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Cell-type specificity of human CYP11A1 TATA box ☆

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Abstract

Expression of the CYP11A1 (SCC) genes, which encode the enzyme important for the first step of steroid biosynthesis, occurs in the adrenal gland and gonads, and is stimulated by cAMP. Transfection of serial deletions of the SCC promoter, which drives reporter gene expression, showed that a minimal promoter containing only the TATA box could direct cAMP-dependent transcription. Transcription factor SF1, which binds to a site next to the TATA box, can stimulate basal transcription but not cAMP response, either in adrenal cell lines or in COS-1 co-transfected with the SF1 expression plasmid. These data lead to the conclusion that the minimal promoter containing only the TATA box can drive cell type-specific, cAMP-dependent transcription. Additional experiments replacing the TATA sequence of SCC with other TATA sequences suggested that the TATA sequence itself is important for this cAMP-dependent transcription. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: P450scc; Steroidogenesis; Promoter context; OVEC system; Adrenal; Y1 cell line; H295 cell line

1. Introduction

The first and rate-limiting step for the synthesis of steroid hormones is the conversion of cholesterol into pregnenolone. This step is catalyzed by a mitochondrial electron transport system consisting of ferredoxin reductase, ferredoxin, and cytochrome P450scc (cholesterol side-chain cleavage enzyme) [1,2]. This reaction is regulated in a tissue-specific and hormonally regulated fashion [3]. The expression of both ferredoxin and P450scc is regulated by peptide hormones using cAMP as an intracellular messenger [4], while the level of ferredoxin reductase remains unchanged after stimulation [5]. Only steroidogenic tissues, including adrenal, gonad, placenta, and some parts of brain contain P450scc [6]. Ferredoxin has a wider tissue distribution; it is also found in the kidney and liver in addition to the above steroidogenic tissues.

Regulation of P450scc and ferredoxin genes is important for the maintenance of steroid secretion and many physiological processes. Regulatory elements at the 5′-flanking region of the genes have been dissected [7,8]. For the ferredoxin gene, controlling elements are located close to the basal promoter [9,10]. Besides the TATA box, two Sp1 sites play an important role in gene transcription [11]. The regulation pattern for the CYP11A1 (SCC) gene, which encodes P450scc, is more complex. Both upstream and proximal elements controlling tissue-specific transcriptional enhancement have been identified [12,13]. There are also other elements required for placental and neural specific gene expression [14,15]. The steroidogenic cell-specific transcription factor SF1 (also termed Ad4BP) [16–18] and the common transcription factor Sp1 [19] plus CREB [20] have been identified to function in cAMP-dependent transcription of the CYP11A1 gene.

Abbreviations: CAT, chloramphenicol acetyltransferase; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; TK, thymidine kinase; PCR, polymerase chain reaction; bp; base pair; the SCC gene, the CYP11A1 gene encoding cholesterol side cleavage enzyme; hC21, the oligo derived from -126/-113 of the human CYP21A1 gene; CRE, cAMP-responsive element; RSV, Rous Sarcoma Virus; TAF, TBP-associate proteins.

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Both CYP11A1 and ferredoxin genes are stimulated by cAMP at the transcriptional level through a delayed time course. It usually requires many hours for the response to take place and is thought to be a secondary effect requiring synthesis of intermediate proteins [3]. Previously we have shown that while the upstream cAMP-responsive sequence of the human SCC gene may need new protein synthesis for the cAMP response, the proximal region can respond to cAMP quickly using constitutively expressed proteins [21]. Important factors governing cAMP-dependent transcription of the CYP11A1 gene have been identified to be SF1 [18], CREB [20], and Sp1 [19]. However the mode of action of these factors in transcription has not been characterized. In this report we showed that CYP11A1 promoter contains TATA sequences that control tissue-specific and cAMP-dependent transcription of the reporter gene. Other transcription factors appear to play only an auxiliary role to enhance the effect of the TATA box.

2. Materials and methods

2.1. Cell culture

Y1 [22], and COS-1 [23] cell lines were maintained in culture as described previously. The H295 [24] cells were grown in DMEM/F12 medium supplemented with 10% fetal calf serum, 100 μ/ml penicillin, 100 μg/ml streptomycin and 2.4 g/l sodium bicarbonate.

