vivo by LH/hCG in the testis and appears to be an important mediator in the action of LH on steroidogenesis *(119)*.

17βHSD. Studies on the regulation of 17βHSD 3 are limited. Baker et al. *(120)* examined the expression of 17β-HSD 3 mRNA during development in normal mice, and mice lacking circulating gonadotropins (*hpg*), or functional androgen receptors (*Tmf*). The results showed that during neonatal development expression of 17β-HSD 3 mRNA is independent of gonadotropin action, but becomes dependent on androgen action at the time of puberty *(120)*.

5α-Steroid Reductase

During puberty in rat *(82)* and mouse *(83,84)* Leydig cells, 5α-steroid reductase is highly expressed resulting in 5α-androstanediol being the major androgen produced. Hypophysectomy of 21-d-old rats elicited a marked decrease in 5α-reductase activity *(121)*. Treatment of the rats with LH, initiated 6 d posthypophysectomy, resulted in a sharp increase in 5α-reductase activity. Treatment with FSH did not increase or prevent the decrease in 5α-reductase activity following hypophysectomy. Similarly, LH, but not FSH, increased 5α-reductase activity in *hpg* mice *(122,123)*.

Additional studies provided evidence that prolactin (PRL) is involved in the maintenance of high 5α-reductase activity in testes of immature mice *(124,125)*. PRL treatment of mice had no effect on 5α-reductase activity, but enhanced the LH-induced increase in activity *(125)*. Murono and Washburn tested several hormones in 25-d-old rat Leydig cells in culture and found only hCG increased 5α-reductase activity *(126)*. Additional studies by Murono et al. showed that the acidic fibroblast growth factor, as well as the platelet-derived growth factor repressed basal and hCG-stimulated 5α-reductase activity in cultured immature rat Leydig cells *(127)*. Basic fibroblast growth factor repressed hCG-stimulated 5α-reductase activity, but had no effect on basal activity *(128)*. In a subsequent study, Viger and Robaire examined the type and developmental expression of 5α-reductase mRNA and protein in the testis of rat *(129)*. At all ages examined, they identified type-1 5α-reductase in Leydig cells. Type-1 5α-reductase mRNA was found to the be most abundant in the immature rat between 21 and 28 d of age. Immunohistochemical staining with a specific antiserum to the type-1 enzyme localized the type-1 protein in the cytoplasm of Leydig cells with the highest expression between 21 and 28 d, followed by a progressive decrease closely paralleling the enzyme activity reported earlier by other investigators.

CLINICAL FEATURES OF MUTATIONS IN STEROIDOGENIC ENZYMES

P450 Enzymes

CYP11A1. Mutations, homozygous in the *CYP11A1* gene in human, are lethal *(130)*. Such mutations result in the inability of the placenta to produce progesterone, which is essential for maintenance of pregnancy beginning at 6–7 wk gestation when production of progesterone by the corpus luteum wanes.

CYP17A1. Numerous reports have been published describing patients with mutations in the *CYP17A1* gene. Mutations have been identified that cause either complete, partial, or isolated 17,20 lyase activity *(131–137)*. The patients exhibit a range of phenotypes depending on the mutation. Both male and female patients are hypertensive because of the overproduction of mineralocorticoids as well as impaired production of cortisol. Affected females exhibit abnormal sexual development resulting in primary amenorrhea. Male patients are phenotypic females due to the deficiency of testosterone production.

CYP19A1. To date five male patients with mutations in *CYP19A1* have been reported *(138–143)*. Defects observed in these patients are not because of the absence of aromatase activity in Leydig cells but, the absence of conversion of testosterone to estradiol in peripheral tissues. Male patients developed very high stature in their late twenties owing to the failure of epiphyseal fusion. Furthermore, they exhibited severely delayed bone age resulting in ostopenia and undermineralization. In addition, these patients experienced marked metabolic defects in carbohydrate and lipid metabolism.

Hydroxysteroid Dehydrogenases

HSD3B. Homozygous mutations in *HSD3B1* are lethal in human because the 3β-HSD I protein is required for progesterone synthesis in the placenta as described above for *CYP11A*. Many mutations in the *HSD3B2* gene have been identified and are summarized in a review by Simard et al. *(72)*. The major defect observed in males with mutations in the *HSD3B2* gene is either perineal hypospadias or perineoscrotal hypospadias and ambiguous external male genitalia or microphallus. These features in the male are because of decreased biosynthesis of testosterone that is required for normal development of external male genitalia *(72)*.

