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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.

Keywords

CYP17; p54^{nrb}; GCN5; SRC-1; cAMP; Steroidogenic factor-1

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, 3 β -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 17 α -hydroxylation of pregnenolone and progesterone for cortisol production and performs a 17, 20 bond scission reaction that results

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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 3 β -hydroxysteroid dehydrogenase (Bassett et al., 2004a,b,c). The ability of P450c17 to differentially carry out 17 α -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 3 β -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 3 β (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 phospholipids (Ingraham and Redinbo, 2005; Krylova et al., 2005; Li et al., 2005; Wang et al., 2005). Moreover, given the large binding pocket (1600 Å³), 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, but depend on protein–protein interactions with Sp1 (Fluck and Miller, 2004). GATA-6 also synergizes with SF-1 to regulate the transcription of cytochrome b5 (Huang et al., 2005) and steroid sulfotransferase 2A1 (Saner et al., 2005) in the human adrenal, further supporting the role of this transcription factor in adrenal development and steroidogenesis.

3.3. Sp1 and Sp3

In addition to associating with GATA proteins (Fluck and Miller, 2004), Sp1 and Sp3 also interact with nuclear factor 1 to control basal expression of CYP17 (Lin et al., 2001). Sp proteins were once thought of as transcription factors that controlled the expression of housekeeping genes, however emerging evidence supports roles for these proteins in regulating transcriptional responses to cell signaling (Li et al., 2004; Wierstra, 2008). Sp1 and Sp3 interact with numerous nuclear receptors to regulate target gene transcription (Safe and Kim, 2004). Intriguingly, both Sp1 and Sp3 can act as transcriptional activators or repressors (Li et al., 2004; Valin and Gill, 2007). Since both proteins are regulated by phosphorylation (Chu and Ferro, 2005) and SUMOylation (Valin and Gill, 2007), it is likely that activated intracellular signaling results in the concurrent post-translational modification of multiple transcription factors that control CYP17 gene expression.

3.4. Sterol regulatory element binding protein (SREBP)

Another transcription factor that regulates the expression of the human *CYP17* gene is sterol regulatory element binding protein 1c (SREBP1c) (Ozbay et al., 2006). SREBPs are transcription factors that not only function as cholesterol sensors (Horton et al., 2002), but also regulate the transcription of steroidogenic genes, including steroidogenic acute regulatory protein (Shea-Eaton et al., 2001). We have shown that in response to sphingosine-1-phosphate (S1P), SREBP1c is cleaved and transported to the nucleus where it activates CYP17 transcription (Ozbay et al., 2006). Interestingly, activation of the ACTH signaling cascade promotes the rapid metabolism of complex sphingolipid species and promotes the secretion of S1P into the cell culture media (Ozbay et al., 2004; Ozbay et al., 2006). Since S1P is a ligand for a family of G-protein coupled receptors (Lee et al., 1998; Spiegel and Milstien, 2002; van Brocklyn et al., 1998, 2000), increased release of this bioactive lipid into extracellular space provides evidence for ACTH-induced paracrine signaling. Of note, given that we have recently found that S1P promotes migration of H295R adrenocortical cells and increases the trafficking of mitochondria (Li and Sewer, unpublished observations), it is probable that S1P will be found to regulate steroidogenesis and adrenal function by multiple mechanisms.

4. Regulation by coregulators

The ability of transcription factors to regulate the expression of target genes is controlled by complex formation with coregulatory proteins. Coregulators act to repress or activate target gene transcription by facilitating transcription factors to adopt either an active (mediated by coactivators) or inactive (corepressor-dependent) conformation (Lonard et al., 2007; Perissi and Rosenfeld, 2005). In general, agonist binding to a nuclear receptor induces a conformational change that increases the receptor's ability to bind to coactivator proteins that contain Leu-x-x-Leu-Leu motifs (also called nuclear receptor boxes) (Ding et al., 1998; Heery et al., 1997; McInerney et al., 1998). Interestingly, nuclear receptor boxes are required for the interaction between diacylglycerol kinase theta, the enzyme that produces phosphatidic acid, and SF-1 (Li et al., 2007). These findings indicate that the protein machinery required for agonist synthesis forms a complex with the receptor, thus maximizing efficient ligand binding.

