0163-769X/05/$20.00/0
Printed in U.S.A.

Endocrine Reviews 26(4):525–582

doi: 10.1210/er.2002-0050

Molecular Biology of the 3β-Hydroxysteroid Dehydrogenase/Δ^5-Δ^4 Isomerase Gene Family

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The 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) isoenzymes are responsible for the oxidation and isomerization of Δ^5-3β-hydroxysteroid precursors into Δ^4-ketosteroids, thus catalyzing an essential step in the formation of all classes of active steroid hormones. In humans, expression of the type I isoenzyme accounts for the 3β-HSD activity found in placenta and peripheral tissues, whereas the type II 3β-HSD isoenzyme is predominantly expressed in the adrenal gland, ovary, and testis, and its deficiency is responsible for a rare form of congenital adrenal hyperplasia. Phylogeny analyses of the 3β-HSD gene family strongly suggest that the need for different 3β-HSD genes occurred very late in mammals, with subsequent evolution in a similar manner in other lineages. Therefore, to a large extent, the 3β-HSD gene family should have evolved to facilitate differential patterns of tissue- and cell-specific expression and regulation involving multiple signal transduction pathways, which are activated by several growth factors, steroids, and cytokines. Recent studies indicate that *HSD3B2* gene regulation involves the orphan nuclear receptors steroidogenic factor-1 and dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome gene 1 (DAX-1). Other findings suggest a potential regulatory role for STAT5 and STAT6 in transcriptional activation of *HSD3B2* promoter. It was shown that epidermal growth factor (EGF) requires intact STAT5; on the other hand IL-4 induces *HSD3B1* gene expression, along with IL-13, through STAT 6 activation. However, evidence suggests that multiple signal transduction pathways are involved in IL-4 mediated *HSD3B1* gene expression. Indeed, a better understanding of the transcriptional factors responsible for the fine control of 3β-HSD gene expression may provide insight into mechanisms involved in the functional cooperation between STATs and nuclear receptors as well as their potential interaction with other signaling transduction pathways such as GATA proteins. Finally, the elucidation of the molecular basis of 3β-HSD deficiency has highlighted the fact that mutations in the *HSD3B2* gene can result in a wide spectrum of molecular repercussions, which are associated with the different phenotypic manifestations of classical 3β-HSD deficiency and also provide valuable information concerning the structure-function relationships of the 3β-HSD superfamily. Furthermore, several recent studies using type I and type II purified enzymes have elegantly further characterized structure-function relationships responsible for kinetic differences and coenzyme specificity. (*Endocrine Reviews* 26: 525–582, 2005)

I. Introduction

STEROID HORMONES PLAY a crucial role in the differentiation, development, growth, and physiological function of most vertebrate tissues. The major pathways of steroid hormone synthesis are well established, and the sequence of the responsible steroidogenic enzymes has been

elucidated (Refs. 1–7 and references therein) (Fig. 1). For example, in the human, after the conversion of cholesterol to pregnenolone (PREG) by the mitochondrial side-chain cleavage system, the adrenal cortex may direct PREG toward one of three different pathways. First, PREG may remain as a C21,17-deoxysteroid and proceed down the pathway to produce the mineralocorticoid, aldosterone. Second, it may undergo 17α-hydroxylation and proceed down the C21,17-hydroxy pathway to form the principal glucocorticoid, cortisol. The third option is that, after 17α-hydroxylation, it may undergo cleavage of the C17–20 bond to become a C19–17-ketosteroid, leading to the formation of androgens and estrogens. As can be seen in Fig. 1, whichever pathway is followed, the subsequent formation of all classes of steroid hormones relies upon the action of the enzyme 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD) (8–10).

It is also well recognized that humans and certain other primates are unique among animal species in having adrenals that secrete large amounts of the inactive steroid precursors, dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S). These steroids do not bind to the androgen receptor (11) but exert either estrogenic or androgenic action after their conversion into active estrogens and/or androgens in target tissues (12, 13). Indeed, in postmenopausal women, almost all sex steroids are synthesized from precursors of adrenal origin except for a small contribution from ovarian testosterone (T) and Δ^4-androstenedione (Δ^4-DIONE), whereas in adult men, approximately half of androgens are made locally in target tissues (12). Thus, the various types of human enzymes catalyzing 3β-HSD, 17β-hydroxysteroid dehydrogenase (17β-HSD)/ketosteroid reductase (KSR), 5α-reductase activities, and the alternative promoter usage of the aromatase gene, because of their tissue- and/or cell-specific expression and substrate specificity, provide each cell with necessary mechanisms to control the level of intracellular active estrogens and androgens (12, 14–16).

A. The role of 3β-hydroxysteroid dehydrogenase activity in steroid formation and degradation

The nicotinamide adenine dinucleotide $(NAD)^+$-dependent membrane-bound enzyme 3β-HSD, was first described in 1951 by Samuels *et al.* (17). It is located in the endoplasmic reticulum (ER) and mitochondria (18–23), and it catalyzes the sequential 3β-hydroxysteroid dehydrogenation and Δ^5 to Δ^4-isomerization of the Δ^5-steroid precursors PREG, 17α-hydroxypregnenolone (17OH-PREG), DHEA, and androst-5-ene-3β,17β-diol (Δ^5-DIOL) into their respective Δ^4-ketosteroids, namely progesterone (PROG), 17α-hydroxyprogesterone (17OH-PROG), Δ^4-DIONE, and T. Therefore, this bifunctional dimeric enzyme is required for the biosynthesis of all classes of steroid hormones, namely glucocorticoids, mineralocorticoids, PROG, androgens, and estrogens (Fig. 1). In addition, enzymes of the 3β-HSD family also catalyze the formation and/or degradation of 5α-androstanes and 5α-pregnanes, such as dihydrotestosterone (DHT) and dihydroprogesterone (DHP) (8–10). The 3β-HSD isoenzyme therefore controls critical steroidogenic reactions in the adrenal cortex, gonads, placenta, and a variety of peripheral target tissues (24).

Transient expression of human 3β-HSD isoenzymes provided the first direct evidence that the 3β-HSD and Δ^5-Δ^4-isomerase activities reside within a single protein (25–27). However, data obtained from affinity alkylation (28) and inhibition experiments (29) that suggested separate 3β-HSD and isomerase sites are also consistent with a bifunctional catalytic site adopting a different conformation for each activity, as suggested by tryptic peptides associated with both catalytic activities localized using affinity radiolabeled steroids (30–32). Additional studies have supported the hypothesis that reduced NAD (NADH), the coenzyme product of the rate-limiting 3β-HSD reaction, induces a conformational change around the bound 3-oxo-Δ^5-steroid (the 3β-HSD product and the isomerase substrate) to activate the isomerase step (33). Finally, as revealed by site-directed mutagenesis of the human type I (placental) enzyme, His^{261} appears to be a critical amino acid residue for 3β-HSD activity, whereas Tyr^{253} or Tyr^{254} participate in the isomerase activity (23).

B. Subcellular localization

Many of the enzymes of the steroidogenic pathway are localized to the smooth ER with the notable exceptions of P450scc (P450 cholesterol side-chain cleavage; *CYP11A1*), P450c11 (*CYP11B1*), and aldosterone synthase (*CYP11B2*). 3β-HSD subcellular localization patterns are unique in that they show various degrees of ER and mitochondrial distribution. The relevance of dual localization is unclear, yet it can be hypothesized that substrate accessibility could be limited with higher degrees of mitochondrial expression due to reduced mitochondrial transport. This would be analogous to

First Published Online January 4, 2005

Abbreviations: A-II, Angiotensin II; AMG, aminoglutethimide; AP-1, activator protein-1; AR, androgen receptor(s); BAC, bovine adrenocortical; CAH, congenital adrenal hyperplasia; CAT, chloramphenicol acetyltransferase; CL, corpus luteum; CNS, central nervous system; CSF, colony-stimulating factor; DAX-1, dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome gene 1; DEX, dexamethasone; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; DHP, dihydroprogesterone; DHT, dihydrotestosterone; Δ^5-DIOL, androst-5-ene-3β,17β-diol; Δ^4-DIONE, Δ^4-androstenedione; Dlx, distal-less; E_2, estradiol; EGF, epidermal growth factor; ER, endoplasmic reticulum; FGF, fibroblast growth factor; hCG, human chorionic gonadotropin; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; HYPOX, hypophysectomized; IL-4R, IL-4 receptor; IMO, immobilization stress; IRS, insulin receptor substrate; K_m, Michaelis constant; KSR, ketosteroid reductase; 17OH-PREG, 17α-hydroxypregnenolone; 17OH-PROG, 17α-hydroxyprogesterone; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; NADPH, NADH phosphate; NO, nitric oxide; P450c17, P450 17α-hydroxylase/17,20-lyase; P450c21, P450 21α-hydroxylase; P450scc, P450 cholesterol side-chain cleavage; PBR, peripheral benzodiazepine receptor; p.c., post coitum; PCOS, polycystic ovary syndrome; PI3-K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; PR, PROG receptor(s); PrEC, normal human prostate epithelial cells; PREG, pregnenolone; PRL, prolactin; PROG, progesterone; SF-1, steroidogenic factor-1; SNP, single nucleotide polymorphism; StAR, steroidogenic acute regulatory protein; T, testosterone; TDF, thecal differentiating factor; TEF-5, transcription enhancer factor-5; V_{max}, maximal velocity; ZF, zona fasciculata; ZG, zona glomerulosa; ZR, zona reticularis.

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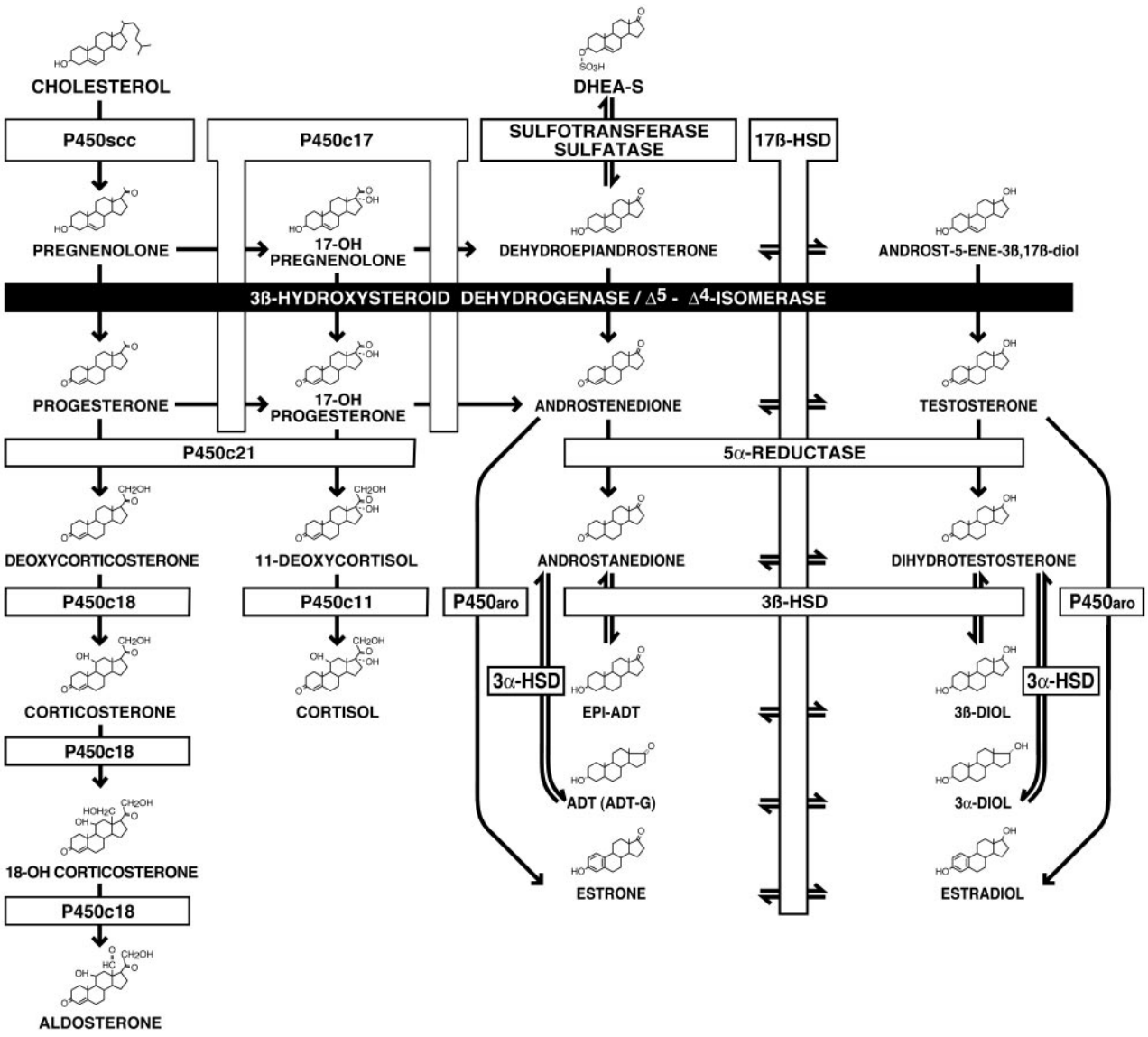
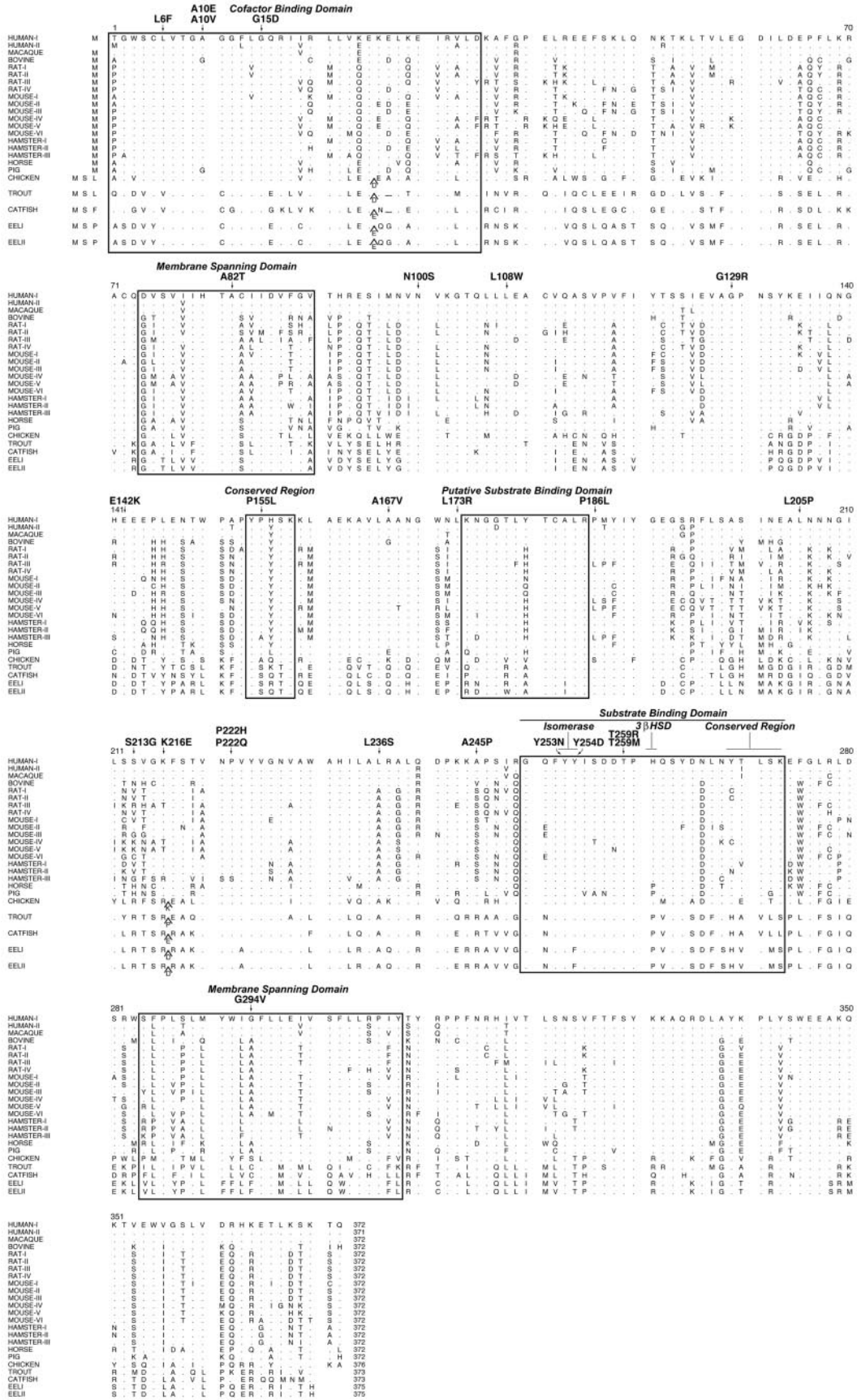


FIG. 1. Schematic representation of the major mammalian steroidogenic pathways. All P450s are cytochrome enzymes. P450c18, This enzyme mediates 11β-hydroxylation and subsequent reactions involved in the biosynthesis of aldosterone; P450c11, 11β-hydroxylase; P450aro, P450 aromatase.

the inability of high-efficiency catalysis of cholesterol by P450scc in the absence of the protein controlling cholesterol shuttling, steroidogenic acute regulatory protein (StAR) (34). Smooth ER localized 3β-HSD presumably would have a greater access to cytosolic steroid precursors, such as DHEA and Δ^5-DIOL.

3β-HSD activity was detected using histochemical techniques as early as 1965 (35, 36). Similar techniques isolated this activity to the smooth ER and mitochondrial cristae (37). Its membrane localization was not known until studies localized 3β-HSD to the microsomal fraction of human adrenal (38) and chorion/amnion fetal membranes (39), suggesting that 3β-HSD is a membrane-associated enzyme. With the development of antibodies against 3β-HSD, the resolution of its localization increased, and it was verified that it is associated with the ER and mitochondria in human placenta (18, 40, 41), bovine adrenocortical cells (20), and rat adrenal tissue (21).

Submitochondrial fractionation studies show that bovine adrenal 3β-HSD is associated with the inner membrane and with a particulate fraction characterized by contact sites between the two membranes. 3β-HSD activity was higher in this fraction than in the inner mitochondrial membrane, suggesting that intermembrane contact sites may facilitate both the access of cholesterol to the inner membrane where P450scc is localized and the rapid conversion of PREG to PROG by 3β-HSD (19). Elegant biochemical studies have confirmed that a significant amount of adrenocortical 3β-HSD is present in the inner mitochondrial membrane (42). Coprecipitation studies have shown that 3β-HSD is in a functional steroidogenic complex with P450scc in the inner mitochondrial membrane (43), which provides the enzyme



with immediate substrate metabolized from cholesterol transported across the mitochondrial membrane. Other work has shown that subcellular distribution in bovine and murine adrenal tissues demonstrated a higher degree of microsomal to mitochondrial localization (44, 45). A similar subcellular distribution was also recently reported in rat ovary, as revealed by immunoelectron microscopic localization, whereas in the testis, the 3β-HSD was restricted to the mitochondria (45).

Although the functional significance of differential 3β-HSD subcellular localization is unknown, studies have been performed to determine whether the dynamics of 3β-HSD subcellular localization can be altered by regulation. Because Ca^{2+} flux mediates K^+ and A-II increases in aldosterone production by zona glomerulosa (ZG) (46), it is possible that Ca^{2+} could affect the mitochondrial to ER ratio of 3β-HSD. However, bovine ZG cells showed that neither Ca^{2+} nor A-II had any effect on the subcellular distribution of 3β-HSD and P450scc, but it did affect StAR localization (22). Another report showed that microsomal 3β-HSD activity in the ovary was unchanged during mouse estrous, yet mitochondrial 3β-HSD activity increased and doubled during diestrous in the mouse (47). These results suggest that 3β-HSD activity could be preferentially distributed to the mitochondria under certain physiological conditions, but this may not be a general phenomenon.

II. Human Type I and II 3β-HSD Genes and Pseudogenes

During the past decade, the structure of the isoenzymes of the 3β-HSD family has been characterized in the human and several other vertebrate species (Fig. 2). Human type I 3β-HSD cDNA was isolated and characterized by Luu-The *et al.* (18, 48, 49) after purification of the 3β-HSD enzyme from human placenta, and this sequence was later confirmed by other workers (25, 50). The second human 3β-HSD isoenzyme, chronologically designated as type II, was isolated from a human adrenal cDNA library (27). The type I 3β-HSD gene (*HSD3B1*) encodes an enzyme of 372 amino acids predominantly expressed in the placenta and peripheral tissues, such as the skin (principally in sebaceous glands), mammary gland, prostate, and several other normal and tumor tissues (27, 51–54). The purified enzyme has a Michaelis constant (K_m) of 3.7 μM and maximal velocity (V_{max}) of 43 nmol/min·mg for 3β-HSD substrate (DHEA) and a K_m of 28 μM and V_{max} of 598 nmol/min·mg for the isomerase substrate (5-androstene-3,17-dione) (54). In comparison, the type II gene (*HSD3B2*), which encodes a protein of 371 amino acids, shares 93.5% identity with the type I and is almost exclusively expressed in the adrenals, the ovary, and testis (27, 54, 55). The purified enzyme has a K_m of 47 μM and V_{max} of 82 nmol/min·mg for 3β-HSD substrate (DHEA) and a K_m of 88 μM and V_{max} of 970 nmol/min·mg for the isomerase substrate (5-androstene-3,17-dione). The higher affinity of type I 3β-HSD could facilitate steroid formation from relatively low concentrations of substrates usually present in peripheral tissues. Based on their differential tissue-specific expression pattern, it is not surprising that classical 3β-HSD deficiency, which will be discussed further in *Section VII*, results from mutations in the *HSD3B2* gene, whereas the *HSD3B1* gene is normal in affected individuals (56–59).

The structure of each of the *HSD3B1* and *HSD3B2* genes consists of four exons which are included within a DNA fragment of 7.8 kb and which share 77.4, 91.8, 94.5, and 91.0% identity, respectively (26, 60, 61). The genes are assigned to chromosome 1p13.1, 1–2 cM from the centromeric marker D1Z5 (Fig. 3) (62, 63). Our initial data suggested that the *HSD3B1* and *HSD3B2* genes and three related pseudogenes (64) are included within a 0.29 megabase *Sac*II DNA fragment, suggesting that the human 3β-HSD gene family exists as a tandem cluster of related genes (63) as observed for the mouse β-HSD genes (65). In support of these findings, in addition to the two expressed genes in the human, five pseudogenes have also been recently cloned and physically mapped (66) (Fig. 3). HSD3Bψ1–5 are unprocessed pseudogenes that are closely related to *HSD3B1* and *HSD3B2* genes, but contain no corresponding open reading frames. Although mRNA is expressed from ψ4 and ψ5 in several tissues, altered splice sites disrupt the reading frames. The two expressed genes, *HSD3B1* and *HSD3B2*, are located in direct repeat, 100 kb apart; however, separation by two pseudogenes, ψ1 and ψ2, prevents them from sharing common promoter elements (66).

III. Structure-Function Relationships

The two-step reaction of the 3β-HSD/isomerase involves the reduction of NAD^+ to NADH by the rate-limiting 3β-HSD activity and the requirement of this NADH for the activation of the isomerase on the same enzyme (41, 67). Stopped-flow spectroscopy studies show that NADH activates the isomerase activity by inducing a time-dependant conformational change in the enzyme, suggesting that the 3β-HSD and isomerase domains of the enzyme are linked by a shared coenzyme domain that functions both as the binding site for NAD^+ during the 3β-HSD reaction and as the coenzyme domain for the allosteric activation of the isomerase reaction (33).

The 3β-HSD isoenzymes belong to the short-chain alcohol dehydrogenase superfamily, mainly determined by the nucleotide-binding site sequence located at the amino terminus. It consists of a β-strand, α-helix, β-strand in a fold that provides a hydrophobic pocket for the AMP part of the nucleotide factor. The turn between the first β-strand and the α-helix is a glycine-rich segment, Gly-X-X-Gly-X-X-Gly, similar to the common Rossmann fold sequence Gly-X-Gly-X-

FIG. 2. Comparison of the amino acid sequences of members of the 3β-HSD gene family: human types I and II; macaque; bovine; rat types I, II, III, and IV; mouse types I, II, III, IV, V, and VI; hamster types I, II, and III; horse; pig; chicken; rainbow trout; catfish; and eel types I and II. Residues common to the human type I 3β-HSD are represented by a *dot*. The members of the mammalian 3β-HSD family have been chronologically designated according to their order of elucidation in each species. The *numbers indicated above* refer to the human type II sequence. The missense mutations associated with 3β-HSD deficiency are shown by an *arrow* indicating their position in the human type II sequence. [Adapted from Ref. 576.]

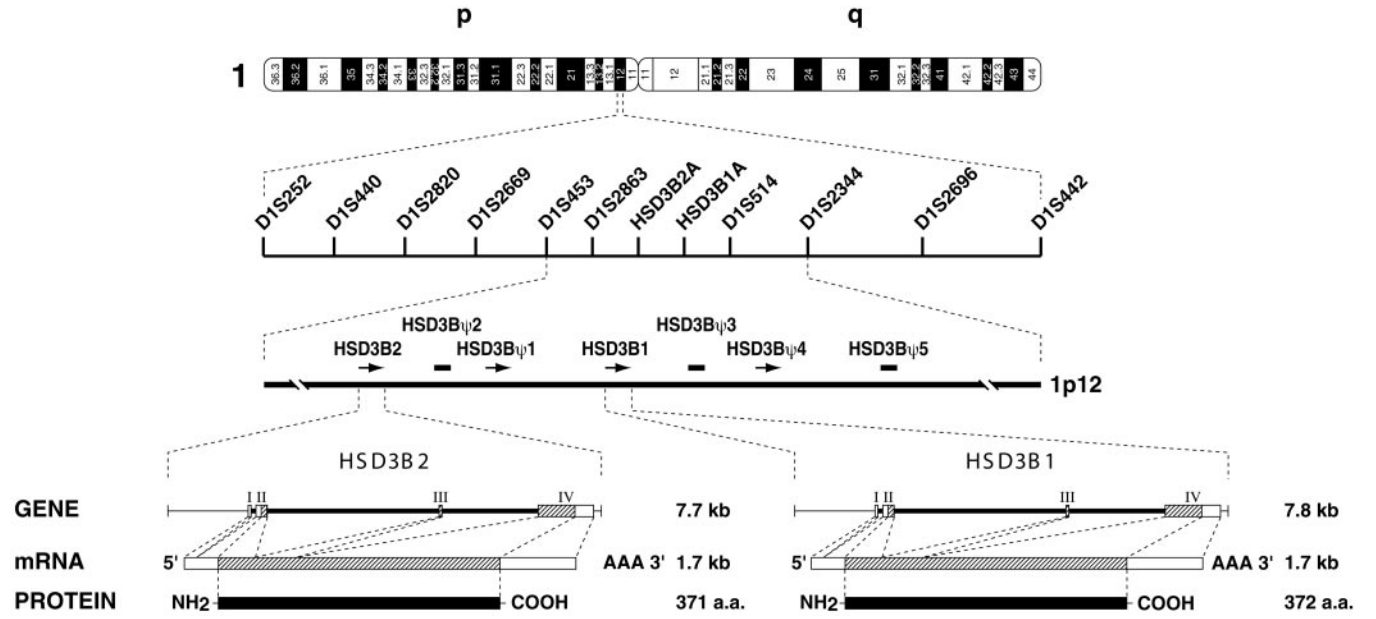


FIG. 3. Chromosomal localization showing the two expressed genes *HSD3B1* and *HSD3B2*, and five pseudogenes, Ψ1–5. The orientation of four genes is shown by the *arrow* that points toward the stop codon or its homolog. Clones of yeast artificial chromosomes (alphanumeric identification) are shown as a contig. The information regarding the order of the markers was obtained from the Whitehead Institute/MIT Center for Genome Research, Cambridge, Massachusetts (http://www-genome.wi.mit.edu/). Structure of human type I and type II 3β-HSD genes, mRNA species, and the corresponding proteins. Exons are represented by *boxes* in which *hatched lines* demarcate the coding regions, whereas *open boxes* represent the noncoding regions. Introns are represented by *black bold lines*. [Adapted from Ref. 576.]

X-Gly conserved among most NAD(H)-binding enzymes (68). This well-conserved glycine-rich fragment forms a hydrophobic pocket that allows close association of the AMP part of the cofactor. A preliminary study of rat type III enzyme has targeted Asp (36) as the amino acid that may be responsible for the strict NAD^+ specificity of the enzyme (8). More recent mutagenesis studies in human type I enzyme demonstrate that the D36A/K37R mutant shifts cofactor preferences of both 3β-HSD and isomerase activities from NAD(H) to NADP(H), thus showing that the two activities utilize a common coenzyme domain (69).

Affinity labeling of purified human type I identified two tryptic peptides, comprising amino acids Asn^{176} to Arg^{186} and Gly^{251} to Lys^{274} that contain residues involved in the putative substrate-binding domain (30). These studies have shown that the Gly^{251} to Lys^{274} peptide was associated with the site of isomerase activity, whereas Tyr^{253} appears to function as the general proton donor in the isomerase reaction (24). His^{261} also appears to be a critical residue for the 3β-HSD activity (23). Additional kinetic analyses of D257L and D258L mutants suggest that this region is part of the isomerase substrate domain (69).

In contrast to other short chain dehydrogenases with a single catalytic Y-X-X-X-K motif (5, 70, 71), there are two potential catalytic motifs (Y^{154}-X-X-X-K^{158} and Y^{269}-X-X-X-K^{273}) in the primary structure of all 3β-HSDs. Human type I and type II only differ at position 156 in this motif, type I having a tyrosine whereas type II has a histidine residue. The H156Y mutant form of the type I enzyme shifts the substrate kinetics for DHEA and PREG to the same K_m and V_{max} values exhibited by the type II enzyme; thus, H^{156} in the type I *vs.* Y^{156} in type II 3β-HSD accounts for the substantially higher affinity of the type I 3β-HSD activity for these substrates and inhibitor epostane relative to the type II enzyme (72).

Two membrane-binding domains lying between residues 72 and 89 in the NH_2-terminal region and between residues 283 and 310 in the COOH-terminal region were identified. Indeed, deletion of the 283–310 region causes the enzyme to localize in the cytosol without affecting its activities (73). The region is therefore a critical membrane domain of 3β-HSD that can be deleted without compromising enzyme function (54, 73). Deletion of residues 72–89 in the NH_2-terminal region produces a mutant protein that is distributed among the microsomes, mitochondria, and cytosol (73). Because 28% of the 3β-HSD and isomerase activities remain in the membranes of microsomes and mitochondria, the presence of the 283–310 domain in this mutant allows the protein to retain significant hydrophobicity. However, a majority (72%) of the protein is shifted into the cytosol, so the 72–89 region does contribute to membrane association. The 8-fold loss of both 3β-HSD and isomerase activity that results from the 72–89 deletion underscores the importance of this region to enzyme function (73). The data obtained by Thomas' group (73) with the human type I enzyme are consistent with one of our previous studies in which the increased polarity of the domain between residues 75 and 91 in the rat type II 3β-HSD/isomerase was responsible for its having much lower activity than the rat type I enzyme (74). Thus, the presence of this highly conserved hydrophobic domain may be crucial to activity in the entire 3β-HSD gene family. The expression of an active soluble 283–310 deletion mutant of the type I enzyme in a baculovirus expression system provides a valuable tool for crystallographic studies that may ultimately determine the tertiary/quaternary structure of the enzyme (73). A

three-dimensional ribbon model has been constructed by Thomas' group (69), based on the homology data for human type I 3β-HSD and UDP-galactose-4-epimerase. This also represents a useful tool for interpreting biochemical data and designing inhibitors (Fig. 4) (69).

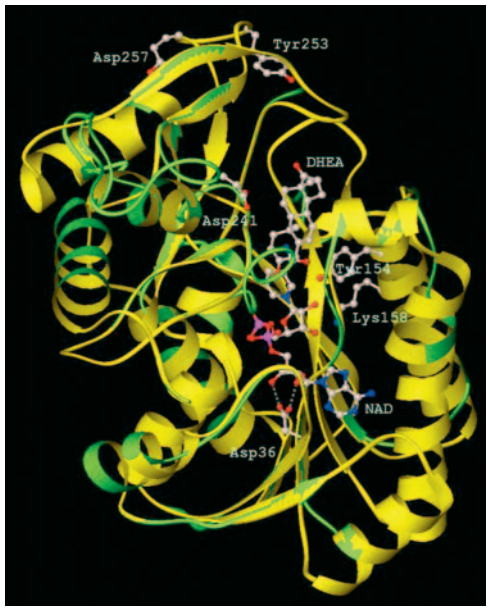


FIG. 4. Ribbon structure of human type I 3β-HSD/isomerase based on homology modeling with key amino acids identified. The primary sequences of 3β-HSD/isomerase (*green*) and UDP-galactose-4-epimerase (*yellow*) were aligned using ClustalX. The NAD and DHEA structures are included. The key Asp^{36} residue is shown hydrogen binding (*gray dotted lines*) to the 2′, 3′-hydroxyl groups of the adenosyl ribose group of NAD. The catalytic Tyr^{154} and Lys^{158} residues for human type I 3β-HSD activity, the catalytic Tyr^{253} and Asp^{257} residues for isomerase activity, and the Asp^{241} residue that bridges the upper isomerase domain with the lower coenzyme domain are also shown. This ribbon model represents the 3β-HSD/isomerase structure in the 3β-HSD conformation. The oxygen atoms are *red*, nitrogen atoms are *blue*, carbon atoms are *gray*, and phosphorus atoms are *pink*. [Reproduced from J.L. Thomas *et al.*: *J Biol Chem* 278:35483–35490, 2003 (69), copyright 2003, with permission from The American Society for Biochemistry and Molecular Biology.]

IV. Evolution of the 3β-HSD Gene Family

Multiple 3β-HSD isoenzymes have been cloned from several other species, further illustrating that the 3β-HSD gene family is conserved in vertebrate species (Fig. 2 and Table 1). The tissue-specific expression of multiple members of the 3β-HSD family was first demonstrated in the rat (75). Other 3β-HSD cDNAs have been cloned using adrenal/gonadal cDNA libraries from six other species, namely the macaque ovary (76), bovine ovary (77), chicken adrenal (78), horse testis (79), rainbow trout ovary (80), and eel ovary (81). It is important to note that in contrast to the human, which is designated as type II, the adrenal/gonadal 3β-HSD isoenzymes in all other vertebrate species have been designated as type I, due to the chronological order in which they were cloned. The only 3β-HSD sequence available from the pig was obtained using a cDNA library from adipose tissue (616).

A. The rat 3β-HSD gene family

The secretion of sex steroids originates exclusively from the gonads in rodents and domestic animals, which is in contrast to humans who in addition to secreting sex steroids from the gonads, secrete the sex precursors DHEA and DHEA-S from the adrenal gland. The structures of four members of the rat 3β-HSD family have been characterized (75, 82, 83). With the exception of type III, all isoenzymes catalyze the transformation of 5-pregnen-3β-ol and 5-androsten-3β-ol steroids into the corresponding Δ^4-3-ketosteroids as well as the interconversion of 3β-hydroxy- and 3-keto-5α-androstane steroids. The various isoenzymes show differences in tissue-specific expression (84) (Fig. 5). The rat type I and II 3β-HSD proteins are expressed in the adrenals, gonads, kidney, placenta, adipose tissue, and uterus and share 93.8% identity. The type III protein shares 80% identity with the type I and II proteins but, in contrast to other types, is a specific 3-KSR. The type III gene is exclusively expressed in

TABLE 1. Kinetic parameters and major expression sites of 3β-HSDs from human, rat, mouse, hamster, macaque, bovine, and rainbow trout

Species	Type	K_m PREG/DHEA (μM)	Cofactor	Major sites of expression	First cloning (Ref. no.)
Human	I	<1	NAD^+	Placenta, skin and mammary gland	18
	II	1 to 4	NAD^+	Adrenals and gonads	27
Rat	I	<1	NAD^+	Adrenals and gonads	82
	II	>10	NAD^+	Adrenals and gonads	82
	III	3-KSR	NADPH	Male liver	75
	IV	<1	NAD^+	Placenta and skin	83
Mouse	I	<1	NAD^+	Adrenals and gonads	87
	II	nd	nd	Kidney and liver	87
	III	<1	NAD^+	Liver > kidney	87
	IV	3-KSR	NADPH	Kidney	88
	V	3-KSR	NADPH	Male liver	91
	VI	<1	NAD^+	Placenta and skin	593
Hamster	I	2 to 5.5	NAD^+	Adrenals and gonads	96
	II	2 to 9	NAD^+	Kidney and liver	96
	III	3-KSR	NADPH	Male liver	96
Macaque		nd	nd	Adrenals and gonads	76
Bovine		>10	NAD^+	Adrenals and gonads	77
Rainbow trout		DHEA > PREG	NAD^+	Ovary	80

nd, Not determined.

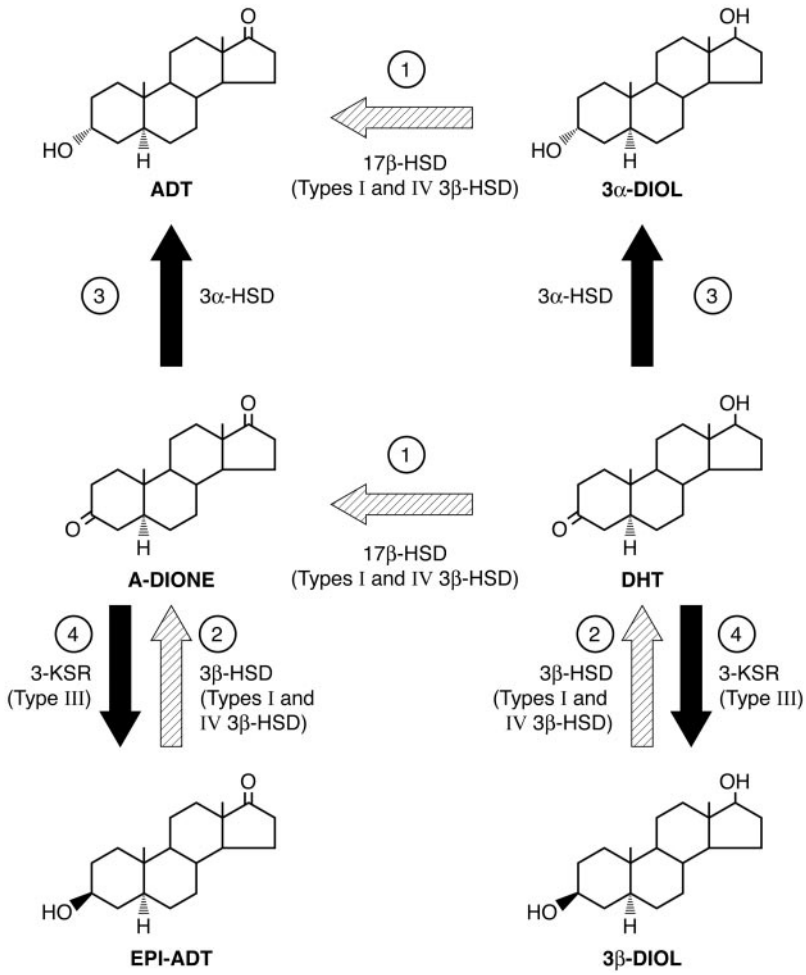


FIG. 5. Enzymatic 17β-HSD and 3β-HSD activities of rat types I and IV 3β-HSD expressed in intact cells. Reaction 1 corresponds to the androgenic 17β-HSD activity measured in cells expressing the rat type I and IV 3β-HSD isoenzymes. Reaction 2 corresponds to the 3β-HSD activity present in cells expressing rat type I or IV 3β-HSD enzymes. Reaction 3 corresponds to 3α-HSD present in some cells. Reaction 4 corresponds to the 3-KSR activity present in liver cells expressing the rat 3-KRS (type III) enzyme. The *hatched arrows* indicate predominant reactions expected to use primarily NAD^+ as cofactor, whereas the *black arrows* indicate the predominant reactions expected to use primarily NADPH as cofactor. [Adapted from Ref. 84.]

male liver, and there is marked sexual dimorphic expression, which results from pituitary hormone-induced gene repression in the female rat liver (75, 85). The rat type IV protein shares 90.9, 87.9, and 78.8% identity with that of types I, II, and III proteins, respectively, and is the prominent mRNA species detectable in the placenta and the skin (83). In this respect, it is therefore possible that the rat type IV and the human type I proteins have conserved *cis*-acting elements in their promoter regions, involved in tissue-specific transcriptional control common to skin and placenta (8). The activities of rat types I and IV are similar (83), whereas there is much lower enzyme activity for the type II compared with the type I, which could be due to a change in four amino acid residues located in a putative membrane-spanning domain, between residues 75 and 91 as described in the previous section (74). Furthermore, types I and IV possess a 17β-HSD activity specific to 5α-androstane-17β-ol steroids, thus suggesting a key role in controlling the bioavailibility of the active androgen DHT (84, 86).

B. The mouse 3β-HSD gene family

To date, six distinct cDNAs encoding murine members of the 3β-HSD family have been cloned (87–92), all of which are highly homologous and encode a protein of 372 amino acids. The murine family of 3β-HSD enzymes has been extensively reviewed in the literature (9, 93) and references therein. The genes encoding the different isoenzymes are found closely linked on mouse chromosome 3 (65). Hybridization by Southern blot analysis of restriction enzyme-digested yeast artificial chromosome DNA using an 18-base oligonucleotide that hybridizes without mismatch to all known *Hsd3b* sequences indicates that there are a total of seven *Hsd3b* genes or pseudogenes in the mouse genome. Additional analysis of mouse genomic DNA by pulse field gel electrophoresis suggests that all of the *Hsd3b* gene family is found within a 400-kb fragment (9, 94). The different forms are expressed in a tissue-specific and developmentally specific manner and fall into two functionally distinct classes of enzymes (92). 3β-HSD types I and III, and most probably type II, function as dehydrogenase/isomerases, and are essential for the biosynthesis of active steroid hormones, whereas 3β-HSD type IV and type V, analogous to rat type III, function as 3-KSRs and are therefore involved in the inactivation of active steroid hormones (88, 91). 3β-HSD I in the adult mouse is expressed in the gonads and the adrenal gland (87), whereas 3β-HSD II and III are expressed in the liver and kidney (87), with much greater expression of type III in the liver than in the kidney. The major site of expression of 3β-HSD IV is in the proximal tubules of the kidney in both the male and female mice (89), with minor expression in the testis (91). The

type V isoenzyme appears to be expressed only in the liver of the male mouse, with expression starting during the latter half of pubertal development (91, 95). The type VI isoenzyme functions as a NAD^+-dependent 3β-HSD and is the earliest isoform to be expressed during the first half of pregnancy in cells of embryonic origin and in uterine tissue (92). In the adult mouse, 3β-HSD type VI appears to be the only isoenzyme expressed in the skin and is also expressed in Leydig cells of the testis, although to a lesser extent than type I 3β-HSD.

It is hypothesized that mouse 3β-HSD type VI cDNA is orthologous to human 3β-HSD type I cDNA, which has been shown to be the only isoenzyme expressed in the placenta and the skin. The demonstration that the type VI isoenzyme in the mouse functions as a 3β-HSD and is the predominant isoenzyme expressed during the first half of pregnancy in uterine tissue and embryonic cells, suggests that this isoenzyme may be involved in the local production of PROG, which is required for the successful implantation of the blastocyst and/or maintenance of pregnancy (92).

C. The hamster 3β-HSD gene family

The hamster is a rodent species, but in contrast to the rat and mouse, in which the principal corticosteroid is corticosterone, the principal corticosteroid in the hamster is cortisol. A study on the regulation of adrenal steroidogenic enzymes suggested that the hamster could be a good model for studying human steroidogenesis. With this in mind, three isoenzymes of 3β-HSD were characterized in the hamster (96, 97). The type I isoenzyme was isolated from an adrenal cDNA library and was identified as being a low K_m 3β-HSD (K_m: PREG, 5.5 μM; DHEA, 2.4 μM). A separate isozyme, designated type II was isolated from the kidney and was also found to be a low K_m 3β-HSD (K_m: PREG, 8.8 μM; DHEA, 2.9 μM). Two cDNAs were isolated from the liver, one which was identical to the type II sequence found in the kidney, and a distinct cDNA encoding an isoform designated as type III, which does not possess any steroid dehydrogenase activity but functions as a 3-KSR. There is sexual dimorphic expression of this liver-specific type III 3β-HSD in the hamster, as seen for the rat liver-specific type III KSR. As is the case for both the rat and mouse, a high affinity 3β-HSD is expressed in the adrenal and gonad of the hamster, consistent with the steroidogenic role of these tissues (96).

D. Phylogeny of the 3β-HSD gene family

McBride *et al.* (66) indicated no evidence for the presence of other members of the human 3β-HSD family within the physical contig of 0.5 Mb by Southern blot analysis, thus suggesting that in humans there is no comparable liver-specific 3-KSR sharing a high percentage of identity with other members of the *HSD3B* cluster. Such a conclusion is also well supported by phylogenetic analysis of the mammalian 3β-HSD gene family. Unexpectedly, the phylogenetic tree strongly suggests that independent gene duplications occurred in different species (66, 91), (V. Laudet, personal communication). As illustrated in Fig. 6, our recent analysis shows a first complex of three genes from primates and suggests that an ancestral gene duplicated specifically in the primate lineage to give rise to human types I and II, whereas the macaque gene is the homolog of human type II. It is very likely that an ortholog of the human type I exists in the macaque genome, but yet remains to be identified. The second complex clusters together the single 3β-HSD species characterized in bovine, pig, and horse. The third complex clusters together three clear classes of rodent 3β-HSD genes; firstly, the rat type I, II, and IV as well as the mouse type I, II, III, and VI; secondly, the mouse type IV and V and rat type III, the specific 3-KSRs; and thirdly the hamster type I, II, and III. Because the hamster type III is a liver-specific 3-KSR (97), it is surprising that it is not included in the second class of rodent genes. These findings strongly suggest that the 3β-HSD genes were independently duplicated or triplicated three times in the lineage of the rat, the mouse, and the hamster. It is difficult to understand why the duplication failed to occur earlier in mammalian evolution if there are physiological needs and/or advantages for the presence of multiple isoenzymes. These data may indicate that the need for different 3β-HSD genes occurred very late in mammals, with subsequent evolution in a similar manner in other lineages.

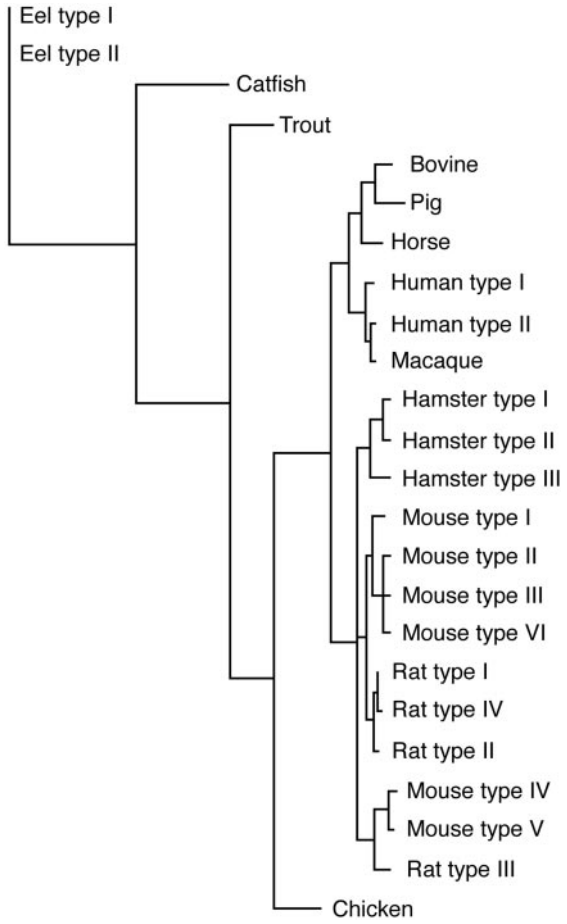


FIG. 6. Unrooted phylogenetic tree constructed by the Neighbor-Joining method using 1000 bootstrap replicates. Multiple nucleotide alignments of 3β-HSDs from different species were obtained using PILEUP (Wisconsin GCG package), and the phylogenetic analysis was performed by PAUPsearch, which provides a GCG interface to the tree-searching options in the PAUP program, version 4.0.0d55 (Phylogenetic Analysis Using Parsimony) (590). [Adapted from Ref. 576.]

