Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3614–3618, June 1987
Biochemistry

Identification of promoter elements required for *in vitro* transcription of hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase gene

(cholesterol synthesis/end-product repression)

TIMOTHY F. OSBORNE, GREGORIO GIL, MICHAEL S. BROWN, ROBERT C. KOWAL, AND JOSEPH L. GOLDSTEIN

Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center, Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX 75235

Contributed by Michael S. Brown, February 9, 1987

ABSTRACT **The 5′-flanking region of the gene for hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is shown to contain promoter sequences that drive transcription *in vitro* in the presence of a HeLa whole-cell extract. DNase I protection studies revealed at least six different regions within the 277-base-pair (bp) promoter that bind nuclear proteins and produce "footprints." The functional significance of these sequences was determined through transcriptional analysis of a series of substitution mutations that scrambled short sequences throughout this region. Two of the footprint sequences were crucial for transcription *in vitro*; one of these contains a match in 6 of 6 bp, with a sequence in the adenovirus type 2 major late promoter that is known to be required for transcription. Scrambling a 26-bp sequence in a third footprint led to a consistent 2-fold increase in transcription, suggesting that this sequence might be a site for negative regulation. These studies define three regions that play a role in regulating transcription of the gene for HMG-CoA reductase, a negatively regulated enzyme in the cholesterol biosynthetic pathway.**

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a classic example of a rate-limiting enzyme in a biosynthetic pathway that is controlled by end-product repression (1). The enzyme produces mevalonate, an early intermediate in the biosynthesis of cholesterol and several other isoprenoid compounds in animal cells. The negative feedback regulation of this pathway occurs primarily as a result of an inhibition of transcription of the reductase gene. The gene is transcribed at a relatively high rate when cells are starved for cholesterol and other mevalonate-derived products (2, 3). Transcription is repressed by >80% when cholesterol and other mevalonate-derived products are supplied (2, 3).

As a first step in understanding the mechanism for control of transcription, we prepared a series of chimeric genes in which the 5′-flanking region of HMG-CoA reductase was fused to the gene for bacterial chloramphenicol acetyltransferase (CAT) and introduced them into mouse L cells by transfection (3). These studies revealed that the cis-acting information necessary for both transcription and repression by sterols was contained within a 509-base-pair (bp) fragment that extended from 277 bp upstream of the transcription initiation sites to 231 bp downstream. Large deletions within the 277-bp upstream segment led to markedly reduced transcription (3).

The 5′ end of the hamster reductase gene is somewhat unusual in that it lacks a classic "TATA" box (4), which is thought to position the site of transcription initiation in most eukaryotic genes (5). *In vivo*, reductase transcripts initiate from at least five different sites that are scattered over 100 bp (4, 6). The 5′-flanking region also lacks a classic CAAT sequence, but it does possess five copies of the sequence CCGCCC or its inverse complement (4). These sequences, known as GC boxes, are important positive elements in the promoters for both the simian virus 40 early region and herpes simplex virus thymidine kinase (5). They have also been found in the 5′-flanking region of several "housekeeping" genes that are transcribed at relatively low rates (7).

In an effort to understand the mechanism by which sterols inhibit transcription of the HMG-CoA reductase gene, we have begun by asking whether the 5′ flanking region of the reductase gene is able to drive transcription *in vitro* in a HeLa whole-cell extract system. This system, originally devised by Manley *et al.* (8), has been shown to be useful in the *in vitro* transcription of several viral and cellular genes (9). Moreover, the *in vitro* system is adequate to detect a requirement for specific promoter sequences, such as the TATA box and GC boxes (9, 10).

In the current studies, we show that HMG-CoA reductase is transcribed *in vitro* in the presence of a HeLa cell extract. We also demonstrate that transcription is either disrupted or enhanced by substitution mutations that alter the sequence of discrete elements located to the 5′ side of the transcription initiation sites.

METHODS

Materials. Male golden Syrian hamsters (80–100 g) were purchased from Sasco, Inc. (Omaha, NE) and kept under a 12-hr light/12-hr dark cycle for at least 7 days prior to use. Animals were fed a normal chow diet (Wayne Research Animal Diets) and sacrificed 3 hr into the light cycle. DNase I (no. LS0006330) was purchased from Worthington. Oligonucleotides were synthesized on a model 380A DNA synthesizer (Applied Biosystems, Foster City, CA).

