A Novel Zinc Finger Protein TReP-132 Interacts with CBP/p300 to Regulate Human CYP11A1 Gene Expression*

Received for publication, January 5, 2001, and in revised form, May 4, 2001
Published, JBC Papers in Press, May 10, 2001, DOI 10.1074/jbc.M100113200

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The human CYP11A1 gene is expressed specifically in steroidogenic tissues and encodes cytochrome P450scc, which catalyzes the first step in steroid synthesis. A region of the 5′-flanking DNA of the gene from nucleotides −155 to −131 (−155f−131) is shown to activate transcription in steroidogenic human placental JEG-3 (1) and adrenal NCI-H295 cells. Using this region of the gene as probe, a cDNA clone of 4.4 kilobase pairs was isolated by screening JEG-3 cell and human placental cDNA expression libraries. The open reading frame encodes three zinc fingers of the C4H2 subtype, and separate regions rich in glutamate, proline, and glutamine, which are indicative of a DNA-binding protein involved in gene transcription. Expression of the cDNA in vitro and in HeLa cells yields a protein of 132 kDa, which concurs with the predicted size. Northern blot analysis demonstrates expression of two TReP-132 transcripts of 4.4 and 7.5 kilobase pairs in the thymus, adrenal cortex, and testis; and expression is also found in the steroidogenic JEG-3, NCI-H295, and MCF-7 cell lines. Immunocytochemistry analysis demonstrates localization of the HA-tagged TReP-132 protein in the nucleolus. Expression of exogenous TReP-132 in HeLa cells was demonstrated to interact with the −155f−131 region in band-shift analysis. Transfection of the cDNA in placent JEG-3 and adrenal NCI-H295 cells increases expression of a reporter construct controlled by the P450scc gene 5′-flanking region from nucleotides −1676 to +49. Moreover, a chimeric protein generated by fusion of TReP-132 with the Gal4 DNA-binding domain was able to significantly increase promoter activity of a reporter construct via Gal4-binding sites upstream of the E1b minimal promoter. Coexpression of CREB-binding protein (CBP)/p300 with TReP-132 has an additive effect on promoter activity, and the proteins were demonstrated to interact physically. Thus, these results together indicate the isolation of a novel zinc-finger transcriptional regulating protein of 132 kDa (TReP-132) involved in the regulation of P450scc gene expression.

Cytochrome P450scc is a mitochondrial enzyme that catalyzes the conversion of cholesterol to pregnenolone and is the first step in the synthesis of all steroid hormones (reviewed in Ref. 2). The CYP11A1 gene, which encodes P450scc, is expressed in steroidogenic tissues, which include the adrenal, ovary, testis, placenta, and brain (3, 4). In each of these tissues, steroid hormones play important physiological roles, which include regulation of tissue development, progression of secondary sexual characteristics, and maintenance of homeostasis. The hormonal regulation and developmental pattern of expression of P450scc are specific to each steroidogenic tissue; adrenocorticotropic increases steroidogenesis and accumulation of P450scc transcript in the human adrenal cortex, and similar effects are seen with luteinizing hormone and follicle stimulating hormone in human ovarian granulosa cells, and with luteinizing hormone and human chorionic gonadotropin in human testicular Leydig cells (5–7). In each case, interaction of the tropic hormone with the cell surface receptor activates G proteins leading to activation of the cAMP-dependent protein kinase A and Ca2+/protein kinase C pathways, which regulate P450scc gene transcription (8).

The human P450scc cDNA has been cloned and the gene mapped to chromosome 15q22.3-q23 (9, 10). Preliminary studies on the 5′-flanking region of the gene demonstrated the ability of a 2.5-kb DNA fragment to confer basal and cAMP responsive activity when transiently transfected into mouse adrenal Y1 tumor cells (11–13). Subsequent studies in mouse Leydig MA-10 (14), I-10 (15), human placenta JEG-3 (16), and adrenal NCI-H295 (17) cells identified regions of the 5′-flanking DNA that conferred basal promoter activity and response to cAMP. However, it is apparent that different elements are utilized in a cell type-specific manner. The cAMP-responsive element identified in human JEG-3 cells differs from the element identified when studies were performed in mouse Y1, MA-10, and I-10 cells (16, 18). In these mouse cell lines, cAMP-responsive elements were localized to sites in the vicinity of nucleotides −1620 and −600, whereas cAMP response in JEG-3 cells was conferred by a proximal region around nucleotide −117 (18, 19); however, neither of these elements were functional in NCI-H295 cells (17). Positive cis-acting elements have also been identified, which confer expression of P450scc gene promoter activity in steroidogenic cell lines, but not in nonsteroidogenic cells. However, it is also apparent that some elements, such as those found between nucleotides −1931 and −1822, can increase promoter activity in Y1 and MA-10 cells,

* This work was supported by Operating Grant MT-12901 from the Medical Research Council of Canada and by Scholarship 980149 from the Fonds de la Recherche en Sante du Quebec (FRSQ) (to D. W. H.).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank$$, EBI Data Bank with accession number(s) AF297872.

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1 The abbreviations used are: kb, kilobase pair(s); bp, base pair(s); PCR, polymerase chain reaction; TBS, Tris-buffered saline; uORF, upstream open reading frame; ORF, open reading frame; UTR, untranslated region; HA, hemagglutinin; EMSA, electrophoretic mobility shift assay; PAGE, polyacrylamide gel electrophoresis; DAPI, diamidino-2-phenylindole; PBS, phosphate-buffered saline; GST, glutathione S-transferase.
but not in NCI-H295 and JEG-3 cells (20). In other studies, the region between −155 and −131 was found to significantly increase promoter activity in JEG-3 cells (1, 16).

These putative cis-acting elements in the 5′-flanking region of the human P450succ gene have been demonstrated to interact with multiple proteins, which include CREB/ATF (19), NF1- and Sp1-like proteins, and in particular the steroidogenic factor-1 (SF-1). The orphan nuclear receptor SF-1 also known as adrenal binding protein (Ad4BP) has been demonstrated to promote cell specific expression of the human P450succ gene promoter in Y1 cells (19, 21, 22). The human P450succ gene promoter is inactive in nonsteroidogenic CV-1 cells, but could be activated by expression of exogenous SF-1 (21, 23). In addition to the interaction of the P450succ gene 5′-flanking DNA with known proteins, the putative cis-acting elements have also been found to interact with unidentified presumably novel transcription factors. The positive element between −155 and −131 (−155/−131) was shown to interact specifically with at least two different proteins; however, the identity and role of neither of these proteins are known. Based on the many previous studies on P450succ gene expression, which demonstrate the cell type specific usage of cis-acting elements and involvement of multiple trans-acting factors, it is obvious that a clear understanding of the mechanisms involved in the regulation of expression of this important gene will require identification and characterization of the transcription factors involved. Recently, Huang et al. (24) identified two proteins, LBP-1b and LBP-9, which interact with the −155/−131 element and regulate reporter activity via this element.