It is also of interest to note that although the N-terminal amino acid sequences of the pig hepatic 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase and the vertebrate 3β-HSD enzymes show some similarities, substrate specificities differ. Although vertebrate 3β-HSD/Δ^5-Δ^4 isomerase enzymes are active on C_{19}/C_{21} steroids, porcine hepatic 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase is active on C_{27} steroids such as 7α-hydroxycholesterol, 7α-25-dihydroxycholesterol, 7α-27-dihydrocholesterol, and 3β-7α-dihydroxy-5-cholestenoic acid, and participates in bile acid biosynthesis (98, 99) (Fig. 7). Furthermore, genetic studies of a kindred affected with 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency, which is associated with hepatic failure in childhood, showed no genetic linkage to the *HSD3B* cluster (100). In fact, such hepatic and extrahepatic activity was practically unaffected by trilostane, a well-known C_{19}/C_{21} 3β-HSD inhibitor (99). Gene structure of *HSD3B7*, as well as positioning of disease-associated mutations on corresponding nucleic and amino acid sequences, is represented in Fig. 8. Moreover, it has recently been suggested that the alcohol dehydrogenase γγ isoenzyme is the sole 3β-HSD using bile acids as a substrate in human liver cytosol (101). Also, it has been demonstrated that the X-linked dominant male-lethal phenotype gene mutated in bare patches and striated mice encodes a novel 3β-HSD (102). This gene encodes an NADPH enzyme, which is likely to be involved in cholesterol biosynthesis and shares only 30% identity with other mammalian 3β-HSD enzymes, thus supporting the phylogenetic divergence between the C_{19}/C_{21} 3β-HSD/Δ^5-Δ^4 isomerase and the other enzymes involved in bile acid metabolism and/or biosynthesis of cholesterol.

E. Enzymatic characteristics of the 3-KSRs (rat liver-specific type III, mouse types IV and V, and hamster type III)

As mentioned briefly above, the rat type III protein (75) does not display oxidative activity for the classical substrates PREG, DHEA, Δ^5-DIOL, and 3β-DIOL, but instead is a specific 3-KSR responsible for the conversion of 3-keto saturated

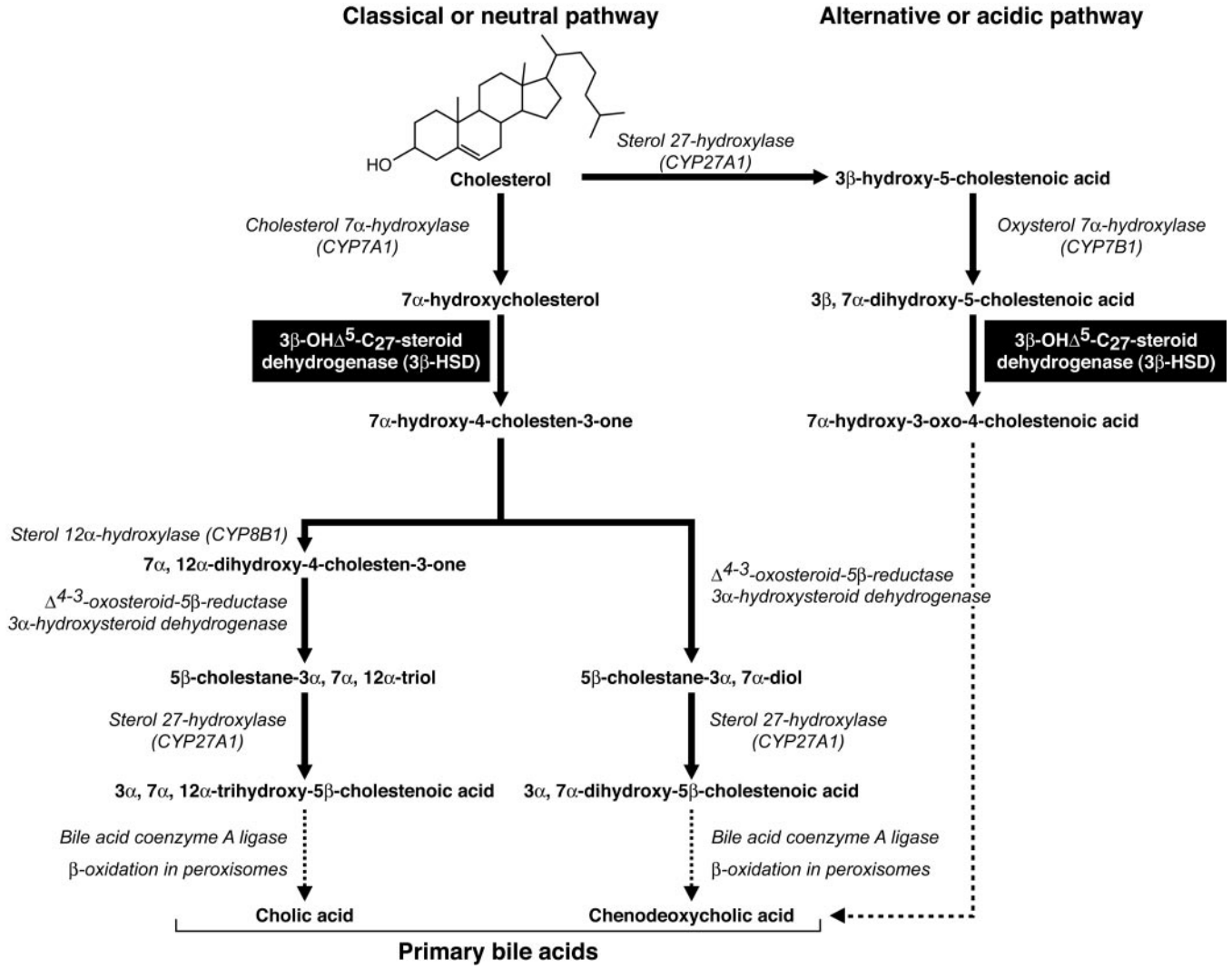


FIG. 7. Major bile acid biosynthesis pathways. Two major bile acid biosynthesis pathways are shown. Only major enzymes and intermediates are shown.

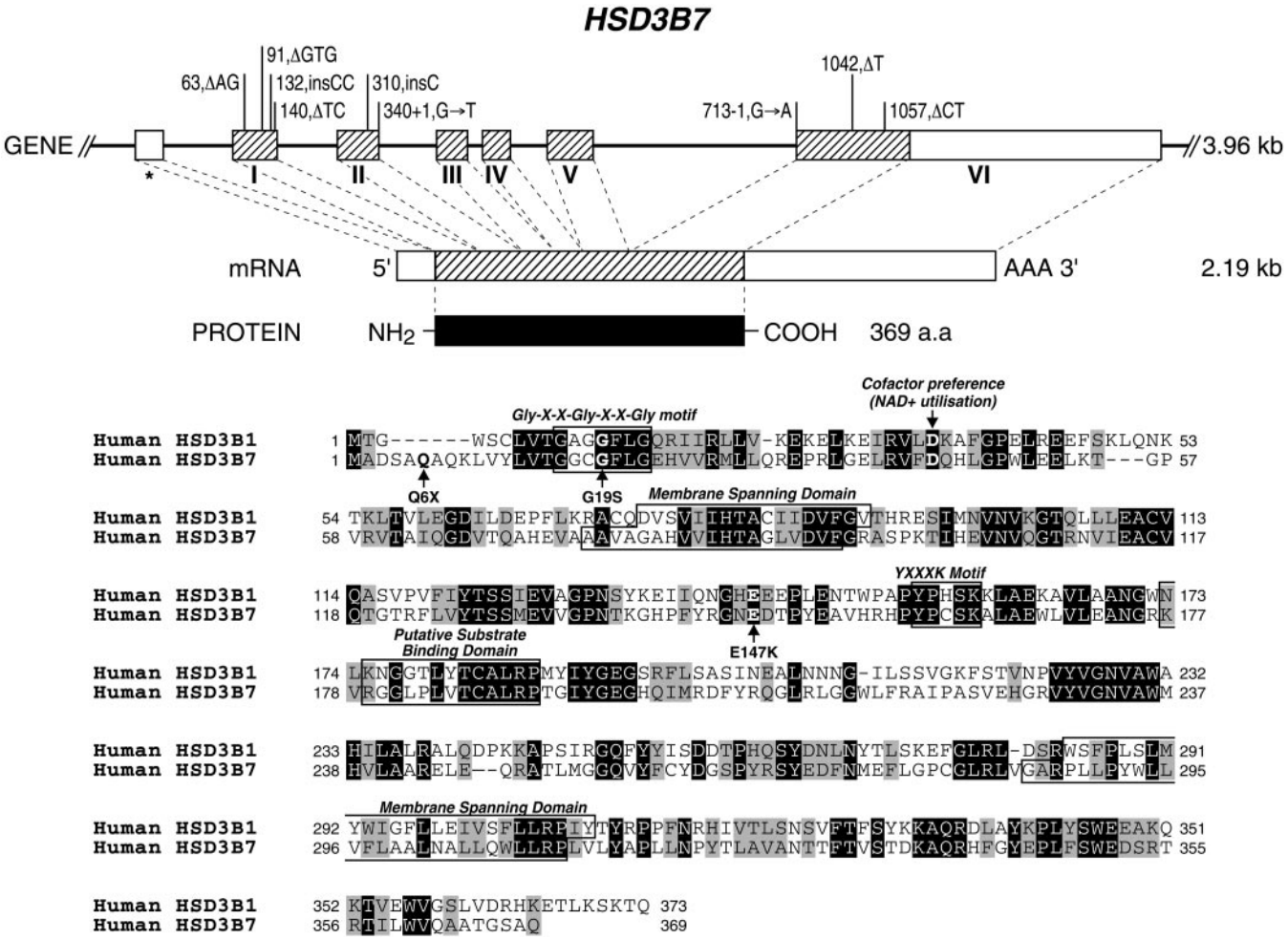


FIG. 8. *Top*, Schematic representation of *HSD3B7* gene, mRNA, and corresponding protein. Exons are represented by *hatched boxes* indicating coding region, whereas *open boxes* represent noncoding regions. *Asterisk* represents an alternative noncoding exon. Introns are represented by *black bold lines*. Mutations causing progressive intrahepatic cholestasis are identified on the gene. The nucleotide numbers indicating the positions of individual mutations refer to C_{27} 3β-HSD cDNA (GenBank accession no. AF277719). *Bottom*, Alignment of amino acid sequences of human type I and type VII 3β-HSD. Residues common to both types are identified by *black areas*, whereas similar residues are identified by *gray areas*. Positions of mutations causing progressive intrahepatic cholestasis are identified by an *arrow* in reference to amino acid sequences on the primary structure. [Adapted from Ref. 591.]

steroids, such as DHT and DHP, into inactive steroids using NADH phosphate (NADPH) as cofactor instead of NADH (86). In addition to using 5α-androstane steroids such as DHT and androstanedione as substrates, the expressed rat 3-KSR also catalyzes the 3β-reduction of DHP into 5α-pregnane-3β,20β-diol (84). The K_m and V_{max} values of the expressed 3-KSR protein using DHP as substrate and NADPH as cofactor were calculated to be 0.24 μM and 0.83 nmol/min·mg protein, respectively. In comparison, the K_m value of the expressed type I 3β-HSD isoenzyme, also using DHP as the substrate in the presence of NADH as cofactor, was 0.55 μM, whereas the calculated V_{max} value was 0.18 nmol/min·mg protein (84).

Examination of the 3β-HSD isoenzymes shows a typical βαβ dinucleotide-binding fold with Asp (36) located in the position predicted for the acidic residue that participates in hydrogen bond formation with the 2′-hydroxyl moiety of the adenosine ribose of all known NAD-dependent dehydrogenases (Fig. 2). Using site-directed mutagenesis, it has been shown that the presence of a Tyr residue instead of an Asp residue at position 36 in the typical βαβ dinucleotide-binding fold of the cofactor binding domain of rat type III is responsible for the difference in cofactor specificity of the rat 3-KSR (type III) protein, but this alteration is not sufficient to explain the low activity of the enzyme with Δ^5-3β-hydroxysteroid substrates (103). The physiological importance of this peculiar member of the rat 3β-HSD family is well supported by the finding that mouse types IV and V and hamster type III also possess this specific 3-KSR activity (88, 91, 96). The 3-KSR activity of these three latter enzymes is most likely due to the presence of Phe (36) in place of Arg (36), as suggested by the data resulting from the study on Asp (36) in the rat type III sequence.

F. 17β-HSD activity of rat type I and IV 3β-HSDs

Using cell homogenate preparations, it was first noted that the rat type I 3β-HSD protein has androgenic 17β-HSD-like

activity. It was found that the affinity for the DHT substrate by the enzyme is similar to that of the substrates for 3β-HSD activity, although with a much lower velocity (86). However, using intact cells transfected with the rat type I, it was found that the enzyme catalyzed almost exclusively the conversion of DHT into androstanedione via the 17β-HSD-like activity. The intrinsic 17β-HSD activity of rat types I and IV 3β-HSD is specific to 5α-androstane-17β-ol steroids, therefore suggesting that it plays a key role in controlling the bioavailability of the active androgen DHT.

The predominance of this secondary 17β-HSD activity over the primary 3β-HSD activity in intact cells most likely results from the high bioavailability of NAD^+ in mammalian cells relative to the low levels of the intracellular pool of NADH. The apparent discrepancy between the oxidoreductase activity of the rat type I and IV proteins, as revealed by the interconversion of DHT and 3β-DIOL using homogenate preparations from mammalian cells expressing the recombinant isoenzymes, and the lack of significant 3-KSR activity, measured in intact cells in culture expressing the same recombinant enzymes, can also be explained by the low levels of the intracellular pool of NADH relative to the high bioavailability of NAD^+ in mammalian cells. In agreement with this explanation, in cultured HeLa, JEG-3, and SW-13 cells expressing rat types I and IV 3β-HSDs, Δ^5-hydroxysteroid precursors are efficiently converted into their corresponding Δ^4-3-keto steroids, which is well known to require NAD^+ as the allosteric cofactor. The highly efficient conversion of DHT into 3β-DIOL in cultured HeLa cells expressing rat 3-KSR (type III) also argues in favor of the bioavailability of NADPH within the cells (84, 86). It is also of interest that 3α-HSD is known to use NADPH as a cofactor, which is in agreement with the predominant metabolic pathways using NAD^+ or NADPH as cofactors postulated to be present in transfected cells in culture. These results also emphasize the important fact that assessment of enzyme activity should always be performed in intact cells to more closely resemble the situation *in vivo*, an issue that will be discussed in more detail in *Section VII*.

In relation to an enzyme having dual activity, such secondary activity could be explained by the binding of the steroid in the inverted substrate orientation, in this case from the C-17 rather than C-3 extremity, to the same active site responsible for the primary activity of the enzyme. The physiological relevance of this secondary 17β-HSD activity is also supported by the observation that the purified bovine adrenal 3β-HSD enzyme (20) also possesses the 17β-HSD-like activity (84, 86). Moreover, it was recently shown that mouse types I and VI 3β-HSD isoforms display significant 17β-HSD-like activity (617).

Another enzyme known to catalyze the oxidation of both 3β- and 17β-hydroxy groups of certain hydroxysteroids is the NAD^+-dependent 3β-17β-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* (104). The elucidation of the cDNA sequence of this enzyme revealed that it is a member of the short-chain dehydrogenase/reductase superfamily, however, sharing more homology with the 17β-HSD than with the 3β-HSD enzymes (105). It is also known that some members of the 17β-HSD family, such as human type 2 (106) and human type 7 (107) 17β-HSDs, possess dual 3β/17β activity.

V. Transcriptional Regulation of Human Type I and II 3β-HSD

The human *HSD3B2* gene is the major form expressed in the adrenal cortex, ovary, and testicular Leydig cells. It is most homologous to the type I gene expressed in mice, rats, and other species. The different gene isoforms are so named because of their chronological isolation. The current state of knowledge concerning the transcriptional control human *HSD3B2* gene will be discussed first.

A. Gonadal/adrenal isoenzyme—type II 3β-HSD

1. Steroidogenic factor-1 (SF-1). Initial studies investigating the transcriptional regulation of the human *HSD3B2* gene primarily focused on the trophic hormones known to regulate expression of other genes, including ACTH in the adrenal cortex, LH/human chorionic gonadotropin (hCG) in theca cells and corpus luteum (CL), and LH in testis Leydig cells. Until fairly recently, data regarding the transcriptional regulation of 3β-HSD was highly limited. After the isolation and sequencing of genomic clones for human type II 3β-HSD, several studies analyzed the promoter and 5′-flanking regions looking for regulatory elements essential for expression and regulation in steroidogenic cells. Initial examinations of the sequence were not very revealing. Because cAMP was a known intracellular mediator of trophic hormone stimulation of 3β-HSD expression, it was interesting that no identifiable cAMP response elements were observed in the proximal 1.3 kb of the 5′-flanking sequence. There were two putative activator protein-1 (AP-1) elements at −576 and −977 that matched functional AP-1 elements in other genes (*e.g.*, the sequence at −977 is an exact match of an AP-1 element in the simian virus 40 promoter). This limited information formed the basis of the initial functional studies of the 3β-HSD promoter by transfection into steroidogenic cells.

The H295R human adrenocortical carcinoma cell line was chosen as a steroidogenic cell model due to its relatively high level of differentiated functions, including responsiveness to adrenal trophic hormones such as ACTH and synthesis of cortisol. Interestingly, these cells have characteristics of all three zones of the normal adrenal gland, the zona reticularis (ZR), zona fasciculata (ZF), and ZG (108). As such, these cells may represent a pluripotent adrenal cortical stem cell that transformed before terminal differentiation. The H295R cells can be cultured under different specific conditions that promote the differentiated characteristics of cells from the individual zones (108). In these studies, the cells were treated with activators of the protein kinase A (PKA) pathway, such as forskolin and dibutyryl cAMP, to increase the expression of enzymes P450 17α-hydroxylase/17,20-lyase (P450c17), 3β-HSD, and P450c11 as well as cortisol production, thereby promoting the characteristics of ZF cells. Promoter-reporter constructs were prepared that used a series of 5′-deletions of the human *HSD3B2* 5′-flanking sequence and promoter, fused to the chloramphenicol acetyltransferase (CAT) gene. Transfections were performed, and the cells were treated in

the presence and absence of phorbol ester stimulation. The surprising finding of these initial studies was that the putative AP-1 elements were nonfunctional with respect to either cAMP or phorbol ester stimulation. Both putative AP-1 elements could be removed without affecting the cAMP- or phorbol ester-stimulated promoter activity, with 5′-deletion down to −100 bp. The promoter-reporter constructs did not lose responsiveness to cAMP or phorbol ester stimulation until a further deletion was made to −52 (109).

The sequence between −100 and −52 contained an element at −64 to −56 that is an 8/9 match for the consensus regulatory element that binds the orphan nuclear receptor SF-1, also referred to as adrenal 4 binding protein. New nomenclature for members of the nuclear receptor superfamily designates this family as NR5A and SF-1 as NR5A1a (110). The essential nature of the SF-1 element was tested further using two approaches. First, promoter-reporter constructs were transfected into a nonsteroidogenic cell type that does not express SF-1 (HeLa cells) in the presence and absence of cotransfection with an SF-1 expression vector. Second, the importance of SF-1 was tested with the use of a 2-bp point mutation in the element (from TCAAGGTAA to TCAATTTAA), whereas all other sequences in the −100-bp *HSD3B2* gene promoter remained as in the wild type. The 2-bp point mutation in the SF-1 element dramatically abrogated the cAMP and phorbol ester response of the promoter (109). These studies indicated that SF-1 was essential for cAMP or phorbol ester-stimulated steroidogenesis and suggested that trophic hormone stimulation of type II 3β-HSD expression involved SF-1 activation.

The mechanisms by which cAMP and phorbol esters stimulate SF-1-mediated transcription of the *HSD3B2* gene are not yet clear. In the GnRH promoter, another SF-1 responsive gene, the essential response of SF-1 involves an interaction between SF-1 and another transcription factor, early growth response protein (111, 112). However, the *HSD3B2* promoter differs from the GnRH promoter in that it lacks proximal early growth response recognition sequences. However, it is possible that the interaction of SF-1 with another yet undescribed transcription factor is a necessary component to the cAMP/phorbol ester stimulation.

2. Stat5. The involvement of transcription factors other than SF-1 in the control of the human *HSD3B2* gene has become evident following the discovery of a regulatory element, which interacts with Stat5. The Stat family of proteins is named for the acronym (signal transducers and activators of transcription). The Stats comprise a family of cytoplasmic transcription factors that are activated by tyrosine kinases followed by nuclear translocation and binding to specific regulatory elements (reviewed in Ref. 113). The Stats are activated by a number of extracellular protein ligands including cytokines, growth factors, and prolactin (PRL)/GH. The activation involves either tyrosine kinase activity that is intrinsic to the receptors for these ligands or tyrosine kinases that associate with the receptors such as the Janus kinases. Seven different Stat genes have been identified (Stats 1, 2, 3, 4, 5a, 5b, and 6). Targeted gene disruptions have been performed for each of these seven Stat genes in mice, resulting in different phenotypes, which include immune deficiencies in Stat 1 knockouts, embryonic lethals in knockouts of Stats 2 and 3, and deficient breast development and lactation in Stat5 knockouts. Interestingly, the Stat5a Stat5b double knockout displays luteal failure (114), which involves one of the key tissues expressing 3β-HSD.

Stat5 was independently identified and cloned in studies concerning a transcription factor important for PRL activation of the β-casein promoter in mammary epithelial cells (115, 116). The potential involvement of PRL and Stat5 in the human 3β-HSD promoter is an intriguing concept. Prolactin and placental lactogens are known to be important luteotropic hormones in many species including bovine, porcine, and rodent (117). The human placenta expresses human placental lactogen, which was previously referred to as human chorionic sommatomammotropin. The involvement of PRL as a potential luteotropic hormone in humans has not been examined in detail, although conditions of hypo- and hyperprolactinemia are known indicators of female infertility (118, 119).

Two independent findings suggested the possibility that Stat5 could be mediating regulation of the 3β-HSD promoter. First, analysis by Western blot showed that Stat5 was induced on d 5 (after hCG) in pseudopregnant rat ovaries approximately 10-fold over controls (120). This was a specific effect because Stat3 levels remained unchanged. These data suggested that Stat5 was being specifically induced during luteinization and that it could be involved in mediating the regulation of luteal function. Because this represents an important site of Stat5-mediated activity, it suggested a role for Stat5 in luteal function. Stat5 is expressed in a wide variety of tissues, and its expression is fairly constitutive. However, it has been shown to be induced in mammary epithelial cells during differentiation of the mammary gland into the active lactation state (121). A second finding was the discovery of a 9/9 match for a Stat5 consensus regulatory element in the human *HSD3B2* gene promoter during the examination of SF-1 action. The sequence TTCTGAGAA at −118 to −110 is a 9/9 match to the consensus regulatory element TTCNNNGAA for Stat5 (Fig. 9). These findings suggested a potential regulatory role for Stat5.

Transfection studies have confirmed that PRL activates Stat5 regulation of the human *HSD3B2* promoter (122). Point mutations of the Stat5 regulatory element abrogate the PRL response, both the fold-stimulation and the levels of stimulated activity. However, disruption of Stat5 action does not totally remove PRL stimulation. It is possible that some of the alternative signaling pathways stimulated by PRL may contribute to PRL-stimulated 3β-HSD expression.

The functional Stat5 element in the *HSD3B2* promoter may also integrate signaling from other ligand-stimulated pathways. For example, angiotensin II has been shown to stimulate Stat5 in cardiac myocytes (123). A similar signaling pathway in adrenal ZG cells would allow angiotensin II stimulation of 3β-HSD expression in the synthesis of mineralocorticoids. Another ligand that could utilize this pathway is EGF. Stat5 has been shown to be the major Stat protein activated in EGF stimulation of the mouse liver (124). Recent studies have demonstrated that EGF stimulates cortisol synthesis in H295 adrenocortical cells as well as stimulating 3β-HSD mRNA levels and promoter activity (125). Further-

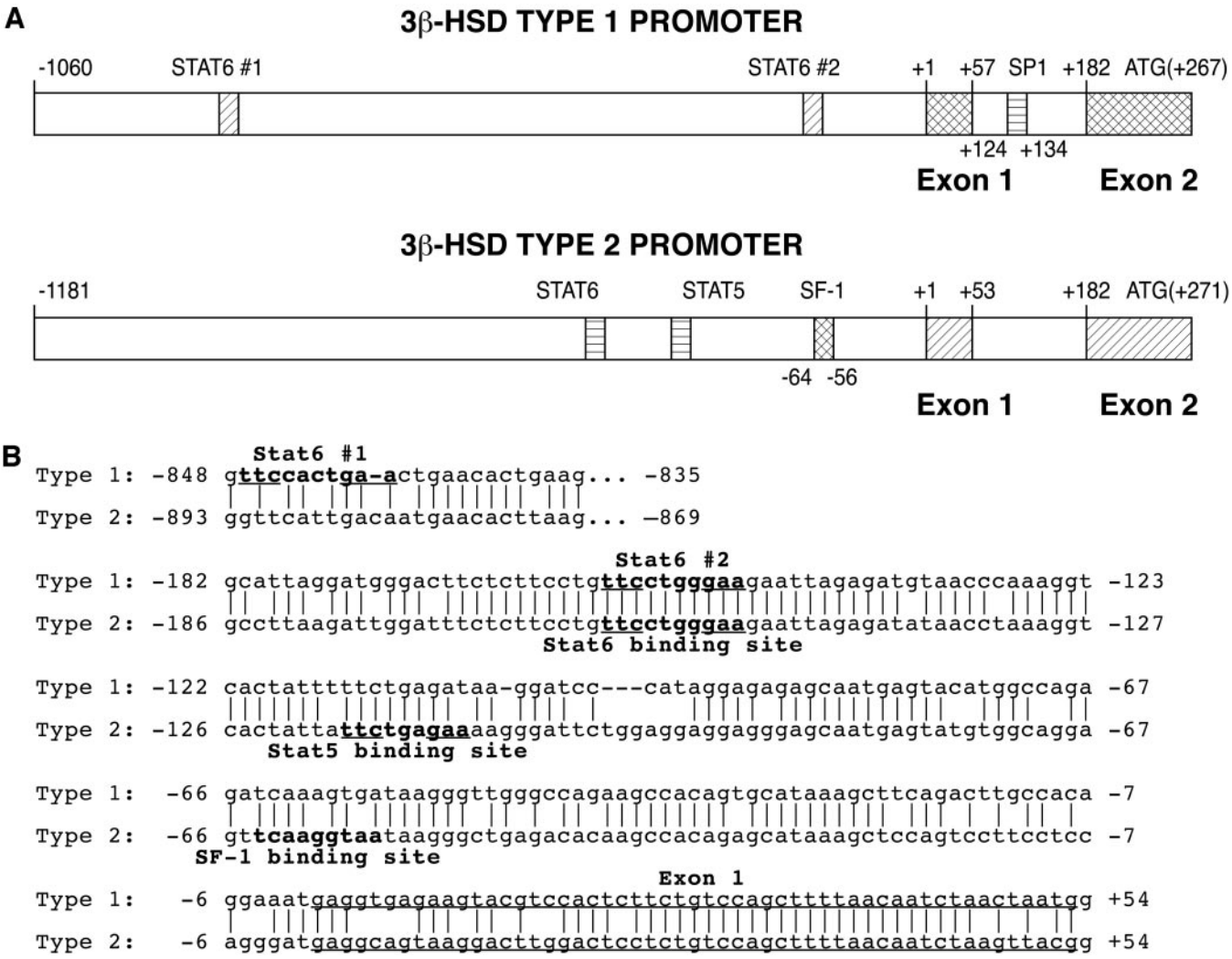


FIG. 9. Schematic representation of type I and II 3β-HSD promoter regions (A) and alignment of the promoter sequences (B). [Reproduced from S. Gingras *et al.*: *J Steroid Biochem Mol Biol* 76:213–225, 2001 (196), copyright 2001, with permission from Elsevier.]

more, Stat5 and a functional Stat5 regulatory element in the *HSD3B2* promoter are required for this stimulation (Fig. 10) (125).

Stat proteins are important for the transcription of other steroidogenic enzyme genes. For example, Stat3 has been shown to be important for aromatase (*CYP19*; $P450_{arom}$) expression in adipocytes (126). The aromatase gene has multiple promoters that are used in tissue-specific expression and regulation (126). Interestingly, the adipocyte promoter activity utilizing the Stat3 element requires glucocorticoids for maximal activity (126). Although the aromatase adipocyte promoter contains a glucocorticoid response element, it is not clear whether additional glucocorticoid effects can occur through interactions between Stat3 and the glucocorticoid receptor.

3. DAX-1. DAX-1 (dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome gene 1) was originally isolated by positional cloning from patients presenting with adrenal congenita hypoplasia associated with hypogonadotropic hypogonadism (127, 128). Mutations in DAX-1 have been found to be the cause of adrenal hypoplasia congenita and hypogonadotropic hypogonadism. The possible role of an atypical member of the nuclear hormone receptor superfamily on 3β-HSD promoter activity has come from studies examining the effects of DAX-1 overexpression on adrenal cell steroidogenesis and steroidogenic enzyme gene expression. The structure of DAX-1 indicates that it lacks the most highly conserved DNA-binding domain typical of members of the nuclear hormone receptor superfamily (127). This has raised questions as to whether this transcription factor binds to DNA. Data have been presented suggesting that DAX-1 has unique mechanisms of DNA binding through putative stem-loop structures (129). An alternative hypothesis is suggested by the sequencing of DAX-1 from another species, which apparently lacks the analogous domain necessary for DNA binding (130). This implies that possible protein-protein interactions between DAX-1 and other transcription factors may be critical to the function of this orphan nuclear receptor. Additional work in this field is needed to determine the mechanisms of DAX-1 actions.

The overexpression of DAX-1 in Y-1 adrenal cells inhibits

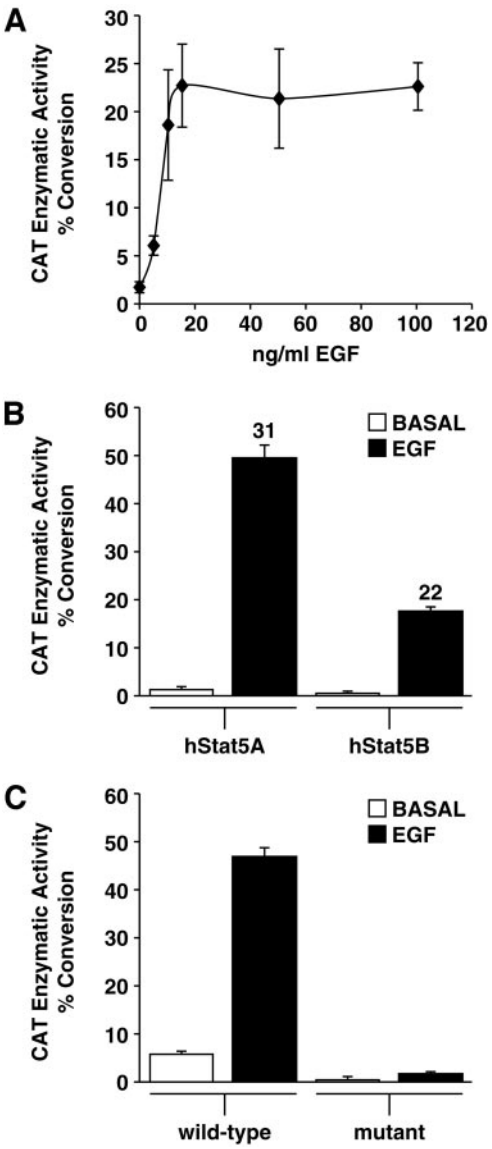


FIG. 10. EGF activates type II 3β-HSD reporter activity through a Stat5-dependent mechanism. A, Increasing concentrations of EGF result in increased type II 3β-HSD reporter activity. HeLa cells were cotransfected with a −301→+45 fragment of the type II 3β-HSD promotor fused to a CAT reporter gene (−301 CAT; 5 μg), ovine Stat5 (5 μg), β-galactosidase (0.5 μg), and control DNA for a total of 15.5 μg using the calcium phosphate precipitation method, followed by treatment for 24 h with increasing doses of EGF (0→100 ng/ml). B, hStat5A and hStat5B isoforms in activation of 3β-HSD reporter activity. HeLa cells were cotransfected with a −301→+45 fragment of the type II 3β-HSD promotor fused to a CAT reporter gene (−301 CAT; 5 μg), hStat5A, or hStat5B (5 μg), β-galactosidase (0.5 μg), and control DNA for a total of 15.5 μg using the calcium phosphate precipitation method, followed by treatment for 24 h with EGF (25 ng/ml). The *number above the bar* is fold activation compared with the identically transfected group minus EGF. C, A Stat5 regulatory element confers EGF responsiveness to the human type II 3β-HSD promotor region. HeLa cells were cotransfected with −301 (wild-type) or −301 (mutant) CAT reporter contructs (5 μg), ovine Stat5 (5 μg), β-galactosidase (0.5 μg), and control DNA for a total of 15.5 μg using the calcium phosphate precipitation method, followed by treatment for 24 h with EGF (25 ng/ml). Data represent the mean ± SE of triplicate cultures after correction for transfection efficiency from a representative experiment of two performed. [Reproduced from F.A. Feltus *et al.*: *Endocrinology* 144: 1847–1853, 2003 (125), copyright 2003, The Endocrine Society.]

steroidogenesis (131). Associated with this inhibition are strong inhibitory effects on the expression of mRNAs for StAR, P450scc, and 3β-HSD (Fig. 11) (131). The exact mechanisms by which DAX-1 overexpression affects 3β-HSD expression remain unclear. Additional studies of these mechanisms are needed to elucidate the factors involved.

4. Steroids. There is growing evidence in the literature that steroid hormones modulate type II 3β-HSD expression. For example, glucocorticoids stimulate the expression of 3β-HSD in adrenal cells (132), whereas androgens inhibit 3β-HSD expression in the adrenal cortical cells and in testicular Leydig cells (133, 134). The transcriptional mechanisms by which this regulation is occurring are of high interest because the *HSD3B2* gene promoter and 5′-flanking sequence lack clear homologous consensus steroid regulatory elements. One possible explanation of these findings is that the steroid hormones exert their effects using posttranscriptional mechanisms. Alternatively, the steroids could exert their actions indirectly, altering the transcription of another transcription factor, which targets the *HSD3B2* gene promoter. Lastly, another possible mechanism is that the steroid hormones and their cognate nuclear receptors are acting via nonclassical mechanisms to alter transcription. Accumulating evidence indicates that nuclear hormone receptors can alter transcription via protein-protein interactions with other classes of transcription factors and that these interactions do not require direct DNA binding by the receptors (135). Steroid receptors have been shown to interact with Stat proteins (136), AP-1 (137), nuclear factor-κB (138), and Sp1 (139) proteins and to alter transcription in a ligand-dependent manner.

It has been recently demonstrated that glucocorticoids stimulate type II 3β-HSD mRNA levels in H295R cells and stimulate *HSD3B2* gene promoter activity (125). The mechanisms by which this stimulation occurs are beginning to be characterized and are linked to Stat5. Inactivating point mutations in the Stat5 regulatory element of the *HSD3B2* gene promoter abolishes glucocorticoid regulation (125). Additionally, point mutations in Stat5, which convert the critical tyrosine phosphorylation residue to a phenylalanine and abolish Stat5 activation, also abolish its regulation by glucocorticoids. These data indicate that Stat5 is critical to the mechanism of *HSD3B2* gene promoter activation by glucocorticoids. The exact mechanisms involved in this action are not yet characterized but could involve protein-protein interactions between glucocorticoid receptor and Stat5, as suggested for the β-casein promoter in mammary glands (136). These data are intriguing because they point to new ways in which nuclear receptors function in transcriptional activation. They also raise a number of questions concerning the established mechanisms of nuclear receptor action. For example, the domains of the nuclear receptor proteins, which are critical for different aspects of their function, have been mapped out in relation to the traditional DNA binding-dependent mechanism of action. With these new nontraditional mechanisms, which domains of the nuclear receptors are critical to their functions? In addition to structure-function questions, what is the influence of known agonists and antagonists on the efficacy of activation? What is the effect of

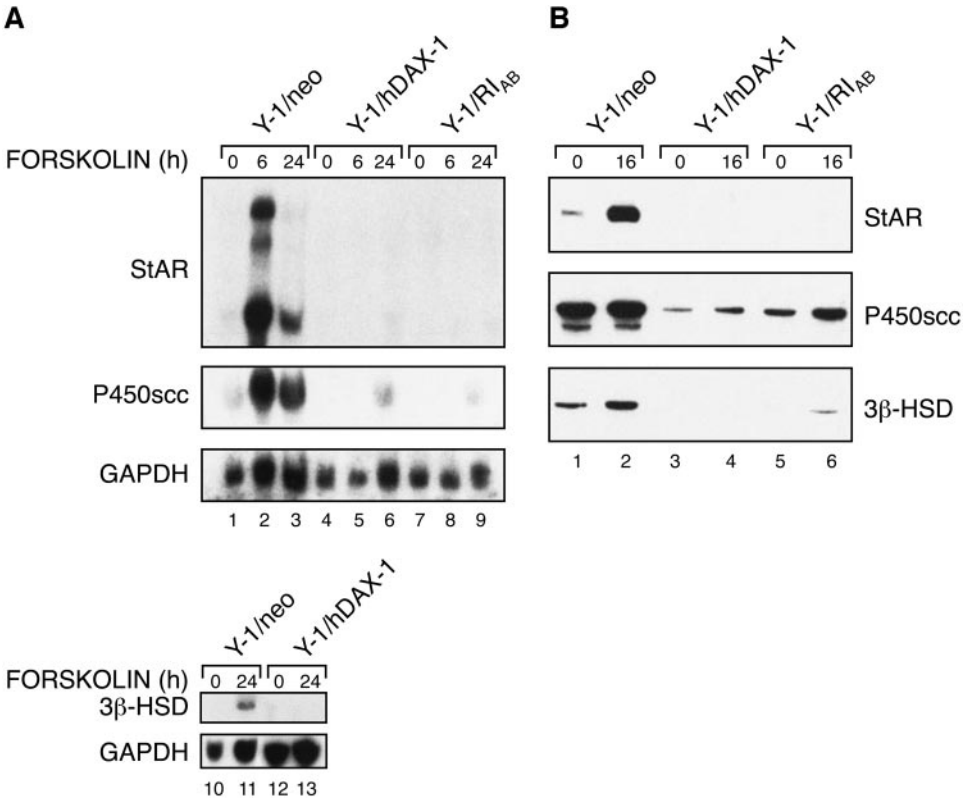


FIG. 11. Effect of DAX-1 on the expression of StAR, P450scc, and 3β-HSD in Y-1 cells. A, Northern blot showing expression of StAR, P450scc, and 3β-HSD RNA transcripts in Y-1/neo, Y-1/hDAX-1 and Y-1/RI$_{AB}$ cells. Total RNA was extracted from each cell line growing in basal conditions (lanes 1, 4, 7, 10, and 12) or after 6 h (lanes 2, 5, and 8) and 24 h (lanes 3, 6, 9, 11, and 13) forskolin (10 μg/ml) stimulation. RNA was transferred on a nylon membrane and hybridized with StAR, P450scc, 3β-HSD, and GAPDH probes. B, Western blot showing expression of StAR, P450scc, and 3β-HSD proteins in Y-1/neo, Y-1/hDAX-1, and Y-1/RI$_{AB}$ cells. Mitochondrial extracts were prepared from cell lines in basal conditions (lanes 1, 3, and 5) and after 16 h forskolin (10 μg/ml) stimulation (lanes 2, 4, and 6). Western blots were sequentially probed with specific antibodies directed to StAR, P450scc, and 3β-HSD, respectively. [Reproduced from E. Lalli *et al.*: *Endocrinology* 139:4237–4243, 1998 (592), copyright 1998, The Endocrine Society.]

other nonsteroid factors, which are known to activate other intracellular signaling pathways on steroid-regulated transcription? With these new mechanisms, each of these questions will need to be explored to characterize the essential processes.

5. IL-4. Immune cell populations in the ovary change during the reproductive cycle and cytokines from these immune cells have been shown to affect steroidogenesis (140). Recent data indicate that IL-4 stimulates 3β-HSD mRNA levels in primary cultures of human granulosa-lutein cells (141). IL-4 has been shown to primarily activate Stat6 through Stat6 regulatory elements, one of which is present in the human *HSD3B2* promoter at −160 to −151 (Fig. 9). However, the activation of Stat protein signaling often involves multiple Stat proteins with some overlap in function (113). Although IL-4 stimulation is associated with Stat6 activation, IL-4 stimulation of *HSD3B2* gene promoter activity requires both an intact Stat5 and an intact Stat6 regulatory element (141).

6. GATA proteins. The GATA proteins are a family of zinc finger transcription factors that bind to GATA regulatory motifs (A/TGATAA/G) in the promoter regions of numerous target genes. Although they were originally identified as crucial regulators of hematopoietic cell differentiation and heart development, the expression of GATA factors is not limited to these two tissues. Interestingly, transcription factors belonging to the GATA family are emerging as novel regulators of steroidogenesis. Indeed, members belonging to this family, namely GATA-4 and GATA-6, are strongly expressed in steroidogenic cells of both the fetal and adult adrenals and gonads (142). In these tissues, several target genes for GATA factors have been identified, such as *StAR* (143–146), *CYP11A* (P450 side-chain cleavage) (147), *SULT2A1* (618), *CYP17* (17α-steroid hydroxylase) (147–150), *HSD17B1* (17β-HSD type 1) (151), *CYP19* (aromatase) (143), human *HSD3B1* (3β-HSD type 1) (152), and most recently human *HSD3B2* (3β-HSD type 2) (153). The human *HSD3B2* promoter contains four consensus GATA elements within the first 1000 bp upstream of the transcription start site and is potently activated by both GATA-4 and GATA-6 in cooperation with the nuclear receptors SF-1 (NR5A1) and liver receptor homolog-1 (NR5A2). This suggests that GATA factors are key regulators of this gene and that deregulated GATA expression and/or activity might be relevant to pathological processes associated with aberrant *HSD3B2* expression such as adrenal insufficiency, male pseudohermaphroditism, and polycystic ovary syndrome (PCOS).

B. Future directions in transcriptional regulation

One area of future studies will be to follow up on new developments in the orphan nuclear receptors SF-1 and DAX-1. The importance and effectiveness of these factors in the regulation of the 3β-HSD expression as well as emerging information on their action can be applied to developing insights into the regulation of 3β-HSD and its potential interaction with other intracellular signaling pathways. For example, there is a growing body of evidence suggesting that SF-1 interacts with other transcription factors such as Egr-1 (111). In addition, SF-1 has been suggested to interact with other transcription factors (154) as well as coactivators and integrating proteins (155, 156). As patterns of interactions

emerge, they may provide insight into mechanisms important for understanding the regulation of 3β-HSD.

Another potential area of future interest relates to regulation by members of the TGFβ family. Members of the TGFβ family including TGFβ have been shown to regulate 3β-HSD mRNA levels in adrenal cells (157). Another potential area of interest relates to regulation by members of the nerve growth factor-induced clone B (NGFI-B) family. Nur77, a member of the NGFI-B family, was shown to regulate type II 3β-HSD mRNA levels in granulosa (158), as well as Leydig and adrenal cells (619). Nur77 stimulates transcription of *HSD3B2* through action at a NBRE site (−124/−131 bp from transcription start site) on the promoter and is only minimally affected by SF-1. Furthermore, Nur77 synergizes with SRC coactivators (SRC-1/2/3) to further enhance *HSD3B2* promoter activity in Leydig and adrenal cells (619). Taken together, the identification of Nur77 as a regulator of *HSD3B2* expression helps better define the tissue-specific and hormonal regulation of *HSD3B2* gene in steroidogenic cells.

C. Regulation of placenta/peripheral tissue type I 3β-HSD

The *HSD3B1* gene encoding the type I 3β-HSD shares a relatively high degree of homology with the *HSD3B2* gene, not only over the coding sequence but also over the promoter and 5′-flanking sequence (Fig. 9). Long stretches of the promoter up to approximately 1 kb upstream of the transcription start site share more than 80% nucleotide sequence homology with the *HSD3B2* promoter. Although this degree of homology might suggest some common regulatory mechanisms, very small changes in critical core nucleotides of regulatory elements can disrupt their function. Therefore, careful functional studies will be needed to map the critical regulatory elements of the *HSD3B1* gene and to compare and contrast these elements to those of its homologous *HSD3B2* gene.

1. Placenta. Initial studies of the *HSD3B1* gene in placental cells indicated that stimulation with phorbol esters increased gene expression and that this effect could not be blocked with protein synthesis inhibitors (159). The regulatory elements important for transcription of the *HSD3B1* gene in human placental cells have not been fully mapped. Some studies have suggested that some of the important regulatory elements for this gene are localized in the first intron of the *HSD3B1* gene (160) with an element overlapping an Sp1 motif. Recently, a 53-bp placental-specific enhancer element located between −2570 and −2518 of the *HSD3B1* promoter was identified. Within this 53-bp element, two potential placental transcription factor binding sites were found, one identified as being a specific binding site for the transcription enhancer factor-5 (TEF-5), which is highly expressed in human placenta, and the other being an overlapping binding site specific for a GATA-like protein. Because the expression of P450scc and *HSD3B1* is essential for the biosynthesis of PROG, one would assume that their expression is coordinated. It will be of interest in future experiments to investigate whether TEF-5 and GATA proteins also determine the human placental-specific expression of P450scc (152). Additional studies are needed in this area, particularly using normal, physiological models. In this regard, it is interesting that expression of type I 3β-HSD mRNA levels increases during placental development, during the process of syncytium formation (161). If this process involves transcriptional changes, this may provide a model for investigations of the critical regulatory elements in placental expression of 3β-HSD.

2. Crucial role of IL-4 and IL-13 in peripheral tissues. In animal species as well as in humans, there are several reports suggesting that cytokines can regulate the expression of several steroidogenic enzymes. For example, IL-1, IL-2, or IL-3 and TNF-α exhibit either stimulatory or inhibitory effects on steroid production in ovaries (140, 162–164). IL-1, IL-4, IL-6, IGF-I, and IL-2 modulate 17β-HSD activity in breast cancer cells lines (165–167). And finally, IL-1 and IL-6 regulate aromatase expression in breast cancer cells and in adipose tissues (168, 169).

IL-4 is produced by T cells, mast cells, and basophils and exerts its activities on many immune cells, including B and T lymphocytes, mast cells, and macrophages. The most specific effects of IL-4 are differentiation of T cells to the Th2 phenotype (secretion of IL-4, IL-5, IL-6, IL-10, and IL-13), and Ig class switching to IgE in B cells (reviewed in Ref. 170). On the other hand, the IL-4 receptor (IL-4R) is also expressed in certain nonimmune cells, thus suggesting that IL-4 may regulate some functions within those cells.

