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Review

Regulation of steroid production: Analysis of *Cyp11a1* promoter

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ABSTRACT

CYP11A1 is a key enzyme in steroid synthesis abundantly expressed in the adrenal, testis, ovary, and placenta. This article reviews recent studies on *cis*-regulatory elements and *trans*-regulators of the *CYP11A1* promoter, with special focus on their tissue-specific regulation. *Trans*-regulators include tissue-specific factors such as SF-1, DAX-1, TReP-132, LBP, and GATA that regulate tissue-specific expression of *CYP11A1*. These tissue-specific factors interact with factors commonly present in most cells like AP-1, Sp1, and AP-2 to bring *CYP11A1* transcription to full potential. These transcription factors stimulate *CYP11A1* transcriptional activity through interaction with their specific *cis*-elements or through protein–protein interaction. The *cis*-element on the *Cyp11a1* promoter was further characterized *in vitro* and *in vivo*. Mutation of the proximal SF-1-binding site results in down regulation of *CYP11A1* in the adrenal and testis but not in the ovary and placenta, leading to attenuated corticosterone circadian rhythms and blunted stress response.

Contents

1. Characteristics of CYP11A1

CYP11A1, also called cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc), is encoded by *Cyp11a1*. CYP11A1 is a type I cytochrome P450 enzyme located in mitochondria. CYP11A1 is the first step and rate limiting enzyme in the steroidogenic pathway. It cleaves the side chain of cholesterol, converting it to pregnenolone. Pregnenolone is further catalyzed by other steroidogenic enzymes such as CYP17, CYP21, CYP11B1, CYP11B2, HSD3B, and CYP19 to form various steroid products. These steroid products can be categorized into three

Abbreviation: U-CRS, upstream cAMP-responsive sequence; AdE, adrenal enhancer; AP-1/AP-2, Activator protein-1/Activator protein-2; CRE, cAMP-responsive element; CREB, cAMP-responsive element-binding protein; LBP, long terminal repeat binding protein; LRH-1, liver receptor homolog-1; mtP, mutant promoter; P450scc, cytochrome P450 cholesterol side-chain cleavage enzyme; SF-1, steroidogenic factor-1; SF1RE, steroidogenic factor-1 response element; TBP, TATA-box-binding protein; WT, wildtype.

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major classes: mineralocorticoids, glucocorticoids, and sex hormones.

2. Functions of steroids

Glucocorticoids are synthesized mainly in the adrenal cortex. In rodents, the major component of glucocorticoids is corticosterone, while in humans the predominant glucocorticoid is cortisol. Glucocorticoids control energy homeostasis, immune modulation, and proper stress response.

Aldosterone is the principal mineralocorticoid, which is synthesized in the zona glomerulosa of the adrenal glands. It regulates the balance of water and electrolytes by facilitating re-absorption of sodium and increasing the rate of potassium-ion excretion from the tubules of the kidneys. Water is passively reabsorbed accompanying sodium retention.

Sex steroids include estrogens and androgens, which are mainly synthesized in the gonads. Sex steroids promote sexual differentiation and sex organ maturation.

3. *Cyp11a1* expression pattern

Cyp11a1 is expressed abundantly in the adrenal, gonad, placenta, and to a lesser extent in the brain, skin, and intestine (Guo et al., 2003). In the adrenal gland, *Cyp11a1* is expressed in the cortex, which is divided into three functional zones: zonae glomerulosa, fasciculata, and reticularis. In the testis, *Cyp11a1* RNA is found in the Leydig cells; whereas in the ovary, it is found in the granulosa cells, theca, and corpora lutea (Hu et al., 1999). In the placenta, *Cyp11a1* is expressed in the trophoblast giant cells (Ben-Zimra et al., 2002a). In the adult brain, CYP11A1 is detected by immunostaining in many places including hippocampus (Kimoto et al., 2001) and cerebellum (Ukena et al., 1998).

4. Transcriptional regulation of *Cyp11a1*

4.1. Cis-regulatory elements

The transcriptional regulation of *Cyp11a1* has been characterized in both cell culture and transgenic mice. Many *cis*-regulatory elements were originally identified by the analyses of promoters deleted at the 5′-flanking region. The studies of these shorter promoter fragments revealed several *cis*-regulatory elements.

4.1.1. Cell culture studies

CYP11A1 promoter contains basal regulating elements extending from the TATA box to about −130, a more distant region from −130 to −500, an upstream cAMP-responsive sequence (U-CRS) at −1600, and an adrenal enhancer (AdE) at around −1850 (Fig. 1).

