Contribution of steroidogenic factor 1 to the regulation of cholesterol synthesis

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Steroidogenic factor 1 (SF-1) is an orphan member of the nuclear receptor family expressed in steroidogenic tissues, where it has an essential role in the regulation of the steroid hormone biosynthesis, adrenal and gonadal development and endocrine responses fundamental for reproduction. Here we show that SF-1 regulates the transcription of cytosolic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase gene, which is essential for the endogenous synthesis of cholesterol. We have identified an element located 365 bp upstream of the gene for cytosolic HMG-CoA synthase; SF-1 binds as a monomer to this element and confers SF-1 responsiveness to homologous and heterologous promoters. It has been shown that in tissues with a high demand for cholesterol to be used in steroid synthesis, there is a lack of correlation between the cholesterol levels and the activity of the limiting enzymes of the mevalonate pathway. In accord with those results, we observed that cholesterol synthesis from acetate and either cytosolic HMG-CoA mRNA expression or transcriptional activity were not changed in response to 25-hydroxycholesterol in the SF-1-expressing steroidogenic Leydig tumour MA-10 cells. Moreover, the overexpression of SF-1 in non-steroidogenic CV-1 cells renders them less sensitive to the regulatory effects of cholesterol. This observation led to the hypothesis that in steroidogenic tissues the expression of SF-1 permits high levels of endogenous synthesis of cholesterol irrespective of the intracellular levels of this metabolite.

Key words: nuclear receptors, steroidogenesis, transcription.

INTRODUCTION

Cytosolic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase catalyses an early step in the biosynthesis of cholesterol, which is regulated by negative feedback from cholesterol. A low cellular concentration of sterols induces the transcription of the gene for cytosolic HMG-CoA synthase [1,2]. This negative feedback is mediated by the sterol-regulatory-element-binding proteins (SREBPs). When the sterol levels decrease, the mature form of the SREBPs is released by a two-step proteolytic process; it then translocates to the nucleus and activates the transcription of genes related to cholesterol and fatty acid metabolism through interaction with sterol-regulatory elements in the promoter of target genes [3].

Steroidogenic factor 1 (SF-1) is a monomeric member of the orphan nuclear receptor family capable of activating a variety of genes involved in steroid biosynthesis [4–7]. These genes all have promoters containing sequences related to the consensus TCAAGGTCA, which is required for maximal gene expression [8]. SF-1 is expressed in adrenal glands and gonads; remarkably, mice deficient in SF-1 lack these organs [9,10]. These results have established a critical role for SF-1 at multiple levels of endocrine development and function. Recent studies have shown that 25-, 26- and 27-hydroxycholesterol enhance SF-1-dependent gene transcription [11]; although this is a controversial result [12,13], it suggests that ligand-dependent transactivation of SF-1 by oxysterols might enhance steroidogenesis *in vivo*.

Results from other laboratories have shown that in tissues with a high demand for cholesterol to be used in steroid synthesis, there is a lack of correlation between the cholesterol levels and the activity of the limiting enzymes of the mevalonate pathway. Thus feeding cholesterol in amounts sufficient to increase the plasma cholesterol level more than 30-fold did not prevent the increase in HMG-CoA reductase activity in the ovary of pregnant rabbits [14]. Moreover, a decrease in the levels of circulating cholesterol did not increase HMG-CoA reductase activity in normal rat testes, or in a Leydig cell tumour grown in mice or in a clonal strain of cultured Leydig tumour cells (MA-10) [15].

Here we show that the cytosolic HMG-CoA synthase gene promoter contains an SF-1-responsive element; this has been localized precisely. In addition, the cytosolic HMG-CoA synthase mRNA levels, the transcriptional activity of the gene and the synthesis of labelled cholesterol from [^{14}C]acetate in the presence of 25-hydroxycholesterol were higher in cells naturally expressing SF-1 (MA-10) than in cells not expressing this factor (CV-1). We hypothesize that, in tissues with a high demand of cholesterol for use in steroid synthesis that also express high levels of SF-1, the endogenous synthesis of cholesterol is turned on in spite of the intracellular levels of this metabolite. Moreover, we hypothesize the existence of a co-ordinated control of the expression of genes involved in cholesterol acquisition {scavenger receptor class B type I (SR-BI) [16–18] and cytosolic HMG-CoA synthase} and metabolism to steroid hormones [steroidogenic acute regulatory protein (StAR)] [13] through common transcription factors.

