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Enzymes in Steroid Synthesis

THE ROLE OF TRANSCRIPTIONAL REGULATION IN STEROID HORMONE BIOSYNTHESIS

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Summary—The regulated expression of the genes encoding the various steroidogenic enzymes is a crucial component in the control of steroid hormone biosynthesis. Tissue-specific transcription of each of the steroidogenic enzyme genes determines the array of enzymes present within a steroidogenic tissue, and therefore the types of steroid hormones the tissue produces. Transcriptional regulation also determines developmental changes in the steroid hormones synthesized by steroidogenic tissues and for the quantitative regulation of steroid hormones necessary for reproduction and for maintaining physiological homeostasis. The molecular mechanisms governing transcriptional regulation of steroidogenic enzyme genes is now being studied. The results so far indicate that, like most other genes, transcription of steroidogenic enzyme genes is regulated by *cis*-elements in the 5′ flanking DNA of the genes that bind *trans*-acting proteins found in the nucleus. Several types of *cis*-elements have been identified: elements responsible for basal transcription, for induction by cAMP, and for both basal and cAMP induction. Some of the basal *cis*-elements identified may have a role in tissue-specific transcription of certain steroidogenic enzyme genes in steroidogenic tissues. We have also identified regions in both the human P450scc and human P450c17 promoters that repress transcription when activated by the Ca^{2+}/protein kinase C intracellular second messenger system used by angiotensin II. This review summarizes our current understanding of transcriptional regulation of the steroidogenic enzyme genes.

INTRODUCTION

Steroid hormones are important regulators of many physiological processes. While the mechanisms by which steroids signal their target cells are relatively well understood (reviewed in Ref. [1]) the equally important regulation of their synthesis is less clear [2, 3]. A key component in the control of steroid hormone synthesis is the regulated expression (i.e. transcription) of the genes encoding enzymes needed to produce a particular steroid hormone. This transcriptional control is responsible for the tissue-specific production of steroids, for the developmental regulation of steroid hormone production and for the quantitative regulation needed for reproduction and maintaining physiological homeostasis.

The isolation of cDNA and genomic clones for most of the enzymes involved in steroidogenesis has provided the reagents necessary for detailed analysis of the molecular mechanisms, particularly gene transcription, responsible for tissue-specific gene expression, developmental programming and quantitative regulation. From studies of the 5′-flanking DNA of a variety of the steroidogenic enzyme genes, a picture is emerging in which a complex array of known *cis*-acting DNA sequence elements (*cis*-elements) and *trans*-acting nuclear proteins, as well as previously undescribed *cis*-elements and proteins, are responsible for transcriptional regulation of these genes.

The following review summarizes this information with a focus on two key enzymes in the pathway of steroidogenesis: P450scc, the cholesterol side chain cleavage enzyme, and P450c17 which mediates 17-hydroxylase and 17,20-lyase activities. P450scc is a mitochondrial cytochrome P450 enzyme that catalyzes the conversion of cholesterol to pregnenelone by three sequential reactions, all mediated on one active site. P450scc is the first and rate-limiting step in steroid hormone synthesis; therefore, it is a critical quantitative regulator

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of steroidogenesis. P450c17 is a microsomal cytochrome P450 enzyme that catalyzes two reactions. 17-hydroxylation and 17,20-carbon bond scission, again on one active site. P450c17 is an important qualitative regulator as it occupies a crucial fork in the pathways of steroidogenesis such that expression and regulation of its two enzymatic activities directs pregnenelone to mineralocorticoids (neither activity); to glucocorticoids (17α-hydroxylation but not C-17,20 cleavage); or to sex steroids (both 17α-hydroxylase and C-17,20 cleavage). The identity, function, hormonal control, and genetics of each of the enzymatic components in steroid hormone synthesis have been reviewed recently [2].