The proximal promoter region controls basal promoter activities of *CYP11A1* in all steroidogenic tissues; it includes a TATA box at −24/−29, a proximal SF1-binding site at −38/−46 termed P and an imperfect Sp1 binding site at −101/−111 (Guo et al., 1993, 1994; Hum et al., 1993; Guo and Chung, 1999; Hu et al., 2001). The TATA sequence binds to TATA-box-binding protein (TBP) as part of general transcription machinery. Replacing the TATA binding site of *CYP11A1* abolishes its response to cAMP (Guo and Chung, 1999). This indicates that this TATA box may bind to factors that contribute to cAMP response depending on the promoter context. The imperfect Sp1-binding site binds to proteins in the Sp1 family (Guo et al., 1994).

The −155/−131 region of *CYP11A1* is critical for placental *CYP11A1* expression. It binds to TReP-132 and/or long terminal repeat binding protein (LBP-1b and LBP-9) to stimulate or modulate *CYP11A1* expression (Gizard et al., 2001; Huang and Miller, 2005).

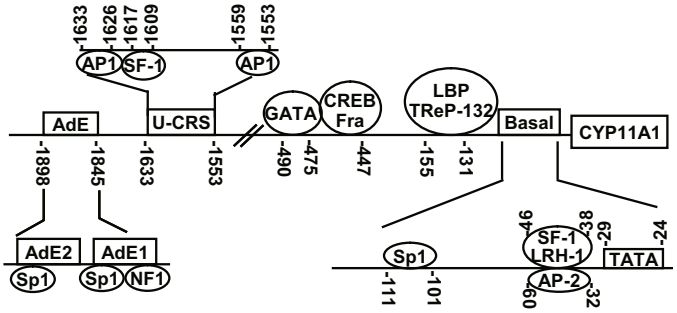

Fig. 1. A diagram of *Cyp11a1* promoter and its binding proteins. The promoter region is represented by a horizontal line and the *Cyp11a1* gene is shown as a box next to the line. Important *cis*-elements are shown as boxes above the line. The proteins that bind to the promoter elements are shown in circles. Basal: basal promoter; U-CRS: upstream cAMP-responsive sequence; AdE: adrenal enhancer.

The −475/−447 sequence is a half-CRE, which binds to CREB-1/Fra-2 or CREB-1 in rodent ovary or placenta, respectively (Sher et al., 2007). Its neighboring −490 sequence is a GATA sequence, which binds to GATA-2 in the placental giant cells and to GATA-4 in the ovarian granulose cells (Sher et al., 2007).

U-CRS is an 80-bp sequence located at the −1600 region of the *CYP11A1* promoter. It contains an SF-1-binding sequence flanked at both sides by sequences that bind to AP-1 family members (Hum et al., 1993; Guo et al., 2007). These sequences are important for hormonal-dependent stimulation of *CYP11A1* transcription via the cAMP signaling pathways (Guo et al., 1994, 2007; Hu et al., 2001).

The −1850 region is an enhancer (AdE), which participates in the control of *CYP11A1* expression in steroidogenic cell lines of adrenal, placenta, and gonad origins, but not in nonsteroidogenic cell lines such as COS-1 and Rat-1 (Guo et al., 1994; Chou et al., 1996). It is composed of two protein-binding regions termed AdE2 and AdE1; the upstream AdE2 contains an imperfect Sp1 binding site and the downstream AdE1 contains an imperfect Sp1 site and an NF1-binding site (Guo et al., 1994; Chou et al., 1996).

4.1.2. In vivo study of proximal SF1RE

The *cis*-regulatory elements and transcription factors described above for *Cyp11a1* transcription were mostly obtained via studies of promoter deletion and analysis of DNA-binding proteins in electrophoretic mobility shift assay. Although this knowledge is valuable, one should caution that these shorter promoter fragments were taken out of endogenous promoter context, therefore the results obtained from these studies need to be evaluated further. Studies evaluating the functions of *cis*-regulatory elements by site-directed mutagenesis in the right promoter context would be more appropriate. So far, only the SF-1-binding site (SF1RE) has been analyzed in this fashion.

SF1RE has the consensus sequence of TCAAGGTCA that binds to several nuclear receptors. These proteins include those in the NR5A family like NR5A1 (SF-1, Ad4BP) and NR5A2 (LRH-1). To analyze the importance of the *cis*-regulatory sequence, two nucleotides in SF1RE at −38/−46 were mutated in the context of the entire 2.3-kb promoter. These wildtype (WT) and mutant (mtP) promoter sequences were separately linked to *LacZ*, and their transcriptional activities as shown by the β-galactosidase activities were measured after transfection into different types of cells (Fig. 2). The mtP promoter activity was reduced to 50% in adrenal Y1 cells, but not changed in placental JEG3 cells. It was also normal in caprine luteal tsCLC-D cells, which was a cell line derived from goat corpora lutea following transformation with temperature sensitive SV40 large T antigen (tsA209) (Chiu et al., 2008). This result indicates that the contribution of the proximal SF1RE to basal *Cyp11a1* transcription is cell type-specific.