In the present study, a novel protein TReP-132 was cloned by screening a placental cDNA expression library with a region of the P450succ gene 5′-flanking region from nucleotides −155 to −131 (−155/−131). Analysis of the predicted primary structure reveals a protein of 1200 residues, which contains three zinc fingers of the Cys2His2 subtype. TReP-132 also has other motifs characteristic of transcriptional regulating proteins including regions rich in glutamine, proline, and acidic residues. The TReP-132 primary structure contains two LXXLL motifs, which suggests the interaction of the protein with nuclear receptors such as SF-1 to regulate gene expression. The encoded protein with an apparent molecular mass of 132 kDa was shown to interact with the −155/−131 element by electrophoretic mobility shift assay (EMSA). Transient expression of this protein increased expression of a reporter construct controlled by the 5′-flanking region of the P450succ gene from nucleotides −1676 to +49. As well, the −155/−131 element was found to confer response to TReP-132. The interaction of TReP-132 with DNA, its subcellular localization in the cell nucleus, and its ability to increase promoter activity indicate its function as a transcriptional regulating protein. To further understand the mechanism by which TReP-132 regulates gene expression, it was shown to interact with the coregulator protein CBP/p300 to synergistically increase promoter activity. Transcripts encoding TReP-132 are expressed predominantly in the adrenal gland and testis, as well as steroidogenic cell lines, which is consistent with the potential role of this factor to regulate steroid synthesis. However, TReP-132 transcripts are also expressed in several other tissues examined, including the thymus, stomach, and heart, which indicates additional roles for TReP-132 other than regulation of steroid synthesis.

MATERIALS AND METHODS

Isolation of TReP-132 cDNA—A human placenta cDNA expression library (catalog no. HL3007b, CLONTECH, Palo Alto, CA) was screened with multimerized copies of a double-stranded oligonucleotide probe corresponding to nucleotides −155 to −131 of the human P450succ gene 5′-flanking DNA. Following hybridization of equal amounts of the sense and antisense oligonucleotides, they were multimerized with T4 DNA ligase and end-labeled with [α-32P]dATP and [α-32P]dGTP (PerkinElmer Life Sciences) using Klenow fragment (New England Biolabs, Beverly, MA). The agt11 library was screened as described (25) using a method for Southwestern blot analysis (1). After screening ~2 × 10^6 recombinants, three positive clones were selected using the oligonucleotide TReP-132 clone (P1). A ZAP Express cDNA library was made using JEG-3 cell mRNA according to the manufacturer's protocol (Stratagene, La Jolla, CA), and an additional 4 × 10^5 recombinants were screened with the P1 cDNA but yielded only 10 partial clones, which did not contain the initiating codon. An additional 2 × 10^6 recombinants from another human placenta agt11 cDNA library (kindly provided by Dr. Morag Park, McGill University) were screened to isolate 6 clones including P1-CL6 which contains the putative initiator codon. To subclone the full-length TReP-132 cDNA for subsequent studies, an KpnIEcoRI fragment from the P1-CL6 cDNA in the Blue-script KS+ vector (Stratagene, La Jolla, CA) was ligated to the EcoRI/XbaI fragment of P1-CL4 in the pcDNA3 vector (Invitrogen, Carlsbad, CA) (Fig. 2A). Both strands of the TReP-132 cDNA were each sequenced at least twice, by dyeoxynucleotide sequencing (Amersham Pharmacia Biotech, Baie d’Urfe, Quebec, Canada). The sequence has been deposited to the GenBank data base and has been assigned accession number AF297872.

Northern Blot Analysis—JEG-3 cell total RNA was isolated by the Tri reagent acid phenol method (Molecular Research Center Inc., Cincinnati, OH). Poly(A) RNA was subcloned into the Bluescript KS+vector. A radiolabeled riboprobe was generated using [α-32P]UTP and the MAXscript in vitro transcription kit from Ambion (Austin, TX) according to the manufacturer's protocol. The probe was generated following lineazation of the TReP-132-HA cDNA in the pcDNA3 vector by digestion with NotI, and transcription from the Sp6 promoter. The transcribed probe was a 226-bp cRNA consisting of 174 bases from the 3′ end of the TReP-132 cDNA and 52 bases from the polylinker including the HA sequences. The 5′ S riboprobe used for normalization was generated from the pTri-18S-Human vector from Ambion and produces a protected fragment of 80 bp. RNase protection assays were performed using the Ribonuclelease Protection Assay II kit from Ambion, according to the manufacturer's protocol. RNA from different cell lines (20 μg) or yeast tRNA (20 μg) was hybridized to the probes at 45 °C, and then digested for 1 h at 37 °C at a dilution of 1:75 (BRL). The protected RNase digestion sample. The samples were resolved on a 6% urea-polyacrylamide gel.

In Vitro Transcription/Translation, Coimmunoprecipitation, and Western Blot Analysis—Proteins were synthesized by in vitro transcription/translation using rabbit reticulocyte lysate (TNT coupled in vitro system, Promega, Madison, WI) from plasmids containing a T7 promoter, according to the manufacturer's protocol with [35S]methionine. [35S]Labeled proteins were separated by SDS-polyacrylamide gel electrophoresis, and the gel was dried and exposed to film for autoradiography.