2.2. Oligonucleotides

Sequences of SCC-117/-94, CAT 27mer, and globin 23mer were reported before [25]. Sequences of other oligos used in this report are as follows:

SCC-76/-52, *TCGACAGGACGTGAACATTTTATCAGCTTG*
SCC-60/-37, *TCGACATCAGCTTCTGGTATGGCCTTGAG*
SCC-55/-29, *TCGACTTCTGGTATGGCCTTGAGCTGGTAG*
SCC-36/-18, *TCGACTGGTAGTTATAATCTTGG*
SCC-13/-35, *CAGGGCCAAGATTATAACTACCA*
-39/-13 RSV, *GAGCTGGTAGTATTTAACTTGGCCCTG*
-13/-39 RSV, *CAGGGCCAAGTTAAATACTACCAGCTC*
CRE sense, *CTAGACCGGCTGACGTCATCAAGCT*
CRE antisense, *CTAGAGCTTGATGACGTCAGCCGGT*

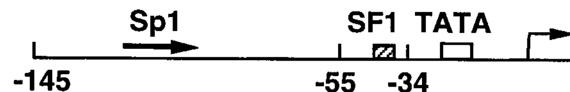

Fig. 1. Diagram of the human CYP11A1 promoter region. The transcription start site is denoted by an arrow. The TATA box and protein-binding sites are also shown.

2.3. Plasmid construction

Expression plasmids for SF1 and the catalytic subunit of protein kinase A [26] are gifts of Keith Parker and Michael Uller, respectively. Oligonucleotides SCC-117/-94, -76/-52, -60/-37, -55/-29, or -36/-18 were paired with CAT27mer in a polymerase chain reaction (PCR) using pSCC145 as a template. The PCR products were digested with XbaI at one end before insertion into the XbaI/SmaI-digested PUC13CAT vector. The inserts of the resulting plasmids, pSCC34, 55, 60, 76, and 117, were sequenced entirely to ensure their sequence and orientation.

2.4. PCR mutagenesis

Two step PCR mutagenesis from pSCC145 was performed as described before [27]. The end primers for the PCR reactions were -117/-94 and CAT27mer. The mutagenesis primers were -39/-13RSV and -13/-39RSV. The resulting PCR product was digested with XbaI at one end and inserted into XbaI/SmaI digested pUC13CAT to create p117RSV. Sequencing was performed to confirm the validity of the entire amplified region.

2.5. Assay of transfection

Effector, reporter, and internal control plasmids were transfected into cell lines using the calcium phosphate precipitation method [28]. Two days after transfection, cells were treated with 40 nM forskolin or 1 mM 8-Br-cAMP for 24 h before they were harvested. Gene expression was analyzed by CAT assay or direct RNA analysis by primer extension using established methods [9,28]. Gel mobility shift assay procedures were described before [25].

3. Results

3.1. Dissection of the CYP11A1 promoter

Since cAMP-dependent transcription appears to depend on the promoter context, we dissected the basal promoter of the CYP11A1 (SCC) gene. The proximal promoter of the SCC gene contains the TATA box, a weak Sp1-binding site [25], and the