It has recently been shown that IL-4 and IL-13 induce 3β-HSD type I gene expression in ZR-75-1, T47-D, and MDA-MB-231 breast cancer cells; in HT-29 colon and ME-180 cervical cancer cell lines; in the HaCaT immortalized keratinocytes; as well as in normal human mammary and prostate epithelial cells in primary culture (Figs. 12 and 13) (51, 52, 171). The IL-4-induced expression of the *HSD3B1* gene requires new mRNA synthesis, but not new protein synthesis, thus suggesting that this induction takes place at the transcriptional level by activating latent transcription factors (Fig. 12) (51).

a. STAT6 activation. Studies of IL signal transduction have clarified the mechanism by which IL-4 functions. Stat6 is an IL-4-activated transcription factor (172, 173). Indeed, recent experiments performed on Stat6-deficient mice demonstrated that Stat6 plays an essential role in IL-4 and IL-13 signaling (174–176). Analysis of the sequences upstream of the *HSD3B1* gene revealed the presence of two sequences matching the Stat6 consensus sequence (TTCNNNNGAA) (Fig. 9). The distal site (Stat6#1) is located at position −847 to −838, and the proximal site (Stat6#2) is located between −156 and −137. Stat6 binds in an IL-4-dependent fashion to the 3β-HSD type 1 Stat6#1 and Stat6#2 probes as revealed using EMSA. No such complexes were formed on mutated probes containing two single-base pair substitutions in the Stat6 consensus sequence at positions that disrupt Stat6-DNA binding (51). IL-4 activated Stat6 in all cell lines studied in which IL-4 also induced 3β-HSD type I expression, but not in those cell lines that failed to respond to IL-4 (51, 52). Furthermore, the lack of a stimulatory effect of IL-4 on 3β-HSD type 1 expression in some cell lines cannot be explained by the absence of Stat6 expression, because this protein was expressed in the IL-4 nonresponsive PC-3, Caco-2, JAR, and JEG-3 cell lines. Rather, our study shows that the cell-specific

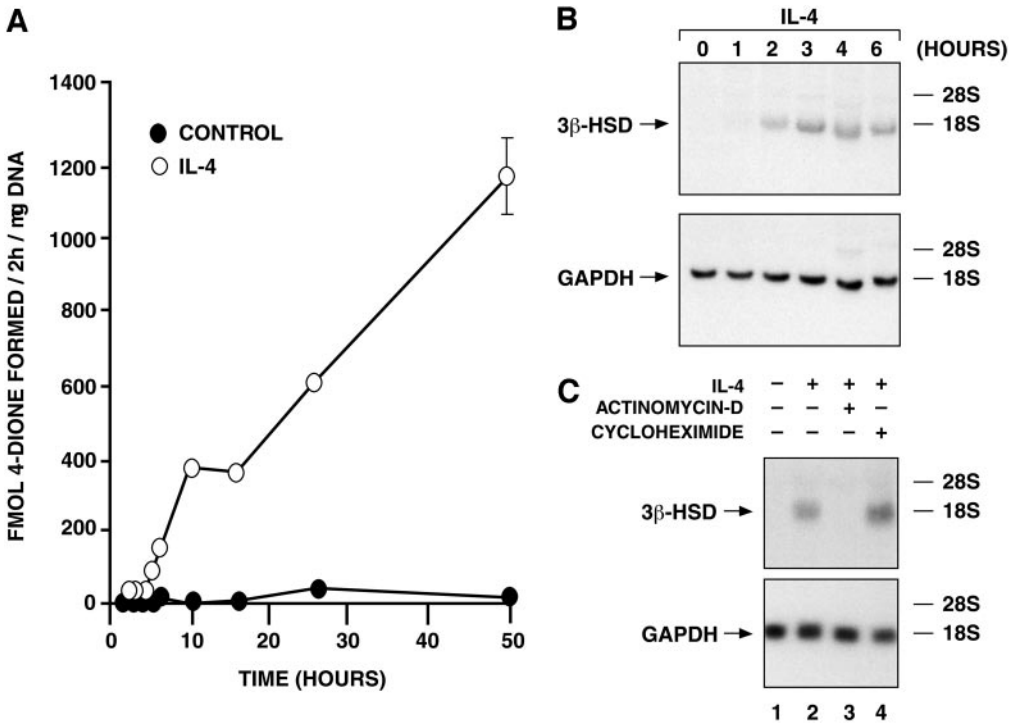


FIG. 12. Rapid induction of 3β-HSD activity and 3β-HSD type I transcripts by IL-4 in ZR-75-1 human breast cancer cells. A, Induction of 3β-HSD activity. Cells were plated at a density of 40,000 cells per well. Three days after plating (time 0), ZR-75-1 cells were incubated for the indicated time periods with or without 140 pM IL-4. During the last 2 h of each incubation period, medium was replaced with fresh medium containing [^{3}H]DHEA in the presence or absence of IL-4. Data are expressed as mean ± SEM of triplicate dishes. B, Increase of type I 3β-HSD mRNA levels. Cells were treated with 140 pM IL-4 for the indicated time periods. Northern analysis was performed, and the membrane was probed with type I 3β-HSD cDNA and exposed for 5 d, then stripped and reprobed with GAPDH, followed by an overnight exposure period. C, Inhibition of IL-4 induced type I 3β-HSD mRNA by actinomycin-D (4 μg/ml) but not by cycloheximide. Cells were treated for 2 h with 140 pM IL-4 in the presence or absence of actinomycin-D (4 μg/ml) or cycloheximide (10 μg/ml) before subsequent incubation for the indicated time periods with or without IL-4. [Reproduced from S. Gingras *et al.*: *Mol Endocrinol* 13:66–81, 1999 (51), copyright 1999, The Endocrine Society.]

action of IL-4 correlates with the activation of Stat6 (Fig. 13) (52).

To determine whether Stat6 DNA-binding activity was modulated by other transduction pathways (see *Section V.C.2.b*), ZR-75-1 cells were incubated with IL-4 in the presence or absence a phosphatidylinositol 3-kinase (PI3-K) inhibitor (wortmannin), a MAPK inhibitor (PD98058), or an activator of protein kinase C (PKC) pathway [phorbol-12-myristate-13-acetate (PMA)]. It has been demonstrated that neither wortmannin nor PD98059 blocked the induction of Stat6 DNA-binding activity by IL-4, whereas PMA did not change the potency of IL-4 to induce Stat6 DNA-binding activity (177). Those results indicated that Stat6 is specifically activated by IL-4 and independently of the PI3-K, MAPK, and PKC pathways. The fact that: 1) Stat6 plays an essential role in IL-4 signaling, 2) Stat6 DNA-binding sequences are present in the *HSD3B1* promoter, and 3) Stat6 is activated by IL-4 in all cell lines studied where IL-4 induced 3β-HSD type 1 expression, but not in those cell lines that failed to respond to IL-4, suggests that Stat6 could be involved in the signal transduction leading to the induction of 3β-HSD type 1 expression by this cytokine. However, we have never been able to show induction by activated-Stat6 of a reporter gene under the control of the *HSD3B1* promoter, so it was considered that other pathways could be involved for the effect of IL-4.

b. Multiple signal transduction pathways. There is evidence that IL-4 stimulates bifurcating signaling pathways in which the Stat6-signal pathway is involved in differentiation and gene regulation, whereas insulin receptor substrate (IRS) proteins mediate the mitogenic action of IL-4 (178, 179). The cytoplasmic protein IRS-1 was first identified as a major substrate for the insulin receptor and IGF-I receptor, whereas IRS-2 (also designated 4PS, IL-4 phosphorylated substrate) was first identified as a substrate for the IL-4R (reviewed in Ref. 180). However, both proteins share extensive structural and functional identities and are tyrosine-phosphorylated in response to IL-4, insulin, and IGF-I (181, 182). Moreover, it has been demonstrated that activated IRS-1 and IRS-2 act as mediators of IL-4 mitogenic signaling (178, 179, 182). Despite the inhibitory effects of IL-4 on estradiol (E_2)-induced proliferation of ZR-75-1 cells (183), we still decided to investigate whether IRS proteins are involved in the induction of 3β-HSD type 1 expression by IL-4. Our study demonstrated that IL-4 caused rapid tyrosine phosphorylation of both IRS-1 and IRS-2, which was readily detectable after a 15-min exposure (177). The phosphorylation of IRS proteins by IL-4 raised the possibility that this pathway could be involved in IL-4 signaling in ZR-75-1 cells.

To determine their involvement, the effect of other factors that induce the phosphorylation of IRS proteins on 3β-HSD

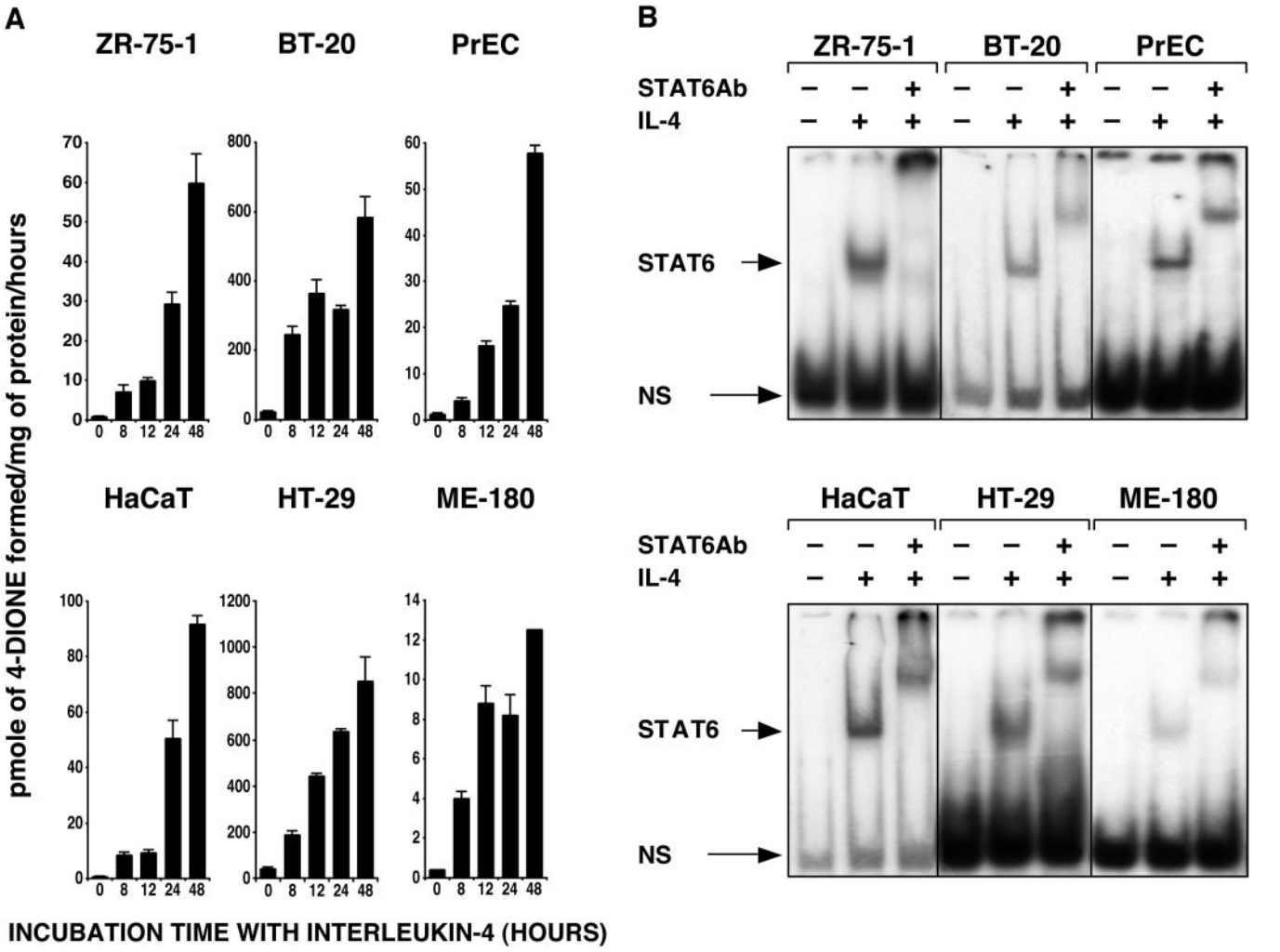


FIG. 13. A, Rapid induction of 3β-HSD activity by IL-4 in a variety of cell types derived from peripheral tissues. ZR-75-1 and BT-20 breast cancer cells, PrEC normal human prostate epithelial cells in primary culture, HaCaT human immortalized keratinocytes, HT-29 human colon cancer cells, and ME-180 human cervix cancer cells were plated at 200,000 cells per well in six-well plates. Two days after plating, cells were incubated for the indicated time periods with 100 pM IL-4. Thereafter, cells were harvested, and 3β-HSD activity was measured, as previously described (52), with 10 nM [^{14}C]DHEA in the presence of 1 mM NAD^+. Data are expressed as mean ± SEM of triplicate dishes. B, Stat6 activation by IL-4. Cell types mentioned in panel A were incubated in the presence or absence of IL-4 (10ng/ml for 30 min). Analysis of Stat6 activation using EMSA was performed as previously described using a well-established Stat6 responsive element derived from the IgE promotor. A Stat6 antibody was included in the binding reaction where indicated. [Adapted from Ref. 52].

type 1 expression was examined. IRS-1 and IRS-2 are also tyrosine phosphorylated by insulin and IGF-I in ZR-75-1, MCF-7, and T47-D breast cancer cell lines (184). As shown in Fig. 8, IGF-I and insulin increase the stimulatory effect of IL-4 on 3β-HSD activity. However, neither IGF-I nor insulin, even at high concentrations, induced 3β-HSD activity in the absence of IL-4, thus indicating that phosphorylation of IRS proteins is not sufficient to induce 3β-HSD type 1 expression. Because both IGF-I and insulin exerted their stimulatory action on IL-4-induced 3β-HSD activity at EC_{50} values in the low nanomolar range (177), and knowing that there is no significant binding of insulin to the IGF-I receptor or of IGF-I to the insulin receptor at concentrations less than 10 nM (185), these data suggest that both factors exert their potentiating action through the binding to their specific receptors.

However, as illustrated in Fig. 14, incubation with PMA increased the maximal stimulatory effect of IL-4. It is interesting to note that PMA, even at high concentrations, was unable to induce 3β-HSD activity in the absence of IL-4. Thus, similar to the IRS pathway, activation of the PKC pathway is not sufficient to induce 3β-HSD type 1 expression, but it suggests that at least one signaling molecule involved in signal transduction of the IL-4-induced 3β-HSD type 1 expression is also a substrate for PKC.

Another means of addressing the role of IRS proteins in the induction of 3β-HSD type 1 expression was to study the involvement of pathways downstream of IRS proteins in IL-4 signaling. To study a possible role in the induction of 3β-HSD type 1 expression by IL-4, specific inhibitors of the PI3-K and MAPK pathways were used. Wortmannin and LY294002 are two structurally unrelated molecules that specifically inhibit PI3-K activity. Both inhibitors completely blocked the IL-4-induced 3β-HSD activity. Because it has been shown that the regulatory subunit of PI3-K associates with phosphorylated

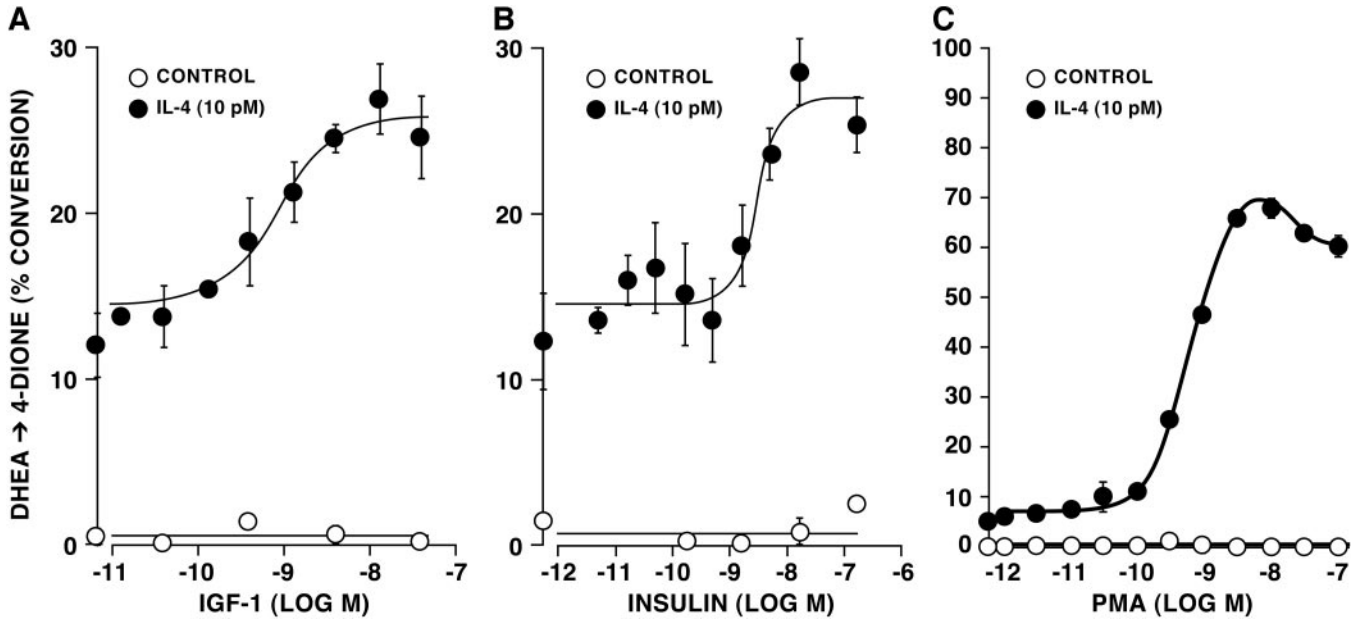


FIG. 14. IGF-I, insulin, and PMA increase IL-4-induced 3β-HSD activity in ZR-75-1 cells. ZR-75-1 cells were incubated for 6 h with increasing concentrations of IGF-I (A), insulin (B), and PMA (C) in the presence or absence of 10 pM IL-4. Cell homogenates equivalent to 100,000 cells were used to measure 3β-HSD activity for 6 h by measuring the conversion of [^{14}C]DHEA into [^{14}C]4-DIONE as previously described. [Adapted from Ref. 177].

IRS proteins in ZR-75-1 (184), the blockade of IL-4 signaling by the PI3-K inhibitors indicates not only the involvement of PI3-K but also the involvement of IRS proteins in IL-4-induced 3β-HSD type 1 expression.

Phosphorylated IRS-1 and IRS-2 can activate the MAPK pathway by binding Grb2 and engaging the Ras/Raf pathway (186–188). The specific inhibitor of MAPK kinase activation, PD98059, inhibits the induction of 3β-HSD expression by IL-4 (177). Moreover, MAPK activity was also found to be increased in cells treated with IL-4, IGF-I, or insulin compared with untreated cells (177). Although IL-4 failed to activate MAPK in lymphohemopoietic cells (189–191), the activation of MAPK in ZR-75-1 cells is in accordance with reports showing that MAPK is activated by IL-4 in human keratinocytes (192) as well as in breast cancer cell lines (184). Wortmannin is able to block the induction of MAPK by all three factors, thus suggesting that MAPK activation is a downstream effector of PI3-K that is activated by IL-4, insulin, or IGF-I (177). In conclusion, the involvement of IRS proteins in the regulation of *HSD3B1* gene expression is supported by the fact that: 1) IL-4 induces the phosphorylation of both IRS-1 and IRS-2; 2) IL-4 activates the MAPK pathway by an PI3-K dependent pathway; and 3) inhibition of downstream targets of IRS proteins (PI3-K and MAPK pathway) block IL-4-induced 3β-HSD type 1 expression.

In accordance with this model, IGF-I and insulin increase the pool of receptors able to phosphorylate IRS proteins and activate MAPK in a PI3-K-dependent manner. Our observation that PMA caused a greater potentiating effect of IL-4-induced 3β-HSD expression than that exerted by IGF-I or insulin may be explained, at least in part, by its capacity to activate a different set of PKC isoforms, *i.e.*, the diacylglycerol-sensitive PKC isoenzymes (193), whereas IL-4, IGF-I, and insulin activate atypical (diacylglycerol-insensitive) PKC (194).

The inability of IGF-I, insulin, and PMA to induce 3β-HSD activity in the absence of IL-4 indicates that IRS-1 and IRS-2 phosphorylated proteins and their downstream effectors may cooperate with another IL-4-specific signaling transduction pathway. Thus, it has been postulated that IL-4-induced *HSD3B1* gene expression requires the independent activation of at least two transcription factors, *i.e.*, Stat6, an IL-4-specific transcription factor, and a second unidentified factor, which can be activated not only by IL-4 but also by IGF-I, insulin, and PMA. The fact that IL-4-induced Stat6 DNA-binding activity is not affected by wortmannin or PD98059 and that PMA does not modify the potency of IL-4 to activate Stat6 indicates that Stat6 is activated independently of IRS proteins as previously suggested in another model system (195). Taking into consideration these findings, Gingras *et al.* (196) proposed the following model for the induction of 3β-HSD type 1 induction by IL-4 (Fig. 15).

D. Species similarity/divergence in mechanisms

The similarities/differences in the transcriptional control mechanisms between humans and other species will need to be carefully examined. The existence of six different expressed 3β-HSD genes in mice is suggestive of some potentially interesting differences in the mechanisms between the two species because sequence divergence in the promoter regions will likely introduce changes in the control of the isoform genes. Many of these potential similarities/differences will need to be examined using functional studies. Sequences that appear to contain homologous regulatory elements are sometimes nonfunctional in the context of a specific promoter, and sequences that appear

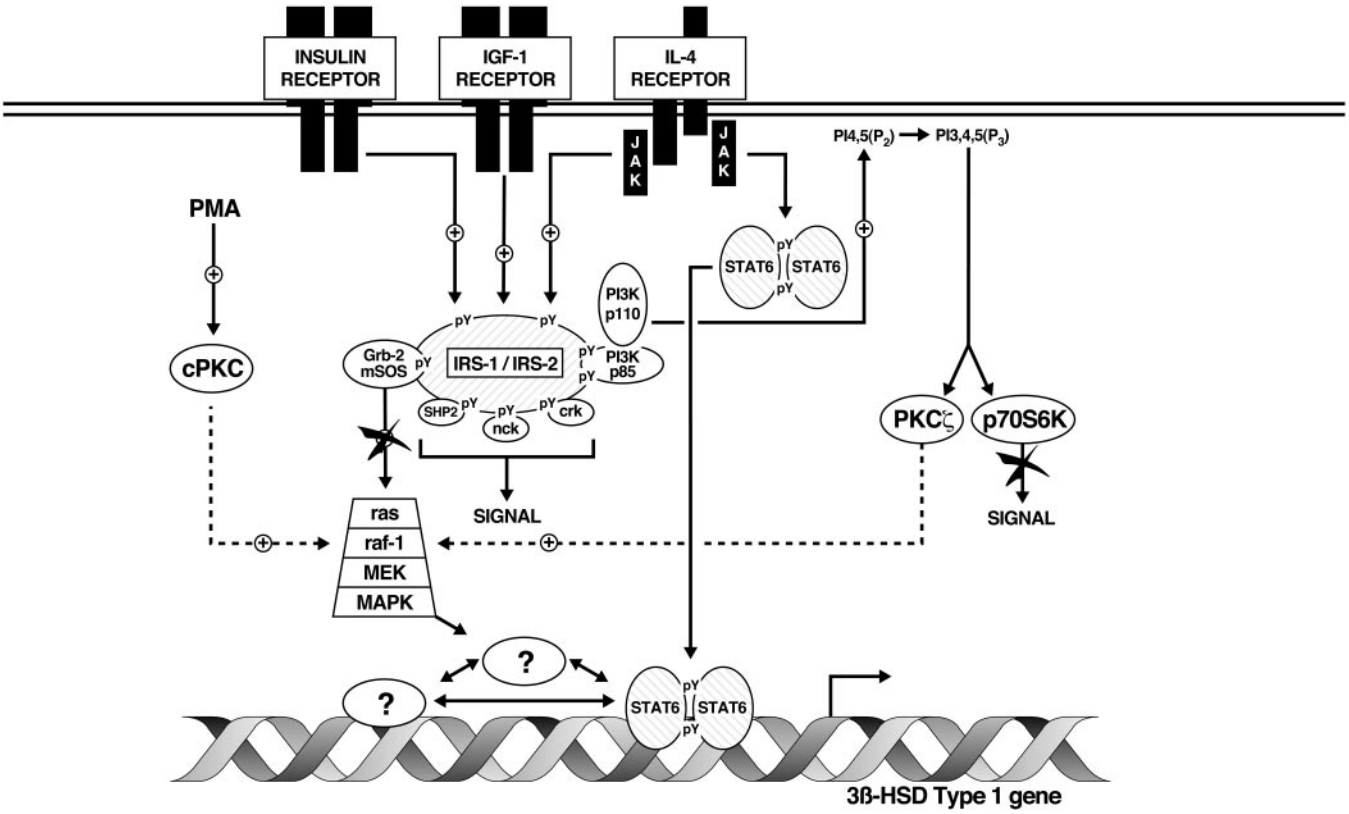


FIG. 15. Working hypothesis for the induction of 3β-HSD type I gene expression by IL-4 in ZR-75-1 breast cancer cells. The steps in the pathway known to occur are represented by *solid lines*, whereas the proposed pathways are shown in *dashed lines*. A *cross* on the signaling pathways indicates those that are not involved in the regulation of 3β-HSD type I gene expression. Phosphorylated tyrosines are represented by pY. [Reproduced from S. Gingras *et al.*: *J Steroid Biochem Mol Biol* 76:213–225, 2001 (196), copyright 2001, with permission from Elsevier.]

to have diverged from the core necessary sequences can sometimes prove functional.

The complexity of this process is seen by comparing the potential SF-1 regulatory element in the human *HSD3B2* gene promoter with the homologous sequence in the human *HSD3B1* gene (placental/peripheral form) and the rat type I gene (gonadal/adrenal form). The human type I gene has a substitution of one nucleotide in the core AAGG sequence resulting in an AAaG core as seen in Fig. 9. A substitution of this kind in the core sequence would predict that the SF-1 element would be nonfunctional in the human *HSD3B1* gene. Alternatively, the rat type I gene that is the major gonadal/adrenal form has diverged significantly in sequence from the SF-1 element in the human *HSD3B2* gene but maintains the core AAGG sequence. Therefore, it will be important to test the function of these elements because the highly divergent sequence of the rat type I could still be functional.

In a recent study on the expression of the mouse orthologous 3β-HSD, 3β-HSD VI, in giant trophoblast cells during midpregnancy, two transcription factors were identified that determined the trophoblast-specific expression of 3β-HSD VI (197). The two transcription factors, AP-2γ and the homeodomain protein distal-less (Dlx-3), were found in both mouse giant trophoblast cells and human placental JEG-3 cells. In addition, it was reported that AP-2γ was the transcription factor required for mouse trophoblast-specific expression of P450scc. Thus, the identification of an AP-2γ and Dlx-3 binding site in the promoter of the human *HSD3B1* gene and the demonstration that these two transcription factor binding sites were located within an enhancer region of the *HSD3B1* promoter led to the belief that the placental-specific expression of human 3β-HSD type 1 was determined by the same transcription factors as those found for the trophoblast-specific expression of the mouse 3β-HSD VI (198). However, neither one of these transcription factors is involved in determining the placental-specific expression of human 3β-HSD type 1. As previously mentioned in *Section V.C.1*, a 53-bp placental-specific enhancer element was identified between −2570 and −2518 of the *HSD3B1* promoter. The sequence comprising this enhancer element identified a consensus binding site for a family of TEFs, TEF-5 as well as an overlapping binding site specific for a GATA protein (152). Although the tissue-specific expression of mouse 3β-HSD type VI and human 3β-HSD type 1 are very similar, it may not be surprising that the placental-specific expression is regulated by different transcription factors in the two species. Placental PROG production in humans, which requires 3β-HSD type 1, is absolutely essential for maintenance of pregnancy, whereas in mouse, trophoblast cells have the capacity for PROG production (197) and there is no evidence that the trophoblast-generated PROG is required for maintenance of mouse pregnancy. The difference in the transcription factors that determine placental-specific expression does not appear to be the result of the absence of expression of the

factors identified that mediate human or mouse trophoblast-specific expression. As discussed above, AP-2γ and Dlx-3 are present in human placental JEG-3 cells as well as in mouse trophoblast cells. TEF-5 (199), GATA-2, and GATA-3 (200) are expressed in mouse giant trophoblast cells. Whether GATA-4 or other GATA-like proteins are expressed in mouse trophoblast cells is not currently known (152).

VI. Ontogeny, Localization, and Regulation of 3β-HSD Expression

A. Adrenal

Morphological differentiation during adrenal development results in two functionally distinct organs: the fetal and adult adrenal cortex. In primates, the fetal adrenal cortex consists of an outer neocortical (definitive) zone that is the primary site of aldosterone synthesis, a cortisol-producing transitional zone, and an inner fetal zone that comprises 80% of fetal adrenal volume and is responsible for the synthesis of DHEA and its sulfated derivative DHEA-S. In the adult adrenal, the outer cortex has differentiated through the process of zonation into three steroidogenically and morphologically distinct zones: ZG, ZF, and ZR). In the primate, these zones are responsible for the production of aldosterone, cortisol, and DHEA/DHEA-S, respectively. The ZR is absent in early infancy and starts to develop around ages 5 to 6 before DHEA-S increases steadily until the end of puberty (201, 202). However, in mice and rats, ZG produces aldosterone, ZR and ZF produce corticosterone, but no production of androgens is observed due to the absence of P450c17 expression. In fact, after castration of rats, guinea pigs, and mice, no androgens can be detected in the serum, suggesting that sex steroids are solely of gonadal origin in subprimate species (203).

The onset of 3β-HSD expression in the fetal adrenal cortex correlates with the ability of the definitive zone to synthesize aldosterone and also allows for cortisol production by transitional zone cells due to coexpression of P450c17 (204–207). Although 3β-HSD is not expressed to a high degree in the fetal zone, P450c17 is expressed, thereby directing the steroidogenic pathway toward Δ^5-hydroxysteroid (*i.e.*, DHEA/DHEA-S) production. This zone-specific steroid secretion pattern is dependent on the relative expression levels of 3β-HSD, P450c17, and P450 21α-hydroxylase (P450c21) that serve as molecular markers of the adrenocortical developmental state. After birth, the neocortex differentiates into the three functional zones of the adult adrenal cortex. The coexpression of 3β-HSD and P450c21 in the ZG leads to aldosterone production, whereas the coexpression of 3β-HSD and P450c17 in the ZF results in the production of cortisol. The expression of P450c17 in the ZR along with the low levels of 3β-HSD expression leads to the synthesis of DHEA and DHEA-S. A third period of adrenal functional differentiation, in which DHEA/DHEA-S serum levels increase, occurs in humans at around age 6–8 yr. This increase in adrenal androgen production is termed adrenarche and correlates with a decline in 3β-HSD expression in the ZR (Fig. 16). The mechanisms underlying the variability of expression are not well understood at this time, and a deeper understanding of the factors involved in the regulation of 3β-HSD is critical to elucidating the control of adrenal steroidogenesis.

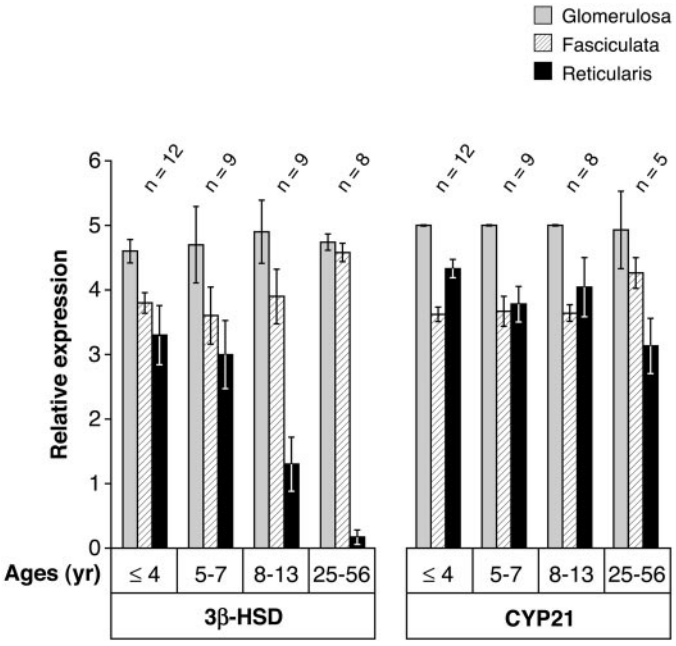


FIG. 16. Relative expression of 3β-HSD and CYP21 in three zones of the human adrenal cortex at different ages. Immunohistochemical staining of 3β-HSD and CYP21 in various zones of the adrenal cortex. *Gray column*, Glomerulosa; *hatched column*, fasciculata; *black column*, reticularis. Arbitrary units, 5 (highest degree of staining) to 0 (no staining present). Data are expressed as mean ± SEM. Reticularis compared with fasciculata within each group. [Adapted from Ref. 271.]

1. Ontogeny and localization. The primate fetal adrenal is derived from the dorsal coelomic epithelium. At 8 wk gestation in the human, the fetal adrenal is composed of chromaffin cells that later form the medulla, fetal, and neocortical zones. The neocortical zone further divides into the ZG, ZF, and ZR, whereas the fetal zone regresses after birth. The fetal zone, which expresses P450c17, catalyzes DHEA synthesis, which serves as a reservoir of precursors for placental estrogen biosynthesis (208). The neocortex also expresses P450c17, as well as 3β-HSD and P450scc, and is the site of fetal cortisol biosynthesis (209). The fetal and neocortical zones have undetectable 3β-HSD protein and mRNA expression at 17–22 wk gestation in humans, whereas P450scc and P450c17 are detected in the fetal and transitional zones (207). At 22 wk gestation, the neocortex starts expressing 3β-HSD, and at 28 wk the enzyme is widely distributed in the neocortex (204). Another study showed that neocortical expression of 3β-HSD could be detected as early as 11 wk gestation, followed by a reduction in expression until 24–25 wk gestation (210). By 35 wk gestation, 3β-HSD expression is observed in differentiated ZG and ZF cells in the mature human fetus and is also seen in the 2- and 8-month-old infant. In the 2-yr-old infant, 3β-HSD staining is seen in all cortical layers (204). Adult adrenal 3β-HSD expression was not seen in the macaque reticularis (211), whereas its expression was low in human reticularis cells accounting for their increased output of DHEA/DHEA-S (212). The highly differential expression of 3β-HSD *vs.* P450scc and P450c17 in the fetal adrenal is

suggestive of unique transcriptional control mechanisms between these steroidogenic enzyme genes.

In the rhesus monkey, 3β-HSD has been localized to the definitive zone of the fetus between 109 and 148 d, whereas at term (155–172 d), 3β-HSD was localized in both definitive and transitional zones (207). Similarly to humans, rhesus monkey 3β-HSD is contained in the outer regions of the adrenal during fetal life. At midgestation, the levels of 3β-HSD increase, which accounts for a rise in the levels of cortisol in circulation (213). In addition to cortisol output by the fetal adrenal, estrogen levels (via conversion of DHEA) and PROG levels fall after fetalectomy in baboons. This suggests that the fetus is directly responsible for steroidogenesis either by fetal steroid production or via secretion of steroidogenesis promoting factors (214).

Detailed ontological studies in the rat have also been performed. 3β-HSD activity studies in the rat adrenal were initially performed using histochemical techniques (215). These studies revealed that 3β-HSD protein and mRNA were not detected in cortical cells until d 16 of gestation. Differential levels of expression could be seen between d 18 and 21 of gestation with high levels of expression in ZF/ZR relative to ZG. Fetal ACTH secretion begins on d 17–18 in the rat, which could account for this induction in ZF/ZR (216, 217). This pattern was maintained until 15 d after birth. Twenty-five-day-old rats then showed adult expression patterns with equal cytoplasmic 3β-HSD immunostaining and *in situ* hybridization patterns in all three cortical zones and no staining in the medulla (218). Other studies have shown by immunohistochemistry that 3β-HSD is present in the rat ZG at low levels in the first few days after birth, peaks at d 20, and then decreases to levels below that seen in the ZF until adulthood at 65 d. In the rat ZF, 3β-HSD staining was high at birth and reached a maximum at 14 d, which was maintained until adulthood. The inner part of ZF/ZR was low before d 20 and then increased to ZF levels and remained so until d 90. Aldosterone secretion paralleled the development of the ZG, whereas corticosterone levels were low for the first 14 postnatal days (219, 220). These data show that 3β-HSD expression is expressed in the ZF/ZR before the ZG. Earlier induction of ACTH relative to angiotensin II (A-II) (221) could account in part for this differential expression.

Ontological studies have also been performed in other animals besides rodents and primates. Adrenal studies in sheep demonstrated 3β-HSD localization to the outer cortical layer from d 43 of gestation until term. 3β-HSD localization was observed in ZF from d 65–130 (222). Adult ovine adrenal ZG stained negatively for P450c17 and only faintly positive for 3β-HSD, whereas the adjacent layer of cells (ZF) stained strongly for P450c17 and 3β-HSD (223). Bovine adrenal development studies show that 3β-HSD appears in a narrow ZG/ZF band at d 80 of gestation (224), and that 3β-HSD mRNA expression is seen in the bovine fetal adrenal with higher expression in early and late gestational stages relative to midgestation (225). Species-specific differences must therefore be taken into account when examining 3β-HSD ontogeny in the adrenal.

2. Regulation

a. ACTH and A-II. The differential expression of the enzymes required for zonal-specific steroid production in the adrenal is under the control of multiple factors. Regulation of 3β-HSD expression in the adult adrenal has long been known to involve ACTH in ZF/ZR and A-II in the ZG. The involvement of ACTH in 3β-HSD induction in the fetal adrenal has been examined. The release of ACTH resulting from metyrapone treatment (an inhibitor of 11β-hydroxylase activity) of rhesus monkeys at 135–137 d gestation caused an induction of 3β-HSD in the transitional zone suggesting that ACTH plays a role in the induction of 3β-HSD at term (226). The transitional zone, which is comprised of cortical cells that express P450c17 and 3β-HSD (*i.e.*, cortisol production), and the definitive zone, which lacks P450c17 expression but does express 3β-HSD (*i.e.*, mineralocorticoid production), develop in the late stages of gestation. It has been shown by immunocytochemistry that induction of 3β-HSD and P450c17 in the transitional zone of baboon adrenals occurs late in gestation and is dependent on ACTH. However, the development of the definitive zone at midgestation occurs in the absence of ACTH (227). ACTH receptor and 3β-HSD mRNA and protein levels were also decreased in the developing definitive zone of the fetal adrenal. These data suggest that ACTH is required for the up-regulation of ACTH responsiveness and steroidogenesis in the fetal definitive zone of the baboon adrenal gland (228). Additional evidence for adrenal differentiation in the absence of ACTH comes from studies of anencephalic human fetuses that lack ACTH production, yet in which the definitive zone appears to develop normally (229). In the fetal sheep adrenal, ACTH administration in the late stages of pregnancy led to an increase in P450c17 but not 3β-HSD, suggesting that control of 3β-HSD levels in the pregnant sheep is more complex (230). These results suggest that the ontogenesis of 3β-HSD expression in the transitional zone is dependent on ACTH, yet these effects appear to be species specific.

The functional development of the rat adrenal cortex is characterized by a triphasic response to ACTH with a nadir in responsiveness around neonatal d 10. Immunoreactive levels of 3β-HSD are low until d 10 relative to adults, although enzymatic activity is similar to adults. ACTH injection on d 10 increased 3β-HSD staining (231). Studies in adult rats have shown that 3β-HSD staining by *in situ* hybridization followed a uniform pattern throughout the adrenal cortex that was unaffected by ACTH injection until responsiveness appeared at d 4 of treatment (232). Other studies have been performed using chronic stimulation (injections twice daily for 9 d) of adult rats with ACTH. Results from these studies showed increased StAR mRNA levels, but decreased 3β-HSD and angiotensin type 1 receptor transcripts in ZG cells, whereas the levels of P450scc and P450C21 mRNA did not differ significantly from the control values, thus suggesting a control of StAR expression by ACTH and 3β-HSD by A-II. Interestingly, Western blotting analysis revealed that StAR protein levels were increased, whereas 3β-HSD protein levels were unchanged. In addition, ACTH suppression via dexamethasone (DEX) treatment for 5 d did not affect either StAR or 3β-HSD. In the ZF/ZR, chronic ACTH stimulation led to increases in StAR, whereas 3β-HSD was unchanged (233). These data demonstrate that: 1) the ability of ACTH to stimulate 3β-HSD follows a temporal pattern in the rat fetal adrenal; 2) differential regulation of steroidogenic enzymes

exists under chronic exposure of adult adrenals to circulating ACTH; and 3) other factors are involved in modulating 3β-HSD expression.

Primary cultures of human adrenal cells have demonstrated the capacity of ACTH and A-II to regulate 3β-HSD. *In vitro* studies of fetal zone cells demonstrate that they are responsive to ACTH with an induction of 3β-HSD, suggesting that an *in vivo* endogenous factor is inhibiting 3β-HSD production (205, 206, 234). Many studies have shown increased receptivity of adrenal cells after the addition of ACTH and A-II as well as second messenger mimetics of ACTH (cAMP/forskolin/cholera toxin induce PKA activity) and A-II (phorbol esters induce PKC activity). In human reticularis-fasciculata cells, the stimulatory effects of ACTH and A-II were additive only on 3β-HSD expression (235). Moreover, in rat adrenal cells in the absence of ACTH, this enzymatic activity was found to decay with a half-life of 3.1 d, which was similar to the half-life of the enzyme activity induced by ACTH *in vitro* (3.5 d) (236). The induction of 3β-HSD activity by ACTH or dibutyryl cAMP required a lag period of approximately 4 h and was dependent on RNA and protein synthesis (236). ACTH and cAMP also up-regulate 3β-HSD protein and mRNA expression in cultured bovine adrenocortical (BAC) cells (157, 237), yet A-II treatment decreases ACTH-induced 3β-HSD expression in BAC cells (157, 238). These *in vitro* model systems have been useful in the examination of ACTH and A-II on steroidogenesis regulation.

Two steroid-producing adrenal cell line models exist: H295R (human adrenocortical carcinoma) and Y1 (mouse adrenocortical carcinoma). In H295R cells, it has been demonstrated that forskolin, A-II, and cAMP treatments stimulated 3β-HSD mRNA; however, this effect could be blocked by cycloheximide treatment (239, 240). H295R studies have also shown that cAMP and phorbol esters induce 3β-HSD (240). K^+, an agent known to increase intracellular Ca^{2+} through the opening of voltage-sensitive Ca^{2+} channels, caused an increase in H295R cells of P450c17 and P450scc, but not of 3β-HSD mRNA levels. This suggests that phorbol ester effects in this system may signal via a pathway independent of that induced by Ca^{2+} alone (241). Interestingly, staurosporine (PKC inhibitor) increases 3β-HSD mRNA levels in mouse Y1 cells, suggesting that PKC is a tonic inhibitor of steroidogenesis in these cells (242). The effects of induction of PKC activity via the employment of phorbol esters may not be indicative of signaling by the angiotensin type 1 receptor due to the differential effects seen in these model systems.

b. TGFβ, IGF, and growth factors. Because the expression of 3β-HSD during adrenal development is not completely under the control of ACTH and A-II, other factors must be involved in the induction and repression of 3β-HSD in the neocortical zone and the fetal zone, respectively. Candidate molecules involved in this regulation include EGF, fibroblast growth factor (FGF), IGF, and TGFβ. EGF and FGF are mitogenic in human fetal adrenals (243), whereas aFGF and bFGF have been shown to be mitogens in BAC and Y1 cells (244, 245). In addition, it has been postulated that the mitogenic effects of GH in the adrenal could be due to IGFs (246). On the other hand, TGFβ has a growth inhibitory effect (247). Therefore, there appears to be a complex interplay of factors controlling adrenal growth and development, and combinations of these factors could be involved in the regulation of 3β-HSD and other steroidogenic enzymes *in vivo*.

Several studies have implicated EGF in the regulation of adrenal development and function. Macaque fetuses treated with EGF show induced expression of 3β-HSD protein in the transitional zone of the fetal adrenal, whereas betamethasone treatment resulted in a reduction of 3β-HSD protein levels (248). Studies by McAllister and Hornsby (249) have shown that forskolin induction of 3β-HSD activity in human adrenocortical cells could be enhanced by A-II, EGF, or FGF treatment, whereas other work has shown that EGF can induce 3β-HSD mRNA and cortisol production in H295R cells (125). Another study has shown that infusion of EGF into ewes for 24 h resulted in a 700% increase in cortisol levels (250). Activation of the EGF receptor is probably not being mediated by EGF in humans because EGF is not expressed in the adrenal cortex. However, TGFα is expressed (251). These data suggest that activation of the EGF receptor in the adrenal cortex can induce steroidogenic enzymes including 3β-HSD, but the precise role of EGF and EGF-like ligands is unclear.

Another factor thought to play a role during adrenal development is TGFβ. Antisense oligonucleotides to $TGF\beta_1$ caused a 2-fold increase of mRNAs for 3β-HSD and P450c17 in BAC cells (252). Similarly, TGFβ decreased P450c17 and 3β-HSD expression, but the effect was more pronounced on P450c17 levels in the cow (157). Interestingly, human adrenocortical cells treated with $TGF\beta_1$ in the presence of ACTH resulted in an increase in 3β-HSD mRNA levels, a decrease in P450c17 mRNA, but no effect on P450scc expression (253). Studies by Naville *et al.* (254) showed that ACTH up-regulates 3β-HSD mRNA and protein expression in ovine adrenocortical cells, whereas P450c17 and 3β-HSD expression were decreased by TGFβ. However, the effect TGFβ was more pronounced on P450c17 levels in the sheep. Furthermore, $TGF\beta_1$ decreased 3β-HSD mRNA and protein levels in mouse Y1 cells (255). Therefore, TGFβ may play an important role in the down-regulation of 3β-HSD and steroidogenesis in the adrenal gland, however the regulatory context in which TGFβ exerts its effects may be lost *in vitro*.

Insulin and IGFs have been known to enhance steroidogenesis in multiple tissues, and these effects have been shown to involve the up-regulation of 3β-HSD mRNA levels. IGF-II enhances ACTH-stimulated, but not basal levels of 3β-HSD mRNA in cultured human fetal adrenals (256). Treatment with insulin and IGF-I increased 3β-HSD and P450c17 mRNA levels in human adrenocortical cells, and this increase was coincident with a decrease in the DHEA/cortisol ratio (257). Although P450c17 and 3β-HSD mRNAs were increased by IGF-I/II treatment, P450scc levels remained unaffected in human adult fasciculata-reticularis cells. In addition, the acute steroidogenic response to ACTH of cells pretreated with IGF-I, IGF-II, or insulin was 3- to 6-fold higher than that of control cells (258). ACTH and IGF-I also increased 3β-HSD and P450c17 mRNA levels in BAC cells (238). Therefore, these studies demonstrate that insulin and IGFs play a role in enhancing adrenal steroid output, and that part of this effect is mediated at the level of 3β-HSD.

c. Steroids. Data from multiple studies have suggested that steroids can regulate adrenal 3β-HSD and possibly steroidogenesis. If verified, this would have important implications on the ability of steroids downstream of 3β-HSD catalysis to feedback or feed-forward on steroid production. For example, DEX can enhance 3β-HSD mRNA levels and cortisol production in H295R cells, and this effect is enhanced with PMA treatment (125). These results suggest that cortisol production by the ZF might enhance the steroidogenic potential of the adrenal gland through direct transcriptional effects. Other work has shown that E_2 decreased 3β-HSD activity in a dose-dependent manner in H295R cells (259). The PROG/glucocorticoid receptor antagonist RU486 administered to hypophysectomized (HYPOX)/castrated/ACTH-replaced rats decreased 3β-HSD activity, yet corticosterone levels were unaffected (260). 3β-HSD activity is greater in female than male adrenals of C57BL/6J and C3 h/HeJ mice. Injection of T or DHT decreased adrenal 3β-HSD activity in both strains (133). These studies therefore open the possibility for complex feedback by androgens, estrogens, and glucocorticoids in the regulation of adrenal steroidogenesis.

d. Other regulatory mechanisms. Several studies have suggested that adrenal 3β-HSD is regulated by factors other than those mentioned. For example, thyroid hormone (T_3) regulation of 3β-HSD has been examined in the adrenal gland as well as in other tissues. T_3 alone had no effect, however cotreatment with T_3 enhanced ACTH up-regulation of 3β-HSD activity in human fetal adrenocortical cells (261). On the other hand, T_3 and T_4 reduced 3β-HSD activity in male rat ZFR cells (262). A detailed examination of T_3 performed in the perch showed that T_3 caused a linear increase of 3β-HSD activity in cells isolated from the ovarian follicles. These studies also demonstrated that T_3 induction required protein synthesis, which could be overcome by the addition of T_3-induced protein (263, 264). Furthermore, piscine gonadotropin and T_3 stimulated 3β-HSD activity in perch testis; however, the kinetics of stimulation differed (265). These studies therefore suggest that the thyroid hormone can also participate in the regulation of steroidogenesis by modulating 3β-HSD activity.

