ACTH modulation of transcription factors responsible for steroid hydroxylase gene expression in the adrenal cortex

ARTICLE *in* MICROSCOPY RESEARCH AND TECHNIQUE · JUNE 2003

Impact Factor: 1.15 · DOI: 10.1002/jemt.10339 · Source: PubMed

2 AUTHORS:

Marion B Sewer
University of California, San Diego
48 PUBLICATIONS **1,465** CITATIONS
SEE PROFILE

Michael R Waterman
Vanderbilt University
429 PUBLICATIONS **16,980** CITATIONS
SEE PROFILE

ACTH Modulation of Transcription Factors Responsible for Steroid Hydroxylase Gene Expression in the Adrenal Cortex

MARION B. SEWER* AND MICHAEL R. WATERMAN
Department of Biochemistry and Center in Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

KEY WORDS ACTH; cAMP; steroid hydroxylases; SF-1; PKA

ABSTRACT Steroid hormone biosynthesis in the adrenal cortex and gonads involves the coordinated transcription of the genes encoding the steroid hydroxylases, 3β-hydroxysteroid dehydrogenase (3βHSD), the steroidogenic acute regulatory protein (StAR), and adrenodoxin (Adx). Transcriptional regulation of steroidogenic genes is multifactorial, entailing developmental, tissue-specific, constitutive, and cAMP-dependent mechanisms. Optimal steroidogenic capacity is achieved by the actions of ACTH which exerts transcriptional pressure on all steroidogenic genes. The actions of ACTH in the adrenal cortex have been studied in great detail and is mediated by cAMP and protein kinase A (PKA) via two temporally distinct pathways. The acute response leads to mobilization of cholesterol, the initial substrate for all steroidogenic pathways, from cellular stores to the inner mitochondrial membrane where cholesterol sidechain cleavage cytochrome P450 (P45011A1) resides. The slower, chronic response of ACTH in the adrenal cortex directs transcription of the genes encoding the steroidogenic enzymes. Although steroidogenic gene transcription in response to ACTH is cAMP-dependent, the consensus cAMP response pathway (CRE/CREB) is not involved. Instead, each steroidogenic gene utilizes unique cAMP-responsive sequences (CRS) found in the promoters of each gene, which bind a diverse array of transcription factors. Moreover, once specific transcription factors are bound to the promoters of the steroidogenic genes, increased gene expression requires posttranslational modification (phosphorylation/dephosphorylation) of the transcription factors and binding of coactivator proteins. This review provides a general view (with emphasis on the human) of the important factors involved in regulating steroidogenic gene expression and ultimately steroid hormone biosynthesis. *Microsc. Res. Tech. 61:300–307, 2003.*

OVERVIEW OF STEROID HYDROXYLASE GENES

Steroid hormones are important regulators of many physiological processes, including, but not limited to, maintenance of homeostasis, development of secondary sex characteristics, and gluconeogenesis. These hormones are synthesized from cholesterol by members of the cytochrome P450 superfamily (steroid hydroxylases) and by steroid dehydrogenases in steroidogenic tissues such as the adrenal cortex, ovaries, testes, and placenta. Once produced, the hormones enter the circulation where they are transported to target tissues to serve as ligands for the zinc-finger nuclear receptor family of transcription factors. The hormone/receptor complexes function as both transcriptional activators and repressors and, as a result, exert a broad array of physiological effects in a wide variety of cell types.

In the adrenal cortex of humans, five distinct steroid hydroxylases participate in the steroidogenic pathways leading to the production of cortisol, aldosterone, and adrenal androgens (dehydroepiandrosterone and androstenedione). Three of these P450s are localized in the mitochondria (P45011A1, P45011B1, P45011B2) and two are microsomal (P450c17 and P450c21). Schematic representations of these enzymes, their CYP (gene that encodes P450) designations, and the reactions they catalyze are presented in Figure 1. The first and rate-limiting step of steroid hormone production is the transfer of cholesterol at the inner mitochondrial membrane. Once at the inner mitochondrial membrane cholesterol is converted to pregnenolone by P45011A1 (P450scc; cholesterol sidechain-cleavage monooxygenase). P45011A1 catalyzes three separate reactions, 20α hydroxylation, 22α hydroxylation, and C20,22 bond scission, converting the C27 cholesterol to the C21 pregnenolone. Once pregnenolone is formed it can be 17α-hydroxylated by P450c17 to form 17-hydroxyprenenolone or converted to progesterone by 3β-hydroxysteroid dehydrogenase (3βHSD), the single non-P450 enzyme in the pathways. P450c17 can also form 17-hydroxyprogesterone from progesterone.

P45017α also catalyzes a 17,20 lyase reaction on 17-hydroxypregenolone and 17-hydroxyprogesterone to form the adrenal androgens dehydroepiandrosterone and androstenedione, respectively. Both progesterone and 17-hydroxyprogesterone can be 21-hydroxylated by P450C21 to yield deoxycorticosterone and 11-deoxycortisol, respectively. The final reactions in adrenocortical

*Correspondence to: Marion B. Sewer, School of Biology, Georgia Institute of Technology, 310 Ferst Drive, Atlanta, GA 30332-0230.
E-mail: marion.sewer@biology.gatech.edu

Received 23 May 2002; accepted in revised form 12 July 2002

Contact grant sponsor: NIH, Contract grant numbers: DK28350, ES00267 (to MRW); Contract grant sponsor: UNCF/Merck Science Initiative Postdoctoral Fellowship (to MBS); Contract grant number: NIH Postdoctoral Training T32 CA09582 (to MBS)

DOI 10.1002/jemt.10339

Published online in Wiley InterScience (www.interscience.wiley.com).

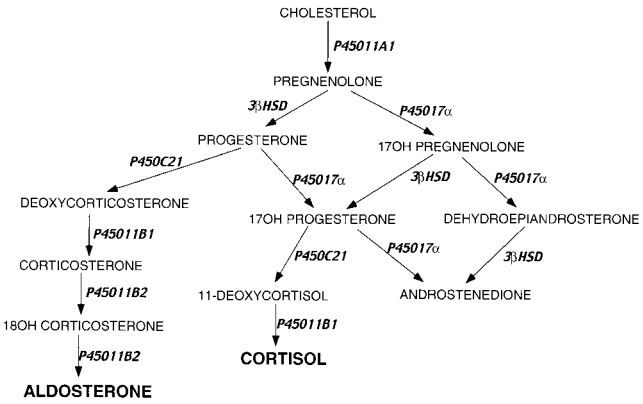

Fig. 1. Pathways of human adrenal steroid hormone biosynthesis.

steroid hormone biosynthesis involve the hydroxylation of 11-deoxycortisol and deoxycorticosterone by P45011B1 to form cortisol and corticosterone, respectively. Corticosterone can then be converted to aldosterone by P45011B2 in a series of hydroxylation steps. In most species, including humans, the fasiculata/reticularis cells of the adrenal cortex produce cortisol. P45011B2 is not present in these cells. In the outer layer of cells in the adrenal cortex (glomerulosa), P450c17 is not present and P45011B2 is; thus, the glomerulosa produces aldosterone.

