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Transcriptional regulation of human *CYP11A1* in gonads and adrenals

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Summary

The *CYP11A1* gene encodes the cholesterol side-chain cleavage enzyme, also termed cytochrome P450scc, which catalyzes the conversion of cholesterol to pregnenolone in the first step of steroid biosynthesis in mitochondria. The adrenal- and gonad-selective, hormonally and developmentally regulated expression of *CYP11A1* is principally driven by its 2.3 kb promoter. Multiple *trans*-acting factors like SF-1, Sp1, AP-2, TReP-132, LBP-1b, LBP-9, AP-1, NF-1, and Ets control *CYP11A1* transcription either through DNA-protein interaction with their specific *cis*-acting elements or through protein-protein interaction between each other, wherein SF-1 plays a central role in adrenals and testes. In addition to binding with its proximal and upstream motifs, SF-1 also physically interacts with TFIIB, CBP/p300, TReP-132, and c-Jun/AP-1 to specifically transmit the regulatory signals of cAMP. Other factors like Sp1 family members, AP-2, and LBP-1b/LBP-9 may be other factors that play a role in *CYP11A1* transcription, particularly in placental cells. The *TATA* sequence could also contribute to tissue-specificity and hormonal regulation of *CYP11A1* transcription. This article reviews recent studies focusing on adrenals and gonads.

Enzymes in steroid biosynthesis

Two categories of enzymes are involved in steroid synthesis, including cytochrome P450 enzymes and hydroxysteroid dehydrogenases (HSD). HSD proteins have multiple isoforms; they can be classified into two major classes, 3βHSD and 17βHSD, and a few other minor classes. Cytochrome P450 enzymes are divided into two types according to their intracellular locations, the mitochondrial type I and the microsomal type II.

Cytochromes P450 constitute a superfamily of membrane-bound heme-containing proteins with a characteristic shift of absorption peak from 420 nm to 450 nm after reduction by CO. These cytochromes P450 function as monooxygenases utilizing electrons donated from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to catalyze the hydroxylation and cleavage of substrates. Type I enzymes are loosely associated with the inner mitochondrial membrane, receiving electrons supplied by an electron-transport system composed of ferredoxin (a 14 kDa iron-sulfur protein previously called adrenodoxin) and ferredoxin reductase (a 54 kDa flavoprotein also named adrenodoxin reductase). These mitochondrial P450s include members of the CYP11 subfamily like CYP11A1, CYP11B1 and CYP11B2. The other steroidogenic P450s belong to the type II enzymes, which are anchored on the inner

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membrane of endoplasmic reticulum and receive electrons of NADPH principally through cytochrome P450 oxidoreductase, an 82 kDa membrane-bound protein. These type II enzymes include CYP17, CYP21 and CYP19. To further understand the characteristics of steroidogenic P450s and their associated electron-transport systems, please read the articles reviewed by Payne and Hales [1] and Miller [2].

Biochemical properties of CYP11A1 in steroidogenesis

All steroid hormones are derived from the same precursor, cholesterol. The conversion of cholesterol to pregnenolone is catalyzed by the CYP11A1 encoded by the *CYP11A1* gene; this enzyme is also termed cytochrome P450scc or cholesterol side-chain cleavage enzyme. It is the first and rate-limiting step of steroid biosynthesis. Pregnenolone is transformed into various forms of mineralocorticoids, glucocorticoids and androgens in adrenal cortices, progestins and estrogens in ovaries and placentae, and androgens in testes through sequential oxidation-reduction reactions catalyzed by other steroidogenic P450 and HSD enzymes [3].

CYP11A1 contains three kinds of enzymatic activities. The 20- and 22-hydroxylase activities of CYP11A1 catalyze hydroxylation of cholesterol at carbon-20 and carbon-22, respectively. The resulting 20*R*-,22*R*-dihydroxycholesterol is then cleaved at the carbon 20–22 bond by the 20,22 desmolase activity of CYP11A1 resulting in the removal of the six-carbon side chain and yielding the C21 steroid pregnenolone and isocaproaldehyde [1]. All these three sequential reactions are catalyzed at a single active site of CYP11A1 receiving three pairs of electrons from NADPH via ferredoxin and ferredoxin reductase [2].

