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Biochemistry

cAMP regulates P450scc gene expression by a cycloheximide-insensitive mechanism in cultured mouse Leydig MA-10 cells

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ABSTRACT **Mouse MA-10 Leydig tumor cells synthesize and secrete progesterone in response to human chorionic gonadotropin, luteinizing hormone, and cAMP but may not synthesize androgens. Maximal doses of human chorionic gonadotropin, ovine luteinizing hormone, forskolin, or 8-bromoadenosine 3′,5′-cyclic monophosphate, stimulated cytochrome P450scc mRNA accumulation 1.5- to 3-fold and progesterone secretion 10- to 100-fold in MA-10 cells. P450scc mRNA increased by 2 hr and was maximal by 8 hr; polymerase run-on experiments showed this was due to increased P450scc gene transcription. MA-10 cells are a hormonally homogeneous population, as all cells expressed P450scc mRNA and responded to cAMP equally. cAMP-stimulated accumulation of P450scc mRNA continued in the presence of cycloheximide. Gonadotropins stimulated testicular steroidogenesis by coordinate cAMP-induced increases in P450scc gene transcription, mRNA accumulation, and P450scc activity. We cloned rat P450c17 cDNA and showed it detected no P450c17 mRNA in control or cAMP-stimulated MA-10 cells by RNA transfer blots or RNase protection assays. Similarly, HPLC detected no 17α-hydroxyprogesterone or testosterone synthesis in MA-10 cells. Thus MA-10 cells, unlike untransformed Leydig cells, do not express detectable amounts of P450c17 mRNA or P450c17 activity.**

Conversion of cholesterol to pregnenolone, the rate-limiting step in steroidogenesis, is mediated by cytochrome P450scc, and its two electron-transport intermediates, adrenodoxin (an iron/sulfur protein) and adrenodoxin reductase (a flavoprotein) (for review, see ref. 1). Tropic hormones, by way of cAMP, stimulate P450scc mRNA accumulation in primary cultures of bovine (2) and human fetal (3, 4) adrenal cells; human fetal testis cells (5); human (4, 6, 7), rat (8), and bovine (9) ovarian cells; human placental cells (4); and transformed JEG-3 choriocarcinoma cells (10). P450scc mRNA accumulates in human granulosa and trophoblast cells by a cycloheximide-insensitive mechanism (7, 11), whereas bovine adrenocortical and human JEG-3 cells are sensitive to cycloheximide (1, 2, 10). The basis of these differences is unknown.

Pregnenolone is converted to dehydroepiandrosterone by a single protein, cytochrome P450c17 (12, 13). Human beings (14) and cattle (15) have a single P450c17 gene, transcribed in adrenal glands and testes (16). Tropic hormones, by way of cAMP, regulate the abundance of P450c17 mRNA in adrenal glands and testes (2–5), but human granulosa cells (6) and trophoblasts (7) and rat adrenal glands (6) lack detectable P450c17 mRNA. Primary cultures of mouse, rat, and pig testicular interstitial cells synthesize P450scc protein in response to cAMP (17–19). In these cells, cAMP stimulates P450scc synthesis about 2-fold and testosterone synthesis about 10-fold. Similarly, cAMP increases P450scc mRNA accumulation 2.5-fold in cultured human fetal testicular cells (5). However, primary cultures of whole testes and of interstitial cells are inherently heterogeneous, thus Leydig cell steroidogenesis may be influenced by other cells. Therefore, we studied P450scc gene expression in homogeneous mouse Leydig MA-10 tumor cells, which synthesize progesterone in response to luteinizing hormone (LH), human chorionic gonadotropin (hCG), and cAMP (20). The completeness of the androgen synthetic pathway in these cells has been uncertain. 17α-Hydroxylase activity and cAMP-stimulated increased secretion of 17α-hydroxyprogesterone, androstenedione, and testosterone have been reported, suggesting P450c17 is present (20). We report that the regulation of P450scc gene transcription and mRNA accumulation by tropic hormones and cAMP occurs by a cycloheximide-insensitive mechanism in MA-10 cells. HPLC analyses of steroids synthesized and secreted from these cells show they do not synthesize or secrete 17α-hydroxyprogesterone, androstenedione, or testosterone, suggesting they lack 17α-hydroxylase activity. This was proven by cloning rat ovarian P450c17 cDNA* and showing that MA-10 cells lack P450c17 mRNA detectable by RNA transfer blot analysis or by RNase protection experiments.