Most of the work described in the following review was done by analyzing the expression and regulation of transfected reporter constructions. Control of transcription is usually mediated by the actions of *trans*-acting proteins, such as steroid hormone receptors, which bind to specific DNA sequences (*cis*-elements) in the 5′-flanking DNA of a gene. This can be studied by fusing portions of the 5′-flanking DNA and promoter from the gene of interest in front of the gene for an easily assayed reporter gene such as bacterial chloramphenicol acetyl transferase (CAT). These constructions are then introduced (transfected) into cultured cells using one of a variety of chemical or physical techniques, usually by coprecipitation with calcium phosphate. When this DNA construction reaches the nucleus of the transfected cells, the DNA of interest, which was fused in front of the CAT sequences, drives transcription of the CAT gene. The resulting CAT mRNA is translated into CAT protein, which can be measured using an easy and very sensitive assay. Since the half-life of the CAT mRNA is very short, the level of CAT activity accurately reflects the transcriptional power of the sequences fused in front of the CAT reporter.

Using this approach several groups have identified regions of the 5′-flanking DNA of various steroidogenic enzyme genes that are important for basal, unstimulated transcription, for regulated expression by either cAMP or Ca^{2+}/protein kinase C (PKC), or for both basal and cAMP regulated transcription.

BASAL TRANSCRIPTION

Reporter constructions driven by varying amounts of 5′-flanking DNA from a number of steroidogenic enzyme genes are expressed when transfected into steroidogenic mouse adrenal Y1 cells. Such expression demonstrates that a *cis*-element(s) that confers basal transcription must exist within the 5′-flanking DNA used; if the DNA used lacks such a *cis*-element no reporter activity would be seen. By deleting portions of the flanking DNA inserted in front of the reporter gene (usually progressively from the 5′-end) the regions necessary for basal and regulated expression of a gene can be identified. The results of such experiments with a variety of steroidogenic enzyme genes are summarized in Fig. 1. In some cases more precise experiments have isolated the *cis*-elements responsible for basal and regulated expression and are summarized in both Fig. 1 and in Table 1. Table 1 also lists *cis*-elements that have a proposed role in transcriptional regulation based on their sequence homology to known *cis*-elements, to regions in the 5′-flanking DNA of the corresponding gene in different species, or to regions in other steroidogenic enzyme genes.

The basal *cis*-elements identified so far fall into two categories. The first category of *cis*-elements only confers basal transcriptional activity. The second category of *cis*-elements is multifunctional, as they confer both basal activity and regulation by cAMP. The role of cAMP regulated *cis*-elements in conferring both basal activity and cAMP regulation was first observed with the classic cAMP response element (CRE) in the promoter for the rat gene encoding PEPCK [4]. This work demonstrated that basal promoter activity was partially determined by the presence of the CRE and by the basal concentration of intracellular cAMP.

To study the role of cAMP in basal expression of steroidogenic enzyme genes, several groups have used a set of mutant cell lines created from the Y1 adrenocortical tumor cell line. These mutant cell lines differ in the ability of cAMP to activate protein kinase A (PKA). In one of these PKA-deficient cell lines, the basal expression of transfected reporter constructions driven by the murine P450c21 promoter was significantly reduced [5]. Basal expression of the endogenous genes for murine P450scc and P450c11 in the set of mutant cell lines was directly correlated with the severity of the PKA mutation [6]. The gene for P450c11 was the most sensitive to reduced PKA activity, as it was not detected in the severest mutants. By contrast, both the endogenous P450scc gene and the transfected P450c21 gene do not have an

