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Review

Complex assembly on the human CYP17 promoter

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ABSTRACT

Optimal steroid hormone biosynthesis occurs via the integration of multiple regulatory processes, one of which entails a coordinate increase in the transcription of all genes required for steroidogenesis. In the human adrenal cortex adrenocorticotropin (ACTH) activates a signaling cascade that promotes the dynamic assembly of protein complexes on the promoters of steroidogenic genes. For CYP17, multiple transcription factors, including steroidogenic factor-1 (SF-1), GATA-6, and sterol regulatory binding protein 1 (SREBP1), are recruited to the promoter during activated transcription. The ability of these factors to increase CYP17 mRNA expression requires the formation of higher order coregulatory complexes, many of which contain enzymatic activities that post-translationally modify both the transcription factors and histones. We discuss the mechanisms by which transcription factors and coregulatory proteins regulate CYP17 transcription and summarize the role of kinases, phosphatases, acetyltransferases, and histone deacetylases in controlling CYP17 mRNA expression.

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1. Introduction

In the human adrenal cortex, glucocorticoids and adrenal androgens are synthesized in response to the trophic hormone adrenocorticotropin (ACTH). ACTH directs increased steroid hormone secretion by regulating multiple cellular processes, including substrate import, trafficking, and delivery, transcriptional activation, pyridine nucleotide metabolism, and electron transfer (Miller, 2005, 2007; Sewer et al., 2007; Stocco et al., 2005). One of the intracellular second messengers that mediates the adrenocortical response to ACTH is cAMP. cAMP, via activation of the cAMP-dependent protein kinase (PKA) evokes rapid changes in the availability of free cholesterol and also acts to coordinately increase the transcription of all genes required for steroid hormone biosynthesis. These genes include adrenodoxin, 3β-hydroxysteroid dehydrogenase, and members of the cytochromes P450 superfamily of monooxygenases. This review will briefly summarize factors that regulate the transcription of CYP17, the gene that encodes P450c17.

2. CYP17

P450c17 is a bifunctional enzyme that catalyzes the 17α-hydroxylation of pregnenolone and progesterone for cortisol

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production and performs a 17, 20 bond scission reaction that results in adrenal androgen biosynthesis. CYP17 (and thus P450c17) is predominantly expressed in the adrenal gland, testicular Leydig cells, and ovarian thecal cells. However, expression has been detected in rat gastrointestinal tract (Dalla Valle et al., 1995; Le Goascogne et al., 1995), brain (Compagnone et al., 2000; Hojo et al., 2004), and liver (Vianello et al., 1997), in zebrafish brain, liver, and intestine (Wang and Ge, 2004), and in avian (Matsunaga et al., 2001) and frog (Do Rego et al., 2007) brain. It has been suggested that expression of CYP17 in the embryonic rodent central nervous system indicates that the enzyme plays a key role in providing androgens for sensory-motor function (Compagnone et al., 1995).

In the adult human adrenal cortex, selective expression of CYP17 in the zona fasciculata and zona reticularis, but not in the zona glomerulosa permits zone-specific production of steroid hormones. In fact, cAMP-dependent transcription of CYP17 is negatively regulated by agents that increase aldosterone production (Bird et al., 1996) and is unresponsive to nuclear receptors that increase the transcription of CYP11B2 and 3β-hydroxysteroid dehydrogenase (Bassett et al., 2004a,b,c). The ability of P450c17 to differentially carry out 17α-hydroxylase versus 17,20 lyase reactions is regulated by protein–protein interactions (Auchus et al., 1998; Geller et al., 1999) and post-translational modification (Pandey et al., 2003; Tee et al., 2008; Zhang et al., 1995). Therefore, in addition to 3β-hydroxysteroid dehydrogenase, the expression and specific enzymatic reaction catalyzed by P450c17 determines functional zonation of the two inner zones of the adult adrenal cortex. Finally, the importance of CYP17 is evidenced by the embryonic lethal phenotype of 127/SvJ mice null for the enzyme (Bair and Mellon, 2004). In contrast, targeted disruption of CYP17 in C57BL/6 mice resulted in male infertility due to androgen imbalance (Liu et al., 2005).

3. Regulation by transcription factors

As mentioned above, like the vast majority of genes involved in adrenal steroidogenesis, the transcription of CYP17 is increased in response to ACTH signaling. Several DNA-binding proteins have been found to be important in conferring increased CYP17 gene expression. Some of these factors act to ensure basal transcription, while others act in response to activation of the ACTH signaling pathway.