MATERIALS AND METHODS

MA-10 cells were cultured as described (20). For experiments, medium containing serum was replaced with serum-free medium with or without hormones (20). For experiments involving cycloheximide, cells were stimulated with cycloheximide (20 μg/ml) 30 min before and during cAMP stimulation.

RNA was isolated from cytoplasmic cell extracts as described (21). mRNA abundances were determined by slot and Northern blots (22), using human P450scc cDNA (23), human P450c17 cDNA (16), and rat P450c17 cDNA probes. Low-stringency hybridizations were in 20% (vol/vol), formamide/5× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate)/5× Denhardt's solution (0.1% bovine serum albumin/0.1% polyvinylpyrrolidone/0.1% Ficoll)/salmon sperm DNA (100 μg/ml)/0.1% $NaDodSO_4$ at 37°C, and blots were washed for two 15-min periods in 0.5× SSC/0.1% $NaDodSO_4$ at 37°C. RNase protection experiments were performed as described (24) using our rat P450c17 cDNA clone.

Nuclear run-on experiments were performed as described (25) except that the hybridization buffer contained 20% formamide. Filters were washed for two 15-min periods at 37°C in 2× SSC/0.1% $NaDodSO_4$, in 0.5× SSC/0.1% $NaDodSO_4$, and in 2× SSC, treated with RNase A (0.1 μg/ml) at 37°C for 15 min, and then washed in 2× SSC/0.1% $NaDodSO_4$ at 20°C and autoradiographed. Transcription was estimated by densitometric scanning of the autoradiograms.

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Abbreviations: hCG, human chorionic gonadotropin; LH, luteinizing hormone; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M27282).

Heat-denatured linearized plasmids were applied to nitrocellulose, baked at 80°C for 2 hr, and prehybridized for at least 16 hr (25).

For *in situ* hybridizations, 10,000 MA-10 cells per cm^2 of a multiwell microscope slide (Miles Scientific) were stimulated with 1 mM 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP), fixed, and treated with protease K (26). Cells were acetylated (27) and then prehybridized in buffer (26) containing heparin (140 μg/ml), *Escherichia coli* DNA (50 μg/ml), and poly(A) (25 μg/ml). Slides were covered with a coverslip, hybridized in the same buffer containing ^3H-labeled human P450scc RNA (100 pg/μl) at 37°C overnight. Slides were washed for two 15-min periods at 37°C in 2× SSC and in 0.5× SSC, for 15 min in RNase buffer (10 mM Tris·HCl, pH 7.9/300 mM NaCl/5 mM EDTA) at 37°C, then treated with RNase A (1.0 μg/ml) in RNase buffer at 37°C for 30 min, washed in 2× SSC at 20°C, rinsed quickly in 70% and 95% ethanol, and air-dried. Slides were coated with Ilford K-5 nuclear emulsion (diluted 1:1 with water), stored at 4°C, and developed in 2 weeks.

We screened a rat ovarian cDNA library in λgt10 (Clontech) using the 285-base-pair *Hin*cII–*Pvu* II fragment of hP450c17 cDNA encompassing the highly conserved steroid- and heme-binding domains (16, 28), under low-stringency conditions. In one million cDNA clones, one partial rat P450c17 cDNA was obtained. This cDNA was subcloned into pBluescript (Stratagene) and analyzed by supercoil sequencing (29).

MA-10 cells were treated with 1 mM 8-Br-cAMP or vehicle for 6 hr and then incubated for 2 hr with 10^6 cpm of HPLC-purified [^3H]progesterone. Medium and cells were extracted separately in 10 ml of methylene chloride for 30 min. The organic phase was washed twice with 1 ml of H_2O and dried under nitrogen, and the residue was resuspended in 100 μl of the appropriate mobile phase for HPLC. Radioactivity in aliquots from 375-μl fractions was measured. Elution times were established by monitoring the absorbance at 210 nm of added unlabeled standards (see Fig. 5). Monitoring at the maximal absorption of testosterone, 240 nm, yielded identical elution times for all added standards. Culture medium was also analyzed for progesterone by using an RIA kit (Cambridge Medical Diagnostics).