To assess the apparent molecular mass of TReP-132, protein was expressed both in vitro and in vivo. Whole cell lysate of HeLa cells transfected with TReP-132 tagged with the HA epitope (TReP-132-HA) or with pcDNA3 alone, and the product of in vitro transcription/translation performed with the TReP-132-HA cDNA as template (no template control) were resuspended in 2× electrophoresis sample buffer. Total protein was resolved by 8% SDS-PAGE and then transferred to nitrocellulose membrane (Bio-Rad). Membranes were preincubated 1 h in blocking solution containing 5% powdered milk in Tris-buffered saline (TBS), then incubated overnight at 4 °C with a rabbit polyclonal anti-HA antibody (1/15,000, Covance, Richmond, CA). After three washes with 0.2% Tween 20 in PBS, membranes were incubated with a horseradish peroxidase-coupled anti-rabbit antibody (1/10,000, Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) for 45 min. After three washes with 0.2% Tween 20 in PBS and one wash with 2% Tween 20 in 10× TBS, development was performed with the Renaissance ECL Plus kit (PerkinElmer Life Sciences) according to manufacturer's protocol.

To assess if TReP-132 coimmunoprecipitates with p300, 1 × 10^6 HeLa cells were plated on a 100-mm dish and incubated for 24 h. Cells were transfected using 40 μl of ExGen 500/5 μg of the p300 construct and 5 μg of the TReP-132 construct tagged with a Flag epitope (TReP-132-Flag). Immunoprecipitation was performed according to the procedure described by Santa Cruz Biotechnology (Santa Cruz, CA). Cells
were performed using 10^6 HeLa cells (2 × 10^5 cells/well) for 2 h at 150 V. Gels were dried and exposed to film for 10 min in the dark at room temperature with 0.1 μl dilactate DAPI (Molecular Probes Inc.). Cells were thoroughly washed with water, allowed to dry and were mounted as described in the Prolong Anti-Fade kit (Molecular Probes Inc.). For visualization, a Leica DMRB epifluorescence microscope (Leica Inc. Deerfield, IL) was used in combination with standard photography. Typically, exposure times varied between 0.5 and 15 s with a Kodak Gold 100 ASA film.

**Tissue Culture**—Human NCI-H295 adrenal tumor cells, choriocarcinoma JEG-3 cells, cervical HeLa cells, and transformed primary embryonal kidney HK-293 cells were obtained from American Type Culture Collection (Rockville, MD). NCI-H295 and JEG-3 cells were cultured in monolayer as described previously (17) in equal amounts of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (Life Technologies, Inc.) supplemented with 1% insulin, transferrin, selenium (Roche Molecular Biochemicals), 1% red phenol, 100 μM E2, and 100 μM HOCortisone. JEG-3 and HeLa cells were cultured in minimal essential medium containing 10% and 5% fetal bovine serum (Hyclone, Logan, UT), respectively. HK-293 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. All medium were supplemented with glutamine (2 mM), penicillin (55 units/ml), and streptomycin (55 μg/ml).

**Plasmids**—The reporter plasmid 3X(−155/−131)TK32Luc comprises three copies of the 155/−131 element generated by annealing and multimerizing the oligonucleotides: 5′-GATCTCGTCTCAAGATGCAGAG-3′ and 5′-GGCGAATGTTTATGCTGAGTGAGTTGCGCATG-3′. Three copies of this element was then cloned upstream from the minimal HSV thymidine kinase promoter (TK32) into the BamHI site of the pLUC plasmid. The P450scc luciferase reporter construct −1676/+48sccLuc contains a fragment of the human P450scc gene, which spans from nucleotide 1676 at the 5′ end to nucleotide +49 at the 3′ end. The DNA fragment was amplified from PCR from a P450scc genomic clone kindly provided by Dr. Bon-chu Chung (Academia Sinica, Nanking, Taipei, Taiwan) using oligonucleotides that introduced a KpnI site and BglII site at the 5′ and 3′ ends, respectively. Following amplification, the PCR product was digested with KpnI and BglII and then subcloned into the pGL3 reporter plasmid (Promega, Madison, WI). The TReP-132 HA vector, containing the entire cDNA of TReP-132 or luciferase tagged with the HA epitope, were created by amplifying by PCR the TReP-132 or luciferase cDNA with oligonucleotides that introduced a KpnI site at the 5′ end and a HA site and an XbaI site at the 3′ end. The PCR products were digested with KpnI and XbaI and subcloned into the pcDNA3 expression vector (Invitrogen, Carlsbad, CA). The pcDNA3-GAL4 vector was generated by subcloning a Haemoglobin DNA binding domain containing the GAL4 DNA binding domain (amino acids 1–147) from the pSG424 plasmid (a gift from Dr Michael R. Green, Howard Hughes Medical Institute Research Laboratories, University of Massachusetts Medical Center, Worcester, MA) into the corresponding sites of the pcDNA3 expression vector. The GAL4-TReP-132 plasmid was created by subcloning a PCR product corresponding to the entire coding region of TReP-132 into the pcDNA3-GAL4 vector.

The P1 clone 2 plasmid used in GST pull-down analysis was created by inserting a PCR fragment corresponding to amino acids 751–1200 of TReP-132, fused in-frame downstream of GST sequences in the pGEX-2TK vector (Amersham Pharmacia Biotech). All the constructs made were verified by dideoxynucleotide sequencing using Sequenase 2.0 (Amersham Pharmacia Biotech).

**Immunofluorescence and Subcellular Localization of TReP-132**—HeLa cells (2 × 10^4) were plated on culture slides (Becton Dickinson, Franklin Lakes, NJ) and incubated for 16 h. Transfection was then performed with Exgen 500 (MBI Fermentas, Flamborough, Ontario, Canada) according to manufacturer’s instructions. Twenty hours after transfection, cells were washed three times with cold PBS, then fixed for 15 min with formalin (Sigma). Cells were washed three times with PBS before permeabilization with PBS containing 0.5% Triton X-100 (Sigma) for 15 min at room temperature. After three washes with PBS, cells were incubated for 1 h in blocking buffer (6% powder milk, 3% bovine serum albumin in TBS) to minimize unspecific binding of antibodies. Slides were then incubated with the rabbit anti-HA primary antibody (Covance) in binding buffer (blocking buffer diluted 1:10 with TBS) at a 1:100 dilution, for overnight at 4°C. Subsequently, the slides were washed twice with PBS containing 0.2% Tween 20 and twice with PBS. The Alexa Fluor 594 goat-anti-rabbit secondary antibody (Molecular Probes Inc., Eugene, OR) was added at a 1:1000 dilution in binding buffer, and slides were incubated for 1 h at room temperature in the dark. Washes were done as described for the primary antibody. Diaminobenzidine-2-phenyindole (DAP) counterstaining was done by incubating slides for 10 min in the dark at room temperature with 0.1 μl dilactate DAPI (Molecular Probes Inc.). Cells were thoroughly washed with water, allowed to dry and were mounted as described in the Prolong Anti-Fade kit (Molecular Probes Inc.). For visualization, a Leica DMR-B epifluorescence microscope (Leica Inc. Deerfield, IL) was used in combination with standard photography. Typically, exposure times varied between 0.5 and 15 s with a Kodak Gold 100 ASA film.
were transfected with ExGen 500 (MBI Fermentas, Flamborough, Ontario, Canada) at a ratio of 8 μl of ExGen 500/1.5 μg of DNA. NCI-H295 cells were transfected with Effectene transfection reagent (Qiagen, Mississauga, Ontario, Canada) at a ratio of 1 μg of DNA to 25 μl of Effectene. Following transfection for 12 h, the cells were incubated in fresh medium for 12–36 h.