REGULATORY LEVELS OF STEROIDOGENIC GENE EXPRESSION

There are multiple levels by which the biosynthesis of steroid hormones is transcriptionally regulated: developmental, tissue-specific, constitutive (cAMP-independent), and cAMP-dependent (reviewed in Sewer and Waterman, 2001; Waterman and Keeney, 1996). The production of steroid hormones is critical during fetal development; thus, timely expression of steroidogenic genes is an essential component of normal human development. Perhaps the best-known need for steroid hormones in development is the requirement for testosterone in establishing the male phenotype. Similarly, impaired synthesis of other steroid hormones during development can lead to multiple metabolic defects. Developmental expression of steroidogenic genes in the adrenal cortex (and gonads) is dependent on the orphan nuclear receptor steroidogenic factor (SF)-1 (Parker and Schimmer, 1997; Morohashi et al., 1992). All genes encoding steroid hydroxylases have one or more SF-1 binding sequence in close proximity to the TATA box (Morohashi et al., 1992). Moreover, SF-1 has been found to interact with coactivator and corepressor proteins which serve as bridges between transcription factors and the basal transcription machinery (Hammer et al., 1999; Ito et al., 1998; Monte et al., 1998; Shibata et al., 2000). Targeted disruption of the gene encoding SF-1 leads to mice lacking gonads and adrenal glands and having impaired function of pituitary gonadotropes (Luo et al., 1994; Sadovsky et al., 1995). Subsequent studies have found more broad roles for SF-1 in the development and function of the endocrine system (Majdie et al., 2002; Ikeda et al., 1995; Ingraham et al., 1994; Shinoda et al., 1995).

Tissue-specific expression is also regulated by SF-1. However, in contrast to the consequences of the absence of the SF-1 gene on the adrenal gland and gonads in the knockout mice, the fetal placenta structure was intact in the embryos of the knockout mice and expressed normal levels of P45011A1 (Sadovsky et al., 1995). In addition, steroidogenic enzymes are expressed in tissues such as the brain (Le Goascogne et al., 1987) and liver and intestines (Vianello et al., 1997) in the absence of SF-1 expression, suggesting that SF-1 does not regulate steroid hydroxylase gene expression in nonsteroidogenic tissues. Studies have implicated SF-1 as a mediator of the differential expression of CYP11B1 and CYP11B2 in the zonae fasciculata and glomerulosa of the human adrenal cortex (Wang et al., 2000). Transient transfection of human CYP11B1 promoter-reporter constructs indentified ATF-2 and SF-1 as essential for mediating ACTH-stimulated transcription, which is in contrast to the lack of effect of SF-1 on the transcription of human CYP11B2 (Wang et al., 2000). It is plausible that there are at least three mechanisms for tissue-specific expression of steroidogenic genes: one dependent on SF-1 in steroidogenic tissues (adrenal cortex and gonads), one independent of SF-1 in steroidogenic tissues (placenta), and one in other sites (brain, liver, etc.) that is independent of SF-1.

Constitutive expression of steroidogenic enzymes has been extensively studied in cell lines derived from steroidogenic tissues of various species. Findings are varied across species, tissues, and genes. In a general sense, however, it is clear that stimuli including interferon (Orava et al., 1989), calcium (Bird et al., 1998; Clark and Combs, 1999), phorbol esters (Bird et al., 1998; Ilvesmaki and Voutilainen, 1991; Leers-Sucheta et al., 1997), and growth factors (Doi et al., 2001; Pestell et al., 1995) modulate transcription of steroidogenic genes and that cAMP-independent mechanisms overlay the fundamental cAMP-dependent process. Once development has taken place cAMP-dependent transcription is the most important level of regulation of steroidogenic genes because it assures optimal steroidogenic capacity throughout the life of the organism. Over the last 25 years considerable attention has been given to elucidating the mechanism by which cAMP exerts transcriptional pressure on the adrenal cortex, allowing for the biosynthesis of cortisol, aldosterone, and adrenal androgens. The rest of this review will focus on cAMP-dependent steroidogenic gene expression in the adrenal cortex.

HISTORICAL OVERVIEW OF SEMINAL FINDINGS ON ACTH/cAMP-DEPENDENT TRANSCRIPTION IN THE ADRENAL CORTEX

As previously mentioned, the cAMP-dependent transcription of steroidogenic genes assures that optimal steroidogenic capacity is maintained in order to meet physiological needs throughout life. The stimulatory actions of ACTH and cAMP on steroidogenic enzyme levels were initially demonstrated using primary cultures of bovine adrenocortical cells that were treated with ACTH or an analog of cAMP (Zuber et al., 1986). Subsequently, it was shown by nuclear run-on assay that the ACTH/cAMP-mediated increase in steroidogenic enzyme activity was predominantly due to increased steroidogenic gene transcription (John et al.,

1986). It was also found that the cAMP-mediated increases in steroidogenic mRNA expression took hours, rather than minutes, to manifest (John et al., 1986; Waterman and Simpson, 1989). This observation suggested that the actions of cAMP on steroidogenic gene transcription were distinct from the rapid cAMP response element (CRE)/cAMP response element binding protein (CREB) transcription pathway that has been shown to induce the expression of the immediate early genes c-Fos and Jun-B within minutes (Roesler et al., 1988; Viard et al., 1992). Another difference between the CRE/CREB pathway and the ACTH/cAMP steroidogenic pathway is the effect of cycloheximide (CHX). CHX acts synergistically with cAMP to superinduce CRE/CREB responsive genes (Roesler et al., 1988), whereas CHX inhibits ACTH/cAMP-mediated transcription of steroidogenic genes (Waterman, 1994). The delayed induction of steroidogenic gene expression following ACTH/cAMP stimulation (as compared to CRE/CREB responsive genes) coupled with the CHX-sensitive nature of the response led to the hypothesis that new protein synthesis may be required prior to increased steroidogenic gene mRNA levels.

