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Adrenal Organogenesis and Steroidogenesis

Role of Nuclear Receptors Steroidogenic Factor-1, DAX-1, and Estrogen Receptor

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INTRODUCTION

Steroid hormone biosynthesis by the fetal adrenal gland is crucial to the integrity and continuation of pregnancy, growth of the fetal and maternal tissues, as well as perinatal homeostatic adaptation for extrauterine survival. The adrenal cortex in the primate undergoes a remarkable morphological and functional remodeling such that the fetal adrenal cortex transforms into an adult adrenal cortex capable of independent glucocorticoid and mineralocorticoid biosynthesis. This architectural and functional transition from a fetal to an adult adrenal cortex ensures self-sufficient existence of the neonate.

The fetal zone of the adrenal cortex, which is unique to the fetal adrenal, atrophies soon after birth (1–5). This predominant zone, which forms 80–90% of the fetal adrenal cortex synthesizes dehydroepiandrosterone sulfate (DHEA-S), the precursor hormone for estrogen biosynthesis by the placenta (6–9). During late gestation, placental estrogen promotes fetal adrenal cortisol biosynthesis, which supports the growth and maturation of various fetal tissues including the lung, thyroid, liver, and the gut (9,10). This placental estrogen is also instrumental in regulating fetal cortisol levels throughout pregnancy. In addition to feto-placental steroidogenesis, estrogen plays a critical role in the maintenance of pregnancy, regulation of maternal cardiovascular system, control of uteroplacental blood flow and neovascularization of the placenta, maintenance of uterine quiescence, and progesterone-mediated immunosuppression to allow implantation of the embryo in the uterus (9).

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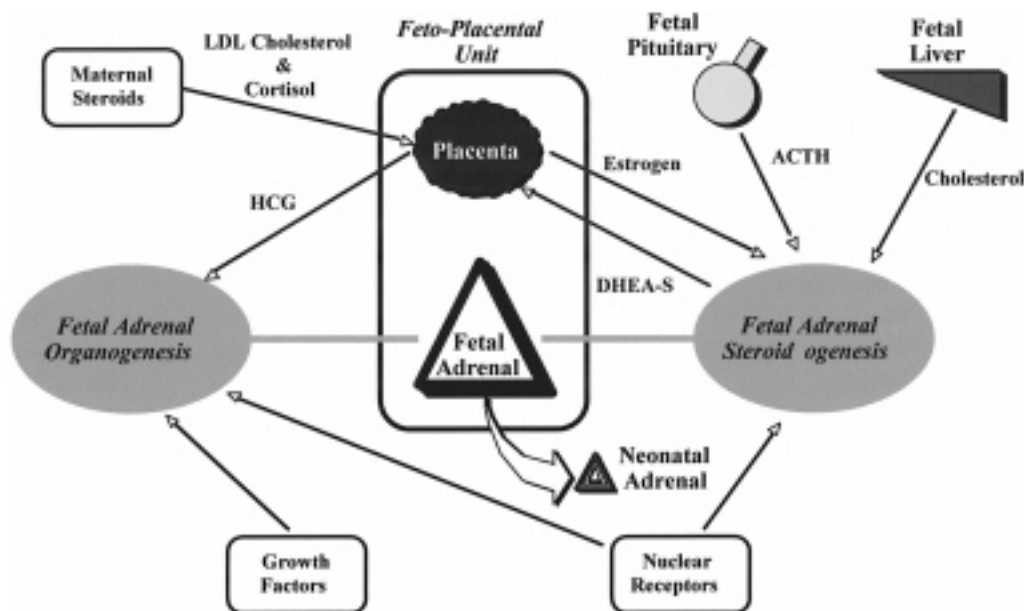


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The steroidogenesis and growth of the fetal adrenal cortex are regulated by endocrine, paracrine and autocrine factors (Fig. 1). These include placental chorionic gonadotropins (4,5,11–13) the fetal pituitary adrenocorticotrophic hormone (ACTH) (4,7,13–18), the local adrenal cortical growth factors (19) such as basic fibroblast growth factor (bFGF) (20–25), epidermal growth factor (EGF) (26), and its homolog—transforming growth factor α (TGF α) (19), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) (27–32), transforming growth factor β (TGF- β) family of peptides including activin, inhibin, and TGF- β 1 (33–40), as well as nuclear receptors—steroidogenic factor 1 (SF-1) (41–47), and dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X-chromosome gene 1 (*DAX-1*) (48,49), and estrogen receptor (ER), which mediates the actions of estrogen (50,51).

Steroidogenic factor 1 (SF-1) is a tissue- and cell-specific orphan nuclear receptor that is pivotal to the transcriptional regulation of several genes encoding steroidogenic enzymes (52–61). Targeted disruption of *SF-1* gene in the mouse has demonstrated that this gene is crucial to adrenal and gonadal development in this species (41,43,62). The newborn mice with the targeted disruption of the *SF-1* gene had adrenal insufficiency secondary to agenesis of the adrenal glands. These newborn mice were rescued following replacement therapy with glucocorticoids and mineralocorticoids. This postnatal picture is similar to the clinical picture of neonates and infants with non-X-linked congenital adrenal hypoplasia. Similar to the *SF-1*-gene-disrupted mice, infants with this disorder have aplastic adrenal glands. Recently, a patient was described with a heterozygous mutation located in the “P” box of the DNA-binding domain of the *hSF-1* gene, which resulted in congenital adrenal hypoplasia, adrenal insufficiency, and XY sex reversal

(63). Thus, similar to its role in the mouse, *SF-1* has a pivotal role in human adrenal and gonadal development.

DAX-1 is also an orphan nuclear receptor, which plays key roles in the development of adrenal gland and the gonads (48,49,64). Studies on patients with X-linked adrenal hypoplasia congenita (AHC) with hypogonadotropic hypogonadism have shown that these patients have mutations and deletions in the *DAX-1* gene (65–69). Targeted disruption of *DAX-1* gene in mice revealed that, similar to the adrenal glands in children with *DAX-1* gene mutations resulting in AHC, the adrenal glands of these mice had persistent fetal adrenal cortical zone. However, unlike the syndrome of X-linked AHC in humans, these mice did not require steroid replacement for survival as their adrenal glands had normal zona glomerulosa and fasciculata (70).

SF-1 and *DAX-1* are both expressed in the tissues of hypothalamic-pituitary-adrenal/gonadal axis (71). In addition, recent studies have demonstrated that these two receptors may interact to direct steroidogenesis by regulating the expression of crucial target genes, such as steroidogenic acute regulatory protein (StAR) (72).

Thus, the development of the fetal adrenal gland and its transition into an adult gland capable of supporting independent existence of the organism is a complex process that involves a multitude of factors. In this chapter, in addition to a brief review of the current literature on adrenal organogenesis and steroidogenesis, the roles of nuclear receptors SF-1, DAX-1, and ER in these processes are discussed. For recent excellent reviews on fetal adrenal and placental steroidogenesis, the reader is referred to the papers on these topics by Pepe and Albrecht (8,9) and Mesaino and Jaffe (7).

FETAL ADRENAL ORGANOGENESIS AND STEROIDOGENESIS

Fetal adrenal development and steroidogenesis are well-orchestrated and temporally regulated processes. The development of fetal adrenal relies on cellular hyperplasia, hypertrophy, migration, and apoptosis (7,13,16,38,73–78). Steroidogenesis by the fetal adrenal is dependent on maternal factors, the feto-placental unit, and most importantly, on the placental estrogen biosynthesis from precursor fetal DHEA-S (9,79). This placental estrogen sustains and maintains the pregnancy, modulates DHEA-S production by the fetal adrenal, and regulates the function of the fetal hypothalamic-pituitary-adrenal-axis (HPAA). Thus, the fetal adrenal organogenesis and steroidogenesis are closely interlinked and coordinated to ensure the maturation and function of the fetal adrenal cortex.