Physiological phenotypes of CYP11A1 deficiency

In addition to CYP11A1, the initial step of steroid biosynthesis requires Steroidogenic Acute Regulatory (StAR) protein, which is responsible for the delivery of cytosolic cholesterol into mitochondria. Mutations of *StAR* and *CYP11A1* cause congenital lipoid adrenal hyperplasia (lipoid CAH) with concomitant male sexual reversal. Patients with lipoid CAH suffer from severe adrenal insufficiency and would not survive in the absence of mineralocorticoid and glucocorticoid replacement [4, 5]. Their plasma ACTH levels are high leading to adrenal hyperplasia. As a result of glucocorticoid and mineralocorticoid deficiency, patients have elevated plasma rennin activity. Similarly the lack of feedback inhibition leads to increased plasma concentrations of gonadotrophins. For more detailed symptoms please refer to several case reports [4–7] and the updated review article [8].

The phenotypes of *Cyp11a1*-null mice are very similar to that of human patients; for example, exceedingly high levels of ACTH but very little corticosterone and aldosterone in blood [9]. Excessive amounts of lipid are accumulated in adrenals and gonads, and these mice die soon after birth if they are not rescued by steroid injection. The male *Cyp11a1*-null mice are feminized with female external genitalia. They have undersized testes, epididymes and vasa deferens. In addition, these male *Cyp11a1*-null mice do not develop male accessory sex organs such as seminal vesicle, prostate, and penis; instead, the upper part of vagina is formed [10]. Some excellent review articles are helpful for readers to further understand how StAR works in steroidogenesis [11] and the role of CYP11A1 in embryonic development [12, 13].

Characteristics of *CYP11A1* promoter

CYP11A1 is expressed in tissue-selective, hormonally and developmentally regulated manners [12, 13]. In transgenic mice, the expression patterns of the *LacZ* reporter gene driven by the 4.4 kb 5′-flanking region of *CYP11A1* are similar to that of *CYP11A1* gene in the adrenal cortex and testis Leydig cells [14]. It appears that the 4.4 kb 5′-flanking region of *CYP11A1* possesses regulatory elements for *CYP11A1* expression in adrenocortical and Leydig cells. The 2.3 kb *CYP11A1* promoter also leads to similar spatio-temporal profiles of β-galactosidase as the 4.4 kb fragment in transgenic mice [14, 15].

We have carefully characterized the 2.3 kb *CYP11A1* promoter in placental JEG-3, adrenal Y1 and NCI-H295 cells, and identified several