EXPERIMENTAL

Plasmids

pCSCAT containing a 559 bp fragment of the hamster gene for cytosolic HMG-CoA synthase (−528 to +31) was constructed by PCR with a pair of oligonucleotide primers, CSF (5′-atgaactgcagGAATTCAAATCAAATATTTCAAACGTTTTA-3′) and CSR (5′-acgctgtcgacGAAAGGAGCAATCCACGGAGC-

Abbreviations used: CAT, chloramphenicol acetyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; SF-1, steroidogenic factor 1; StAR, steroidogenic acute regulatory protein; SR-BI, scavenger receptor class B type I; SREBPs, sterol-regulatory-element-binding proteins; TK, thymidine kinase.

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ACAGTGC-3′), corresponding to coordinates −528 to −499 and +31 to +4 respectively of the hamster cytosolic HMG-CoA synthase gene [2], and CHO (Chinese hamster ovary)-cell genomic DNA as a template. The nucleotides shown in lower case were added to provide the restriction sites for *Pst*I and *Sal*I (underlined) for cloning. The *Pst*I–*Sal*I-digested PCR product was cloned into the *Pst*I–*Sal*I sites of vector pCAT-BASIC (Promega) in front of the chloramphenicol acetyltransferase (CAT) reporter gene. Mutant pCSCATm was generated by site-directed mutagenesis with overlapping extension by using PCR [19]. To confirm the sequence, the PCR-amplified fragments were automatically sequenced with the use of the fluorescent terminator kit (Perkin-Elmer).

Heterologous promoter plasmids were constructed in the herpes virus thymidine kinase (TK)-gene promoter upstream of the CAT reporter gene pBLCAT2 [20]. pTKCAT3xCSSF-1 contained three copies of a fragment corresponding to co-ordinates −366 to −352 of the hamster cytosolic HMG-CoA synthase gene. It was constructed by cloning the oligonucleotide 5′-agcttCTGACCTTCAATTGG-3′ annealed to 5′-tcgaCCAAT-TGAAGGTCAGa-3′ into pBLCAT2 (nucleotides designated in lower case were added to provide cohesive *Hin*dIII–*Sal*I ends at the 5′ and 3′ termini respectively). DNA sequence analysis, by the fluorescent terminator kit, was performed to confirm the number and the orientation of the inserts. A plasmid expressing mouse SF-1 was kindly provided by Dr K. Parker. cDNA encoding this SF-1 was originally cloned in the expression vector pCMV5 [21]. The SF-1 cDNA was digested with *Eco*RI and cloned in pSG5 in both orientations.

Cell culture and transfections

MA-10 cells and CV1 cells were cultured in Waymouth medium supplemented with 15% (v/v) horse serum, and in minimal essential medium supplemented with 10% (v/v) fetal bovine serum respectively. In the experiments with 25-hydroxy-cholesterol the indicated serum was replaced by 10% (v/v) delipidated calf serum (Sigma). Cells were typically co-transfected with 10 μg of the reporter pCSCAT gene construct and, when indicated, with 2 μg of effector plasmids expressing full-length cDNA species for mouse SF-1. Plasmid pCMVβGAL (cytomegalovirus promoter β-galactosidase) (4 μg) was included as internal control in co-transfections. Transfection experiments were performed by the calcium phosphate method as described [22,23]. After removal of the calcium phosphate/DNA precipitate, cells were refed with the appropriate medium. Cells were harvested 48 h after refeeding.

β-Galactosidase and CAT assays

Extracts of harvested cells were prepared by liquid nitrogen freeze–thaw disruption (three times) after resuspension in 100 μl of 0.25 M Tris/HCl, pH 7.5. β-Galactosidase activity was determined [23] in a 10–20 μl volume of extract to normalize for transfection efficiency. All samples assayed for CAT activity were first incubated at 65 °C for 5 min. CAT assays were performed [22] for 60 min. Radioactivity of samples was measured on an LKB-1217 liquid-scintillation counter.

Transcription and translation *in vitro*

cDNA for SF-1 was transcribed and translated by using a commercially available kit in accordance with the instructions of the manufacturer (Promega).