Fetal and Neonatal Organogenesis

To determine the developmental pattern of the human adrenal gland, Sucheston and Cannon (80) studied adrenal glands from 58 autopsy specimens ranging in age from 1 mo to 69 yr. Their study revealed that the adrenal gland in the human is derived from celomic epithelium at 3–4 wk of gestational age. Between 4–10 wk of gestation, these celomic epithelial cells proliferate, migrate, and differentiate into two distinct zones, the inner fetal zone, which forms 80–90% of the cortex and the outer definitive zone, which forms the rest of the fetal adrenal cortex. Most of the fetal adrenal growth and remodeling that starts around the tenth week of intrauterine life and continues until 1 yr of postnatal age. Between 28–30 wk of gestation, the zona glomerulosa, outer

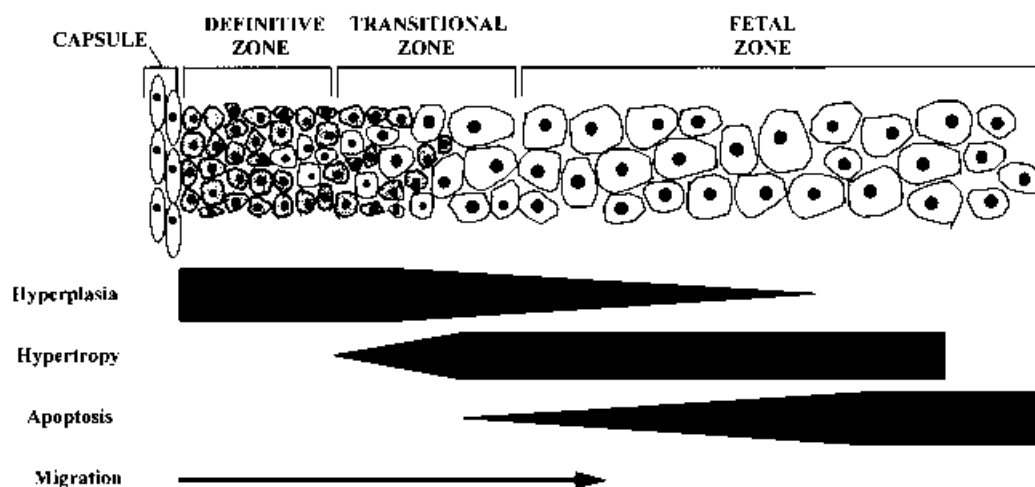


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zona fasciculata, fetal cortex, and medulla are delineated. Soon after birth, the fetal zone atrophies and disappears by 3 mo of postnatal life. During the second year of life, a poorly organized zona reticularis is discernable and attains its permanent characteristics by 11–12 yr of age. The fetal adrenal does not have an adrenal medulla as a distinct entity. The adrenal medulla appears in the first few postnatal weeks following the involution of the fetal zone. By the fourth week of postnatal life, the chromaffin cells cluster at the center of the gland, and it is not until 12–18 mo of age that the infant has the medulla with the adult-type architecture (81). The adrenal gland attains its adult architecture by 15 yr of age.

Johannisson studied 57 human fetal adrenal cortices at various gestational ages by both light and electron microscopy (73). Adrenal glands from three full-term anencephalics were included in these 57 cases. These studies show that in 1–1.5-cm fetus, corresponding to a gestational age of 5–6 wk, the adrenocortical cells are immature and show poor differentiation of the endoplasmic reticulum, the Golgi apparatus, and the mitochondria. Between 6–7 wk of gestation, these cells form two distinct zones—an outer zone and an inner zone. Although the cells of the outer zone remain immature, those of the inner zone show an increase in the cytoplasmic organelles, which indicates both a functional and structural differentiation and maturation. During the second trimester, a transitional zone located between the outer and inner zone appears, and it consists of two types of “dark” cells with differences in the agranular endoplasmic reticulum. In the second and third trimester of pregnancy, the definitive zone cells show maturation, which correlates with increasing functional activity of this zone. The cells of this transitional zone are capable of synthesizing cortisol and are the precursor cells of the zona fasciculata of the adult adrenal (13).

The cellular processes of, hyperplasia, cell-migration, hypertrophy, and apoptosis govern the growth and remodeling of the human adrenal gland (Fig. 2). By the eighth week of gestation, when the two zones of the fetal adrenal are discernible, cellular

mitosis and hyperplasia are limited to the definitive zone (73). This zone is thought to be the germinal/stem-cell compartment, which gives rise to inner cortical zones. Studies by Keene and Hewer, Crowder, and Jirasek show that cells from the definitive zone migrate in a centripetal fashion to invade the outer layers of the fetal zone (1,81,82). However, recent studies by Morley et al. in the mouse embryo using mouse 21-hydroxylase/ β -galactosidase transgene experiments show that in this species the centripetal migration of cells in the fetal adrenal is only established in the later stages of embryonic life or early postnatal life (78).

In contrast to the definitive zone, the fetal zone shows mostly hypertrophy and minimal mitosis, forms the bulk of the fetal adrenal gland, and accounts for 80% of its weight. Unlike other species, the fetal adrenal in the humans and other higher primates shows maximal rate of growth during mid- and late gestation. Most of this growth occurs in the inner fetal zone of the adrenal cortex. By 20 wk of gestation, the fetal adrenal weight is similar to that of the fetal kidney. By 30 wk, the gland rapidly enlarges and becomes 10–20 times the size of the adult adrenal gland. Between 30 wk and term, it doubles in size and weighs 3–4 grams at birth (1,82). In addition to cellular hyperplasia, centripetal migration, and hypertrophy, apoptosis also contributes to the remodeling of fetal adrenal into an adult organ. Both morphologic and DNA-based techniques show that apoptosis, which occurs mainly in the central part of the cortex, is more marked in the fetal zone as compared to the definitive zone (38,82). During the first postnatal week, the fetal zone undergoes rapid involution and disappears by the third month of postnatal life. As the fetal zone involutes by apoptosis, the fetal definitive and transitional zones form the zona glomerulosa and fasciculata, respectively (13). The innermost cells of the transitional zone form the zona reticularis of the adult adrenal cortex. This remodeling continues all through the first year of life.

The growth, development, and function of the fetal adrenal cortex is governed not only by the various cellular processes, aforementioned, but also by the feto-placental steroidogenesis regulated by endocrine factors, a multitude of paracrine growth factors, and autocrine nuclear receptors.

Fetal Adrenal Steroidogenesis

The complex coordination of fetal adrenal, placental, and maternal steroidogenesis is the hallmark of the feto-placental unit. The feto-placental unit ensures the survival and maturation of the fetus. Several studies have shown that baboon and human pregnancy share a great degree of similarity in the structure, function, and steroidogenesis of the feto-placental unit (8,9,83–86). Therefore, studies on regulation of placental and fetal steroidogenesis in primate pregnancy provide an excellent model to understand temporal events related to steroidogenesis of the human feto-placental unit. The discussion that follows is based on in vitro and in vivo studies on primate and human pregnancy.

Morphological features of steroidogenesis in the fetal adrenal cells is first observed at 6–8 wk of gestational age (7,74). The temporal relationship between ambiguous external genital development in female infants with congenital adrenal hyperplasia because of 21-hydroxylase deficiency and the development of the fetal adrenal cortex indicates that the feedback loop of the fetal HPAA is functional prior to the tenth week of gestational life. Between 8–10 wk of gestation, the fetal zone, which forms 80% of the fetal adrenal cortex, synthesizes DHEA-S, the main precursor of estrogen synthesis

by the placenta (79). This placental estrogen is crucial to the maintenance of pregnancy, the maturation of the fetal and maternal tissues, and immunosuppression leading to implementation of the placenta and the fetus (9).

Estrogen also plays a very significant role in placental and fetal steroidogenesis through stimulation of placental progesterone production, modulation of DHEA-S production by the fetal adrenal, and regulation of the HPPA, all of which ensure integrity of the pregnancy and neonatal self-sufficiency (87).

In addition to estrogen, fetal steroidogenesis is essential for the maintenance of pregnancy and maturation of fetal organs (9,10,19). In humans as in the other primates, there is substantial transplacental transfer of maternal cortisol to the fetus throughout gestation. The fetal cortisol level is dependent on the fetal cortisol production and metabolic clearance rate, the transplacental transfer of maternal cortisol, as well as the estrogen-mediated conversion of cortisol to cortisone by both the fetus and the placenta. Whereas the fetal zone is the principal site of DHEA-S synthesis, the outer definitive zone of the fetal adrenal mainly produces cortisol (16,88). The fetal cortisol level, which increases with gestational age, plays a significant role in the maturation of the fetal organs (8).