RESULTS

Hormonal Stimulation of P450scc. When MA-10 cells were stimulated with maximally effective doses of hCG (25 ng/ml), forskolin (10 μM), or cAMP (1 mM), they accumulated a single 2.0-kilobase P450scc mRNA (Fig. 1). Stimulation with cAMP (3-fold) was greater than that with hCG (1.6-fold). 8-Br-cAMP stimulated progesterone synthesis 100-fold, whereas hCG and ovine LH stimulated progesterone synthesis only 20-fold (Table 1). This amount of hCG and ovine LH stimulation of progesterone secretion was less than that described (20, 31). The MA-10 cells used in these studies also bound less ^{125}I-labeled hCG than described (20). Therefore, the lesser effect of hCG compared to cAMP was due to cellular desensitization after long-term culture. 8-Br-cAMP induced P450scc mRNA accumulation and progesterone secretion rapidly. Both increased significantly by 2 hr and reached maximal values by 8 hr (Fig. 2). The effect of cAMP on progesterone secretion was acute, whereas the effect on P450scc mRNA was long-term and did not immediately result in increased progesterone secretion.

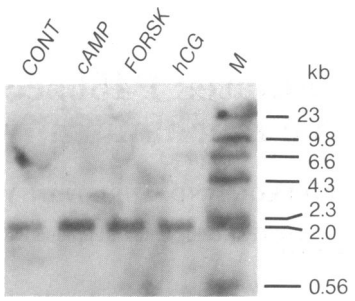

FIG. 1. Transfer blot of P450scc mRNA from MA-10 cells hormonally stimulated for 8 hr. Lanes: CONT, control, no added hormone; cAMP, 1 mM 8-Br-cAMP; FORSK, 10 μM forskolin; hCG, hCG (25 ng/ml). Each lane contains 15 μg of cytoplasmic RNA. Molecular size markers (lane M, *Hin*dIII-digested bacteriophage λ) are indicated. Blots initially probed with ^{32}P-labeled human P450scc cDNA were reprobed with human γ-actin cDNA (30); actin mRNA was equal in all samples. Radioactive probes were gel-purified cDNA inserts labeled with random primers. kb, Kilobase(s).

cAMP increased P450scc mRNA accumulation by increasing P450scc gene transcription. One hour of cAMP stimulation doubled the amount of labeled nuclear RNA hybridizing to P450scc DNA, while the amount of radioactivity hybridizing to actin DNA remained constant (Table 2).

Since transformed hormonal cell lines may not maintain a stable phenotype (ref. 32 and references therein) and since the MA-10 cells we used had experienced desensitization to hCG, we determined if our cell population was homogeneous. *In situ* hybridization showed that all cells synthesized steroids and responded to cAMP stimulation equally (Fig. 3). All control cells contained P450scc mRNA equally (Fig. 3*A*) and all cells responded to cAMP equally, so that each cell accumulated P450scc mRNA approximately 2-fold (Fig. 3*B*). Only probe complementary to P450scc mRNA hybridized to RNA in the cells; the opposite-strand probe equivalent to P450scc mRNA detected no mRNA either in control (Fig. 3*C*) or cAMP-stimulated (Fig. 3*D*) cells.

Protein synthesis was not required for cAMP-mediated accumulation of P450scc mRNA in MA-10 cells. Incubation of MA-10 cells with cycloheximide (20 μg/ml) for 30 min before and during an 8-hr induction with 1 mM 8-Br-cAMP abolished greater than 96% of protein synthesis. Although the basal concentration of P450scc mRNA decreased with cycloheximide treatment, cAMP still stimulated the accumulation of P450scc mRNA in MA-10 cells (Fig. 4); similar results were obtained when 200 μM puromycin was substituted for cycloheximide (data not shown).

Table 1. Secretion of progesterone from MA-10 cells and accumulation of P450scc mRNA in MA-10 cells after 4 and 8 hr of stimulation

	Progesterone, ng per 10^4 cells		P450scc mRNA, arbitrary units	
Treatment	4 hr	8 hr	4 hr	8 hr
Control	0.27 ± 0.92	0.25 ± 0.04	0.55 ± 0.13	0.72 ± 0.05
8-Br-cAMP	15.66 ± 1.52	27.34 ± 0.80	1.01 ± 0.21	2.12 ± 0.27
Forskolin	6.80 ± 0.57	12.10 ± 0.25	0.83 ± 0.10	1.73 ± 0.18
hCG	3.91 ± 0.39	2.76 ± 0.38	0.94 ± 0.35	1.18 ± 0.27
LH	1.18 ± 0.07	1.76 ± 0.45	0.90 ± 0.13	1.46 ± 0.20

Values are the mean ± SEM of triplicate incubations.