Buffers. Buffer A: 50 mM Tris·HCl, pH 7.9 at 25°C/12.5 mM $MgCl_2$/1 mM EDTA/20% (vol/vol) glycerol. Buffer B: 10 mM Hepes-NaOH, pH 7.9/0.88 M sucrose/2 mM $Mg(OAc)_2$/1 mM EDTA/1.5 mM $CaCl_2$. Buffer C: Same as buffer B plus 20% glycerol. Buffer D: 10 mM Hepes-NaOH, pH 7.9/2.2 M sucrose/5 mM $Mg(OAc)_2$/1 mM EDTA. Buffer E: 10 mM Tris·HCl, pH 7.9/100 mM KCl/2 mM $MgCl_2$/0.1 mM EDTA. All buffers contained 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 50 μM leupeptin added just prior to use.

HeLa Cell Extracts. A suspension culture of HeLa cells, obtained from Robert Tjian (University of California at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAT, chloramphenicol acetyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MLP, adenovirus type 2 major late promoter; nt, nucleotide(s).

Berkeley), was grown at 37°C in minimum essential medium (GIBCO) supplemented with 5% (vol/vol) newborn calf serum/2 mM glutamine/100 units of penicillin per ml/100 μg of streptomycin per ml. Cultures were maintained between 3.5×10^5 and 7×10^5 cells per ml. Two days prior to harvest, the cells were collected by centrifugation, washed once with phosphate-buffered saline, and reseeded at 3.5×10^5 cells per ml in medium supplemented with 5% newborn calf lipoprotein-deficient serum (3), glutamine, and penicillin/streptomycin. HeLa whole-cell extracts were prepared by the procedure of Manley *et al.* (8). The final ammonium sulfate pellet was resuspended in buffer A containing 100 mM KCl and dialyzed at 4°C against 500 vol of the same buffer for 12–24 hr with one change. The extract (8–18 mg of protein per ml) was divided into 0.5-ml aliquots and stored at −70°C.

Preparation of Hamster Liver Nuclear Extracts. All operations were carried out at 0°C–4°C. Livers from male Syrian hamsters were perfused with 150 mM NaCl/15 mM sodium citrate to remove erythrocytes. Tissue was mixed (6 ml per g of tissue) with buffer B, homogenized in a Potter–Elvehjem grinder fitted with a motor-driven Teflon pestle, and filtered through cheesecloth. The filtrate (850 ml) was layered over 150 ml of buffer C in Sorvall GC3C centrifuge bottles. After centrifugation at 3500 × *g* for 20 min, the pellets were resuspended in buffer B (0.6 ml per g of original tissue). An equal volume of buffer D was added, and this mixture was layered over 10 ml of buffer D in Sorvall AH 627 centrifuge tubes. After centrifugation at 100,000 × *g*, for 1 hr, the purified nuclei (pellet) were washed three times by resuspension in buffer B containing 0.1 M KCl followed by centrifugation at 8000 × *g* for 10 min. The final nuclear pellet was stored frozen at −70°C. The yield was $\approx 10^8$ nuclei per g of liver. The purified nuclei were resuspended in buffer E (0.1 ml per g of original tissue) to which 5 M NaCl was added to give a final concentration of 0.4 M. Nuclei were lysed by gentle stirring for 30 min and centrifuged at 100,000 × *g* for 30 min. The resulting supernate was dialyzed against two changes of buffer A, centrifuged for 15 min at 100,000 × *g*, and the supernate was retained.

Plasmids. pRedCAT-1 contains the hamster HMG-CoA reductase promoter [nucleotides (nt) −277 to +231] cloned into the *Hin*dIII site of pSV0CAT (3). Oligonucleotide-directed mutagenesis (11) was performed with oligonucleotides that hybridized to 15 nt of the wild-type sequence on either side of the target sequence. Mutants A (18 nt different from wild type), B (18 nt), C (22 nt), D (26 nt), and F (6 nt) were made by substituting random nucleotides for the indicated number of nucleotides in the wild-type sequence. Mutants E and G–P were made by substituting the same 10 nt (5′ TGGTCGACCA 3′) for 10 nt of the wild-type sequence. The sequence that was introduced contains a *Sal* I site. All mutant promoters were reinserted into pSV0CAT. The position of each mutation is diagramed in Figs. 4 and 5. pALP (obtained from R. Tjian) contains the human adenovirus type 2 major late promoter (MLP) (−404 to +33 relative to the RNA cap site) cloned into the *Pvu* II site of pBR322.