The uptake of maternal low-density lipoproteins by the syncytiotrophoblast provides the substrate necessary for placental steroidogenesis (89,90). The placental synthesis of pregnenolone and progesterone from this maternal cholesterol, is regulated by the estrogen derived from fetal DHEA-S. During early and midgestation, the fetus obtains its cortisol from maternal sources, either through transplacental transfer of maternal cortisol or through the conversion of placental progesterone to cortisol by the fetal adrenal. The fetal zone of the adrenal cortex produces the DHEA-S under the regulation of fetal ACTH (16,88,91). Carr and Simpson (92) have demonstrated that in the fetus, the liver produces significant amounts of cholesterol, which is provided as circulating LDL substrate to the adrenal for steroidogenesis. The fetal zone produces DHEA-S from this cholesterol. These investigators have proposed that the positive feedback system that includes the fetal liver, fetal adrenal, and the placenta is responsible for the exponential increase in steroidogenesis by the fetoplacental unit (90). The fetal DHEA-S forms the main substrate for estrogen biosynthesis through placental aromatization of fetal DHEA-S to estrogen (7,93). This fetal DHEA-S-based estrogen production by the placenta is a delicately balanced mechanism. Studies in baboon pregnancy show that estrogen exerts a negative feedback control on the fetal DHEA-S synthesis by suppressing the fetal adrenal responsiveness to ACTH (83,94,95).

Estrogen upregulates both low-density lipoprotein (LDL) receptor and *P450scc* enzyme expression in the placenta, thus increasing placental syncytiotrophoblast LDL uptake and *p450scc* activity (85,96). These estrogen-mediated actions are developmentally regulated in an autocrine and/or paracrine manner in the placenta. Thus, although in early and midgestational periods the fetal adrenal does not significantly contribute to direct cortisol synthesis, it does so indirectly by producing DHEA-S.

In contrast, during late gestation and prior to birth, the fetal adrenal is the major source of cortisol biosynthesis (88). The major substrate for cortisol synthesis by the fetal adrenal is LDLs derived from the fetal liver (97). In addition, placental progesterone also contributes to this cortisol biosynthesis by the fetal adrenal, although in late gestation the conversion of placental progesterone to cortisol is minimal (98). The estrogen-induced regulation of placental and fetal 11 β -hydroxysteroid dehydrogenase

(11 β HSD) increases with advancing age and leads to increased oxidation of cortisol to cortisone, thereby effectively decreasing fetal cortisol levels. This, in turn, regulates the fetal HPAA resulting in an increase in ACTH-stimulated cortisol synthesis by the fetal adrenal. In addition, estrogen through regulation of 11 β HSD and cortisol synthesis, plays an important role in the maturation of the hypothalamic-pituitary adrenal axis (9,99,100) (Fig. 3).

The differences in steroid biosynthesis by the fetal zone and definitive zone are based on the temporal and spatial expression of branch-point enzymes *P450 17 α -hydroxylase/17,20 lyase* (*P450c17*) and 3 β -hydroxysteroid dehydrogenase (3 β HSD) (7,16,101,102) (Fig. 4a, b). The differential expression of these enzymes in the fetal zone and the definitive zone determines whether the fetal adrenal gland converts pregnenolone to DHEA-S or cortisol. *In situ* hybridization, as well as immunocytochemistry studies, show that, all through gestation *P450scc* is expressed in all of the fetal adrenal cortex. Throughout gestation, the fetal zone does not show expression of 3 β HSD *in vivo*, but does show *p450c17* expression. Interestingly, the cells of this zone do show expression of this enzyme when they are exposed to supraphysiological doses of ACTH *in vitro*. In contrast, the definitive zone cells do not express *p450c17* enzyme, but between 22–24 wk of gestation show 3 β HSD expression. By 28 wk, this enzyme is expressed in the entire definitive zone and extends into the transitional zone. Thus, the fetal zone mainly produces DHEA-S and is unable to synthesize cortisol due to a lack or block of 3 β HSD. On the other hand, the cortisol synthesis, which mainly occurs during late gestation and near-term, is limited to the definitive zone. Thus, the temporal expression of steroid hydroxylase enzymes in the definitive, transitional, and fetal zones of the adrenal cortex is central to steroidogenesis by these zones (103,104).

MOLECULAR MECHANISMS

The twin processes of adrenal organogenesis and steroidogenesis are both governed by underlying molecular mechanisms that not only regulate the growth and function of the fetal adrenal, but also have a pivotal role in the remodeling of the fetal adrenal into a self-sufficient and life-sustaining adult adrenal cortex. Endocrine, paracrine, and autocrine factors are central to these molecular mechanisms. These factors include human chorionic gonadotropin (HCG) and ACTH, several growth factors including bFGF, EGF, TGF- α , IGF-1 and -2), and members belonging to the TGF- β family of proteins including activin, inhibin, and TGF- β 1, and the nuclear receptors *SF-1*, *DAX-1*, and *ER*.

HCG and ACTH

The growth of the fetal adrenal in the first trimester of pregnancy is regulated by HCG (4,5). Although evidence suggests that the HPAA axis is functional by about the tenth week of intrauterine life, HCG appears to play a significant role in adrenal growth during early gestation. *In vitro* studies by Seron-Ferre et al. on fetal adrenals between 12–17 wk gestational age show that HCG significantly increased DHEA-S secretion (11,16). Furthermore, studies in anencephalic fetuses have shown that the adrenal gland develops normally up to the fifth month of gestation (105), thus supporting the role of HCG in early fetal adrenal cortical development.

ACTH plays a pivotal role in the growth, differentiation, and steroidogenesis of the

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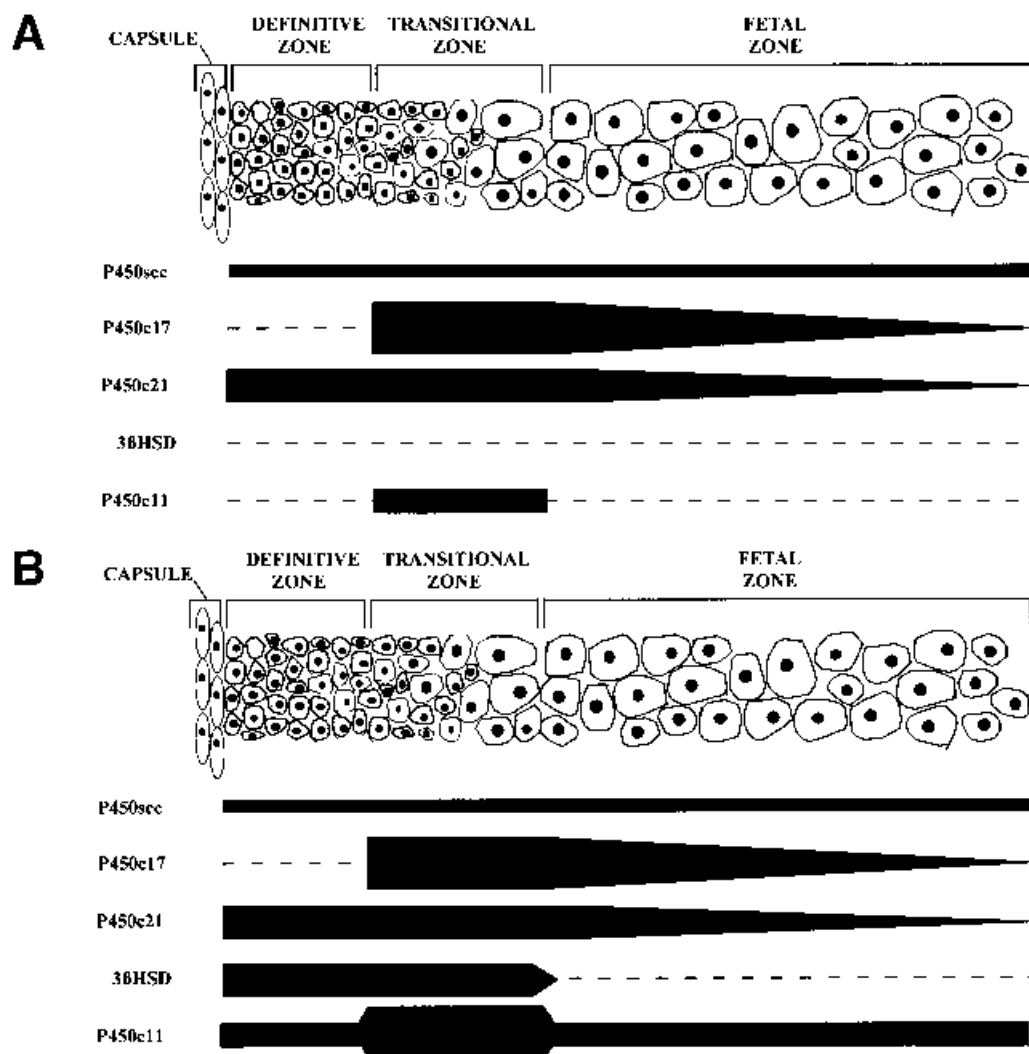


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fetal adrenal cells (11,13,15–18,88,106–111). After the twentieth week of gestational age, ACTH-mediated effects are of paramount importance in further growth of the fetal adrenal (88). Studies of anencephalic fetuses shows that after the first 15 wk of gestation in the absence of ACTH, the fetal zone of the adrenal gland fails to develop, which results in a marked decrease in maternal estrogen levels. The growth of anencephalic fetal adrenals can be partially restored by administration of ACTH to these fetuses (73). In contrast, syndromes of excessive ACTH production such as Cushing's disease caused by ACTH producing tumors of the pituitary and congenital adrenal hyperplasia caused by steroidogenic enzyme deficiencies lead to hyperplastic adrenal glands. Although in vitro studies show that ACTH is not a direct mitogen for adrenocortical cells grown in culture (110,112), it indirectly influences the growth of these cells in

vivo (113–115). These indirect effects of ACTH on fetal adrenal glands are governed by growth factors, which, in addition to mediating the effects of ACTH, also act directly on the fetal adrenal to modulate its growth.