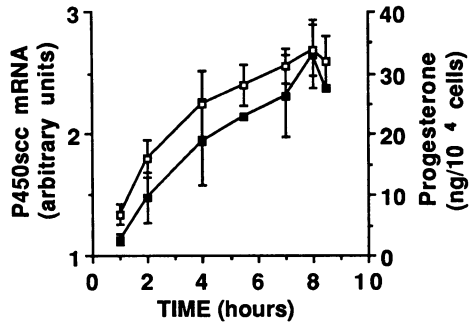

FIG. 2. Induction of P450scc mRNA by cAMP in MA-10 cells. Autoradiographs of slot blots hybridized to human P450scc and human γ-actin cDNA were scanned by laser densitometry. P450scc mRNA was normalized to actin mRNA in the same sample; data are expressed as the ratio of cAMP-induced to uninduced control P450scc mRNA (■). Progesterone secretion (ng per 10^4 cells) was measured from the same cells (□). Progesterone concentrations from control cells at all times were between 0.1 and 0.3 (±0.04) ng per 10^4 cells. Data are mean ± SEM of four experiments.

P450c17 mRNA Analysis. MA-10 cells were generated from a mouse Leydig cell tumor that lacked 17α-hydroxylase activity (20, 33), leading to the general opinion that MA-10 cells also lack 17α-hydroxylase activity. However, these cells have been reported to synthesize immunoassayable testosterone and androstenedione, and this androgen synthesis could be increased by incubation with cAMP or hCG (20). We found that MA-10 cells stimulated for 8 hr with 1 mM 8-Br-cAMP secreted less than 5 pg of testosterone per 10^6 cells per ml of medium, a concentration at the detection limit of the immunoassay. Using radioactive precursors and HPLC analysis of steroids synthesized, a procedure that could detect as little as 0.6 pg of steroid produced from a radioactive precursor, we found that these cells did not secrete radioactive testosterone. Medium from cells incubated for 2 hr with 10^6 cpm of [^{3}H]progesterone was extracted and the secreted ^{3}H-labeled steroids were chromatographed using two solvent systems. Eight percent of the total radioactivity comigrated with testosterone and 17α-hydroxyprogesterone in one solvent system (Fig. 5*A*) but the radioactivity was cleanly separated from both of these 17α-hydroxylated compounds in the second system (Fig. 5*B*). Equivalent results were obtained when the cells were analyzed for tritiated steroids. Thus MA-10 cells synthesize neither 17α-hydroxyprogesterone nor testosterone from progesterone, with or without cAMP stimulation.

MA-10 cells cannot synthesize testosterone because they lack P450c17 mRNA. We cloned and sequenced rat P450c17 cDNA (Fig. 6) to analyze RNA from control and cAMP-stimulated MA-10 cells. This rat P450c17 cDNA probe did not hybridize to RNA from control or cAMP-stimulated MA-10 cells in either slot or RNA transfer blots, even under low-stringency conditions that detect sequences having only 60% homology.

Using a riboprobe containing a 120-base *Eco*RI–*Bam*HI rat P450c17 cDNA sequence in an RNase protection experiment, we detected P450c17 mRNA in pig, bovine, rat, and mouse testes but not in MA-10 cells (Fig. 7). The intensity of the band corresponding to P450c17 RNA from MA-10 cells was less than 4% of that from whole mouse testis, which is only 2% Leydig cells (35). Therefore, the abundance of P450c17 mRNA in MA-10 cells was less than 0.08% of that found in normal Leydig cells. Thus a variety of experimental approaches indicate that MA-10 cells lack significant quantities of P450c17 mRNA.

Table 2. Nuclear run-on gene transcription in MA-10 cells stimulated with 8-Br-cAMP for 1 hr

	Relative amount of transcription, densitometric scan/input cpm		
RNA	Control	+ cAMP	Fold-increase
P450scc	305 ± 59	542 ± 160	1.78
Actin	919 ± 102	988 ± 72	1.08

Radiographic signal from control plasmid DNA was subtracted from P450scc and actin signals. Values are the mean ± SEM of three experiments.