Other factors tested include CRH, prostaglandins, and hypoxemia. CRH increased DHEA-S/cortisol production by cultured human adrenal fetal zone and neocortical zone cells in a dose-dependent manner (266). This suggests a direct role for the pituitary factor in the regulation of adrenal steroidogenesis. Chronic treatment of BAC cells with PGE_2 induced 3β-HSD mRNA and protein expression (267). Prostaglandin signaling in the adrenal might therefore be involved in the regulation of 3β-HSD. Finally, hypoxemia increased the cortisol to Δ^4-DIONE ratio in pregnant sheep fetuses and also increased adrenal P450scc, P450c21, and 3β-HSD, although P450c17 remained unaffected (268). These factors may therefore play a role in the regulation of adrenal steroidogenesis at the level of 3β-HSD; however, more work needs to be done to determine whether these effects are a general phenomenon or a specific effect of the model systems tested.

3. Adrenarche. Adrenarche is characterized by an increase in adrenal androgen secretion (DHEA/DHEA-S), which is not accompanied by an increase in cortisol and is independent of the gonads or gonadotropins. Adrenarche is limited to Old World primates (269) and occurs at around age 6–8 yr in humans. After this period, P450c17 expression continues in the ZR, whereas that of 3β-HSD declines. Several studies have shown that 3β-HSD expression decreases in the ZR in humans over 8 yr old (Fig. 16) (270–272). However, the mechanisms underlying the decrease in 3β-HSD expression are still unknown. Another proposed mechanism that could explain the increase in DHEA/DHEA-S output during adrenarche is an alteration of the ratio of 17α-OH/17,20-lyase activities of P450c17 (211, 273, 274). Increases in the coupling of the allosteric factor, cytochrome b5, to P450c17 promote 17,20-lyase activity and androgen synthesis by P450c17 independent of increases in P450c17 expression. Moreover, an increase in SULT2A1 expression is observed, which results in an increased conversion of DHEA to DHEA-S, further attenuating Δ^4-steroid production (620). Adrenarche may therefore result from an increase in 17,20-lyase activity of P450c17 due to an increase in cytochrome b5 association, an increase in SULT2A1 expression along with a decrease in 3β-HSD expression, which results in an increase of adrenal androgen output.

B. Ovary

At birth, the ovary consists of steroidogenically inert primordial follicles that during adulthood are recruited to proliferate and differentiate into distinct layers with specialized functions. These follicles pass through preantral, antral, and ovulatory stages under gonadotropin and intracrine control in which the outer androgen-producing theca layer and the inner estrogen-producing granulosa layer develop. After ovulation, follicles differentiate into the PROG-secreting CL. Each of these structures possesses a different steroidogenic profile that is the result of altered steroidogenic enzyme expression. Differential regulation of 3β-HSD plays an important role in the steroidogenic profile of ovarian tissue, and a complex interplay of pituitary factors and ovarian factors maintains a tight control over steroidogenic enzyme control.

Ontological studies have shown that 3β-HSD is expressed during some fetal stages in human ovaries, but then disappears until puberty. Therefore, fetal human ovaries are steroidogenically quiescent except for a window late in gestation (275), so most estrogens seen by the primate fetus are of placental origin (276). This is in contrast to testicular expression because androgen production by the male embryo is critical for male sexual development (277). In humans, hilar cells of the ovary express 3β-HSD at 26 wk gestation as detected by immunohistochemistry (210), whereas 3β-HSD protein could be seen in the theca and interstitial layers of the human fetal ovary from 28–34 wk. No 3β-HSD staining was seen from birth until puberty (275). The role of steroid production in the human fetal ovary is unclear at this time. In contrast to humans, 3β-HSD protein cannot be detected in the fetal rat ovary and is not seen until 6 d after birth in the developing theca (278, 279). Similarly, 3β-HSD is not expressed in the fetal mouse ovary (280). Steroidogenesis by the ovary does not appear to be critical for female development,

with the caveat that there is an unknown function of steroid production during the development of the human ovary.

1. Follicle

a. Follicular localization. 3β-HSD localization studies track the molecular machinery required for PROG and androgen production by the thecal layer during primate follicular development. Androgens are then further metabolized into estrogens by the granulosa cells (281). 3β-HSD mRNA has been localized to the theca interna of preantral, antral, and atretic follicles as well as the CL (282–285). Preantral/antral expression studies show 3β-HSD mRNA and protein expression in the human ovary to be initially in the theca and then developing in the granulosa layer as folliculogenesis continues. Interestingly, some layers of theca interna cells next to the basement membrane showed no 3β-HSD expression (282, 283). Low levels of 3β-HSD relative to P450c17 in the theca during the proliferative phase of the menstrual cycle lead to more Δ^5-steroid precursors being produced, which results in higher estrogen output by the follicle (286).

In nonprimate species, 3β-HSD has been shown to have different expression patterns. In the rat, 3β-HSD mRNA is elevated throughout the estrous cycle. Preantral, antral, and preovulatory rat follicles showed 3β-HSD expression in the theca, but no expression was seen in the granulosa layer (287). During gestation, 3β-HSD could not be detected at d 10, but increased on d 14, 17, and 21 (288). Similarly to the rat, 3β-HSD is localized to the theca-interna layer of the pig follicle (289–291). In contrast to rodents, pigs, and primates, 3β-HSD expression in the cow was seen in all stages of the preovulatory follicle in both theca and granulosa layers (292), and mRNA and protein levels peaked in bovine ovaries on d 16–17 after estrous (293). After formation of theca interna around the granulosa cells, mRNA for 3β-HSD is expressed in thecal cells about the time of antrum formation, and expression increases with the growth of early antral follicles (294–296). Moreover, granulosa cells of preantral and early antral follicles (less than 4 mm) do not express 3β-HSD, indicating that bovine follicles less than 4 mm in diameter are not able to convert cholesterol to PREG and subsequently to PROG (297). Selection of dominant follicle occurs between 36 and 48 h after initiation of the first follicular wave and is associated with mRNA expression for 3β-HSD in granulosa cells (295, 296, 298). Expression in thecal and granulosa cells decreases dramatically in preovulatory follicles after the preovulatory LH surge (292, 299). Other studies in the cow have localized 3β-HSD mRNA to theca at 12 h after the first follicular wave, and expression in the theca was maximal between 24 and 96 h (298). These studies suggest that the primary site of 3β-HSD expression is in the thecal layer of the follicle, yet expression in the granulosa layer is seen depending upon the stage of folliculogenesis and species examined.

b. Pituitary control. Pituitary hormones are a primary means of regulating steroidogenesis in the ovary. Follicular binding of the gonadotropins FSH and LH causes an increase in cAMP and phosphatidylinositol-turnover, which in turn results in an increase in 3β-HSD expression concomitantly with other steroidogenic enzymes under the similar control by PKA and PKC. *In vitro* studies have shown that human theca-interna cells responded to cAMP with an induction of 3β-HSD (300). Similarly, 3β-HSD activity was increased by cAMP in fetal rat ovaries (301), and 3β-HSD mRNA levels in pseudopregnant rats rose dramatically 2 d after induction of ovulation (302). The induction of rat 3β-HSD by FSH can be attenuated by AG18 (a tyrosine kinase inhibitor), suggesting that an undetermined tyrosine kinase pathway is involved in the cAMP-dependent signal transduction pathway of FSH action (303). Activation of the PKB/Akt kinase pathway by FSH has been reported to occur in terminally differentiated granulosa cells, in a cAMP-dependent and PKA-independent manner (304). In these cells, FSH also activates the p38 (305) and ERK MAPKs (306, 307), indicating that the FSH-receptor owns the structural requirements for coupling to these pathways.

Work in the pig parallels 3β-HSD regulation seen in rats and primates. The porcine granulosa tumor cell line, JC-410, responded to cholera toxin with an increase in 3β-HSD and P450scc mRNA, but not P450arom (308). LH/hCG treatment increases 3β-HSD expression in porcine granulosa cells, which can be attenuated with PMA treatment (309). 3β-HSD protein levels increased linearly between d 3 and 7 in preovulatory follicles of the pig (290). These studies show that cAMP production induced by gonadotropins is an important regulatory mechanism for the control of 3β-HSD, yet modulation by other factors is evident. Another pituitary factor critical to the regulation of ovarian steroidogenesis in rodents and possibly primates is PRL. Although the direct control of 3β-HSD by PRL is unclear, studies have been performed that suggest its ability to alter 3β-HSD enzyme levels. Martel *et al.* (310) have demonstrated that induction of 3β-HSD mRNA levels in the follicle of hypophysectomized rats can be accomplished by hCG but not FSH treatment. In addition, PRL was shown to be inhibitory, yet the inhibition could be counteracted with hCG treatment. Additional studies using *in situ* hybridization showed that the administration of hCG for 9 d induced increases of 146% in 3β-HSD mRNA levels in ovarian interstitial cells of pseudopregnant rats. Treatment with PRL caused a 78% decrease in 3β-HSD mRNA levels in CL after 9 d of treatment (311). Although the levels of PRL used in these studies were high, PRL was effective in the control of ovarian steroidogenesis.

Although declines in serum gonadotropin levels during menopause limit the capacity of the ovary to produce steroids, some steroid production continues. In postmenopausal women, for example, 30% of circulating Δ^4-DIONE is of ovarian origin (312, 313). 3β-HSD expression continues in menopausal and postmenopausal women and is localized to dispersed interstitial cells (275). These studies suggest that ovarian steroid production in postmenopausal women continues, but the decline in pituitary control dramatically changes the steroid profile. Due to the continued expression of steroidogenic enzymes, the postmenopausal ovary could be viewed as a site of both local synthesis and peripheral conversion of steroids produced by the adrenal.

c. Nonpituitary control. Other factors of extrapituitary origin have been examined for their capacity to regulate follicular 3β-HSD. These factors, which include PROG, IGFs, thecal differentiating factor (TDF), GnRH, EGF, and β-

adrenergic agents, may modulate gonadotropic and lactogenic control of 3β-HSD expression. Furthermore, 3β-HSD autoantibodies are present in patients with premature ovarian failure, potentially affecting ovarian steroid biosynthesis (314). However, the relative role of these factors in controlling follicular steroid production independent of trophic factors is a matter of debate.

PROG is a critical component in the ovulatory process in rodents and primates (315). However, the role that PROG plays in the regulation of steroidogenic enzymes in the ovary is less well defined. Treatment of macaques with trilostane (a competitive inhibitor of 3β-HSD) allows for the examination of 3β-HSD regulation in the absence of PROG and other active steroids. For example, an hCG bolus administered to macaques resulted in a time-dependent decrease in P450scc mRNA, whereas 3β-HSD increased in a steroid-independent manner (316). Although these studies do not implicate PROG as a major inducer of 3β-HSD in primates, the effects on basal production of steroids have yet to be examined.

In the rat, however, PROG does appear to have a positive effect on the transcription of 3β-HSD. Onapristone, which is an antiprogestin, blocks ovulation and decreases PROG receptor (PR) and 3β-HSD protein expression in pseudopregnant rats (317). Furthermore, RU486 (a PROG/glucocorticoid antagonist) administered before hCG injection in the pseudopregnant rat model attenuated the hCG induction of 3β-HSD activity (318). R5020 (a PROG agonist) and PROG in the presence of the 3β-HSD inhibitor, cyanoketone, augmented the FSH induction of 3β-HSD activity in a dose-dependent manner in rat granulosa cells (319). Progesterone may therefore play a role in the feedback regulation of 3β-HSD in the rat ovary.

There is evidence that the IGFs and their binding proteins play a role in ovarian steroidogenesis. Studies in the rat have shown that intrafollicular IGF-I amplifies granulosa cell steroidogenesis in this species (320). Similar to results obtained in the adrenal gland, IGF-I appears to augment trophic hormone stimulation of ovarian steroidogenesis. Cultured rat granulosa cells showed a 3.8-fold basal and 1.8-fold FSH-stimulated increase in 3β-HSD activity and mRNA levels by IGF-I treatment (321). IGF-I also increased 3β-HSD mRNA by 2-fold in theca-interstitial cells isolated from HYPOX immature rats (322). The relative importance of an autocrine effect of IGF on ovarian function has yet to be fully determined.

TDF, which is secreted by the preantral follicle, stimulates theca-interna androgen production in a LH-independent manner. TDF is induced by FSH in the rat and increases 3β-HSD, P450scc, P450c17, and LH receptor mRNA levels (323). 3β-HSD mRNA is also expressed in the theca-interna layer of the rat follicle before LH-responsiveness. Studies showed that the treatment of dispersed cells from 4-d-old rat ovaries with media from preantral follicles containing TDF activity caused an induction of 3β-HSD and other steroidogenic enzymes in these cells along with an increased androgen production (324). Therefore, FSH-induced TDF may enhance the steroidogenic capacity of the rat follicle.

Other nonpituitary factors shown to have effects on 3β-HSD are GnRH, EGF, and β-adrenergic agonists. The GnRH receptor has been shown to be expressed in the follicle (325). Moreover, the GnRH agonist, buserelin, increased 3β-HSD activity in pseudopregnant rat ovarian cells (326), suggesting a role for GnRH outside of pituitary in the control of ovarian steroidogenesis. Another factor that might have an intraovarian role in the regulation of 3β-HSD is EGF. In rat granulosa cells, EGF increases 3β-HSD in the presence or absence of TGFβ and increases FSH induction of this parameter (327). Finally, studies have suggested a role for direct innervation and local production of neurotransmitters within the ovary in the control of 3β-HSD and ovarian steroidogenesis (328, 329). Indeed, terbutaline and isoproterenol (β-adrenergic agonists) produced dose-dependent increases in 3β-HSD mRNA in porcine granulosa cells (330). The regulation of 3β-HSD and ovarian steroid output involves an interplay of multiple factors (intrinsic and extrinsic) of which the complexity has yet to be fully ascertained.

2. CL

a. CL localization. After ovulation, luteinization ensues in which the follicle becomes highly vascularized and secretes copious amounts of PROG. PROG output is controlled in part by the amount of 3β-HSD enzyme. Macaque localization studies demonstrate high levels of 3β-HSD expression in the early luteal cycle with a decline in expression thereafter (331, 332). A more detailed temporal examination showed expression of 3β-HSD mRNA from d 1–9 of an induced cycle in CL of macaques (333). In the sheep, however, 3β-HSD mRNA was not detected in ovine CL until 48 h after onset of estrous (334). These results suggest that 3β-HSD localization to the CL is a marker of the PROG production capacity of the CL.

Several studies have correlated the luteal expression of 3β-HSD with that of androgen receptors (AR) and PR. These receptors may alter luteal function based upon local PROG and androgen concentrations. For example, 3β-HSD, AR, and PR are all expressed in human CL (284, 335). In addition, 3β-HSD and PR, but not ER, were present at the highest levels during the midluteal phase of the baboon menstrual cycle (336, 337). As in the baboon, 3β-HSD and PR show the highest expression levels in the midluteal phase of the macaque cycle (338), and 3β-HSD was also detected in the CL of macaques with coexpression of AR (339). These studies suggest a potential role of steroids in the modulation of 3β-HSD levels, and this potential will be discussed further.

Luteal cells are derived from the theca and granulosa layers of the follicle. Two types of luteal cells have been identified in multiple species: large and small. Large luteal cells contain more 3β-HSD mRNA per microgram than small ovine luteal cells (340). Small, medium, and large 3β-HSD positive cells are also found in rhesus monkeys (341). In the mouse, 3β-HSD staining was seen in large and small luteal cells. Although the functional significance of luteal cell types is unclear, the two cell types are regulated differentially as is the case in the ovine CL (334).

b. CL regulation. The CL is a transient organ that is under the control of luteotropic factors that maintain PROG output. In primates, LH/hCG action through the LH receptor provides a primary means of luteotropic support. For example, hCG has been shown to maintain 3β-HSD mRNA expression in humans (342) and macaque CL (343). Furthermore, LH, hCG, and forskolin increased 3β-HSD activity in human

granulosa-lutein cell culture (344), and induction of 3β-HSD mRNA was observed by cAMP or hCG treatment of porcine granulosa-lutein cells (309). In addition, FSH has been shown to increase 3β-HSD protein and mRNA levels in human granulosa-lutein cells, and this effect could be enhanced via cotreatment with insulin (345). In rodents, both PRL and LH are important luteotropic factors. PRL potentiated hCG induction of 3β-HSD mRNA in rat CL (346), and treatment with mPL-1 (a placental lactogen) resulted in a dose-dependent increase in PROG production (347). Owing to the luteotrophic nature of PRL in the rat CL, it is possible that PRL might play a contributing role in the primate CL. Prolactin has been shown to increase basal PROG production in antral follicles (348), dispersed CL cells (349), and in granulosa-lutein cell cultures obtained from women undergoing egg retrieval in *in vitro* fertilization procedures (350, 351). Increases in PROG production occurred at physiological doses of PRL, but these effects were reversed when doses approached levels seen in hyperprolactinemic patients (348). Therefore, it is postulated that PRL might be capable of regulating genes involved in the PROG biosynthetic pathway. Although the direct control of 3β-HSD by PRL in humans has yet to be demonstrated, PRL has been shown to up-regulate 3β-HSD transcriptional activity *in vitro* (122). The activation of LH and PRL receptors is therefore an important event in the maintenance of 3β-HSD activity and PROG secretion by the CL.

As is seen in other steroidogenic tissues, increases in PKA and PKC activities modify 3β-HSD levels in the CL. Interestingly, PKA activity induced by increases in cAMP is affected by cross-talk from PKC pathways mimicked by treatment with phorbol esters. It has been shown that tetradecanoyl phorbol acetate decreased 3β-HSD activity in human granulosa-lutein cells (352). Forskolin treatment increased PROG production in small ovine luteal cells, whereas PMA inhibited PROG production in large and small cells, yet neither PMA nor forskolin altered 3β-HSD activity (353). Other studies have shown that PMA inhibited hCG/cAMP/LH/forskolin induction of 3β-HSD mRNA in porcine granulosa-lutein cells (309). These studies thus suggest that cross-talk exists between PKA and PKC pathways in the control of 3β-HSD expression in the CL.

Although LH is a critical control factor for PROG output, there is evidence that LH may be, at least partially, uncoupled to 3β-HSD mRNA levels. Administration of a GnRH antagonist to macaques suggested an uncoupling of PROG production from LH stimulation. Although PROG secretion was markedly diminished after 24 h of antagonist treatment, there were no differences in P450scc and 3β-HSD mRNA levels between antagonist-treated and control animals. These data suggest that P450scc and 3β-HSD mRNA regulation are uncoupled from LH-induced PROG production (354).

Rothchild (355) and others have suggested that PROG output by the CL could be regulated by endogenous and circulating steroids. Presumably, these intrinsic factors could work independently of external luteotropins at the level of 3β-HSD regulation. For example, trilostane treatment of female macaques reduced PROG production, but not relaxin output by the CL, suggesting that PROG is important in regulating PROG biosynthesis in the primate and this effect is uncoupled from luteolysis (356–358). RU486 induced luteolysis in rats with a decrease in 3β-HSD activity and an increase in 20α-HSD activity (359–362). On the other hand, R5020 but not hydrocortisone increased PROG output of rat luteal cells from d 19 of pregnancy (363). E_2 decreased 3β-HSD activity in isolated human (364, 365) and rat (366) luteal cells. Results from these studies suggest interplay in the steroid modulation of PROG output by the CL.

Other factors besides LH/hCG, PRL, and steroids have been shown to affect 3β-HSD regulation in the CL. These include growth factors, LHRH, catecholestrogens, and adrenergic innervation. In the case of growth factors, cAMP-stimulated 3β-HSD activity was enhanced by FGF, EGF, and TGFβ in cultured human granulosa-lutein cells (352), suggesting that these factors may play an autocrine role in the regulation of 3β-HSD in the CL. Similarly, the administration of GH to normally cycling women resulted in an increase of 3β-HSD mRNA levels in granulosa-lutein cells (367), although these effects may be mediated indirectly through the action of IGFs. On the other hand, GnRH antagonist decreased 3β-HSD content in rat luteal cells in culture (368), demonstrating the ability of GnRH to have a direct role in the regulation of luteal PROG production. Finally, catecholestrogen treatment has been shown to down-regulate 3β-HSD expression in rat luteal cells (369), whereas adrenergic innervation of the cow increases 3β-HSD activity and PROG secretion (370). These studies again suggest that multiple luteotrophic agents are involved in the regulation of 3β-HSD activity and PROG output by the CL, yet their relative importance has not been determined.

c. Luteolysis. Luteolytic factors are involved in the controlled destruction of the CL, a process termed luteolysis. Evidence for the induction of luteolysis by prostaglandins is extensive, and evidence also exists for direct regulation of 3β-HSD by PGF_{2a} during this process. A 50% reduction in 3β-HSD mRNA and protein levels was observed 4 h after injection of $PGF_{2\alpha}$ in pseudopregnant rats. This effect was transient because 3β-HSD levels returned to normal 8 h after injection (371). Along with a decrease in 3β-HSD activity, $PGF_{2\alpha}$ injection into whole rats resulted in an increase in 20α-HSD activity, and this effect could be overcome with a dose of PROG on d 19 of pregnancy (363). In isolated porcine granulosa cells, $PGF_{2\alpha}$ caused a reduction of P450scc and 3β-HSD mRNA levels (372). $PGF_{2\alpha}$ has also been shown to induce luteolysis in cows and decrease 3β-HSD expression (373–375). In ewes, $PGF_{2\alpha}$ injection at d 11 of estrous resulted in a 95% reduction in 3β-HSD mRNA after 48 h of treatment in large luteal cells (376). Although the role of prostaglandins in primate luteolysis is unclear, PGF_{2a} is clearly a mediator of luteal regression in other species.

Another factor known to induce luteolysis in rodent and play a role in 3β-HSD regulation is LH. LH-induced luteolysis on d 19 of pregnancy resulted in a decrease in 3β-HSD activity and an increase in 20α-HSD activity 48 h after treatment. This effect can be blocked with LH antiserum given on d 19 of pregnancy. The decrease in 3β-HSD could not be rescued by the treatment with diclofenac (prostaglandin H2 synthase inhibitor) or PROG, yet this treatment did reduce 20α-HSD activity (377, 378). Therefore, these results dem-

onstrate that LH signaling plays a role in luteal regression in rodents.

3. PCOS. PCOS is an ovarian disorder associated with hyperthecosis of the ovary and elevated serum LH, insulin, and androgen levels. Several studies have put forth evidence of aberrant 3β-HSD regulation in polycystic thecal cells. Troglitozone (an insulin-sensitizing agent) lowers androgen concentrations in women with PCOS. It was also shown that troglitazone reduced PROG production in porcine granulosa cells and interfered with forskolin- and FSH-induced PROG production. In addition, 3β-HSD enzymatic activity was reduced 60% after treatment (379). A reduction in 3β-HSD activity was also observed in rat cystic ovaries (380), and it has been hypothesized that excess adrenal androgens might inhibit 3β-HSD activity and increase DHEA levels (381). PCOS thecal cells exhibited both higher basal and forskolin-induced P450c17, 3β-HSD, P450scc, and 17β-HSD, but not StAR activities, relative to normal thecal cells (382). The mechanisms of aberrant steroidogenic enzyme expression in PCOS theca cells are unclear, but it has been suggested that hyperphosphorylation of insulin or high circulating insulin levels may be involved in the etiology of the condition.

C. Testis

1. Ontogeny and localization. In the human fetus, Leydig cells of the testis secrete T, which promotes differentiation of the Wolffian ducts, urogenital sinus, and external genitalia. Leydig cells first appear at 8 wk gestation in humans. T secretion in humans begins around 8 wk gestation, peaks around 14–16 wk, and then begins to fall, and these low levels are then maintained until late gestation when they decrease even further (383–385). Plasma T levels are high at birth in boys, fall within 2 to 5 d, and then rise again at 2–3 wk of life. They decrease correlatively with time from the third month to the sixth month of life, where minimal levels are reached and maintained until the onset of puberty (386–389). The induction of several steroidogenic enzymes, including 3β-HSD, is associated with these changes in T production.

Ontology studies on testis have been performed to examine T production in relation to 3β-HSD activity. Immunohistochemistry studies have revealed that human Leydig cells express 3β-HSD as early as 18 wk gestation (210). During gestation in human, 3β-HSD expression in the testis parallels androgen production and is detected at the second and third trimesters. Eight-month-old and 11-yr-old boys showed no testicular 3β-HSD staining, and levels remained low until puberty (390). In the rodent, 3β-HSD protein was detected in fetal rat testis on d 14.5 post coitum (p.c.) (391). Mouse type I/VI 3β-HSD is expressed in adult testis, and RT-PCR analysis showed that type I was expressed from embryonic d 13 until adulthood, whereas type VI was first detected at d 11 postnatally and was the predominant isoform in the adult animal (280, 392). Clearly, expression of 3β-HSD during development is an indicator of testicular androgen production.

Adult Leydig cells arise postnatally and encompass three developmental stages: progenitor, immature, and adult cells (393). Rat testes of postnatal d 15 showed 3β-HSD localization to the smooth ER of precursor Leydig cells and endothelial cells in the vicinity of 3β-HSD positive Leydig cells (394, 395). Large (12 μm) rat Leydig cells showed higher staining of 3β-HSD than smaller cells (8 μm) (396). Furthermore, cells from the rat H540 Leydig cell tumor line express 3β-HSD, P450c17, P450scc, P450arom, and 5α-reductase (397, 398). Other localization studies have examined 3β-HSD by immunocytochemistry in cynomolgus monkey testis, showing that 3β-HSD expression is seen in both Leydig and Sertoli cells (399). The immunoreactive 3β-HSD was labeled in part of Leydig cells and in all Sertoli cells in neonatal, late infantile, pubertal, and adult testes, whereas only a few Leydig cells, but no Sertoli cells, were labeled in early infantile testes (400). Thus, the predominate site of 3β-HSD expression in the testis is in Leydig cells, but evidence exists for some localization in Sertoli cells, at least in some primate species.

2. LH. During development, testicular 3β-HSD expression appears to be uncoupled from LH regulation. T production is first detected in humans at 9 wk (383), yet LH receptors do not appear until 12 wk (277). Studies in normal mice have shown that LH levels can be seen at d 17 p.c., which is after induction of 3β-HSD in fetal Leydig cells. 3β-HSD expression was also shown to be normal and independent of circulating GnRH during development (401). In rats, 3β-HSD protein was detected in fetal testis on d 14.5 p.c., whereas LH receptor expression was seen on d 16.5 p.c., also suggesting an independent mechanism of 3β-HSD regulation in the fetal rat testis (391).

In adult rat testis, however, primary control of 3β-HSD expression occurs through the action of the LH receptor and the induction of the cAMP second messenger system. LH, forskolin, cAMP, and cholera toxin all induced 3β-HSD mRNA in adult rat Leydig cells (402, 403), whereas hCG and cAMP induced 3β-HSD activity in immature rat Leydig cells (404). hCG also induced 3β-HSD activity in adult rat testis (405, 406). However, desensitizing doses of hCG down-regulated 3β-HSD mRNA in adult rat Leydig cells, whereas it had no effect on 3β-HSD mRNA in pseudopregnant rat ovaries (407). Furthermore, K9 mouse Leydig cells respond to hCG with a 4-fold increase in 3β-HSD mRNA (408). LH secretion by the pituitary is therefore a critical control pathway in adult rodent testicular steroidogenesis, which is in contrast to fetal regulatory mechanisms.

3. Steroids. Studies have suggested that androgens can inhibit androgen production by the testis and that this repression could occur at the level of 3β-HSD regulation. Rats treated with hCG showed an increase in 3β-HSD activity, but R1881 (androgen agonist) decreased hCG induction, whereas cyproterone acetate (androgen antagonist) increased activity (405). Similarly, DHT and methyltrienolone (androgen agonist) inhibited 3β-HSD activity in adult rat Leydig cells (409), and T decreased 3β-HSD activity in Leydig cells from C57BL/6J mice (134, 410). Disruption of steroidogenesis with aminoglutethimide (AMG) treatment increased 3β-HSD and P450c17 mRNA levels, whereas treatment with T and cAMP reversed this effect (411). DEX (glucocorticoid agonist) addition in the presence of AMG reduced both basal and cAMP stimulatory effect in 3β-HSD mRNA, but not P450c17 in

mouse Leydig cells (411). AMG has also been shown to increase 3β-HSD mRNA in porcine Leydig cells; however, in contrast to mice this effect could not be rescued by T treatment (412). DEX increased P450 content and decreased 3β-HSD activity in rat Leydig cells, and older rats appeared more sensitive than younger rats (413). The data concerning down-regulation of 3β-HSD by androgens and up-regulation by glucocorticoids suggests that steroid regulation occurs in testicular Leydig cells and differs between species.

4. Stress. A study by Orr and Mann (414) showed that immobilization stress (IMO) of rats results in a reduction of serum androgen but not of serum LH concentrations. For example, 3 h of IMO to rats showed a 7-fold increase in serum corticosterone along with a reduction in serum T concentrations. Western blots of testicular fractions revealed reduced amounts of P450scc and 3β-HSD, but not P450c17 (415). Treatment with an opioid antagonist, naltrexone-methobromide, reversed this effect, suggesting that endogenous opioid peptides might play a role in regulating testicular steroidogenesis (416–418). Another study has suggested that IMO of rats results in the down-regulation of P450c17 and 3β-HSD and is mediated through nitric oxide (NO) synthase (419). Testicular injection of aminoguanidine hydrochloride (inducible NO synthase inhibitor) prevented the sustained, but not the acute, stress-induced decrease in serum T. In addition, aminoguanidine hydrochloride added *in vivo* and *in vitro* blocked the sustained decrease in 3β-HSD and P450c17 activities. These data seem to suggest that P450c17 and 3β-HSD are regulated in the testis independently of LH and that opioids and/or NO production might mediate this effect. In addition, the direct role of glucocorticoids in the modulation of testicular 3β-HSD transcription has yet to be examined. It should however be kept in mind that the effect of stress on serum LH concentrations varies according to the type of and length of stress.

5. Growth factors. Studies have been performed to determine whether 3β-HSD regulation in fetal testis might be mediated through growth factors. EGF, TGFβ, activin A, and GH have been shown to increase 3β-HSD activity. It has been demonstrated that EGF treatment of porcine Leydig cells increased 3β-HSD activity (420), whereas TGFβ and activin A treatment increased 3β-HSD activity in immature porcine Leydig cells (421). GH induced StAR and 3β-HSD mRNA expression as well as androgen production in progenitor Leydig cells isolated from 28-d-old rats, whereas cycloheximide treatment altered this 3β-HSD induction (422). FGF and platelet-derived growth factor have been shown to reduce 3β-HSD activity in immature rat Leydig cells. aFGF in immature rat Leydig cells decreased 3β-HSD activity (423), whereas insulin/IGF-I treatment reversed the bFGF inhibitory effect in immature rat Leydig cells (423). Another report has shown that platelet-derived growth factor inhibits 3β-HSD activity induction by cAMP/hCG but not basal activity in cultured immature rat Leydig cells (424). Candidate growth factors might play a role in the regulation of 3β-HSD in the developing testis; however, the precise regulators of testicular 3β-HSD *in vivo* remain to be fully defined.

6. Other regulatory mechanisms. Other factors besides LH, steroids, stress, and growth factors have been tested for their ability to regulate 3β-HSD. Some of these include immune cytokines, neurotransmitter agonists, and oxidative metabolites. Amphetamine treatment of rat Leydig cells decreases 3β-HSD (425), whereas H_2O_2 decreased 3β-HSD activity in mouse MA-10 cells in a dose-dependent manner (426). Cl^- ion removal from culture media of rat Leydig cells enhances LH-stimulated steroidogenesis, and this is independent of 3β-HSD, but may involve an increase of StAR (427). CSF-1 deficient mouse Leydig cells have reduced amounts of 3β-HSD, P450scc, P450c17, and 17β-HSD protein (428). TNFα and IL-1 decreased cAMP-stimulated 3β-HSD mRNA and protein in mouse Leydig cells, however only TNFα reduced basal levels of 3β-HSD and T output (429). Taken together, these studies suggest that a complex interplay of extrinsic ligands (*i.e.*, LH) and intrinsic factors modulates androgen production by the testis.

D. Placenta

The placenta is responsible for the production of the majority of progestins and estrogens in primates during later stages of pregnancy. The 3β-HSD expressed in human placenta is the peripheral isoform, type I 3β-HSD, and is under differential regulatory control than the adrenal/gonadal isoform, type II 3β-HSD as previously described. It should be noted that the catalytic efficiency of human type I 3β-HSD is 5.9-, 4.5-, and 2.8-fold higher than the catalytic efficiency (V_{max}/K_m) of the human type II 3β-HSD (adrenal/gonadal) isoform using PREG, DHEA, and DHT, respectively, as substrates (27). The higher K_m for type II 3β-HSD is correlative to the higher amounts of substrate produced in these tissues (430). A detailed understanding of the regulation of 3β-HSD in the placenta and other peripheral tissues must take into consideration the catalytic differences between the two isoforms present in humans.

1. Ontogeny and localization. It is generally considered that human 3β-HSD expression in the placenta is localized to syncytiotrophoblastic cells. 3β-HSD mRNA and protein were detected in uninucleate cytotrophoblasts in chorion laeve similar to that in the syncytia, but not in cytotrophoblast placenta. The expression of 3β-HSD was not induced either temporally or by cAMP in laeve cytotrophoblasts, whereas villous cytotrophoblasts spontaneously demonstrated progressive increases in 3β-HSD expression (161). Another study in humans has shown that 3β-HSD protein was localized to syncytiotrophoblast and intermediate trophoblast cells at both villous and extravillous sites, but not in cytotrophoblast cells from 6 wk gestation to term (431). Although several aspects of the baboon placenta are different from that of human, syncytiotrophoblast expression of 3β-HSD mRNA remained constant during all phases of gestation (432).

In contrast to primates, the mouse placenta is not of crucial importance in the *de novo* synthesis of steroids in the second half of pregnancy (347). However, recent reports have shown a role for the local production of steroids in the maternal decidua and trophoblast layers surrounding the endometrial

cavity. Mouse type VI 3β-HSD is expressed in the uterine wall during decidualization (433). Expression is initially in the antimesometrial cells of the decidua on d 6.5–7.5 p.c. and then shifts to embryonal giant trophoblast cells. 3β-HSD VI then declines between d 10.4 and 14.5 p.c. (433). These data thus suggest that maternal expression of placental steroidogenic enzymes may play a determining role in maintenance of pregnancy and/or embryogenesis in a localized manner.

2. Regulation. Activation of PKA and PKC pathways in human placental *in vivo* models results in an increase in 3β-HSD activity. PMA and cAMP induces 3β-HSD mRNA in human JEG-3 choriocarcinoma cells; however, the phorbol ester response is mediated by PKC (159). A23187 increased basal 17β-HSD-I and 3β-HSD-I, but not P450scc in human JEG-3 cells. Furthermore, A23187 decreased cAMP stimulated type I 3β-HSD and P450scc, but synergistically activated type I 17β-HSD (434). Cross-talk between calcium flux/PKC and cAMP/PKA signaling pathways in the regulation of placental steroidogenesis is thus evident.

Growth factors have been tested for their ability to induce 3β-HSD in the human placenta. For example, insulin and IGF-I treatment of human cytotrophoblasts increased 3β-HSD activity (435). IGF-II also increased 3β-HSD activity in human placental cytotrophoblasts, and this effect could be inhibited via cycloheximide or actinomycin D treatment (436). In addition, Morrish *et al.* (437) have examined the effects of cytotrophoblast differentiation on gene expression. EGF, colony-stimulating factor (CSF)-1, and granulocyte-macrophage CSF stimulate, whereas TGFβ inhibits human cytotrophoblast differentiation, and subtractive hybridization experiments between undifferentiated and differentiating cytotrophoblasts identified an increase in the hCG α-subunit, β1-glyocoprotein, 3β-HSD, and plasminogen activator inhibitor-1 mRNAs during differentiation (437). These results suggest that growth factors involved in placental differentiation might play a role in the regulation of 3β-HSD and other factors. Although species- and placental-specific mechanisms are observed, similar regulatory pathways as those seen in other tissues exist for 3β-HSD expression in the placenta.

It is interesting that sex steroids have been shown to regulate 3β-HSD mRNA levels in trophoblast cells (438). Treatment of human trophoblast cell cultures with PROG and E_2 led to increases in P450scc and 3β-HSD mRNA levels, but had no effects on 3β-HSD protein levels (438). The potential transcriptional regulation of the human type I 3β-HSD gene by PROG or E_2 is interesting in light of the lack of palindromic steroid regulatory elements in the promoter and 5′-flanking sequence. In this regard, the human *HSD3B1* gene may share some similar mechanisms with the *HSD3B2* gene that has recently been shown to respond to PROG and glucocorticoids, although it is also lacking palindromic steroid regulatory elements (132).

The murine ortholog, type VI 3β-HSD, is the only isoform expressed in giant trophoblast cells during the first half of mouse pregnancy. Transfection studies in placental and nonplacental cells identified a novel 66-bp trophoblast-specific enhancer element located between −2896 and −2831 of type VI 3β-HSD promoter (197). As revealed by DNase protection analysis, three trophoblast-specific binding sites, FPI, FPII, and FPIII, were identified. EMSAs with oligonucleotides representing the protected sequences, FPI and FPIII, and nuclear extracts isolated from human JEG-3 cells and from mouse trophoblast cells demonstrated the same binding pattern that was different from the binding pattern with mouse Leydig cell nuclear proteins. Additional assays identified AP-2γ and the homeodomain protein, Dlx-3, as the transcription factors that specifically bind to FPI and FPIII, respectively. Site-specific mutations in each of the binding sites eliminated enhancer activity, indicating that AP-2γ and Dlx-3, together with an additional transcription factor(s) that is conserved between humans and mice, are required for trophoblast-specific expression of type VI 3β-HSD (197).

E. Liver

Hepatic 3β-HSD expression is presumed to be important in the metabolism and inactivation of steroids. 3β-HSD has been purified from human liver (439, 440), and 3β-HSD activity in human liver microsomes was shown to be three times higher for the reduction of DHT to 3β-DIOL than the reverse reaction (441). The adult mouse liver expresses 3β-HSD types II, III, and IV, with type III predominating. Mouse type I 3β-HSD (analogous to human type II) is the predominant form in the fetal liver during development until postnatal d 1, when type III is induced. Mouse type I expression ends at postnatal d 20, while type V is detected at postnatal d 40 and is male specific, whereas type II is expressed at low levels throughout development. These data suggest that the mouse liver might play a key role during fetal development (95). Rat type III is expressed in the male rat liver and is a 3-KSR implicating its function in inactivating steroids in this tissue (75, 85, 442). Immunohistochemical studies localized 3β-HSD protein to the bile duct epithelium and microsomal fraction in the pig liver (98, 99).

Studies have also examined the regulation of 3β-HSD in the rodent liver. It appears that isoform-specific, sexually dimorphic regulation of 3β-HSD occurs in rats and mice. Castration of rats led to an 80% reduction in type III 3-KSR in rat liver after 15 d, and this effect could be rescued by the administration of DHT. Similarly, DHT increased levels in ovariectomized females to levels seen in the male (85). T increased, whereas E_2 decreased type III 3-KSR in gonadectomized rats, but these effects were not seen in HYPOX animals. Also, PRL exerted a sex-specific blockade of T/E_2 regulation in HYPOX females (85). A down-regulation of type III 3-KSR by GH was also observed in rats (85, 443). In the mouse liver, sexually dimorphic GH expression regulates many steroidogenic enzymes. GH expression in transgenic mice driven by the phosphoenolpyruvate-carboxykinase promoter down-regulates the 42-kDa male-specific isoform without affecting the 47-kDa nondimorphic isoform (444). These data suggest that circulating levels of steroids might affect regulation of 3β-HSD activity in the liver, principally through altering GH and PRL levels, and thereby resulting in feedback on steroid degradation.

F. Breast

Sex steroids are well recognized to play a predominant role in the regulation of cell growth and differentiation of normal mammary gland as well as in hormone-sensitive breast carcinomas. Estrogens stimulate cell growth of hormone-sensitive breast cancer cells (445–448), whereas androgens exert an antiproliferative action in breast cancer cells (12, 448–450). The role of estrogen formation in peripheral intracrine tissues is well illustrated in women by the important benefits on breast cancer observed in postmenopausal women treated by a series of aromatase inhibitors (451). Moreover, the recent observation that postmenopausal women who received the antiestrogen raloxifene for only 3 yr led to a 76% decrease in the incidence of breast cancer (452) is also consistent with the role of extra ovarian estrogens in the development and growth of breast cancer. 3β-HSD activity was detected in breast tissue in humans as early as 1990 (453), and 3β-HSD activity was demonstrated in human adipose stromal tissue (454). Expression in breast cancer tissue has also been investigated. Stage II/III infiltrating ductal primary breast tumors demonstrated 3β-HSD activity (455), and 3β-HSD protein was seen in 36% of breast carcinoma samples tested (456). Similarly, 3β-HSD activity was detected in ZR-75-1 breast cancer cells (457).

Both endocrine and paracrine influences on the proliferation of human breast cancer cells are well recognized, thus supporting the suggestion that breast tumor growth is modulated by the hormonal environment (458, 459). The presence in breast tumors of considerable numbers of tumor-associated macrophages and tumor-infiltrating lymphocytes secreting a wide spectrum of cytokines also suggests a key role for these factors in neoplastic cell activity (460, 461). Moreover, it has been observed that Natural Killer cells isolated directly from ductal invasive breast tumors secrete increased amounts of interferon-γ and IL-4 (462). The rationale to investigate the potential action of IL-4 and IL-13 in breast cancer cells pertains to the recent demonstration of high-affinity IL-4 receptors in human breast carcinomas (463), the inhibitory effect of IL-4 and IL-13 on the estrogen-induced breast cancer cell proliferation (171, 183, 464, 465) and their potent stimulatory effect on GCDFP-15/PIP gene expression (183), as well as on spermidine transport in ZR-75-1 cells (466). Furthermore, it has been reported that some cytokines can regulate the expression of several enzymes involved in sex steroid formation and inactivation in breast cells. For example, IL-6 regulates the expression of 17β-HSD/KSR (171, 467), estrone sulfatase (468), and P450 aromatase (15, 126), whereas IL-4 regulates 17β-HSD/KSR (171) activity as well as 3β-HSD type 1 activity (51, 52, 171).

Under basal growth condition, there is no detectable 3β-HSD activity in ZR-75-1 cells; however, incubation with IL-4 or IL-13 caused a potent up-regulation of 3β-HSD activity (51). It should be noted that IL-1α, IL-2, IL-3, IL-6, IL-8, IL-10, interferon-γ, and EGF all failed to induce 3β-HSD activity in ZR-75-1 cells. In the ER^- MDA-MB-231 cells, IL-4 increased this enzymatic activity by 3.3-fold as measured by the conversion of [^{3}H]DHEA into both [^{3}H]-4-DIONE and [^{3}H]-A-DIONE, thus indicating that the IL-4 action on 3β-HSD activity was not restricted to ER^+ ZR-75-1 and T-47D human breast cancer cells. Furthermore, the 5.5-fold increase in 3β-HSD activity after exposure to IL-4 in normal human mammary epithelial cell in primary culture suggests the physiological relevance of our finding.

As shown in Fig. 14, IGF-I and insulin increase the stimulatory effect of IL-4 on 3β-HSD activity. The stimulatory effect of IGF-I and insulin on the IL-4-induced 3β-HSD activity was exerted at EC_{50} values of 0.8 and 3 nM, respectively, thus providing the first evidence of potentiation of an IL-4 response by IGF-I and/or insulin. The relevance of this finding also pertains to the observation that most invasive breast tumors appear to express IGF-I receptor, and their growth is stimulated *in vitro* in response to exogenous IGF-I (469). Furthermore, it is of interest to note that the amplitude of the effect of insulin or IGF-I on IL-4-induced 3β-HSD activity was more striking in the presence of submaximal concentrations of the cytokine. It is thus tempting to speculate that the action of these growth factors could be significant under physiological conditions when IL-4 is present at low concentrations.

The similar effects exerted by IL-4 and IL-13 are explained by the fact that their receptors share at least one subunit (470, 471). One type of IL-4R is composed of the IL-4Rα chain and the common γ chain from the IL-2 receptor, whereas the IL-13 receptor α1 chain can heterodimerize with the IL-4Rα chain to form receptors for both IL-4 and IL-13 (472).

Because aromatase is expressed in a large proportion of human breast carcinomas (15, 473, 474), induction of 3β-HSD activity by IL-4 and IL-13 could markedly increase the formation of estrogen precursors, namely Δ^4-DIONE and T, and therefore may well have a significant impact on the estrogen synthesis in breast tumors. However, it should be taken into consideration that androgens are well recognized to exert an antiproliferative action in breast cancer cells (446, 447). Thus, the IL-4-induced 3β-HSD activity would first increase the intracellular levels of T that in return would inhibit breast cancer cell growth. In support of this hypothesis, DHEA exerts an inhibitory effect on the development of ZR-75-1 human breast cancer cell xenografts in ovariectomized nude mice (450). Moreover, DHEA exerts a potent inhibitory effect on the development of dimethylbenzanthracene-induced mammary carcinoma in the rat (475). Finally, DHEA exerts an almost exclusive androgenic effect in the rat mammary gland (476).

Δ^5-DIOL is well recognized to exert an estrogenic action at physiological concentrations due to its relatively high affinity for estrogen receptors, while it also possesses a lower affinity for the androgen receptor (446, 448, 449, 477, 478). Thus, as demonstrated by an experiment using both estrogen- and androgen-sensitive reporter constructs, the induction of type I 3β-HSD expression by IL-4 may indeed modulate the balance between estrogenic and androgenic biological responses when incubating ZR-75-1 cells with Δ^5-DIOL (51). Our findings thus strongly support the relevance of the action of IL-4 and IL-13 in the biosynthesis of active sex steroids from adrenal precursors (DHEA and Δ^5-DIOL) in both normal and tumoral human breast tissues.

G. Prostate

In men, the importance of intracrine formation of active androgens is in concordance with the observation that after

elimination of testicular androgens by medical or surgical castration, the intraprostatic concentration of DHT remains at approximately 40% of that measured in the prostate in intact 65-yr-old men, thus leaving important amounts of free androgens to continue stimulating growth of the prostate cancer (13, 479, 480). Indeed, an important source of androgens in the human prostate is androgens synthesized locally by the conversion of adrenal-derived DHEA and DHEA-S by 3β-HSD and 17β-HSD type V into Δ^4-DIONE and T, respectively.

It has been shown that 3β-HSD expression colocalizes by immunocytochemistry and *in situ* hybridization with 17β-HSD type V in the glandular epithelium of the prostate with the highest levels seen in basal cells relative to luminal cells (481). Other studies have found 3β-HSD in human hyperplastic prostate tissue (482, 483). These studies demonstrate the capacity of the human prostate to locally produce androgens, which increase the hypertrophic potential of the organ.

More recently, IL-4 and IL-13 were shown to induce 3β-HSD activity and mRNA levels in normal human prostate epithelial cells (PrEC) (52). The 3β-HSD activity was not detectable in PrEC under basal growth conditions, whereas incubation with IL-4 or IL-13 induced a potent up-regulation in 3β-HSD activity observed at EC_{50} values of 20 and 170, respectively. Moreover, IL-4 induced 3β-HSD gene expression as early as 3 h after exposure (52), whereas the 3β-HSD activity was detectable after a short incubation period of 8 h with IL-4 and continued to increase for at least 48 h, thus showing a rapid induction of 3β-HSD activity by IL-4. Thus, the induction of 3β-HSD activity by IL-4 and IL-13 would markedly increase the formation, from DHEA, of the type V 17β-HSD/KSR substrates Δ^4-DIONE and A-DIONE, which would lead to the synthesis of T and DHT, respectively. This may well have a significant impact on the development of prostate cancer because the effect of prolonged presence of androgens that stimulate prostate cancer growth is well established (13, 484, 485). The relevance of the IL-4 action in prostate cells also pertains to the observation that in an immortalized human prostate cell line derived from primary cultures, the gene expression of the tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 was regulated by IL-4 (486). It was suggested that IL-4 might control the molar ratio of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 to influence the level of protease activity and perhaps the invasive behavior of malignant cells *in vivo*.