For luciferase assays, cells were lysed by the addition of 250 μl of lysis buffer (0.8% Triton X-100, 25 mM glycylglycine, pH 7.8, 15 mM MgSO₄, 4 mM EGTA) and incubated for 15 min. Fifteen μl of the cell lysate was assayed using the Luciferase assay system from Promega Corp. (Madison, WI) in a Berthold Lumat LB9501 luminometer. All experiments were normalized by cotransfecting 0.1 μg of a β-galactosidase expression plasmid driven by the Rous sarcoma virus promoter. β-Galactosidase assays were performed using 10 μl of lysate in the Galacto-Light Plus assay system (Tropix, Bedford, MA).

For the transfection of each cell type, the efficiency of transfection

FIG. 2. A, schematic of a subset of the cDNA clones isolated. P1, the initial partial cDNA purified, was used as a probe to isolate the subsequent clones. The clones represented by a bar are denoted by names on the left. The shaded area on the right represents the portion of the 3′-untranslated region that is found only in the longer transcript. The regions denoted I and II were amplified by PCR using oligonucleotides denoted by the arrows. The resulting amplified cDNA products were radiolabeled and used as probes in Northern blot analysis. The shaded region denoted by II was amplified by PCR to assess the size of the 3′-untranslated region in clone P1-CL1 and was found to be ~2 kb in length. The EcoRI site used to construct the full-length TReP-132 cDNA from clones P1-CL4 and P1-CL6 is denoted by the vertical arrows. The initiation codon (ATG) and the stop codon (TAA) are denoted and the location of the C₂H₂ zinc finger motifs are represented by the closed black boxes. The lengths of the cDNAs are drawn to scale. B, Northern blot analysis of TReP-132 transcripts. 2 μg of JEG-3 cell mRNA was separated on a 1.1% agarose formaldehyde gel, and the blot was hybridized with a 5′ probe and a 3′ probe, depicted in panel A as regions I and II, respectively.

FIG. 3. A, Northern blot analysis of mRNA from human tissues. Each lane contains 2 μg of mRNA, and the blot was hybridized with a radiolabeled probe, which contains nucleotides 532–1035 of TReP-132. The blot on the left was exposed for 48 h and the blot on the right for 96 h to X-Omat film at ~70 °C. The panels on the bottom show the results of hybridization of the same blots with a β-actin probe. B, RNase protection analysis of TReP-132 transcripts in cell lines. 20 μg of total RNA from the different cell lines were hybridized with a radiolabeled TReP-132 riboprobe in combination with a 18 S riboprobe for normalization. Following digestion with RNase, samples were separated on a 6% urea-polyacrylamide gel to resolve the protected fragments. Transcript expression was detected in all the steroidogenic cell lines examined with the highest level in JEG-3 cells.
was verified by a green fluorescence protein expression plasmid, and the efficiencies obtained were 60% or better.

**In Vitro Protein Binding Assay**—To ascertain the interaction between CBP/p300 and TReP-132 in vitro, fragments of CBP polypeptides from different regions of the protein were expressed as GST fusion proteins and were immobilized on glutathione-coupled Sepharose as described by Frangioni and Nell (29) prior to incubation with radiolabeled TReP-132 protein. The 35S-labeled TReP-132 protein was produced from the P1 clone 2 expression construct (as described above) using rabbit reticulocyte lysate and T7 RNA polymerase (Promega). GST-CBP fusion proteins were produced from cDNA constructs in E. coli BL21 following induction with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside. Equal quantities of GST-CBP fusion proteins were incubated with glutathione-coupled Sepharose for 30 min at 4°C in binding buffer (100 mM NaCl, 1 mM EDTA, pH 8, 20 mM Tris, pH 8.0, 0.5% Nonidet P-40). The beads were then washed three times with PBS and once with binding buffer, and were then incubated with radiolabeled TReP-132 for 2 h at 4°C in the same buffer. After incubation, the beads were washed five times with washing buffer (20 mM Tris, pH 8, 500 mM NaCl, 5 mM EDTA, pH 8.0, and 0.1% Triton X-100). Bound proteins were released from the Sepharose beads by boiling in SDS sample buffer and were analyzed by SDS-PAGE (30). The gels were stained with Coomassie Blue to ascertain that equal amounts of GST proteins were loaded, after which the gels were incubated for 30 min with Amplify (Amersham Pharmacia Biotech), dried, and exposed to film.