HSD17B3. Autosomal-recessive mutations in *HSD17B3* have been identified and characterized in numerous male patients *(144)*. The product of the *HSD17B3* gene is essential for Leydig cell biosynthesis

of testosterone during fetal development. Mutations result in phenotypic males with female external genitalia. These males have abdominal testes, epididymides, vas deferentia, seminal vesicles, and ejaculatory ducts. Plasma testosterone concentrations rise at the time of puberty resulting in many of these individuals undergoing marked virilization. The explanation for this phenomenon is most likely result of the peripheral conversion of circulating androstenedione by one of the other 17β-HSD isoforms *(145).*

5α-Reductases

In males, SDR5A2 is expressed in external genitalia. Thus, mutations in *SDR5A2* result in various degrees of male pseudohermaphroditism with undermasculinized external genitalia (*see* Chapter 12).

ACKNOWLEDGMENTS

I am very grateful to Dr. Dale B. Hales, University of Illinois, Chicago for the figure illustrating the steroidogeneic pathway in Leydig cells. I wish to thank Caren Spencer for editorial assistance.

REFERENCES

1. Normington K, Russell DW. Tissue distribution and kinetic characteristics of rat steroid 5 α-reductase isozymes. Evidence for distinct physiological functions. J Biol Chem 1992;267:19,548–19,554.
2. Payne AH, Kelch RP, Musich SS, Halpern ME. Intratesticular site of aromatization in the human. J Clin Endocrinol Metab 1976;42:1081–1087.
3. Valladares LE, Payne AH. Acute stimulation of aromatization in Leydig cells by human chorionic gonadotropin in vitro. Proc Natl Acad Sci USA 1979;76:4460–4463.
4. George F, Wilson J. Sex-determination and differentiation. In: Knobil E, Neil D, eds. The Physiology of Reproduction. New York: Raven Press, 1994;3–28.
5. Holdcraft RW, Braun RE. Hormonal regulation of spermatogenesis. Int J Androl 2004;27:335–342.
6. Stocco DM. Tracking the role of a star in the sky of the new millennium. Mol Endocrinol 2001;15:1245–1254.
7. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev 2004;25:947–970.
8. Nelson DR, Koymans L, Kamataki T, et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 1996;6:1–42.
9. Boyd G, Simpson ER. Studies on the conversion of cholesterol to pregnenolone to bovine adrenal mitochondria. In: KW M, ed. Functions of the Adrenal Cortex. Vol. 1. New York: Appleton-Century-Crofts, 1968;49–76.
10. Burstein S, Gut M. Intermediates in the conversion of cholesterol to pregnenolone: kinetics and mechanisms. Steroids 1976;28:115–131.
11. Schulster D, Burstein S, Cooke B. Biosynthesis of steroid hormones. In: Shulster D, Burstein S, Cooke B, eds. Molecular endocrinology of the steroid hormones. New York, Wiley and Sons, 1976;44–77.
12. Shikita M, Hall PF. Cytochrome P-450 from bovine adrenocortical mitochondria: an enzyme for the side chain cleavage of cholesterol. I. Purification and properties. J Biol Chem 1973;248:5598–5604.
13. Watanuki M, Granger GA, Hall PF. Cytochrome P-450 from bovine adrenocortical mitochondria. Immunochemical properties and purity. J Biol Chem 1978;253:2927–2931.
14. Simpson ER. Cholesterol side-chain cleavage, cytochrome P450, and the control of steroidogenesis. Mol Cell Endocrinol 1979;13:213–227.
15. Waterman MR, Simpson ER. Regulation of Steroid hydroxylase gene expression is multifactorial in nature. Recent Prog Horm Res 1989;45:533–566.
16. Black SM, Harikrishna JA, Szklarz GD, Miller WL. The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450scc. Proceedings of the National Acadamy of Science USA 1994;91:7247–7251.
17. Vickery LE. Molecular recognition and electron transfer in mitochondrial steroid hydroxylase systems. Steroids 1997; 62:124–127.
18. Morohashi K, Fujii-Kuriyama Y, Okada Y, et al. Molecular cloning and nucleotide sequence of cDNA for mRNA of mitochondrial cytochrome P-450scc of bovine adrenal cortex. Proceedings of the National Academy of Science USA 1984;81:4647–4651.
19. Chung BC, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL. 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 1986;83:8962–8966.
20. Oonk RB, Krasnow JS, Beattie WG, Richards JS. Cyclic AMP-dependent and -independent regulation of cholesterol side chain cleavage cytochrome P-450 (P-450scc) in rat ovarian granulosa cells and corpora lutea. J Biol Chem 1989; 264:21,934–21,942.
21. Arensburg J, Payne AH, Orly J. Expression of steroidogenic genes in maternal and extraembryonic cells during early pregnancy in mice. Endocrinology 1999;140:5220–5232.
22. Morohashi K, Sogawa K, Omura T, Fujii-Kuriyama Y. Gene structure of human cytochrome P-450(SCC), cholesterol desmolase. J Biochem (Tokyo) 1987;101:879–887.
23. Okuyama E, Okazaki T, Furukawa A, Wu RF, Ichikawa Y. Molecular cloning and nucleotide sequences of cDNA clones of sheep and goat adrenocortical cytochromes P450scc (CYP11A1). J Steroid Biochem Mol Biol 1996;57: 179–185.
24. Oonk RB, Parker KL, Gibson JL, Richards JS. Rat cholesterol side-chain cleavage cytochrome P-450 ($P\text{-}450_{scc}$) gene. Structure and regulation by cAMP in vitro. J Biol Chem 1990;265:22,392–22,401.
25. Youngblood GL, Nesbitt MN, Payne AH. The structural genes encoding P450scc and P450arom are closely linked on mouse chromosome 9. Endocrinology 1989;125:2784–2786.
26. Nakajin S, Hall PF. Microsomal cytochrome P-450 from neonatal pig testis. J Biol Chem 1981;256:3871–3876.
27. Nakajin S, Shively JE, Yuan PM, Hall PF. Microsomal cytochrome P-450 from neonatal pig testis: two enzymatic activities (17 α-hydroxylase and c17,20-lyase) associated with one protein. Biochemistry 1981;20:4037–4042.
28. Zuber MX, Simpson ER, Waterman MR. Expression of bovine 17α-hydroxylase cytochrome P-450 cDNA in nonsteroidogenic (COS1) cells. Science 1986;234:1258–1261.