Once it was established that cAMP induced all steroidogenic genes, several laboratories attempted to localize the regions of the promoters of individual steroidogenic genes that acted to convey cAMP-responsive transcriptional activity. Due to the coordinate nature of the ACTH/cAMP effect on steroidogenic gene transcription (John et al., 1986), it was postulated that a common cAMP-responsive sequence (CRS) would be found in all genes, however, various transcription factors, including adrenal-specific protein (ASP) (Kagawa and Waterman, 1992), homeodomain proteins (Bischof et al., 1998; Kagawa et al., 1994), Sp family members (Ahlgren et al., 1999; Cheng et al., 2000; Lin et al., 2001), CREBP (Bassett et al., 2000; Rice et al., 1989), and SF-1 (Clark and Combs, 1999; Leers-Sucheta et al., 1997; Morohashi et al., 1993; Sewer et. al., 2002) have all been found to be required for increased transcription of different steroidogenic genes. As previously mentioned, one shared feature of the cAMP-dependent transcription of steroidogenic genes is the CHX-sensitive nature of the event. The site of CHX sensitivity in cAMP-stimulated gene transcription has yet to be elucidated. Due to the wide variety of transcription factors identified to be essential for cAMP-dependent transcription, it is likely that the CHX sensitive factor in the ACTH/cAMP pathway occurs prior to the binding of these diverse transcription factors to CRS elements in steroidogenic genes. Nevertheless, despite the seemingly complex and gene-specific nature of the actions of ACTH, the coordinate increase in transcription and cAMP-dependent transcriptional pressure is cognate for all steroidogenic genes.

ACTH/cAMP PATHWAY FOR STEROIDOGENIC GENE EXPRESSION

Although it was determined that the increase in steroid hormone biosynthesis in response to ACTH/cAMP involved the delayed increase in steroidogenic gene transcription, it was also realized that the actions of ACTH/cAMP in the adrenal cortex contained two distinct components. Upon ACTH release from the anterior pituitary, the peptide hormone binds to its specific, cell surface receptor on adrenocortical cells. This binding activates adenylyl cyclase, leading to elevated levels of intracellular cAMP. Increased intracellular cAMP results in the activation of the cAMP-dependent protein kinase (PKA). Once PKA is activated there are two temporal responses (acute and chronic) that occur to increase steroid hormone biosynthesis. During the acute response, an essential site of phosphorylation by PKA is cholesterol ester hydroxylase, which upon activation catalyzes the conversion of cholesterol esters to free cholesterol (Jefcoate et al., 1992). The acute response leads to mobilization of cholesterol, the initial substrate for all steroidogenic pathways, from cellular stores to the inner mitochondrial membrane where P45011A1 resides. This rapid response also involves the increase in gene transcription of the steroidogenic acute regulatory protein (StAR), which acts to facilitate cholesterol movement in the mitochondria (Clark et al., 1994; Stocco, 2001).

StAR is an acutely regulated, cycloheximide-sensitive, mitochondrial protein that was originally identified because of the requirement that cholesterol be delivered to the inner mitochondrial membrane to undergo conversion to pregnenolone in the first and rate-limiting step of steroid hormone biosynthesis. The acute stimulation of steroid hormone production in response to ACTH/cAMP is accompanied by rapid increases in StAR mRNA levels in the steroidogenic cells of the adrenal cortex, testis, and ovary (Clark et al., 1995; Stocco, 2001). StAR's essential role in the regulation of steroidogenesis became solidified after the discovery of mutations leading to premature stop codons in the StAR gene of patients with congenital lipoid adrenal hyperplasia (Lin et al., 1995; Tee et al., 1995). Moreover, targeted disruption of the StAR gene in mice resulted in defects in steroid hormone biosynthesis, with consequent male pseudohermaphroditism, and ultimately lethality within 1 week after birth (Caron et al., 1997b).

PKA phosphorylation leading to the chronic response activates transcription of all other steroidogenic genes (Sewer and Waterman, 2002b), including the steroid hydroxylases, 3βHSD, and the mitochondrial electron transport protein adrenodoxin (Adx): however, the precise proteins phosphorylated are not known. Although the target of PKA has yet to be determined, several transcription factors have been shown to be required for conveying cAMP-dependent transcription. In the next section, ACTH/cAMP modulated transcription factors will be highlighted for the various steroidogenic genes expressed in the adrenal cortex, with an emphasis on the human (Fig. 2).

ACTH/cAMP-MODULATED TRANSCRIPTION FACTORS

CYP11A1

CYP11A1 encodes the mitochondrial enzyme P45011A1 (P450scc) which, as discussed previously, converts cholesterol to pregnenolone. Transcription of human CYP11A1 in response to ACTH/cAMP stimulation requires binding of SF-1 (Fig. 2) at two sites (−40 bp and −1600 bp) on the promoter (Hu et al., 2001). Interestingly, c-Jun was found to potentiate SF-1 dependent transcription of human CYP11A1 promoter-reporter construct transfected into the human chorio-

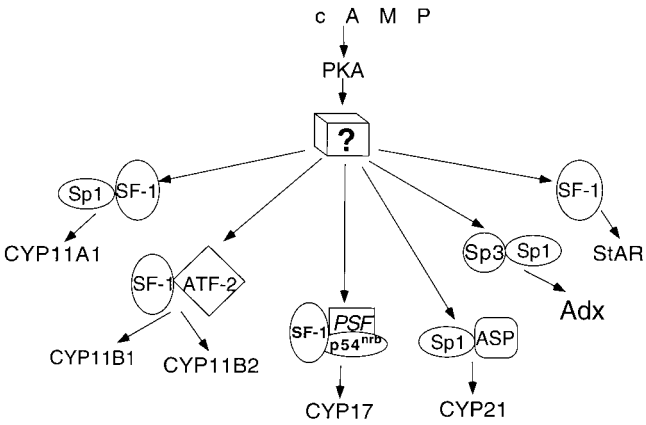

Fig. 2. Overview of cAMP-dependent transcription of steroidogenic genes in the human adrenal cortex.

carcinoma Jeg-3 cell line (Huang et al., 2001). Recently, a novel zinc finger protein, TReP-132, was identified as a positive regulator of human CYP11A gene expression (Gizard et al., 2001). The stimulatory actions of TReP-132 were found to be due to interaction with the co-regulatory protein CBP/p300 (Gizard et al., 2001). The ubiquitously expressed transcription factor Sp1 also regulates the cAMP-dependent transcription of CYP11A1 in human (Chou et al., 1996), bovine (Ahlgren et al., 1999; Liu and Simpson, 1999), and porcine (Urban et al., 2000) adrenals. The Sp family of transcription factors are ubiquitously expressed zinc finger-containing proteins that bind to GC- or GT-rich sequences and either activate or repress transcription (Lania et al., 1997). Sp1, Sp3, and Sp4 have similar structural features, the DNA binding domain of these three proteins being highly conserved (Hagen et al., 1997; Kingsley and Winoto, 1992), whereas Sp2 has different DNA binding specificities (Kingsley and Winoto, 1992). In the bovine adrenal, SF-1 and Sp1 act cooperatively to mediate cAMP-dependent transcription of CYP11A1 (Liu and Simpson, 1999). Further, the stimulatory actions of SF-1 and Sp1 on bovine CYP11A1 transcriptional activity is enhanced by the coactivator CBP (Liu and Simpson, 1999).