In the general population, we observe an interindividual variability in the susceptibility to cancer; however, little is known about the underlying genes contributing to this variability. Although a number of rare highly penetrant loci contribute to the Mendelian inheritance of prostate cancer as recently described (487), some of the familial risks may be due to shared environment and more specifically to common low-penetrance genetic variants, which alter predisposition to prostate cancer. It is not surprising that analyses of genes encoding key proteins involved in androgen biosynthesis and action, led to the observation of a significant association between common genetic variants and a susceptibility to prostate cancer (488–491). Such analyses provided some understanding of how common low-penetrance polymorphisms present in a number of these candidate genes were involved in prostate cancer onset, progression, and response to treatment for the disease. To evaluate the possible role of *HSD3B* genes in prostate cancer susceptibility, a recent study used a panel of DNA samples collected from 96 men with or without prostate cancer for sequence variants in the putative promoter region, exons, exon-intron junctions, and 3′-untranslated region of *HSD3B1* and *HSD3B2* genes by direct sequencing (492). Four of the 11 single nucleotide polymorphisms (SNPs) were informative. These four SNPs were further genotyped in a total of 159 hereditary prostate cancer probands, 245 sporadic prostate cancer cases, and 222 unaffected controls. Although a weak association between prostate cancer risk and a missense SNP (B1-N367T) was found, stronger evidence for association was found when the joint effect of the two genes was considered. Indeed, men with the variant genotypes at either B1-N367T or B2-c7519g had a relative risk of 1.76 (95% confidence interval, 1.21–2.57; $P = 0.003$) to develop prostate cancer compared with men who were homozygous wild type at both genes. The risk for the hereditary type of prostate cancer was stronger, with a relative risk of 2.17 (95% confidence interval, 1.29–3.65; $P = 0.003$). Most importantly, the subset of hereditary prostate cancer probands, whose families provided evidence for linkage at 1p13, predominantly contributed to the observed association (492). Additional studies are warranted to confirm these findings.

The rat provides a useful model for the study of prostate steroid production. 3β-HSD activity was seen in the rat ventral prostate (493) with higher activity in epithelial *vs.* stromal cells (494). In addition, the 5α-reductase inhibitor, 4MA, was shown to decrease 3β-HSD activity in the prostate of rats (495), thus suggesting that androgens may play a role in the regulation of prostatic steroidogenesis; however, more work needs to be done to verify these effects.

H. Skin

Sharp *et al.* (496) have shown that 3β-HSD activity in human fetal skin is low at 12 wk, whereas 28-, 38-, and 41-wk human fetal skin samples showed high 3β-HSD activity. It was also observed that 90% of 3β-HSD activity from human fetuses was found to be in sebaceous glands (497). Thereafter, it has been demonstrated that human 3β-HSD is found in sebaceous gland ducts with highest expression in fully differentiated ducts (53, 498, 499). 3β-HSD expression in sebaceous glands provides the ability of glands to control local concentrations of DHEA and other steroids in the skin.

It has been demonstrated recently that IL-4 and IL-13 caused a rapid induction of 3β-HSD activity in HaCaT human immortalized keratinocytes (Fig. 13) (52, 500). The physiological relevance of this finding is well supported by the observations that: 1) human skin can convert DHEA into T (501); 2) DHEA can stimulate sebaceous gland secretion (502, 503); 3) the *in vivo* sebum secretion rate in humans is closely correlated with 3β-HSD activity (504); and 4) 3β-HSD type 1 gene is expressed in human skin (27, 53). The relevance of this finding also pertains to the observation that IL-4 and IL-13 stimulate IL-6 expression in normal keratinocytes and ker-

atinocyte cell lines of human origin (505), and that IL-4 induces proliferation of normal human keratinocytes, which is associated with c-*myc* gene expression (506). Therefore, it is likely that these cytokines play a role in the regulation of inflammation at both systemic and local levels. Finally, human keratinocytes constitutively express IL-4R, and its expression is also increased in some epidermal proliferative diseases (507, 508), whereas IL-4 can be detected in atopic dermatitis and during the elicitation phase of contact sensitivity (507, 509). Thus, it would be relevant to investigate the potential effect of androgens or estrogens in those pathologies as well as in the physiological functions of keratinocytes.

In rats, type IV is the major isoform in skin (83) with much lower expression of types II and I, whereas the only 3β-HSD transcript found in mouse skin is the type VI (91, 92). HYPOX rats showed an increase of 3β-HSD activity in female animals and a decrease in males. Moreover, treatment of HYPOX animals with L-T_4 demonstrated elevated 3β-HSD mRNA in a male-specific manner, corticosterone showed a decrease in mRNA in both sexes, and elevated PRL levels caused a stimulation of type IV in both sexes (510).

I. Brain

Steroid hormones exert important functions in the control of growth, maturation, and differentiation of the central and peripheral nervous systems. These actions have long been attributed exclusively to steroid hormones secreted by adrenal glands and gonads. However, during the past decade, it has been demonstrated that both neurons and glial cells are capable of synthesizing bioactive steroids, now called neurosteroids, which also participate in the control of various functions in the central nervous system (CNS). Several recent studies have made possible the establishment of the neuroanatomical distribution of key enzymes (see Ref. 511 for a review). Levels of PROG are higher in the peripheral nerves of rats and mice than in plasma, and these levels persist after removal of steroidogenic endocrine glands (512). It should be noted that examination of PROG production must be coupled with relative levels of PROG metabolites to fully understand the neurosteroid milieu present in specific regions of the brain.

The presence of 3β-HSD activity in the CNS was first demonstrated in homogenates of rat amygdala and septum (513), whereas the first immunohistochemical localization of 3β-HSD in the CNS was performed in the European green frog *Rana ridibunda* (514). Multiple 3β-HSD localization studies in rats have characterized expression at the gross neuroanatomical level. For instance, P450scc and 3β-HSD are expressed as demonstrated by *in situ* hybridization in the hippocampus, dentate gyrus, cerebellar granular layer, olfactory bulb, and Purkinje cells of the rat brain (515). Other studies showed by RT-PCR that 3β-HSD expression occurs in the cerebellum and cerebrum of the rat brain (516). 3β-HSD mRNA was also localized to the olfactory bulb, striatum, cortex, thalamus, hypothalamus, septum, habenula, hippocampus, and cerebellum in the rat brain. The cerebellum showed the highest levels of expression of a 1.8-kb transcript (517). Rat 3β-HSD mRNA was also localized in the medulla ventrally and laterally bordering the fourth ventricle (518). *In situ* studies showed 3β-HSD expression in neonatal rat Purkinje cells and external granule cells throughout the cerebellum at 10 d of age (519). In the peripheral nervous system, rat 3β-HSD was localized in the sciatic nerve (520, 521) as well as in dorsal root ganglion, where its expression is increased after peripheral nerve injury (522). Although expression patterns of steroid metabolizing enzymes in the brain are emerging, the functional implications of local steroid production are only beginning to be examined.

Other studies have demonstrated 3β-HSD expression in cell culture. Rat Schwann cells and sensory neurons from the embryonic dorsal root ganglia express 3β-HSD protein and activity (523). RT-PCR studies have shown that rat cerebellar glial and cerebellar granule primary cell cultures expressed 3β-HSD and P450scc (524). Rat astrocytes express 3β-HSD activity (525, 526), whereas 3β-HSD, 3α-HSD, and 5α-reductase activities can be detected in fetal rat brain cells in culture (527). 3β-HSD was increased 10-fold in rat Schwann cell/neuronal cocultures, suggesting that 3β-HSD regulation in isolated cells differs from that seen *in vivo*. Moreover, 3β-HSD and P450scc mRNAs were induced during the differentiation of O-2A precursor cells to oligodendrocytes (528). Astrocytes appear to be the most steroidogenically active cells in the brain as determined from cells isolated from the neonatal rat cerebral cortex (529). Finally, it has been reported that in frog hypothalamic explants that the endozepine triakontatetraneuropeptide stimulates 3β-HSD activity. This effect was mimicked by a peripheral benzodiazepine receptor (PBR) agonist and inhibited by the PBR antagonist PK11195, whereas the central-type PBR antagonist, flumazenil, did not affect the triakontatetraneuropeptide-evoked neurosteroid secretion (530). These cell culture models therefore provide systems in which regulatory factors for the control of neurosteroidogenesis can be identified.

J. Other expression sites

The importance of local steroid production by peripheral isoforms of 3β-HSD is easily envisioned. However, far too little is known about the regulation of 3β-HSD in additional peripheral tissues, namely, kidney (88), vas deferens (531), bone (532, 533), cardiovascular tissues (534, 535), adipose tissue (454), *etc*. Nevertheless, as one example supporting the key role of 3β-HSD activity in peripheral tissues, it has been recently observed that 3β-HSD is expressed in mouse megakaryocytes in which it is essential for the initiation of proplatelet formation. A recent study showed that 3β-HSD expression is up-regulated by the megakaryocyte/erythrocyte specific transcription factor p45 NF-E2 (536). This up-regulation induces biosynthesis of estrogens in matured megakaryocytes, which in turn, by autocrine action on the estrogen receptor, triggers proplatelet formation. Because platelet transfusions are presently the only established therapy for preventing bleeding complications in severely thrombocytopenic individuals, these results provide an interesting venue for the development of an effective treatment of thrombocytopenia (536). The molecular mechanisms involved in the regulation of 3β-HSD in classical steroidogenic tissue appears to have little overlap due to the lack of control by trophic factors in these tissues, but cytokines, GH, and

PRL are good candidates to first investigate. Thus, the mapping of hormones and transcription factors involved in the control of each of these tissues points to a potentially rich source of regulatory pathways involved in peripheral steroidogenesis that have yet to be elucidated. An understanding of these pathways will allow for controlled modulation of steroidogenesis and its effects in these tissues.

VII. Molecular Genetics of Human 3β-HSD Deficiency

Classical 3β-HSD deficiency is a rare form of congenital adrenal hyperplasia (CAH) accounting for about 1–10% of cases of CAH (537, 538). The salt-losing forms of CAH are a group of life-threatening diseases that require prompt recognition and treatment. Indeed, autosomal recessive mutations in the *CYP21*, *CYP17*, *CYP11B1*, and *HSD3B2* genes that encode steroidogenic enzymes, in addition to mutations in the gene encoding the intracellular cholesterol transport protein, StAR, can cause CAH, each resulting in different biochemical consequences and clinical features (277, 539–541). These five biochemical defects impair cortisol secretion, which results in compensatory hypersecretion of ACTH and consequent hyperplasia of the adrenal cortex. However, only deficiencies in 21-hydroxylase (*CYP21*) and 11β-hydroxylase (*CYP11B1*) predominantly result in virilizing disorders. Indeed, in patients with the classical form of these two defects, the most noticeable abnormality in the sexual phenotype is the masculinization of the female fetus due to oversynthesis of adrenal DHEA and DHEA-S, whereas in comparison, affected males do not have ambiguous genitalia. In contrast to these two most frequent causes of CAH, which are exclusive adrenal defects, 3β-HSD, P450c17, and StAR deficiencies impair steroidogenesis in both the adrenals and the gonads, resulting in the diminished formation of sex steroids in addition to cortisol and aldosterone.

A. Clinical features

Since the first reports by Bongiovanni (542, 543), many patients of both sexes have been described with various heterogeneity upon clinical presentation (Table 2). Classical 3β-HSD deficiency results from mutations in the *HSD3B2* gene, whereas the *HSD3B1* gene in these patients is normal (56–59, 539, 544–547) (also Ref. 548 and references therein). The classical form of 3β-HSD deficiency can be divided, depending upon the severity of the salt-wasting associated with the disorder, into the salt-wasting or non-salt-wasting forms. There is no correlation between the impairment in male sexual differentiation and salt wasting.

Male individuals suffering from classical 3β-HSD deficiency present with either perineal hypospadias or perineoscrotal hypospadias as shown in Table 2. On the other hand, complete or partial inhibition of 3β-HSD activity in the adrenals and ovaries was not accompanied by a noticeable alteration in the differentiation of the external genitalia of female patients, as indicated by the absence of ambiguous external genitalia. The reason for this striking difference in phenotype between the male and female individuals is that deficiency of 3β-HSD in the fetal testis results in a lowering of the levels of T below the levels required for the normal development of external genitalia (56–58, 539, 544–547). However, males affected with pseudohermaphroditism and complete or partial 3β-HSD deficiency have intact Wolffian duct structures, including vas deferens. This is also the case in 17β-HSD type III deficiency as well as 5α-reductase type II deficiency, which is consistent with the hypothesis that a major effect of 3β-HSD deficiency is to reduce the formation of DHT below the level required for normal development of external genitalia (545). However, it is important to keep in mind that the decreased DHT results only from reduced T, knowing that the 5α-reductase activity is intact in these patients.

The salt-losing form of classical 3β-HSD deficiency is usually diagnosed during the first few months of life due to insufficient biosynthesis of aldosterone and consequent salt loss, which may be fatal if not diagnosed and treated early (542, 547, 549–554). In contrast, the non-salt-losing form of 3β-HSD deficiency may be diagnosed either at a young age in the presence of indicating factors, such as a family history of death during early infancy (555), perineal hypospadias in male newborns (556, 557), or failure to gain weight (553), or the diagnosis may be made at a later date (554, 558–560). It would be expected that death in early infancy is more indicative of salt loss; however, the family history of a patient diagnosed with the non-salt-losing form of 3β-HSD deficiency indicated that four of seven children died in early infancy (554). Because sexual differentiation is normal in female newborns affected by non-salt-losing 3β-HSD deficiency, the correct diagnosis is delayed until adrenarche (559, 561) or puberty (560) (Table 2).

Interestingly, the patient recently reported by Van Vliet and colleagues (561) underwent progressive feminization starting between 8 and 9 yr of age and was the first female with severe salt-wasting 3β-HSD deficiency reported to have progressive breast development, regular menses, and evidence of PROG secretion. This patient is homozygous for the A10E mutation that results in the complete loss of type II 3β-HSD activity (561). One possible explanation for the development of breasts and the endometrium in the patient reported by Van Vliet is that there is local conversion of inactive adrenal precursors into estrogens. On the other hand, it is possible that, as suggested for the testes (562), pubertal levels of gonadotropins may induce sufficient ovarian 3β-HSD activity by increasing the normally low levels of type I 3β-HSD, thereby allowing significant ovarian production of E_2. The rise in the patient's plasma PROG levels favors the latter hypothesis and raises the possibility that this individual may be fertile (561). It should be noted that a male affected with proven severe 3β-HSD deficiency has in fact fathered children (56, 549). These recent findings further illustrate that genotype may not always predict the phenotype in patients with this disorder (548).

B. Biological diagnosis

The basal plasma levels of Δ^5-3β-hydroxy steroids such as PREG, 17OH-PREG, and DHEA are elevated in affected individuals. An elevated ratio of Δ^5/Δ^4-steroids is considered to be the best biological parameter for the diagnosis of 3β-HSD deficiency (560, 563). The best criteria for the correct

TABLE 2. Genotype-phenotype relationships of patients with classical 3β-HSD deficiency bearing a missense, nonsense, frameshift, or an in-frame deletion mutation in the *HSD3B2* gene

Family	Origin	Patient	Karyotype	Age at diagnosis	Phenotype	Salt-wasting	Mutant alleles	Apparent activity in intact cells	Evidence of protein instability	Ref. no.
Homozygotes										
1	Pakistani	1	46 XY	Birth	Hypospadias, hyperpigmented and bifid scrotum	No (normal renin activity)	L6F /L6F	54.75 ± 8%	No	580
2	French-Canadian	2	46 XX	3 wk	Normal genitalia, breast development and menses at puberty	Yes	A10E/A10E	No detectable activity	Yes	561
3	French-Canadian	3	46 XY	13 d	Ambiguous genitalia, palpable testes in bifid scrotum	Yes	A10E/A10E	No detectable activity	Yes	561
4	Egyptian	4	46 XY	6 months	Perineoscrotal hypospadias	No (normal renin activity)	A10V/A10V	29.1 ± 0.6%	No	59
		5	46 XY	4 months	Perineoscrotal hypospadias					
5	Algerian	6	46 XY	Birth	Perineoscrotal hypospadias, bifid scrotum	Yes	G15D/G15D	No detectable activity	Yes	555, 582
6	Brazilian	7	46 XX	31 yr	Clinically normal	No (normal renin activity)	A82T/A82T	7.6 ± 0.5%	No	59, 559, 577
		8	46 XY	Birth	Ambiguous genitalia					
		9	46 XY	Birth	Ambiguous genitalia					
7	Brazilian	10	46 XX	5 yr	Premature pubarche	No (normal renin activity)	A82T/A82T	7.6 ± 0.5%	No	59, 577
8	French	11	46 XY	20 months	Perineal hypospadias	No (elevated renin activity)	N100S/N100S	2.8 ± 0.07%	No	553, 575
9	Chilian	12	46 XX	Birth	Hyperpigmentation, normal genitalia	Yes	E135X/E135X	No predicted activity	N/A	594
10	Swiss	13	46 XX	14.7 yr	Lack of spontaneous breast development	Yes	W171X/W171X	No predicted activity	N/A	56, 550, 578
11	Swiss	14	46 XX	?	Lack of spontaneous breast development	Yes	W171X/W171X	No predicted activity	N/A	56, 595
12	Scottish	15	46 XY	5 yr	Perineal hypospadias	No (normal renin activity)	L173R/L173R	52.8 ± 0.6%	No	59, 545
		16	46 XX	2 yr	Clinically normal					
13	Japanese	17	46 XY	3 months	Hyperpigmentation, severe hypospadias, bifid scrotum	Yes	L205P/L205P	No detectable activity	No	59, 596
		18	46 XX	11 months	Hyperpigmentation, mild clitoromegaly					
14	Algerian	19	46 XY	Birth	Perineal hypospadias with micropenis, palpable testes in scrotum	Yes	P222Q/P222Q	No detectable activity	No	59
		20	46 XX	1 month	Mild clitoromegaly, first diagnosis 21-hydroxylase deficiency					
15	Eastern European	21	46 XX	4 wk	Normal external genitalia, first diagnosis 21-hydroxylase deficiency	Yes	P222T/P222T	No detectable activity	Yes	597
16	Turkish	22	46 XY	4 yr	Scrotal hypospadias, bifid scrotum	No (normal renin activity)	A245P/A245P	35.4 ± 0.2%	Yes	57, 554
17	Japanese	23	46 XY	N/A	Pigmentation, hypospadias, bifid scrotum	Yes	R249X/R249X	No predicted activity	N/A	598
18	Japanese	24	46 XY	N/A	Pigmentation, mild clitomegaly	Yes	R249X/R249X	No predicted activity	N/A	598
19	Japanese	25	46 XY	Birth	Hypospadias with micropenis and bifid scrotum	Yes	R249X/R249X	No predicted activity	N/A	562, 599
20	Japanese	26	46 XY	N/A	Pigmentation, hypospadias, bifid scrotum	Yes	T259R/T259R	No detectable activity	Yes	59, 598
		27	46 XX	2 wk	Normal genitalia with severe pigmentation					
21	Taiwanese	28	46 XY	Birth	Hypospadias, microphallus	Yes	T259M/T259M	Very weak activity	Yes	59, 580
22	Brazilian	29	46 XX	31 yr	Clitoromegaly, severe virilization	No (normal renin activity)	T259M/T259M	Very weak activity	Yes	59, 600
23	Brazilian	30	46 XX	7.8 yr	Mild clitoromegaly	No (normal renin activity)	T259M/T259M	Very weak activity	Yes	59, 594
24	Sri-Lankan	31	46 XY	Birth	Perineal hypospadias with micropenis, palpable testes in scrotum	Yes	687del27/687del27	No detectable activity	Yes	59
25	Sri-Lankan	32	46 XY	Birth	Perineal hypospadias	Yes	687del27/687del27	No detectable activity	Yes	59
26	Afghan/Pakistani	33	46 XY	2 wk	Perineal hypospadias	Yes	818 del AA/818 del AA	No predicted activity	N/A	601
27	Afghan/Pakistani	34	46 XY	Birth	Ambiguous genitalia, hypospadias, bifid scrotum	Yes	818 del AA/818 del AA	No predicted activity	N/A	601
28	Afghan/Pakistani	35	46 XY	Birth	Hypospadias, bifid scrotum	Yes	818 del AA/818 del AA	No predicted activity	N/A	601

Compound heterozygotes										
29	English	36	46 XY	9 yr	Perineal hypospadias with normal testes, initial diagnosis of 21-hydroxylase deficiency (elevated renin)	No (elevated renin activity)	N100S	1.3%	No	575, 602, 603
		37	46 XY	11 yr	Perineal hypospadias, micropenis with normal testes (normal renin)		797delA	No predicted activity	N/A	
30	Spanish/Portugese	38	46 XY	Birth	Perineal hypospadias, bifid scrotum	Yes	L108W	No detectable activity	Yes	553, 604, 605
							P186L	No detectable activity	Yes	
31	N/A	39	46 XX	43 months	Minor virilization of external genitalia, premature pubarche, accelerated growth	No	S123G	N/A	N/A	606
	N/A	40	46 XX	23 months	Minor virilization of external genitalia, premature pubarche, mild acne		P155L	No detectable activity	No	59, 555
32	American	41	46 XX	18 yr	Premature pubarche, acne, mild clitoromegaly	No (normal renin activity)	G129R	11.7 ± 0.1%	No	558, 574, 607
		42	46 XY	16 yr	Perineal hypospadias, bilateral cryptorchidism		6651(G to A)	No predicted activity	N/A	
33	Brazilian	43	46 XX	6.7 yr	Normal genitalia, premature pubarche	No (elevated renin activity)	G129R	11.7 ± 0.1%	No	59, 574, 594
		44	46 XX	7 yr	Normal genitalia, premature pubarche		P222H	No detectable activity	No	
34	American	45	46 XY	1 wk	Perineal hypospadias, bifid scrotum	Yes	E142K	No detectable activity	No	57, 560
							W171X	No predicted activity	N/A	
35	Caucasian	46	46 XX	7.3 yr	Normal genitalia, premature pubarche at 5.5 yr, acne	No	E142K	No detectable activity	No	57, 560
							X373C	27%	Yes	597
36	French	47	46 XY	Birth	Perineal hypospadias, palpable testes in scrotum	No (elevated renin activity)	P155L	No detectable activity	No	59, 555
		48	46 XY	Birth	Perineal hypospadias, palpable testes in scrotum		G294V	20.5 ± 4.6%	No	
37	American	49	46 XY	34 yr	Hypospadias (1 month), gynecomastia (13 yr)	Yes	W171X	No predicted activity	N/A	56, 608
							558/insC	No predicted activity	N/A	
38	French	50	46 XY	Birth	Perineal hypospadias with micropenis, no palpable testes	No (normal renin activity)	L236S	100%	No	59
							867delG	No predicted activity	N/A	
39	Dutch	51	46 XY	Birth	Urethral diverticula and hypospadias	Yes	Y253N	No detectable activity	Yes	57, 609, 610
							558/insC	No predicted activity	N/A	
40	French	52	46 XY	Birth	Perineal hypospadias, palpable testes in bifid scrotum	Yes	T259M	Very weak activity	Yes	59
		53	46 XX	Birth	Normal genitalia		867delG	No predicted activity	N/A	
41	Pakistani	54	46 XX	Birth	Pigmentation, mildly enlarged clitoris	Yes	818 del AA	No predicted activity	N/A	611
							953 del C	Not determined	N/A	
42	American	55	46 XX	Puberty	Primary amenorrhea, slight clitoromegaly, moderate hirsutism, enlarged polycystic ovaries	No (normal renin activity)	Y254D/?	No detectable activity	No	557, 612–615
43	Japanese	56	46 XY		Pigmentation, perineal hypospadias	Yes	Y308X/?	No predicted activity	N/A	598
Sequence variants										
44	American	57	46 XX	15.6 yr	Hirsutism and/or oligomenorrhea	No	A167V	81.45 ± 0.03%	No	59, 585
45	American	58	46 XX	7 yr	Premature pubarche at 4 yr, growth acceleration	No	S213G	58.4 ± 0.6%	No	59, 586
							K216E	58.9 ± 0.2%	No	
46	American	59	46 XX	9 yr	Premature pubic hair	No	L236S	100%	No	59, 585
47	American	60	46 XX	17 yr	Hirsutism and/or oligomenorrhea	No	L236S	100%	No	59, 585

The ethnic origin, phenotypic characteristics, and clinical features of the patients are indicated as well as a summary of the functional consequence of mutations or sequence variants in the *HSD3B2* gene. The mutations previously designated 186/insC/187, 266ΔA, 273ΔAA, and 318ΔC have been changed to 558insC, 818delAA, 797delA, and 953delC, respectively, to follow the current international nomenclature, where the first nucleotide used to assign the name of the mutant is the A of the ATG codon. The apparent activity of mutant enzymes in intact cells was calculated after a 1-h incubation. The values indicated are the mean ± SEM of two independent experiments performed in triplicate. N/A, Not available; ?, undetermined.

diagnosis of this disorder now appears to be a plasma level of 17OH-PREG greater than 100 nmol/liter after stimulation with ACTH. However, it is well recognized that plasma levels of 17OH-PROG and Δ^4-DIONE and other Δ^4-steroids are frequently elevated in 3β-HSD-deficient patients (13, 56–58, 539, 543–547, 550, 551, 558–560). The T response to hCG is usually poor in infancy (553) but may be substantial in pubertal boys (564). Such observations are consistent with a functional type I 3β-HSD enzyme that is expressed in peripheral tissues. However, the peripheral type I 3β-HSD activity could explain why certain patients were initially misdiagnosed as suffering from 21-hydroxylase deficiency, in view of the elevated levels of 17OH-PROG and mild virilization seen in girls at birth (544). Nevertheless, the significant accumulation of precursors, for instance 17OH-PREG, may also interfere with the RIAs used in routine laboratories to measure levels of 17OH-PROG, although in practice this elevated level of 17OH-PROG is considered beneficial. It has been suggested by Morel *et al.* (544) that an increased level of 17OH-PROG observed when screening neonates for 21-hydroxylase deficiency should help in making the correct diagnosis of 3β-HSD deficiency. Therefore, measurement of the levels of 17OH-PREG should be performed when an elevated level of 17OH-PROG has been observed in a female neonate without ambiguity of external genitalia or if the patient is a male pseudohermaphrodite (544).

In the presence of premature pubarche with high DHEA-S levels in girls, the diagnosis of attenuated or late-onset 3β-HSD deficiency was often evoked. The late-onset form of 3β-HSD deficiency has been described in older females with hyperandrogenism beginning at adulthood and in children with premature pubarche (565–569). No mutation was found in the *HSD3B1* and/or *HSD3B2* genes in these patients (569–572), and upon reexamination certain patients no longer showed an elevated Δ^5/Δ^4 ratio (569, 572).

C. Molecular diagnosis

To date, a total of 37 mutations (including five frameshift, four nonsense, one in-frame deletion, one splicing, and 26 missense mutations) have been identified in the *HSD3B2* gene in 60 individuals from 47 families suffering from classical 3β-HSD deficiency as shown in Table 2. In almost all the cases, the characterization of *HSD3B2* mutations has provided a molecular explanation for the heterogeneous clinical presentation of this disorder (59). As described above, the high production rate of PROG by the placenta, due to type I 3β-HSD activity is essential for the maintenance of human pregnancy. Consequently, it was suggested that in a fetus homozygous for an *HSD3B1* gene defect, the absence of the activity of the placental enzyme would lead to interruption of pregnancy before the end of the first trimester (56–58). The recent data presented by Moisan *et al.* (59) supports the notion that it is more appropriate to assess the enzymatic activity of transiently expressed mutant proteins using intact HEK293 cells rather than homogenates from cells, because addition of exogenous cofactor can drive a reaction that may not occur *in vivo* (Fig. 17). For example, enzyme activity can be detected for mutants G15D, L108W, and P186L when assessed in cell homogenates, using PREG as the substrate, whereas no activity can be detected in intact transfected cells using DHEA as the substrate (548). Moreover, this study provides biochemical evidence supporting the involvement of a new molecular mechanism in classical 3βHSD deficiency involving protein instability (Fig. 18). Although the exact molecular and cellular explanation for the apparent instability of various mutant recombinant 3β-HSD proteins in intact transfected HEK293 cells remains to be elucidated, our recent results illustrate that it might be difficult, if not impossible, to rigorously measure the levels of expression of some of these mutant proteins to obtain an accurate estimate of their V_{max} value. We are thus suggesting that the varying degrees of protein instability may explain, at least in part, not only the observed decrease in the V_{max} values for several mutant proteins and more specifically for those with the L173R or G294V substitution, but also the absence of activity observed in HEK293 cells transiently expressing mutant recombinant proteins A10E, G15D, L108W, P186L, A245P, Y253N, T259M, and T259R.

D. Genotype-phenotype relationships

The current knowledge on the molecular basis of CAH due to 3β-HSD deficiency is in agreement with the prediction that no functional type II 3β-HSD isoenzyme is expressed in the adrenals and gonads of the patients suffering from a severe salt-wasting form (Table 2 and Fig. 17). Furthermore, the missense mutations associated with the severe salt-wasting form of 3β-HSD deficiency (Fig. 17) result in mutant 3β-HSD proteins that retain no detectable enzyme activity, *e.g.*, A10E, G15D, L108W, E142K, P186L, L205P, P222Q, Y253N, and T259R (Table 2). Taken together, all of these results are in perfect agreement with the severity of this form of CAH.

In addition, the current data demonstrates that the non-salt-losing form of classical 3β-HSD deficiency also results from missense mutation(s) in the *HSD3B2* gene, which causes an incomplete loss in enzyme activity, thus leaving sufficient enzymatic activity to prevent salt wasting (57–59, 546, 573–575). Indeed, for example, the hormonal profile of two of the individuals bearing the N100S mutation (patients 11, 36, and 37) suggests that salt loss was compensated for by a limited capacity of aldosterone biosynthesis at the price of high renin synthesis (Table 2) (575).

In general, the present functional and biochemical data (Table 2 and Fig. 17) are in close agreement with the severity of the disease in patients suffering from the non-salt-wasting form of 3β-HSD deficiency (576). On the other hand, knowing that all heterozygote carriers of a deleterious mutation in the *HSD3B2* gene are asymptomatic, it was unexpected to observe such a relatively high activity of L173R, *i.e.*, 52.8% (Table 2), although the apparent instability of the L173R mutant protein would most likely be involved in further reducing the activity catalyzed by the mutant type II 3β-HSD protein in the cells of the adrenals and gonads in patients 15 and 16. It is also possible that the apparent instability of A245P (patient 22) and G294V (patients 47 and 48) will play a role in further decreasing the activity in these patients, as also suggested by their V_{max}/K_m values.

Although the results described above illustrate the almost perfect genotype-phenotype relationship associated with

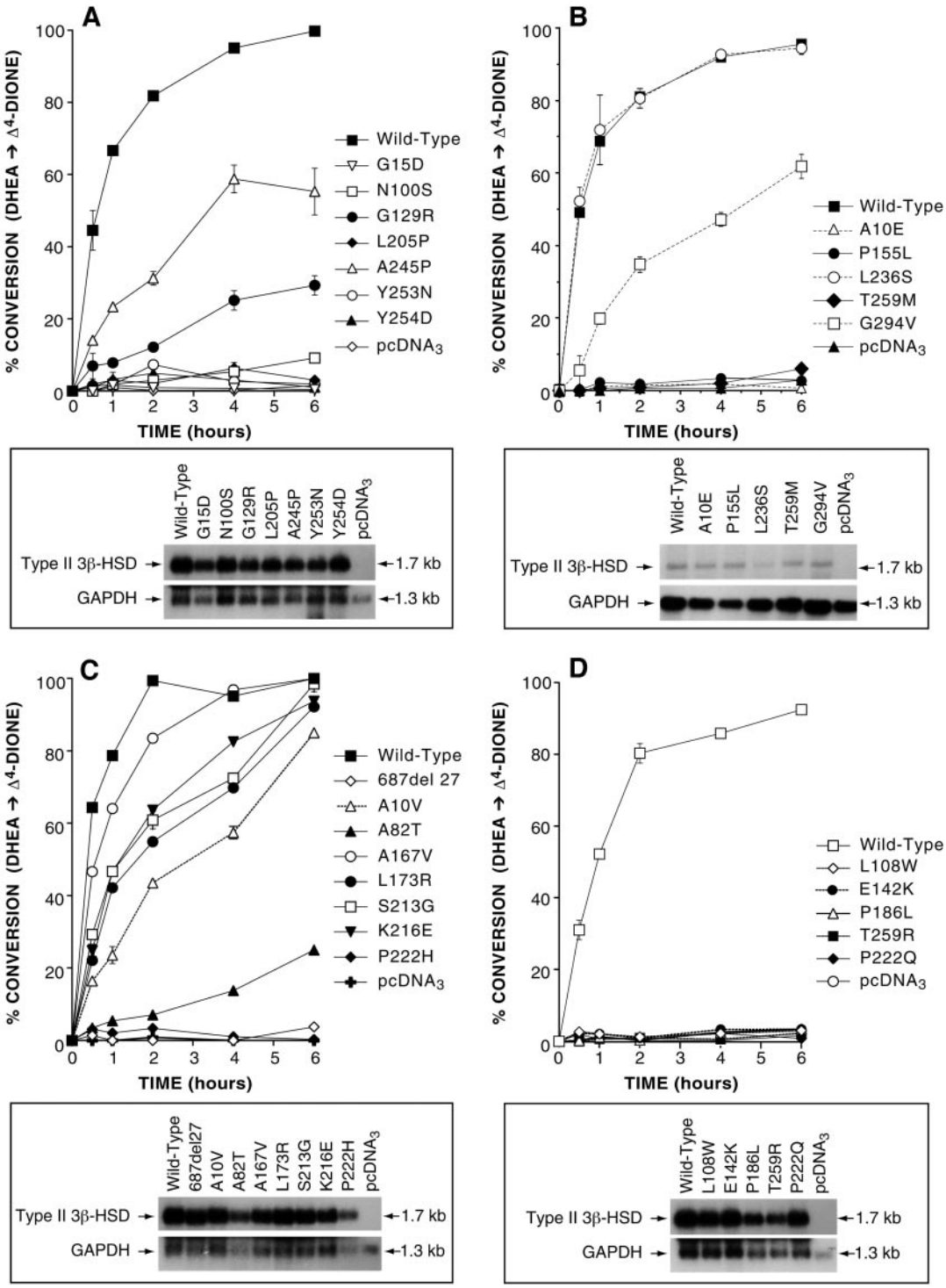


FIG. 17. Comparison of the time course of enzymatic conversion of [^{14}C]DHEA into [^{14}C]Δ^4-DIONE in intact HEK293 cells in culture transfected with the indicated type II 3β-HSD wild-type or mutant expression vectors. The results are presented as the mean ± SEM (n = 3). When the SEM overlaps with the *symbol* used, only the *symbol* is illustrated. *Inset*, Northern blot analysis demonstrating that following transient expression with the indicated expression vector constructs, all transcripts were expressed at equivalent levels in transfected HEK293 cells. The cells were transfected with the pcDNA3 vector alone to show no endogenous expression of type II 3βHSD mRNA. Hybridization to human GAPDH is also shown as a control. [Reproduced from A.M. Moisan *et al.*: *J Clin Endocrinol Metab* 84:4410–4425, 1999 (59), copyright 1999, The Endocrine Society.]

this disorder, there are several examples supporting the notion that there are exceptions to every rule. One of these exceptions is the homozygous A82T mutation that was previously reported in Brazilian patients 7, 8, 9, and 10 (559, 577). In family 7, the homozygous A82T mutation was associated with precocious puberty, whereas in an unrelated family,

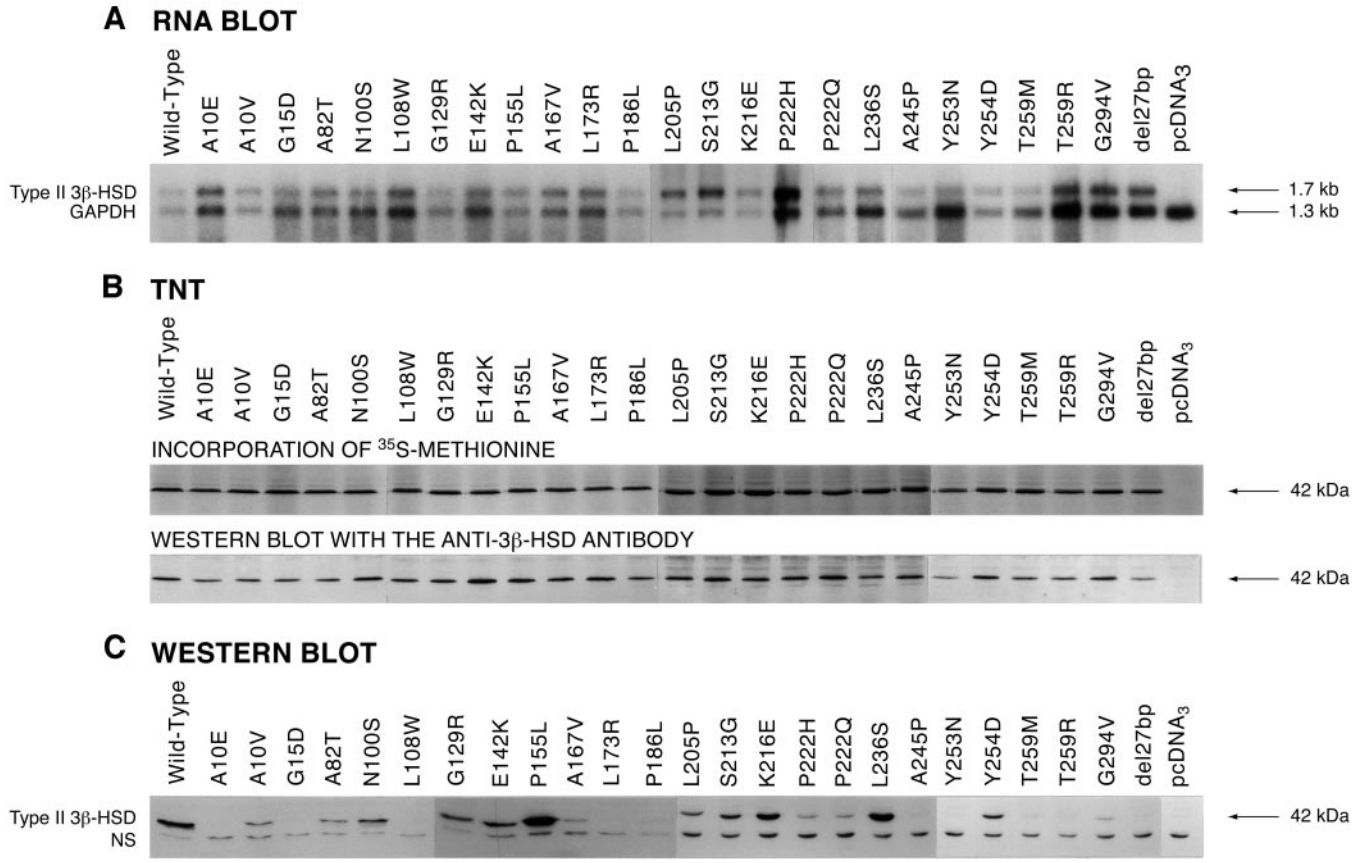


FIG. 18. Comparison of the levels of expression and stability of 25 mutant recombinant type II 3β-HSD proteins. A, Northern blot analysis demonstrating that after transient expression with the indicated expression vector constructs, all transcripts were expressed in transfected HEK293 cells. The cells were transfected with the pcDNA3 vector alone to show no endogenous expression of type II 3βHSD mRNA. Hybridization to GAPDH is also shown as a control of transfection efficiency. B, Representation of an *in vitro* transcription/translation (TNT) rabbit reticulocyte lysate assay using the mutant cDNA constructs showing that each pcDNA3 construct is adequately translated into a [^{35}S]-labeled 42-kDa protein, indicative of the normal expression levels of mutant recombinant type II 3β-HSD proteins. Translation was assessed by separation on a 12% SDS-PAGE gel. To determine whether all mutant proteins are recognized by the polyclonal antibody, Western blot analysis of the corresponding samples from the TNT assay, probed with an antihuman type I 3β-HSD polyclonal antibody at 1:2000 dilution, was performed as described (59). C, Western blot analysis of the homogenates purified from the same corresponding transiently transfected HEK293 cells with the indicated expression vectors, which have been used for the RNA blot analysis illustrated in panel A. A 42-kDa band corresponding to the type II 3β-HSD protein was detectable in several but not all homogenate preparations from HEK293 transfected cells expressing the indicated wild-type or mutant recombinant proteins, whereas no 42-kDa protein is detected in cells transfected with the mock pcDNA3 vector alone. The nonspecific band (NS) observed may also be used as an internal control for loading. [Reproduced from A.M. Moisan *et al.*: *J Clin Endocrinol Metab* 84:4410–4425, 1999 (59), copyright 1999, The Endocrine Society.]

family 6, it was found to be associated with male pseudohermaphroditism, although it had no effect in the homozygous female relative. However, it has recently been demonstrated that although the 46XY individual bearing the A10E homozygous mutation, patient 3, presented with the typical phenotype of ambiguous genitalia at birth with normal virilization at puberty, the female patient, patient 2, also harboring this homozygous mutation, presented with spontaneous feminization and menarche (561), which is in contrast to the 46XX patient 14 with severe 3β-HSD deficiency (due to a homozygous W171X mutation) who was hypogonadal (56, 578). These latter findings demonstrate the complex relationships between the genotype and the gonadal phenotype in severe 3β-HSD deficiency and the difficulty in predicting fertility (561). Another good example is the observation that the Brazilian patients 29 and 30 bearing the homozygous mutation T259M suffer from a non-salt-wasting form of the disease (579), whereas the homozygous Taiwanese patient 28 also bearing the T259M mutation and the compound heterozygous French patients 52 and 53 with the heterozygous T259M/867delG mutation, as well as the homozygous Japanese patients 26 and 27 bearing the T259R mutation were all found to be affected by the severe salt-wasting form of the disease. Moreover, the current data strongly indicate instability of the T259M mutant protein (59, 580). Nevertheless, as illustrated in Fig. 17, it was possible to observe a very low residual 3β-HSD activity after a longer incubation period in intact cells expressing the mutant T259M protein when using DHEA as the substrate (59); however, this low activity was more evident when using PREG as substrate (580). It can be speculated that the Brazilian patients possess a different steroid responsiveness, but additional experiments would be needed to better understand this apparent discrepancy.

It was also unexpected to observe that the L236S mutation, which was found in the compound heterozygous non-salt-losing patient 50 (L236S/867delG), possesses the same enzyme activity as the wild-type enzyme with no evidence suggesting that this mutation affects the stability of the protein. Nevertheless, this hypothesis is difficult to reconcile with the well-established fact that heterozygous carriers are asymptomatic (8, 58, 544, 545). On the other hand, it cannot be ruled out that the L236S mutation could be in linkage disequilibrium with another deleterious mutation affecting the expression or the splicing of this gene.

E. Structure-function relationships

Finally, the functional characterization of these mutant enzymes also generated valuable information concerning the structure-function relationships of the 3β-HSD superfamily. Indeed, it is of special interest to note that the amino acid residues that are the sites of missense mutations are generally in highly conserved regions in members of the vertebrate 3β-HSD isoenzymes characterized thus far (Fig. 2). This finding strongly suggests the crucial role of these residues for the catalytic activity of these enzymes. It is also of interest to mention that mutations A10E, A10V, and G15D alter amino acid residues in the highly conserved Gly-X-X-Gly-X-X-Gly region found in all members of the 3β-HSD superfamily (544, 581, 582), which is similar to the common Gly-X-Gly-X-X-Gly conserved sequence present in most NAD(H)-binding enzymes (68, 583). Furthermore, the striking phenotypic differences observed between the homozygous salt-losing patients 2 and 3, bearing the A10E mutation (561), and the non-salt-losing patients 4 and 5, bearing the A10V mutation, are in accordance with their respective enzymatic properties, as determined in intact cells (Fig. 17). Such a difference may be the result of the observed apparent instability of the A10E protein coupled with the fact that Glu is a negatively charged residue, whereas Val, like Ala, is a nonpolar residue.

Moreover, mutations A82T and G294V create a substitution in each of the two predicted membrane-spanning domains (8, 74). Furthermore, mutation P155L is located in the first of the two characteristic Y-X-X-X-K sequences located in the region from Tyr^{154} to Lys^{158} and Tyr^{269} to Lys^{273}, which is found in the active site of short-chain alcohol dehydrogenases (68, 72, 584). Affinity labeling of purified human type I 3β-HSD identified two tryptic peptides, comprising amino acids Asn^{176} to Arg^{186} and Gly^{251} to Lys^{274} that should contain residues involved in the putative substrate-binding domain (30). Consequently, the exact role of the first YXXXK motif in the 3β-HSD family remains to be confirmed. Finally, recent findings have shown that His^{261} is a critical amino acid residue for 3β-HSD activity and Tyr^{253} or Tyr^{254} participates in the isomerase activity of the human type I 3β-HSD enzyme (23), in addition to providing evidence indicating that Tyr^{253} functions as the general acid (proton donor) in the isomerase reaction (24). Consequently mutations located within this area will inevitably have a major effect on enzyme activity, as exemplified in the case of the deleterious mutations Y253N, Y254D, T259M, and T259R.

F. Sequence variants in the HSD3B2 gene vs. nonclassical 3β-HSD deficiency

Although, as previously mentioned, no mutations have been identified in the *HSD3B1* and *HSD3B2* genes in patients diagnosed to be suffering from late-onset 3β-HSD deficiency, several sequence variants have been identified in these individuals (A167V, S213G, K216E, and L236S) (585, 586). As illustrated in Fig. 17, in addition to the L236S mutation, the heterozygous A167V sequence variant leads to a protein that has a similar activity to the native enzyme, whereas mutant S213G and K216E proteins have only a minor impact on the activity, retaining 58.4 and 58.95% of the activity of the wild type, respectively (Table 2, patients 57–60) (59). It should also be noted that the mother of patient 57 was also a heterozygous A167V carrier, but did not have any symptoms of hyperandrogenism (585). However, as indicated previously, all reported heterozygous carriers bearing a deleterious mutation in the *HSD3B2* gene were typically asymptomatic; these results provide additional molecular proof in support of the conclusion that other genetic or environmental/hormonal influences may contribute to the expression of the observed symptoms (569–572, 587, 588). Thus, the functional data concerning these sequence variants, coupled with the previous studies reporting that no mutations were found in *HSD3B1* and/or *HSD3B2* genes in the patients affected by premature pubarche or hyperandrogenism (570–572, 587–589), strongly support the conclusion that this disorder does not result from a mutant 3β-HSD isoenzyme. Although the possibility that inherited mutation(s) could be located farther upstream in the putative promoter region of the *HSD3B2* gene, leading to an aberrant level of expression of a normal type II 3β-HSD protein cannot be refuted, this hypothesis is markedly weakened by the fact that all patients come from unrelated pedigrees and diverse ethnic origins. In contrast to the 21-hydroxylase deficiency, in which the nonclassical form is an allelic variant of the classical form, the so-called late-onset 3β-HSD deficiency is not an allelic variant of the classical form. On the other hand, because 3β-HSD gene expression and activity are under multiple complex hormonal regulation, it cannot be ruled out that at least some forms of late-onset 3β-HSD deficiency result from a genetic or acquired origin acting indirectly on these modulatory parameters. There is also the possibility of the implication of a steroidogenic enzyme different from known 3β-HSD isoenzymes.

Acknowledgments

The authors thank Dr. Robert S. Viger and Dr. Jacques J. Tremblay for helpful discussion and contribution to the manuscript on the role of GATA proteins in 3β-HSO regulation.