29. Brock BJ, Waterman MR. Biochemical differences between rat and human cytochrome P450c17 support the different steroidogenic needs of these two species. Biochemistry 1999;38:1598–1606.
30. Fluck CE, Miller WL, Auchus RJ. The 17, 20-lyase activity of cytochrome p450c17 from human fetal testis favors the delta5 steroidogenic pathway. J Clin Endocrinol Metab 2003;88:3762–3766.
31. Yanagibashi K, Hall PF. Role of electron transport in the regulation of the lyase activity of C21 side-chain cleavage P-450 from porcine adrenal and testicular microsomes. J Biol Chem 1986;261:8429–8433.
32. Lin D, Black SM, Nagahama Y, Miller WL. Steroid 17α-hydroxylase and 17,20-lyase activities of P450c17: contributions of serine^{106} and P450 reductase. Endocrinology 1993; 132:2498–2506.
33. Zhang LH, Rodriguez H, Ohno S, Miller WL. Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. Proc Natl Acad Sci USA 1995;92:10,619–10,623.
34. Pandey AV, Miller WL. Regulation of 17,20 lyase activity by cytochrome b5 and by serine phosphorylation of P450c17. J Biol Chem 2005;280:13,265–13,271.
35. Auchus RJ, Lee TC, Miller WL. Cytochrome b_5 augments the 17,20-lyase activity of humn P450c17 without direct electron transfer. J Biol Chem 1998;273:3158–3165.
36. Swart P, Swart AC, Waterman MR, Estabrook RW, Mason JI. Progesterone 16α-hydroxylase activity is catalyzed by human cytochrome P450 17α-hydroxylase. J Clin Endocrinol Metab 1993;77:98–102.
37. Liu Y, Yao ZX, Papadopoulos V. Cytochrome P450 17α-hydroxylase/17,20 lyase (CYP17) function in cholesterol biosynthesis: identification of squalene monooxygenase (epoxidase) activity associated with CYP17 in Leydig cells. Mol Endocrinol 2005;19:1918–1931.
38. Lieberman S, Warne PA. 17-Hydroxylase: an evaluation of the present view of its catalytic role in steroidogenesis. J Steroid Biochem Mol Biol 2001;78:299–312.
39. Youngblood GL, Sartorius C, Taylor BA, Payne AH. Isolation, characterization, and chromosomal mapping of mouse P450 17α-hydroxylase/$C_{17\text{-}20}$ lyase. Genomics 1991;10:270–275.
40. Fevold HR, Lorence MC, McCarthy JL, et al. Rat P450(17α) from testis: characterization of a full-length cDNA encoding a unique steroid hydroxylase capable of catalyzing both delta 4- and delta 5-steroid-17,20-lyase reactions. Mol Endocrinol 1989;3:968–975.
41. Picado-Leonard J, Miller WL. Cloning and sequence of the human gene for P450c17 (steroid 17 α-hydroxylase/17,20 lyase): similarity with the gene for P450c21. DNA 1987; 6:439–448.
42. Kagimoto K, Waterman MR, Kagimoto M, Ferreira P, Simpson ER, Winter JS. Identification of a common molecular basis for combined 17 α-hydroxylase/17,20-lyase deficiency in two Mennonite families. Hum Genet 1989; 82:285–286.
43. Bhasker CR, Adler BS, Dee A, et al. Structural characterization of the bovine CYP17 (17 α-hydroxylase) gene. Arch Biochem Biophys 1989;271:479–487.
44. Zhang P, Nason TF, Han XG, Hall PF. Gene for 17 α-hydroxylase/C (17-20) lyase P-450: complete nucleotide sequence of the porcine gene and 5′ upstream sequence of the rat gene. Biochim Biophys Acta 1992;1131:345–348.