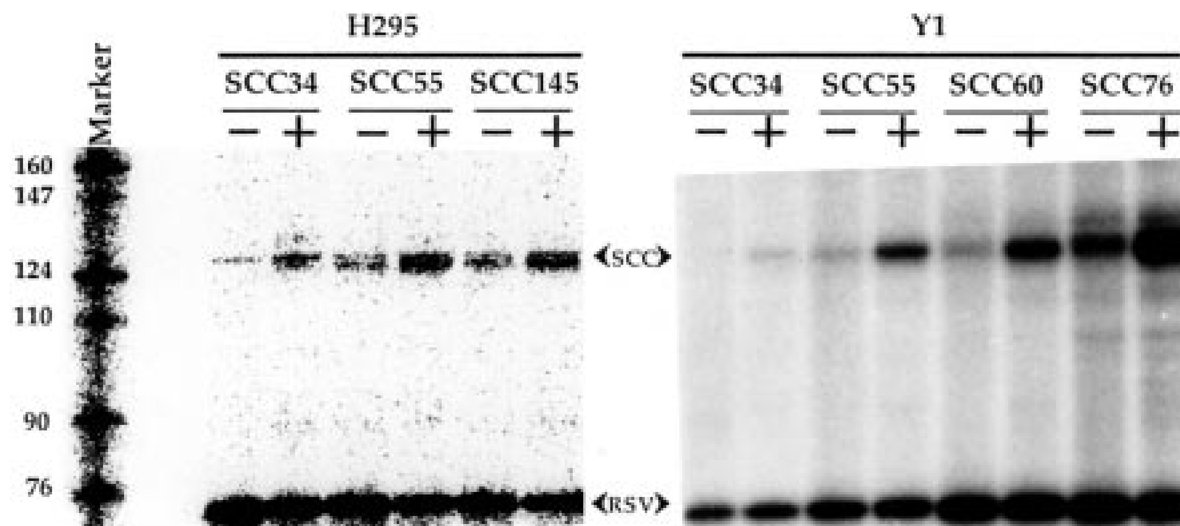

Fig. 2. Transcriptional activity of the CYP11A1 promoter with decreasing lengths of 5′-flanking sequence in Y1 and H295 cells. Test plasmids containing different lengths of the CYP11A1 promoter linked to the reporter gene CAT were co-transfected with an internal control plasmid RSVCAT into H295 or Y1 cells. Results of primer extension from the CAT RNA are shown. Transcripts initiated from the SCC and the RSV promoters are marked. Size markers are at the side of the gel.

sequence which matches the SF1-binding site (Fig. 1). Different lengths of the SCC promoter was linked to a CAT reporter gene for transfection into mouse adrenal Y1 and human adrenal H295 cells (Fig. 2). The pattern of CAT RNA expression in both cell lines was similar, with a low but detectable level of transcription driven by 34 bp of the SCC promoter. This transcription was more prominent after 8-Br-cAMP stimulation. The basal expression was enhanced when the SCC promoter was increased to 55 bp, which includes the SF1-binding site. Therefore, SF1 appeared to contribute to basal transcription of the SCC gene. This basal expression became more prominent as the length of the promoter was increased. This result indicated that the basal promoter within 34 bp of the sequence was able to direct cAMP-dependent transcription.

3.2. Function of the SCC promoter in nonsteroidogenic cells

We have shown earlier that ferredoxin expression is stimulated by cAMP only in steroidogenic cells [29]. Since P450scc is expressed only in steroidogenic cells, we tested whether cAMP induction of the SCC gene is also cell type-specific. Transcription driven by different lengths of the SCC promoter was assayed by transfection into non-steroidogenic cell line COS-1 (Fig. 3). Co-transfection of the expression plasmid for SF1 was required for the expression of the reporter gene, demonstrating the requirement of SF1 for SCC basal transcription. Co-transfection of the catalytic subunit of the cAMP-dependent protein kinase (PKA), however, could not stimulate reporter gene expression controlled by the SCC promoter, when it was 34, 55, 117, or 145 bp long (Fig. 3). This failure of cAMP induction was not an experimental artifact, as the expression of the CAT gene controlled by CRE (cAMP-responsive element) linked to the TK promoter was strongly enhanced by increasing concentrations of the PKA expression plasmid. Therefore the lack of cAMP induction in COS-1 cells indicated that the SCC promoter responded to cAMP in a cell type-specific manner.

3.3. Requirement of the TATA sequence for cAMP induction

Fig. 2 shows that 34 bp of the SCC promoter could direct cAMP-dependent transcription. Since the only important sequence in this short fragment appears to be the TATA box, we mutated the TATA sequence of the SCC promoter into that of RSV, which differs from the SCC TATA by four nucleotides (Fig. 4). RSV promoter does not respond to cAMP and are frequently used as internal controls. As shown in Fig. 4, while expression from pSCC117 was still stimulated by 8-Br-cAMP, the expression from 117RSV lost cAMP response in H295 cells. Since the sequence of p117RSV are identical to that of pSCC117 except the four nucleotides in the TATA sequence, the lack of response in p117RSV indicated that the TATA sequence per se may be important for cAMP response.

4. Discussion

In this report, we have dissected the minimal promoter of the human CYP11A1 gene. This minimal promoter contains the SF1-binding site and the TATA