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References

1. **Payne AH, Hales DB** 2004 Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev 25:947–970

2. **Labrie F, Belanger A, Simard J, Luu-The V, Labrie C** 1996 DHEA and sex steroids: intracrinology in target tissues. J Endocrinol 150: S1–S265
3. **Miller WL** 1998 Early steps in androgen biosynthesis: from cholesterol to DHEA. Baillieres Clin Endocrinol Metab 12:67–81
4. **Grumbach MM, Auchus RJ** 1999 Estrogen: consequences and implications of human mutations in synthesis and action. J Clin Endocrinol Metab 84:4677–4694
5. **Penning TM** 1997 Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 18:281–305
6. **Mahendroo MS, Russell DW** 1999 Male and female isoenzymes of steroid 5α-reductase. Rev Reprod 4:179–183
7. **Labrie F, Luu-The V, Labrie C, Belanger A, Simard J, Lin SX, Pelletier G** 2003 Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor, dehydroepiandrosterone. Endocr Rev 24:152–182
8. **Simard J, Durocher F, Mebarki F, Turgeon C, Sanchez R, Labrie Y, Couet J, Trudel C, Rheaume E, Morel Y, Luu-The V, Labrie F** 1996 Molecular biology and genetics of the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene family. J Endocrinol 150 (Suppl):S189–S207
9. **Payne AH, Abbaszade IG, Clarke TR, Bain PA, Park CH** 1997 The multiple murine 3β-hydroxysteroid dehydrogenase isoforms: structure, function, and tissue- and developmentally specific expression. Steroids 62:169–175
10. **Mason JI, Keeney DS, Bird IM, Rainey WE, Morohashi K, Leers-Sucheta S, Melner MH** 1997 The regulation of 3β-hydroxysteroid dehydrogenase expression. Steroids 62:164–168
11. **Grover PK, Odell WD** 1975 Correlation of in vivo and in vitro activities of some naturally occurring androgens using a radioreceptor assay for 5α-dihydrotestosterone with rat prostate cytosol receptor protein. J Steroid Biochem 6:1373–1379
12. **Labrie F** 1991 Intracrinology. Mol Cell Endocrinol 78:C113–C118
13. **Labrie F, Belanger A, Luu-The V, Simard J, Lin SX, Cusan L, Labrie C** 1999 Role of DHEA transformation into androgens and estrogens in peripheral intracrine tissues. In: Thijssen J, Nieuwenhuyse H, eds. DHEA: a comprehensive review. New York: Parthenon Publishing; 69–103
14. **Labrie F, Luu-The V, Labrie C, Simard J** 2001 DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. Front Neuroendocrinol 22:185–212
15. **Simpson ER, Michael MD, Agarwal VR, Hinshelwood MM, Bulun SE, Zhao Y** 1997 Cytochromes P450 11: expression of the CYP19 (aromatase) gene: an unusual case of alternative promoter usage. FASEB J 11:29–36
16. **Peltoketo H, Luu-The V, Simard J, Adamski J** 1999 17β-hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. J Mol Endocrinol 23:1–11
17. **Samuels L, Helmreich M, Lassater N, Reich M** 1951 An enzyme in endocrine tissues which oxidizes 5-3 hydroxy steroids to Δ 5-3 unsaturated ketones. Science 113:490–491
18. **Luu-The V, Lachance Y, Labrie C, Leblanc G, Thomas JL, Strickler RC, Labrie F** 1989 Full length cDNA structure and deduced amino acid sequence of human 3β-hydroxy-5-ene steroid dehydrogenase. Mol Endocrinol 3:1310–1312
19. **Cherradi N, Defaye G, Chambaz EM** 1993 Dual subcellular localization of the 3β-hydroxysteroid dehydrogenase isomerase: characterization of the mitochondrial enzyme in the bovine adrenal cortex. J Steroid Biochem Mol Biol 46:773–779
20. **Cherradi N, Defaye G, Chambaz EM** 1994 Characterization of the 3β-hydroxysteroid dehydrogenase activity associated with bovine adrenocortical mitochondria. Endocrinology 134:1358–1364
21. **Sauer LA, Chapman JC, Dauchy RT** 1994 Topology of 3β-hydroxy-5-ene-steroid dehydrogenase/Δ^5-Δ^4 isomerase in adrenal cortex mitochondria and microsomes. Endocrinology 134:751–759
22. **Cherradi N, Rossier MF, Vallotton MB, Timberg R, Friedberg I, Orly J, Wang XJ, Stocco DM, Capponi AM** 1997 Submitochondrial distribution of three key steroidogenic proteins (steroidogenic acute regulatory protein and cytochrome p450scc and 3β-hydroxysteroid dehydrogenase isomerase enzymes) upon stimulation by intracellular calcium in adrenal glomerulosa cells. J Biol Chem 272:7899–7907
23. **Thomas JL, Evans BW, Blanco G, Mercer RW, Mason JI, Adler S, Nash WE, Isenberg KE, Strickler RC** 1998 Site-directed mutagenesis identifies amino acid residues associated with the dehydrogenase and isomerase activities of human type I (placental) 3β-hydroxysteroid dehydrogenase/isomerase. J Steroid Biochem Mol Biol 66:327–334
24. **Mason JI, Naville D, Evans BW, Thomas JL** 1998 Functional activity of 3β-hydroxysteroid dehydrogenase/isomerase. Endocr Res 24:549–557
25. **Lorence MC, Murry BA, Trant JM, Mason JI** 1990 Human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase from placenta: expression in nonsteroidogenic cells of a protein that catalyzes the dehydrogenation/isomerization of C21 and C19 steroids. Endocrinology 126:2493–2498
26. **Lachance Y, Luu-The V, Labrie C, Simard J, Dumont M, de Launoit Y, Guerin S, Leblanc G, Labrie F** 1990 Characterization of human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene and its expression in mammalian cells. J Biol Chem [Erratum 267: 3551 (1992)] 265:20469–20475
27. **Rheaume E, Lachance Y, Zhao HF, Breton N, Dumont M, de Launoit Y, Trudel C, Luu-The V, Simard J, Labrie F** 1991 Structure and expression of a new complementary DNA encoding the almost exclusive 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in human adrenals and gonads. Mol Endocrinol 5:1147–1157
28. **Thomas JL, Myers RP, Rosik LO, Strickler RC** 1990 Affinity alkylation of human placental 3β-hydroxy-5-ene-steroid dehydrogenase and steroid 5–4-ene-isomerase by 2α-bromoacetoxyprogesterone: evidence for separate dehydrogenase and isomerase sites on one protein. J Steroid Biochem 36:117–123
29. **Luu-The V, Takahashi M, de Launoit Y, Dumont M, Lachance Y, Labrie F** 1991 Evidence for distinct dehydrogenase and isomerase sites within a single 3β-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase protein. Biochemistry 30:8861–8865
30. **Thomas JL, Nash WE, Myers RP, Crankshaw MW, Strickler RC** 1993 Affinity radiolabeling identifies peptides and amino acids associated with substrate binding in human placental 3β-hydroxy-Δ^5-steroid dehydrogenase. J Biol Chem 268:18507–18512
31. **Thomas JL, Nash WE, Crankshaw MW, Strickler RC** 1994 Affinity labeling in the presence of the reduced diphosphopyridine nucleotide NADH identifies peptides associated with the activities of human placental 3β-hydroxy-Δ^5-steroid dehydrogenase/isomerase. J Soc Gynecol Investig 1:155–163
32. **Thomas JL, Evans BW, Strickler RC** 1997 Affinity radiolabeling identifies peptides associated with the isomerase activity of human type I (placental) 3β-hydroxysteroid dehydrogenase/isomerase. Biochemistry 36:9029–9034
33. **Thomas JL, Frieden C, Nash WE, Strickler RC** 1995 An NADH-induced conformational change that mediates the sequential 3β-hydroxysteroid dehydrogenase/isomerase activities is supported by affinity labeling and the time-dependent activation of isomerase. J Biol Chem 270:21003–21008
34. **Clark BJ, Wells J, King SR, Stocco DM** 1994 The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 269:28314–28322
35. **Chieffi G, La Torretta G, Del Bianco C, Tramontana S** 1965 [Histochemical distribution of 3-β-, 17-α- and 17-β-hydroxysteroid dehydrogenase in the human ovary during the menstrual cycle]. Arch Ostet Ginecol 70:492–500
36. **Tapia Freses A, Diaz J, Falen J** 1965 [Histochemical study of the effect of progesterone on the activity of the enzyme, Δ^5, 3β-hydroxysteroid dehydrogenase]. Rev Iber Endocrinol 12:393–408
37. **Berchtold JP** 1977 Ultracytochemical demonstration and probable localization of 3β-hydroxysteroid dehydrogenase activity with a ferricyanide technique. Histochemistry 50:175–190
38. **Headon DR, Hsiao J, Ungar F** 1978 The intracellular localization of adrenal 3β-hydroxysteroid dehydrogenase/Δ^5-isomerase by density gradient perturbation. Biochem Biophys Res Commun 82: 1006–1012

39. **Gibb W, Lavoie JC, Roux JF** 1978 3β-Hydroxysteroid dehydrogenase activity in human fetal membranes. Steroids 32:365–372
40. **Alvarez CI, Genti-Raimondi S, Patrito LC, Flury A** 1994 Topography of human placental 3β-hydroxysteroid dehydrogenase/Δ5–4 isomerase in microsomal membrane. A study using limited proteolysis and immunoblotting. Biochim Biophys Acta 1207:102–108
41. **Thomas JL, Myers RP, Strickler RC** 1989 Human placental 3β-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 33:209–217
42. **Chapman JC, Sauer LA** 1979 Intracellular localization and properties of 3β-hydroxysteroid dehydrogenase/isomerase in the adrenal cortex. J Biol Chem 254:6624–6630
43. **Cherradi N, Chambaz EM, Defaye G** 1995 Organization of 3β-hydroxysteroid dehydrogenase/isomerase and cytochrome P450scc into a catalytically active molecular complex in bovine adrenocortical mitochondria. J Steroid Biochem Mol Biol 55:507–514
44. **Perry JE, Ishii-Ohba H, Stalvey JR** 1991 Subcellular distribution of 3β-hydroxysteroid dehydrogenase-isomerase in bovine and murine adrenocortical tissue: species differences in the localization of activity and immunoreactivity. Steroids 56:329–336
45. **Pelletier G, Li S, Luu-The V, Tremblay Y, Belanger A, Labrie F** 2001 Immunoelectron microscopic localization of three key steroidogenic enzymes (cytochrome P450(scc), 3β-hydroxysteroid dehydrogenase and cytochrome P450(c17)) in rat adrenal cortex and gonads. J Endocrinol 171:373–383
46. **Capponi AM, Lew PD, Jornot L, Vallotton MB** 1984 Correlation between cytosolic free Ca2+ and aldosterone production in bovine adrenal glomerulosa cells. Evidence for a difference in the mode of action of angiotensin II and potassium. J Biol Chem 259:8863–8869
47. **Chapman JC, Waterhouse TB, Michael SD** 1992 Changes in mitochondrial and microsomal 3β-hydroxysteroid dehydrogenase activity in mouse ovary over the course of the estrous cycle. Biol Reprod 47:992–997
48. **Luu-The V, Coté J, Labrie F** 1988 Purification and characterization of human placental 3β-hydroxysteroid dehydrogenase/5–4-isomerase. Clin Invest Med 11(Suppl):C32 (Abstract)
49. **Luu-The V, Coté J, Labrie F** 1990 Purification of microsomal 3β-hydroxysteroid dehydrogenase/5–4-isomerase from human placenta. Ann NY Acad Sci 595:386–388
50. **Nickson DA, McBride MW, Zeinali S, Hawes CS, Petropoulos A, Mueller UW, Sutcliffe RG** 1991 Molecular cloning and expression of human trophoblast antigen FDO161G and its identification as 3β-hydroxy-5-ene steroid dehydrogenase. J Reprod Fertil 93:149–156
51. **Gingras S, Moriggl R, Groner B, Simard J** 1999 Induction of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13. Mol Endocrinol 13:66–81
52. **Gingras S, Simard J** 1999 Induction of 3β-hydroxysteroid dehydrogenase/isomerase type 1 expression by interleukin-4 in human normal prostate epithelial cells, immortalized keratinocytes, colon, and cervix cancer cell lines. Endocrinology 140:4573–4584
53. **Dumont M, Luu-The V, Dupont E, Pelletier G, Labrie F** 1992 Characterization, expression, and immunohistochemical localization of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in human skin. J Invest Dermatol 99:415–421
54. **Thomas JL, Mason JI, Blanco G, Veisaga ML** 2001 The engineered, cytosolic form of human type I 3β-hydroxysteroid dehydrogenase/isomerase: purification, characterization and crystallization. J Mol Endocrinol 27:77–83
55. **Lachance Y, Luu-The V, Labrie C, Simard J, Dumont M, de Launoit Y, Guerin S, Leblanc G, Labrie F** 1992 Characterization of human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene and its expression in mammalian cells. J Biol Chem 267:3551
56. **Rheaume E, Simard J, Morel Y, Mebarki F, Zachmann M, Forest MG, New MI, Labrie F** 1992 Congenital adrenal hyperplasia due to point mutations in the type II 3β-hydroxysteroid dehydrogenase gene. Nat Genet 1:239–245
57. **Simard J, Rheaume E, Sanchez R, Laflamme N, de Launoit Y, Luu-The V, Van Seters AP, Gordon RD, Bettendorf M, Heinrich U, Moshang T, New M-I, Labrie F** 1993 Molecular basis of congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency. Mol Endocrinol 7:716–728
58. **Simard J, Rheaume E, Mebarki F, Sanchez R, New MI, Morel Y, Labrie F** 1995 Molecular basis of human 3β-hydroxysteroid dehydrogenase deficiency. J Steroid Biochem Mol Biol 53:127–138
59. **Moisan AM, Ricketts ML, Tardy V, Desrochers M, Mebarki F, Chaussain JL, Cabrol S, Raux-Demay MC, Forest MG, Sippell WG, Peter M, Morel Y, Simard J** 1999 New insight into the molecular basis of 3β-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. J Clin Endocrinol Metab 84:4410–4425
60. **Lachance Y, Luu-The V, Verreault H, Dumont M, Rheaume E, Leblanc G, Labrie F** 1991 Structure of the human type II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) gene: adrenal and gonadal specificity. DNA Cell Biol 10:701–711
61. **Lorence MC, Corbin CJ, Kamimura N, Mahendroo MS, Mason JI** 1990 Structural analysis of the gene encoding human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase. Mol Endocrinol 4:1850–1855
62. **Berube D, Luu The V, Lachance Y, Gagne R, Labrie F** 1989 Assignment of the human 3β-hydroxysteroid dehydrogenase gene (HSDB3) to the p13 band of chromosome 1. Cytogenet Cell Genet 52:199–200
63. **Morissette J, Rheaume E, Leblanc JF, Luu-The V, Labrie F, Simard J** 1995 Genetic linkage mapping of HSD3B1 and HSD3B2 encoding human types I and II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase close to D1S514 and the centromeric D1Z5 locus. Cytogenet Cell Genet 69:59–62
64. **Luu-The V, Lachance Y, Leblanc G, Labrie F** 1992 Human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase: characterization of three additional related genes. Program of the 74th Annual Meeting of The Endocrine Society, San Antonio, TX, 1992, p 426 (Abstract 1499)
65. **Bain PA, Meisler MH, Taylor BA, Payne AH** 1993 The genes encoding gonadal and nongonadal forms of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase are closely linked on mouse chromosome 3. Genomics 16:219–223
66. **McBride MW, McVie AJ, Burridge SM, Brintnell B, Craig N, Wallace AM, Wilson RH, Varley J, Sutcliffe RG** 1999 Cloning, expression, and physical mapping of the 3β-hydroxysteroid dehydrogenase gene cluster (HSD3BP1-HSD3BP5) in human. Genomics 61:277–284
67. **Thomas JL, Berko EA, Faustino A, Myers RP, Strickler RC** 1988 Human placental 3β-hydroxy-5-ene-steroid dehydrogenase and steroid 5–4-ene-isomerase: purification from microsomes, substrate kinetics, and inhibition by product steroids. J Steroid Biochem 31:785–793
68. **Scrutton NS, Berry A, Perham RN** 1990 Redesign of the coenzyme specificity of a dehydrogenase by protein engineering. Nature 343:38–43
69. **Thomas JL, Duax WL, Addlagatta A, Brandt S, Fuller RR, Norris W** 2003 Structure/function relationships responsible for coenzyme specificity and the isomerase activity of human type 1 3β-hydroxysteroid dehydrogenase/isomerase. J Biol Chem 278:35483–35490
70. **Chen Z, Jiang JC, Lin ZG, Lee WR, Baker ME, Chang SH** 1993 Site-specific mutagenesis of *Drosophila* alcohol dehydrogenase: evidence for involvement of tyrosine-152 and lysine-156 in catalysis. Biochemistry 32:3342–3346
71. **Tanabe T, Tanaka N, Uchikawa K, Kabashima T, Ito K, Nonaka T, Mitsui Y, Tsuru M, Yoshimoto T** 1998 Roles of the Ser146, Tyr159, and Lys163 residues in the catalytic action of 7α-hydroxysteroid dehydrogenase from *Escherichia coli*. J Biochem (Tokyo) [Erratum 126:456 (1999)] 124:634–641
72. **Thomas JL, Mason JI, Brandt S, Spencer Jr BR, Norris W** 2002 Structure/function relationships responsible for the kinetic differences between human type 1 and type 2 3β-hydroxysteroid dehydrogenase and for the catalysis of the type 1 activity. J Biol Chem 277:42795–42801
73. **Thomas JL, Evans BW, Blanco G, Mason JI, Strickler RC** 1999

Creation of a fully active, cytosolic form of human type I 3β-hydroxysteroid dehydrogenase/isomerase by the deletion of a membrane- spanning domain. J Mol Endocrinol 23:231–239
74. **Simard J, de Launoit Y, Labrie F** 1991 Characterization of the structure-activity relationships of rat types I and II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase by site-directed mutagenesis and expression in HeLa cells. J Biol Chem 266:14842–14845
75. **Zhao HF, Rheaume E, Trudel C, Couet J, Labrie F, Simard J** 1990 Structure and sexual dimorphic expression of a liver-specific rat 3β-hydroxysteroid dehydrogenase/isomerase. Endocrinology 127: 3237–3239
76. **Simard J, Melner MH, Breton N, Low KG, Zhao HF, Periman LM, Labrie F** 1991 Characterization of macaque 3β-hydroxy-5-ene steroid dehydrogenase/Δ^5-Δ^4 isomerase: structure and expression in steroidogenic and peripheral tissues in primate. Mol Cell Endocrinol 75:101–110
77. **Zhao HF, Simard J, Labrie C, Breton N, Rheaume E, Luu-The V, Labrie F** 1989 Molecular cloning, cDNA structure and predicted amino acid sequence of bovine 3β-hydroxy-5-ene steroid dehydrogenase/Δ^5-Δ^4 isomerase. FEBS Lett 259:153–157
78. **Nakabayashi O, Nomura O, Nishimori K, Mizuno S** 1995 The cDNA cloning and transient expression of a chicken gene encoding a 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase unique to major steroidogenic tissues. Gene 162:261–265
79. **Hasegawa T, Ishida M, Harigaya T, Ishida N, Mukoyama H** 1998 Molecular cloning, nucleotide sequence and tissue distribution of equine testicular 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase messenger ribonucleic acid. J Equine Sci 9:45–52
80. **Sakai N, Tanaka M, Takahashi M, Fukada S, Mason JI, Nagahama Y** 1994 Ovarian 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase of rainbow trout: its cDNA cloning and properties of the enzyme expressed in a mammalian cell. FEBS Lett 350:309–313
81. **Kazeto Y, Ijiri S, Matsubara H, Adachi S, Yamauchi K** 2003 Molecular cloning and characterization of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase cDNAs from Japanese eel ovary. J Steroid Biochem Mol Biol 85:49–56
82. **Zhao HF, Labrie C, Simard J, de Launoit Y, Trudel C, Martel C, Rheaume E, Dupont E, Luu-The V, Pelletier G** 1991 Characterization of rat 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase cDNAs and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. J Biol Chem 266:583–593
83. **Simard J, Couet J, Durocher F, Labrie Y, Sanchez R, Breton N, Turgeon C, Labrie F** 1993 Structure and tissue-specific expression of a novel member of the rat 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) family. The exclusive 3β-HSD gene expression in the skin. J Biol Chem 268:19659–19668
84. **Sanchez R, de Launoit Y, Durocher F, Belanger A, Labrie F, Simard J** 1994 Formation and degradation of dihydrotestosterone by recombinant members of the rat 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase family. Mol Cell Endocrinol 103:29–38
85. **Couet J, Simard J, Martel C, Trudel C, Labrie Y, Labrie F** 1992 Regulation of 3-ketosteroid reductase messenger ribonucleic acid levels and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase activity in rat liver by sex steroids and pituitary hormones. Endocrinology 131:3034–3044
86. **de Launoit Y, Simard J, Durocher F, Labrie F** 1992 Androgenic 17β-hydroxysteroid dehydrogenase activity of expressed rat type I 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase. Endocrinology 130:553–555
87. **Bain PA, Yoo M, Clarke T, Hammond SH, Payne AH** 1991 Multiple forms of mouse 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase and differential expression in gonads, adrenal glands, liver, and kidneys of both sexes. Proc Natl Acad Sci USA 88:8870–8874
88. **Clarke TR, Bain PA, Greco TL, Payne AH** 1993 A novel mouse kidney 3β-hydroxysteroid dehydrogenase complementary DNA encodes a 3-ketosteroid reductase instead of a 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase. Mol Endocrinol 7:1569–1578
89. **Clarke TR, Bain PA, Sha L, Payne AH** 1993 Enzyme characteristics of two distinct forms of mouse 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase complementary deoxyribonucleic acids expressed in COS-1 cells. Endocrinology 132:1971–1976
90. **Keeney DS, Naville D, Milewich L, Bartke A, Mason JI** 1993 Multiple isoforms of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in mouse tissues: male-specific isoforms are expressed in the gonads and liver. Endocrinology 133:39–45
91. **Abbaszade IG, Clarke TR, Park CH, Payne AH** 1995 The mouse 3β-hydroxysteroid dehydrogenase multigene family includes two functionally distinct groups of proteins. Mol Endocrinol 9:1214–1222
92. **Abbaszade IG, Arensburg J, Park CH, Kasa-Vubu JZ, Orly J, Payne AH** 1997 Isolation of a new mouse 3β-hydroxysteroid dehydrogenase isoform, 3β-HSD VI, expressed during early pregnancy. Endocrinology [Erratum 139:218 (1998)] 138:1392–1399
93. **Payne AH, Clarke TR, Bain PA** 1995 The murine 3β-hydroxysteroid dehydrogenase multigene family: structure, function and tissue-specific expression. J Steroid Biochem Mol Biol 53:111–118
94. **Clarke TR, Bain PA, Burmeister M, Payne AH** 1996 Isolation and characterization of several members of the murine Hsd3b gene family. DNA Cell Biol 15:387–399
95. **Park CH, Abbaszade IG, Payne AH** 1996 Expression of multiple forms of 3β-hydroxysteroid dehydrogenase in the mouse liver during fetal and postnatal development. Mol Cell Endocrinol 116:157–164
96. **Rogerson FM, LeHoux JG, Mason JI** 1995 Expression and characterization of isoforms of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in the hamster. J Steroid Biochem Mol Biol 55: 481–487
97. **Rogerson FM, Courtemanche J, Fleury A, Head JR, LeHoux JG, Mason JI** 1998 Characterization of cDNAs encoding isoforms of hamster 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase. J Mol Endocrinol 20:99–110
98. **Furster C, Zhang J, Toll A** 1996 Purification of a 3β-hydroxy-Δ5-C27-steroid dehydrogenase from pig liver microsomes active in major and alternative pathways of bile acid biosynthesis. J Biol Chem 271:20903–20907
99. **Furster C** 1999 Hepatic and extrahepatic dehydrogenation/isomerization of 5-cholestene-3β,7α-diol: localization of 3β-hydroxy-Δ5-C27-steroid dehydrogenase in pig tissues and subcellular fractions. Biochim Biophys Acta 1436:343–353
100. **Russell A, Nazer H, Shams A, Sjovall J, Sutcliffe R** 1995 No linkage to the 3β-HSD gene cluster in a kindred affected with 3β-hydroxy-Δ5-C27-steroid dehydrogenase deficiency and early onset hepatic failure. Hum Genet 95:586–588
101. **Filling C, Marschall HU, Prozorovski T, Nordling E, Persson B, Jornvall H, Oppermann UC** 1999 Structure-function relationships of 3β-hydroxysteroid dehydrogenases involved in bile acid metabolism. Adv Exp Med Biol 463:389–394
102. **Liu XY, Dangel AW, Kelley RI, Zhao W, Denny P, Botcherby M, Cattanach B, Peters J, Hunsicker PR, Mallon AM, Strivens MA, Bate R, Miller W, Rhodes M, Brown SD, Herman GE** 1999 The gene mutated in bare patches and striated mice encodes a novel 3β-hydroxysteroid dehydrogenase. Nat Genet 22:182–187
103. **Turgeon C, Sanchez R, Rheaume E, Labrie F, Simard J** 1995 Characterization of the unique cofactor specificity of liver-specific rat 3β-hydroxysteroid dehydrogenase by site-directed mutagenesis. Program of the 77th Annual Meeting of The Endocrine Society, Washington, DC, 1995, p 619 (Abstract P613-603)
104. **Minard P, Legoy MD, Thomas D** 1985 3β, 17β-hydroxysteroid dehydrogenase of *Pseudomonas testosteroni*. Kinetic evidence for the bifunctional activity at a common catalytic site. FEBS Lett 188:85–90
105. **Abalain JH, Distefano S, Amet Y, Quemener E, Abalaincolloc ML, Floch HH** 1993 Cloning, DNA sequencing and expression of (3–17)β-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni*. J Steroid Biochem Mol Biol 44:133–139
106. **Suzuki T, Sasano H, Andersson S, Mason JI** 2000 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase activity associated with the human 17β-hydroxysteroid dehydrogenase type 2 isoform. J Clin Endocrinol Metab 85:3669–3672
107. **Torn S, Nokelainen P, Kurkela R, Pulkka A, Menjivar M, Ghosh S, Coca-Prados M, Peltoketo H, Isomaa V, Vihko P** 2003 Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. Biochem Biophys Res Commun 305:37–45
108. **Rainey WE, Bird IM, Mason JI** 1994 The NCI-H295 cell line: a

pluripotent model for human adrenocortical studies. Mol Cell Endocrinol 100:45–50
109. **Leers-Sucheta S, Morohashi K, Mason JI, Melner MH** 1997 Synergistic activation of the human type II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase promoter by the transcription factor steroidogenic factor-1/adrenal 4-binding protein and phorbol ester. J Biol Chem 272:7960–7967
110. **Nuclear Receptors Nomenclature Committee** 1999 A unified nomenclature system for the nuclear receptor superfamily. Cell 97: 161–163
111. **Halvorson LM, Kaiser UB, Chin WW** 1999 The protein kinase C system acts through the early growth response protein 1 to increase LHβ gene expression in synergy with steroidogenic factor-1. Mol Endocrinol 13:106–116
112. **Dorn C, Ou Q, Svaren J, Crawford PA, Sadovsky Y** 1999 Activation of luteinizing hormone β gene by gonadotropin-releasing hormone requires the synergy of early growth response-1 and steroidogenic factor-1. J Biol Chem 274:13870–13876
113. **Darnell Jr JE** 1997 STATs and gene regulation. Science 277:1630–1635
114. **Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN** 1998 Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 93:841–850
115. **Wakao H, Gouilleux F, Groner B** 1994 Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO J [Erratum 14:854–855 (1995)] 13:2182–2191
116. **Liu X, Robinson GW, Gouilleux F, Groner B, Hennighausen L** 1995 Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal transduction in mouse mammary tissue. Proc Natl Acad Sci USA 92:8831–8835
117. **Albarracin CT, Gibori G** 1991 Prolactin action on luteal protein expression in the corpus luteum. Endocrinology 129:1821–1830
118. **Kauppila A, Martikainen H, Puistola U, Reinila M, Ronnberg L** 1988 Hypoprolactinemia and ovarian function. Fertil Steril 49:437–441
119. **Blackwell RE** 1992 Hyperprolactinemia. Evaluation and management. Endocrinol Metab Clin North Am 21:105–124
120. **Ruff SJ, Leers-Sucheta S, Melner MH, Cohen S** 1996 Induction and activation of Stat 5 in the ovaries of pseudopregnant rats. Endocrinology 137:4095–4099
121. **Kazansky AV, Raught B, Lindsey SM, Wang YF, Rosen JM** 1995 Regulation of mammary gland factor/Stat5a during mammary gland development. Mol Endocrinol 9:1598–1609
122. **Feltus FA, Groner B, Melner MH** 1999 Stat5-mediated regulation of the human type II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene: activation by prolactin. Mol Endocrinol 13:1084–1093
123. **McWhinney CD, Dostal D, Baker K** 1998 Angiotensin II activates Stat5 through Jak2 kinase in cardiac myocytes. J Mol Cell Cardiol 30:751–761
124. **Ruff-Jamison S, Chen K, Cohen S** 1995 Epidermal growth factor induces the tyrosine phosphorylation and nuclear translocation of Stat 5 in mouse liver. Proc Natl Acad Sci USA 92:4215–4218
125. **Feltus FA, Kovacs WJ, Nicholson W, Silva CM, Nagdas SK, Ducharme NA, Melner MH** 2003 Epidermal growth factor increases cortisol production and type II 3β-hydroxysteroid dehydrogenase/Δ5- Δ4-isomerase expression in human adrenocortical carcinoma cells: evidence for a Stat5-dependent mechanism. Endocrinology 144:1847–1853
126. **Zhao Y, Nichols JE, Bulun SE, Mendelson CR, Simpson ER** 1995 Aromatase P450 gene expression in human adipose tissue. Role of a Jak/STAT pathway in regulation of the adipose-specific promoter. J Biol Chem 270:16449–16457
127. **Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER, Meitinger T, Monaco AP, Sassone-Corsi P, Camerino G** 1994 An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 372:635–641
128. **Muscatelli F, Strom TM, Walker AP, Zanaria E, Recan D, Meindl A, Bardoni B, Guioli S, Zehetner G, Rabl W, Schwarz HP, Kaplan J-C, Camerino G, Meitinger T, Monaco AP** 1994 Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Nature 372:672–676
129. **Zazopoulos E, Lalli E, Stocco DM, Sassone-Corsi P** 1997 DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. Nature 390:311–315
130. **Smith CA, Clifford V, Western PS, Wilcox SA, Bell KS, Sinclair AH** 2000 Cloning and expression of a DAX1 homologue in the chicken embryo. J Mol Endocrinol 24:23–32
131. **Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, Moras D, Sassone-Corsi P** 1997 A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. Mol Endocrinol 11:1950–1960
132. **Feltus FA, Cote S, Simard J, Gingras S, Kovacs WJ, Nicholson WE, Clark BJ, Melner MH** 2002 Glucocorticoids enhance activation of the human type II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene. J Steroid Biochem Mol Biol 82:55–63
133. **Perry JE, Stalvey JR** 1992 Gonadal steroids modulate adrenal fasciculata 3β-hydroxysteroid dehydrogenase-isomerase activity in mice. Biol Reprod 46:73–82
134. **Stalvey JR, Clavey SM** 1992 Evidence that testosterone regulates Leydig cell 3β-hydroxysteroid dehydrogenase-isomerase activity by a trans-acting factor distal to the androgen receptor. J Androl 13:93–99
135. **Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schutz G** 1998 DNA binding of the glucocorticoid receptor is not essential for survival [see comments]. Cell 93:531–541
136. **Stocklin E, Wissler M, Gouilleux F, Groner B** 1996 Functional interactions between Stat5 and the glucocorticoid receptor. Nature 383:726–728
137. **Tuckermann JP, Reichardt HM, Arribas R, Richter KH, Schutz G, Angel P** 1999 The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1-dependent genes in skin. J Cell Biol 147:1365–1370
138. **McKay LI, Cidlowski JA** 1998 Cross-talk between nuclear factor-κB and the steroid hormone receptors: mechanisms of mutual antagonism. Mol Endocrinol 12:45–56
139. **Saville B, Wormke M, Wang F, Nguyen T, Enmark E, Kuiper G, Gustafsson JA, Safe S** 2000 Ligand-, cell-, and estrogen receptor subtype (α/β)-dependent activation at GC-rich (Sp1) promoter elements. J Biol Chem 275:5379–5387
140. **Adashi EY** 1990 The potential relevance of cytokines to ovarian physiology: the emerging role of resident ovarian cells of the white blood cell series. Endocr Rev 11:454–464
141. **Cote S, Feltus AF, Gingras S, Freeman M, Melner MH, Simard J** 2000 IL-4 stimulation of ovarian 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase type II gene expression: mechanisms of activation. Program of the 82nd Annual Meeting of The Endocrine Society, Toronto, Ontario, 2000, p 313 (Abstract 1295)
142. **Tremblay JJ, Viger RS** 2003 Novel roles for GATA transcription factors in the regulation of steroidogenesis. J Steroid Biochem Mol Biol 85:291–298
143. **Tremblay JJ, Viger RS** 2001 GATA factors differentially activate multiple gonadal promoters through conserved GATA regulatory elements. Endocrinology 142:977–986
144. **Silverman E, Eimerl S, Orly J** 1999 CCAAT enhancer-binding protein β and GATA-4 binding regions within the promoter of the steroidogenic acute regulatory protein (StAR) gene are required for transcription in rat ovarian cells. J Biol Chem 274:17987–17996
145. **Wooton-Kee CR, Clark BJ** 2000 Steroidogenic factor-1 influences protein-deoxyribonucleic acid interactions within the cyclic adenosine 3,5-monophosphate-responsive regions of the murine steroidogenic acute regulatory protein gene. Endocrinology 141:1345–1355
146. **LaVoie HA, Singh D, Hui YY** 2004 Concerted regulation of the porcine steroidogenic acute regulatory protein gene promoter activity by follicle-stimulating hormone and insulin-like growth factor I in granulosa cells involves GATA-4 and CCAAT/enhancer binding protein β. Endocrinology 145:3122–3134
147. **Jimenez P, Saner K, Mayhew B, Rainey WE** 2003 GATA-6 is expressed in the human adrenal and regulates transcription of genes required for adrenal androgen biosynthesis. Endocrinology 144:4285–4288

148. **Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, Urbanek M, McAllister JM, Mosselman S, Strauss 3rd JF** 2003 The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 278:26380–26390
149. **Tremblay JJ, Viger RS** 2003 Transcription factor GATA-4 is activated by phosphorylation of serine 261 via the cAMP/protein kinase a signaling pathway in gonadal cells. J Biol Chem 278: 22128–22135
150. **Fluck CE, Miller WL** 2004 GATA-4 and GATA-6 modulate tissue-specific transcription of the human gene for P450c17 by direct interaction with Sp1. Mol Endocrinol 18:1144–1157
151. **Piao YS, Peltoketo H, Vihko P, Vihko R** 1997 The proximal promoter region of the gene encoding human 17β-hydroxysteroid dehydrogenase type 1 contains GATA, AP-2, and Sp1 response elements: analysis of promoter function in choriocarcinoma cells. Endocrinology 138:3417–3425
152. **Peng L, Huang Y, Jin F, Jiang SW, Payne AH** 2004 Transcription enhancer factor-5 and a GATA-like protein determine placental-specific expression of the type I human 3β-hydroxysteroid dehydrogenase gene, HSD3B1. Mol Endocrinol 18:2049–2060
153. **Tremblay JJ, Martin LJ, Taniguchi H, Simard J, Viger RS** 2004 GATA factors and orphan nuclear receptors cooperate to synergistically activate the human HSD3B2 promoter. Program of the 83rd Annual Meeting of The Endocrine Society, New Orleans, LA, 2004, p 110–111 (Abstract OR26-6)
154. **Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA** 1998 Wilms' tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. Cell 93:445–454
155. **Ito M, Yu RN, Jameson JL** 1998 Steroidogenic factor-1 contains a carboxy-terminal transcriptional activation domain that interacts with steroid receptor coactivator-1. Mol Endocrinol 12:290–301
156. **Monte D, DeWitte F, Hum DW** 1998 Regulation of the human P450scc gene by steroidogenic factor 1 is mediated by CBP/p300. J Biol Chem 273:4585–4591
157. **Rainey WE, Naville D, Mason JI** 1991 Regulation of 3β-hydroxysteroid dehydrogenase in adrenocortical cells: effects of angiotensin-II and transforming growth factor β. Endocr Res 17:281–296
158. **Havelock JC, Smith AL, Seely JB, Dooley CA, Rodgers RJ, Rainey WE, Carr BR** 2005 The NGFI-B family of transcription factors regulates expression of 3β-hydroxysteroid dehydrogenase type 2 in the human ovary. Mol Hum Reprod 11:79–85
159. **Tremblay Y, Beaudoin C** 1993 Regulation of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase messenger ribonucleic acid levels by cyclic adenosine 3′,5′-monophosphate and phorbol myristate acetate in human choriocarcinoma cells. Mol Endocrinol 7:355–364
160. **Guerin SL, Leclerc S, Verreault H, Labrie F, Luu-The V** 1995 Overlapping *cis*-acting elements located in the first intron of the gene for type I 3β-hydroxysteroid dehydrogenase modulate its transcriptional activity. Mol Endocrinol 9:1583–1597
161. **Mason JI, Ushijima K, Doody KM, Nagai K, Naville D, Head JR, Milewich L, Rainey WE, Ralph MM** 1993 Regulation of expression of the 3β-hydroxysteroid dehydrogenases of human placenta and fetal adrenal. J Steroid Biochem Mol Biol 47:151–159
162. **Fukuoka M, Yasuda K, Emi N, Fujiwara H, Iwai M, Takakura K, Kanzaki H, Mori T** 1992 Cytokine modulation of progesterone and estradiol secretion in cultures of luteinized human granulosa cells. J Clin Endocrinol Metab 75:254–258
163. **Evagelatou M, Peterson SL, Cooke BA** 1997 Leukocytes modulate 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in human granulosa-lutein cell cultures. Mol Cell Endocrinol 133:81–88
164. **Van der Hoek KH, Woodhouse CM, Brannstrom M, Norman RJ** 1998 Effects of interleukin (IL)-6 on luteinizing hormone- and IL-1β-induced ovulation and steroidogenesis in the rat ovary. Biol Reprod 58:1266–1271
165. **Duncan LJ, Coldham NG, Reed MJ** 1994 The interaction of cytokines in regulating oestradiol 17β-hydroxysteroid dehydrogenase activity in MCF-7 cells. J Steroid Biochem Mol Biol 49:63–68
166. **Speirs V, Adams EF, Rafferty B, White MC** 1993 Interactive effects of interleukin-6, 17β-estradiol and progesterone on growth and 17β-hydroxysteroid dehydrogenase activity in human breast carcinoma cells. J Steroid Biochem Mol Biol 46:11–15
167. **Singh A, Reed MJ** 1991 Insulin-like growth factor type I and insulin-like growth factor type II stimulate oestradiol-17β-hydroxysteroid dehydrogenase (reductive) activity in breast cancer cells. J Endocrinol 129:R5–R8
168. **Reed MJ, Coldham NG, Patel SR, Ghilchik MW, James VH** 1992 Interleukin-1 and interleukin-6 in breast cyst fluid: their role in regulating aromatase activity in breast cancer cells. J Endocrinol 132:R5–R8
169. **Simpson E, Rubin G, Clyne C, Robertson K, O'Donnell L, Jones M, Davis S** 2000 The role of local estrogen biosynthesis in males and females. Trends Endocrinol Metab 11:184–188
170. **Paul WE** 1991 Interleukin-4: a prototypic immunoregulatory lymphokine. Blood 77:1859–1870
171. **Turgeon C, Gingras S, Carriere MC, Blais Y, Labrie F, Simard J** 1998 Regulation of sex steroid formation by interleukin-4 and interleukin-6 in breast cancer cells. J Steroid Biochem Mol Biol 65: 151–162
172. **Quelle FW, Shimoda K, Thierfelder W, Fischer C, Kim A, Ruben SM, Cleveland JL, Pierce JH, Keegan AD, Nelms K** 1995 Cloning of murine Stat6 and human Stat6, Stat proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis. Mol Cell Biol 15:3336–3343
173. **Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL** 1994 An interleukin-4-induced transcription factor: IL-4 Stat. Science 265:1701–1706
174. **Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE, Ihle JN** 1996 Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 380:630–633
175. **Kaplan MH, Schindler U, Smiley ST, Grusby MJ** 1996 Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity 4:313–319
176. **Takeda K, Kishimoto T, Akira S** 1997 STAT6: its role in interleukin 4-mediated biological functions. J Mol Med 75:317–326
177. **Gingras S, Cote S, Simard J** 2000 Multiple signaling pathways mediate interleukin-4-induced 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase type 1 gene expression in human breast cancer cells. Mol Endocrinol 14:229–240
178. **Keegan AD, Nelms K, White M, Wang LM, Pierce JH, Paul WE** 1994 An IL-4 receptor region containing an insulin receptor motif is important for IL-4-mediated IRS-1 phosphorylation and cell growth. Cell 76:811–820
179. **Wang LM, Myers Jr MG, Sun XJ, Aaronson SA, White M, Pierce JH** 1993 IRS-1: essential for insulin- and IL-4-stimulated mitogenesis in hematopoietic cells. Science 261:1591–1594
180. **Myers Jr MG, White MF** 1996 Insulin signal transduction and the IRS proteins. Annu Rev Pharmacol Toxicol 36:615–658
181. **Wang LM, Keegan AD, Li W, Lienhard GE, Pacini S, Gutkind JS, Myers Jr MG, Sun X, White MF, Aaronson SA, Paul WE, Pierce JH** 1993 Common elements in interleukin 4 and insulin signaling pathways in factor-dependent hematopoietic cells. Proc Natl Acad Sci USA 90:4032–4036
182. **Sun XJ, Wang LM, Zhang Y, Yenush L, Myers Jr MG, Glasheen E, Lane WS, Pierce JH, White MF** 1995 Role of IRS-2 in insulin and cytokine signalling. Nature 377:173–177
183. **Blais Y, Gingras S, Haagensen DE, Labrie F, Simard J** 1996 Interleukin-4 and interleukin-13 inhibit estrogen-induced breast cancer cell proliferation and stimulate GCDFP-15 expression in human breast cancer cells. Mol Cell Endocrinol 121:11–18
184. **Jackson JG, White MF, Yee D** 1998 Insulin receptor substrate-1 is the predominant signaling molecule activated by insulin-like growth factor-I, insulin, and interleukin-4 in estrogen receptor-positive human breast cancer cells. J Biol Chem 273:9994–10003
185. **Kjeldsen T, Andersen AS, Wiberg FC, Rasmussen JS, Schaffer L, Balschmidt P, Moller KB, Moller NP** 1991 The ligand specificities of the insulin receptor and the insulin-like growth factor I receptor reside in different regions of a common binding site. Proc Natl Acad Sci USA 88:4404–4408
186. **Myers Jr MG, Wang LM, Sun XJ, Zhang Y, Yenush L, Schlessinger**

J, Pierce JH, White MF 1994 Role of IRS-1-GRB-2 complexes in insulin signaling. Mol Cell Biol 14:3577–3587
187. **Skolnik EY, Batzer A, Li N, Lee CH, Lowenstein E, Mohammadi M, Margolis B, Schlessinger J** 1993 The function of GRB2 in linking the insulin receptor to Ras signaling pathways. Science 260:1953–1955
188. **Skolnik EY, Lee CH, Batzer A, Vicentini LM, Zhou M, Daly R, Myers Jr MJ, Backer JM, Ullrich A, White MF** 1993 The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine- phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. EMBO J 12:1929–1936
189. **Welham MJ, Learmonth L, Bone H, Schrader JW** 1995 Interleukin-13 signal transduction in lymphohemopoietic cells. Similarities and differences in signal transduction with interleukin-4 and insulin. J Biol Chem 270:12286–12296
190. **Satoh T, Nakafuku M, Miyajima A, Kaziro Y** 1991 Involvement of ras p21 protein in signal-transduction pathways from interleukin 2, interleukin 3, and granulocyte/macrophage colony-stimulating factor, but not from interleukin 4. Proc Natl Acad Sci USA 88: 3314–3318
191. **Foltz IN, Lee JC, Young PR, Schrader JW** 1997 Hemopoietic growth factors with the exception of interleukin-4 activate the p38 mitogen-activated protein kinase pathway. J Biol Chem 272:3296–3301
192. **Wery S, Letourneur M, Bertoglio J, Pierre J** 1996 Interleukin-4 induces activation of mitogen-activated protein kinase and phosphorylation of shc in human keratinocytes. J Biol Chem 271:8529–8532
193. **Liu JP** 1996 Protein kinase C and its substrates. Mol Cell Endocrinol 116:1–29
194. **Standaert ML, Bandyopadhyay G, Galloway L, Farese RV** 1996 Effects of phorbol esters on insulin-induced activation of phosphatidylinositol 3-kinase, glucose transport, and glycogen synthase in rat adipocytes. FEBS Lett 388:26–28
195. **Kotanides H, Moczygemba M, White MF, Reich NC** 1995 Characterization of the interleukin-4 nuclear activated factor/STAT and its activation independent of the insulin receptor substrate proteins. J Biol Chem 270:19481–19486
196. **Gingras S, Cote S, Simard J** 2001 Multiple signal transduction pathways mediate interleukin-4-induced 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in normal and tumoral target tissues. J Steroid Biochem Mol Biol 76:213–225
197. **Peng L, Payne AH** 2002 AP-2γ and the homeodomain protein distal-less 3 are required for placental-specific expression of the murine 3β-hydroxysteroid dehydrogenase VI gene, Hsd3b6. J Biol Chem 277:7945–7954
198. **Ben-Zimra M, Koler M, Orly J** 2002 Transcription of cholesterol side-chain cleavage cytochrome P450 in the placenta: activating protein-2 assumes the role of steroidogenic factor-1 by binding to an overlapping promoter element. Mol Endocrinol 16:1864–1880
199. **Jacquemin P, Sapin V, Alsat E, Evain-Brion D, Dolle P, Davidson I** 1998 Differential expression of the TEF family of transcription factors in the murine placenta and during differentiation of primary human trophoblasts in vitro. Dev Dyn 212:423–436
200. **Ma GT, Roth ME, Groskopf JC, Tsai FY, Orkin SH, Grosveld F, Engel JD, Linzer DI** 1997 GATA-2 and GATA-3 regulate trophoblast-specific gene expression in vivo. Development 124:907–914
201. **Dhom G** 1973 The prepuberal and puberal growth of the adrenal (adrenarche). Beitr Pathol 150:357–377
202. **Dhom G** 1973 Morphology of the human adrenarche. Acta Endocrinol Suppl (Copenh) 173:27
203. **Belanger B, Belanger A, Labrie F, Dupont A, Cusan L, Monfette G** 1989 Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. J Steroid Biochem 32:695–698
204. **Dupont E, Luu-The V, Labrie F, Pelletier G** 1990 Ontogeny of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) in human adrenal gland performed by immunocytochemistry. Mol Cell Endocrinol 74:R7–R10
205. **Doody KM, Carr BR, Rainey WE, Byrd W, Murry BA, Strickler RC, Thomas JL, Mason JI** 1990 3β-hydroxysteroid dehydrogenase/isomerase in the fetal zone and neocortex of the human fetal adrenal gland. Endocrinology 126:2487–2492
206. **Voutilainen R, Ilvesmaki V, Miettinen PJ** 1991 Low expression of 3β-hydroxy-5-ene steroid dehydrogenase gene in human fetal adrenals *in vivo*; adrenocorticotropin and protein kinase C-dependent regulation in adrenocortical cultures. J Clin Endocrinol Metab 72:761–767
207. **Mesiano S, Coulter CL, Jaffe RB** 1993 Localization of cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17α-hydroxylase/17, 20-lyase, and 3β-hydroxysteroid dehydrogenase isomerase steroidogenic enzymes in human and rhesus monkey fetal adrenal glands: reappraisal of functional zonation. J Clin Endocrinol Metab 77:1184–1189
208. **Siiteri PL, MacDonald PC** 1963 The utilization of circulating dehydroepiandrosterone sulfate for estrogen synthesis during human pregnancy. Steroids 2:713–716
209. **Seron-Ferre M, Lawrence C, Jaffe R** 1978 Steroid production by definitive and fetal zones of the human fetal adrenal gland. J Clin Endocrinol Metab 46:603–609
210. **Parker Jr CR, Faye-Petersen O, Stankovic AK, Mason JI, Grizzle WE** 1995 Immunohistochemical evaluation of the cellular localization and ontogeny of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in the human fetal adrenal gland. Endocr Res 21:69–80
211. **Mapes S, Corbin CJ, Tarantal A, Conley A** 1999 The primate adrenal zona reticularis is defined by expression of cytochrome b5, 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17) and NADPH-cytochrome P450 reductase (reductase) but not 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD). J Clin Endocrinol Metab 84:3382–3385
212. **Endoh A, Kristiansen SB, Casson PR, Buster JE, Hornsby PJ** 1996 The zona reticularis is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult human adrenal cortex resulting from its low expression of 3β-hydroxysteroid dehydrogenase. J Clin Endocrinol Metab 81:3558–3565
213. **Migeon CJ, Kenny FM, Taylor FH** 1968 Cortisol production rate. 8. Pregnancy. J Clin Endocrinol Metab 28:661–666
214. **Albrecht ED, Haskins AL, Pepe GJ** 1980 The influence of fetectomy at midgestation upon the serum concentrations of progesterone, estrone, and estradiol in baboons. Endocrinology 107:766–770
215. **Farcnik K, Auersperg N** 1984 Distribution of 3β-hydroxysteroid dehydrogenase during development of the rat adrenal cortex and capsule. Differentiation 27:46–52
216. **Klepac R, Milkovic K, Milkovic S** 1977 Development of steroidogenesis in the fetal rat adrenal gland: an in vitro study. J Steroid Biochem 8:841–845
217. **Roos TB** 1967 Steroid synthesis in embryonic and fetal rat adrenal tissue. Endocrinology 81:716–728
218. **Dupont E, Rheaume E, Simard J, Luu-The V, Labrie F, Pelletier G** 1991 Ontogenesis of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in the rat adrenal as revealed by immunocytochemistry and *in situ* hybridization. Endocrinology 129:2687–2692
219. **Pignatelli D, Bento MJ, Maia M, Magalhaes MM, Magalhaes MC, Mason JI** 1998 Ontogeny of 3β-hydroxysteroid dehydrogenase expression in the rat adrenal gland as studied by immunohistochemistry. Endocr Res 24:613–614
220. **Pignatelli D, Bento MJ, Maia M, Magalhaes MM, Magalhaes MC, Mason JI** 1999 Ontogeny of 3β-hydroxysteroid dehydrogenase expression in the rat adrenal gland as studied by immunohistochemistry. Endocr Res 25:21–27
221. **Wallace KB, Hook JB, Bailie MD** 1980 Postnatal development of the renin-angiotensin system in rats. Am J Physiol 238:R432–R437
222. **Riley SC, Boshier DP, Luu-The V, Labrie F, Challis JR** 1992 Immunohistochemical localization of 3β-hydroxysteroid/Δ^5-Δ^4 isomerase, tyrosine hydroxylase and phenylethanolamine *N*-methyl transferase in adrenal glands of sheep fetuses throughout gestation and in neonates. J Reprod Fertil 96:127–134
223. **Bird IM, Pasquarette MM, Rainey WE, Mason JI** 1996 Differential control of 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase expression in human adrenocortical H295R cells. J Clin Endocrinol Metab 81:2171–2178
224. **Wrobel KH, Suss F** 1999 On the origin and prenatal development of the bovine adrenal gland. Anat Embryol (Berl) [Erratum 200:123 (1999)] 199:301–318