45. Youngblood GL, Payne AH. Isolation and characterization of the mouse P450 17 α-hydroxylase/C17-20-lyase gene (Cyp17): transcriptional regulation of the gene by cyclic adenosine 3′,5′-monophosphate in MA-10 Leydig cells. Mol Endocrinol 1992;6:927–934.
46. Fevold HR, Ivanovitch JD, Zanger UM, Waterman MR. The sequence of the 5′-end of the rat CYP17 gene, the transcription initiation site and a comparison with the homologous genes of other species. Mol Cellular Endocrinol 1993; 95:95–100.
47. Matteson KJ, Picado-Leonard J, Chung BC, Mohandas TK, Miller WL. Assignment of the gene for adrenal P450c17 (steroid 17 α-hydroxylase/17,20 lyase) to human chromosome 10. J Clin Endocrinol Metab 1986;63:789–791.
48. Fan YS, Sasi R, Lee C, Winter JS, Waterman MR, Lin CC. Localization of the human CYP17 gene (cytochrome P450 17α) to 10q24.3 by fluorescence in situ hybridization and simultaneous chromosome banding. Genomics 1992;14:1110–1111.
49. Chung BC, Picado-Leonard J, Haniu M, et al. Cytochrome P450c17 (steroid 17 α-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 1987; 84:407–411.
50. Yanase T, Simpson ER, Waterman MR. 17 α-hydroxylase/17,20-lyase deficiency: from clinical investigation to molecular definition. Endocr Rev 1991;12:91–108.
51. Ozols J, Heinemann FS. Amino acid sequence of an analogous peptide from two forms of cytochrome P-450. J Biol Chem 1981;256:11,405–11,408.
52. Zuber MX, John ME, Okamura T, Simpson ER, Waterman MR. Bovine adrenocortical cytochrome P-450(17α). Regulation of gene expression by ACTH and elucidation of primary sequence. J Biol Chem 1986;261:2475–2482.
53. Ono H, Iwasaki M, Sakamoto N, Mizuno S. cDNA cloning and sequence analysis of a chicken gene expressed during the gonadal development and homologous to mammalian cytochrome P-450c17. Gene 1988;66:77–85.
54. Kitamura M, Buczko E, Dufau ML. Dissociation of hydroxylase and lyase activities by site-directed mutagenesis of the rat P45017α. Mol Endocrinol 1991;5:1373–1380.
55. Lin D, Zhang L-h, Chiao E, Miller WL. Modeling and Mutagenesis of the active site of human P450c17. Mol Endocrinol 1994;8:392–402.
56. Simpson ER, Mahendroo MS, Means GD, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocrine Rev 1994;15:342–355.
57. Simpson ER, Clyne C, Rubin G, et al. Aromatase—a brief overview. Annu Rev Physiol 2002;64:93–127.
58. Terashima M, Toda K, Kawamoto T, et al. Isolation of a full-length cDNA encoding mouse aromatase P450. Arch Biochem Biophys 1991;285:231–237.
59. Hickey GJ, Krasnow JS, Beattie WG, Richards JS. Aromatase cytochrome P450 in rat ovarian granulosa cells before and after luteinization: adenosine 3′,5′-monophosphate-dependent and independent regulation. Cloning and sequencing of rat aromatase cDNA and 5′ genomic DNA. Mol Endocrinol 1990;4:3–12.
60. Chen SA, Besman MJ, Sparkes RS, et al. Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. DNA 1988;7:27–38.
61. Jenkins C, Michael D, Mahendroo M, Simpson E. Exon-specific northern analysis and rapid amplification of cDNA