CYP11B1 and CYP11B2

In humans, the final steps in corticosteroid biosynthesis results from the activity of P45011β (11β-hydoxylase) in the fasciculata and P45011B2 (aldosterone synthase) in the glomerulosa. These enzymes are encoded by CYP11B1 and CYP11B2, respectively. ACTH-stimulated transcription of human CYP11B1 is dependent on a CREBP family member, ATF-2, in addition to SF-1 (Fig. 2) (Wang et al., 2000). Human CYP11B1 is also positively regulated by the liver receptor homolog (LHR)-1 (Wang et al., 2001). It was shown that LHR1 can substitute for SF-1 to enhance transcription of human CYP11B1 (Wang et al., 2001). ACTH/cAMP, angiotensin II, and potassium all regulated human CYP11B2 via two elements: an SF-1 binding site and a CRE (Clyne et al., 1997). The murine CYP11B2 gene has also been found to bind CREBP in response to ACTH/cAMP (Rice et al., 1989). In the bovine adrenal, only one CYP11B gene, whose promoter contains both an SF-1 and a CRE-like binding site that are required for cAMP-dependent transcription, is expressed (Hashimoto et al., 1992; Morohashi et al., 1993).

CYP17

CYP17 encodes P450c17, which catalyzes both the 17α-hydroxylation of pregnenolone and progesterone required for cortisol biosynthesis and the 17,20-lyase activity of 17α-hydroxylated steroids producing androgens. Studies of transcriptional regulation of CYP17 in cows and humans has been actively pursued because of the gene product's key role in a branch point of adrenocortical steroidogenesis. Transcriptional control of hCYP17 gene expression in human adrenocortical H295R cells reveals that both basal and cAMP-responsive elements lie within the first 63 bp upstream of the transcriptional initiation site and that a second basal transcription element lies between −184 to −206 bp (Rodriguez et al., 1997). A complex comprised of SF-1, p54nrb/NonO, and polypyrimidine tract-binding protein-associated splicing factor (PSF) bind within this first 60 bp and confer cAMP-dependent transcriptional activity (Fig. 2) (Sewer et al., 2002). Recently, Sp1, Sp3, and NF-1C have been shown to bind the second basal element and are essential for optimal basal transcription (Lin et al., 2001). Studies of cultured bovine adrenocortical cells (Bischof et al., 1998; Lund et al., 1990) and bovine fetal adrenals (Lund et al., 1988) indicate that transcription of the bovine CYP17 (bCYP17) gene is regulated by ACTH/cAMP via two cAMP-regulatory sequences (CRS1 and CRS2) in the 5′ flanking region of the gene, which bind to the homeodomain proteins Pbx1, Meis1, and Pknox (Bischof et al., 1998) and the orphan nuclear receptors chicken ovalbumin promoter-transcription factor (COUP-TF)-1 and SF-1, respectively. COUP-TF1 acts through CRS2 to suppress transcription and SF-1 stimulates gene expression at the same site (Bakke and Lund, 1995b).

CYP21

CYP21 encodes P450c21, a key enzyme in the production of cortisol and aldosterone. Studies aimed at locating cAMP-responsive elements within the human CYP21 gene identified a region within a 200-bp proximal promoter adjacent to the transcriptional start site that binds an adrenal specific protein (ASP) (Kagawa and Waterman, 1992). However, in depth characterization of ASP and its precise role in mediating ACTH/cAMP-dependent transcription of human CYP21 has not been undertaken. Interestingly, adrenal-specific expression of human CYP21 is driven by a distal region (lying approximately 4.8 kb upstream of the CYP21 start site) that binds SF-1 (Wijesuriya et al., 1999). ACTH/cAMP-dependent transcription of murine CYP21 is dependent on regulatory elements within the first 300 bp upstream of the transcriptional initiation site (Parissenti et al., 1993).

Adrenodoxin

Adx is a mitochondrial electron transport protein that shuttles electrons from NADPH (via Adx reductase) to mitochondrial P450s (Kimura et al., 1969). In the cow, Sp1 and Sp3 along with a homolog of ZBP-89 convey transcription of the bovine Adx gene (Cheng et

al., 2000). Sp1 and Sp3 confer basal transcriptional activity, Sp1 confers cAMP-dependent transcription, and ZBP-89 represses basal transcription (Cheng et al., 2000). The CRS of the human Adx gene is located adjacent to an essential Sp1 binding site (Chang et al., 1992). Interestingly, SF-1 has not been found to play a role in the basal or ACTH/cAMP-dependent transcription of Adx. Since Adx is ubiquitously expressed, it is likely that the stimulatory effects of ACTH/cAMP on Adx gene transcription are mediated in a manner distinct from that of steroid hydroxylase genes, StAR and 3βHSD. Perhaps Sp1 can function as the cAMP-responsive transcription factor. In vitro assays have shown Sp1 to be stimulated by PKA (Rohlff et al., 1997); however, whether PKA directly phosphorylates Sp1 (or other Sp family members) in the adrenal cortex resulting in steroidogenic gene transcription has yet to be elucidated.

StAR

The acute stimulation of steroid hormone production in response to ACTH/cAMP is accompanied by rapid increases in StAR mRNA levels. Deletion mutagenesis of the mouse, human, porcine, bovine, and rat StAR promoters indicate that the cAMP-responsive region is within the first 350 bp upstream of the transcription initiation site (Caron et al., 1997a; LaVoie et al., 1999; Sandhoff et al., 1998; Sugawara et al., 1996). In the human adrenocortical cell line H295R, both angiotensin II and cAMP-induced transcription of the human StAR gene occurs via binding of SF-1 to the same response element (Clark and Combs, 1999). In contrast, mutation of the SF-1 elements in the mouse promoter did not affect cAMP induction in MA-10 mouse Leydig cells or Y1 adrenocortical cells (Caron et al., 1997a). Within the cAMP-responsive region (first 350 bp upstream of transcriptional start site) of StAR genes there are also binding sites for the CCAAT enhancer binding protein C/EBP. Transient transfection studies using the murine StAR promoter have found that C/EBPβ can activate transcription (Reinhart et al., 1999). Further, SF-1 transactivation of the StAR promoter is dependent on the presence of functional C/EBP binding sites, but cAMP-inducibility was unaffected by mutations in C/EBP binding sites (Reinhart et al., 1999). Thus, it appears that C/EBP may play a role in StAR expression under basal conditions but not in cAMP-dependent transcription of StAR. Recently, cAMP-dependent transcription of StAR in MA-10 mouse Leydig cells has been shown to be regulated by CREM (Manna et al., 2002), providing evidence for cAMP-dependent expression of a steroidogenic gene by a CREB family member.