Growth Factors

Several growth factors including, bFGF, EGF, IGF 1 and 2, TGF- α , and members belonging to the TGF- β family of proteins including activin, inhibin, and TGF- β 1 play a role in steroidogenesis, growth, and development of the adrenal gland (19). These peptide growth factors are produced both by the placenta and the fetus. In conjunction with ACTH, these growth factors play a role in the differential steroid biosynthesis by the two zones of the fetal adrenal.

Basic Fibroblast Growth Factor

bFGF belongs to a family of mitogenic proteins. Cultured fetal adrenal cells from midgestational human fetus showed that bFGF increases the proliferation of both the definitive and fetal zone cells (21,22), as well as adrenal cortex-derived capillary endothelial cells (23). However, its effect on cells of the definitive zone is twice that on the cells of the fetal zone (21). In addition to direct mitogenic effects on fetal adrenal cells, bFGF has indirect mitogenic effects, which are mediated through ACTH (25). ACTH not only increases the expression of bFGF leading to increased cell proliferation, but it also increases angiogenesis and vascularization of the fetal adrenal cortex (19).

EGF, TGF- α , and EGF Receptor

EGF and TGF- α are paracrine intracellular signaling molecules that belong to a larger family of mitogenic proteins (116–118). These two growth factors share sequence homology, activate the EGF receptor, and have similar biological functions.

Studies have shown that EGF is mitogenic to the midgestational cultured fetal adrenal cortical and definitive zone cells (21,22), but not to cultures of adult bovine adrenal cortical cells (22,119). Both EGF and TGF- α and other EGF receptor ligands mediate their actions through the EGF receptor. Whereas, the expression of EGF, TGF- α , and EGF receptor in human fetal adrenals was detected by RT-PCR, immunostaining only showed the expression of TGF- α and EGF receptor. These studies indicate that in the fetal adrenal instead of EGF, TGF- α may be the main peptide growth factor acting through the EGF receptor (19,120). In vivo studies in late gestational-rhesus monkeys show that treatment with EGF significantly increases the weight and width of the definitive zones, as well as the amount of 3 β HSD protein in both the definitive and transitional zones of the fetal adrenal (121). However, this increase in weight is caused by cellular hypertrophy and not hyperplasia. Luger et al. examined the effects of EGF on primate HPAA by giving mouse EGF to rhesus monkeys. They determined that mEGF increased the plasma levels of ACTH and cortisol in a dose-dependent manner. However, further studies showed that EGF stimulates hypothalamic CRH release, but does not cause pituitary ACTH release (26). Thus, in addition to its direct effects on the fetal adrenal cells, EGF may influence the growth and steroidogenic activity of these cells by increasing the 3 β HSD protein and regulating the HPAA.

Insulin-Like Growth Factors 1 and 2

The endocrine, paracrine, and autocrine roles of IGF-1 and IGF-2 in proliferation and differentiation of steroidogenic cells is well established (30,122). Growth hormone

regulates IGF-1 levels, which mediates many of the somatotropic effects of growth hormone (123). Northern blot and RT-PCR studies show that IGF-1 and 2, their receptors, as well as binding proteins, are all expressed in human fetal adrenals (27,32). However, *in situ* hybridization studies by Mesiano et al. have shown IGF-1 is expressed only in the adrenal capsule, whereas IGF-2 is expressed by all cortical cells (124). Recent studies on adult bovine adrenal cortical cells show that IGF-1 increases the effects of ACTH on these cells (28). Specifically, in the bovine species, IGF-1 enhances adrenal responsiveness to ACTH by increasing the ACTH receptors (125). Human studies have shown that in addition to increasing the adrenal responsiveness to ACTH, IGF-1 also increases the activity of 17α , 21 -, and 11β -hydroxylases, thereby enhancing steroidogenic activity of the adrenal (31).

Whereas IGF-1 has important roles in the postnatal steroidogenic tissues, IGF-2 has a central role in the growth and development of the fetal tissues. In most fetal tissues including the fetal adrenals where IGF-2 is detectable, its level of expression is higher than that of IGF-1 (27,32). ACTH and IGF-2 are closely interlinked in the growth and development of the adrenal cortex. *In situ* hybridization studies show that IGF-2 mRNA is highly expressed in the cortical cells. Studies using cultured fetal adrenal cells have shown that these cells retain their ability to express IGF-2 in response to ACTH (13,126). This effect of ACTH on IGF-2 is limited to the fetal cortex and is not seen in early postnatal period. Whereas ACTH increases IGF-2 expression, IGF-2 increases the responsiveness of the adrenal cortical cells to ACTH. Studies also show that in conjunction with estrogen, IGF-2 promotes ACTH-stimulated DHEA-S synthesis. In addition, it also regulates the steroidogenic enzymes *p450scc*, *p450c17*, and 3β HSD in the fetal adrenal. These key enzymes are central to both cortisol and androgen biosynthesis by the fetal adrenal (127). Thus, IGF-2 is a key growth factor in the fetal adrenal development and steroidogenesis.

Transforming Growth Factor β Family of Growth Factors: Activin, Inhibin, and TGF β 1

Activin and inhibin are glycoproteins that belong to the TGF β family of proteins (35). These proteins form homo- or heterodimers and are composed of α -, β A-, and β B-subunits. The α -subunit, which is a part of the inhibin molecule only, heterodimerizes with β A- and β B-subunits to form inhibin A and B, respectively. Whereas the subunits β A, β B homodimerize to form activin-A (β A β A) and activin-B (β B β B) they heterodimerize to form activin-AB (β A β B). Immunocytochemistry and *in situ* hybridization studies show that all the three subunits— α , β and β B, are expressed in the fetal and adult adrenal cortex (36,37). *In situ* hybridization studies on cultured fetal adrenal cortical cells show that ACTH upregulates the expression of the α , and β A subunit-mRNA whereas, the β B unit mRNA expression is not affected. These data suggest that ACTH stimulates the production of inhibin-A, as well as that of activin-A.

In addition to regulating the secretion of FSH by the pituitary, activin also has a paracrine role in the granulosa cells of the ovary and in the fetal adrenal cortical cells, which are both derived from the celomic epithelial cells. Although both of these tissues show expression of activin, the function of activin in the adrenal cortical and ovarian granulosa cells is not the same. Whereas, recombinant human activin-A promotes granulosa cell proliferation, it inhibits fetal zone cell proliferation (36,37). Activin also increases the ACTH-stimulated production of cortisol by the fetal zone cells, however,

it has no effect on DHEA-S production by these cells. In contrast, activin had no effect on growth or steroidogenesis in definitive or adult adrenal cortical cells. Interestingly, recombinant human inhibin had no effect on either the growth or function of these cells. A recent study by Spencer et al. also shows that activin promotes apoptosis in the inner-cortical compartment of the adrenal suggesting that it may be responsible for the involution of the fetal adrenal cortex during the postnatal period (38).

The paracrine/autocrine role of TGF- β 1 in the adrenal cortex is well-established. TGF- β 1 acts by binding to its specific receptor on the fetal adrenal cells and this binding is upregulated by ACTH (40). In the human fetal adrenal, TGF- β 1 appears to be a negative regulator of fetal and definitive zone cell proliferation and steroidogenesis (128). Studies in bovine and ovine adrenal cortical cells show that it decreases the expression of *p450scc* and 17 α -hydroxylase expression in both basal, as well as the ACTH-stimulated cells (33,34). Studies by Stankovic et al. show that this peptide factor inhibits basal, as well as ACTH-stimulated steroid biosynthesis by the fetal adrenal cells including DHEA-S and cortisol production in response to forskolin and dibutyryl cAMP (39). In addition, these authors also show that it interferes with the ACTH-stimulated expression of *p45017 α* mRNA in both the fetal and definitive zone cells. Interestingly, whereas TGF- β 1 has no effect on ACTH receptor or *p450scc* expression, it promotes the ACTH-stimulated expression of 3 β HSD (129). Thus, both activin and TGF- β 1 are negative paracrine regulators of growth and steroidogenesis in the fetal adrenal cortex.