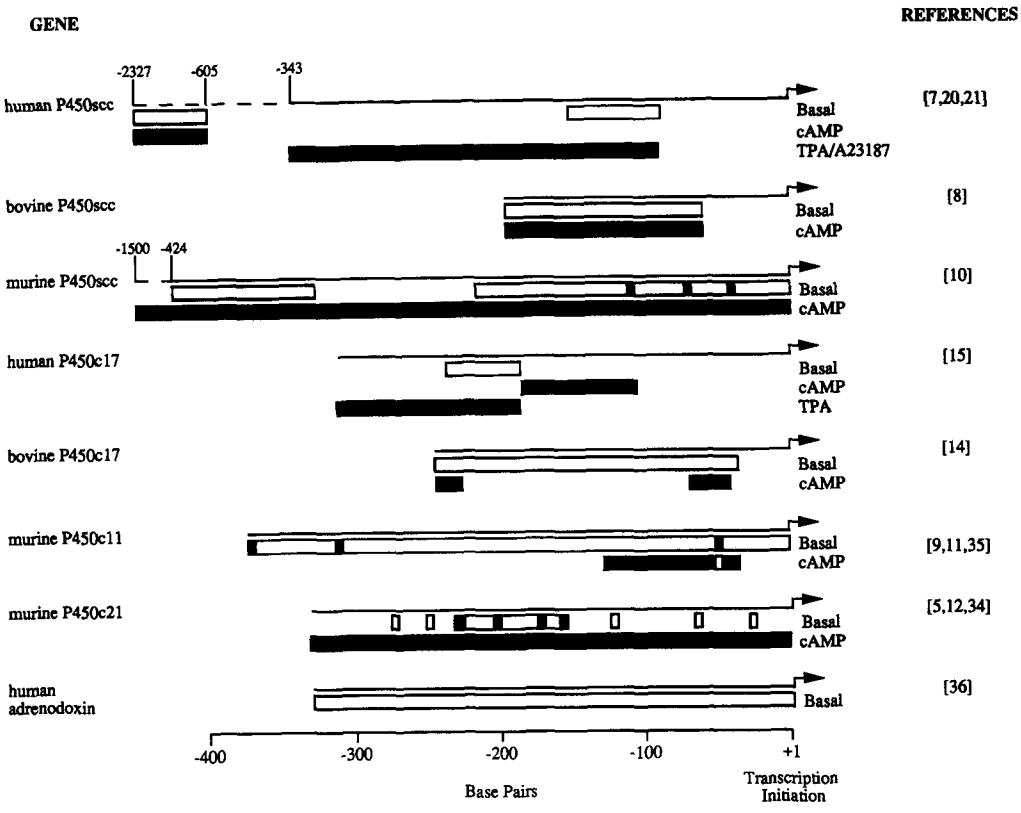

Fig. 1. Regions of the steroidogenic enzyme promoter important for transcriptional regulation. The boxed regions confer basal or regulated transcriptional activity to transfected DNA constructions containing a reporter gene. Most of the regions were identified by progressive deletions through the 5′-flanking DNA. Smaller boxes were shown to mediate the designated activity by a functional assay and are also listed in Table 1.

absolute requirement for PKA activity as their basal expression was reduced, but not eliminated, in the same cell lines that did not express P450c11 [5, 6]. These results strongly suggest that the basal state of the cAMP intracellular signalling pathway is important for basal expression of these steroidogenic enzyme genes.

While basal activity and cAMP-inducibility have been co-localized to the same regions of 5′-flanking DNA (see Fig. 1) of the human [7] and bovine [8] P450scc genes and the murine P450c21 gene, the *cis*-elements responsible have not been isolated so this dual function has not yet been studied. This is not the case for the murine P450c11 gene, in which mutation of the classic CRE element (described below) markedly decreased basal reporter activity in addition to eliminating cAMP-inducibility [9].

When a reporter construction driven by the murine P450scc promoter was transfected into the Y1 cell lines with PKA mutations, the basal activity was not reduced relative to that seen in the wild type Y1 cells [10]. As expression of the endogenous murine P450scc gene was reduced in these mutants another cAMP-regulated *cis*-element with basal activity must lie outside the 5′-flanking DNA used in the reporter construction. One possibility is that the *cis*-element is further upstream, as is seen in the human P450scc gene [7].

Basal *cis*-elements belonging to the first category (cAMP-independent) have been identified at bases −50, −73 and −126 of the murine P450scc promoter (Table 1) [10]. (Note that bases upstream from the transcription initiation site (+1) are assigned negative numbers, while those downstream receive positive numbers.) Mutations of these *cis*-elements severely reduced basal expression, but had no apparent effect on regulation by cAMP [10]. Similarly, the *cis*-elements in the murine P450c11 promoter at −380 and −320 (Table 1) also belong to this category, as mutations in these *cis*-elements reduced basal expression but had no effect on cAMP regulation [11]. In the murine P450c21 promoter a large number of basal *cis*-elements have been identified. However, none of these *cis*-elements was directly tested for a role in cAMP regulation so they may or may not be cAMP-independent basal *cis*-elements [12].