**RESULTS**

**Isolation of TReP-132 cDNA Clone**—The promoter region of the human P450scc gene contains a sequence between nucleotides 155 and 131 (155/131) that was previously demonstrated to confer increased transcriptional activity when fused to a heterologous HSV thymidine kinase minimal promoter (TK32). Three copies of this element conferred activation of TK32 promoter activity of 500- and 80-fold when the reporter construct was transfected transiently in human placental JEG-3 (1) or human adrenal NCI-H295 cells, respectively (Fig. 1). However, promoter activation was significantly less in non-steroidogenic cervical HeLa cells and was not observed in kidney HK293 cells. In a previous study, this element was also demonstrated to interact specifically with at least two proteins in JEG-3 cell nuclear extract, as determined by electrophoretic mobility shift assay (EMSA) (1, 16). To isolate cDNA clones that encode these putative transcription factors, a human placenta cDNA expression library was screened with multimerized copies of the 155/131 DNA fragment, and a positive clone (P1) of 1.6 kb was isolated (Fig. 2A). The P1 cDNA has a coding sequence in frame with the β-galactosidase fusion protein in the gt11 cloning vector. The open reading frame in P1 encodes 451 residues with a primary structure that contains an acidic region, a region rich in prolines, and two zinc finger motifs, which suggest the isolation of a DNA-binding protein involved in gene transcription.
The TReP-132 protein tagged with the HA epitope at the carboxyl end. Total protein was resolved by 8% SDS-PAGE, and bands were revealed with an anti-HA polyclonal antibody. The first lane (left to right) contains HeLa cell lysate obtained from cells transfected with the TReP-132-HA cDNA in the pcDNA3 vector; the second lane is the negative control of HeLa cell lysate obtained from cells transfected with the empty pcDNA3 vector; the third lane is the negative control of in vitro transcribed/translated protein in the presence of the pcDNA3 vector only, and the fourth lane contains in vitro transcribed/translated protein produced by rabbit reticulocyte lysate from the TReP-132-HA cDNA subclone in the pcDNA3 vector. B, translation of truncated TReP-132 protein from templates containing a wild type or mutated initiator AUG. Translation of TReP-132 cDNA containing the wild type or mutated initiator codon was truncated by restriction with either SmaI or PstI as indicated, and proteins were produced by in vitro transcription/translation. Mutation of the AUG codon to AUC yielded shorter protein, which is indicative of the mutated AUG codon being the initiator methionine. There are 44 residues between the initiator AUG and the next in-frame AUG in the coding region. The expected number of residues in the predicted proteins are as indicated in the parentheses on the right of the bottom panel.

Northern blot analysis of JEG-3 cell mRNA probed with the 1.6-kb P1 cDNA revealed two hybridizing transcripts of ∼4.4 and 7.5 kb (data not shown). To isolate the full-length cDNA, the initial library and a subsequent human placenta cDNA library (kindly provided by Dr. Morag Park, McGill University) were screened using the 1.6-kb P1 cDNA as probe to obtain a clone that contains the entire coding region. From analysis of the 22 clones isolated (of which only a subset is depicted in Fig. 2A), it is apparent that two classes of clones were isolated. Based on the sizes of the clones and the overlap of sequences, it can be deduced that some clones contain a 3'-untranslated region, which is ∼2.5 kb longer. To determine if different lengths of the 3'-untranslated region can explain the two bands seen in Northern blots, a probe from the 5’ end of clone P1-CL4 hybridized to the same sized transcripts of 4.4 and 7.5 kb as seen with the P1 cDNA, whereas a probe from the 3’ end of clone P1-CL13 hybridized only to the transcript of 7.5 kb (Fig. 2B). As well, PCR amplification of the 3’ end of clones P1-CL13 and P1-CLF using a 5’ primer immediately upstream of the poly(A) tail in P1-CL4 and a 3’ primer immediately upstream of the poly(A) tail of P1-CL13 yielded a product of 2 kb (data not shown), thus indicating that this region accounts at least in part for the larger transcript observed in Northern blots. In support of the coexistence of these two transcripts, which may arise from differential polyadenylation sites, a consensus adenylation signal AAATAAA is found 20 and 21 nucleotides upstream of the poly(A) tail in P1-CL4 and a 3’ primer immediately upstream of the poly(A) tail of P1-CL13 yielded a product of 2 kb (data not shown), thus indicating that this region accounts at least in part for the larger transcript observed in Northern blots. In support of the coexistence of these two transcripts, which may arise from differential polyadenylation sites, a consensus adenylation signal AAATAAA is found 20 and 21 nucleotides upstream of the poly(A) tail in P1-CL4 and a 3’ primer immediately upstream of the poly(A) tail of P1-CL13 yielded a product of 2 kb (data not shown), thus indicating that this region accounts at least in part for the larger transcript observed in Northern blots.
tain the expression of TReP-132 transcript in established cell lines, RNase protection assays were performed with a probe of 226 bp derived from the 3′ end of the cDNA clone. As found in the tissues, varying levels of transcript are expressed in the cell lines examined. However, expression is most prominent in the steroidogenic JEG-3 and MCF-7 cells, and very low expression was detected in the non-steroidogenic HepG2 and HK293 cells (Fig. 3B).

**Primary Structure of TReP-132**—The open reading frame of TReP-132 comprises 3600 nucleotides, which encode a polypeptide of 1200 residues with a predicted molecular mass of 132 kDa. Analysis of the primary structure of TReP-132, which was predicted from nucleotide sequence, revealed several motifs that suggest the function of this protein as a DNA-binding protein involved in gene transcription (Fig. 4). The region between residues 252 and 343 contains several glutamine-rich sequences, the regions between residues 553 and 574 and between residues 252 and 343 contains several glutamine-rich motifs, which in other proteins have been shown to be involved in protein interaction and DNA binding. Two sequences LRQLL and LEMLL are also found, which match the canonical LXXLL sequence that has been implicated in interaction with nuclear receptors. Based on the primary structure of TReP-132, it would be reasonable to predict that the protein interacts with other factors to regulate gene transcription.

When expressed in *vitro* using rabbit reticulocyte lysates, the P1-CL4 cDNA encodes a protein with an apparent molecular mass of 132 kDa. Analysis of the primary structure of TReP-132, which was predicted from nucleotide sequence, revealed several motifs that suggest the function of this protein as a DNA-binding protein involved in gene transcription (Fig. 4). The region between residues 252 and 343 contains several glutamine-rich sequences, the regions between residues 553 and 574 and between residues 984 and 1003 contain many prolines, and the region between amino acids 956 and 982 contains 17 glutamate residues, all of which have been found in other proteins to be involved in transcriptional activation. TReP-132 contains three C6H2-type zinc finger motifs, which in other proteins have been shown to be involved in protein interaction and DNA binding. Two sequences LRQLL and LEMLL are also found, which match the canonical LXXLL sequence that has been implicated in interaction with nuclear receptors. Based on the primary structure of TReP-132, it would be reasonable to predict that the protein interacts with other factors to regulate gene transcription.

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**Subcellular Localization of TReP-132 Protein**—Considering that TReP-132 is a putative DNA-binding protein, fluorescent immunocytochemistry was performed to establish its subcellular localization. A construct that expresses a chimeric TReP-132 protein with an HA tag at its carboxyl terminus was transfected into HeLa cells, and subcellular localization of the protein was detected by an anti-HA antibody. In agreement with this, RNase protection analysis demonstrates the expression of endogenous TReP-132 in HeLa and JEG-3 cells.