Electrophoretic mobility-shift analysis

SF1 (0.5 μl) synthesized *in vitro* or nuclear extracts (5 μg) were preincubated on ice for 10 min in 10 mM Tris/HCl (pH 8.0)/100 mM KCl/0.05% (v/v) Nonidet P40/6% (v/v) glycerol/1 mM dithiothreitol containing 1 μg of poly(dI-dC). The total amount of reticulocyte lysate was kept constant in each reaction (2 μl) by the addition of unprogrammed lysate. For competition experiments, a 50–150-fold molar excess of unlabelled double-stranded probes, relative to the labelled probe, was included during preincubation. For supershift experiments, 1 μl (2.4 μg/μl) of an antibody specific for SF-1 (Upstate Biotechnology) was included when indicated. Then 2 ng of SF1-SC, ^{32}P-labelled by fill-in with Klenow polymerase, was added and the incubation continued for 15 min at room temperature. The final volume for all reactions was 20 μl. Samples were subjected to electrophoresis at 4 °C on a 4.5% (w/v) polyacrylamide gel in 0.5 × TBE buffer [45 mM Tris/HCl (pH 8.0)/45 mM boric acid/1 mM EDTA]. SF1-SC is the fragment corresponding to coordinates −365 to −357 of the hamster cytosolic HMG-CoA synthase gene; two complementary oligonucleotides, agctTGACCTTCA and tcgaTGAAGGTCA, were used (the nucleotides added for cloning and labelling are indicated in lower-case letters). SF1-SCm is a probe of the same size and with the same nucleotides added for cloning and labelling in which the sequence TGACCTTCA had been changed to TGA-CCTggg, indicating in lower-case letters the nucleotides corresponding to those that had been changed from the wild-type sequence. LSF1-SC is a 145 bp fragment of the hamster cytosolic HMG-CoA synthase gene from coordinates −383 to −238. MSF1-SC is the fragment corresponding to coordinates −366 to −352 of the hamster cytosolic HMG-CoA synthase gene; two complementary oligonucleotides, agctCTGACCTTCAATTGG and tcgaCCAATTGAAGGTCAG, were used (the nucleotides added for cloning and labelling are indicated in lower-case letters).

RNA blot analysis

Total RNA was prepared from MA-10 and CV-1 cells by extraction with guanidine isothiocyanate and centrifugation through a CsCl cushion [23]. Aliquots of 15 and 25 μg respectively (determined by A_{260}) were fractionated on 1% (w/v) agarose/formaldehyde gels, transferred to Nytran-N membranes (Schleicher & Schuell) and cross-linked with UV. Hybridizations were performed as described [23], with a 1.1 kb specific fragment (*Apa*I–*Sac*I) of the rat liver cytosolic HMG-CoA synthase cDNA as a probe; washes were performed at 55 °C with 0.1 × SSC and 0.1 × SDS [1 × SSC is 0.15 M NaCl/0.015 M Na citrate (pH 7.0)]. A 14 h exposure of this blot was analysed for attenuance in a Bioprofil computing densitometer (Vilber Lourmat).

Incorporation of labelled acetate into cholesterol

CV-1 or MA10 cells (5×10^5) were seeded on 35 mm plates and incubated overnight in minimal essential medium or Waymouth medium respectively, supplemented with 10% (v/v) delipidated calf serum. The medium was then changed and 2 μCi of [1-^{14}C]acetate (59 mCi/mmol; Amersham) was added. After incubation for 24 h, cells were harvested by being scraped into PBS; cellular lipids were extracted [24], saponified [25] and separated by silica-gel TLC in chloroform/methanol (19:1, v/v). The identity of the cholesterol formed was determined by using pure [^{14}C]cholesterol as a standard. After localization of the

radioactive bands by autoradiography, the silica gel on the region with the same R_F as standard cholesterol was measured on an LXB-1217 liquid-scintillation counter.

RESULTS

Hamster cytosolic HMG-CoA synthase gene 5′-flanking region contains an SF-1 binding site