POSTTRANSLATIONAL MODIFICATION OF TRANSCRIPTION FACTORS

With the completion of the human genome project, it has become apparent that the number of genes present in humans is less than initially estimated. Thus, the importance of posttranslational modification as a means to elicit specific responses in various cellular systems has emerged. Several studies have recently examined the role of posttranslational modification (specifically, phosphorylation/dephosphorylation) in the ACTH/cAMP-dependent regulation of steroid hormone biosynthesis. Studies by Hammer et al. (1999) have demonstrated that SF-1 can be phosphorylated on serine 203 by MAPK in a PKA-independent manner and enhance basal transcriptional activity. It is possible that this phosphorylation leads to increased cofactor recruitment, which then modulates the coordinate regulation of multiple SF-1 target genes (steroid hydroxylases) in response to hormone stimulation. As previously mentioned, cAMP stimulation does not result in SF-1 phosphorylation (Hammer et al., 1999). Rather, SF-1 has been shown to be dephosphorylated in response to cAMP (Sewer and Waterman, 2002a). Moreover, inhibition of the MAPK cascade can mimic the cAMP-stimulated increase in human CYP17 gene transcription (Sewer and Waterman, 2002a). Thus, it appears that the transcription factors involved in ACTH/cAMP-dependent regulation of steroidogenic gene expression may undergo changes in phosphorylation state, and that these changes (phosphorylation/dephosphorylation) may involve cross-talk between the ACTH/cAMP signaling pathway and the MAPK cascade.

The role of dephosphorylation has been given considerable attention in the field of steroidogenic gene expression. cAMP-induced increases in StAR mRNA has also been found to be dependent on phosphoprotein phosphatase activities (Jones et al., 2000). Using competitive reverse transcription-polymerase chain reaction, it was found that inhibition of phosphoprotein phosphatase 1 and 2A activities attenuated the forskolin-induced expression of StAR mRNA (Jones et al., 2000). Similarly, Paz et al., (2000) found that ACTH regulation of steroidogenesis in the rodent depends on PKA-dependent serine/threonine phosphorylation and also on the activity of protein tyrosine phosphatases. Rocchi et al., (2000) have shown that ACTH stimulates phosphotyrosine phosphatase SHP2 in bovine adrenocortical cells.

Recently, it has also been found that both serine/threonine and tyrosine protein phosphatase activities were essential for cAMP-dependent expression of human CYP17 mRNA in human H295R adrenocortical cells (Sewer and Waterman, 2002a). Subsequently, using both serine/threonine and phosphotyrosine selective phosphatase inhibitors, the cAMP-mediated induction of multiple steroidogenic genes in H295R cells requires a dual-specificity phosphatase (DSP) (Sewer and Waterman, 2002b). The specific DSP has been identified as mitogen-activated protein phosphatase 1 (MKP-1) (Sewer and Waterman, 2003). Since SF-1 has been found to be dephosphorylated in response to cAMP-stimulation, it is likely that SF-1 is the steroidogenic target of MKP-1. The emerging importance of dephosphorylation is exemplified in the model pathway for ACTH/cAMP-dependent expression of hCYP17 in the adrenal cortex (Fig. 3).

RECRUITMENT OF COACTIVATORS AND COREPRESSORS

Several studies have demonstrated the role of coactivators and corepressors in the transcriptional regulation of steroid hydroxylase genes and StAR (Hammer et al., 1999; Monte et al., 1998). Coactivators are proteins that are recruited to regulatory regions by DNA-bound transcription factors. Many coactivator proteins,

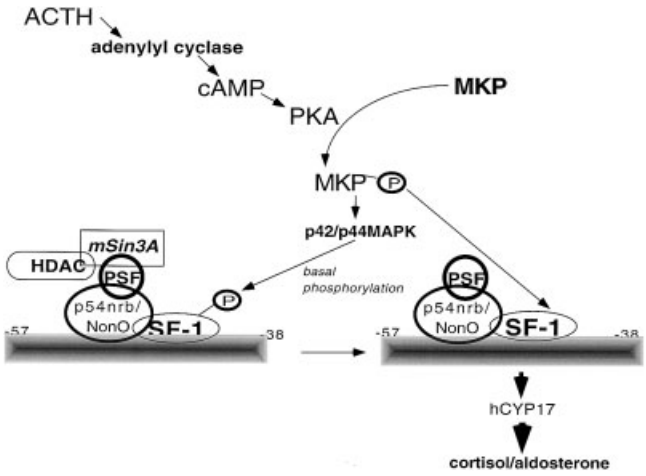

Fig. 3. Model signal transduction pathway for CYP17 transcription in the human adrenal cortex.

including steroid receptor coactivator (SRC)-1 and CREB binding protein/p300 (CBP/p300), have been found to possess intrinsic histone acetyltransferase activity (Bannister and Kouzarides, 1996; Spencer et al., 1997). The acetylation of histones increases the accessibility of transcription factors to nucleosomal DNA and correlates with transcriptional activity (Struhl, 1998). It was shown that the regulation of the human CYP11A gene by SF-1 and TReP-132 is mediated by CBP/p300 (Gizard et al., 2001).

Similarly, the coactivators SRC-1, TIF (transcriptional intermediary factor)-2, and CBP/p300 were found to potentiate the SF-1 mediated induction of bovine CYP17 transcription, whereas the corepressors N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) were found to potentiate the repressive activity of chicken ovalbumin upstream-transcription factor (COUP-TF)-1 (Shibata et al., 2000). A proline-rich nuclear receptor coregulatory protein was also identified and shown to interact with SF-1 and enhance the SF-1-mediated transcriptional activation of the human aromatase (CYP19) gene (Zhou et al., 2000). Another corepressor, mSin3A, mediates the inhibition of human CYP17 gene transcription by recruiting a histone deacetylase to the SF-1/NonO/PSF complex that binds to the promoter of CYP17 (Sewer et al., 2002). It is thought that ACTH/cAMP alleviates the repression exerted by mSin3A and the histone deacetylase by increasing the affinity of the complex for the DNA, dephosphorylating SF-1, and/or recruiting coactivators to the complex.