Nuclear Receptors

SF-1, DAX-1, and ER are members of the nuclear hormone receptor superfamily of proteins that have a common modular architecture (130–133). These nuclear receptors have six functional domains. The A/B domain has a transactivation function and is highly variable in both sequence and length. The C domain is highly conserved and it is the DNA-binding domain (DBD), which is characterized by the presence of two zinc-fingers. The highly variable D domain may have nuclear localization signals and/or a transactivation function. The E domain is complex in function. In addition to ligand binding, it also has specific regions for dimerization, nuclear localization, transactivation, intermolecular silencing, and repression. Although the specific function of the F region remains to be established, research has shown that this region is highly variable. The members of this family are transcription factors, which in addition to maintaining biological function, govern the expression of genes that regulate cellular growth, differentiation, and apoptosis.

SF-1 and DAX-1 are crucial to adrenal organogenesis and steroidogenesis. These nuclear receptors are classified as orphan receptors as their ligands are unknown (134). DAX-1 is a unique member of the nuclear receptor family of proteins. Unlike other members of this family, the transactivating A/B domain, the DNA-binding C domain, and the hinge region or the D domain of the DAX-1 protein are replaced by 3.5 tandem repeats of a 65–67 amino acid motif (135,136). Although DAX-1 differs markedly in its N-terminal domain, it is also included in this family of proteins as it has a well-conserved ligand-binding domain, which is similar to that of other members of this superfamily. ER regulates gene transcription by binding to its ligand estrogen (137,138), although recent studies show that ER can also regulate gene transcription in a ligand-

independent manner through the transactivating function located in its A/B domain (139,140).

Steroidogenic Factor 1 (SF-1)

Steroidogenic factor 1 (SF-1) is the mouse homolog of *fushi tarazu* factor 1 (52,141), a cell-specific orphan nuclear receptor in the *Drosophila*, proposed to regulate the expression of *fushi tarazu* (*ftz-F1*) homeobox gene (142,143). The gene encoding SF-1 in the mouse was also named *ftz-F1*. *In situ* hybridization studies in the mouse embryos demonstrated that SF-1 was expressed in the gonads, the diencephalon, the urogenital sinus, and the developing adrenal cortex (54,60,144,145). In the adult animal, this gene is expressed in the steroidogenic cells of the adrenal glands and the gonads (45,146), in the gonadotropic cells of the pituitary (147,148), and in the ventromedial nucleus of the hypothalamus (VMH) (149,150). Targeted disruption of the *ftz-F1* gene in mice established its essential role in the organogenesis of these tissues (41–43,151). Thus, these mice had agenesis of their adrenal glands, gonads, and the VMH, resulting in complete congenital adrenal insufficiency and male-to-female sex reversal.

Homologs of *SF-1* gene have been identified in several species (52,152–154) including the human (155,156). It is significant to note that all SF-1 cDNAs identified and characterized to date from various species showed a very high degree of sequence conservation in their various functional domains (155,156). In several species, *ftz-F1* genes encode at two or more transcripts (154,157–159). It is noteworthy that in the mouse four distinct alternatively spliced products are derived from the *ftz-F1* gene (154). These four different transcripts, ELPs 1, 2, 3, and SF-1, are generated by alternative use of nested promoters and splice sites. Functional studies of these transcripts, using cotransfection experiments in NIH-3T3 cells, showed that although ELP 1 isoform, which lacks the AF2 domain, repressed the transcription of a reporter construct containing the SF-1/ELP response element, ELP Isoforms 2 and 3 activated transcription of this construct. Also, the ELP3 isoform in this species, which is expressed in the pituitary, is controlled by a promoter different than the one that controls the SF-1 isoform, which is expressed in the steroidogenic tissues. In the zebra fish (159), two isoforms of this receptor function differently. The nontruncated form of this receptor (zFF1A) not only stimulates the gonadotropin β -subunit promoter, but also synergizes with ER to further activate this promoter. The C-terminally truncated version (zFF1B), however, does not synergize with ER to regulate the gonadotropin β -subunit promoter, but it does function as repressor. These data suggest that the isoforms have differential species-specific tissue expression, regulation, and function.

To determine the role of SF-1 in humans, we recently isolated and characterized human steroidogenic factor 1 (hSF-1) by heterologous probing of a λ gt11 fetal adrenal cDNA library, using a mouse SF-1 cDNA probe that did not include the region coding for the zinc finger domain (155). The human cDNA sequence showed a high degree of homology (>95%) found in both the bovine and murine sequences. The zinc fingers, the FTZ-1 box and the AF2 domains showed 100% conservation of the derived amino acid residues with a lesser degree of homology in the ligand-binding/dimerization domains (Fig. 5).

Following the cloning and sequencing of hSF-1 cDNA, we defined the sites of hSF-1 mRNA expression in human tissues by both Northern blot and *in situ* hybridization

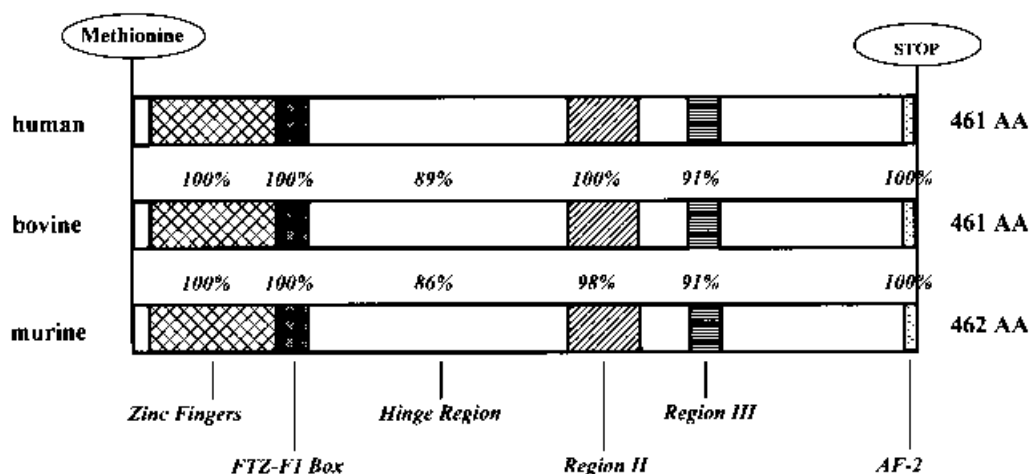


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analyses (160). These studies revealed high hSF-1 mRNA expression in the adrenal cortex, ovaries, testes, and the spleen. Northern blot analysis of these tissues revealed a main message of 3.5 kb. Interestingly, the spleen showed three additional transcripts of 2.4 kb, 4.4 kb, and 8.0 kb. Specifically, the additional 4.4 kb transcript was also seen in several peripheral tissues, the CNS and several components of the limbic system, as well as the myeloid and lymphoid cancer cell lines. The human gene encoding SF-1 is highly homologous to that of other species, and the pattern of hSF-1 expression in the adrenal glands and gonads is similar to that seen in the mouse and the cow. The expression of SF-1 in the steroidogenic tissues in the human parallels that of the mouse. Although in the human CNS, unlike in the mouse, the expression of hSF-1 mRNA is not limited to the hypothalamus, as is the case in the mouse. The species-specific widespread distribution of SF-1 in the human CNS and the strong expression of SF-1 in the reticuloendothelial network of the human spleen suggest that SF-1 may have a more comprehensive role in the human than in other species.

Northern blot analysis of the human placenta did not reveal hSF-1 message after a 16-h exposure, however, a weak signal was noted after 8 wk of exposure. SF-1 message expression in the bovine and human placenta was previously reported, using the highly sensitive RT-PCR technique (161). The apparently low expression of hSF-1 in human placenta suggests that it may not have a major role in placental steroidogenesis. This view is supported by previous studies that demonstrated expression of SF-1 mRNA in BeWo human choriocarcinoma cells only by the highly sensitive RT-PCR technique, nonexpression of the *StAR* gene in the placenta (162), expression of *P450* side-chain-cleavage enzyme in the placenta of SF-1-deficient mice (43), use of SFRE-deficient aromatase promoter 1.1 for placental aromatase gene transcription (163), and regulation of placental *p450scc* gene transcription by a 55-kDa protein that is expressed in the placenta, but not in the adrenal cortex (164). The data from these studies along with our own data suggest that alternative pathways of steroid metabolism or functional homologs of hSF-1 may be operational in human placental steroidogenesis.