The analysis of the 5′-flanking region of the hamster cytosolic HMG-CoA synthase gene by the TF-SEARCH routine, performed with the Kyoto Center's GenomeNet WWW Server, revealed a putative monomeric nuclear-receptor binding sequence. The analysis of this sequence shows a coincidence of eight out of nine bases with the consensus binding sequence proposed for SF-1. We performed gel mobility-shift assays to analyse whether SF-1 binds to this sequence. Incubation of a probe containing this sequence (SF1-SC) with SF-1 transcribed and translated *in vitro* resulted in a prominent complex (Figure 1A, lane 3). The specificity of this complex was demonstrated by competition with a 50–150-fold molar excess of unlabelled probe (Figure 1A, lanes 4–6), or a probe containing the consensus TCAAGGTCA SF-1-binding sequence (results not shown), whereas the same molar excess of a probe containing a mutation that changed 3 bp just 5′ of the AGGTCA (SF1-SCm) did not lead to the disappearance of the complex (lanes 7–9). A gel shift experiment performed with a 145 bp fragment of the cytosolic HMG-CoA promoter (from −383 to −238) showed the presence of a unique retardation complex (Figure 1A, lane 12). As shown in Figure 1(B), the specific SF-1 complex was present in MA-10 cell extracts but not in CV-1 cells. Even though the binding was not totally promiscuous, several members of the nuclear receptor family bound to this sequence (results not shown).

Cytosolic HMG-CoA synthase SF-1-binding site increases gene transcription

We decided to investigate the effect of the observed binding of SF-1 to the cytosolic HMG-CoA synthase gene promoter on its transcriptional activity. We made constructs in which the 5′-flanking region of this gene was linked to a promoterless bacterial CAT gene (pCSCAT). This plasmid was introduced into cultured SF-1-deficient CV-1 cells by the calcium phosphate method, with or without an expression vector for SF-1. CAT expression from this plasmid greatly exceeded the activity observed for a promoterless CAT construct; co-transfection of SF-1 expression vector led to an increase in CAT activity (Figure 2A).

Next, three copies of a pair of oligonucleotides containing the cytosolic HMG-CoA synthase gene SF-1-binding site were inserted into pBLCAT2, a plasmid containing the CAT gene under the control of the TK gene promoter. This sequence conferred SF-1 responsiveness to the otherwise unresponsive TK gene promoter (Figure 2B). This result demonstrates that the cytosolic HMG-CoA synthase SF-1-responsive element conferred SF-1 responsiveness both in its natural context and on a normally SF-1-unresponsive promoter.

The functional role of the SF-1-binding site was analysed in MA-10 cells, Leydig tumour cell line that endogenously expresses high levels of SF-1, and in CV-1 cells, which, as shown above and confirmed by Northern blot experiments (results not shown), do not express SF-1. The basal level of expression of the cytosolic HMG-CoA synthase promoter construct was higher in MA-10 than in CV-1 cells; mutation of the SF-1-binding site decreased

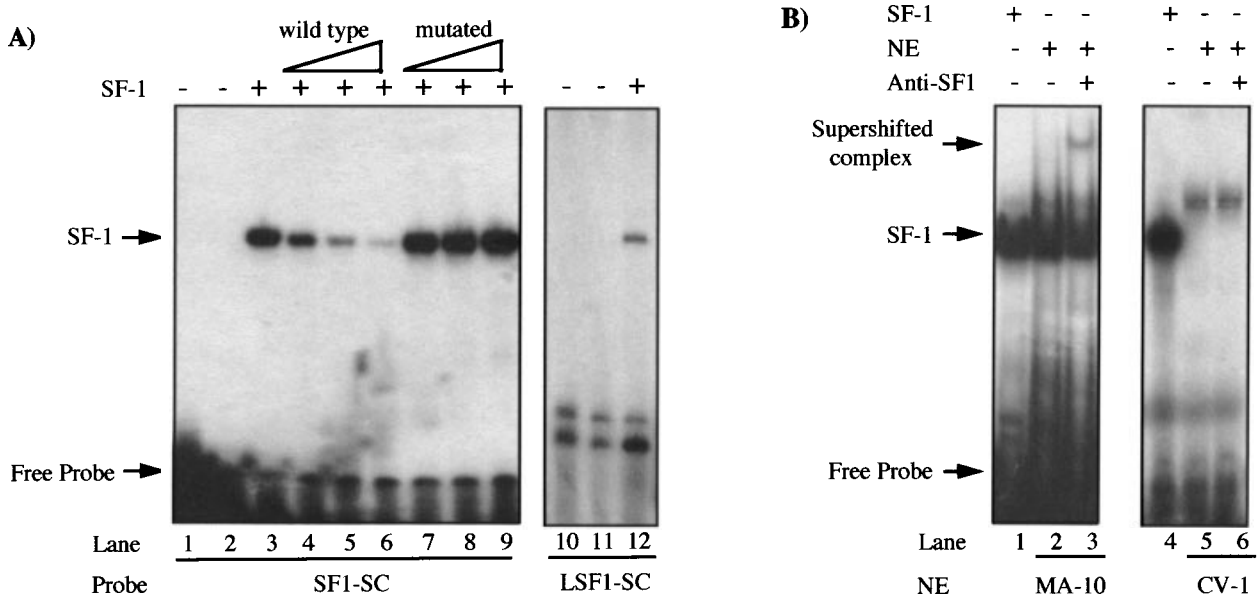

Figure 1 Electrophoretic mobility-shift assay of the cytosolic HMG-CoA synthase SF-1-binding site with SF-1 transcribed and translated *in vitro*