Table 1. Functional and potentially functional *cis*-acting DNA elements in the promoters of steroidogenic enzyme genes. The sequences are presented 5′ to 3′ reading towards the transcriptional initiation site (+1). The number preceding the sequence is the 5′ most base of the sequence. Bases underlined indicate that sequence homology exists with other sequences listed in the table. Such homologous sequences are grouped together

Gene	Sequence	Function[1]	Reference
Bovine P450scc	−68 CCGCCC	Homology to SP1 consensus	[8]
Human P450scc	−117 GGGGAGGAGC	Homology to SP1 consensus	[7]
Bovine P450scc	−109 TGGGAGGAGC	Homology to SP1 consensus and to sequence in human P450scc	[7]
Human P450scc	−1633TGATGTCA	Homology to CRE	[7]
	−611 TGAGTCA	Homology to TRE	[7]
Murine P450scc	−50 CTCTTAGCCTTGAGCTG	Basal, binds SF-1	[10]
Murine P450scc	−126 TCTGAGTTTGGGAG	Basal, binds SF-3	[10]
Murine P450scc	−325 TAGCCATGAGTCAGGAG	Homology to TRE consensus and binds protein in Y1 extracts	[10]
Human P450scc	−86 AGCAGGAGGAAGGAC	These sequences resemble each other and sequences in xenobiotic P450 genes.	[13]
Human P450c21	−67 AGCAGGAGGGATGGC		
Human P450c17	−287 GGAGTCA	Homology to TRE and to negative regulatory element in HIV LTR	[15]
	−256 GGAGTCA		[15]
	−164 TGAGCCAG	Homology to TRE	[15]
	−110 TGAGCTCA	Homology to CRE	[15]
Bovine P450scc	−166 TTGATG	Homology to sequence in bovine P450c17	[8]
Bovine P450c17	−243 TTGATGGACAGTGAGCAAG	Lies within cAMP responsive region and interacts with nuclear proteins	[14]
Murine P450c11	−380 CCTTAGATTTATTTTCACT	Basal	[11]
Murine P450c11	−56 TGACGTGA	cAMP regulation	[9]
Murine P450c21	−74 AGGTCA	Basal	[12]
Murine P450c21	−214 AGGTCA	Basal	
Murine P450scc	−73 GGGGAGGTCACCGCTCCA	Basal, binds SF-2	[10]
Murine P450c21	−150 GATTCTCCAAGGCT	Footprint	[12]
Murine P450c11	−320 ACCAAGGTCTTCTATGATATC	Basal. These sequences are all homologous to each other and to elements in murine c11, scc and c21 with basal activity [10–12]. In the case of the c21 genes the homology extends further than shown across species [34]	
Bovine P450c21	−251 AGGTCA		
Human P450c21	−241 AGGTCA		
Bovine P450scc	−834 AAGGTCA		
Human P450scc	−772 CCAAGGTCTT		
Murine P450c21	−128 CTGTGCCAATGTGAAA	Basal	[12]
Murine P450c21	−177 GATGGTCCCATC	Basal	[12]
Murine P450c21	−185 TTCTTCTTGA	Basal	[12]
Murine P450c21	−237 AGTTAGGAAACT	Basal	[12]
Murine P450c21	−259 TCCGCCTACT	Basal	[12]
Murine P450c21	−288 TGGTGGGAGGGG	Basal	[12]

[1]If the function has been demonstrated it is listed, otherwise the homology to known elements, or to regions of other steroidogenic genes, is given and functionality awaits demonstration.

Other candidates for cAMP-independent basal *cis*-elements include the potential SP1 binding sites in the human P450scc [7] and bovine P450scc [8] promoters. SP1 is a ubiquitous *trans*-acting protein with a role in the transcription of a number of viral and cellular genes, usually (but not exclusively) associated with "housekeeping" functions. SP1 has not been reported to mediate regulation by cAMP. In the human P450scc promoter the possible SP1 sequence is at −117 [7]; in the bovine P450scc promoter a homologous sequence is found at −109 (Table 1) and a second consensus sequence is also found at −68 [8]. It is not yet known if these putative SP1 sites are functional.

Another sequence with homology to a known basal transcription *cis*-element is found in the promoters for both human P450scc at −86 and human P450c21 at −67 [13]. These *cis*-elements resemble the basal transcription element (BTE) identified in the rat gene for xenobiotic-metabolizing P450c [13]. This BTE confers basal transcriptional activity and is also required for full induction by the *cis*-acting xenobiotic response elements [13]. The functional role of these *cis*-elements in the basal transcription of the genes for steroidogenic P450s is not yet known.