In situ hybridization studies of normal architecture adrenal gland showed similar distribution of SF-1 mRNA signal in all the three zones of the adrenal cortex (160). However, within each cortical zone, the signal distribution was heterogeneous. Interestingly, in our *in situ* hybridization studies of a normal nodular variant of the adrenal gland, we detected a very high *SF-1* gene expression in the proliferative nodules. Sasano et al., using immunohistochemistry also demonstrated the heterogeneous distribution of the signal of Ad4BP, the bovine homolog of SF-1, within each of the three cortical zones of normal, neoplastic, and atrophied human adrenal glands (165). These data suggest that, in addition to regulation of steroidogenesis, SF-1 may also have a role in regulating the growth and proliferation of adult adrenal cortical cells.

In situ hybridization studies in the ovary demonstrated that hSF-1 mRNA was abundant in granulosa cells at all stages of follicular development, except for primordial follicles, and was also present in corpora lutea. Both the theca internal and theca external cells surrounding the graafian follicles also expressed hSF-1 mRNA. hSF-1 mRNA, however, was also seen in atretic follicles, which normally are not steroidogenic. A recent study showed that enhanced SF-1 expression is associated with GC differentiation, and that it inhibits TPA-induced mitosis of these cells (166). Given its role in GC cell differentiation and its increased expression in the adrenal nodules, as seen in our *in situ* hybridization studies, it is conceivable that SF-1 may also govern the fetal adrenal cell proliferation, differentiation, and apoptosis.

In the adult testis, hSF-1 expression was seen in both the interstitial cells and the inner border of the seminiferous tubules, suggesting expression in the steroidogenic Leydig cells and the germinal epithelium. Our results suggest that in the human testis, in addition to steroidogenesis, hSF-1 may also have a role in the function of spermatogenesis.

The data in other species and our human tissue expression studies of this gene suggest that SF-1 isoforms may be present in the human, and it is conceivable that these isoforms have differential tissue expression, function, and regulation. It is therefore also conceivable that mutations, deletions, or rearrangement of the genes encoding hSF-1 or putative hSF-1 isoforms may result in aberrant or truncated proteins with disrupted function leading to defective fetal adrenal organogenesis and steroidogenesis. Ultimately, this could manifest as the clinical syndrome of non-X-linked autosomal recessive congenital adrenal hypoplasia, or dysregulated steroidogenesis. Thus, SF-1 may be pivotal to the organogenesis of the fetal adrenal gland, including its remodeling into a self-sufficient neonatal organ.

Previous studies have revealed that SF-1 plays a pivotal role in the transcriptional regulation of several genes coding for steroidogenic enzymes (56,61,167–179). It is becoming increasingly clear that as a transcription factor SF-1 is crucial to adrenal steroidogenesis at more than one level. In addition to its role as a regulator of steroid hydroxylase enzyme expression, SF-1 also has a role in stimulating the promoter activities of genes encoding the ACTH receptor (180,181), steroidogenic acute regulatory (StAR) protein (182,183), and the scavenger receptor-type class BI (SR-BI) (184,185). Whereas the StAR protein is crucial to the translocation of cholesterol from the outer to the inner mitochondrial membrane for its conversion to pregnenolone, the SR-BI/CLA-1 protein mediates the selective transport of lipids from high-density lipoproteins (HDL) to steroidogenic cells and thus, provides these cells with the cholesterol substrate for steroidogenesis (184). Similar to its expression in the rodents, in the

human, SR-BI mRNA is highly expressed in the adrenal and the ovary. Interestingly, its expression in the fetal adrenal is estimated to be 50 times greater than its expression in the adult adrenal gland. SR-BI receptor is highly expressed in the fetal zone of the adrenal cortex, where DHEAS is synthesized. Fetal adrenal gland also expresses LDL and LDL receptor (LDLR). Although the relative role of SR-BI and LDLR in providing cholesterol to fetal adrenal cells for steroidogenesis is not known, studies show that in addition to HDL, SR-BI also binds to LDL (186,187). Thus, SR-BI may also have a role in providing LDL to the fetal adrenal for steroidogenesis. Clearly, not only is SF-1 critical for the constitutive activity of the human ACTH receptor-gene promoter, but it also regulates the genes that are important in providing the substrate, cholesterol, for adrenal and gonadal steroid biosynthesis. Furthermore, SF-1 is a global regulator of steroid hydroxylase enzymes. Collectively, these studies establish SF-1's role at multiple levels of the HPA axis and underscore the central role of SF-1 in fetal adrenal steroidogenesis.

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SF-1 also plays a critical role in the regulation of genes crucial to development, and maintenance of the reproductive function. SF-1 regulates the genes coding for aromatase (53,188–192), Mullerian inhibiting substance (193,194), oxytocin (195,196), prolactin receptor (197), the α -subunit of the glycoprotein hormones (198), the β -subunit of luteinizing hormone (199,202), gonadotropin-releasing hormone receptor (203). SF-1 may regulate the expression of DAX-1. Recent cotransfection studies in NCI-H295 cells using wild-type and deletional SF-1 mutant expression vectors show that a functional SF-1 response element is present in the DAX-1 promoter, which enhances the activity of this promoter (204). Also, SF-1 and chicken ovalbumin upstream promoter-transcription factor (COUP-TF) modulate the expression of DAX-1 (205). Whereas SF-1 stimulates murine DAX-1 promoter, COUP-TF inhibits its activity.

Recent studies suggest that similar to other members of the nuclear receptor superfamily SF-1 also interacts with cofactors such as SRC-1 and CBP/p300 to regulate gene transcription. Most nuclear receptors contain two transactivation domains, called AF1 and AF2 domains, located in the N-terminal and C-terminal regions, respectively (206). Unlike other members of this superfamily, our studies show that in the human, as in other species, SF-1 does not have an AF-1 domain in the N-terminal region (155). However, it has a unique region termed the Ftz-F1 box followed by a proline-rich region located just downstream from the DNA-binding domain. Whereas the Ftz-F1 box facilitates the binding of SF-1 to its response elements in the target DNA (207), the proline-rich region is proposed to contribute to transcriptional activation (208). More recently, Li and colleagues have shown that the Ftz-F1 box together with the proline-rich region termed the FP region, functions in nuclear localization and interaction with basic transcription factor TFIIB and *c-jun* (209). Our studies also show that in the human SF-1, the carboxy-terminal-AF2 domain, which forms an amphipathic α -helix, is conserved. The AF2 region, which is essential for protein–protein interaction between SF-1 and the cofactors (210, 209, 211), is also important in protein kinase-C potentiation of SF-1-regulated reporter gene activity (212). Jacob et al. show that expression of AF2 mutants of SF-1 in the presence of PKA-C drastically inhibited the transcriptional activation of endogenous SF-1 (212). Thus, the AF2 mutant has a dominant negative effect suggesting that the AF2 domain of SF-1 is essential for the activation of SF-1 by cAMP-dependent PKA mediated signaling pathway. Ito et al., using cotransfection experiments in JEG-3 cells, show that although SRC-1 and CBP/p300 act synergistically

to potentiate the transcription by SF-1, CBP/p300 in of itself is unable to enhance SF-1 mediated activity of the reporter gene (213). However, Monte et al. show that in NCI-H295 cells the SF-1-mediated activity of *p450scc* gene promoter, which has multiple SF-1 binding sites, is enhanced by CBP/p300. These experiments suggest that SRC-1 is also a cofactor for SF-1 and that the interactions between SF-1 and CBP/p300 are cell and promoter specific (214). The interaction of SF-1 with other proteins, as well as cofactors, may play a significant role in adrenal steroidogenesis. Liu and colleagues have shown that an interaction between SF-1 and Sp1 is necessary for the regulation of the cholesterol side chain cleavage enzyme (*p450scc*) gene expression. This may either be a direct protein–protein interaction between these two transcription factors, or it may be mediated through coactivator CBP/p300 (215). SF-1 and DAX-1 interactions may involve competition for shared coactivator proteins, such as SRC-1 and CBP/p300 (213,216). Thus, the interactions of SF-1 with cofactor proteins expands SF-1's repertoire of strategies for target gene regulation.