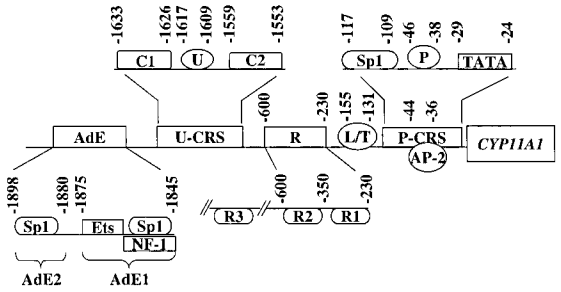

Figure 1. Locations of *cis*-acting elements within 2.3 kb 5′-flanking region of human *CYP11A1*. The cAMP-inducible basal promoter within −155 bp upstream from the transcription start site harbors the proximal cAMP-responsive sequences (*P-CRS*), an AP-2-binding motif (GCCTTGAGC) at −44/−36, and the TReP-132-binding sequence at −155/−131. *P-CRS* is composed of the *TATA* box (TTATAA) at −29/−24, an inverted SF-1-binding site (TGGCCTTGA, termed *P*) at −46/−38, and an imperfect Sp1-binding site (GGGGAGGAG) at −117/−109. SF-1 directly associates with TFIIB. AP-2 perhaps competes with SF-1 for binding to its motif that largely overlaps with *P* site; otherwise, it is also capable of interacting with Sp1/Sp3 independently of its binding site. TReP-132, in addition to binding with the −155/−131 sequence, also physically interacts with SF-1 and CBP/p300. In placental cells, the −155/−131 TReP-132-binding sequence is alternatively bound with the homo- or hetero-dimers of LBP-1b and LBP-9. The −117/−109 Sp1-binding site may be also recognized by Sp3 and Sp4. At least three negative controlling elements scatter within −350/−230 (R1), −600/−350 (R2), and −2300/−600 (R3), respectively; interestingly, the repressor at −2300/−600 is active in JEG-3 but not in Y1. The upstream cAMP-responsive sequences (*U-CRS*) consists of a core SF-1-binding site (TCAAGGTCA, termed *U*) at −1617/−1609, and two flanking *TRE*/*CRE*-like sequences, *C1* (TGATGTCA) at −1633/−1626 and *C2* (TGACTGA) at −1559/−1553. *U-CRS* does not function in placental cells that lack SF-1 expression. Two adrenal-selective enhancers (*AdE*), which function selectively in steroidogenic cells, include the downstream −1875/−1845 *AdE1* harboring an imperfect Sp1-binding site (GGGGTGG), a NF1-binding motif (TGG(C/A)(N)$_5$GCCAA) and a consensus Ets-binding sequence (CGGAAGT), and the upstream *AdE2* at −1898/−1880 with another imperfect Sp1-binding site (GGGGAGG).

important *cis*-elements as shown in Figure 1. Basically, four regions of *cis*-acting elements have been analyzed. To unify nucleotide positions, this review uses the deposited sequences of *CYP11A1* promoter as a standard to describe their locations (GenBank, Accession no.: M60421) [16]. The cAMP-inducible basal promoter, termed proximal cAMP-responsive sequences (*P-CRS*), is located within −120 bp upstream from the transcription start site, and contains the TATA box (TTATAA) at −29/−24, a inverted SF-1-binding site (TGGCCTTGA, termed *P*) at −46/−38, and an imperfect Sp1-binding site (GGGGAGGAG) at −117/−109 [17]. At least three negative controlling elements scatter within −350/−230, −600/−350, and −2300/−600, respectively; interestingly, the repressor at −2300/−600 is active in JEG-3 but not in Y1 [18]. An upstream cAMP-responsive sequences (*U-CRS*) consists of a core SF-1-binding site (*U* element, TCAAGGTCA) at −1617/−1609, and two flanking AP1/CREB-binding sites, *C1* (TGATGTCA) at −1633/−1626 and *C2* (TGACTGA) at −1559/−1553 [19, 20]. Two adrenal-selective enhancers (*AdE*) include the downstream −1875/−1845 *AdE1* harboring an imperfect Sp1-binding site (GGGGTGG), a NF1-binding motif (TGG(C/A)(N)$_5$GCCAA) and a consensus Ets-binding sequence (CGGAAGT), and the upstream *AdE2* at −1898/−1880 with another imperfect Sp1-binding site (GGGGAGG) [21].

Besides our results, several groups have found additional elements in *CYP11A1* promoter. The −155/−131 element interacts with TReP-132 (transcriptional regulating protein of 132 kDa), which is expressed in a broad spectrum of tissues and physically binds to SF-1 together with CBP/p300 synergistically activating *CYP11A1* promoter [22, 23]. The same sequence is also bound to two long terminal repeat binding proteins, LBP-1b and LBP-9; which form homo- or hetero-dimers to modulate *CYP11A1* transcription [24]. The GCCTTGAGC motif of rat *Cyp11a1* promoter identical to −44/−36 of human *CYP11A1* also interacts with alpha isoform of activating protein-2 (AP-2) to govern *Cyp11a1* promoter in human placental cells [25].

Physiological and intracellular regulations of steroid biosynthesis

Steroids are synthesized mainly from the adrenals and gonads. In the adrenals, steroidal secretion in response to environmental stimuli or physiological conditions is stimulated by pituitary adrenocorticotropin (ACTH) in the hypothalamic-pituitary-adrenal axis (H-P-A axis). ACTH is again regulated by hypothalamic corticotropin-releasing hormone (CRH). Similarly, synthesis of sex steroid hormones in testes and ovaries is controlled by the hypothalamic-pituitary-gonadal axis (H-P-G axis). The components of the H-P-G axis include the hypothalamic gonadotropin-releasing hormone (GnRH), pituitary gonadotropins (GTH), and gonadal steroids.