(**A**) SF-1 translated *in vitro* was incubated with a labelled probe containing the proposed SF-1-binding site, SF1-SC (lanes 1–9) or LSF1-SC (lanes 10–12), and analysed by electrophoretic mobility-shift assay. Additions were as indicated at the top. Lanes 1 and 10, and 2 and 11 contained protein-free and empty-vector-(pSG5)-programmed lysate incubations respectively. Lanes 3–9 and 12 contained an SF-1-programmed translation reaction. The specific SF-1 complex is indicated by an arrow. Lanes 4–9 contained a competition of the complex with 50-fold, 100-fold and 150-fold molar excesses of unlabelled probe containing the proposed SF-1-binding sites (lanes 4–6) or a mutated version (SF1-SCm) (lanes 7–9). (**B**) SF-1 translated *in vitro* (lanes 1 and 4) or nuclear extracts (NE) from MA-10 (lanes 2–3) or CV-1 cells (lanes 5 and 6) were incubated with a labelled probe containing the proposed SF-1-binding site (MSF1-SC) and analysed by electrophoretic mobility-shift assay. Additions were as indicated at the top. The specific SF-1 and the supershifted complexes are indicated by arrows.

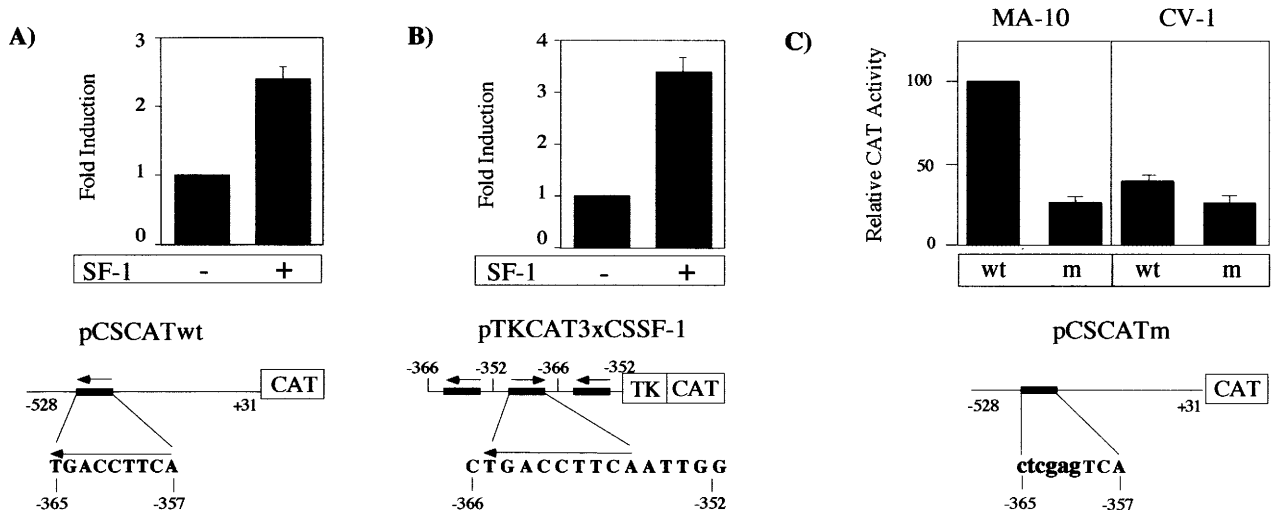

Figure 2 SF-1 mediates the activation of the hamster cytosolic HMG-CoA synthase gene promoter in SF-1-deficient CV-1 cells (A, B) or SF-1-expressing MA-10 cells (C)