DAX-1

Adrenal hypoplasia congenita (AHC) was mapped to *Xp21* by studies in male patients with contiguous gene deletion syndrome (217–220). These patients have complex glycerol kinase deficiency, X-linked AHC, and/or Duchenne muscular dystrophy caused by gene deletions in the *Xp21* locus. Following the isolation of *DAX-1* gene, mutations in this gene were identified in patients with X-linked AHC and hypogonadotropic hypogonadism (48,221). The gene-encoding DAX-1 (*Ahch* gene) consists of two exons separated by an intron. DAX-1 is a unique member of the nuclear receptor family of proteins. Unlike other members of this family it differs markedly in its N-terminal region, which does not have the classical DNA-binding domain. In the DAX-1 protein, the transactivating A/B domain, the DNA-binding C domain and the hinge region or the D domain are replaced by a 3.5 tandem repeats of a 65–67 amino acid motif whereas the ligand-binding domain or the E domain is homologous to the E domain of other family members. Within this E domain are regions II and III that are thought to play important roles in ligand binding, dimerization, and transactivation. These regions are also well-conserved in the DAX-1 protein (48,135,136,222). In addition, similar to other members of this family such as ER, retinoic acid receptor (RAR), retinoic X receptor (RXR), thyroid receptor (TR), and SF-1 (155,223–226), the AF2 domain in the carboxy terminal region of DAX-1 is well-conserved (49). Recent studies in patients with X-linked AHC, have revealed truncations or mutations of *DAX-1* gene in this C-terminal region (216,227,228), thus establishing its critical role in adrenal organogenesis.

DAX-1 gene expression is tissue specific and this expression is developmentally regulated. It is highly expressed in the fetal and the adult adrenal gland, ovaries, testes, pituitary, and the hypothalamus (48,49,64,229,230). It is significant that SF-1 and DAX-1 show parallel tissue expression, which suggests that these two transcription factors may coregulate adrenal and gonadal organogenesis, as well as steroidogenesis (71).

The mitochondrial StAR protein, which plays a pivotal role in the translocation of cholesterol from the outer to the inner mitochondrial membrane was recently isolated and characterized from LH-induced mouse MA-10 Leydig tumor cells (231,232). In gonadal and adrenal cells, there is a direct correlation between the expression of *StAR*

gene and steroidogenic activity (231). Tropic hormones, ACTH and LH, regulate steroid biosynthesis by increasing the translocation of cholesterol from the outer to the inner mitochondrial membrane. Subsequently, the cholesterol side chain cleavage enzyme (*p450scc*), which resides in the inner mitochondrial membrane, catalyses the conversion of cholesterol to pregnenolone. Although the exact mechanism underlying this tropic hormone-stimulated acute steroidogenic response is not clear, recent studies have shown that the tropic hormone-stimulated expression of StAR mRNA and protein was within a time frame concomitant with acute steroid biosynthesis (231,233). Furthermore, recent studies on patients with lipoid congenital adrenal hyperplasia showed that these patients are unable to synthesize adrenal and gonadal steroids due to mutations in the StAR gene, thus confirming the critical role of this gene in steroid biosynthesis (234,235).

Recent studies have shown that DAX-1 blocks steroidogenesis not only by inhibiting the activity of StAR, but also by inhibiting the expression of *p450scc* and 3 β -HSD expression (236). Using transient cotransfection experiments, Zazapoulos et al. have recently demonstrated that despite the unique N-terminal region of DAX-1, which does not have any known DNA-binding motif, DAX-1 binds to the StAR promoter to act as a powerful repressor of both basal and cAMP-stimulated activity of the StAR promoter (72). These investigators also show that DAX-1 represses *StAR* gene expression by binding with equal efficiency to both hairpin structures and stems composed of 10–24 nucleotides in the StAR promoter. It is interesting to note that even though the presence of the loop structure in the StAR promoter is crucial to binding of the DAX-1 protein to the promoter, the sequence of the loop itself influences the binding efficiency of DAX-1. Loops rich in thymines or cytosines show increased binding as compared to loops rich in adenines. This study directly links DAX-1 to the StAR protein-mediated regulation of acute steroid biosynthesis in the adrenals and the gonads.

X-linked AHC, is a life-threatening disorder that mostly presents in infancy although it can also present later in childhood. It is characterized by adrenal insufficiency manifesting as glucocorticoid and mineralocorticoid deficiency in males and these infants show a blunted or absent adrenal steroid response to ACTH stimulation. Appropriate replacement therapy with glucocorticoids and mineralocorticoids ensures survival of these children. These children also develop hypogonadotropic hypogonadism (HH) at puberty. The permanent zone of the adrenal cortex is absent in AHC, and there is structural disorganization of the adrenal gland. The abnormally large fetal adrenal cells persist resulting in adrenal insufficiency (48,65,221). Studies in families with AHC show that DAX-1 mutations are responsible for both AHC and HH (66). Microdeletions, insertions, point mutations, microduplications, and base substitutions in the *DAX-1* gene resulting in frameshifts and truncated DAX-1 proteins have all been described in patients with isolated AHC and hypogonadotropic hypogonadism. In addition to inherited forms of this disorder, *de novo* mutations of the *DAX-1* gene have also been reported in this disorder (237). Mutations in the *DAX-1* gene have been found both in the unique N-terminal, as well as the C-terminal domains of the DAX-1 protein (48,49,67,68,238). Recent studies have shown that the silencing activity of the DAX-1 protein, which resides in the C-terminal region may be crucial to the pathogenesis of X-linked AHC (216,228). It is significant to note that in patients with AHC all the naturally occurring DAX-1 deletional mutants reported to date show deletions of this silencing domain of the DAX-1 protein. Interestingly, even among patients harboring

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the same DAX-1 mutation, there is a great degree of variability in expression of adrenal insufficiency including the age of onset and the severity of clinical symptoms. This variability in the clinical expression of the disease could be explained on the basis of the variability of adrenal organogenesis found in patients within the same kindred with the same DAX-1 mutation. Later in life, these children also develop hypogonadotropic hypogonadism (HHG). This HHG is either caused by a pituitary defect in gonadotropin production or caused by a hypothalamic defect in GnRH production or a combination of the two (66).

Targeted disruption of *DAX-1* gene in the mouse has revealed that the development of the fetal and adrenal cortical zones in the wild-type and the *DAX-1* gene deleted mouse are similar until sexual maturation (70). At puberty in the *DAX-1* gene-deleted adult mouse, the fetal adrenal zone fails to regress. This failure of the fetal zone to regress is similar to the persistence of fetal zone cells in adrenals of patients with DAX-1 mutations. However, unlike these patients, the *DAX-1* gene knockout mice have normal *zona glomerulosa* and *fasciculata* and do not require corticosteroid replacement for survival. Thus, the role of DAX-1 in mice and humans in adrenal organogenesis is not identical.

Estrogen Receptor (ER)

Estrogens have a key role in cell growth, differentiation, and function of diverse tissues, including bone, liver, the cardiovascular system, central nervous system, and the reproductive system. Estrogen actions are mediated through ERs, which are members of the steroid-receptor superfamily (137,239,240). In addition to the previously characterized ER, now called ER α , a homolog of ER α , called ER β , was recently characterized both in the rat and the human (241–243). These ERs are expressed in a tissue-specific manner. ER α is expressed in the female reproductive system as well as in the placenta (9). In adult humans, ER β expression was found in the testes, ovary, and pituitary gland (243–246). In the human fetus, semiquantitative RT-PCR revealed that ER β was highly expressed not only in the ovaries and testes, but also in the spleen and adrenal glands (247). In contrast, the expression of ER α was very low in the fetal spleen and absent in the fetal adrenal. Recent transfection studies show not only that these two subtypes of estrogen receptor signal differently, based on the ligand and the response element, but also that they may have different roles in gene regulation (248,249). These ERs, primarily nuclear proteins, bind to their recognition sites either as homodimers or as ER α and ER β heterodimers (137,138,250). Studies have shown that ER α can bind to its palindromic DNA recognition sequence both in the presence and in the absence of its ligand, estrogen (139,251). This ligand-independent ER α action is cell- and promoter-specific, and it is attributed to transactivation function 1 (TAF-1), located in the A/B region of the receptor (139,252–254). Transactivation function 2 (TAF-2) of this receptor is located in the carboxy terminal end of the E-domain and is ligand-dependent (252). ER can also modulate its target gene transcription by participating in protein–protein interaction with activator or repressor proteins (255–258).

As discussed earlier in this chapter under the section on steroidogenesis, estrogen is central to fetal steroidogenesis. Through this role, it also regulates fetal adrenal organogenesis and maturation. These actions of estrogen are mediated through its receptor ER isoforms that are expressed both in the placenta and the fetal adrenal.