Many of these coactivator proteins harbor innate catalytic properties, such as lysine acetyltransferase and arginine methyl-transferase activities. Collectively, the goal of coactivator proteins is to establish a transcriptionally competent environment at the promoter of the target gene. This is achieved by post-translationally modifying histones and transcription factors, displacing nucleosomes, and providing interaction domains for RNA polymerase II and the general transcriptional machinery. In contrast, when a nuclear receptor, such as SF-1, is unliganded or occupied by antagonist (e.g. sphingosine), the activation function 2 domain interacts more favorably with proteins that contain a CoRNR (corepressor nuclear receptor)

box motif (Hu et al., 2001). CoRNR boxes are typically found on nuclear receptor corepressors such as N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) (Hu and Lazar, 1999; Li et al., 1997). Indeed, sphingosine represses CYP17 transcription by promoting the interaction between SF-1 and SMRT (Urs et al., 2006).

Using temporal chromatin immunoprecipitation (ChIP) in synchronized H295R cells, we have characterized the components of the protein complexes that associate with the CYP17 promoter during cAMP-stimulated transcriptional activation (Dammer et al., 2007). Like temporal ChIP studies carried out to interrogate other genes (Metivier et al., 2003; Winnay and Hammer, 2006), our experiments have revealed that complexes containing corepressors or coactivators are reciprocally recruited to the promoter (Dammer et al., 2007). Moreover, within 60 min after cAMP treatment, a complex containing steroid receptor coactivator 1 (SRC1), general control nonderepressed 5 (GCN5), and SF-1 forms on the CYP17 promoter. Given that GCN5 is a histone acetyltransferase that has been shown to acetylate SF-1 (Jacob et al., 2001), it is possible that formation of this trimer facilitates the acetylation of SF-1 and proximally localized histones. An increase in acetylated histone H4 at the 60-min time point supports a role for GCN5 in modifying adjacent nucleosomes (Dammer et al., 2007). Interestingly, we have data that suggests a role for acetylation of SF-1 in controlling ligand binding to the receptor (Dammer et al., unpublished observations). Dissociation of the SF-1/SRC1/GCN5 trimer is closely followed by recruitment of histone deacetylase (HDAC) 1 and the corepressors RIP140 (receptor interacting protein 140) and Sin3A. These findings confirm previous studies demonstrating that Sin3A represses CYP17 transcription (Sewer et al., 2002). Another important event that occurs in response to cAMP stimulation is the recruitment of ATPase-containing chromatin remodeling complexes, which coincides with the rapid loss of histone H2 from nucleosomes within this region of the CYP17 promoter (Dammer et al., 2007).

Because our initial characterization of factors interacting with the CYP17 promoter identified p54^{nrb} and PSF as members of a complex with SF-1 (Sewer et al., 2002), we also assessed the kinetics of binding of these two proteins to the promoter and found that in contrast to SF-1/GCN5/SRC1 trimer that forms early in transcriptional cycling, p54^{nrb} and PSF are enriched at the promoter at a later time point (Dammer et al., 2007). At this later time point (180 min post-cAMP stimulation), SF-1 and RNA polymerase II re-associate with the promoter, thus enabling a second cycle of transcription. p54^{nrb} is a multi-functional protein that contains both RNA (Peng et al., 2002) and DNA-binding domains (Yang et al., 1993), as well as motifs that facilitate interaction with proteins such as the spliceosome (Kameoka et al., 2004) and RNA polymerase II (Emili et al., 2002). p54^{nrb} also promotes the binding of transcription factors to their cognate response elements (Yang et al., 1997) and regulates the transactivation potential of several nuclear receptors (Amelio et al., 2007; Fox et al., 2005). The presence of diverse functional domains in p54^{nrb} has led to the hypothesis that the protein plays an integral role in coupling transcription and pre-mRNA splicing (Emili et al., 2002; Kameoka et al., 2004; Liang and Lutz, 2006). Of note, we have recently found that p54^{nrb} binds to CYP17 pre-mRNA and to spliceosome proteins (Jagarlapudi and Sewer, unpublished observations). Further, the ability of p54^{nrb} to differentially interact with nucleic acids and proteins is regulated by phosphorylation (Jagarlapudi and Sewer, unpublished observations).