Pituitary GTH is composed of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Within both H-P-G and H-P-A axes, two negative feedback loops operate to prevent excessive secretion of tropic signals. The short loop is executed by pituitary hormones that inhibit the release of hypothalamic releasing hormones; and the long loop is accomplished by steroid hormones that inhibit the release of both pituitary tropic hormones and hypothalamic releasing hormones. These negative feedback loops prevent target steroidogenic tissues from being over-stimulated by tropic signals.

ACTH and GTH are the major tropic hormones secreted from the pituitary for the stimulation of adrenal cortices and gonads, respectively. When binding to their cognate G-coupled receptors, these tropic hormones activate the membrane-bound adenylyl cyclase that catalyzes the synthesis of cyclic AMP, which functions as the second messenger to transmit tropic signal of the first messenger, ACTH or GTH.

The activated cAMP signaling acutely stimulates steroid biosynthesis by increasing the availability of cholesterol for the CYP11A1 enzyme. Both cholesterol esterase and StAR are activated leading to facilitated conversion of cholesterol ester to the free form and the delivery of cytosolic cholesterol into mitochondria, respectively. The long-term effect of cAMP signaling takes hours to occur. It stimulates expression of steroidogenic genes to maintain long-term secretion of steroid hormones. A recent article reviewing the interplay between H-P-A and H-P-G axes is recommended for further reading [26].

Tissue selectivity and hormonal regulation of *CYP11A1* promoter activity

Differential functions of P-CRS and U-CRS

The expression of *CYP11A1* typically responds to chronic effect of cAMP. Both *CYP11A1* protein and mRNA levels are increased several hours after cAMP treatment in both adrenal Y1 and placental JEG-3 cells, but the mechanisms for this cAMP-stimulated *CYP11A1* expression in these two cell types are different [27, 28].

Two cAMP-responsive sequences, *P-CRS* and *U-CRS*, are responsible for basal and cAMP-responsive *CYP11A1* expression. *P-CRS* functions as the basal promoter with cAMP-inducible activity in both Y1 and JEG-3, and *U-CRS* mainly confers cAMP-responsiveness in Y1 [18]. Both *CRS* sequences contain an *SF-1* site, *P* site in *P-CRS* and *U* site in *U-CRS*. The basal *CYP11A1* promoter activity is lost in *P* mutants but retained in *U* mutants in Y1 and transgenic adrenals and testes; moreover, the adrenals and testes of transgenic *U* mutants lose response to ACTH and hCG stimulations simultaneously [29].

SF-1 sites in both *CRS* sequences are important for *CYP11A1* expression; the *P-CRS* is responsible for the basal activity, and the *U-CRS* is associated with hormonal regulation. However, why is the same *SF-1* motif associated with apparently different functions in both *CRS*? It is probably because *SF-1* element alone does not efficiently activate transcription; instead, the multiple proteins interacting with SF-1 also contribute to function. Sequences surrounding *SF-1* sites in *P-CRS* and *U-CRS* are very different. In *P-CRS*, the *Sp1* site and other sites as well as *TATA* box are present, while in *U-CRS* two *TRE/CRE*-like elements are present (Fig. 1). Depending on its promoter context and the neighboring interacting proteins, SF-1 functions in *P-CRS* and *U-CRS* may not be identical.

Role of P-CRS in tissue selectivity of CYP11A1 promoter

The *CYP11A1* promoter is active in steroidogenic tissues; on the contrary, they are inactive in kidney COS-1 cells [15, 17]. That is why kidney does not secret steroids. The lack of SF-1 may be one of reasons for the silence of *CYP11A1* promoter in non-steroidogenic tissues [29]. We have shown that SF-1 interact with transcription factor IIB (TFIIB) physically [30]. This interaction will probably stabilize the binding of transcription factor IID (TFIID) to the *TATA* box and the assembly of other general transcription factors and RNA polymerase II to form the transcription pre-initiation complexes. When COS-1 is supplemented with SF-1, the *CYP11A1* promoter becomes active [15, 17].