ends (RACE) reveal that the proximal promoter II (PII) is responsible for aromatase cytochrome P450 (CYP19) expression in human ovary. Mol Cell Endocrinol 1993;97:R1–R6.
62. Bulun SE, Rosenthal IM, Brodie AMH, et al. Use of tissue-specific promoters in the regulation of aromatase cytochrome P450 gene expression in human testicular and ovarian sex cord tumors, as well as in normal fetal and adult gonads. J Clin Endocrinol Metab 1993;77:1616P–1621P.
63. Rheaume E, Lachance Y, Zhao H-F, et al. Structure and expression of a new complementary DNA encoding the almost exclusive 3-hydroxysteroid dehydrogenase/Δ5–Δ4 isomerase in human adrenals and gonads. Mol Endocrinol 1991;5:1147–1157.
64. Bain PA, Yoo M, Clarke TR, Hammond SH, Payne AH. Multiple forms of mouse 3-hydroxysteroid dehydrogenase/Δ5–Δ4 isomerase and differential expression in gonads, adrenal glands, liver and kidneys of both sexes. Proc Natl Acad Sci USA 1991;88:8870–8874.
65. Abbaszade IG, Arensburg J, Park CH, Kasa-Vubu JZ, Orly J, Payne AH. Isolation of a new mouse 3β-hydroxysteroid dehydrogenase isoform, 3-HSD VI, expressed during early pregnancy [published erratum appears in Endocrinology 1998 Jan;139(1):218]. Endocrinology 1997;138:1392–1399.
66. Zhao H-F, Labrie C, Simard J, et al. Characterization of rat 3-ydrhoxysteroid dehydrogenase/Δ5–Δ4 isomerase cDNAs and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. J Biol Chem 1991;266:583–593.
67. Geissler WM, Davis DL, Wu L, et al. Male pseudohermaphroditism caused by mutations of testicular 17-hydroxysteroid dehydrogenase 3. Nat Genet 1994;7:34–39.
68. Sha JA, Dudley K, Rajapaksha WR, O'Shaughnessy PJ. Sequence of mouse 17-hydroxysteroid dehydrogenase type 3 cDNA and tissue distribution of the type 1 and type 3 isoform mRNAs. J Steroid Biochem Mol Biol 1997;60:19–24.
69. Andersson S. Molecular genetics of androgenic 17-hydroxysteroid dehydrogenases. J Steroid Biochem Mol Biol 1995; 55:533–534.
70. Thomas JL, Myers RP, Strickler RC. Human placental 3-hydroxy-5-ene-steroid dehydrogenase and steroid 5–4-ene-isomerase: purification from mitochondria and kinetic profiles, biophysical characterization of the purified mitochondrial and microsomal enzymes. J Steroid Biochem 1989;33: 209–217.
71. Thomas JL, Duax WL, Addlagatta A, Brandt S, Fuller RR, Norris W. Structure/function relationships responsible for coenzyme specificity and the isomerase activity of human type-1 3-hydroxysteroid dehydrogenase/isomerase. J Biol Chem 2003;278:35,483–35,490.
72. Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the 3-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocr Rev 2005;26:525–582.
73. Morissette J, Rheaume E, Leblanc J-F, Luu-The V, Labrie F, Simard J. Genetic linkage mapping of HSD3B1 and HSD3B2 encoding human types I and II 3-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase close to D1S514 and the centromeric D1Z5 locus. Cytogenet Cell Genet 1995;69:59–62.
74. Bain PA, Meisler MH, Taylor BA, Payne AH. The genes encoding gonadal and nongonadal forms of 3-hydroxysteroid dehydrogenase/Δ5–Δ4 isomerase are closely linked on mouse chromosome 3. Genomics 1993;16:219–223.
75. Clarke TR, Bain PA, Burmeister M, Payne AH. Isolation and characterization of several members of the murine Hsd3b gene family. DNA Cell Biol 1996;15:387–399.
76. Peng L, Payne AH. AP-2γ and the homeodomain protein distal-less 3 are required for placental-specific expression of the murine 3-hydroxysteroid dehydrogenase VI gene, Hsd3b6. J Biol Chem 2002;277:7945–7954.
77. Abbaszade IG, Clarke TR, Park C-HJ, Payne AH. The Mouse 3–Hydroxysteroid Dehydrogenase Multigene Family Includes Two Functionally Distinct Groups of Proteins. Mol Endocrinol 1995;9:1214–1222.
78. Payne AH, Abbaszade IG, Clarke TR, Bain PA, Park CH. The multiple murine 3-hydroxysteroid dehydrogenase isoforms: structure, function, and tissue- and developmentally specific expression. Steroids 1997;62:169–175.
79. Thomas JL, Mason JI, Brandt S, Spencer BR, Jr., Norris W. Structure/function relationships responsible for the kinetic differences between human type 1 and type 2 3-hydroxysteroid dehydrogenase and for the catalysis of the type 1 activity. J Biol Chem 2002;277:42,795–42,801.
80. McKeever BM, Hawkins BK, Geissler WM, et al. Amino acid substitution of arginine 80 in 17-hydroxysteroid dehydrogenase type 3 and its effect on NADPH cofactor binding and oxidation/reduction kinetics. Biochim Biophys Acta 2002; 1601:29–37.
81. Ficher M, Steinberger E. In vitro progesterone metabolism by rat testicular tissue at different stages of development. Acta Endocrinol (Copenh) 1971;68:285–292.
82. Chase DJ, Payne AH. Changes in distribution and androgen production of Leydig cells of two populations during sexual maturation in the rat. Endocrinology 1983;112:29–34.
83. Chase DJ, Payne AH. Changes in Leydig cell function during sexual maturation in the mouse. Biol Reprod 1983;29: 1194–1200.
84. Sheffield JW, O'Shaughnessy PJ. Testicular steroid metabolism during development in the normal and hypogonadal mouse. J Endocrinol 1988;119:257–264.
85. Russell DW, Wilson JD. Steroid 5 α-reductase: two genes/two enzymes. Annu Rev Biochem 1994;63:25–61.
86. Mahendroo MS, Russell DW. Male and female isoenzymes of steroid 5α-reductase. Rev Reprod 1999;4:179–183.
87. Jenkins EP, Hsieh CL, Milatovich A, et al. Characterization and chromosomal mapping of a human steroid 5α-reductase gene and pseudogene and mapping of the mouse homologue. Genomics 1991;11:1102–1112.
88. Thigpen AE, Davis DL, Milatovich A, et al. Molecular genetics of steroid 5α-reductase 2 deficiency. J Clin Invest 1992; 90:799–809.
89. Strausberg RL, Feingold EA, Grouse LH, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 2002;99:16,899–16,903.
90. Mahendroo M, Wilson JD, Richardson JA, Auchus RJ. Steroid 5α-reductase 1 promotes 5α-androstane-3α,17-diol synthesis in immature mouse testes by two pathways. Mol Cell Endocrinol 2004;222:113–120.
91. Lala DS, Rice DA, Parker KL Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of *fushi tarazu*-factor I. Mol Endocrinol 1992;6: 1249–1258.
92. Morohashi K-i, Honda S-i, Inomata Y, Handa H, Omura T. A common trans-acting factor, Ad4-binding protein, to the