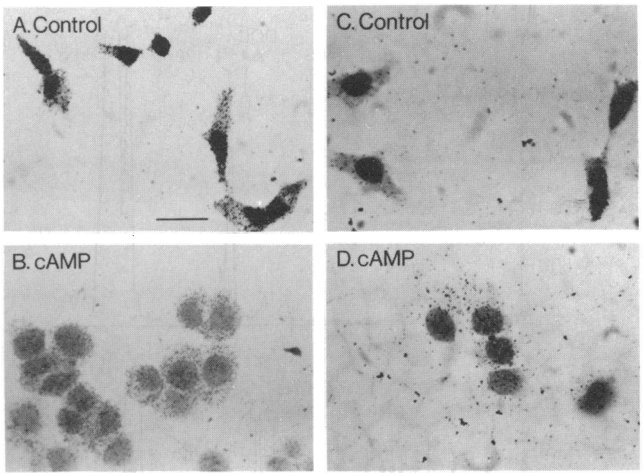

FIG. 3. *In situ* hybridization of P450scc mRNA in MA-10 cells. Control (*A*) and cAMP-stimulated (*B*) cells were hybridized to a ^{3}H-labeled human P450scc cRNA riboprobe; control (*C*) and cAMP-stimulated (*D*) cells were hybridized to a ^{3}H-labeled human P450scc mRNA riboprobe. (Bar = 25 μm; all panels are at the same magnification.)

DISCUSSION

Cultured mouse MA-10 Leydig tumor cells provide an excellent system for studying hormonal regulation of ste-

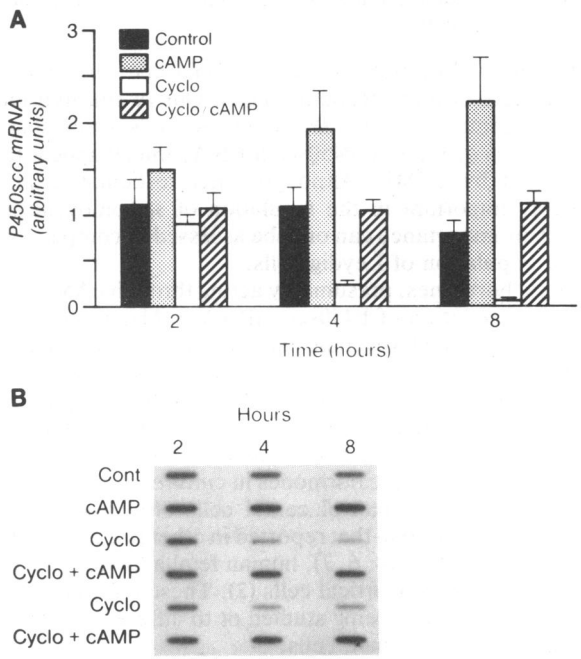

FIG. 4. Effect of cycloheximide on basal and cAMP-stimulated P450scc mRNA accumulation in MA-10 cells. (*A*) Data are mean ± SEM of triplicate experiments. P450scc mRNA is indicated as arbitrary units and is corrected for the abundance of actin mRNA in each sample. (*B*) Autoradiograph of a representative blot yielding the data in *A*. Cont, control; Cyclo, cycloheximide.