The basal transcription *cis*-elements have not yet been defined in either the human or bovine P450c17 genes, either by functional assays or by sequence homology to known *cis*-elements. However, basal expression is clearly seen with the reporter constructions used in transfection studies for both these genes [14, 15]; therefore, the 5′-flanking DNA used must contain basal transcription *cis*-elements.

TISSUE-SPECIFIC TRANSCRIPTION

The presence or absence of a given steroidogenic enzyme in a specific steroidogenic tissue is determined at the level of transcription. Northern blots and more sensitive RNase protection assays for a variety of the key steroidogenic enzymes demonstrate that they

are found only in the specific tissues that require their presence for the synthesis of the appropriate steroids [16, 17]. It is this regulated expression that results in the correct array of enzymes to produce cortisol in the human adrenal, while the rat adrenal produces corticosterone, or that is responsible for the different steroidogenic potentials of ovarian granulosa and theca cells.

Very little is known about the mechanisms that determine the tissue-specific expression of the individual steroidogenic enzymes. In other systems, such as somatotrope-specific expression of growth hormone, the presence or absence of one *trans*-acting protein, termed GHF-1 or Pit-1, confers tissue-specific expression [18, 19]. To explain the various arrays of steroidogenic enzymes in different steroidogenic tissues by this mechanism would require a complex array of such *trans*-acting proteins whose presence, absence, and possibly abundance would determine which array of steroidogenic enzymes is expressed.

The best system for studying tissue-specific expression of steroidogenic enzyme genes is P450scc, because various transformed steroidogenic cell lines continue to express it, while non-steroidogenic cell lines do not. Several groups have shown that reporter constructions driven by human [7, 20, 21], murine [10], and bovine [8] P450scc 5′-flanking DNA are expressed when transfected into adrenal Y1 cells. Such constructions also work in other steroidogenic cell lines such as human JEG-3 choriocarcinoma cells [21 and C. C. D. Moore and W. L. Miller, unpublished observations] and in testicular MA-10 cells [10 and C. C. D. Moore and W. L. Miller, unpublished observations]. By contrast, transient transfection of a variety of non-steroidogenic cell lines such as L929, HTC, Hepa I, HeLa I, CHO or Cos-I cells [20, 21 and C. C. D. Moore and W. L. Miller, unpublished observations] results in low or undetectable expression of the P450scc promoter. These results suggest that tissue-specific regulation of P450scc expression is determined, at least in part, by a *cis*-element(s) lying within the 5′-flanking DNA of the P450scc gene used in the reporter constructions, as the reporters are expressed only in the appropriate cell lines.

While the transient transfection strategy used in the studies described above can provide informative results, variations in transfection efficiency and in the expression of control constructions in different cell lines can diminish the sensitivity and reliability of the data. To avoid these problems, we are using pools of stably transfected cell lines to study the tissue-specificity of the human P450scc promoter. As the selection process results in a final population only of transfected cells, the efficiency of the initial transfection no longer influences the reporter assay. Our preliminary experiments show that constructions containing up to −2327 bp of 5′-flanking DNA from the human P450scc gene are expressed very well in Y1 cells, but that expression is undetectable in Cos-I cells [C. C. D. Moore and W. L. Miller, unpub. obs.]. This confirms our previous results with transient transfection analysis [7]. Rice *et al.* [10] found that Y1 cells stably transfected with reporter constructions driven by the murine P450scc promoter expressed this construction efficiently and that it was responsive to cAMP. By contrast, this same construction was expressed relatively poorly and was not responsive to cAMP when stably transfected into testicular MA-10 cells. Thus tissue specificity is probably conferred by multiple *cis*-elements interacting with different *trans*-acting proteins in the various steroidogenic tissues, although a role for *cis*-elements and proteins common to multiple steroidogenic tissues remains possible, and is discussed below.