SUMMARY AND FUTURE DIRECTIONS

The studies outlined in this review have provided the field with a clearer understanding of how ACTH/cAMP exerts transcriptional pressure on steroidogenic gene expression, although it is clear that there is still a great deal of further study to be carried out in order to completely understand the details of the ACTH/cAMP signaling pathway. Investigation of the biochemical details of transcriptional regulation for all genes is necessary and will provide greater insight into the role of protein–protein interactions and the relationship of transcription factor binding to chromatin structure. Elucidating the mechanism by which ACTH/cAMP controls the balance between activation and repression is essential. This will involve determining the identity of the molecular switch that allows for the shift from repression to activation upon increased intracellular cAMP and what factors play a role in cAMP-dependent gene activation. There are several other areas that warrant investigation, including, but not limited to, further examination of posttranslational modifications and how ACTH/cAMP activates kinases and phosphatases which modify transcription factors shown to be important for steroidogenic gene expression. Nevertheless, it is clear that the mechanism by which ACTH conveys the signal for increased cortisol and aldosterone production in the adrenal cortex is complex and involves the coordinated action of numerous proteins.

REFERENCES

Ahlgren R, Suske G, Waterman MR, Lund J. 1999. Role of Sp1 in cAMP-dependent transcriptional regulation of the bovine CYP11A gene. J Biol Chem 274:19422–19428.

Bakke M, Lund J. 1995. Transcriptional regulation of the bovine CYP17 gene: two nuclear orphan receptors determine activity of cAMP-responsive sequence 2. Endocr Res 21:509–516.

Bannister AJ, Kouzarides T. 1996. The CBP co-activator is a histone acetyltransferase. Nature 384:641–643.

Bassett MH, Zhang Y, White PC, Rainey WE. 2000. Regulation of human CYP11B2 and CYP11B1: comparing the role of the common CRE/Ad1 element. Endocr Res 26:941–951.

Bird IM, Mason JI, Rainey WE. 1998. Protein kinase A, protein kinase C, and Ca(2+)-regulated expression of 21-hydroxylase cytochrome P450 in H295R human adrenocortical cells. J Clin Endocrinol Metab 83:1592–1597.

Bischof LJ, Kagawa N, Moskow JJ, Takahashi Y, Iwamatsu A, Buchberg AM, Waterman MR. 1998. Members of the meis1 and pbx homeodomain protein families cooperatively bind a cAMP-responsive sequence (CRS1) from bovine CYP17. J Biol Chem 273: 7941–7948.

Caron KM, Ikeda Y, Soo SC, Stocco DM, Parker KL, Clark BJ. 1997a. Characterization of the promoter region of the mouse gene encoding the steroidogenic acute regulatory protein. Mol Endocrinol 11:137–147.

Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. 1997b. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. Proc Natl Acad Sci USA 94:11540–11545.

Chang C.-Y, Huang C, Guo I.-C, Tsai H.-M, Wu D.-A, Chung B. 1992. Transcription of the human ferredoxin gene through a single promoter which contains the 3′,5′-cyclic adenosine monophosphate-responsive sequence and Sp1 binding site. Mol Endocrinol 6:1362–1370.

Cheng PY, Kagawa N, Takahashi Y, Waterman MR. 2000. Three zinc finger nuclear proteins, Sp1, Sp3, and a ZBP-89 homologue, bind to the cyclic adenosine monophosphate-responsive sequence of the bovine adrenodoxin gene and regulate transcription. Biochemistry 39:4347–4357.

Chou SJ, Lai KN, Chung BC. 1996. Characterization of the upstream sequence of the human CYP11A1 gene for cell type-specific expression. J Biol Chem 271:22125–22129.

Clark BJ, Combs R. 1999. Angiotensin II and cyclic adenosine 3′,5′-monophosphate induce human steroidogenic acute regulatory protein transcription through a common steroidogenic factor-1 element. Endocrinology 140:4390–4398.

Clark BJ, Wells J, King SR, Stocco DM. 1994. The purification, cloning, and expression of a novel leutenizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 269:28314–28322.

Clark BJ, Soo SC, Caron KM, Ikeda Y, Parker KL, Stocco DM. 1995. Hormonal and developmental regulation of the steroidogenic acute regulatory (StAR) protein. Mol Endocrinol 9:1346–1355.

Clyne CD, Zhang Y, Slutsker L, Mathis JM, White PC, Rainey WE. 1997. Angiotensin II and potassium regulate human CYP11B2 transcription through common cis-elements. Mol Endocrinol 11: 638–649.

Doi J, Takemori H, Ohta M, Nonaka Y, Okamoto M. 2001. Differential regulation of 3β-hydroxysteroid dehydrogenase type II and 17α-hydroxylase/lyase P450 in human adrenocortical carcinoma cells by epidermal growth factor and basic fibroblast growth factor. J Endocrinol 168:87–94.

Gizard F, Lavallee B, DeWitte F, Hum DW. 2001. A novel zinc finger protein TReP-132 interacts with CBP/p300 to regulate human CYP11A1 gene expression. J Biol Chem 276: 33881–33892.

Hagen G, Muller S, Beato M, Suske G. 1997. Cloning by recognition site screening of two novel GT box binding proteins: a family of Sp1 related genes. Nucleic Acids Res 20:5519–5525.

Hammer GD, Krylova I, Zhang Y, Darimont BD, Simpson K, Weigel NL, Ingraham HA. 1999. Phosphorylation of the nuclear receptor SF-1 modulates cofactor recruitment: integration of hormone signaling in reproduction and stress. Mol Cell 3:521–526.

Hashimoto T, Morohashi K, Takayama K, Honda S, Wada T, Handa H, Omura T. 1992. Cooperative transcription activation between Ad1, CRE-like element, and other elements in the CYP11B gene promoter. J Biochem 12:573–575.

Hu MC, Hsu NC, Pai CI, Wang CK, Chung B. 2001. 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 15:812–828.

Huang Y, Hu M, Hsu N, Wang CL, Chung B. 2001. Action of hormone responsive sequence in 2.3 kb promoter of CYP11A1. Mol Cell Endocrinol 175:205–210.

Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL, 1995. The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 9:478–486.

Ilvesmaki V, Voutilainen R. 1991. Interaction of phorbol ester and adrenocorticotropin in the regulation of steroidogenic P450 genes in human fetal and adult adrenal cell cultures. Endocrinology 128: 1450–1458.

Ingraham HA, Lala DS, Ikeda Y, Luo X, Shen W, Nachtigal MW, Abbud R, Nilson JH, Parker KL. 1994. The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. Genes Dev 8:2302–2312.

Ito M, Yu RN, Jameson JL. 1998. Steroidogenic factor-I contains a carboxy-terminal transcriptional activation domain that interacts with steroid receptor coactivator-1. Mol Endocrinol 12:290–301.