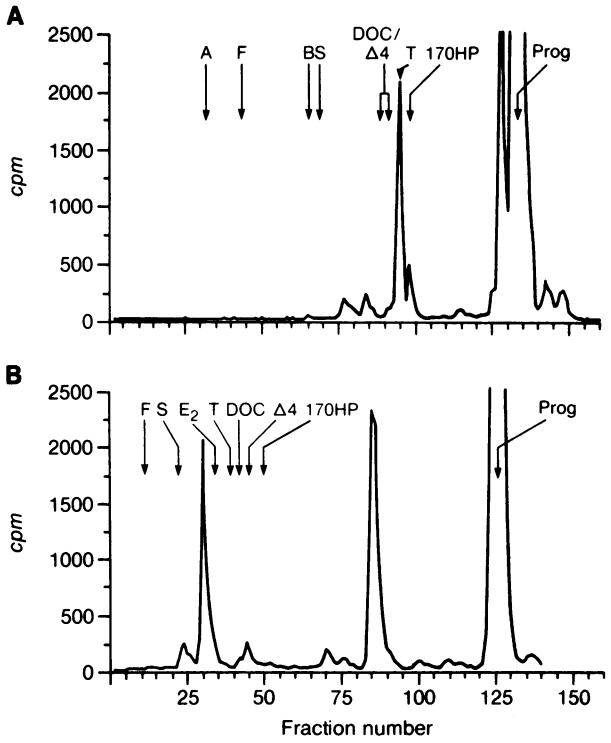

FIG. 5. HPLC profile of steroids secreted from MA-10 cells incubated with [^{3}H]progesterone. A reversed-phase C_{18} column (4.6 × 250 mm, Applied Biosystems) was used with two solvent systems: the gradient system for the separation of natural glucocorticoids and progestins (34) (*A*) and an isocratic separation utilizing 0.01 M KH_2PO_4/acetonitrile/isopropanol, 300:175:25 (vol/vol), (*B*) applied at a flow rate of 1.5 ml/min. The positions where unlabeled steroid standards eluted were determined by monitoring at 210 nm and are indicated in each graph. T, testosterone; Δ4, androstenedione; DOC, deoxycorticosterone; P, progesterone; 17OHP, 17α-hydroxyprogesterone; F, cortisol; B, corticosterone; S, 11-deoxycortisol; A, aldosterone; E_2, estradiol. Monitoring at 240 nm yielded identical elution times (data not shown).

roidogenesis. Because they are a clonal line, they are free from effects of other testicular cells. Our *in situ* hybridizations of control and cAMP-stimulated cells show that all MA-10 cells synthesize P450scc mRNA, and all appear to be stimulated by cAMP. Although other testicular cells are probably important in the regulation of steroidogenesis *in vivo*, their importance can only be assessed in comparison to a pure population of Leydig cells.

Tropic hormones, presumably acting through cAMP, stimulate accumulation of P450scc mRNA in MA-10 cells. This stimulation is rapid, reaching maximum by 8 hr. The maximal accumulation of P450scc mRNA in these cells was 3-fold; this is similar to the 2-fold stimulation of P450scc protein synthesis by LH in primary cultures of rat and mouse interstitial cells (17, 18) and to the 2.5-fold stimulation of P450scc mRNA accumulation by tropic hormones in cultured fetal testis cells (5) and cultured human placental cells (4). However, this stimulation is less than that reported in other primary cultures of human granulosa (4, 6, 7), human fetal adrenal (3, 4), and adult bovine adrenocortical cells (2). These differences may be due to the tissues being studied or to differences between primary and transformed cells.

Nuclear run-on assays show that the cAMP-mediated increase in P450scc mRNA accumulation is due to increased P450scc gene transcription. This increased transcription occurs within 1 hr and is specific; transcription of actin was unaltered by cAMP stimulation. Increased P450scc mRNA accumulation in bovine adrenocortical cells is also due to