Direct testing for *cis*-elements that confer tissue-specific expression of the steroidogenic enzyme genes has not been done; however, a variety of *cis*-elements necessary for basal expression has been described. As *cis*-elements that confer tissue specificity will also confer basal activity, the basal *cis*-elements described above are good candidates for tissue specificity elements. Strong candidates for steroidogenic tissue specificity are the basal *cis*-elements found in the murine genes for both P450scc (at −73) [10] and P450c21 (at −74 and −214) [12] and in the murine P450c11 gene (at −32) [11]. These *cis*-elements also have sequence homology to regions in the genes for human P450scc at −772 and P450c11 at −241 and in bovine P450scc at −834 (see Table 1).

To determine if these *cis*-elements confer tissue specificity by interacting with *trans*-acting proteins expressed only in steroidogenic cell lines, several groups have used very sensitive techniques for detecting protein–DNA interactions. Although only a limited number of cell lines was assayed, the *cis*-element at −320 that confers basal activity in the murine P450c11 gene binds a protein(s) found only in extracts

from adrenal Y1 and murine testicular MA-10 cells [11]. In addition, a *cis*-element at −140 in the murine P450c21 promoter that has a sequence similar to the P450c11 −320 *cis*-element, also binds protein only from Y1 and MA-10 cells [12]. However, similar *cis*-elements from murine P450scc at −73 and the *cis*-elements at −74 and −214 in murine P450c21 (see Table 1) also bind a protein(s) from HeLa and PC12 cells [10, 12]. The amount of this protein appears to be reduced in the non-steroidogenic HeLa and PC12 cells compared to the steroidogenic Y1 and MA-10 cells. Whether all these *cis*-elements with homologous sequence bind the same protein(s) but with varying affinities (and thus confusing the issue of tissue specificity) or to different proteins is unknown.

Other basal *cis*-elements found in the human P450scc and murine P450c11 genes may also have a role in tissue specificity. For example, the protein(s) that binds the murine P450scc *cis*-element at −50 is found only in Y1 and MA-10 cell extracts [10], and the *cis*-element at −380 in the murine P450c11 gene apparently binds a protein found only in Y1 cell extracts. However, as the authors note, the weakness of this interaction makes this conclusion suspect [11].

Some of the remaining *cis*-elements with basal activity are unlikely to have a role in tissue specificity. For example, sequences in the human and bovine P450scc promoters resemble the consensus DNA binding site for SP1. However, since SP1 is expressed in all cell types it may be necessary but not sufficient for tissue specificity. Similarly, the CRE element in the P450c11 gene that confers both basal activity and responsiveness to cAMP is also unlikely to confer tissue specificity since CREB, the *trans*-acting protein that binds to the CRE, is expressed in a wide variety of tissues. The regions responsible for the combined basal and cAMP responsive activity in the genes for murine P450c21, murine and bovine P450scc, and bovine P450c17 do not contain *cis*-elements with significant homology to the CRE and may interact with another tissue-specific protein(s) that confers both cAMP regulation and basal activity.

HORMONAL REGULATION

The production of steroids is regulated by several hormones in a pattern specific to each steroidogenic tissue. Examples exist for hormonal regulation by steroids themselves and by several intracellular signalling pathways; cGMP by atrial natriueretic peptide, cAMP by tropic hormones such as ACTH and gonadotropins, and intracellular Ca^{2+} by angiotensin II. The regulation of steroidogenic enzyme gene transcription by cAMP is the best studied of these systems, although we have recently described regions of the human P450scc and P450c17 promoters that are repressed by agents that mimic the action of angiotensin II [7, 15].

Regulation by both cAMP and Ca^{2+} occurs at many levels and can be divided into two phases. The first is an acute phase where steroid hormone production is altered rapidly, within minutes, and depends upon activation of pre-existing proteins to alter the synthesis of steroids from their precursors. The second, chronic phase, involves altered abundance of the steroidogenic enzymes and other components needed to increase or decrease the capacity of the tissue to produce the appropriate steroids.

Although mechanisms other than transcription can account for alterations in the abundance of a protein, direct evidence for increased transcription for a number of steroidogenic enzyme genes (P450scc, P450c21, P450c11, and P450c17) was initially described in bovine adrenal cells treated with ACTH [22]. More recently transcriptional runoff assays demonstrate that transcription of P450scc is also regulated by 8-Br-cAMP in testicular MA-10 cells [23]. In addition, transfection analysis of a reporter gene driven by either human P450scc [7] or P450c17 [15] 5′-flanking DNA revealed that their transcription is regulated by agents that mimic the actions of ACTH and angiotensin II in adrenal Y1 cells.