Jefcoate CR, McNamara BC, Artemenko I, Yamazaki T. 1992. Regulation of cholesterol movement to mitochondrial cytochrome P450scc in steroid hormone synthesis. J Steroid Biochem Mol Biol 43:751–767.

John ME, John MC, Boggaram V, Simpson ER, Waterman MR. 1986. Transcriptional regulation of steroid hydroxylase genes by corticotropin. Proc Natl Acad Sci USA 83:4715–4719.

Jones PM, Sayed SB, Persaud SJ, Burns CJ, Gyles S, Whitehouse BJ. 2000 Cyclic AMP-induced expression of steroidogenic acute regulatory protein is dependent upon phosphoprotein phosphatase activities. J Mol Endocrinol 24:233–239.

Kagawa N, Waterman MR, 1992. Purification and characterization of a transcription factor which appears to regulate cAMP responsiveness of the human CYP21B gene. J Biol Chem 267:25213–25219.

Kagawa N, Ogo A, Takahashi Y, Iwamatsu A, Waterman MR. 1994. A cAMP-regulatory sequence (CRS1) of CYP17 is a cellular target for the homeodomain protein Pbx1. J Biol Chem 269:18716–18719.

Kimura T, Suzuki K, Padmanabhan R, Samejima T, Tarutani O, Ui N. 1969. Studies on steroid hydroxylase. Molecular properties of adrenal iron-sulfur protein. Biochemistry 8:4027–4031.

Kingsley C, Winoto A. 1992. Cloning of GT box-binding proteins: a novel Sp1 multigene family regulating T-cell receptor gene expression. Mol. Cell Biol 12:4251–4261.

Lania L, Majello B, De Luca P. 1997. Transcriptional regulation by the Sp family proteins. Int J Biochem Cell Biol 29:1313–1323.

LaVoie HA, Garmey JC, Veldhuis JD, 1999. Mechanisms of insulin-like growth factor 1 augmentation of follicle-stimulating hormone-induced porcine steroidogenic acute regulatory protein gene promoter activity in granulose cells. Endocrinology 140:146–153.

Le Goascogne C, Robel P, Gouezou M, Sananes N, Baulieu EE, Waterman MR, 1987. Neurosteroids: cytochrome P450scc in rat brain. Science 237:1212–1215.

Leers-Sucheta S, Morohashi K, Mason JI, Melner MH, 1997. Synergistic activation of the human type II 3β-hydroxysteroid dehydrogenase/5-4 isomerase promoter by the transcription factor steroidogenic factor-1/adrenal 4-binding protein and phorbol ester. J Biol Chem 272: 7960–7967.

Lin D, Sugawara T, Strauss JR, III, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL. 1995. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 267:1828–1831.

Lin CJ, Martens JWM, Miller WL. 2001. NF-1C, Sp1, and Sp3 are essential for transcription of the human gene for P450c17 (steroid 17α-hydroxylase/17,20 lyase) in human adrenal NCl-H295R cells. Mol Endocrinol 15:1277–1293.

Liu Z, Simpson ER. 1999. Molecular mechanism for cooperation between Sp1 and steroidogenic factor-1 (SF-1) to regulate bovine CYP11A gene expression. Mol Cell Endocrinol 153:183–196.

Lund J, Faucher DJ, Ford SP, Porter JC, Waterman MR, Mason JI. 1988. Developmental expression of bovine adrenocortical steroid hydroxylases. J Biol Chem 263: 16195–16201.

Lund J, Ahlgren R, Wu DH, Kagimoto M, Simpson ER, Waterman MR. 1990. Transcriptional regulation of the bovine CYP17 (P-450(17)alpha) gene. Identification of two cAMP regulatory regions lacking the consensus cAMP-responsive element (CRE). J Biol Chem 265:3304–3312.

Luo X, Ikeda Y, Parker KL. 1994. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 77:481–490.

Majdic G, Young M, Gomez-Sanchez, Anderson P, Szcepaniak LS, Dobbins RL, McGarry JD, Parker KL. 2002. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 143:607–614.

Manna PR, Dyson MT, Darrell WE, Clark BJ, Lalli E, Sassone-Corsi P, Zeleznik AJ, Stocco DM. 2002. Regulation of steroidogenesis and the steroidogenic acute regulatory protein by a member of the cAMP response-element binding protein family. Mol Endocrinol 16:184–199.

Monte D, DeWitte F, Hum DW. 1998. Regulation of the human P450scc gene by steroidogenic factor 1 is mediated by CBP/p300. J Biol Chem 273:4585–4591.

Morohashi K, Honda S, Inomata Y, Handa H, Omura T, 1992. A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450s. J Biol Chem 267:17913–17919.

Morohashi K, Zanger UM, Honda S, Hara M, Waterman MR, Omura T, 1993. Activation of CYP11A and CYP11B gene promoters by the steroidogenic cell-specific transcription factor. Ad4BP. Mol Endocrinol 7:1196–1204.

Orava M, Voutilainen R, Vihko R. 1989. Interferon-g inhibits steroidogenesis and accumulation of mRNA of the steroidogenic enzymes P450scc and P450c17 in cultured porcine Leydig cells. Mol Endocrinol 3:887–894.

Parissenti AM, Parker KL, Schimmer BP. 1993. Identification of promoter elements in the mouse 21-hydroxylase (Cyp21) gene that require a functional cyclic adenosine 3′,5′-monophosphate-dependent protein kinase. Mol Endocrinol 7:283–290.

Parker KL, Schimmer BP. 1997. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 18:391–377.

Paz C, Maciel FC, Poderoso C, Gorostizaga A, Podesta EJ. 2000. An ACTH-activated protein tyrosine phosphatase is modulated by PKA-mediated phosphorylation. Endocr Res 26: 604–614.

Pestell RG, Albanese C, Watanabe G, Johnson J. 1995. Epidermal growth factor and c-jun act via a common DNA regulatory element to stimulate transcription of the ovine P-450 cholesterol side chain cleavage (CYP11A1) promoter. J Biol Chem 270:18301–18308.

Reinhart A, Williams S, Clark B, Stocco D. 1999. SF-1 and C/EBPβ cooperate to regulate the murine StAR promoter. Mol Endocrinol 13:729–741.

Rice DA, Aitken LD, Vandenbark GR, Mouw AR, Franklin A, Schimmer BP, Parker KL. 1989. A cAMP-responsive element regulates expression of the mouse steroid 11 beta-hydroxylase gene. J Biol Chem 264:14011–14015.

Rocchi S, Gaillard I, Obberghen EV, Chambaz EM, Vilgrain I. 2000. Adrenocorticotropic hormone stimulates phosphotyrosine phosphatase SHP2 in bovine adrenocortical cells: phosphorylation and activation by cAMP-dependent protein kinase. Biochem J 352:483–490.