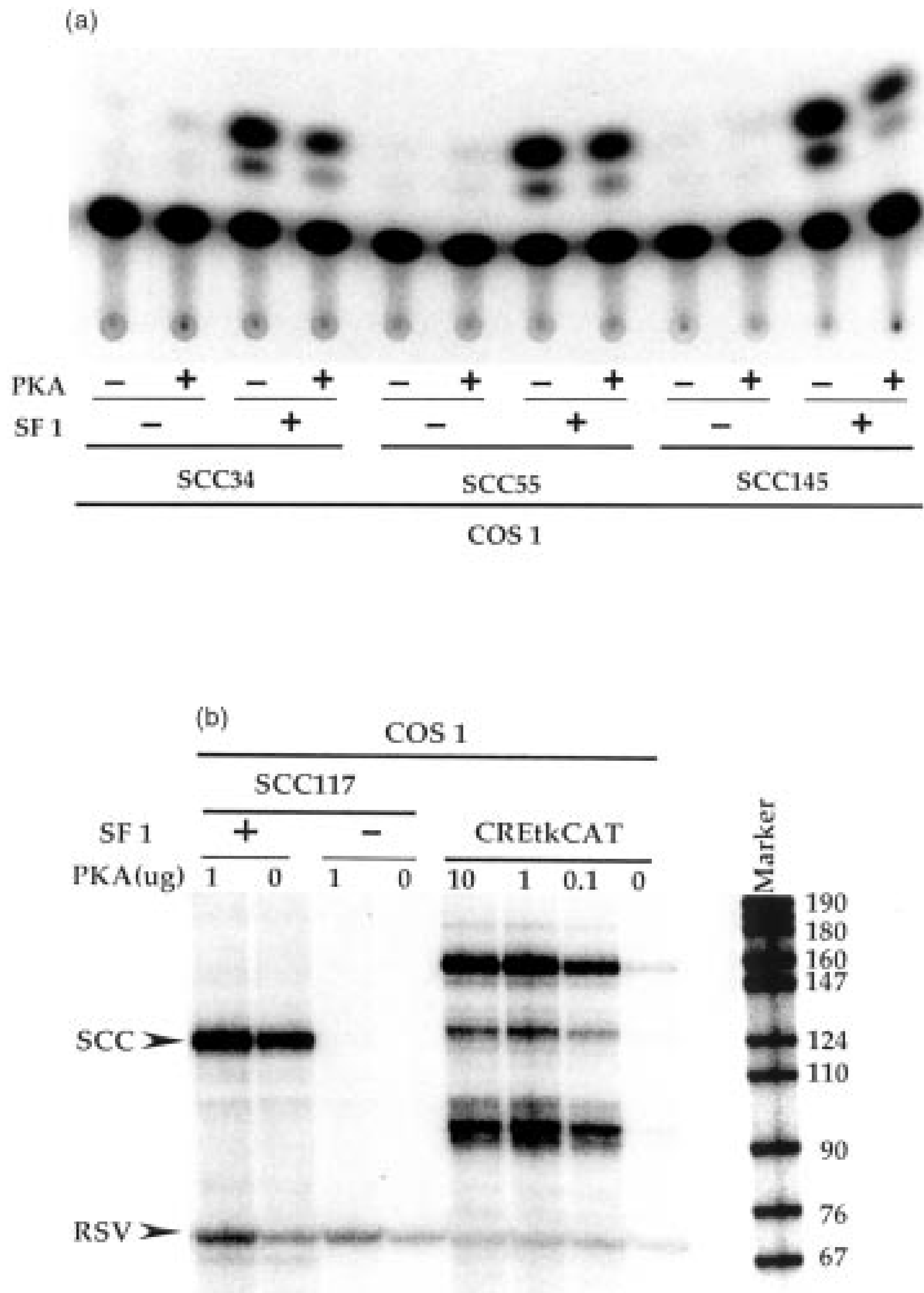

Fig. 3. Transfection of minimal promoter into COS-1 cells. Plasmids containing the CAT reporter gene under the control of 34, 55, 145, or 117 bp of the SCC promoter were co-transfected with internal control plasmid RSVCAT or RSV-β-gal in the presence or absence of expression plasmids for SF1 or the catalytic subunit of the cAMP-dependent protein kinase (PKA). Reporter gene expression was analyzed by (A) CAT activity assay or (B) primer extension of CAT RNA.

sequence. SF1 is a tissue-specific transcription factor which regulates expression of many steroid hydroxylase genes [30,31]. We showed that by supplementing COS-1 cells with SF1, the SCC promoter increased greatly in strength. SF1, however, failed to restore cAMP induction of the SCC gene in COS-1 cells. SF1 appears to augment the effect of the minimal promoter.

While SF1 controls expression of the SCC gene in the adrenal cell lines, it is absent in placental JEG-3 cells (our unpublished result). Being another steroidogenic cell line, JEG-3 apparently uses a different mechanism requiring no SF1 for the expression of SCC. This is similar to the situation in neuronal cells which also need no SF1 for SCC gene expression [15].