(**A**) CV-1 cells were transiently transfected with pCSCAT, a reporter construct containing the 5′-flanking region of the hamster gene for cytosolic HMG-CoA synthase, from nt −528 to +31. The promoter reporter construct was co-transfected with plasmids expressing SF-1 (+) or anti-sense SF-1 (−). (**B**) CV-1 cells were co-transfected with pTKCAT3xCSSF-1, a reporter plasmid containing the CAT gene under the control of the TK gene promoter, containing three copies of the proposed SF-1-binding site and an empty (pSG5) or expression vector for SF-1. (**C**) MA-10 or CV-1 cells were co-transfected with pCSCAT or pCSCATm, a reporter construct containing the 5′-flanking region of the hamster gene for cytosolic HMG-CoA synthase, from nt −528 to +31, in which the SF-1-binding sequence had been changed between positions −365 and −360. Average values of β-galactosidase-normalized CAT activity (means ± S.D.), from three independent transfections with two plates each, are expressed as fold induction relative to the activity of the reporter construct with the plasmid expressing anti-sense SF-1 (**A**) or in the absence of SF-1 (**B**), or as relative CAT activity, the activity of the pCSCAT in MA-10 cells being set at 100 (**C**). Black boxes in the lower panels indicate the SF-1-binding site of the cytosolic HMG-CoA synthase (−365 to −357) or the mutated sequence. Arrows over the black boxes represent the orientation of this sequence with respect to the wild-type (wt) promoter. The nucleotide sequence between −366 and −352 is shown. Arrows over the sequence indicate the motif and its orientation.

the promoter activity in MA-10 cells much more severely than in CV-1 cells, (75% compared with 35% decrease respectively) (Figure 2C).

Expression of the cytosolic HMG-CoA synthase gene and synthesis of labelled cholesterol from [^{14}C]acetate in the presence of 25-hydroxycholesterol is higher in steroidogenic cells containing SF-1

To show that cholesterol metabolism was less dependent on cholesterol levels in steroidogenic cells containing SF-1 than in SF-1-deficient cells, we examined the effect of 25-hydroxycholesterol on several parameters: (1) the HMG-CoA synthase mRNA levels, (2) the transcriptional activity of this gene and (3) the synthesis of cholesterol from labelled acetate. The mRNA levels in CV-1 cells are regulated in response to the cholesterol levels of the cell; as expected, they increased in lipoprotein-deficient serum and this increase was abolished by the addition of 25-hydroxycholesterol (Figure 3A). In contrast, in MA-10 cells, the mRNA levels were higher and independent of the presence of lipoprotein or 25-hydroxycholesterol. The transcriptional activity of a chimaeric construct (Figure 3B) and the cholesterol synthesis measured as the incorporation of [^{14}C]acetate into cholesterol (Figure 3C) are less diminished by 25-hydroxycholesterol in steroidogenic SF-1-containing MA-10 cells than in SF-1-deficient CV-1 cells.

Overexpression of SF-1 in CV-1 cells decreases the inhibitory effect of 25-hydroxycholesterol

To assess whether the different effects of 25-hydroxycholesterol in MA-10 and CV-1 cells were due to SF-1 expression levels, CV-1 cells were co-transfected with pCSCAT plus either an empty vector (pSG5) or an SF-1 expression vector and incubated in medium with 10% (v/v) delipidated fetal calf serum in the presence or absence of 25-hydroxycholesterol. As expected, 25-hydroxycholesterol repressed the cytosolic HMG-CoA synthase transcriptional activity in the absence of SF-1 (Figure 4). However, this repression was less pronounced in the presence of SF-1.

DISCUSSION

In steroidogenic tissues there is a lack of correlation between cholesterol levels and the activity of HMG-CoA reductase, the limiting enzyme of the mevalonate pathway [14,15]. In this paper we show that, in accordance with those results, the transcriptional activity and mRNA levels of the cytosolic HMG-CoA synthase gene and the synthesis of labelled cholesterol from [^{14}C]acetate do not seem to be regulated in cultured MA-10 cells by the presence of 25-hydroxycholesterol in the culture medium.

We also provide evidence that the gene encoding cytosolic HMG-CoA synthase contains a functional SF-1-responsive element. This is a TGAAGGTCA sequence that shows a coincidence of eight out of nine bases with the TCAAGGTCA consensus binding site proposed for SF-1. This sequence confers SF-1 responsiveness both in its natural context and on a normally unresponsive promoter; when it was altered by mutation, the transcriptional activity of the cytosolic HMG-CoA synthase promoter in MA-10 cells was severely decreased.