**Effect of TReP-132 on Human P450scc Gene Promoter Activity**—To determine if TReP-132 has an intrinsic ability to regulate transcription, a chimeric protein was generated by fusion of the yeast Gal4 DNA-binding domain to the amino terminus of full-length TReP-132. Transient expression of this protein significantly increased promoter activity of a reporter plasmid controlled by five Gal4-binding sites upstream of the E1b minimal promoter. This activation was not observed with expression of the Gal4 DNA-binding domain or TReP-132 proteins alone, thus demonstrating the ability of the DNA interacting Gal4-TReP-132 fusion protein to specifically activate transcription (Fig. 8A).

To determine if TReP-132 can affect promoter activity of the P450scc gene, the cDNA subcloned into pCDNA3 was cotransfected transiently with a luciferase reporter construct (−1676/−49sccLuce) in human placental JEG-3 and adrenal NCI-H295 cells. The 5′-flanking region of the human P450scc gene from nucleotides −1676 to −49 conferred basal promoter activity in both cell types; and coexpression of the 132-kDa protein had a dose-dependent stimulatory effect, which activated transcrip-
A, intrinsic transcriptional activity of TReP-132. A chimeric construction composed of the GAL4 DNA-binding domain fused to the amino end of TReP-132 (GAL4-TReP-132) was generated and cotransfected into HeLa cells with 1 μg of the pFR-LUC reporter plasmid containing five GAL4 elements in front of the minimal E1b promoter. Control transfections were performed with the GAL4 DNA-binding domain alone (GAL4) or the native TReP-132 expression construct. The results are expressed as relative light units of luciferase activity. Transfections were normalized to β-galactosidase activity, expressed from a cotransfected plasmid. Values are the mean of five independent experiments ± S.D.

B, regulation of human P450scc gene promoter activity by TReP-132. Human placenta JEG-3 (left panel) and adrenal NCI-H295 (right panel) cells were transiently cotransfected with the 1676/+49sccLuc reporter construct and increasing amounts of the construct expressing TReP-132. The results are expressed as relative light units of luciferase activity. Transfections were normalized to β-galactosidase activity, expressed from a cotransfected plasmid. The values are the mean of four independent experiments each performed in triplicate ± S.D.

C, activation of the heterologous TK32 promoter via the cis-acting element. Human placenta JEG-3 (left panel) and adrenal NCI-H295 (right panel) cells were transiently cotransfected with the 33888 inefficient TK32Luc reporter construct and increasing amounts of the construct expressing TReP-132. The results are expressed as -fold increase of luciferase activity over the value obtained from the empty TK32 vector alone. Transfections were normalized to β-galactosidase activity, expressed from a cotransfected plasmid. The values are the mean of four independent experiments ± S.D.
tion of transcriptional activity. Human placenta JEG-3 cells were transiently cotransfected with 0.1 μg of the 3x(-155/-131)/TK32Luc reporter construct and TReP-132, p300, E1A, RG2, or a combination of these proteins as indicated on the bottom of the graph. The results are expressed as fold increase of luciferase activity over the value obtained from the empty ATK32 vector alone. Transfections were normalized to β-galactosidase activity, expressed from a cotransfected plasmid. The values are the mean of four independent experiments ± S.D. p300 interacts with TReP-132 to increase promoter activity. p300-mediated transactivation was inhibited by coexpressed E1A, but not by the E1A-defective mutant RG2.

Interaction of TReP-132 with CBP/p300—Several well characterized transcription factors have been shown to interact with the coregulators CBP/p300 in vivo. To ascertain if TReP-132 can functionally interact with the coregulators, it was coexpressed with p300 in JEG-3 cells. Expression of TReP-132 or p300 separately increases expression of 3x(-155/-131)/TK32Luc. However, the coexpression of both proteins interact additively to further promote expression via the -155/-131 element (Fig. 9). Similar results were also obtained in NCI-H295 cells (data not shown). The specificity of p300-induced activation was demonstrated by co-expression of E1A with TReP-132, which inhibited the effect of p300. As well, the co-expression of R2G (E1A mutated at residue 2, which abolishes interaction with TReP-132) did not affect the activating function of p300.

To determine if TReP-132 physically interacts with CBP/p300, GST fusion proteins, which contain different regions of CBP, were bound to glutathione-coupled Sepharose and incubated with 35S-labeled protein produced from the P1-CL2 cDNA. The protein encoded by P1-CL2, which contains residues 499–1200 of TReP-132 interacts with the region from amino acids 1460 to 1891 of CBP, but no interaction was observed with the following regions: 1–451, 451–721, 721–1100, 1099–1460, and 1892–2441 (Fig. 10A).

To assess if TReP-132 interacts with p300 in intact cells, expression plasmids encoding p300 and TReP-132 tagged with the FLAG epitope were cotransfected into HeLa cells. Immunoprecipitation of p300 from total cell extract using an anti-p300 antiserum followed by Western blot analysis using an anti-FLAG antibody revealed the interaction between these two proteins in vivo (Fig. 10B).

DISCUSSION

The regulation of P450scc gene expression is a key determinant of steroid synthesis in steroidogenic tissues, considering that the encoded cytochrome P450scc catalyzes the first step in the steroid synthesis pathway. The tissue-specific expression of P450scc transcript (2, 31) and its regulated expression in response to peptide hormones (8, 32, 33) demonstrate the importance of transcriptional regulation on P450scc levels. Previous analysis of the 5′-flanking region of the human P450scc gene isolated a cis-acting element between nucleotides −155 and −131, which activated transcription from the P450scc gene promoter and from the heterologous hsa thymidine kinase gene promoter, when reporter plasmids were transfected into human placental JEG-3 cells. As well, proteins from JEG-3 cell nuclear extract interacted with the −155/-131 element to form protein/DNA complexes in EMSAs (1, 16). To characterize the transcription factors, which are potentially involved in P450scc gene regulation, human placenta and JEG-3 cell cDNA expression libraries were screened in the present study to isolate clones that interact with the −155/-131 cis-acting element. A first clone P1 was isolated based on DNA interaction, and subsequent expression of the full-length TReP-132 cDNA in HeLa cells confirmed interaction of this protein with the −155/-131 DNA fragment by EMSA.