Placental JEG-3 represents a different situation. JEG-3 cells express *CYP11A1* and produce steroid hormones, but they do not express SF-1 [18, 28]. JEG-3 perhaps uses alternative factors to

Table 1. The studied interactions of *trans*-acting factors regulating *CYP11A1* transcription.

Interaction	*Trans*-acting factor	Reference
DNA-Protein	TATA-binding protein	[17]
	SF-1[a]	[20], [29]
	AP-2	[25]
	Sp1, Sp3, Sp4	[18], [21]
	TreP-132	[22]
	LBP-1b[b], LBP-9[b]	[24]
	AP-1, CREB/ATF	[20], [3]
	NF-1	[21]
	Ets	[21]
Protein-Protein	SF-1, TFIIB	[30]
	SF-1, TReP-132, CBP/p300	[22], [23]
	SF-1, c-Jun/AP-1	[30]
	Sp1/Sp3, AP-2	[32]

[a]SF-1 does not express in placental cells.
[a]LBP-1b and LBP-9 are placenta-specific proteins.

replace the role of SF-1. Several nuclear factors have been shown to exhibit placenta specificity to replace SF-1 function. LBP-1b bound to −155/−131 is suggested to stimulate *CYP11A1* transcription in JEG-3 under the modulation by its dimer partner, LBP-9 [31]. TReP-132, another transcription factor binding to −155/−131 and physically interacting with CBP/p300, may be another transactivator stimulating *CYP11A1* expression [22, 23]. AP-2 also controls rat *Cyp11a1* promoter activity in human placental cells by interacting with the motif identical to the −44/−36 sequence of human *CYP11A1* that largely overlaps the SF-1-binding sequence of −46/−38 in adrenals and testes [25]. A study of ovine *CYP11A1* in JEG-3 showed that AP-2 can induce Sp1/Sp3 transactivation and this activity is independent of AP-2 binding site [32]. About the interactions of *trans*-acting factors with their DNA motifs and between each other to regulate *CYP11A1* transcription are summarized in Table 1.

Another possibility for the cell type-specific expression of *CYP11A1* may be controlled by its *TATA* sequence of *CYP11A1*. It is known that the distinct *TATA* sequences constitute individual promoters with discrete functions by interacting with the cell type-specific general transcription factors [33]. We have changed the *TATA* sequence of cAMP-inducible *CYP11A1* into that of cAMP-insensitive *RSV* (Rous sarcoma virus) by site-direct mutagenesis; this completely abolishes the cAMP responsiveness of *P-CRS* in adrenal NCI-H295 cells [17]. The presence of SF-1 does not enhance cAMP response in NCI-H295 cells when the *TATA* box of *CYP11A1* is mutated. This result indicates that selective types of general transcription factors participating in *TATA*-assembled RNA polymerase II complexes might be the other determinants than SF-1 for the tissue-specific activity of *CYP11A1* promoter.

Role of U-CRS in hormonal regulation of CYP11A1 promoter

U-CRS of the *CYP11A1* promoter contains an SF-1-binding *U* site and two *TRE/CRE*-like sites, *C1* and *C2*, that bind to AP1/CREB-like proteins in response to cAMP [34]; it functions in an ACTH/cAMP-regulated manner [35]. The fact that *U-CRS* has no function in JEG-3 that lacks SF-1 indicates the indispensable role of SF-1 in *U-CRS* [18, 19]. The *U* mutants in transgenic mice cannot be stimulated by hormones, confirming the essential role of *U-CRS* in hormonal regulation of *CYP11A1* promoter in testes and adrenals [29].