The minimal promoter with the TATA box of the

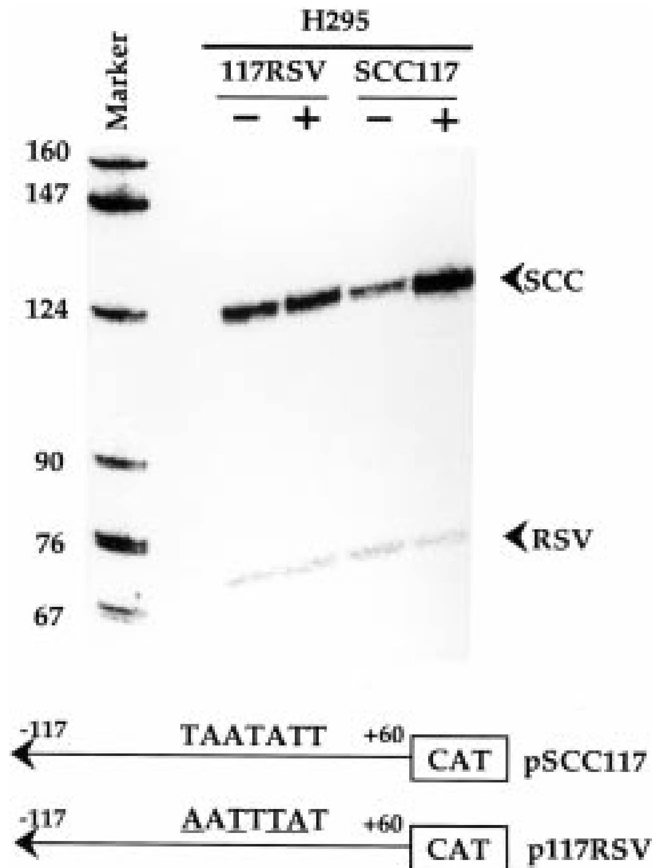

Fig. 4. Transfection of pSCC117 and p117RSV into H295 cells. Primer extension analysis of CAT RNA from H295 cells treated with (+) or without (−) 8-Br-cAMP after transfection of internal control plasmid RSVCAT and test plasmid (pSCC117 or p117RSV) are shown. Both pSCC117 and p117RSV contain the CAT reporter gene under the control of the −117 to +60 sequence of the SCC gene. The only difference between them is their TATA sequences which are spelled out with the variant nucleotides underlined. CAT RNA initiated from the RSV and SCC promoters are marked.

SCC gene sufficed for a low level of cAMP-dependent transcription. This transcription is cell type-specific, as the SCC promoter sequence does not respond to cAMP in COS-1 cells (Fig. 3). TATA sequences directing cell type-specific transcription have been described in many genes, including those encoding myoglobin [32], hsp70 [33], myelin basic protein [34], and the pituitary-specific factor GHF1 [35]. For the GHF1 gene, the TATA and its surrounding sequences are both important determinants of cell type-specific transcription [35]. The TATA sequence of the SCC gene is functionally important because replacing the SCC TATA sequence with RSV TATA abolished cAMP response (Fig. 4). This, however, does not rule out the possibility that sequences surrounding TATA may also contribute to cAMP-dependent transcription of the SCC gene.

What contributes to the specificity of the TATA box has been the focus of intensive studies. One possibility is the preference of the upstream sequence-specific transcription factors to interact with certain promoter context to bring about specificity. Some transcription factors, ATF is one example, can sequester the core promoter complex and its activity altered by changing the TATA motif [36]. Another example is the well-characterized muscle specificity of the myoglobin and the MyoD genes due to synergistic interaction between various muscle-specific transcription factors and the TATA sequence [37,38].

The other possibility is that cell type specific proteins interacting directly with the TATA box for specificity. That different tissues contain functionally non-equivalent TFIID activities has long been suggested [34]. The best known proteins binding to TATA are TBP (TATA-binding protein) and its associated proteins TAF [39,40]. There are also other proteins known to bind to AT-rich sequences [37]. The putative TATA-binding protein could either be associated with the TAF complex, or bind to DNA individually. All of the proteins in the TAF complex characterized to date are core components which are present ubiquitously [41]. The tissue-specific TAF proteins have not been characterized although their existence has been suggested. It is likely that the TATA-binding proteins present in a tissue-specific manner could play a role in activating SCC gene transcription.

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