Nucleotide sequence analysis of the TReP-132 cDNA clone revealed an open reading frame of 3600 bases preceded by a 5′-untranslated region (5′-UTR) of 563 nucleotides, which contains 10 ATG sequences prior to the putative initiator codon. However, the nucleotide context of the predicted initiator AUG (AGAGACAGCAUGG) is the most homologous to the consensus sequence GCCGCGCA/GCCAUGG for initiator codons, as defined by Kozak (34–36). Eight of the 10 upstream open reading frames (uORFs) in the 5′-UTR also provide a termination site before the main ORF; and the ninth uORF has a termination site TGA found 3 nucleotides after the initiator codon. Since 9 of the 10 ATGs in the 5′-UTR lie in poor sequence contexts for translation initiation, it is possible that they are inefficiently recognized or ignored by scanning ribosomes (37, 38). However, it is also possible that these upstream ATGs are recognized by scanning ribosomes, in which case they can modulate initiation of the main ORF (39–41). Alternatively, as has been shown for S-adenosyltransferasemethionine decarboxylase (42, 43) and yeast GCN4, peptides from the uORF can modulate initiation of translation. To confirm that the main ORF of TReP-132 starts at the predicted initiator AUG, mutation of this codon in the context of the 5′-UTR led to translation of a shortened protein.

Upon analysis of the different cDNA clones isolated during the screening process to obtain full-length TReP-132, it was apparent that there exist at least two types of TReP-132 transcripts, which differ in length at the 3′-UTR. The shorter of the 3′-UTRs is 217 nucleotides in length between the stop codon and the poly(A) tail, and the other is ~2.5 kb longer (data not shown). In both cases, a consensus nuclear polyadenylation signal is found at the appropriate position upstream of the poly(A) tail. On Northern blot analysis, a cDNA probe from the coding region hybridized to two transcripts of ~4.4 and 7.5 kb. However, a probe from the longer 3′-UTR hybridized only to the 7.5-kb mRNA, which indicates that this region accounts for at least part of the longer transcript. The 3′-UTR of several transcripts have been shown to be involved in modulating mRNA stability as well as affecting the efficiency of translation (44, 45); thus, it will be interesting to decipher the role of the two 3′-UTRs in the TReP-132 transcripts. Considering the existence of different 3′-UTRs and the usual length of the 5′-UTR, it is tempting to speculate that the two ends in the
TReP-132 transcripts may interact to affect the efficiency of translation (46–48). Northern blot analysis of mRNA from different human tissues shows differential levels of the two TReP-132 transcripts; however, the physiological relevance of two transcripts of different lengths encoding TReP-132 remains to be elucidated.

During the screening process to isolate the full-length TReP-132 cDNA, a partial clone that was missing a region of the 5’ end was isolated, but which also had a deletion of the codons encoding residues 546–628. A recent examination of the GenBank data base revealed the homology of TReP-132 with a sequence (accession no. AJ277275) that is identical except for the extra sequences in TReP-132 encoding residues 546–628. Thus, it is apparent that there are isoforms of TReP-132; however, the functional and physiological significance of these will require further studies. As well, it is tempting to speculate that the presence of the insert in TReP-132 is the result of alternative splicing of pre-mRNA.

Transient expression of exogenous TReP-132 increased expression of the −1676/+49sccLuc reporter plasmid in a dose-dependent manner in JEG-3 and NCI-H295 cells, indicating the ability of this protein to activate P450scc gene transcription. Transient transfection of the TReP-132 cDNA in these cells also significantly increased, in a dose-dependent manner, the expression of the 3×(−155/−131)TK32Luc reporter plasmid, thus demonstrating that the −155/−131 element confers response to TReP-132. The fusion of TReP-132 with the DNA-binding domain of the Gal4 protein was also able to activate transcription from the E1b promoter, via interaction with upstream Gal4 binding sites, thus demonstrating the intrinsic activity of TReP-132 to induce promoter activity. These results, along with the subcellular localization of TReP-132 in the nucleus, indicate the ability of this novel protein to regulate gene transcription.

Analysis of the TReP-132 primary structure of 1200 residues revealed sequences that further support the role of this protein in regulating gene expression. The protein contains three zinc finger motifs of the C2H2 type, which is found in many transcription regulatory proteins (49) including Sp1 (50), WT1 (51), NGFI-A (52), MBP-1 (53), MBP-2 (54), and Krox-20 (55), and have been demonstrated to interact with DNA. The zinc finger motifs are usually found as concatemers in distinct domains in many proteins; however, TReP-132 contains a single motif in the central region of the protein and two at the carboxyl terminus. Interestingly, polypeptides with only one (56) or two zinc fingers have also been demonstrated to complex with DNA (57). In addition to DNA binding, domains containing CzH2 zinc fingers have been implicated in nuclear localization (58), pro-
tein-protein interaction (59, 60), and DNA bending (61). Interestingly, mutations in the C-terminal zinc fingers of the Ikaros proteins, which are also of the C4-H2 subtype, ablate Ikaros protein interactions and decrease the ability of these proteins to bind DNA and activate transcription (62). In addition to the zinc finger domains, TReP-132 contains motifs that have been implicated in transcriptional activation in other proteins. A large region between amino acids 252 and 343 is rich in glutamine residues, which have been shown in several proteins including Sp1 (50) and Oct-1 (63) to confer activation of transcription. The region between residues 553 and 574 is proline-rich, which has been shown to form an important activation domain in CTF (64) and Oct-2 (65); and the region between residues 956 and 1003 contains acidic residues and is rich in prolines, where such a domain is shown to be important in p53 function (66). These characteristic regions, which form activation domains in various proteins, have been shown to mediate increased transcription by interacting with basal transcription proteins of the RNA polymerase II protein complex or with other intermediary transcription factors. The presence of these sequences in TReP-132 suggests its potential interaction with other proteins, required to regulate gene expression.