Steroid Receptor Interactions in the Regulation of Target Genes

Recent studies have demonstrated that the mechanism of steroid-receptor interaction plays a significant role in the regulation of target gene expression. These nuclear hormone receptors use several mechanisms to elicit their actions (132,259–262). They mediate gene transcription by binding to their respective highly conserved and specific enhancer sequences called hormone response elements (HREs), located in the target genes (130,263). Although they primarily recognize specific HREs, these receptors can also bind competitively to other steroid receptor HREs that are similar to their own. Furthermore, they can also bind to overlapping response elements or they can regulate gene transcription through protein–protein interaction with other transcription factors or with a common cofactor (256,264–267). Finally, the generation of protein isoforms, either by alternative splicing of the pre-mRNA or by gene homologs, expands steroid receptors' repertoire of strategies to fine-tune gene expression (268–270).

Oxytocin and *c-fos* gene promoters, which are regulated by a number of steroid receptors, illustrate the mechanism of steroid-receptor interactions in target gene regulation. In rat and human oxytocin genes, a composite hormone response element confers responsiveness to ER, retinoic acid receptor (RAR), thyroid receptor (TR), and orphan nuclear receptors SF-1 and COUP-TF (196). Proto-oncogene *c-fos* functions as a master switch, directs cell proliferation, differentiation, and apoptosis; integrates cytoplasmic signal transduction pathways with gene transcription; and governs signal processing in neuronal cells (271,272). Whereas steroid receptor ER, retinoic acid receptor(s), and vitamin D receptors regulate the *c-fos* gene at a transcriptional level, other steroid receptors, such as the glucocorticoid receptor, interact with the protein products of these proto-oncogenes to regulate gene transcription (271). Similarly, the modulation of murine *DAX-1* gene promoter by SF-1 and COUP-TF is yet another example of coregulation of target genes by nuclear receptors (205). Synergistic interaction between SF-1 and ER in the regulation of the salmon $\text{II}\beta$ subunit gene has been recently reported (199). Also, recent studies with SF-1-expressing R2C (Leydig tumor) cells cotransfected with murine StAR promoter luciferase construct, showed increased reporter gene activity with the addition of estradiol (273). These reports point to SF-1 and ER interactions in the regulation of their target genes.

SF-1 and ER Interactions. As aforementioned, protein regulates the key first step in acute steroidogenic response to tropic hormone stimulation by translocating cholesterol from the outer to the inner mitochondrial membrane. Furthermore, mutations in the *StAR* gene lead to congenital lipoid adrenal hyperplasia. Recent studies on the transcriptional regulation of *StAR* gene have established hSF-1 as a key regulator of basal and cAMP-mediated *StAR* gene expression (183,274). The human *StAR* promoter has multiple hSF-1 response elements (SFREs), which are essential for cAMP-dependent activation of the *StAR* gene (161,182,183). Mutation of both the proximal and distal sites in the *StAR* promoter abolishes SF-1-directed *StAR* activity. In addition, electromobility shift assays reveal that whereas the distal site, which has a SF-1 consensus sequence, binds to SF-1 expressed in COS-1 cells, the mutated distal site does not do so. The proximal site, an ER consensus half-site, also binds to SF-1, but with lesser degree of affinity than the distal site. However, cotransfection studies reveal that the mutated proximal site abolishes SF-1-stimulated *StAR* promoter activity by 91%, whereas mutated distal site reduced this activity by only 80% (161).

Our transient cotransfection studies done in HeLa cells using the human SF-1 expres-

sion vector and human StAR promoter also show that hSF-1 is a regulator of the human StAR promoter through these SFREs. Data from these studies also show that in the absence of either hSF-1 or cAMP, human ER α (hER α) stimulates StAR promoter. These studies suggest that hSF-1 and ER not only independently stimulate the StAR promoter-activity, but also that hSF-1 has a downregulating effect on ER's ability to stimulate StAR promoter activity (46). Thus, hSF-1 and ER may coregulate StAR promoter activity, thereby regulating adrenal steroidogenesis and organogenesis. Furthermore, StAR may not be the only common target gene that hSF-1 and ER coregulate in fetal adrenal steroidogenesis.

Estrogen modulates fetal adrenal steroidogenesis in several ways. Whereas estradiol indirectly increases the fetal adrenal DHEAS production by enhancing the ACTH-stimulated production of this estrogen precursor (127), it directly inhibits the production of DHEAS by downregulating the steroidogenic enzyme *p450c17* (275). Interestingly, recent studies have shown that SF-1 is also a transcription factor for the gene encoding this enzyme (61,173,176,276,277). Also, both SF-1 (41,54) and ER β (247) are expressed in the fetal adrenal gland. Therefore, SF-1 and ER may potentially coregulate *p450c17* gene expression and modulate DHEA synthesis in the fetal adrenal. In addition, ER also upregulates the expression of *p450scc* (278), which is also a SF-1 target gene (167,169,171). Therefore, these two receptors may coregulate fetal adrenal steroid biosynthesis at multiple levels.

SF-1 and DAX-1 Interactions. Orphan nuclear receptors SF-1 and DAX-1 are coexpressed in the developing hypothalamus, pituitary, gonads, as well as the adrenal glands (71). *SF-1* and *DAX-1* gene knockout studies in the mouse and mutations of human SF-1 (63) and the DAX-1 (48,221) genes resulting in the syndrome of non-X-linked and X-linked AHC, respectively, suggest that these two transcription factors are closely connected to the development and function of steroidogenic organs. Recent studies suggest that SF-1 and DAX-1 act in consort to control steroidogenesis by regulating the *StAR* gene promoter (213). Although SF-1 stimulates the StAR promoter activity by binding to the multiple SFREs (183), the DAX-1 protein acts as a repressor of StAR gene activity by binding to hairpin structure and loops in the StAR promoter (72). Furthermore, recent studies using cotransfection experiments and deletion constructs of DAX-1, suggest that DAX-1 inhibits SF-1-mediated transactivation of target genes (216). In addition, these studies also suggest that the carboxy terminal domain of the DAX-1 is responsible for this inhibitory function. These studies also suggest that a protein-protein interaction between SF-1 and DAX-1 or competition for a common coactivator are possible mechanisms responsible for DAX-1 mediated inhibition of SF-1 responsive gene activity.

A large number of the DAX-1 truncated mutants have been identified in patients with X-linked AHC. What is most interesting is that in all these truncated mutants the putative carboxy terminal inhibitory domain is deleted (216,227). Furthermore, two naturally occurring mutations of *DAX-1* gene: one with amino acid substitution (R267P) and the other with single-amino-acid deletion (Δ V269) also showed a reduced DAX-1 inhibitory activity. These patients with DAX-1 mutations resulting in AHC provide compelling evidence that loss of this inhibitory effect of DAX-1 on developmentally regulated target genes may contribute to the pathogenesis of AHC (228). A recent study suggests that DAX-1 recruits N-CoR (nuclear receptor corepressor) to SF-1 (279). Whereas the naturally occurring mutations of DAX-1 allow DAX-1 and SF-1

interactions they markedly diminish the recruitment of corepressor. Thus, it is conceivable that impaired DAX-1-mediated inhibition of SF-1 responsive genes during development may play a significant role in organogenesis of the adrenal gland and, therefore, the syndrome of AHC.

SUMMARY

The development and remodeling of the fetal adrenal gland and its transition to a self-sufficient adult organ capable of sustaining life is a complex and temporally orchestrated process that is regulated by endocrine, paracrine, and autocrine factors that are derived both from maternal and fetal sources. The twin processes of fetal organogenesis and steroidogenesis are closely intertwined. The key cellular functions of proliferation, differentiation and apoptosis, which govern organogenesis of the fetal adrenal, are all regulated by HCG and ACTH, the growth factors, and the nuclear receptors SF-1, DAX-1, and ER. In addition, the fetoplacental unit, which is pivotal to the fetal adrenal steroidogenesis, is also the source of and at the same time is regulated by these endocrine, paracrine, and autocrine factors. Although the role of HCG and ACTH has been well-known for the past two to three decades, more recent studies have clearly established the seminal role of growth factors and nuclear receptors in both organogenesis and steroidogenesis of the fetal adrenal. Gene knockout studies in mice as well as transient transfection experiments using cell-culture systems have increased and enhanced our understanding of the molecular mechanisms underlying the cellular functions of growth, remodeling as well as steroidogenesis. Furthermore, studies on patients with X-linked AHC, which showed that *DAX-1* gene mutations result in both AHC, as well as hypogonadotropic hypogonadism, and a recent study on a patient with non-X-linked AHC have established the fundamental role of nuclear receptors, DAX-1, and SF-1 in adrenal organogenesis. The increasing body of knowledge about the underlying molecular mechanisms that regulate fundamental cellular functions will enable us in the future to design novel therapies for disorders of the adrenal organogenesis and steroidogenesis.

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