225. **Conley AJ, Head JR, Stirling DT, Mason JI** 1992 Expression of steroidogenic enzymes in the bovine placenta and fetal adrenal glands throughout gestation. Endocrinology 130:2641–2650
226. **Coulter CL, Goldsmith PC, Mesiano S, Voytek CC, Martin MC, Mason JI, Jaffe RB** 1996 Functional maturation of the primate fetal adrenal *in vivo*. II. Ontogeny of corticosteroid synthesis is dependent upon specific zonal expression of 3β-hydroxysteroid dehydrogenase/isomerase. Endocrinology 137:4953–4959
227. **Leavitt MG, Albrecht ED, Pepe GJ** 1999 Development of the baboon fetal adrenal gland: regulation of the ontogenesis of the definitive and transitional zones by adrenocorticotropin. J Clin Endocrinol Metab 84:3831–3835
228. **Leavitt MG, Aberdeen GW, Burch MG, Albrecht ED, Pepe GJ** 1997 Inhibition of fetal adrenal adrenocorticotropin receptor messenger ribonucleic acid expression by betamethasone administration to the baboon fetus in late gestation. Endocrinology 138:2705–2712
229. **Benirschke K** 1956 Adrenals in anencephaly and hydrocephaly. Obstet Gynecol 8:412–425
230. **Han X, Berdusco ET, Lu F, Challis JR** 1997 Immunolocalisation of P450(C17) in the fetal sheep adrenal gland during gestation and in response to ACTH and glucocorticoid administration. Equine Vet J Suppl 24:62–67
231. **Nagaya M, Arai M, Widmaier EP** 1995 Ontogeny of immunoreactive and bioactive microsomal steroidogenic enzymes during adrenocortical development in rats. Mol Cell Endocrinol 114:27–34
232. **Engeland WC, Levay-Young BK, Rogers LM, Fitzgerald D** 1997 Differential gene expression of cytochrome P450 11β-hydroxylase in rat adrenal cortex after *in vivo* activation. Endocrinology 138:2338–2346
233. **Lehoux JG, Fleury A, Ducharme L** 1998 The acute and chronic effects of adrenocorticotropin on the levels of messenger ribonucleic acid and protein of steroidogenic enzymes in rat adrenal *in vivo*. Endocrinology 139:3913–3922
234. **Lebrethon MC, Jaillard C, Naville D, Begeot M, Saez JM** 1994 Regulation of corticotropin and steroidogenic enzyme mRNAs in human fetal adrenal cells by corticotropin, angiotensin-II and transforming growth factor β 1. Mol Cell Endocrinol 106:137–143
235. **Lebrethon MC, Jaillard C, Defayes G, Begeot M, Saez JM** 1994 Human cultured adrenal fasciculata-reticularis cells are targets for angiotensin-II: effects on cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17α-hydroxylase, and 3β-hydroxysteroid-dehydrogenase messenger ribonucleic acid and proteins and on steroidogenic responsiveness to corticotropin and angiotensin-II. J Clin Endocrinol Metab 78:1212–1219
236. **Rybak SM, Ramachandran J** 1982 Mechanism of induction of Δ5–3β-hydroxysteroid dehydrogenase-isomerase activity in rat adrenocortical cells by corticotropin. Endocrinology 111:427–433
237. **Naville D, Rainey WE, Milewich L, Mason JI** 1991 Regulation of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase expression by adrenocorticotropin in bovine adrenocortical cells. Endocrinology 128:139–145
238. **Penhoat A, Ouali R, Viard I, Langlois D, Saez JM** 1996 Regulation of primary response and specific genes in adrenal cells by peptide hormones and growth factors. Steroids 61:176–183
239. **Bird IM, Imaishi K, Pasquarette MM, Rainey WE, Mason JI** 1996 Regulation of 3β-hydroxysteroid dehydrogenase expression in human adrenocortical H295R cells. J Endocrinol 150 (Suppl):S165–S173
240. **Bird IM, Mason JI, Rainey WE** 1998 Protein kinase A, protein kinase C, and Ca(2+)-regulated expression of 21-hydroxylase cytochrome P450 in H295R human adrenocortical cells. J Clin Endocrinol Metab 83:1592–1597
241. **Bird IM, Mathis JM, Mason JI, Rainey WE** 1995 Ca(2+)-regulated expression of steroid hydroxylases in H295R human adrenocortical cells. Endocrinology 136:5677–5684
242. **Reyland ME** 1993 Protein kinase C is a tonic negative regulator of steroidogenesis and steroid hydroxylase gene expression in Y1 adrenal cells and functions independently of protein kinase A. Mol Endocrinol 7:1021–1030
243. **Crickard K, Ill CR, Jaffe RB** 1981 Control of proliferation of human fetal adrenal cells *in vitro*. J Clin Endocrinol Metab 53:790–796
244. **Gospodarowicz D, Ill CR, Hornsby PJ, Gill GN** 1977 Control of bovine adrenal cortical cell proliferation by fibroblast growth factor. Lack of effect of epidermal growth factor. Endocrinology 100:1080–1089
245. **Gospodarowicz D, Handley HH** 1975 Stimulation of division of Y1 adrenal cells by a growth factor isolated from bovine pituitary glands. Endocrinology 97:102–107
246. **Dallman MF, Makara GB, Roberts JL, Levin N, Blum M** 1985 Corticotrope response to removal of releasing factors and corticosteroids *in vivo*. Endocrinology 117:2190–2197
247. **Riopel L, Branchaud CL, Goodyer CG, Adkar V, Lefebvre Y** 1989 Growth-inhibitory effect of TGF-β on human fetal adrenal cells in primary monolayer culture. J Cell Physiol 140:233–238
248. **Coulter CL, Read LC, Carr BR, Tarantal AF, Barry S, Styne DM** 1996 A role for epidermal growth factor in the morphological and functional maturation of the adrenal gland in the fetal rhesus monkey *in vivo*. J Clin Endocrinol Metab 81:1254–1260
249. **McAllister JM, Hornsby PJ** 1988 Dual regulation of 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and dehydroepiandrosterone sulfotransferase by adenosine 3′,5′-monophosphate and activators of protein kinase C in cultured human adrenocortical cells. Endocrinology 122:2012–2018
250. **Singh-Asa P, Waters MJ** 1983 Stimulation of adrenal cortisol biosynthesis by epidermal growth factor. Mol Cell Endocrinol 30:189–199
251. **Sasano H, Suzuki T, Shizawa S, Kato K, Nagura H** 1994 Transforming growth factor α, epidermal growth factor, and epidermal growth factor receptor expression in normal and diseased human adrenal cortex by immunohistochemistry and in situ hybridization. Mod Pathol 7:741–746
252. **Le Roy C, Leduque P, Dubois PM, Saez JM, Langlois D** 1996 Repression of transforming growth factor β 1 protein by antisense oligonucleotide-induced increase of adrenal cell differentiated functions. J Biol Chem 271:11027–11033
253. **Lebrethon MC, Jaillard C, Naville D, Begeot M, Saez JM** 1994 Effects of transforming growth factor-β 1 on human adrenocortical fasciculata-reticularis cell differentiated functions. J Clin Endocrinol Metab 79:1033–1039
254. **Naville D, Rainey WE, Mason JI** 1991 Corticotropin regulation of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in ovine adrenocortical cells: inhibition by transforming growth factor β. Mol Cell Endocrinol 75:257–263
255. **Cherradi N, Chambaz EM, Defaye G** 1995 Type β 1 transforming growth factor is an inhibitor of 3β-hydroxysteroid dehydrogenase isomerase in mouse adrenal tumor cell line Y1. Endocr Res 21:61–66
256. **Mesiano S, Jaffe RB** 1993 Interaction of insulin-like growth factor-II and estradiol directs steroidogenesis in the human fetal adrenal toward dehydroepiandrosterone sulfate production. J Clin Endocrinol Metab 77:754–758
257. **Kristiansen SB, Endoh A, Casson PR, Buster JE, Hornsby PJ** 1997 Induction of steroidogenic enzyme genes by insulin and IGF-I in cultured adult human adrenocortical cells. Steroids 62:258–265
258. **I'Allemand D, Penhoat A, Lebrethon MC, Ardevol R, Baehr V, Oelkers W, Saez JM** 1996 Insulin-like growth factors enhance steroidogenic enzyme and corticotropin receptor messenger ribonucleic acid levels and corticotropin steroidogenic responsiveness in cultured human adrenocortical cells. J Clin Endocrinol Metab 81:3892–3897
259. **Gell JS, Oh J, Rainey WE, Carr BR** 1998 Effect of estradiol on DHEAS production in the human adrenocortical cell line, H295R. J Soc Gynecol Investig 5:144–148
260. **Albertson BD, Hill RB, Sprague KA, Wood KE, Nieman LK, Loriaux DL** 1994 Effect of the antiglucocorticoid RU486 on adrenal steroidogenic enzyme activity and steroidogenesis. Eur J Endocrinol 130:195–200
261. **Simonian MH** 1986 ACTH and thyroid hormone regulation of 3β-hydroxysteroid dehydrogenase activity in human fetal adrenocortical cells. J Steroid Biochem 25:1001–1006
262. **Lo MJ, Kau MM, Chen YH, Tsai SC, Chiao YC, Chen JJ, Liaw C, Lu CC, Lee BP, Chen SC, Fang VS, Ho LT, Wang PS** 1998 Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats. Am J Physiol 274:E238–E245
263. **Bhattacharya S, Guin S, Bandyopadhyay A, Jana NR, Halder S** 1996 Thyroid hormone induces the generation of a novel putative

protein in piscine ovarian follicle that stimulates the conversion of pregnenolone to progesterone. Eur J Endocrinol 134:128–135
264. **Datta M, Nagendra Prasad RJ, Bhattacharya S** 1999 Thyroid hormone regulation of perch ovarian 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase activity: involvement of a 52-kDa protein. Gen Comp Endocrinol 113:212–220
265. **Nagendra Prasad RJ, Datta M, Bhattacharya S** 1999 Differential regulation of Leydig cell 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase activity by gonadotropin and thyroid hormone in a freshwater perch, *Anabas testudineus* (Bloch). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 124:165–173
266. **Parker Jr CR, Stankovic AM, Goland RS** 1999 Corticotropin-releasing hormone stimulates steroidogenesis in cultured human adrenal cells. Mol Cell Endocrinol 155:19–25
267. **Rainey WE, Naville D, Cline N, Mason JI** 1991 Prostaglandin E2 is a positive regulator of adrenocorticotropin receptors, 3β-hydroxysteroid dehydrogenase, and 17α-hydroxylase expression in bovine adrenocortical cells. Endocrinology 129:1333–1339
268. **Braems GA, Han VK, Challis JR** 1998 Gestational age-dependent changes in the levels of mRNAs encoding cortisol biosynthetic enzymes and IGF-II in the adrenal gland of fetal sheep during prolonged hypoxemia. J Endocrinol 159:257–264
269. **Cutler Jr GB, Glenn M, Bush M, Hodgen GD, Graham CE, Loriaux DL** 1978 Adrenarche: a survey of rodents, domestic animals, and primates. Endocrinology 103:2112–2118
270. **Gell JS, Atkins B, Margraf L, Mason JI, Sasano H, Rainey WE, Carr BR** 1996 Adrenarche is associated with decreased 3β-hydroxysteroid dehydrogenase expression in the adrenal reticularis. Endocr Res 22:723–728
271. **Gell JS, Carr BR, Sasano H, Atkins B, Margraf L, Mason JI, Rainey WE** 1998 Adrenarche results from development of a 3β-hydroxysteroid dehydrogenase-deficient adrenal reticularis. J Clin Endocrinol Metab 83:3695–3701
272. **Dardis A, Saraco N, Rivarola MA, Belgorosky A** 1999 Decrease in the expression of the 3β-hydroxysteroid dehydrogenase gene in human adrenal tissue during prepuberty and early puberty: implications for the mechanism of adrenarche. Pediatr Res 45:384–388
273. **Miller W** 1997 The regulation of 17,20 lyase activity. Steroids 62: 133–142
274. **Conley AJ, Bird IM** 1997 The role of cytochrome P450 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the Δ5 and Δ4 pathways of steroidogenesis in mammals. Biol Reprod 56:789–799
275. **Dupont E, Labrie F, Luu-The V, Pelletier G** 1992 Immunocytochemical localization of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in human ovary. J Clin Endocrinol Metab 74:994–998
276. **Kaplan S, Grumach M** 1978 Pituitary and placental gonadotropin and sex steroids in the human and sub-human primate fetus. J Clin Endocrinol Metab 7:487–511
277. **Grumbach M, Conte F** 1999 Disorders of sex differentitiation. In: Wilson JD, Foster EW, ed. Williams textbook of endocrinology. Philadelphia: W. B. Saunders; 1303–1425
278. **Dupont E, Zhao HF, Rheaume E, Simard J, Luu-The V, Labrie F, Pelletier G** 1990 Localization of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in rat gonads and adrenal glands by immunocytochemistry and *in situ* hybridization. Endocrinology 127:1394–1403
279. **Juneau C, Dupont E, Luu-The V, Labrie F, Pelletier G** 1993 Ontogenesis of 3β-hydroxysteroid dehydrogenase Δ^5-Δ^4 isomerase in the rat ovary as studied by immunocytochemistry and in situ hybridization. Biol Reprod 48:226–234
280. **Greco TL, Payne AH** 1994 Ontogeny of expression of the genes for steroidogenic enzymes P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase, P450 17α-hydroxylase/C17–20 lyase, and P450 aromatase in fetal mouse gonads. Endocrinology 135:262–268
281. **Hillier SG** 1981 Regulation of follicular oestrogen biosynthesis: a survey of current concepts. J Endocrinol 89:3P–18P
282. **Sasano H, Mori T, Sasano N, Nagura H, Mason JI** 1990 Immunolocalization of 3β-hydroxysteroid dehydrogenase in human ovary. J Reprod Fertil 89:743–751
283. **Suzuki T, Sasano H, Tamura M, Aoki H, Fukaya T, Yajima A, Nagura H, Mason JI** 1993 Temporal and spatial localization of steroidogenic enzymes in premenopausal human ovaries: in situ hybridization and immunohistochemical study. Mol Cell Endocrinol 97:135–143
284. **Suzuki T, Sasano H, Kimura N, Tamura M, Fukaya T, Yajima A, Nagura H** 1994 Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. Hum Reprod 9:1589–1595
285. **Fukaya T** 1995 [Human folliculogenesis and local regulation]. Nippon Sanka Fujinka Gakkai Zasshi 47:726–737
286. **Doody KJ, Lorence MC, Mason JI, Simpson ER** 1990 Expression of messenger ribonucleic acid species encoding steroidogenic enzymes in human follicles and corpora lutea throughout the menstrual cycle. J Clin Endocrinol Metab 70:1041–1045
287. **Teerds KJ, Dorrington JH** 1993 Immunohistochemical localization of 3β-hydroxysteroid dehydrogenase in the rat ovary during follicular development and atresia. Biol Reprod 49:989–996
288. **Doody KJ, Lephart ED, Stirling D, Lorence MC, Magness RR, McPhaul MJ, Simpson ER** 1991 Expression of mRNA species encoding steroidogenic enzymes in the rat ovary. J Mol Endocrinol 6:153–162
289. **Conley AJ, Christenson RK, Ford SP, Geisert RD, Mason JI** 1992 Steroidogenic enzyme expression in porcine conceptuses during and after elongation. Endocrinology 131:896–902
290. **Garrett WM, Guthrie HD** 1997 Steroidogenic enzyme expression during preovulatory follicle maturation in pigs. Biol Reprod 56: 1424–1431
291. **Yuan W, Lucy MC** 1996 Messenger ribonucleic acid expression for growth hormone receptor, luteinizing hormone receptor, and steroidogenic enzymes during the estrous cycle and pregnancy in porcine and bovine corpora lutea. Domest Anim Endocrinol 13: 431–444
292. **Voss AK, Fortune JE** 1993 Levels of messenger ribonucleic acid for cholesterol side-chain cleavage cytochrome P-450 and 3β-hydroxysteroid dehydrogenase in bovine preovulatory follicles decrease after the luteinizing hormone surge. Endocrinology 132:888–894
293. **Couet J, Martel C, Dupont E, Luu-The V, Sirard MA, Zhao HF, Pelletier G, Labrie F** 1990 Changes in 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase messenger ribonucleic acid, activity and protein levels during the estrous cycle in the bovine ovary. Endocrinology 127:2141–2148
294. **Xu Z, Garverick HA, Smith GW, Smith MF, Hamilton SA, Youngquist RS** 1995 Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. Biol Reprod 53:951–957
295. **Xu Z, Garverick HA, Smith GW, Smith MF, Hamilton SA, Youngquist RS** 1995 Expression of messenger ribonucleic acid encoding cytochrome P450 side-chain cleavage, cytochrome p450 17α-hydroxylase, and cytochrome P450 aromatase in bovine follicles during the first follicular wave. Endocrinology 136:981–989
296. **Bao B, Garverick HA, Smith GW, Smith MF, Salfen BE, Youngquist RS** 1997 Changes in messenger ribonucleic acid encoding luteinizing hormone receptor, cytochrome P450-side chain cleavage, and aromatase are associated with recruitment and selection of bovine ovarian follicles. Biol Reprod 56:1158–1168
297. **Bao B, Garverick HA** 1998 Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. J Anim Sci 76:1903–1921
298. **Bao B, Garverick HA, Smith GW, Smith MF, Salfen BE, Youngquist RS** 1997 Expression of messenger ribonucleic acid (mRNA) encoding 3β-hydroxysteroid dehydrogenase Δ4, Δ5 isomerase (3β-HSD) during recruitment and selection of bovine ovarian follicles: identification of dominant follicles by expression of 3β-HSD mRNA within the granulosa cell layer. Biol Reprod 56:1466–1473
299. **Voss AK, Fortune JE** 1993 Levels of messenger ribonucleic acid for cytochrome P450 17α-hydroxylase and P450 aromatase in preovulatory bovine follicles decrease after the luteinizing hormone surge. Endocrinology 132:2239–2245
300. **McAllister JM, Kerin JF, Trant JM, Estabrook RW, Mason JI, Waterman MR, Simpson ER** 1989 Regulation of cholesterol side-chain cleavage and 17α-hydroxylase/lyase activities in proliferating human theca interna cells in long term monolayer culture. Endocrinology 125:1959–1966
301. **Picon R, Darmoul D, Rouiller V, Duranteau L** 1988 Activity of

3β-hydroxysteroid dehydrogenase/isomerase in the fetal rat ovary. J Steroid Biochem 31:839–843
302. **Kaynard AH, Periman LM, Simard J, Melner MH** 1992 Ovarian 3β-hydroxysteroid dehydrogenase and sulfated glycoprotein-2 gene expression are differentially regulated by the induction of ovulation, pseudopregnancy, and luteolysis in the immature rat. Endocrinology 130:2192–2200
303. **Orly J, Rei Z, Greenberg NM, Richards JS** 1994 Tyrosine kinase inhibitor AG18 arrests follicle-stimulating hormone-induced granulosa cell differentiation: use of reverse transcriptase-polymerase chain reaction assay for multiple messenger ribonucleic acids. Endocrinology 134:2336–2346
304. **Gonzalez-Robayna IJ, Falender AE, Ochsner S, Firestone GL, Richards JS** 2000 Follicle-stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-induced kinase (Sgk): evidence for a kinase-independent signaling by FSH in granulosa cells. Mol Endocrinol 14:1283–1300
305. **Maizels ET, Cottom J, Jones JC, Hunzicker-Dunn M** 1998 Follicle stimulating hormone (FSH) activates the p38 mitogen-activated protein kinase pathway, inducing small heat shock protein phosphorylation and cell rounding in immature rat ovarian granulosa cells. Endocrinology 139:3353–3356
306. **Cameron MR, Foster JS, Bukovsky A, Wimalasena J** 1996 Activation of mitogen-activated protein kinases by gonadotropins and cyclic adenosine 5′-monophosphates in porcine granulosa cells. Biol Reprod 55:111–119
307. **Das S, Maizels ET, DeManno D, St Clair E, Adam SA, Hunzicker-Dunn M** 1996 A stimulatory role of cyclic adenosine 3′,5′-monophosphate in follicle-stimulating hormone-activated mitogen-activated protein kinase signaling pathway in rat ovarian granulosa cells. Endocrinology 137:967–974
308. **Rodway MR, Swan CL, Crellin NK, Gillio-Meina C, Chedrese PJ** 1999 Steroid regulation of progesterone synthesis in a stable porcine granulosa cell line: a role for progestins. J Steroid Biochem Mol Biol 68:173–180
309. **Chedrese PJ, Zhang D, Luu The V, Labrie F, Juorio AV, Murphy BD** 1990 Regulation of mRNA expression of 3β-hydroxy-5-ene steroid dehydrogenase in porcine granulosa cells in culture: a role for the protein kinase-C pathway. Mol Endocrinol 4:1532–1538
310. **Martel C, Labrie C, Dupont E, Couet J, Trudel C, Rheaume E, Simard J, Luu-The V, Pelletier G, Labrie F** 1990 Regulation of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase expression and activity in the hypophysectomized rat ovary: interactions between the stimulatory effect of human chorionic gonadotropin and the luteolytic effect of prolactin. Endocrinology 127:2726–2737
311. **Martel C, Gagne D, Couet J, Labrie Y, Simard J, Labrie F** 1994 Rapid modulation of ovarian 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene expression by prolactin and human chorionic gonadotropin in the hypophysectomized rat. Mol Cell Endocrinol 99:63–71
312. **Vermeulen A** 1976 The hormonal activity of the postmenopausal ovary. J Clin Endocrinol Metab 42:247–253
313. **Labrie F, Belanger A, Cusan L, Gomez JL, Candas B** 1997 Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 82:2396–2402
314. **Arif S, Varela-Calvino R, Conway GS, Peakman M** 2001 3β-hydroxysteroid dehydrogenase autoantibodies in patients with idiopathic premature ovarian failure target N- and C-terminal epitopes. J Clin Endocrinol Metab 86:5892–5897
315. **Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery Jr CA, Shyamala G, Conneely OM, O'Malley BW** 1995 Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev 9:2266–2278
316. **Chaffin CL, Dissen GA, Stouffer RL** 2000 Hormonal regulation of steroidogenic enzyme expression in granulosa cells during the peri-ovulatory interval in monkeys. Mol Hum Reprod 6:11–18
317. **Donath J, Michna H, Nishino Y** 1997 The antiovulatory effect of the antiprogestin onapristone could be related to down-regulation of intraovarian progesterone (receptors). J Steroid Biochem Mol Biol 62:107–118
318. **Tanaka N, Iwamasa J, Matsuura K, Okamura H** 1993 Effects of progesterone and anti-progesterone RU486 on ovarian 3β-hydroxysteroid dehydrogenase activity during ovulation in the gonadotrophin-primed immature rat. J Reprod Fertil 97:167–172
319. **Ruiz de Galarreta CM, Fanjul LF, Hsueh AJ** 1985 Progestin regulation of progesterone biosynthetic enzymes in cultured rat granulosa cells. Steroids 46:987–1002
320. **Willis DS, Mason HD, Watson H, Franks S** 1998 Developmentally regulated responses of human granulosa cells to insulin-like growth factors (IGFs): IGF-I and IGF-II action mediated via the type-I IGF receptor. J Clin Endocrinol Metab 83:1256–1259
321. **deMoura MD, Choi D, Adashi EY, Payne DW** 1997 Insulin-like growth factor-I-mediated amplification of follicle-stimulating hormone-supported progesterone accumulation by cultured rat granulosa cells: enhancement of steroidogenic enzyme activity and expression. Biol Reprod 56:946–953
322. **Magoffin DA, Weitsman SR** 1993 Insulin-like growth factor-I stimulates the expression of 3β-hydroxysteroid dehydrogenase messenger ribonucleic acid in ovarian theca-interstitial cells. Biol Reprod 48:1166–1173
323. **Magarelli PC, Zachow RJ, Magoffin DA** 1996 Developmental and hormonal regulation of rat theca-cell differentiation factor secretion in ovarian follicles. Biol Reprod 55:416–420
324. **Gelety TJ, Magoffin DA** 1997 Ontogeny of steroidogenic enzyme gene expression in ovarian theca-interstitial cells in the rat: regulation by a paracrine theca-differentiating factor prior to achieving luteinizing hormone responsiveness. Biol Reprod 56:938–945
325. **Reinhart J, Mertz LM, Catt KJ** 1992 Molecular cloning and expression of cDNA encoding the murine gonadotropin-releasing hormone receptor. J Biol Chem 267:21281–21284
326. **Yago N** 1994 [The effects of GnRH agonist on steroidogenesis in the rat ovary]. Nippon Naibunpi Gakkai Zasshi 70:543–554
327. **Bendell JJ, Dorrington JH** 1990 Epidermal growth factor influences growth and differentiation of rat granulosa cells. Endocrinology 127:533–540
328. **Mayerhofer A, Smith GD, Danilchik M, Levine JE, Wolf DP, Dissen GA, Ojeda SR** 1998 Oocytes are a source of catecholamines in the primate ovary: evidence for a cell-cell regulatory loop. Proc Natl Acad Sci USA 95:10990–10995
329. **Stefenson A, Owman C, Sjoberg NO, Sporrong B, Walles B** 1981 Comparative study of the autonomic innervation of the mammalian ovary, with particular regard to the follicular system. Cell Tissue Res 215:47–62
330. **Li XM, Juorio AV, Chedrese PJ, Murphy BD** 1993 Alterations in 3β-hydroxysteroid dehydrogenase (3β-HSD) mRNA accumulation induced by β-adrenergic stimulation in cultured porcine granulosa cells. Gen Pharmacol 24:715–720
331. **Bassett SG, Little-Ihrig LL, Mason JI, Zeleznik AJ** 1991 Expression of messenger ribonucleic acids that encode for 3β-hydroxysteroid dehydrogenase and cholesterol side-chain cleavage enzyme throughout the luteal phase of the macaque menstrual cycle. J Clin Endocrinol Metab 72:362–366
332. **Ravindranath N, Little-Ihrig LL, Zeleznik AJ** 1992 Characterization of the levels of messenger ribonucleic acid that encode for luteinizing hormone receptor during the luteal phase of the primate menstrual cycle. J Clin Endocrinol Metab 74:779–785
333. **Sanders SL, Stouffer RL** 1997 Localization of steroidogenic enzymes in macaque luteal tissue during the menstrual cycle and simulated early pregnancy: immunohistochemical evidence supporting the two-cell model for estrogen production in the primate corpus luteum. Biol Reprod 56:1077–1087
334. **Juengel JL, Guy MK, Tandeski TR, McGuire WJ, Niswender GD** 1994 Steady-state concentrations of messenger ribonucleic acid encoding cytochrome P450 side-chain cleavage and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in ovine corpora lutea during the estrous cycle. Biol Reprod 51:380–384
335. **Sasano H, Suzuki T** 1997 Localization of steroidogenesis and steroid receptors in human corpus luteum. Classification of human corpus luteum (CL) into estrogen- producing degenerating CL, and nonsteroid-producing degenerating CL. Semin Reprod Endocrinol 15:345–351
336. **Hild-Petito S, Stouffer RL, Brenner RM** 1988 Immunocytochemical localization of estradiol and progesterone receptors in the mon-

key ovary throughout the menstrual cycle. Endocrinology 123: 2896–2905
337. **Hild-Petito S, Fazleabas AT** 1997 Expression of steroid receptors and steroidogenic enzymes in the baboon (*Papio anubis*) corpus luteum during the menstrual cycle and early pregnancy. J Clin Endocrinol Metab 82:955–962
338. **Duffy DM, Stouffer RL** 1995 Progesterone receptor messenger ribonucleic acid in the primate corpus luteum during the menstrual cycle: possible regulation by progesterone. Endocrinology 136:1869–1876
339. **Hild-Petito S, West NB, Brenner RM, Stouffer RL** 1991 Localization of androgen receptor in the follicle and corpus luteum of the primate ovary during the menstrual cycle. Biol Reprod 44:561–568
340. **Hawkins DE, Belfiore CJ, Kile JP, Niswender GD** 1993 Regulation of messenger ribonucleic acid encoding 3 β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in the ovine corpus luteum. Biol Reprod 48:1185–1190
341. **Hild-Petito SA, Shiigi SM, Stouffer RL** 1989 Isolation and characterization of cell subpopulations from the monkey corpus luteum of the menstrual cycle. Biol Reprod 40:1075–1085
342. **Duncan WC, Cowen GM, Illingworth PJ** 1999 Steroidogenic enzyme expression in human corpora lutea in the presence and absence of exogenous human chorionic gonadotrophin (HCG). Mol Hum Reprod 5:291–298
343. **Benyo DF, Little-Ihrig L, Zeleznik AJ** 1993 Noncoordinated expression of luteal cell messenger ribonucleic acids during human chorionic gonadotropin stimulation of the primate corpus luteum. Endocrinology 133:699–704
344. **McAllister JM, Mason JI, Byrd W, Trant JM, Waterman MR, Simpson ER** 1990 Proliferating human granulosa-lutein cells in long term monolayer culture: expression of aromatase, cholesterol side-chain cleavage, and 3β-hydroxysteroid dehydrogenase. J Clin Endocrinol Metab 71:26–33
345. **McGee E, Sawetawan C, Bird I, Rainey WE, Carr BR** 1995 The effects of insulin on 3β-hydroxysteroid dehydrogenase expression in human luteinized granulosa cells. J Soc Gynecol Investig 2:535–541
346. **Martel C, Labrie C, Couet J, Dupont E, Trudel C, Luu-The V, Takahashi M, Pelletier G, Labrie F** 1990 Effects of human chorionic gonadotropin (hCG) and prolactin (PRL) on 3β-hydroxy-5-ene-steroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) expression and activity in the rat ovary. Mol Cell Endocrinol 72:R7–R13
347. **Galosy SS, Talamantes F** 1995 Luteotropic actions of placental lactogens at midpregnancy in the mouse. Endocrinology 136:3993–4003
348. **McNatty KP, Sawers RS, McNeilly AS** 1974 A possible role for prolactin in control of steroid secretion by the human Graafian follicle. Nature 250:653–655
349. **Alila HW, Rogo KO, Gombe S** 1987 Effects of prolactin on steroidogenesis by human luteal cells in culture. Fertil Steril 47:947–955
350. **Lee MS, Ben-Rafael Z, Meloni F, Mastroianni Jr L, Flickinger GL** 1986 Effects of prolactin on steroidogenesis by human luteinized granulosa cells. Fertil Steril 46:32–36
351. **Soto EA, Tureck RW, Strauss 3rd JF** 1985 Effects of prolactin on progestin secretion by human granulosa cells in culture. Biol Reprod 32:541–545.
352. **McAllister JM, Byrd W, Simpson ER** 1994 The effects of growth factors and phorbol esters on steroid biosynthesis in isolated human theca interna and granulosa-lutein cells in long term culture. J Clin Endocrinol Metab 79:106–112
353. **Wiltbank MC, Belfiore CJ, Niswender GD** 1993 Steroidogenic enzyme activity after acute activation of protein kinase (PK) A and PKC in ovine small and large luteal cells. Mol Cell Endocrinol 97:1–7
354. **Ravindranath N, Little-Ihrig L, Benyo DF, Zeleznik AJ** 1992 Role of luteinizing hormone in the expression of cholesterol side-chain cleavage cytochrome P450 and 3β-hydroxysteroid dehydrogenase, Δ^5-Δ^4 isomerase messenger ribonucleic acids in the primate corpus luteum. Endocrinology 131:2065–2070
355. **Rothchild I** 1981 The regulation of the mammalian corpus luteum. Recent Prog Horm Res 37:183–298
356. **Duffy DM, Hess DL, Stouffer RL** 1994 Acute administration of a 3β-hydroxysteroid dehydrogenase inhibitor to rhesus monkeys at the midluteal phase of the menstrual cycle: evidence for possible autocrine regulation of the primate corpus luteum by progesterone. J Clin Endocrinol Metab 79:1587–1594
357. **Duffy DM, Stouffer RL, Stewart DR** 1995 Dissociation of relaxin and progesterone secretion from the primate corpus luteum by acute administration of a 3β-hydroxysteroid dehydrogenase inhibitor during the menstrual cycle. Biol Reprod 53:447–453
358. **Duffy DM, Hutchison JS, Stewart DR, Stouffer RL** 1996 Stimulation of primate luteal function by recombinant human chorionic gonadotropin and modulation of steroid, but not relaxin, production by an inhibitor of 3β-hydroxysteroid dehydrogenase during simulated early pregnancy. J Clin Endocrinol Metab 81:2307–2313
359. **Arakawa S, Kambegawa A, Okinaga S, Arai K** 1990 Luteolytic effect of the antiprogestin and antiglucocorticoid agent RU486 in rats. J Steroid Biochem 36:479–483
360. **Iwamasa J** 1991 [The effect of progesterone and RU486 on progesterone production in the ovulatory process of rats]. Nippon Sanka Fujinka Gakkai Zasshi 43:321–327
361. **Telleria CM, Deis RP** 1994 Effect of RU486 on ovarian progesterone production at pro-oestrus and during pregnancy: a possible dual regulation of the biosynthesis of progesterone. J Reprod Fertil 102:379–384
362. **Telleria CM, Stocco CO, Stati AO, Rastrilla AM, Carrizo DG, Aguado LI, Deis RP** 1995 Dual regulation of luteal progesterone production by androstenedione during spontaneous and RU486-induced luteolysis in pregnant rats. J Steroid Biochem Mol Biol 55:385–393
363. **Telleria CM, Stocco CO, Stati AO, Deis RP** 1999 Progesterone receptor is not required for progesterone action in the rat corpus luteum of pregnancy. Steroids 64:760–766
364. **Vega M, Devoto L, Castro O, Kohen P** 1994 Progesterone synthesis by human luteal cells: modulation by estradiol. J Clin Endocrinol Metab 79:466–469
365. **Fisch B, Rose MP, Elder MG, Winston RM, Margara RA, Hillier SG** 1994 Effects of oestrogen on progesterone synthesis and arachidonic acid metabolism in human luteal cells. Clin Endocrinol (Oxf) 40:21–32
366. **Rodway RG, Rothchild I** 1980 Effects of oestradiol on steroid dehydrogenase activities in rat corpora lutea. J Endocrinol 84:391–395
367. **Tapanainen J, Martikainen H, Voutilainen R, Orava M, Ruokonen A, Ronnberg L** 1992 Effect of growth hormone administration on human ovarian function and steroidogenic gene expression in granulosa-luteal cells. Fertil Steril 58:726–732
368. **Srivastava RK, Sridaran R** 1994 Inhibition of luteal steroidogenesis by two LHRH antagonists (Nal-Glu and Nal-Arg antagonists) in the pregnant rat. Endocr Res 20:365–376
369. **Tekpetey FR, Armstrong DT** 1994 Catecholestrogen modulation of steroid production by rat luteal cells: mechanism of action. Mol Cell Endocrinol 101:49–57
370. **Kotwica J, Bogacki M** 1999 Physiological importance of dopamine as a noradrenaline precursor in the corpus luteum. Clin Exp Pharmacol Physiol Suppl 26:S29–S35
371. **McLean MP, Billheimer JT, Warden KJ, Irby RB** 1995 Prostaglandin F2 α mediates ovarian sterol carrier protein-2 expression during luteolysis. Endocrinology 136:4963–4972
372. **Li XM, Juorio AV, Murphy BD** 1993 Prostaglandins alter the abundance of messenger ribonucleic acid for steroidogenic enzymes in cultured porcine granulosa cells. Biol Reprod 48:1360–1366
373. **Rodgers RJ, Vella CA, Young FM, Tian XC, Fortune JE** 1995 Concentrations of cytochrome P450 cholesterol side-chain cleavage enzyme and 3β-hydroxysteroid dehydrogenase during prostaglandin F2 α-induced luteal regression in cattle. Reprod Fertil Dev [Erratum 8:1215 (1996)] 7:1213–1216
374. **Tian XC, Berndtson AK, Fortune JE** 1995 Differentiation of bovine preovulatory follicles during the follicular phase is associated with increases in messenger ribonucleic acid for cytochrome P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase, and P450 17α-hydroxylase, but not P450 aromatase. Endocrinology 136:5102–5110
375. **Tian XC, Berndtson AK, Fortune JE** 1994 Changes in levels of

messenger ribonucleic acid for cytochrome P450 side-chain cleavage and 3β-hydroxysteroid dehydrogenase during prostaglandin F2α-induced luteolysis in cattle. Biol Reprod 50:349–356
376. **Juengel JL, Larrick TL, Meberg BM, Niswender GD** 1998 Luteal expression of steroidogenic factor-1 mRNA during the estrous cycle and in response to luteotropic and luteolytic stimuli in ewes. Endocrine 9:227–232
377. **Stocco CO, Deis RP** 1996 Luteolytic effect of LH: inhibition of 3β-hydroxysteroid dehydrogenase and stimulation of 20α-hydroxysteroid dehydrogenase luteal activities in late pregnant rats. J Endocrinol 150:423–429
378. **Stocco CO, Deis RP** 1998 Participation of intraluteal progesterone and prostaglandin F2α in LH-induced luteolysis in pregnant rat. J Endocrinol 156:253–259
379. **Gasic S, Bodenburg Y, Nagamani M, Green A, Urban RJ** 1998 Troglitazone inhibits progesterone production in porcine granulosa cells. Endocrinology 139:4962–4966
380. **Cooke GM, Brawer JR, Farookhi R** 1993 Steroidogenic enzyme activities in rat polycystic ovaries. Can J Physiol Pharmacol 71: 247–253
381. **Gonzalez F, Hatala DA, Speroff L** 1991 Adrenal and ovarian steroid hormone responses to gonadotropin-releasing hormone agonist treatment in polycystic ovary syndrome. Am J Obstet Gynecol 165:535–545
382. **Nelson VL, Legro RS, Strauss 3rd JF, McAllister JM** 1999 Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 13:946–957
383. **Siiteri PK, Wilson, JD** 1974 Testosterone formation and metabolism during male sexual differentiation in human embryo. J Clin Endocrinol Metab 38:113–125
384. **Reyes FI, Boroditsky RS, Winter JS, Faiman C** 1974 Studies on human sexual development. II. Fetal and maternal serum gonadotropin and sex steroid concentrations. J Clin Endocrinol Metab 38:612–617
385. **Tapanainen J, Kellokumpu-Lehtinen P, Pelliniemi L, Huhtaniemi I** 1981 Age-related changes in endogenous steroids of human fetal testis during early and midpregnancy. J Clin Endocrinol Metab 52:98–102
386. **Bidlingmainer F, Dorr H, Eisenmenger W, Kuhnle U, Knorr D** 1983 Testosterone and androstenedione concentrations in human testis and epididymis during first two years of life. J Clin Endocrinol Metab 57:311–315
387. **Forest M, Cathiard A** 1975 Pattern of plasma testosterone and Δ4-androstenedione in normal newborns: evidence for testicular activity at birth. J Clin Endocrinol Metab 41:977–984
388. **Frasier SD, Gafford F, Horton R** 1969 Plasma androgens in childhood and adolescence. J Clin Endocrinol Metab 29:1404–1408
389. **Winter J, Hughes I, Reyes F** 1976 Pituitary-gonadal relations in infancy. 2: Patterns of serum gonadal steroid concentrations in man from birth to two years of age. J Clin Endocrinol Metab 42:679–686
390. **Dupont E, Luu-The V, Labrie F, Pelletier G** 1991 Ontogeny of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) in human testis as studied by immunocytochemistry. J Androl 12: 161–164
391. **Majdic G, Saunders PT, Teerds KJ** 1998 Immunoexpression of the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase, C17,20 lyase and the receptor for luteinizing hormone (LH) in the fetal rat testis suggests that the onset of Leydig cell steroid production is independent of LH action. Biol Reprod 58:520–525
392. **Baker PJ, Sha JA, McBride MW, Peng L, Payne AH, O'Shaughnessy PJ** 1999 Expression of 3β-hydroxysteroid dehydrogenase type I and type VI isoforms in the mouse testis during development. Eur J Biochem 260:911–917
393. **Benton L, Shan LX, Hardy MP** 1995 Differentiation of adult Leydig cells. J Steroid Biochem Mol Biol 53:61–68
394. **Majdic G, Millar MR, Saunders PT** 1995 Immunolocalisation of androgen receptor to interstitial cells in fetal rat testes and to mesenchymal and epithelial cells of associated ducts. J Endocrinol 147:285–293
395. **Haider SG, Servos G** 1998 Ultracytochemistry of 3β-hydroxysteroid dehydrogenase in Leydig cell precursors and vascular endothelial cells of the postnatal rat testis. Anat Embryol (Berl) 198: 101–110
396. **Contreras H, Ronco AM** 1994 Leydig cell heterogeneity as judged by quantitative cytochemistry of 3β-hydroxysteroid dehydrogenase activity in individual rat Leydig cells. J Steroid Biochem Mol Biol 51:73–79
397. **Mack SO, Lorence MC, Andersson S, Mason JI** 1990 Expression of cytochrome P-450(17)α, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase, and steroid 5α-reductase in rat H540 Leydig tumor cells. Mol Cell Endocrinol 74:R11–R17
398. **Young M, McPhaul MJ** 1997 Definition of the elements required for the activity of the rat aromatase promoter in steroidogenic cell lines. J Steroid Biochem Mol Biol 61:341–348
399. **Liang JH, Sankai T, Yoshida T, Cho F, Yoshikawa Y** 1998 Localization of testosterone and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in cynomolgus monkey (*Macaca fascicularis*) testes. J Med Primatol 27:10–14
400. **Liang JH, Sankai T, Yoshida T, Cho F, Yoshikawa Y** 1999 Localization of immunoreactive testosterone and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in cynomolgus monkey (*Macaca fascicularis*) testes during postnatal development. J Med Primatol 28: 62–66
401. **O'Shaughnessy PJ, Baker P, Sohnius U, Haavisto AM, Charlton HM, Huhtaniemi I** 1998 Fetal development of Leydig cell activity in the mouse is independent of pituitary gonadotroph function. Endocrinology 139:1141–1146
402. **Keeney DS, Mason JI** 1992 Expression of testicular 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase: regulation by luteinizing hormone and forskolin in Leydig cells of adult rats. Endocrinology 130:2007–2015
403. **Saez JM** 1994 Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev 15:574–626
404. **Murono EP, Washburn AL** 1991 Δ5–3β-hydroxysteroid dehydrogenase-isomerase activity in two distinct density Leydig cells from immature rats. Differences in responsiveness to human chorionic gonadotropin or 8-bromoadenosine 3′,5′-monophosphate. Biochim Biophys Acta 1091:55–62
405. **Ruiz de Galarreta CM, Fanjul LF, Meidan R, Hsueh AJ** 1983 Regulation of 3β-hydroxysteroid dehydrogenase activity by human chorionic gonadotropin, androgens, and anti-androgens in cultured testicular cells. J Biol Chem 258:10988–10996
406. **Wiebe JP** 1978 Steroidogenesis in rat Leydig cells: effect of gonadotropins on the activity of 5-ane and 5-ene 3α- and 3β-hydroxysteroid dehydrogenases during sexual maturation. Endocrinology 102:775–784
407. **Tang PZ, Tsai-Morris CH, Dufau ML** 1998 Regulation of 3β-hydroxysteroid dehydrogenase in gonadotropin- induced steroidogenic desensitization of Leydig cells. Endocrinology 139:4496–4505
408. **Lefevre A, Rogier E, Astraudo C, Duquenne C, Finaz C** 1994 Regulation by retinoids of luteinizing hormone/chorionic gonadotropin receptor, cholesterol side-chain cleavage cytochrome P-450, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase and 17α-hydroxylase/C17–20 lyase cytochrome P-450 messenger ribonucleic acid levels in the K9 mouse Leydig cell line. Mol Cell Endocrinol 106:31–39
409. **Fanjul LF, Quintana J, Gonzalez J, Santana P, Estevez F, Ruiz de Galarreta CM** 1992 Testicular 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in the hypophysectomized rat: effect of treatment with 5α-dihydrotestosterone. J Endocrinol 133:237–243
410. **Heggland SJ, Signs SA, Stalvey JR** 1997 Testosterone decreases 3β-hydroxysteroid dehydrogenase-isomerase messenger ribonucleic acid in cultured mouse Leydig cells by a strain-specific mechanism. J Androl 18:646–655
411. **Payne AH, Sha LL** 1991 Multiple mechanisms for regulation of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase, 17α-hydroxylase/C17–20 lyase cytochrome P450, and cholesterol side-chain cleavage cytochrome P450 messenger ribonucleic acid levels in primary cultures of mouse Leydig cells. Endocrinology 129:1429–1435
412. **Clark AM, Chuzel F, Sanchez P, Saez JM** 1996 Regulation by gonadotropins of the messenger ribonucleic acid for P450 side-chain cleavage, P450(17)α-hydroxylase/C17,20-lyase, and 3β-hy-