promoters of steroidogenic P-450s. J Biol Chem 1992;267: 17,913–17,919.
93. Parker KL, Schimmer BP. Steroidogenic factor 1: A key determinant of endocrine development and function. Endocrine Rev 1997;18:361–377.
94. Parker KL, Rice DA, Lala DS, et al. Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog Horm Res 2002;57:19–36.
95. Fitzpatrick SL, Richards JS. Identification of a cyclic adenosine 3′,5′-monophosphate-response element in the rat aromatase promoter that is required for transcriptional activation in rat granulosa cells and R2C Leydig cells. Mol Endocrinol 1994;8:1309–1319.
96. Michael MD, Michael LF, Simpson ER. A CRE-like sequence that binds CREB and contributes to cAMP-dependent regulation of the proximal promoter of the human aromatase P450 (CYP19) gene. Mol Cell Endocrinol 1997;134:147–156.
97. Parker KL, Schimmer BP. Transcriptional regulation of the genes encoding the cytochrome P-450 steroid hydroxylases. Vitam Horm 1995;51:339–370.
98. Tremblay J, Viger R. GATA Factors Differentially Activate Multiple Gonadal Promoters through Conserved GATA Regulatory Elements. Endocrinology 2001;142:977–986.
99. Tremblay JJ, Viger RS. Novel roles for GATA transcription factors in the regulation of steroidogenesis. J Steroid Biochem Mol Biol 2003;85:291–298.
100. Viger RS, Taniguchi H, Robert NM, Tremblay JJ. Role of the GATA family of transcription factors in andrology. J Androl 2004;25:441–452.
101. Mason JI, MacDonald AA, Laptook A. The activity and biosynthesis of cholesterol side-chain cleavage enzyme in cultured immature pig testis cells. Biochim et Biophys Acta 1984;795:504–512.
102. Anderson CM, Mendelson CR. Regulation of steroidogenesis in rat Leydig cells in culture: effect of human chorionic gonadotropin and dibutyryl cyclic AMP on the synthesis of cholesterol side chain cleavage cytochrome P-450 and adrenodoxin. Archives of Biochemistry & Biophysics 1985;238:378–387.
103. Malaska T, Payne AH. Luteinizing hormone and cyclic AMP-mediated induction of microsomal cytochrome P-450 enzymes in cultured mouse Leydig cells. J Biol Chem 1984; 259:11,654–11,657.
104. Rani CSS, Payne AH. Adenosine 3′,5′-monophosphate-mediated induction of 17α-hydroxylase and C_{17-20} Lyase activities in cultured mouse Leydig cells is enhanced by inhibition of steroid biosynthesis. Endocrinology 1986;118:1222–1228.
105. Anakwe OO, Payne AH. Noncoordinate regulation of *de novo* synthesis of cytochrome P450 cholesterol side-chain cleavage and cytochrome P450 17α-hydroxylase/C17-20 lyase in mouse Leydig cell cultures: relation to steroid production. Mol Endocrinol 1987;1:595–603.
106. Hales DB, Sha LL, Payne AH. Testosterone inhibits cAMP-induced de Novo synthesis of Leydig cell cytochrome P-450 (17α) by an androgen receptor-mediated mechanism. J Biol Chem 1987;262:11,200–11,206.
107. Payne AH, Sha L. Multiple mechanisms for regulation of 3-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase, 17α-hydroxylase/C_{17-20} lyase cytochrome P450, and cholesterol side-chain cleavage cytochrome P450 messenger ribonucleic acid levels in primary cultures of mouse Leydig cells. Endocrinology 1991;129:1429–1435.