Playing a pivotal role, SF-1 interacts with AP-1 for full *U-CRS* activity [15, 34]. In JEG-3 and COS-1, the 2.3-kb *CYP11A1* promoter is stimulated by exogenous SF-1. In addition, c-Jun synergistically interacts with SF-1 to activate the 2.3-kb *CYP11A1* promoter in JEG-3, but not in COS-1 cells [15]. The interaction of SF-1 and c-Jun is through physical association between the amino acids 1–169 of c-Jun and the Ftz-F1 box-Pro

cluster (FP region) of SF-1 [30]. The synergism of SF-1 and AP-1 seems steroidogenic cell-specific. What contribute to the tissue-specificity of *U-CRS*? The factors interacting with *AdE* and *P-CRS*, particularly *TATA* box, are all good candidates. Further detailed studies are expected to answer the question.

Role of Sp1 in CYP11A1 promoter

In *P-CRS*, an element at −117/−109 binds to Sp1, which is ubiquitously distributed in a variety of tissues. The *Sp1* site of *P-CRS* enables the minimal promoter of β-globin, including an *SF-1* site and the *TATA* sequence, to respond to cAMP stimulation [18]. In general, Sp1 assists gene transcription. Beside −117/−109 of *P-CRS*, two *Sp1* sites are located within *AdE1* and *AdE2* of −1900/−1840. The *AdE* region enhances *CYP11A1* basal promoter activity in steroidogenic cells like adrenal Y1 and NCI-H295, testis MA10 and placental JEG-3, but not in non-steroidogenic cells like kidney COS-1 and fibroblast Rat-1 [21]. Collectively, Sp1 seems to cooperate with steroidogenic transcription factors to enhance the basal and cAMP-inducible activities of *CYP11A1* promoter. Similarly, the transcription of ferredoxin, a partner of CYP11A1, is also controlled by two Sp1 sites proximal to RNA initiation site [36, 37].

Sp1 is a protein family consists of many members [38]. All these members can potentially bind to the same *Sp1* site in the promoter; for example, Sp1, Sp3 and Sp4 can bind to the −118/−100 element of bovine *CYP11A* promoter [39], and Sp1/Sp3 bind to the −112/−89 and −80/−57 elements of ovine *CYP11A1* [32]. This adds another layer of complexity for regulated *CYP11A1* expression.

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References

1. Payne A.H. and Hales D.B., Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr. Rev. 25(6) 947–970, 2004.
2. Miller W.L., Minireview: regulation of steroidogenesis by electron transfer. Endocrinology 146(6) 2544–2550, 2005.
3. Guo I.-C., Hu M.-C. and Chung B.-C, Transcriptional regulation of CYP11A1. J. Biomed. Sci. 10(6 Pt 1) 593–598, 2003.
4. Hauffa B.P., Miller W.L., Grumbach M.M., Conte F.A. and Kaplan S.L., Congenital adrenal hyperplasia due to deficient cholesterol side-chain cleavage activity (20, 22-desmolase) in a patient treated for 18 years. Clin. Endocrinol. (Oxf) 23(5) 481–493, 1985.
5. Muller J., Torsson A., Damkjaer Nielsen M., Petersen K.E., Christoffersen J. and Skakkebaek N.E., Gonadal development and growth in 46,XX and 46,XY individuals with P450scc deficiency (congenital lipoid adrenal hyperplasia). Horm. Res. 36(5–6) 203–208, 1991.
6. Hiort O., Holterhus P.M., Werner R., Marschke C., Hoppe U., Partsch C.J., Riepe F.G., Achermann J.C. and Struve D., Homozygous disruption of P450 side-chain cleavage (CYP11A1) is associated with prematurity, complete 46,XY sex reversal, and severe adrenal failure. J. Clin. Endocrinol. Metab. 90(1) 538–541, 2005.
7. Katsumata N., Ohtake M., Hojo T., Ogawa E., Hara T., Sato N. and Tanaka T., Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. J. Clin. Endocrinol. Metab. 87(8) 3808–3813, 2002.
8. Bhangoo A., Anhalt H., Ten S. and King S.R., Phenotypic variations in lipoid congenital adrenal hyperplasia. Pediatr. Endocrinol. Rev. 3(3) 258–271, 2006.
9. Hu M.-C., Hsu N.-C., El Hadj N.B., Pai C.-I., Chu H.-P., Wang C.-K.L. and Chung B.-C., Steroid deficiency syndromes in mice with targeted disruption of Cyp11a1. Mol. Endocrinol. 16(8) 1943–1950, 2002.
10. Ben El Hadj N., Hu M.-C., Chu H.-P., Wang C.-K.L., Chung B.-C., Phenotypic analysis of mice with steroid deficiency. In: Sundberg J.P., Ichiki T. (Eds), Handbook on Genetically Engineered Mice. CRC Press, Chapter 19, 2005, pp. 253–262.
11. Manna P.R. and Stocco D.M., Regulation of the steroidogenic acute regulatory protein expression: functional and physiological consequences. Curr. Drug Targets Immune Endocr. Metabol. Disord. 5(1) 93–108, 2005.
12. Hsu H.-J., Hsu N.-C., Hu M.-C. and Chung B.-C., Steroidogenesis in zebrafish and mouse models. Mol. Cell. Endocrinol. 248(1–2) 160–163, 2006.
13. Hu M.-C., Hsu H.-J., Guo I.-C. and Chung B.-C., Function of Cyp11a1 in animal models. Mol. Cell. Endocrinol. 215(1–2) 95–100, 2004.
14. Hu M.-C., Chou S.-J., Huang Y.-Y., Hsu N.-C., Li H. and Chung B.-C., Tissue-specific, hormonal, and developmental regulation of SCC-LacZ expression in transgenic mice leads to adrenocortical zone characterization. Endocrinology 140(12) 5609–5618, 1999.