droxysteroid dehydrogenase in cultured pig Leydig cells. Biol Reprod 55:347–354
413. **Agular BM, Vind C** 1995 Effects of dexamethasone on steroidogenesis in Leydig cells from rats of different ages. J Steroid Biochem Mol Biol 54:75–81
414. **Orr TE, Mann DR** 1990 Effects of restraint stress on plasma LH and testosterone concentrations, Leydig cell LH/hCG receptors, and in vitro testicular steroidogenesis in adult rats. Horm Behav 24:324–341
415. **Akinbami MA, Philip GH, Sridaran R, Mahesh VB, Mann DR** 1999 Expression of mRNA and proteins for testicular steroidogenic enzymes and brain and pituitary mRNA for glutamate receptors in rats exposed to immobilization stress. J Steroid Biochem Mol Biol 70:143–149
416. **Spratt DI, Longcope C, Cox PM, Bigos ST, Wilbur-Welling C** 1993 Differential changes in serum concentrations of androgens and estrogens (in relation with cortisol) in postmenopausal women with acute illness. J Clin Endocrinol Metab 76:1542–1547
417. **Maric D, Kostic T, Kovacevic R** 1996 Effects of acute and chronic immobilization stress on rat Leydig cell steroidogenesis. J Steroid Biochem Mol Biol 58:351–355
418. **Kostic T, Andric S, Kovacevic R, Maric D** 1997 The effect of opioid antagonists in local regulation of testicular response to acute stress in adult rats. Steroids 62:703–708
419. **Kostic TS, Andric SA, Maric D, Stojilkovic SS, Kovacevic R** 1999 Involvement of inducible nitric oxide synthase in stress-impaired testicular steroidogenesis. J Endocrinol 163:409–416
420. **Sordoillet C, Chauvin MA, de Peretti E, Morera AM, Benahmed M** 1991 Epidermal growth factor directly stimulates steroidogenesis in primary cultures of porcine Leydig cells: actions and sites of action. Endocrinology 128:2160–2168
421. **Mauduit C, Chauvin MA, de Peretti E, Morera AM, Benahmed M** 1991 Effect of activin A on dehydroepiandrosterone and testosterone secretion by primary immature porcine Leydig cells. Biol Reprod 45:101–109
422. **Kanzaki M, Morris PL** 1999 Growth hormone regulates steroidogenic acute regulatory protein expression and steroidogenesis in Leydig cell progenitors. Endocrinology 140:1681–1686
423. **Murono EP, Washburn AL, Goforth DP, Wu N** 1993 Effects of acidic fibroblast growth factor on 5-ene-3β-hydroxysteroid dehydrogenase-isomerase and 5α-reductase activities and [^{125}I]human chorionic gonadotrophin binding in cultured immature Leydig cells. J Steroid Biochem Mol Biol 45:477–483
424. **Murono EP, Washburn AL** 1990 Platelet derived growth factor inhibits 5α-reductase and Δ5–3β-hydroxysteroid dehydrogenase activities in cultured immature Leydig cells. Biochem Biophys Res Commun 169:1229–1234
425. **Tsai SC, Chen JJ, Chiao YC, Lu CC, Lin H, Yeh JY, Lo MJ, Kau MM, Wang SW, Wang PS** 1997 The role of cyclic AMP production, calcium channel activation and enzyme activities in the inhibition of testosterone secretion by amphetamine. Br J Pharmacol 122:949–955
426. **Stocco DM, Wells J, Clark BJ** 1993 The effects of hydrogen peroxide on steroidogenesis in mouse Leydig tumor cells. Endocrinology 133:2827–2832
427. **Ramnath HI, Peterson S, Michael AE, Stocco DM, Cooke BA** 1997 Modulation of steroidogenesis by chloride ions in MA-10 mouse tumor Leydig cells: roles of calcium, protein synthesis, and the steroidogenic acute regulatory protein. Endocrinology 138:2308–2314
428. **Cohen PE, Hardy MP, Pollard JW** 1997 Colony-stimulating factor-1 plays a major role in the development of reproductive function in male mice. Mol Endocrinol 11:1636–1650
429. **Xiong Y, Hales DB** 1997 Differential effects of tumor necrosis factor-α and interleukin-1 on 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase expression in mouse Leydig cells. Endocrine 7:295–301
430. **Labrie F, Simard J, Luu-The V, Pelletier G** 1994 Molecular genetics, structure-function relationships, and tissue-specific expression and regulation of the 3β-HSD gene family. In: Bartke A, ed. Function of somatic cells in the testis. Norwell, MA: Springer-Verlag; 126–150
431. **Riley SC, Dupont E, Walton JC, Luu-The V, Labrie F, Pelletier G, Challis JR** 1992 Immunohistochemical localization of 3β-hydroxy-5-ene-steroid dehydrogenase/Δ^5-Δ^4 isomerase in human placenta and fetal membranes throughout gestation. J Clin Endocrinol Metab 75:956–961
432. **Babischkin JS, Pepe GJ, Albrecht ED** 1996 Developmental expression of placental trophoblast P-450 cholesterol side-chain cleavage, adrenodoxin and Δ5–3β-hydroxysteroid dehydrogenase/isomerase messenger ribonucleic acids during baboon pregnancy. Placenta 17:595–602
433. **Arensburg J, Payne AH, Orly J** 1999 Expression of steroidogenic genes in maternal and extraembryonic cells during early pregnancy in mice. Endocrinology 140:5220–5232
434. **Beaudoin C, Bonenfant M, Tremblay Y** 1997 Regulation of cytochrome P450 cholesterol side-chain cleavage, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase type 1 and estradiol-17β-hydroxysteroid dehydrogenase mRNA levels by calcium in human choriocarcinoma JEG-3 cells. Mol Cell Endocrinol 133:63–71
435. **Nestler JE** 1989 Insulin and insulin-like growth factor-I stimulate the 3β-hydroxysteroid dehydrogenase activity of human placental cytotrophoblasts. Endocrinology 125:2127–2133
436. **Nestler JE** 1990 Insulin-like growth factor II is a potent inhibitor of the aromatase activity of human placental cytotrophoblasts. Endocrinology 127:2064–2070
437. **Morrish DW, Linetsky E, Bhardwaj D, Li H, Dakour J, Marsh RG, Paterson MC, Godbout R** 1996 Identification by subtractive hybridization of a spectrum of novel and unexpected genes associated with in vitro differentiation of human cytotrophoblast cells. Placenta 17:431–441
438. **Beaudoin C, Blomquist CH, Bonenfant M, Tremblay Y** 1997 Expression of the genes for 3β-hydroxysteroid dehydrogenase type 1 and cytochrome P450scc during syncytium formation by human placental cytotrophoblast cells in culture and the regulation by progesterone and estradiol. J Endocrinol 154:379–387
439. **Takikawa H, Stolz A, Sugiyama Y, Yoshida H, Yamanaka M, Kaplowitz N** 1990 Relationship between the newly identified bile acid binder and bile acid oxidoreductases in human liver. J Biol Chem 265:2132–2136
440. **Takikawa H, Fujiyoshi M, Nishikawa K, Yamanaka M** 1992 Purification of 3α-hydroxysteroid and 3β-hydroxysteroid dehydrogenases from human liver cytosol. Hepatology 16:365–371
441. **Pirog EC, Collins DC** 1999 Metabolism of dihydrotestosterone in human liver: importance of 3α- and 3β-hydroxysteroid dehydrogenase. J Clin Endocrinol Metab 84:3217–3221
442. **de Launoit Y, Zhao HF, Belanger A, Labrie F, Simard J** 1992 Expression of liver-specific member of the 3β-hydroxysteroid dehydrogenase family, an isoform possessing an almost exclusive 3-ketosteroid reductase activity. J Biol Chem 267:4513–4517
443. **Naville D, Keeney DS, Jenkin G, Murry BA, Head JR, Mason JI** 1991 Regulation of expression of male-specific rat liver microsomal 3β-hydroxysteroid dehydrogenase. Mol Endocrinol 5:1090–1100
444. **Keeney DS, Murry BA, Bartke A, Wagner TE, Mason JI** 1993 Growth hormone transgenes regulate the expression of sex-specific isoforms of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in mouse liver and gonads. Endocrinology 133:1131–1138
445. **Bernstein L, Ross RK** 1993 Endogenous hormones and breast cancer risk. Epidemiol Rev 15:48–65
446. **Poulin R, Labrie F** 1986 Stimulation of cell proliferation and estrogenic response by adrenal C19-Δ5-steroids in the ZR-75-1 human breast cancer cell line. Cancer Res 46:4933–4937
447. **Poulin R, Baker D, Labrie F** 1988 Androgens inhibit basal and estrogen-induced cell proliferation in the ZR-75-1 human breast cancer cell line. Breast Cancer Res Treat 12:213–225
448. **Labrie F, Simard J, de Launoit Y, Poulin R, Theriault C, Dumont M, Dauvois S, Martel C, Li SM** 1992 Androgens and breast cancer. Cancer Detect Prev 16:31–38
449. **Adams JB** 1985 Control of secretion and the function of C19-Δ5-steroids of the human adrenal gland. Mol Cell Endocrinol 41:1–17
450. **Couillard S, Labrie C, Belanger A, Candas B, Pouliot F, Labrie F** 1998 Effect of dehydroepiandrosterone and the antiestrogen EM-800 on growth of human ZR-75-1 breast cancer xenografts. J Natl Cancer Inst 90:772–778
451. **Byrne GC, Perry YS, Winter JS** 1985 Kinetic analysis of adrenal

3β-hydroxysteroid dehydrogenase activity during human development. J Clin Endocrinol Metab 60:934–939
452. **Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, Norton L, Nickelsen T, Bjarnason NH, Morrow M, Lippman ME, Black D, Glusman JE, Costa A, Jordan VC** 1999 The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. JAMA 281:2189–2197
453. **Belanger A, Labrie F, Angeli A** 1990 Unconjugated and glucuronide steroid levels in human breast cyst fluid. Ann NY Acad Sci 586:93–100
454. **Killinger DW, Strutt BJ, Roncari DA, Khalil MW** 1995 Estrone formation from dehydroepiandrosterone in cultured human breast adipose stromal cells. J Steroid Biochem Mol Biol 52:195–201
455. **Gunasegaram R, Peh KL, Loganath A, Ratnam SS** 1998 Expression of 3β-hydroxysteroid dehydrogenase-5,4-ene isomerase activity by infiltrating ductal human breast carcinoma in vitro. Breast Cancer Res Treat 50:117–123
456. **Sasano H, Nagura H, Harada N, Goukon Y, Kimura M** 1994 Immunolocalization of aromatase and other steroidogenic enzymes in human breast disorders. Hum Pathol 25:530–535
457. **Theriault C, Labrie F** 1991 Multiple steroid metabolic pathways in ZR-75-1 human breast cancer cells. J Steroid Biochem Mol Biol 38:155–164
458. **Adams EF, Newton CJ, Tait GH, Braunsberg H, Reed MJ, James VH** 1988 Paracrine influence of human breast stromal fibroblasts on breast epithelial cells: secretion of a polypeptide which stimulates reductive 17β-oestradiol dehydrogenase activity. Int J Cancer 42: 119–122
459. **Dickson RB, Lippman ME** 1996 Autocrine and paracrine growth factors in the normal and neoplastic breast. In: Harris JR, Lippman ME, Morrow M, Hellman S, eds. Diseases of the breast. Philadelphia: Lippincott-Raven Publishers; 272–292
460. **Kelly PM, Davison RS, Bliss E, McGee JO** 1988 Macrophages in human breast disease: a quantitative immunohistochemical study. Br J Cancer 57:174–177
461. **Whitford P, Mallon EA, George WD, Campbell AM** 1990 Flow cytometric analysis of tumour infiltrating lymphocytes in breast cancer. Br J Cancer 62:971–975
462. **Tamm I, Cardinale I, Krueger J, Murphy JS, May LT, Sehgal PB** 1989 Interleukin 6 decreases cell-cell association and increases motility of ductal breast carcinoma cells. J Exp Med 170:1649–1669
463. **Obiri NI, Siegel JP, Varricchio F, Puri RK** 1994 Expression of high-affinity IL-4 receptors on human melanoma, ovarian and breast carcinoma cells. Clin Exp Immunol 95:148–155
464. **Toi M, Bicknell R, Harris AL** 1992 Inhibition of colon and breast carcinoma cell growth by interleukin-4. Cancer Res 52:275–279
465. **Serve H, Oelmann E, Herweg A, Oberberg D, Serve S, Reufi B, Mucke C, Minty A, Thiel E, Berdel WE** 1996 Inhibition of proliferation and clonal growth of human breast cancer cells by interleukin 13. Cancer Res 56:3583–3588
466. **Blais Y, Zhao C, Huber M, Audette M, Labrie F, Poulin R** 1996 Growth-independent induction of spermidine transport by IL-4 and IL-13 in ZR-75-1 human breast cancer cells. Int J Cancer 67: 532–538
467. **Adams EF, Rafferty B, White MC** 1991 Interleukin 6 is secreted by breast fibroblasts and stimulates 17β-oestradiol oxidoreductase activity of MCF-7 cells: possible paracrine regulation of breast 17β-oestradiol levels. Int J Cancer 49:118–121
468. **Purohit A, Wang DY, Ghilchik MW, Reed MJ** 1996 Regulation of aromatase and sulphatase in breast tumour cells. J Endocrinol 150 Suppl:S65–S71
469. **Pilichowska M, Kimura N, Fujiwara H, Nagura H** 1997 Immunohistochemical study of TGF-α, TGF-β1, EGFR, and IGF-1 expression in human breast carcinoma. Mod Pathol 10:969–975
470. **Zurawski G, de Vries JE** 1994 Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 15:19–26
471. **Zurawski SM, Vega Jr F, Huyghe B, Zurawski G** 1993 Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. EMBO J 12:2663–2670
472. **Murata T, Taguchi J, Puri RK** 1998 Interleukin-13 receptor α′ but not α chain: a functional component of interleukin-4 receptors. Blood 91:3884–3891
473. **Reed MJ, Purohit A** 1997 Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis. Endocr Rev 18:701–715
474. **Sasano H, Frost AR, Saitoh R, Harada N, Poutanen M, Vihko R, Bulun SE, Silverberg SG, Nagura H** 1996 Aromatase and 17β-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. J Clin Endocrinol Metab 81:4042–4046
475. **Luo S, Labrie C, Belanger A, Labrie F** 1997 Effect of dehydroepiandrosterone on bone mass, serum lipids, and dimethylbenz(a) anthracene-induced mammary carcinoma in the rat. Endocrinology 138:3387–3394
476. **Sourla A, Martel C, Labrie C, Labrie F** 1998 Almost exclusive androgenic action of dehydroepiandrosterone in the rat mammary gland. Endocrinology 139:753–764
477. **Seymour-Munn K, Adams J** 1983 Estrogenic effects of 5-androstene-3β, 17β-diol at physiological concentrations and its possible implication in the etiology of breast cancer. Endocrinology 112:486–491
478. **Rochefort H, Garcia M** 1983 The estrogenic and antiestrogenic activities of androgens in female target tissues. Pharmacol Ther 23:193–216
479. **Labrie F, Belanger A, Candas B, Cusan L, Gomez J, Labrie C, Luu-The V, Simard J** 2005 Gonadotropin-releasing hormone agonists in the treatment of prostate cancer. Endocr Rev 26:361–379
480. **Labrie F, Bélanger A, Cusan, L, Candas B** 1997 Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. J Clin Endocrinol Metab 82:2403–2409
481. **El-Alfy M, Luu-The V, Huang XF, Berger L, Labrie F, Pelletier G** 1999 Localization of type 5 17β-hydroxysteroid dehydrogenase, 3β-hydroxysteroid dehydrogenase, and androgen receptor in the human prostate by *in situ* hybridization and immunocytochemistry. Endocrinology 140:1481–1491
482. **Trapp T, Tunn S, Krieg M** 1992 Purification and properties of the 5α-dihydrotestosterone 3α(β)-hydroxysteroid dehydrogenase from human prostatic cytosol. J Steroid Biochem Mol Biol 42:321–327
483. **Amet Y, Simon B, Quemener E, Mangin P, Floch HH, Abalain JH** 1992 Partial purification of 3α- and 3β-hydroxysteroid dehydrogenases from human hyperplastic prostate. Comparison between the two enzymes. J Steroid Biochem Mol Biol 41:689–692
484. **Labrie F, Belanger A, Cusan L, Labrie C, Simard J** 1996 History of LHRH agonist and combination therapy in prostate cancer. Endocr Relat Cancer 3:243–278
485. **Wilding G** 1992 The importance of steroid hormones in prostate cancer. Cancer Surv 14:113–130
486. **Wang M, Fudge K, Rhim JS, Stearns ME** 1996 Cytokine regulation of the matrix metalloproteinases and their inhibitors in human papillomavirus-18 transformed human prostatic tumor cell lines. Oncol Res 8:303–315
487. **Simard J, Dumont D, Soucy P, Labrie F** 2002 Prostate cancer susceptibility genes. Endocrinology 143:2029–2040
488. **Ross RK, Coetzee GA, Pearce CL, Reichardt JK, Bretsky P, Kolonel LN, Henderson BE, Lander E, Altshuler D, Daley G** 1999 Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. Eur Urol 35:355–361
489. **Makridakis NM, Reichardt JK** 2001 Molecular epidemiology of hormone-metabolic loci in prostate cancer. Epidemiol Rev 23:24–29
490. **Singh R, Eeles RA, Durocher F, Simard J, Edwards S, Badzioch M, Kote-Jarai Z, Teare D, Ford D, Dearnaley D, Ardern-Jones A, Murkin A, Dowe A, Shearer R, Kelly J, Labrie F, Easton D, Narod SA, Tonin PN, Foulkes WD** 2000 High risk genes predisposing to prostate cancer. Do they exist? Prostate Cancer Prostat Dis 3:241–247
491. **Nwosu V, Carpten J, Trent JM, Sheridan R** 2001 Heterogeneity of genetic alterations in prostate cancer: evidence of the complex nature of the disease. Hum Mol Genet 10:2313–2318
492. **Chang Bl BL, Zheng SL, Hawkins GA, Isaacs SD, Wiley KE, Turner A, Carpten JD, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB, Xu J** 2002 Joint effect of HSD3B1 and HSD3B2 genes is associated with hereditary and sporadic prostate cancer susceptibility. Cancer Res 62:1784–1789
493. **Levy C, Marchut M, Baulieu EE, Robel P** 1974 Studies of the

3β-hydroxysteroid oxidoreductase activity in rat ventral prostate. Steroids 23:291–300
494. **Orlowski J, Clark AF** 1991 Epithelial-stromal interactions in the regulation of rat ventral prostate function: identification and characterization of pathways for androgen metabolism in isolated cell types. Endocrinology 128:872–884
495. **Geldof AA, Dijkstra I, Newling DW, Rao BR** 1995 Inhibition of 3β-hydroxysteroid-dehydrogenase: an approach for prostate cancer treatment? Anticancer Res 15:1349–1354
496. **Sharp F, Hay JB, Hodgins MB** 1976 Metabolism of androgens in vitro by human foetal skin. J Endocrinol 70:491–499
497. **Hay JB, Hodgins MB** 1978 Distribution of androgen metabolizing enzymes in isolated tissues of human forehead and axillary skin. J Endocrinol 79:29–39
498. **Sawaya ME, Honig LS, Garland LD, Hsia SL** 1988 Δ5–3β-hydroxysteroid dehydrogenase activity in sebaceous glands of scalp in male-pattern baldness [see comments]. J Invest Dermatol 91:101–105
499. **Sawaya ME, Penneys NS** 1992 Immunohistochemical distribution of aromatase and 3B-hydroxysteroid dehydrogenase in human hair follicle and sebaceous gland. J Cutan Pathol 19:309–314
500. **Gingras S, Turgeon C, Brochu N, Soucy P, Labrie F, Simard J** 2003 Characterization and modulation of sex steroid metabolizing activity in normal human keratinocytes in primary culture and HaCaT cells. J Steroid Biochem Mol Biol 87:167–179
501. **Thomas JP, Oake RJ** 1974 Androgen metabolism in the skin of hirsute women. J Clin Endocrinol Metab 38:19–22
502. **Pochi PE, Strauss JS** 1969 Sebaceous gland response in man to the administration of testosterone, Δ-4-androstenedione, and dehydroisoandrosterone. J Invest Dermatol 52:32–36
503. **Drucker WD, Blumberg JM, Gandy HM, David RR, Verde AL** 1972 Biologic activity of dehydroepiandrosterone sulfate in man. J Clin Endocrinol Metab 35:48–54
504. **Simpton NB, Cunliffe WJ, Hodgins MB** 1983 The relationship between the in vitro activity of 3β-hydroxysteroid dehydrogenase Δ4–5-isomerase in human sebaceous glands and their secretory activity in vivo. J Invest Dermatol 81:139–144
505. **Derocq JM, Segui M, Poinot-Chazel C, Minty A, Caput D, Ferrara P, Casellas P** 1994 Interleukin-13 stimulates interleukin-6 production by human keratinocytes. Similarity with interleukin-4. FEBS Lett 343:32–36
506. **Yang Y, Yoo HM, Choi I, Pyun KH, Byun SM, Ha H** 1996 Interleukin 4-induced proliferation in normal human keratinocytes is associated with c-myc gene expression and inhibited by genistein. J Invest Dermatol 107:367–372
507. **Junghans V, Jung T, Neumann C** 1996 Human keratinocytes constitutively express IL-4 receptor molecules and respond to IL-4 with an increase in B7/BB1 expression. Exp Dermatol 5:316–324
508. **Prens E, Hegmans J, Lien RC, Debets R, Troost R, van Joost T, Benner R** 1996 Increased expression of interleukin-4 receptors on psoriatic epidermal cells. Am J Pathol 148:1493–1502
509. **Asada H, Linton J, Katz SI** 1997 Cytokine gene expression during the elicitation phase of contact sensitivity: regulation by endogenous IL-4. J Invest Dermatol 108:406–411
510. **Couet J, Martel C, Labrie Y, Luo S, Simard J, Labrie F** 1994 Opposite effects of prolactin and corticosterone on the expression and activity of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in rat skin. J Invest Dermatol 103:60–64
511. **Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, Vaudry H** 1999 Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. Pharmacol Rev 51:63–81
512. **Baulieu EE, Schumacher M** 1997 Neurosteroids, with special reference to the effect of progesterone on myelination in peripheral nerves. Mult Scler 3:105–112
513. **Weidenfeld J, Siegel RA, Chowers I** 1980 In vitro conversion of pregnenolone to progesterone by discrete brain areas of the male rat. J Steroid Biochem 13:961–963
514. **Mensah-Nyagan AG, Feuilloley M, Dupont E, Do-Rego JL, Leboulenger F, Pelletier G, Vaudry H** 1994 Immunocytochemical localization and biological activity of 3β-hydroxysteroid dehydrogenase in the central nervous system of the frog. J Neurosci 14:7306–7318
515. **Furukawa A, Miyatake A, Ohnishi T, Ichikawa Y** 1998 Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: colocalization of StAR, cytochrome P-450SCC (CYP XIA1), and 3β-hydroxysteroid dehydrogenase in the rat brain. J Neurochem 71:2231–2238
516. **Kohchi C, Ukena K, Tsutsui K** 1998 Age- and region-specific expressions of the messenger RNAs encoding for steroidogenic enzymes p450scc, P450c17 and 3β-HSD in the postnatal rat brain. Brain Res 801:233–238
517. **Guennoun R, Fiddes RJ, Gouezou M, Lombes M, Baulieu EE** 1995 A key enzyme in the biosynthesis of neurosteroids, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD), is expressed in rat brain. Brain Res Mol Brain Res 30:287–300
518. **Dupont E, Simard J, Luu-The V, Labrie F, Pelletier G** 1994 Localization of 3β-hydroxysteroid dehydrogenase in rat brain as studied by in situ hybridization. Mol Cell Neurosci 5:119–123
519. **Ukena K, Honda Y, Inai Y, Kohchi C, Lea RW, Tsutsui K** 1999 Expression and activity of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in different regions of the avian brain. Brain Res 818:536–542
520. **Coirini H, Gouezou M, Delespierre B, Liere P, Pianos A, Eychenne B, Schumacher M, Guennoun R** 2003 Characterization and regulation of the 3β-hydroxysteroid dehydrogenase isomerase enzyme in the rat sciatic nerve. J Neurochem 84:119–126
521. **Coirini H, Gouezou M, Delespierre B, Schumacher M, Guennoun R** 2003 3β-Hydroxysteroid dehydrogenase isomerase (3β-HSD) activity in the rat sciatic nerve: kinetic analysis and regulation by steroids. J Steroid Biochem Mol Biol 85:89–94
522. **Hashimoto N, Yamanaka H, Mizushima T, Noguchi K** 2003 Increased expression of 3β-hydroxysteroid dehydrogenase mRNA in dorsal root ganglion neurons of adult rats following peripheral nerve injury. Neurosci Lett 340:45–48
523. **Guennoun R, Schumacher M, Robert F, Delespierre B, Gouezou M, Eychenne B, Akwa Y, Robel P, Baulieu EE** 1997 Neurosteroids: expression of functional 3β-hydroxysteroid dehydrogenase by rat sensory neurons and Schwann cells. Eur J Neurosci 9:2236–2247
524. **Sanne JL, Krueger KE** 1995 Expression of cytochrome P450 side-chain cleavage enzyme and 3β-hydroxysteroid dehydrogenase in the rat central nervous system: a study by polymerase chain reaction and in situ hybridization. J Neurochem 65:528–536
525. **Kabbadj K, el-Etr M, Baulieu EE, Robel P** 1993 Pregnenolone metabolism in rodent embryonic neurons and astrocytes. Glia 7:170–175
526. **Akwa Y, Sananes N, Gouezou M, Robel P, Baulieu EE, Le Goascogne C** 1993 Astrocytes and neurosteroids: metabolism of pregnenolone and dehydroepiandrosterone. Regulation by cell density. J Cell Biol 121:135–143
527. **Barnea A, Hajibeigi A, Trant JM, Mason JI** 1990 Expression of steroid metabolizing enzymes by aggregating fetal brain cells in culture: a model for developmental regulation of the progesterone 5α-reductase pathway. Endocrinology 127:500–502
528. **Chan JR, Phillips 2nd LJ, Glaser M** 1998 Glucocorticoids and progestins signal the initiation and enhance the rate of myelin formation. Proc Natl Acad Sci USA 95:10459–10464
529. **Zwain IH, Yen SS** 1999 Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. Endocrinology 140:3843–3852
530. **Do-Rego JL, Mensah-Nyagan AG, Feuilloley M, Ferrara P, Pelletier G, Vaudry H** 1998 The endozepine triakontatetraneuropeptide diazepam-binding inhibitor [17–50] stimulates neurosteroid biosynthesis in the frog hypothalamus. Neuroscience 83:555–570
531. **Andonian S, Hermo L** 1999 Principal cells of the vas deferens are involved in water transport and steroid synthesis in the adult rat. J Androl 20:158–176
532. **Kuwano Y, Fujikawa H, Watanabe A, Shimodaira K, Sekizawa A, Saito H, Yanaihara T** 1997 3β-Hydroxysteroid dehydrogenase activity in human osteoblast-like cells. Endocr J 44:847–853
533. **Saito H, Yanaihara T** 1998 Steroid formation in osteoblast-like cells. J Int Med Res 26:1–12
534. **Hatakeyama H, Miyamori I, Takeda Y, Yamamoto H, Mabuchi H** 1996 The expression of steroidogenic enzyme genes in human vascular cells. Biochem Mol Biol Int 40:639–645
535. **Oh SH, Oh JM, Kim JJ, Choi MK, Park ST, Park OK, Chung YT**

1998 Immunohistochemical study of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in the rat cardiovascular system. Arch Histol Cytol 61:297–303
536. **Nagata Y, Yoshikawa J, Hashimoto A, Yamamoto M, Payne AH, Todokoro K** 2003 Proplatelet formation of megakaryocytes is triggered by autocrine-synthesized estradiol. Genes Dev 17:2864–2869
537. **Bois E, Mornet E, Chompret A, Feingold J, Hochez J, Goulet V** 1985 Congenital adrenal hyperplasia (21-OH) in France. Population genetics. Arch Fr Pediatr 42:175–179
538. **Thilen A, Larsson A** 1990 Congenital adrenal hyperplasia in Sweden 1969–1986. Prevalence, symptoms and age at diagnosis. Acta Paediatr Scand 79:168–175
539. **Wilson R, New MI** 1998 Congenital adrenal hyperplasia. In: Jameson JL, ed. Principles of molecular medicine. Totawa, NJ: Humana Press; 481–493
540. **Forest MG** 1995 Diagnosis and treatment of disorders of sexual development. In: Degroot LJ, ed. Endocrinology. 3rd ed. Montreal: W. B. Saunders Co.; 1901–1937
541. **Donohoue P, Parker K, Migeon C** 1995 Congenital adrenal hyperplasia. In: Scriver C, Beaudet A, Sly W, Valle D, eds. 7th ed. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill, Inc.; 2929–2966
542. **Bongiovanni AM** 1961 Unusual steroid pattern in congenital adrenal hyperplasia: deficiency of 3β-hydroxysteroid dehydrogenase. J Clin Endocr 21:860–862
543. **Bongiovanni AM** 1962 The adrenogenital syndrome with deficiency of 3β-hydroxy dehydrogenase. J Clin Invest 41:2086–2092
544. **Morel Y, Mebarki F, Rheaume E, Sanchez R, Forest MG, Simard J** 1997 Structure-function relationships of 3β-hydroxysteroid dehydrogenase: contribution made by the molecular genetics of 3β-hydroxysteroid dehydrogenase deficiency. Steroids 62:176–184
545. **Russell AJ, Wallace AM, Forest MG, Donaldson MD, Edwards CR, Sutcliffe RG** 1994 Mutation in the human gene for 3β-hydroxysteroid dehydrogenase type II leading to male pseudohermaphroditism without salt loss. J Mol Endocrinol 12:225–237
546. **Pang S** 1998 The molecular and clinical spectrum of 3β-hydroxysteroid dehydrogenase deficiency disorder. Trends Endocrinol Metab 9:82–86
547. **Quigley CA** 1998 Disorders of sex determination and differentiation. In: Jameson JL, ed. Principles of molecular medicine. Totawa, NJ: Humana Press; 527–559
548. **Simard J, Moisan AM, Calemard Michel L, Morel Y** 2002 Molecular genetics of 17β-hydroxysteroid dehydrogenase and 5α-reductase deficiencies. In: Mason I, ed. Genetics of steroid biosynthesis and function, modern genetics. London and New York: Taylor and Francis; 297–338
549. **Parks GA, Bermudez JA, Anast CS, Bongiovanni AM, New MI** 1971 Pubertal boy with the 3β-hydroxysteroid dehydrogenase defect. J Clin Endocrinol Metab 33:269–278
550. **Zachmann M, Vollmin JA, Murset G, Curtius HC, Prader A** 1970 Unusual type of congenital adrenal hyperplasia probably due to deficiency of 3β-hydroxysteroid dehydrogenase. Case report of a surviving girl and steroid studies. J Clin Endocrinol Metab 30:719–726
551. **Janne O, Perheentupa J, Vihko R** 1970 Plasma and urinary steroids in an eight-year-old boy with 3β-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 31:162–165
552. **Kenny FM, Reynolds JW, Green OC** 1971 Partial 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency in a family with congenital adrenal hyperplasia: evidence for increasing 3β-HSD activity with age. Pediatrics 48:756–765
553. **de Peretti E, Forest MG, Feit JP, David M** 1980 Endocrine studies in two children with male pseudohermaphroditism due to 3β-hydroxysteroid dehydrogenase (3β-HSD) defect. In: Genazzani AR, Thijssen JHH, Siiteri PK, eds. Adrenal androgens. New York: Raven Press; 141–145
554. **Heinrich UE, Bettendorf M, Vecsei P** 1993 Male pseudohermaphroditism caused by nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency. J Steroid Biochem Mol Biol 45:83–85
555. **Gendrel D, Chaussain JL, Roger M, Job JC** 1979 [Congenital adrenal hyperplasia due to blockade of 3β-hydroxysteroid dehydrogenase]. Arch Fr Pediatr 36:647–655
556. **Nahoul K, Perrin C, Leymarie P, Job JC** 1989 [Elevated levels of plasma 4-ene steroids in a case of congenital deficiency of 3β-hydroxysteroid dehydrogenase]. Ann Endocrinol 50:58–63
557. **Rosenfield RL, Rich BH, Wolfsdorf JI, Cassorla F, Parks JS, Bongiovanni AM, Wu CH, Shackleton CH** 1980 Pubertal presentation of congenital Δ5–3β-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 51:345–353
558. **Pang S, Levine LS, Stoner E, Opitz JM, Pollack MS, Dupont B, New MI** 1983 Nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency with normal glomerulosa function. J Clin Endocrinol Metab 56:808–818
559. **Mendonca BB, Bloise W, Arnhold IJ, Batista MC, Toledo SP, Drummond MC, Nicolau W, Mattar E** 1987 Male pseudohermaphroditism due to nonsalt-losing 3β-hydroxysteroid dehydrogenase deficiency: gender role change and absence of gynecomastia at puberty. J Steroid Biochem 28:669–675
560. **Cara JF, Moshang Jr T, Bongiovanni AM, Marx BS** 1985 Elevated 17-hydroxyprogesterone and testosterone in a newborn with 3β-hydroxysteroid dehydrogenase deficiency. N Engl J Med 313:618–621
561. **Alos N, Moisan AM, Ward L, Desrochers M, Legault L, Leboeuf G, Van Vliet G, Simard J** 2000 A novel A10E homozygous mutation in the HSD3B2 gene causing severe salt-wasting 3β-hydroxysteroid dehydrogenase deficiency in 46,XX and 46,XY French-Canadians: evaluation of gonadal function after puberty. J Clin Endocrinol Metab 85:1968–1974
562. **Yoshimoto M, Kawaguchi T, Mori R, Kinoshita E, Baba T, Tajima T, Fujieda K, Suzuki T, Sasano H** 1997 Pubertal changes in testicular 3β-hydroxysteroid dehydrogenase activity in a male with classical 3β-hydroxysteroid dehydrogenase deficiency showing spontaneous secondary sexual maturation. Horm Res 48:83–87
563. **de Peretti E, Forest MG** 1982 Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period. Horm Res 16:10–22
564. **Rosenfield RL, Barmach de Niepomniszsze A, Kenny FM, Genel M** 1974 The response to human chorionic gonadotropin (HCG) administration in boys with and without Δ5–3β-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 39:370–374
565. **Pang SY, Lerner AJ, Stoner E, Levine LS, Oberfield SE, Engel I, New MI** 1985 Late-onset adrenal steroid 3β-hydroxysteroid dehydrogenase deficiency. I. A cause of hirsutism in pubertal and postpubertal women. J Clin Endocrinol Metab 60:428–439
566. **Schram P, Zerah M, Mani P, Jewelewicz R, Jaffe S, New MI** 1992 Nonclassical 3β-hydroxysteroid dehydrogenase deficiency: a review of our experience with 25 female patients. Fertil Steril 58:129–136
567. **Medina M, Herrera J, Flores M, Martin O, Bermudez JA, Zarate A** 1986 Normal ovarian function in a mild form of late-onset 3β-hydroxysteroid dehydrogenase deficiency. Fertil Steril 46:1021–1025
568. **Eldar-Geva T, Hurwitz A, Vecsei P, Palti Z, Milwidsky A, Rosler A** 1990 Secondary biosynthetic defects in women with late-onset congenital adrenal hyperplasia [see comments]. N Engl J Med 323:855–863
569. **Zerah M, Rheaume E, Mani P, Schram P, Simard J, Labrie F, New MI** 1994 No evidence of mutations in the genes for type I and type II 3β-hydroxysteroid dehydrogenase (3β-HSD) in nonclassical 3β-HSD deficiency. J Clin Endocrinol Metab 79:1811–1817
570. **Chang YT, Zhang L, Alkaddour HS, Mason JI, Lin K, Yang X, Garibaldi LR, Bourdony CJ, Dolan LM, Donaldson DL** 1995 Absence of molecular defect in the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene in premature pubarche children and hirsute female patients with moderately decreased adrenal 3β-HSD activity. Pediatr Res 37:820–824
571. **Sakkal-Alkaddour H, Zhang L, Yang X, Chang YT, Kappy M, Slover RS, Jorgensen V, Pang S** 1996 Studies of 3β-hydroxysteroid dehydrogenase genes in infants and children manifesting premature pubarche and increased adrenocorticotropin-stimulated Δ5-steroid levels. J Clin Endocrinol Metab 81:3961–3965
572. **Forest MG, Mebatki F, David A, Bureau L, Morel Y** 1995 Diagnosis of partial 3β-hydroxysteroid dehydrogenase (3β-HSD) questioned: lessons from long-term study and molecular biology. Horm Res 44:55
573. **Sutcliffe RG, Russell AJ, Edwards CR, Wallace AM** 1996 Human 3β-hydroxysteroid dehydrogenase: genes and phenotypes. J Mol Endocrinol 17:1–5

574. **Rheaume E, Sanchez R, Simard J, Chang YT, Wang J, Pang S, Labrie F** 1994 Molecular basis of congenital adrenal hyperplasia in two siblings with classical nonsalt-losing 3β-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 79:1012–1018
575. **Mebarki F, Sanchez R, Rheaume E, Laflamme N, Simard J, Forest MG, Bey-Omar F, David M, Labrie F, Morel Y** 1995 Nonsalt-losing male pseudohermaphroditism due to the novel homozygous N100S mutation in the type II 3β-hydroxysteroid dehydrogenase gene. J Clin Endocrinol Metab 80:2127–2134
576. **Simard J, Ricketts ML, Moisan AM, Morel Y** 2002 3β-Hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase deficiency. In: Mason JI, ed. Genetics of steroid biosynthesis and function. London and New York: Taylor and Francis; 209–257
577. **Mendonca BB, Russell AJ, Vasconcelos-Leite M, Arnhold IJ, Bloise W, Wajchenberg BL, Nicolau W, Sutcliffe RG, Wallace AM** 1994 Mutation in 3β-hydroxysteroid dehydrogenase type II associated with pseudohermaphroditism in males and premature pubarche or cryptic expression in females. J Mol Endocrinol 12:119–122
578. **Zachmann M, Forest MG, De Peretti E** 1979 3β-Hydroxysteroid dehydrogenase deficiency. Follow-up study in a girl with pubertal bone age. Horm Res 11:292–302
579. **Marui S, Castro M, Latronico AC, Elias LL, Arhnold IJP, Moreira AC, Mendoca BB** 2000 Mutations in type II 3β-hydroxysteroid dehydrogenase (HSD3B2) gene can cause premature pubarche in girls. Clin Endocrinol (Oxf) 52:67–75
580. **Zhang L, Mason JI, Naiki Y, Copeland KC, Castro-Magana M, Gordon-Walker TT, Chang YT, Pang S** 2000 Characterization of two novel homozygous missense mutations involving codon 6 and 259 of type II 3β-hydroxysteroid dehydrogenase (3βHSD) gene causing, respectively, nonsalt-wasting and salt-wasting 3βHSD deficiency disorder. J Clin Endocrinol Metab 85:1678–1685
581. **Baker ME, Blasco R** 1992 Expansion of the mammalian 3β-hydroxysteroid dehydrogenase/plant dihydroflavonol reductase superfamily to include a bacterial cholesterol dehydrogenase, a bacterial UDP-galactose-4-epimerase, and open reading frames in vaccinia virus and fish lymphocystis disease virus. FEBS Lett 301:89–93
582. **Rheaume E, Sanchez R, Mebarki F, Gagnon E, Carel JC, Chaussain JL, Morel Y, Labrie F, Simard J** 1995 Identification and characterization of the G15D mutation found in a male patient with 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency: alteration of the putative NAD-binding domain of type II 3β-HSD. Biochemistry 34:2893–2900
583. **Lesk AM** 1995 NAD-binding domains of dehydrogenases. Curr Opin Struct Biol 5:775–783
584. **Krozowski Z** 1992 11β-Hydroxysteroid dehydrogenase and the short-chain alcohol dehydrogenase (SCAD) superfamily. Mol Cell Endocrinol 84:C25–C31
585. **Nayak S, Lee PA, Witchel SF** 1998 Variants of the type II 3β-hydroxysteroid dehydrogenase gene in children with premature pubic hair and hyperandrogenic adolescents. Mol Genet Metab 64:184–192
586. **Chang Y, Wang J, Pang S**, Molecular basis of the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene in patients with mild nonclassic (late-onset) 3β-HSD deficiency congenital adrenal hyperplasia (CAH). Program of the 75th Annual Meeting of The Endocrine Society, Las Vegas, Nevada, 1993, p 396 (Abstract 1384B)
587. **Mebarki F** 1995 Etude moléculaire de causes de pseudohermaphrodisme masculin le syndrome d'insensibilité aux androgenes, le deficit en enzyme de la stériodogénese 3β-HSD. Lyon, France: Université Claude-Bernard-Lyon 1
588. **Tajima T, Nishi Y, Takase A, Nakae J, Murashita M, Fujieda K** 1997 No genetic mutation in type II 3β-hydroxysteroid dehydrogenase gene in patients with biochemical evidence of enzyme deficiency. Horm Res 47:49–53
589. **Moran C, Knochenhauer ES, Azziz R** 1998 Non-classic adrenal hyperplasia in hyperandrogenism: a reappraisal. J Endocrinol Invest 21:707–720
590. **Swofford DL, Olsen GJ** 1990 Phylogeny reconstruction. In: Hillis DM, Moritz C, eds. Molecular systematics. Sunderland, MA: Sinauer Associates, Inc.; 411–501
591. **Cheng JB, Jacquemin E, Gerhardt M, Nazer H, Cresteil D, Heubi JE, Setchell KDR, Russell DW** 2003 Molecular genetics of 3β-hydroxy-Δ5-C27-steroid oxidoreductase deficiency in 16 patients with loss of bile acid synthesis and liver disease. J Clin Endocrinol Metab 88:1833–1841
592. **Lalli E, Melner MH, Stocco DM, Sassone-Corsi P** 1998 DAX-1 blocks steroid production at multiple levels. Endocrinology 139: 4237–4243
593. **Abbaszade L, Arensburg Y, Clarke T, Kasa-Vubu J, Orly J, Payne A** 1995 Isolation of mouse 3β-hydroxysteroid dehydrogenase isoform that may be critical for maintenance of early pregnancy. Program of the 77th Annual Meeting of The Endocrine Society, Washington, DC, 1995, p 167 (Abstract P163-601)
594. **Marui S, Torrealba IM, Russell AJ, Latronico AC, Sutcliffe RG, Mendonca BB** 1998 A novel homozygous nonsense mutations E135* in the type II 3β-hydroxysteroid dehydrogenase gene in a girl with salt-losing congenital adrenal hyperplasia. Mutations in brief no. 168. Online. Hum Mutat 12:139
595. **Zachmann M, Kempken B, Anner I, Pezzoli V** 1988 Age-related differences in the excretion of Δ5 steroids (D5S) in two related girls with 3β-hydroxysteroid dehydrogenase deficiency. Pediatr Res 24:543
596. **Katsumata N, Tanae A, Yasunaga T, Horikawa R, Tanaka T, Hibi I** 1995 A novel missense mutation in the type II 3β-hydroxysteroid dehydrogenase gene in a family with classical salt-wasting congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency. Hum Mol Genet 4:745–746
597. **Pang S, Wang W, Rich B, David R, Chang YT, Carbunaru G, Myers SE, Howie AF, Smillie KJ, Mason JI** 2002 A novel nonstop mutation in the stop codon and a novel missense mutation in the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene causing, respectively, nonclassic and classic 3β-HSD deficiency congenital adrenal hyperplasia. J Clin Endocrinol Metab 87:2556–2563
598. **Tajima T, Fujieda K, Nakae J, Shinohara N, Yoshimoto M, Baba T, Kinoshita E, Igarashi Y, Oomura T** 1995 Molecular analysis of type II 3β-hydroxysteroid dehydrogenase gene in Japanese patients with classical 3β-hydroxysteroid dehydrogenase deficiency. Hum Mol Genet 4:969–971
599. **Yoshimoto M, Baba T, Yokoo T, Matsumoto T, Hayashi S, Yasui M, Fukuda S, Yanagi T** 1988 Case of salt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency: The first Japanese patient. Acta Paediatr Jpn 92:1964–1970
600. **Paula FJ, Dick-de-Paula I, Pontes A, Schmitt FC, Mendonca BB, Foss MC** 1994 Hyperandrogenism due to 3β-hydroxysteroid dehydrogenase deficiency with accessory adrenocortical tissue: a hormonal and metabolic evaluation. Braz J Med Biol Res 27:1149–1158
601. **Simard J, Rheaume E, Leblanc JF, Wallis SC, Joplin GF, Gilbey S, Allanson J, Mettler G, Bettendorf M, Heinrich U** 1994 Congenital adrenal hyperplasia caused by a novel homozygous frameshift mutation 273ΔAA in type II 3β-hydroxysteroid dehydrogenase gene (HSD3B2) in three male patients of Afghan/Pakistani origin. Hum Mol Genet 3:327–330
602. **McCartin S, Russell AJ, Fisher RA, Wallace AM, Arnhold IJ, Mason JI, Varley J, Mendonca BB, Sutcliffe RG** 2000 Phenotypic variability and origins of mutations in the gene encoding 3β-hydroxysteroid dehydrogenase type II. J Mol Endocrinol 24:75–82
603. **Fisher R, Eades S, Taylor N** 1988 Two brothers with congenital adrenal hyperplasia due to partial 3β-hydroxysteroid dehydrogenase deficiency. Ann Clin Biochem 25 (Suppl):133–135
604. **Lafont M** 1984 Le déficit en 3β-ol-déshydrogénase chez l'enfant. Lyon, France: Université Claude-Bernard-Lyon I
605. **Sanchez R, Mebarki F, Rheaume E, Laflamme N, Forest MG, Bey-Omard F, David M, Morel Y, Labrie F, Simard J** 1994 Functional characterization of the novel L108W and P186L mutations detected in the type II 3β-hydroxysteroid dehydrogenase gene of a male pseudohermaphrodite with congenital adrenal hyperplasia. Hum Mol Genet 3:1639–1645
606. **Reiter JC, Leveau P, Tardy V, Forest MG, Morel Y** 2000 Diagnostic criteria for the diagnosis of 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency: lessons from hormonal and molecular studies in 2 girls presenting with premature pubarche. Horm Res 53(Suppl 2):76 (Abstract P-257)
607. **Chang YT, Kulin HE, Garibaldi L, Suriano MJ, Bracki K, Pang S** 1993 Hypothalamic-pituitary-gonadal axis function in pubertal male and female siblings with glucocorticoid-treated nonsalt-wasting 3β-hydroxysteroid dehydrogenase deficiency congenital adrenal hyperplasia. J Clin Endocrinol Metab 77:1251–1257

608. **Park OK, Mayo KE** 1991 Transient expression of progesterone receptor messenger RNA in ovarian granulosa cells after the preovulatory luteinizing hormone surge. Mol Endocrinol 5:967–978
609. **Van Seters A, Degenhart H, Moolenaar AJ** 1989 Biochemical concealment of 3βHSD deficiency by long-term steroid substitution. J Steroid Biochem 36(Suppl):174
610. **Wolthers BG, de Vries IJ, Volmer M, Nagel GT** 1987 Detection of 3β-hydroxysteroid dehydrogenase deficiency in a newborn by means of urinary steroid analysis. Clin Chim Acta 169:109–116
611. **Zhang L, Sakkal-Alkaddour H, Chang YT, Yang X, Pang S** 1996 A new compound heterozygous frameshift mutation in the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene causes salt-wasting 3β-HSD deficiency congenital adrenal hyperplasia. J Clin Endocrinol Metab 81:291–295
612. **Babalola GO, Shapiro BH** 1992 Changes in the expression of cytochrome P450c17 associated with ovarian cystic follicles. An immunocytochemical and enzymatic analysis of porcine ovaries. J Steroid Biochem Mol Biol 42:581–587
613. **Barnes RB, Rosenfield RL, Burstein S, Ehrmann DA** 1989 Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome. N Engl J Med 320:559–565
614. **Barnes RB, Ehrmann DA, Brigell DF, Rosenfield RL** 1993 Ovarian steroidogenic responses to gonadotropin-releasing hormone agonist testing with nafarelin in hirsute women with adrenal responses to adrenocorticotropin suggestive of 3β-hydroxy-Δ5-steroid dehydrogenase deficiency. J Clin Endocrinol Metab 76:450–455
615. **Sanchez R, Rheaume E, Laflamme N, Rosenfield RL, Labrie F, Simard J** 1994 Detection and functional characterization of the novel missense mutation Y254D in type II 3β-hydroxysteroid dehydrogenase (3βHSD) gene of a female patient with nonsalt-losing 3βHSD deficiency. J Clin Endocrinol Metab 78:561–567
616. **von Teichman A, Joerg H, Werner P, Brenig B, Stranzinger G** 2001 cDNA cloning and physical mapping of porcine 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase. Anim Genet 32:298–302
617. **Mason JI, Howe BE, Howie AF, Morley SD, Nicol MR, Payne AH** 2004 Promiscuous 3β-hydroxysteroid dehydrogenases: testosterone 17β-hydroxysteroid dehydrogenase activities of mouse type I and VI 3β-hydroxysteroid dehydrogenases. Endocr Res 30:709–714
618. **Saner KJ, Suzuki T, Sasano H, Pizzey J, Ho C, Strauss 3rd JF, Carr BR, Rainey WE** 2005 Steroid sulfotransferase 2A1 gene transcription is regulated by steroidogenic factor 1 and GATA-6 in the human adrenal. Mol Endocrinol 19:184–197
619. **Martin LJ, Tremblay J** 2005 The human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase type 2 promoter is a novel target for the immediate early orphan nuclear receptor Nur77 in steroidogenic cells. Endocrinology 146:861–869
620. **Havelock JC, Auchus RJ, Rainey WE** 2004 The rise in adrenal androgen biosynthesis: adrenarche. Semin Reprod Med 22:337–347

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