108. Waterman MR, Keeney DS. Signal transduction pathways combining peptide hormones and steroidogenesis. Vitam Horm 1996;52:129–148.
109. Hales DB, Payne AH. Glucocorticoid-mediated repression of $P450_{scc}$ mRNA and *de novo* synthesis in cultured Leydig cells. Endocrinology 1989;124:2099–2104.
110. Mellon SH, Vaisse C. cAMP regulates P450scc gene expression by a cycloheximide-insensitive mechanism in cultured mouse Leydig MA-10 cells. Proc Natl Acad Sci USA 1989; 86:7775–7779.
111. Burgos-Trinidad M, Youngblood GL, Maroto MR, Scheller A, Robins DM, Payne AH. Repression of cAMP-induced expression of the mouse P450 17 α-hydroxylase/C17-20 lyase gene (Cyp17) by androgens. Mol Endocrinol 1997;11:87–96.
112. Vierhapper H, Nowotny P, Waldhausl W. Production rates of testosterone in patients with Cushing's syndrome. Metabolism 2000;49:229–231.
113. Stalker A, Hermo L, Antakly T. Covalent affinity labeling, radioautography, and immunocytochemistry localize the glucocorticoid receptor in rat testicular Leydig cells. Am J Anat 1989;186:369–377.
114. Leers-Sucheta S, Morohashi, K, and Melner M. Interaction of the transcription factor Ad4BP/SF-1 with the human 3-hydroxysteroid dehydrogenase/D5-D4-isomerase (3bHSD) promoter, The 77th annual meeting of the Endocrine Society, Washington, DC, 1995. P3–P607.
115. Leers-Sucheta S, Morohashi-Ken-ichirou, Mason JI, Melner MH. Synergistic activation of the human type II 3-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase promoter by the transcription factor steroidogenic factor-1/adrenal 4-binding protein and phorbol ester. J Biol Chem 1997;272:7960–7967.
116. Baker PJ, Johnston H, Abel M, HM C, O'Shaughnessy PJ. Differentiation of adult-type Leydig cells occurs in gonadotropin-deficient mice. Reprod Biol Endocrinol 2003; 1:1–9.
117. Martin LJ, Taniguchi H, Robert NM, Simard J, Tremblay JJ, Viger RS. GATA Factors and the Nuclear Receptors, Steroidogenic Factor 1/Liver Receptor Homolog 1, Are Key Mutual Partners in the Regulation of the Human 3-Hydroxysteroid Dehydrogenase Type 2 Promoter. Mol Endocrinol 2005;19:2358–2370.
118. Martin LJ, Tremblay JJ. The human 3-hydroxysteroid dehydrogenase/Delta5-Delta4 isomerase type 2 promoter is a novel target for the immediate early orphan nuclear receptor Nur77 in steroidogenic cells. Endocrinology 2005;146:861–869.
119. Song KH, Park JI, Lee MO, Soh J, Lee K, Choi HS. LH induces orphan nuclear receptor Nur77 gene expression in testicular Leydig cells. Endocrinology 2001;142: 5116–5123.
120. Baker PJ, Sha JH, O'Shaughnessy PJ. Localisation and regulation of 17-hydroxysteroid dehydrogenase type 3 mRNA during development in the mouse testis. Mol Cell Endocrinol 1997;133:127–133.
121. Murono EP, Payne AH. Testicular maturation in the rat. In vivo effect of gonadotropins on steroidogenic enzymes in the hypophysectomized immature rat. Biol Reprod 1979; 20:911–917.
122. O'Shaughnessy PJ. Steroidogenic enzyme activity in the hypogonadal (hpg) mouse testis and effect of treatment with luteinizing hormone. J Steroid Biochem Mol Biol 1991; 39:921–928.