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    274                                                 285
    Asp Asn Asn Asn Ser Cys Glu Gly Arg Asp Pro Asp Val Phe
  A GAC AAC AAC AAC AGC TGT GAA GGC CGG GAC CCA GAT GTG TTT
        290                                             300
Ser Asp Arg His Ile Leu Ala Thr Val Gly Asp Ile Phe Gly Ala
TCA GAT AGG CAC ATC CTT GCC ACG GTG GGA GAC ATC TTT GGG GCG
        305                                             315
Gly Ile Glu Thr Thr Thr Thr Val Leu Lys Trp Ile Leu Ala Phe
GGC ATA GAG ACA ACT ACC ACT GTG CTC AAG TGG ATC CTG GCT TTC
        320                                             330
Leu Val His Asn Pro Glu Val Lys Lys Lys Ile Gln Lys Glu Ile
CTG GTG CAC AAT CCT GAG GTG AAG AAG AAG ATC CAA AAG GAG ATT
        335                                             345
Asp Gln Tyr Val Gly Phe Ser Arg Thr Pro Thr Phe Asn Asp Arg
GAC CAG TAC GTA GGC TTC AGC CGA ACA CCA ACT TTC AAT GAC CGG
        350                                             360
Ser His Leu Leu Met Leu Glu Ala Thr Ile Arg Glu Val Leu Arg
TCT CAC CTC CTC ATG CTG GAG GCC ACT ATC CGA GAA GTG CTG CGT
        365                                             375
Ile Arg Pro Val Ala Pro Met Leu Ile Pro His Lys Ala Asn Val
ATC AGG CCG GTG GCT CCC ATG CTC ATC CCC CAC AAG GCT AAC GTT
        380                                             390
Asp Ser Ser Ile Gly Glu Phe Thr Val Pro Lys Asp Thr His Val
GAC TCC AGC ATT GGA GAG TTT ACT GTC CCC AAG GAC ACA CAT GTG
        395                                             405
Val Val Asn Leu Trp Ala Leu His His Asp Glu Asn Glu Trp Asp
GTC GTC AAT CTC TGG GCA CTG CAT CAC GAT GAG AAT GAA TGG GAC
        410                                             420
Gln Pro Asp Gln Phe Met Pro Glu Arg Phe Leu Asp Pro Thr Gly
CAG CCA GAT CAG TTC ATG CCT GAA CGC TTC TTA GAT CCA ACG GGA
        425                                             435
Ser His Leu Ile Thr Pro Thr Gln Ser Tyr Leu Pro Phe Gly Ala
AGC CAT CTC ATT ACA CCC ACG CAG AGT TAC TTG CCC TTC GGA GCT
        440                                             450
Gly Pro Arg Ser Cys Ile Gly Glu Ala Leu Ala Arg Gln Glu Leu
GGT CCC CGA TCC TGC ATC GGA GAG GCT CTG GCC CGT CAG GAG CTC
        455                                             465
Phe Val Phe Thr Ala Leu Leu Leu Gln Arg Phe Asp Leu Asp Val
TTT GTC TTC ACG GCC TTG CTA CTG CAG AGG TTT GAC TTG GAT GTG
        470                                             480
Ser Asp Asp Lys Gln Leu Pro Arg Leu Glu Gly Asp Pro Lys Val
TCA GAT GAT AAA CAA CTG CCC CGC CTG GAG GGT GAT CCC AAG GTA
        485                                             495
Val Phe Leu Ile Asp Pro Phe Lys Val Lys Ile Thr Val Arg Gln
GTC TTT CTG ATC GAC CCT TTC AAA GTA AAG ATC ACG GTG CGC CAG
        500                                     508
Ala Trp Met Asp Ala Gln Ala Glu Val Ser Thr AM
GCA TGG ATG GAT GCA CAG GCT GAG GTT AGC ACC TAG AGGCCACAACTA
ACATCCCCGATCCATACCTCAACACCCACAGTACAATCTTAGAGGTGCTAGTCCCAGTGC
CTCCTACGGTCCTCCTTCCTCCTACCCCATTTTCTAGTTGGCAGCAATGGGGGGTGATAC
ACATAAATTAAAGTTTTTCAATAAACATC (poly A)
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FIG. 6. Sequence of the rat ovarian P450c17 cDNA clone. Codon numbers are derived from human P450c17 cDNA (16).

increased transcription of the P450scc gene (2); mRNA stability may be unaffected by cAMP treatment.

The mechanism by which cAMP stimulates P450scc gene transcription is unclear. In bovine adrenocortical cells (2) and human JEG-3 choriocarcinoma cells (10), cycloheximide inhibits the cAMP-mediated stimulation of P450scc mRNA accumulation, implying that protein synthesis is required for this stimulation. However, mRNA for the P450scc electron transport protein adrenodoxin is not inhibited by cycloheximide in JEG-3 cells (10) whereas it is in bovine adrenal cells (2). Furthermore, cycloheximide does not inhibit the cAMP-mediated induction of P450scc mRNA accumulation in human granulosa cells (7). These differences cannot be attributed to differences between primary and transformed cell cultures as both JEG-3 cells and normal trophoblasts respond similarly (11). Steroidogenesis, therefore, may be regulated by different mechanisms in different endocrine tissues. We found that in cultured mouse Leydig cells, the cAMP stimulation of P450scc mRNA accumulation is direct and does not require the synthesis of other proteins. Steroidogenesis in the testis and ovary may thus be regulated by similar mechanisms, whereas steroidogenesis in the adrenal and placenta may be regulated differently.