Rodriguez H, Hum DW, Staels B, Miller WL. 1997. Transcription of the human genes for cytochrome P450scc and P450c17 is regulated differently in human adrenal NCI-H295 cells than in mouse adrenal Y1 cells. J Clin Endocrinol Metab 82:365–371.

Roesler WJ, Vandenbar GR, Hanson RW, 1988. Cyclic AMP and the induction of eukaryotic gene transcription. J Biol Chem 263:9063–9066.

Rohlff C, Ahmad S, Borellini F, Lei J, Glazer RI. 1997. Modulation of transcription factor Sp1 by cAMP-dependent protein kinase. J Biol Chem 272:21137–21141.

Sadovsky Y, Crawford PA, Woodson KG, Polish JA, Clements MA, Tourtellotte LM, K. S, Milbrandt J. 1995. Mice deficient in the orphan nuclear receptor steroidogenic factor 1 lack adrenal glands and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. Proc Natl Acad Sci USA 92:10939–10943.

Sandhoff TW, Hales DB, Hales KH, McLean MP. 1998. Transcriptional regulation of steroidogenic acute regulatory protein gene by steroidogenic factor 1. Endocrinology 139:4820–4831.

Sewer MB, Waterman MR. 2001. Insights into the transcriptional regulation of steroidogenic enzymes and StAR. Rev Endocr Metab Disord 2:269–274.

Sewer MB, Waterman MR. 2002a ACTH/cAMP-mediated transcription of the human CYP17 gene in the adrenal cortex is dependent on phosphatase activity. Endocrinology 143:1369–1377.

Sewer MB, Waterman MR. 2002b. cAMP-Dependent transcription of steroidogenic genes in the human adrenal cortex requires a dual-specificity phosphatase in addition to PKA. J Mol Endocrinol 29: 163–174.

Sewer MB, Nguyen V, Huang CJ, Tucker PW, Kagawa N, Waterman MR. 2002. Transcriptional activation of human CYP17 in H295R adrenocortical cells depends on complex formation between p54nrb/NonO, PSF and SF-1, a complex which also participates in repression of transcription. Endocrinology 143:1280–1290.

Sewer MB, Waterman MR. 2003. cAMP-dependent protein kinase enhances CYP17 transcription via MKP-1 activation in H295R human adrenocortical cells. J Biol Chem 278:8106–8111.

Shibata H, Ando T, Kurihara I, Suzuki T, Lund J, Morohashi K, Sasano H, Hayashi K, Hayashi M, Saito Y, Saruta T. 2000. Functional role of COUP-TF1, SF-1, and nuclear receptor coregulators in the steroidogenesis of adrenocortical adenomas. In: Molecular steroidogenesis. Okamoto MIY, Nawata H, ed 1 to 19. Tokyo: J Universal Academy Press. pp. 345–348.

Shinoda K, Lei H, Yoshii H, Nomura M, Nagano M, Shiba H, Sasaki H, Osawa Y, Ninomiya Y, Niwa O, Morohashi K-I, Li E. 1995. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. Dev Dyn 204:22–29.

Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ, O'Malley BW. 1997. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194–198.

Stocco DM. 2001. Tracking the role of a StAR in the sky of the new millenium. Mol Endocrinol 15:1245–1254.

Struhl K. 1998. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 12:599–606.

Sugawara T, Holt JA, Kiriakidou M, Strauss JR III. 1996. Steroidogenic factor 1-dependent promoter activity of the human steroidogenic acute regulatory protein (StAR) gene. Biochemistry 35:9052–9059.

Tee M, Lin D, Sugawara T, Holt JA, Guigen Y, Buckingham B, Strauss JR, III, Miller WL. 1995. T-A transversion 11 bp from a splice acceptor site in the human gene for steroidogenic acute regulatory protein causes congenital lipoid adrenal hyperplasia. Mol Genet 4:2299–2305.

Urban RJ, Bodenburg Y, Kurosky A, Wood TG, Gasic S. 2000. Polypyrimidine tract-binding protein-associated splicing factor is a negative regulator of transcriptional activity of the porcine P450scc insulin-like growth factor response element. Mol Endocrinol 14: 774–782.

Vianello S, Waterman MR, Valle LD, Colombo L. 1997. Developmentally regulated expression and activity of 17α-hydroxylase/C-17,20-lyase cytochrome P450 in rat liver. Endocrinology 138:3166–3174.

Viard I, Hall SH, Jaillard C, Berthelon MC, Saez JM. 1992. Regulation of c-fos, c-jun, and jun-b messenger ribonucleic acids by angiotensin-II and corticotropin in ovine and bovine adrenocortical cells. Endocrinology 130:1193–1200.

Wang X-L, Basett M, Zhang Y, Yin S, Clyne C, White PC, Rainey WE. 2000. Transcriptional regulation of human 11beta-hydroxylase (hCYP11B1). Endocrinology 141:3587–3594.

Wang Z-N, Basett M, Rainey WE. 2001. Liver receptor homologue-1 is expressed in the adrenal and can regulate transcription of 11β-hydroxylase. J Mol Endocrinol 27:255–258.

Waterman MR. 1994. Biochemical diversity of cAMP-dependent transcription of steroid hydroxylase genes in the adrenal cortex. J Biol Chem 269:27783–27786.

Waterman MR, Keeney DS. 1996. Diverse molecular mechanisms regulate the expression of steroid hydroxylase genes required for production of ligands for nuclear receptors. In: Physiological functions of cytochrome P450 in relation to structure and regulation. Jefcoate CR, editor. Greenwich, CT: JAI Press, pp. 81–102.

Waterman MR, Simpson ER. 1989. Regulation of steroid hydroxylase gene expression is multifactorial in nature. Recent Prog Horm Res 45:533–563.

Wijesuriya SD, Zhang G, Dardis A, Miller WL. 1999. Transcriptional regulatory elements of the human gene for cytochrome P450c21 (steroid 21-hydroxylase) lie within intron 35 of the linked C4B gene. J Biol Chem 274:38097–38106.

Zhou D, Quach KM, Yang C, Lee SY, Pohajdak B, Chen S. 2000. PNRC: a proline-rich nuclear receptor coregulatory protein that modulates transcriptional activation of multiple nuclear receptors including orphan receptors SF1 (steroidogenic factor 1) and ERRalpha1 (estrogen related receptor alpha-1). Mol Endocrinol 14:986–998.

Zuber MX, John ME, Okamura T, Simpson ER, Waterman MR. 1986. Bovine adrenocortical cytochrome $P\text{-}450_{17a}$: regulation of gene expression by ACTH and elucidation of primary sequence. J Biol Chem 261:2475–2485.