Another important event in cAMP-dependent transcription of the CYP17 promoter is the association of carboxy terminal binding proteins (CtBPs) (Dammer et al., 2007). The enrichment of CtBPs at the promoter corresponds with the exchange of transcriptional activators for repressors. CtBP corepressors were initially identified and characterized based on their ability to suppress transformation by the E1A viral oncoprotein (Schaeper et al., 1995). It was subsequently found that CtBP corepressors bind to and are regulated by NADH (Kumar et al., 2002; Zhang et al., 2002). We have recently shown that ACTH signaling rapidly

promotes the metabolism of pyridine nucleotides in H295R adrenocortical cells and that this change in the nuclear ratio of NADH/NAD⁺ regulates the ability of CtBPs to repress CYP17 transcription (Dammer and Sewer, 2008). These studies also demonstrated that PKA phosphorylates CtBPs and that phosphorylation regulates their dimerization and nuclear localization (Dammer and Sewer, 2008). In addition to defining the mechanism by which CtBPs repress CYP17 transcription, this work identified a role for ACTH in regulating pyridine nucleotide metabolism and provided support for the coupling of transcription to metabolism.

5. Summary

This brief review summarizes some of the proteins that regulate CYP17 transcription by associating with the promoter. As shown in Fig. 1, some of these proteins bind directly to DNA (e.g. SF-1), while others modulate CYP17 gene expression by forming higher order protein complexes. Additionally, it is clear that post-translational modification of nucleosomes, transcription factors, and coregulators modulate transcriptional potential during ACTH/cAMP signaling. Future studies are likely to uncover additional facets to the complex regulatory pathways involved in controlling CYP17 expression in the human adrenal cortex.

Acknowledgments

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Fig. 1.

Model for transcriptional regulation of human CYP17. In response to ACTH, PKA activation stimulates multiple proteins, including SK1 (sphingosine kinase 1), DGKθ (diacylglycerol kinase theta). It is likely that PKA modulates the activity of several other proteins in adrenocortical cells. Activated DGKθ phosphorylates diacylglycerol (DAG) to form phosphatidic acid (PA). Increased nuclear concentrations of PA facilitate dissociation of sphingosine (SPH) and corepressor proteins [Sin3A, histone deacetylase (HDAC), silencing mediator of retinoid and thyroid receptors (SMRT)]. Binding of PA to steroidogenic factor-1 (SF-1) promotes the assembly of a transcription activator complex containing SRC1 (steroid receptor coactivator 1), the histone acetyltransferase GCN5 (general control nonderepressed 5), p54, and polypyrimidine-tract binding protein-associated splicing factor (PSF). PKA also activates SK1, which converts SPH to sphingosine-1-phosphate (S1P). S1P activates the cleavage and nuclear import of sterol regulatory element binding protein 1c (SREBP1c) via a Ca²⁺-dependent pathway. Constitutive CYP17 expression is mediated by a complex containing specificity protein (Sp) 1, Sp3, and nuclear factor 1C (NF1C). Basal transcription is also mediated by GATA-6, which binds to Sp1. In response to cAMP GATA-6 synergizes with SF-1 to activate CYP17 transcription.