We also studied the regulation of P450c17 in MA-10 cells using a rat P450c17 cDNA. The clone we obtained extends from amino acid 274 (nucleotide 860) of the human P450c17 sequence to the poly(A) tail. In general, there is greater sequence conservation among rat, human (16), bovine (15), and porcine (16) P450c17 in the carboxyl-terminal half of the protein than at the amino-terminal half. This carboxyl-terminal region contains the steroid- and heme-binding domains (28). In the steroid-binding domain (28) (amino acids

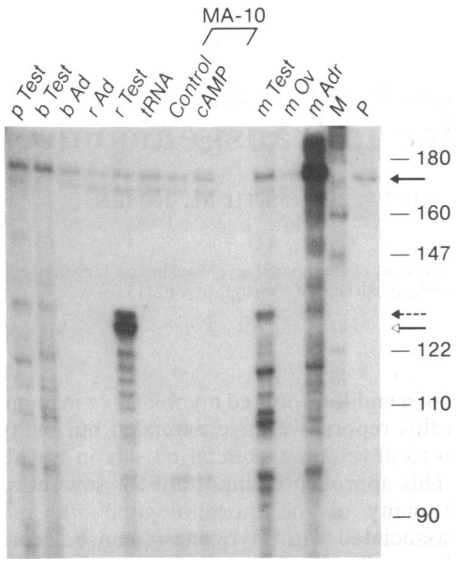

FIG. 7. RNase protection of RNAs with rat P450c17 cDNA riboprobe. RNA was hybridized overnight with 10^6 cpm of ^{32}P-labeled rat P450c17 riboprobe (antisense strand) in 80% formamide/400 mM NaCl/40 mM Pipes, pH 6.4/1 mM EDTA at 37°C, treated with RNase A (rat RNA at 10 μg/ml; other RNAs at 1 μg/ml) for 30 min. Lanes: p Test, 5 μg of pig testis RNA; b Test, 1 μg of bovine testis RNA; b Ad, 5 μg of bovine adrenal RNA; r Ad, 25 μg of rat adrenal RNA; r Test, 25 μg of rat testis RNA; tRNA, 25 μg; Control, 25 μg of MA-10 RNA from control cells; cAMP, 25 μg of MA-10 RNA from cAMP-treated cells; m Test, 10 μg of mouse testis RNA; m Ov, 25 μg of mouse ovary RNA; m Adr, 25 μg of mouse adrenal RNA; M, ^{32}P-labeled *Msp* I-digested pBR322 DNA; P, unprotected ^{32}P-labeled rat P450c17 riboprobe. Arrows on the right correspond to the 171-nucleotide unprotected probe, 132-nucleotide protected RNA band from mouse testis, and 129-nucleotide protected RNA band from rat testis.

350–366), 14 of 17 amino acids are identical among rats, cattle, pigs, and human beings. The rat substitutes methionine for leucine at amino acid 352 in the human sequence and isoleucine for leucine at amino acid 363. In the heme-binding domain (amino acids 433–453) (28), 16 of 17 amino acids are identical to those found in the human sequence; the difference is at amino acid 446 where the rat has alanine and the human sequence has isoleucine.

HPLC analysis of steroids synthesized and secreted by MA-10 cells demonstrates that these cells do not synthesize 17α-hydroxylated steroids. Therefore, previous reports of immunoassayable testosterone and 17α-hydroxyprogesterone secreted from these cells probably represent other, cross-reacting steroids. MA-10 cells cannot synthesize 17α-hydroxylated steroids because they lack P450c17 mRNA. Slot blots, Northern blots, and RNase protection experiments were unable to detect significant amounts of P450c17 mRNA in these cells. The lack of detectable amounts of P450c17 mRNA in MA-10 cells is not a general change from a normal to tumor Leydig cell. We compared the abundance of P450scc mRNA in MA-10 cells with its abundance in normal mouse testes. We found the concentration of P450scc mRNA in MA-10 cells was equal to that from equivalent amounts of total mouse testis RNA. Since the mouse testes are only 2% Leydig cells, we conclude that the amount of P450scc mRNA in MA-10 cells is about 2% of that found in normal Leydig cells. Therefore, the very small concentration of P450c17 mRNA in MA-10 cells, less than 0.01% of the concentration found in normal mouse Leydig cells, is not due to a general decrease in all P450 mRNAs, but rather is due to a specific decrease in the expression of the P450c17 gene in MA-10 cells.

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