***In Vitro* Transcription Assays.** HeLa whole-cell extract (150–250 μg of protein), pALP (50 ng), and the indicated supercoiled reductase plasmid template (250 ng) were preincubated at 25°C in a buffer consisting of 25 mM Tris·HCl, pH 7.9/16 mM $MgCl_2$/65 mM KCl/0.5 mM EDTA/1 mM dithiothreitol/0.1 mM phenylmethylsulfonyl fluoride/10% (vol/vol) glycerol/2% (vol/vol) polyvinyl alcohol. After 45 min, transcription was started with the addition of 2 μl of a solution containing ATP, UTP, CTP, and GTP (final concentration, 0.25 mM each). After 45 min at 25°C, transcription was terminated by addition of 150 μl of stop solution (200 mM NaCl/20 mM EDTA/1% $NaDodSO_4$/125 μg of wheat germ tRNA per ml). The RNA was extracted with phenol/$CHCl_3$, precipitated with ethanol, and subjected to S1 nuclease analysis (3). Gels were exposed to XAR-5 film for 12–16 hr at −20°C with intensifying screens.

Preparation of Single-Stranded End-Labeled ^{32}P Probes for S1 Nuclease Analysis. The probe for HMG-CoA reductase was prepared by cloning the noncoding strand of the *Hin*dIII fragment from pRedCAT-1, which contains the promoter and part of the 5′ untranslated sequence of the mRNA, into M13 mp19 (3). For the MLP, we cloned the noncoding strand of the *Sau*3A1 fragment from pALP containing the entire MLP sequence (12) into M13 mp19. Complementary oligonucleotides were 5′-end-labeled with [γ-^{32}P]ATP and polynucleotide kinase and hybridized to the appropriate M13 clone. The hybrid was extended and truncated with a restriction endonuclease that cuts in the polylinker sequence distal to the promoter insert, and the resulting single-stranded ^{32}P probes were gel-purified. The 5′ end of each oligonucleotide was positioned so that after hybridization to the complementary mRNA S1 nuclease cleavage would result in a protected fragment of 125 nt for reductase mRNA and 77 nt for MLP mRNA. ^{32}P probes for DNase I footprinting were prepared by labeling double-stranded DNA fragments with [γ-^{32}P]ATP and polynucleotide kinase.

DNase I Footprinting. DNase I footprinting was performed as described by Dynan and Tjian (13) with minor modifications. Nuclear extracts of hamster liver were incubated with 10 fmol of the ^{32}P-labeled DNA probe ($\approx 2.5 \times 10^4$ cpm) on ice for 15 min in 50 μl of a buffer containing 25 mM Tris·HCl (pH 7.9), 6.25 mM $MgCl_2$, 50 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 10% glycerol, and 2% polyvinyl alcohol. A solution containing 5 mM $CaCl_2$ and 10 mM $MgCl_2$ (50 μl) was added, followed by incubation at room temperature for 60 s. DNase I (25–375 ng in 1–15 μl) was added, and the samples were incubated for an additional 60 s, after which 150 μl of stop solution (see above)