3.1. Steroidogenic factor-1 (SF-1)

SF-1 is a nuclear receptor that is essential not only for increased steroidogenic gene expression (including CYP17), but also for development and sex determination, as germ line disruption of the receptor results in postnatal lethality and male–female sex reversal (Luo et al., 1994). Recently, several elegant studies using mice generated to ablate SF-1 expression in a tissue-specific manner have revealed novel roles for the receptor in modulating neural function. Conditional knockout of SF-1 in the central nervous system increases anxiety-like behavior (Zhao et al., 2008) and mice lacking expression of the receptor in the anterior pituitary display hypogonadotropism (Zhao et al., 2001a,b). Additionally, SF-1 has been recently found to regulate the expression of the cannabinoid receptor 1 in the ventromedial hypothalamic nucleus (Kim et al., 2008) and has been implicated in regulating energy homeostasis (Bingham et al., 2008; Dhillon et al., 2006). Collectively, these studies have expanded the role for SF-1 as a regulator of varied physiological processes.

Initial studies using reporter gene constructs to define ACTH-dependent transcription of the human CYP17 gene identified that the first 63 base pairs upstream of the transcription initiation site are required for cAMP-stimulated gene expression (Rodriguez et al., 1997). It was subsequently shown that activation of the ACTH signaling pathway promoted the binding of SF-1 to this region (Sewer et al., 2002). cAMP stimulation resulted in the assembly of a complex containing SF-1, the RNA and DNA-binding protein $p54^{nrb}$, and the polypyrimidine-tract-binding protein associated factor (PSF) on the promoter (Sewer et al., 2002). Notably, the affinity of this complex for the human CYP17 promoter was found to be regulated by phosphatase activity (Sewer and Waterman, 2002). Although SF-1 is integral for activating the transcription of the CYP17 gene in other species (Zhang and Mellon, 1996), studies characterizing the bovine gene have demonstrated that in adrenocortical cells SF-1 acts in a reciprocal manner with chicken ovalbumin upstream promoter-transcription factor I which, when bound, represses CYP17 transcription (Bakke and Lund, 1995a).

It has become evident that post-translational modifications play a central role in controlling the transactivation potential of SF-1. In murine Y1 cells, SF-1 is phosphorylated at Ser-203 in response to epidermal growth factor signaling by extracellular related kinase (Hammer et al., 1999). Analyses of kinases that target Ser-203 have recently identified that cyclin-dependent kinase 7 phosphorylates this residue (Lewis et al., 2008). We have recently found that cAMP signaling increases phosphorylation of SF-1 in human H295R cells in a manner that requires glycogen synthase kinase 3β (Dammer et al., unpublished results). SF-1 is also acetylated (Chen et al., 2005; Ishihara and Morohashi, 2005; Jacob et al., 2001) and SUMOylated (Chen et al., 2004; Komatsu et al., 2004), adding to the complexity of post-translational mechanisms that control receptor function.

The ability of SF-1 to activate the transcription of human CYP17 is also regulated by ligand binding. Using mass spectrometry to identify ligands for SF-1, we found that sphingosine bound to the endogenous receptor and antagonized transactivation of CYP17 (Urs et al., 2006). In response to cAMP, sphingosine dissociates from the receptor, concomitant with phosphatidic acid binding and subsequent receptor activation (Li et al., 2007). These findings are supported by crystallographic studies carried out by three independent laboratories have found that the bacterially expressed receptor binds various phosphoplipids (Ingraham and Redinbo, 2005; Krylova et al., 2005; Li et al., 2005; Wang et al., 2005). Moreover, given the large binding pocket (1600 $Å^3$), it is likely that different sphingolipid and phospholipid species will be found to act as antagonists and agonists in specific tissues and/or in response to activation of varied signaling pathways.

3.2. GATA

Another family of transcription factors that regulates CYP17 transcription in the human adrenal cortex is the GATA family of transcription factors. These DNA-binding proteins control gene expression, cell differentiation, and tumorigenesis in diverse cell types, including the gonads and adrenal gland (Parviainen et al., 2007; Shimizu and Yamamoto, 2005; Tong et al., 2003; Tremblay and Viger, 2003; Viger et al., 2004). GATA-6 is highly expressed in the adrenal cortex, primarily in the zona reticularis of adults (Kiiveri et al., 2002). A role for GATA-6 in regulating the transcription of CYP17 in the H295R human adrenocortical cell line has been established, where synergy between GATA-6 and SF-1 directs adrenal androgen biosynthesis (Jimenez et al., 2003). Interestingly, the expression of GATA-6 is positively regulated by cAMP (Kiiveri et al., 2004), suggesting a role for trophic hormone stimulation in fine-tuning the function of this transcription factor in steroidogenic tissues. Complex formation between GATA-6 or GATA-4 and specificity protein (Sp) 1 mediates constitutive expression of CYP17 (Fluck and Miller, 2004). Significantly, the stimulatory actions of GATA-6 on CYP17 transcription are independent of DNA binding,