123. O'Shaughnessy PJ, Bennett MK, Scott IS, Charlton HM. Effects of FSH on Leydig cell morphology and function in the hypogonadal mouse. J Endocrinol 1992;135:517–525.
124. Payne AH, Chase DJ, O'Shaughnessy PJ. Regulation of steroidogenesis in Leydig cells. In: Conn PM, ed. Cellular Regulation of Secretion and Release. New York: Academic Press, 1982;355–408.
125. Chase DJ, Payne AH. Prolactin involvement in regulation of testicular 5 α-reductase activity in the immature rat. Biol Reprod 1985;33:637–643.
126. Murono EP, Washburn AL. Regulation of 5 α-reductase activity in cultured immature leydig cells by human chorionic gonadotropin. J Steroid Biochem 1990;35:715–721.
127. Murono EP, Washburn AL, Goforth DP, Wu N. Effects of acidic fibroblast growth factor on 5-ene-3-hydroxysteroid dehydrogenase-isomerase and 5 α-reductase activities and [125I]human chorionic gonadotrophin binding in cultured immature Leydig cells. J Steroid Biochem Mol Biol 1993;45:477–483.
128. Murono EP, Washburn AL. Fibroblast growth factor inhibits 5 α-reductase activity in cultured immature Leydig cells. Mol Cell Endocrinol 1990;68:R19–R23.
129. Viger RS, Robaire B. Steady state steroid 5 α-reductase messenger ribonucleic acid levels and immunocytochemical localization of the type 1 protein in the rat testis during postnatal development. Endocrinology 1995;136:5409–5415.
130. Miller WL. Why nobody has P450scc(20,22 desmolase deficiency). J Clin Endocr Metab 1998;83:1399–1400.
131. Fardella CE, Hum DW, Homoki J, Miller WL. Point mutation of Arg440 to His in cytochrome P450c17 causes severe 17α-hydroxylase deficiency. J Clin Endocrinol Metab 1994;79:160–164.
132. Yanase T. 17 α-Hydroxylase/17,20-lyase defects. J Steroid Biochem Mol Biol 1995;53:153–157.
133. Auchus RJ. The genetics, pathophysiology, and management of human deficiencies of P450c17. Endocrinol Metab Clin North Am 2001;30:101–119.
134. Martin RM, Lin CJ, Costa EM, et al. P450c17 deficiency in Brazilian patients: biochemical diagnosis through progesterone levels confirmed by CYP17 genotyping. J Clin Endocrinol Metab 2003;88:5739–5746.
135. Geller DH, Auchus RJ, Mendonca BB, Miller WL. The genetic and functional basis of isolated 17,20-lyase deficiency. Nature Genetics 1997;17:201–205.
136. Van Den Akker EL, Koper JW, Boehmer AL, et al. Differential inhibition of 17 α-hydroxylase and 17,20-lyase activities by three novel missense CYP17 mutations identified in patients with P450c17 deficiency. J Clin Endocrinol Metab 2002;87:5714–5721.
137. Sherbet DP, Tiosano D, Kwist KM, Hochberg Z, Auchus RJ. CYP17 mutation E305G causes isolated 17,20-lyase deficiency by selectively altering substrate binding. J Biol Chem 2003;278:48,563–48,569.
138. Simpson ER, Misso M, Hewitt KN, et al. Estrogen—the good, the bad, and the unexpected. Endocr Rev 2005;26: 322–330.
139. Carani C, Qin K, Simoni M, et al. Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 1997;337:91–95.
140. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 1995;80:3689–3698.
141. Bilezikian JP, Morishima A, Bell J, Grumbach MM. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 1998;339:599–603.
142. Herrmann BL, Saller B, Janssen OE, et al. Impact of estrogen replacement therapy in a male with congenital aromatase deficiency caused by a novel mutation in the CYP19 gene. J Clin Endocrinol Metab 2002;87:5476–5484.
143. Maffei L, Murata Y, Rochira V, et al. Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. J Clin Endocrinol Metab 2004;89:61–70.
144. Andersson Se. Molecular genetics and pathophysiology of 17-hydroxysteroid dehydrogenase 3 deficiency. J Clin Endocrinol Metab 1996;81:130–136.
145. Labrie F, Luu-The V, Lin SX, et al. Intracrinology: role of the family of 17-hydroxysteroid dehydrogenases in human physiology and disease. J Mol Endocrinol 2000;25:1–16.
146. Pelletier G, Li S, Luu-The V, Tremblay Y, Belanger A, Labrie F. Immunoelectron microscopic localization of three key steroidogenic enzymes (cytochrome P450scc), 3-hydroxysteroid dehydrogenase and cytochrome P450 c17) in rat adrenal cortex and gonads. J Endocrinol 2001;171:373–383.
147. Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 1997;18:281–305.
148. Andersson S, Russell DW. Structural and biochemical properties of cloned and expressed human and rat steroid 5 α-reductases. Proc Natl Acad Sci USA 1990;87:3640–3644.