In most systems studied, the hormonal regulation of gene transcription is mediated by *trans*-acting proteins that bind to *cis*-elements in the 5′-flanking DNA of the gene to alter transcription by RNA polymerase II. Such appears to be the case for hormonal regulation of the steroidogenic enzyme genes studied so far. Figure 1 and Table 1 summarize the regions and *cis*-elements identified so far that confer regulation by cAMP and Ca^{2+}/PKC on the various steroidogenic enzyme promoters.

The cAMP-regulated transcription of many genes involves one or more *cis*-elements that resemble the consensus, TGACGTCA. These cAMP response elements (CRE) are high-affinity binding sites for the *trans*-acting protein CREB (cAMP response element binding

protein) (reviewed in Ref. [24]). CREB is apparently phosphorylated by protein kinase A in response to increased intracellular cAMP [25], in turn CREB increases gene transcription by RNA polymerase II by an as yet undetermined mechanism. Among the steroidogenic enzyme genes studied to date, a functional, classic CRE element has been identified only in the promoter of the murine P450c11 gene [9]. The region of the human P450scc promoter conferring inducibility by cAMP contains the sequence (TGATGTCA) that matches the CRE consensus at 7 out of 8 bases [7], where the underlined base indicates the mismatch. In one study this sequence bound CREB poorly [26], however the sequences flanking this altered CRE in that study differ from those in the human P450scc gene, and are also important for determining CRE function [27]. Thus the role of this CRE-like element in the human P450scc gene is unknown. cAMP regulation of the human P450c17 gene may also utilize a CRE-like sequence. The similar sequence TGAGCTCA lies within the 80 bases of the promoter in a region that confers some induction by cAMP, as shown by transient transfection analysis [15].

In other steroidogenic enzyme genes that respond to cAMP, no *cis*-elements with significant homology to the CRE, or to other *cis*-elements that confer cAMP regulation, have been found in the promoter regions responsible for cAMP induction. Within these regions only the similar *cis*-elements at −166 and −243 in the bovine genes for P450scc and P450c17, respectively were proposed to have a role in cAMP regulation but function of these *cis*-elements has not yet been demonstrated [8].

In contrast to the direct mechanism by which cAMP regulates transcription of genes with the classic CRE element that binds CREB, an indirect mechanism was proposed for the action of cAMP on bovine steroidogenic enzyme gene transcription (reviewed in Ref. [3]). In this model, cAMP would induce the expression of a labile protein(s), which in turn induced transcription of these genes. This was proposed to explain the apparently delayed cAMP induction kinetics of steroidogenic enzyme genes and the sensitivity of this induction in bovine adrenal cells to inhibition of protein synthesis by cycloheximide [22]. However, recent experiments rule out this model. cAMP induction of P450scc is not sensitive to inhibitors of protein synthesis in either human granulosa cell [28] or in mouse testicular Leydig MA-10 cells [23] and cAMP induction of adrenodoxin is similarly insensitive in placental JEG-3 cells [29]. Also, cAMP induces transcription from the promoters of human [7] or bovine [8] P450scc and bovine P450c17 [14] with very rapid kinetics that are more consistent with a direct effect of cAMP. Furthermore, inhibiting protein synthesis with cycloheximide did not block cAMP induction of the transiently transfected reporter constructions driven by either the P450scc [8] or P450c17 [14] promoters in either Y1 cells or in primary cultures of bovine adrenal cells. Thus, direct induction of P450scc and P450c17 transcription by a pre-existing protein(s) is probably responsible for cAMP regulation.

The role of protein kinase A (PKA) in induction by cAMP was studied using the adrenal Y1 PKA mutant cell lines described above. The degree of induction by cAMP for the endogenous genes for P450scc and P450c11 was significantly impaired in the mutant cell lines relative to the wild type Y1 cells [6]. Most of the induction by cAMP could be recovered by reverting the mutant phenotype by transfecting the cells with functional copies of the PKA subunits [6]. Thus, PKA is a critical component in the regulation of steroidogenic enzyme transcription in adrenal cells.