15. Huang Y.-Y., Hu M.-C., Hsu N.-C., Wang C.-K.L. and Chung B.-C., Action of hormone responsive sequence in 2.3 kb promoter of CYP11A1. Mol. Cell. Endocrinol. 175(1–2) 205–210, 2001.
16. Moore C.C., Brentano S.T. and Miller W.L., Human P450scc gene transcription is induced by cyclic AMP and repressed by 12-O-tetradecanoylphorbol-13-acetate and A23187 through independent cis elements. Mol. Cell. Biol. 10(11) 6013–6023, 1990.
17. Guo I.-C. and Chung B.-C., Cell-type specificity of human CYP11A1 TATA box. J. Steroid Biochem. Mol. Biol. 69(1–6) 329–334, 1999.
18. Guo I.-C., Tsai H.-M. and Chung B.-C., Actions of two different cAMP-responsive sequences and an enhancer of the human CYP11A1 (P450scc) gene in adrenal Y1 and placental JEG-3 cells. J. Biol. Chem. 269(9) 6362–6369, 1994.
19. Chung B.-C., Guo I.-C. and Chou S.-J., Transcriptional regulation of the CYP11A1 and ferredoxin genes. Steroids 62(1) 37–42, 1997.
20. Watanabe N., Inoue H. and Fujii-Kuriyama Y., Regulatory mechanisms of cAMP-dependent and cell-specific expression of human steroidogenic cytochrome P450scc (CYP11A1) gene. Eur. J. Biochem. 222(3) 825–834, 1994.
21. Chou S.-J., Lai K.-N. and Chung B.-C., Characterization of the upstream sequence of the human CYP11A1 gene for cell type-specific expression. J. Biol. Chem. 271(36) 22125–22129, 1996.
22. Gizard F., Lavallee B., DeWitte F. and Hum D.W., A novel zinc finger protein TReP-132 interacts with CBP/p300 to regulate human CYP11A1 gene expression. J. Biol. Chem. 276(36) 33881–33892, 2001.
23. Gizard F., Lavallee B., DeWitte F., Teissier E., Staels B. and Hum D.W., The transcriptional regulating protein of 132 kDa (TReP-132) enhances P450scc gene transcription through interaction with steroidogenic factor-1 in human adrenal cells. J. Biol. Chem. 277(42) 39144–39155, 2002.
24. Huang N. and Miller W.L., LBP proteins modulate SF1-independent expression of P450scc in human placental JEG-3 cells. Mol. Endocrinol. 19(2) 409–420, 2005.
25. Ben-Zimra M., Koler M. and Orly J., 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(8) 1864–1880, 2002.
26. Mastorakos G., Pavlatou M.G. and Mizamtsidi M., The hypothalamic-pituitary-adrenal and the hypothalamic-pituitary-gonadal axes interplay. Pediatr. Endocrinol. Rev. 1(3) 172–181, 2006.
27. Guo I.-C., Huang C. and Chung B.-C., Differential regulation of the CYP11A1 (P450scc) and ferredoxin genes in adrenal and placental cells. DNA Cell Biol. 12(9) 849–860, 1993.
28. Hu M.-C., Guo I.-C., Lin J.-H. and Chung B.-C., Regulated expression of cytochrome P-450scc (cholesterol-side-chain cleavage enzyme) in cultured cell lines detected by antibody against bacterially expressed human protein. Biochem. J. 274: 813–817, 1991.