It has recently been shown that CBP/p300 is involved in the regulation of human P450sec gene promoter activity (67); thus, the present study also addressed the potential interaction between TReP-132 and CBP/p300 involved to regulate gene expression. CBP and p300 are ubiquitous transcriptional integrator proteins, which have been demonstrated to mediate the activity of many factors such as MyoD (68), AP-1 (69), SRC-1 (70, 71) p65 (72), and the nuclear receptors estrogen receptor, (73), RAR, RXR, and TR (74, 75). The coexpression of exogenous TReP-132 and p300 in JEG-3 cells demonstrated at least an additive activation of the 3×(−155/−131)/TK32Luc reporter plasmid, which is indicative of a functional interaction between the two proteins to regulate P450sec gene expression via the −155/−131 element. Human p300 was identified initially by its ability to bind the adenosivial E1A oncoprotein (76) that can transform primary cells, block cellular differentiation, and inhibit certain transcriptional enhancer elements. Binding of E1A to CBP/p300 has been shown to abolish or down-regulate the stimulatory effects of c-Fos (77), c-Jun, JunB (78), MyoD (68), and CREB (79, 80). In this study, the specificity of activation by p300 was ascertainment by cotransfection of E1A, which inhibited the effects of exogenous p300 and TReP-132 expressed individually or in combination. The positive effect of only TReP-132 alone on 3×(−155/−131)/TK32Luc was also inhibited by E1A, which most likely is mediated by inhibition of endogenous p300. In agreement with previous studies, which show that mutation of E1A at position 2 can abolish interaction with p300 and alleviate its inhibitory effect, RG2 is shown to be unable to inhibit the stimulatory effect of TReP-132 and p300 on 3×(−155/−131)/TK32Luc expression. The results of GST pull-down and immunoprecipitation analyses further demonstrate the interaction between TReP-132 and CBP. In pull-down experiments, the region of TReP-132 from residues 439 to 1200 (P1-CL2) interacts with the region of CBP between residues 1460 and 1891, which has also shown to interact with numerous other transcription factors including SF-1 (67, 81).

In addition to having domains potentially implicated in DNA binding and activation of transcription, TReP-132 also has several regions rich in leucine residues including two LXXL sequences, which have been proposed to interact with nuclear receptors. It has been reported that the proteins including p/CIP, NCoA-1, NCoA-2, RIP-140, SRC-1, CBP, and RAC3, which interact with nuclear receptors, contain a consensus core LXXL sequence motif in their nuclear receptor interaction domains (82–87). The presence of the LXXL sequences in TReP-132 suggests that it can also interact with nuclear receptors such as SF-1, which has been demonstrated to regulate P450sec gene expression (67, 88–95). Thus, with the recent finding that SF-1 interacts with CBP/p300 (67, 96), and the present demonstration of TReP-132–CBP/p300 interaction, it is tempting to speculate on the interaction between these three factors.

Considering that nuclear receptor-associated coactivators such as SRC-1 (97), TIF2 (98), and RAC3 (99) are capable of mediating the activity of several receptors, it is possible that TReP-132 can associate with different nuclear receptors to regulate gene expression. It has been postulated that the negative cross-talk observed between nuclear hormone receptors and AP-1, which all interact with CBP/p300, may be the result of competition for interaction with limiting amounts of integrator protein (69). However, recent studies have also indicated a role of CBP/p300 for mediating the positive cross-talk between nuclear hormone receptors and p45/NF-E2 (100), as well as mediating the activity of SRC-1 to enhance steroid receptor-dependent transcription (70, 73). Similarly to SRC-1, which interacts with CBP/p300 and the estrogen receptor to synergistically stimulate receptor-dependent transcription, an interaction of TReP-132 with SF-1 and CBP/p300 may be a mechanism by which this complex can exert an effect on gene expression.

The expression of TReP-132 predominately in the adult human adrenal gland and testis, is consistent with its ability to regulate P450acc gene expression. However, it remains to be determined if TReP-132 can also regulate the expression of other genes involved in steroid synthesis such as P450c21, P450c11AS, P450c17, P450 aromatase, and 3β-HSD II (101). Ongoing experiments show that expression of the human P450c17 gene promoter is activated by TReP-132 and that the activation by SF-1 is also mediated by CBP/p300 in NCI-H295 cells. Although TReP-132 is expressed at high levels in the adrenal gland and testis, the detection of high levels of transcripts in the thymus and of lower levels in the other tissues examined suggests additional roles for this factor. Considering that there are apparently several isoforms of TReP-132, it will be important to determine if there is tissue type or cell type specific expression of the different TReP-132 proteins. It is possible that the isoforms are differentially expressed in different tissues and have distinct physiological functions.

REFERENCES

1. Hum, D. W., Aza Blane, P., and Miller, W. L. (1995) DNA Cell Biol. 14, 451–463
2. Miller, W. L. (1988) Endocr. Rev. 9, 295–318
3. Mellon, S. H., and Descheppe, C. F. (1993) Brain Res. 629, 283–292
4. Stromstedt, M., and Waterman, M. R. (1995) Brain Res. Mol. Brain Res. 34, 75–88
5. Waterman, M. R., John, M. E., and Simpson, E. R. (1998) in Cytochrome P-450 (Ortiz de Montellano, P. R., ed) pp. 345–396, Plenum Press, New York
6. Simpson, E. R., and Waterman, M. R. (1988) Annu. Rev. Physiol. 50, 427–440
7. Hanakoglou, I. (1989) in Follicular Development and the Ovulatory Response (Tafriri, A., and Dekel, N., eds) pp. 233–252, Ares-Serono Symposia, Rome
8. Simpson, E. R., Lund, J., Ahlgren, J., and Waterman, M. R. (1990) Mol. Cell. Endocrinol. 70, C25–C28
9. Chung, B., Matteson, K. J., Voutilainen, R., Mohandas, T. K., and Miller, W. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8962–8966
10. Durocher, F., Morissette, J., and Simard, J. (1998) Pharmacogenetics 8, 49–53
11. Inoue, H., Higashi, Y., Morohashi, K., and Fujii-Kuriyama, Y. (1988) Eur. J. Biochem. 171, 435–440
12. Moore, C. C. D., Brentano, S. T., and Miller, W. L. (1990) Mol. Cell. Biol. 10, 6013–6023
13. Inoue, H., Watanabe, N., Higashi, Y., and Fujii-Kuriyama, Y. (1991) Eur. J. Biochem. 195, 563–569
14. Hum, D. W., Staels, B., Black, S. M., and Miller, W. L. (1993) Endocrinology 132, 546–552

2. D. W. Hum, P. Gizard, unpublished data.
A Novel Zinc Finger Protein TReP-132 Interacts with CBP/p300 to Regulate Human CYP11A1 Gene Expression

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J. Biol. Chem. 2001, 276:33881-33892.
doi: 10.1074/jbc.M100113200 originally published online May 10, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100113200

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