Another *cis*-acting element that can confer cAMP induction is the TPA-response element (TRE), the DNA sequence that responds to both phorbol esters such as TPA and cAMP (reviewed in Ref. [24]). TRE's bind transcription factor AP1, a heterodimer of the c-*jun* and c-*fos* proteins. The cAMP-responsive region of the human P450scc gene contains a sequence identical to the TRE consensus, TGAG/CTCA at −611 [7]. A role for this *cis*-element, or for the potential CRE at −1633, in cAMP regulation of the human P450scc gene will remain unknown until we complete further functional analysis of this region of the promoter. The absence of sequences homologous to the *cis*-elements known to confer cAMP regulation in the cAMP regulated promoters for bovine P450c17, bovine P450scc, and murine P450c21 genes suggests that cAMP regulation of these genes involves other *cis*-acting elements that may or may not bind presently known *trans*-acting proteins.

Angiotensin II regulates adrenal steroidogenesis by altering intracellular Ca^{2+} concentrations and activating protein kinase C. In studies of both the human P450scc and human P450c17 promoters we used agents that mimic

the actions of angiotensin II in the adrenal (reviewed in Ref. [30]). Treatment of Y1 adrenal cells with a combination of the Ca^{2+} ionophore A23187 and the phorbol ester TPA markedly reduced P450scc mRNA abundance after 12 h [7]. Treatment with TPA and A23187 of Y1 adrenal cells transiently transfected with a CAT reporter driven by the human P450scc promoter also repressed CAT activity. Similarly, the human P450c17 promoter was repressed by treatment with TPA [15]. Thus both the human P450scc and P450c17 promoters contain *cis*-elements that repress transcription after prolonged exposure to agonists of the PKC pathway.

While it might appear surprising that agents mimicking the action of angiotensin II would repress transcription of steroidogenic enzyme genes, our results are consistent with recent experiments studying the chronic effects of angiotensin II on adrenal steroidogenesis [31–33]. Our time-course experiment revealed that short-term, acute treatment with these agents increased transcription of the P450scc reporter construction at 60 min [7], consistent with numerous short-term studies that studied the effects of angiotensin II on adrenal steroidogenesis. The molecular mechanism for this regulation is complex. The temporal regulation described above first involves an acute increase in transcription followed by repression of transcription after chronic treatment with A23187 and TPA. The sequences of the human P450scc gene responsible for this complex behavior are not confined to one region but are spread over a large region between −343 and −89 bp. This suggests that multiple *cis*-elements are involved. No common *cis*-elements are found within this region of the human P450scc promoter, nor are any homologies apparent between this region and the region that confers repression by TPA on the human P450c17 promoter.

Transcriptional repression is a poorly understood phenomenon in higher eukaryots; few examples exist, especially for hormonally regulated repression. The repressive *cis*-elements in the human P450scc gene mapped to regions different from those responsible for basal or cAMP regulated expression. Thus, a simple model in which the *trans*-acting proteins are competing for the same *cis*-elements cannot explain this repression. The results are consistent with protein–protein interactions between the *trans*-acting proteins mediating repression and those conferring basal promoter activity [7].

CONCLUSIONS

The regulation of steroid hormone biosynthesis is an important component for the endocrine function of steroids. Hormonal control of steroidogenesis includes an acute, immediate response and a chronic, prolonged response. The acute response to a tropic hormone occurs within minutes and relies on pre-existing proteins. The chronic response of a steroidogenic tissue to prolonged tropic stimulation involves altering the expression of the genes encoding the steroidogenic enzymes, primarily by regulating transcription. Transcriptional regulation is also responsible for the tissue-specific expression and developmental regulation that results in the ability of a steroidogenic tissue to produce the correct steroids in the appropriate tissue at the right time. We are just beginning to understand the molecular mechanisms that regulate steroidogenic enzyme gene transcription and the role of *trans*-acting protein factors with their cognate *cis*-acting DNA elements within their promoters. Further understanding of the mechanisms that control transcription of these genes will require more detailed analysis of these *trans*-acting proteins and *cis*-acting DNA elements using many of the techniques mentioned in this review. Better understanding of the control for each of the individual promoters will provide the groundwork for understanding the complex, integrated regulation of the enzyme arrays required for tissue-specific, developmental, and quantitative regulation of steroid hormone production in individual steroidogenic tissues.

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