29. Hu M.-C., Hsu N.-C., Pai C.-I., Wang C.-K.L. and Chung B.-C., Functions of the upstream and proximal steroidogenic factor 1 (SF-1)-binding sites in the CYP11A1 promoter in basal transcription and hormonal response. Mol. Endocrinol. 15(5) 812–818, 2001.
30. Li L.-A., Chiang E.F.-L., Chen J.-C., Hsu N.-C., Chen Y.-J. and Chung B.-C., Function of steroidogenic factor 1 domains in nuclear localization, transactivation, and interaction with transcription factor TFIIB and c-Jun. Mol. Endocrinol. 13(9) 1588–1598, 1999.
31. Huang N. and Miller W.L., Cloning of factors related to HIV-inducible LBP proteins that regulate steroidogenic factor-1-independent human placental transcription of the cholesterol side-chain cleavage enzyme, P450scc. J. Biol. Chem. 275(4) 2852–2858, 2000.
32. Pena P., Reutens A.T., Albanese C., D'Amico M., Watanabe G., Donner A., Shu I.W., Williams T. and Pestell R.G., Activator protein-2 mediates transcriptional activation of the CYP11A1 gene by interaction with Sp1 rather than binding to DNA. Mol. Endocrinol. 13(8) 1402–1416, 1999.
33. Roeder R.G., The role of general initiation factors in transcription by RNA polymerase II. Trends. Biochem. Sci. 21(9) 327–335, 1996.
34. Chen C. and Guo I.-C., Effect of cAMP on protein binding activities of three elements in upstream promoter of human CYP11A1 gene. Life Sci. 67(17) 2045–2049, 2000.
35. Hsu H.-T., Chang Y.-C., Chiu Y.-N., Liu C.-L., Chang K.-J. and Guo I.-C., Leptin interferes with adrenocorticotropin/3′,5′-cyclic adenosine monophosphate (cAMP) signaling, possibly through a Janus kinase 2-phosphatidylinositol 3-kinase/Akt-phosphodiesterase 3-cAMP pathway, to down-regulate cholesterol side-chain cleavage cytochrome P450 enzyme in human adrenocortical NCI-H295 cell line. J. Clin. Endocrinol. Metab. 91(7) 2761–2769, 2006.
36. Chang C.-Y., Huang C., Guo I.-C., Tsai H.-M., Wu D.-A. and Chung B.-C., Transcription of the human ferredoxin gene through a single promoter which contains the 3′,5′-cyclic adenosine monophosphate-responsive sequence and Sp 1-binding site. Mol. Endocrinol. 6(9) 1362–1370, 1992.
37. Yeh J.-R., Huang C., Wu D.-A., Guo I.-C., Rainey W.E. and Chung B.-C., Regulation of ferredoxin gene in steroidogenic and nonsteroidogenic cells. J. Steroid Biochem. Mol. Biol. 53(1–6) 47–51, 1995.
38. Zhao C. and Meng A., Sp1-like transcription factors are regulators of embryonic development in vertebrates. Dev. Growth Differ. 47(4) 201–211, 2005.
39. Ahlgren R., Suske G., Waterman M.R. and Lund J., Role of Sp1 in cAMP-dependent transcriptional regulation of the bovine CYP11A gene. J. Biol. Chem. 274(27) 19422–19428, 1999.