SF-1 is a member of the nuclear hormone receptor gene family that is constitutively expressed in adrenal cortical cells, the

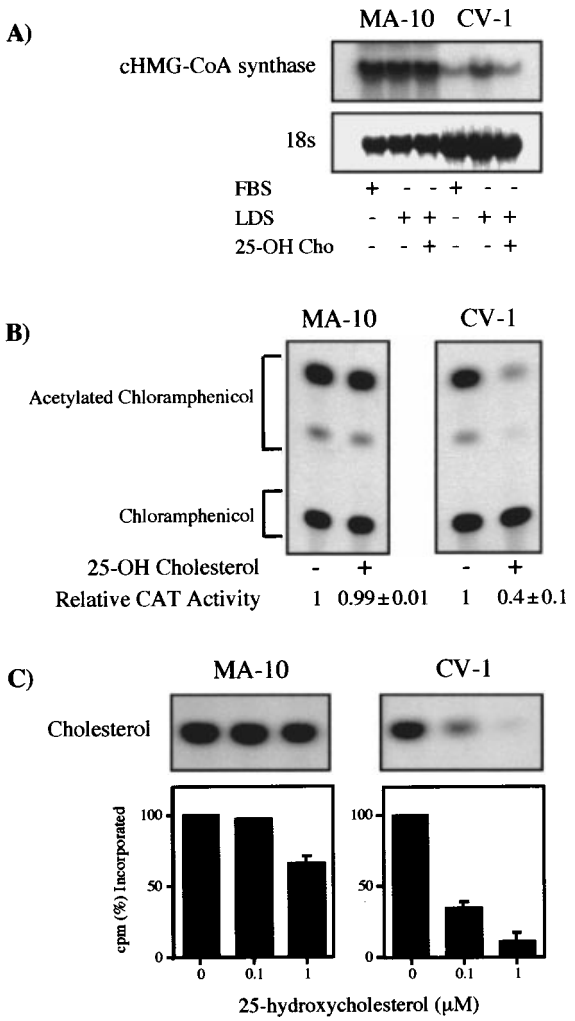

Figure 3 Effect of 25-hydroxycholesterol on the expression of mRNA for cytosolic HMG-CoA synthase and cholesterol synthesis from acetate, in MA-10 or CV-1 cells

(**A**) Cytosolic HMG-CoA synthase mRNA levels were analysed by Northern blot with a heterologous probe corresponding to the rat cDNA and total RNA from MA-10 cells (15 μg) or CV-1 cells (25 μg). Total RNA was obtained from cells incubated in 10% (v/v) fetal bovine serum (FBS) or 10% (v/v) lipoprotein-deficient serum (LDS) in the absence or presence of 1 μM 25-hydroxycholesterol (25-OH Cho), as indicated. The ethidium bromide staining of the 18 S rRNA is shown (18s) for normalization of the RNA loading. (**B**) Transcriptional activity was measured by the transfection of pSCCAT in MA-10 or CV-1 cells, which were incubated in a delipidated medium in the presence or the absence of 1 μM 25-hydroxycholesterol, as indicated. Average values of β-galactosidase-normalized CAT activity (means $\pm$ S.D.), from three independent transfections with two plates each, are expressed as relative CAT activity; the activity in the absence of 25-hydroxycholesterol was set at 1. (**C**) Cholesterol synthesis was measured in 35 mm plates in which CV-1 or MA10 cells (5×10^5) were incubated overnight in the appropriate medium. The medium was then changed to 10% (v/v) delipidated calf serum containing increasing amounts of 25-hydroxycholesterol, as indicated, and 2 μCi of [^{14}C]acetate (59 mCi/mmol; Amersham). After incubation for 24 h, the incorporation of radioactivity into cholesterol was measured as indicated in the Experimental section. The results are expressed as percentages of the radioactivity incorporated in the absence of 25-hydroxycholesterol. A representative autoradiograph of the lipid extracts is shown.

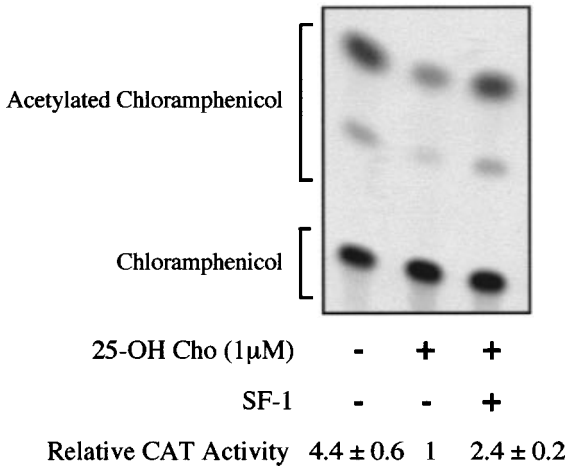

Figure 4 SF-1 expression in CV-1 cells diminishes the inhibitory effect of 25-hydroxycholesterol on the cytosolic HMG-CoA synthase transcriptional activity

CV-1 cells were transfected with pCSCAT and co-transfected or not with an expression vector for SF-1. The cells were incubated in delipidated serum in the presence or the absence of 1 μM 25-hydroxycholesterol. Average values of β-galactosidase-normalized CAT activity (means $\pm$ S.D.), from three independent transfections with two plates each, are expressed as relative CAT activity; the activity of the pCSCAT in the absence of SF-1 and in the presence of 25-hydroxycholesterol was set at 1. An autoradiograph showing a representative experiment is shown.

Leydig cells of the testes, and the granulosa, thecal and corpus luteal cells of the ovary. Although it has recently been suggested that cholesterol-derived compounds might act as ligands for this orphan receptor [11], this effect could be cell-specific [13]; it has been demonstrated that, in steroidogenic cells, transcriptional regulation of the steroid hydroxylase genes by SF-1 does not depend on the presence of 25-hydroxycholesterol and is not modified by its presence [12]. In this context we did not see 25-hydroxycholesterol dependence in the activation of the cytosolic HMG-CoA synthase gene by SF-1 in CV-1 cells at the concentration that we used in our experiments (1 μM) (results not shown); this effect might be dependent on the reporter construct used. The natural ligand for SF-1 remains undetermined.

Steroidogenic cells require cholesterol for membrane and steroid hormone synthesis. In steroidogenic cells, cholesterol is obtained from circulating lipoproteins or from endogenous synthesis. We hypothesize that the decrease in the 25-hydroxycholesterol inhibitory effect on cholesterol synthesis in MA-10 cells that we have shown here is due to the presence of SF-1 in those cells and to the activation by this transcription factor of, at least, the cytosolic HMG-CoA synthase gene, whose co-ordinate regulation controls the rate of cholesterol synthesis. Thus SF-1 has a key role in the regulation of both the endogenous synthesis and the supply of cholesterol to selected tissues, thus making this substrate available to the steroidogenic cell (Scheme 1). These results suggest a co-ordinated control of the expression of the genes involved in cholesterol acquisition (SR-BI [16–18] and cytosolic HMG-CoA synthase) and metabolism to steroid hormones (StAR [13]) through common transcription factors, SREBPs and SF-1.

Several papers have described the role of nuclear receptors such as LXR [26–30] or FXR [26,31–33] in the control of cholesterol catabolism. The present study offers the first link between nuclear receptors and cholesterol synthesis and adds one more component to the complex interplay of metabolites and transcription factors that control cholesterol homeostasis.

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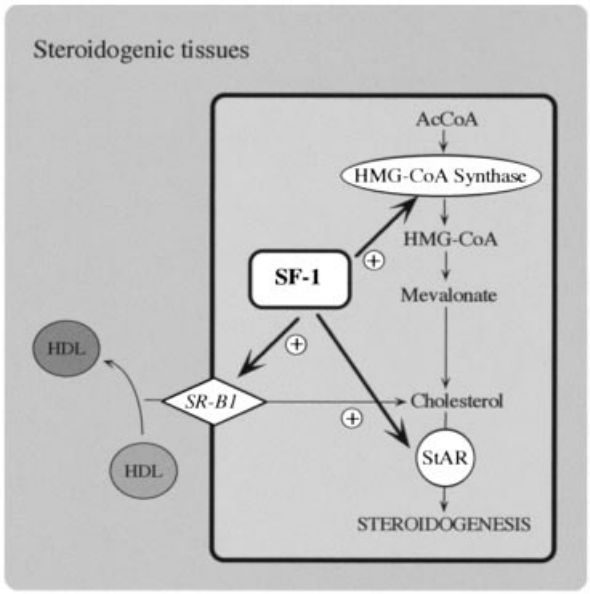

Scheme 1 Contribution of SF-1 to cholesterol homoeostasis

Abbreviations: AcCoA, acetyl-CoA; HDL, high-density lipoprotein.

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