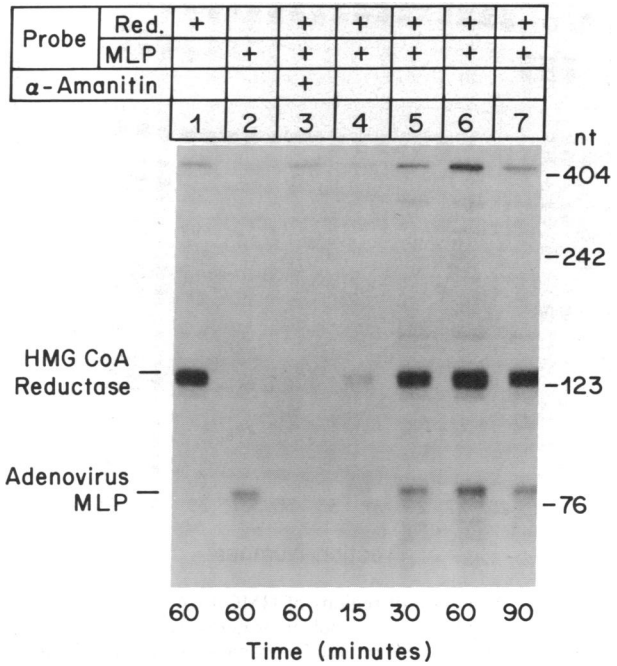

FIG. 1. *In vitro* transcription driven by HMG-CoA reductase promoter (pRedCAT-1) and adenovirus MLP. Transcription was carried out with a HeLa cell extract as described in *Methods*, and the S1 nuclease-resistant DNA fragments specific for the two mRNA transcripts were separated on a denaturing acrylamide gel. Probes for reductase and MLP transcripts were added separately (lanes 1 and 2) or together (lanes 3–7). Lanes 4–7, time of incubation varied from 15 to 90 min. α-Amanitin (1 μg/ml) was added to the reaction mixture in lane 3. Numbers on right indicate the position of migration of fragments of *Msp* I-digested ^{32}P-labeled pBR322 DNA.

was used to terminate the reaction. The samples were extracted with phenol/$CHCl_3$, precipitated with ethanol, and analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography. Gels were exposed to XAR-5 film for 16 hr at −20°C with intensifying screens.

RESULTS

A plasmid containing 509 bp from the HMG-CoA reductase promoter fused to the coding region of the bacterial CAT gene was efficiently transcribed when incubated with a HeLa cell extract *in vitro* (Fig. 1, lane 1). The mRNA transcript gave rise to a single S1 nuclease-protected fragment of 125 nt, indicating a single site of transcription initiation that corresponded to position +1 of the reductase gene. This is the most upstream of the multiple initiation sites for reductase that have been observed *in vivo* (3, 4, 6). As an internal control, we measured the synthesis of the adenovirus late transcript driven by the MLP (lane 2). This transcript generated an S1 nuclease-protected fragment of 77 nt. Transcription of both plasmids was abolished by α-amanitin, confirming that it was dependent on RNA polymerase II (lane 3). Transcription of both plasmids reached a maximum between 30 and 60 min (lanes 5 and 6). In subsequent assays, we used a 45-min time point.

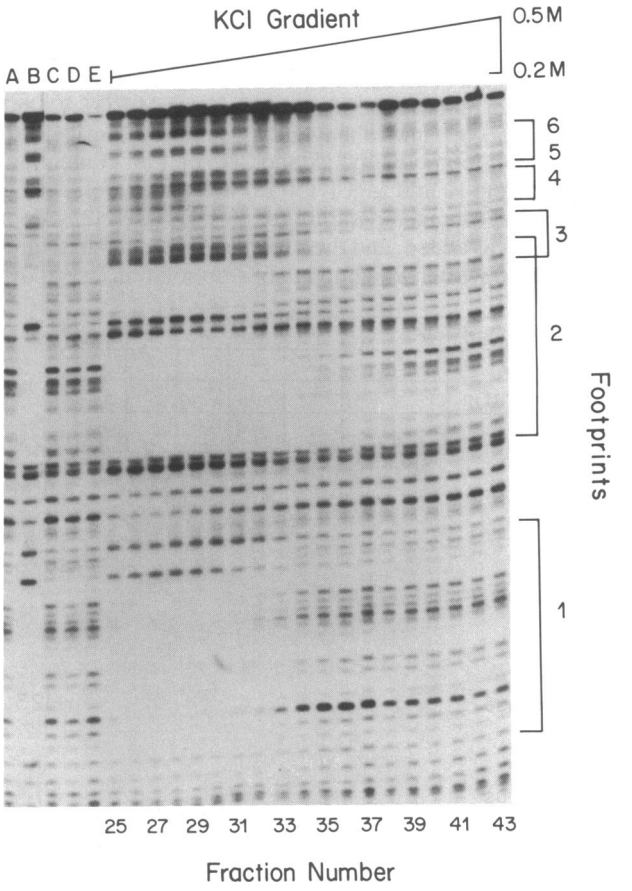

FIG. 2. Protection of regions of HMG-CoA reductase promoter from DNase I digestion by nuclear proteins from hamster liver. A DNA fragment from the reductase promoter region of pRedCAT-1 (nt −276 to +38) was 5′-end-labeled with ^{32}P at −276, incubated with various nuclear extracts, and subjected to DNase I footprinting analysis. Lane A, no nuclear protein; lane B, 16 μg of crude nuclear protein. All other reactions were performed with aliquots (10 μl) of column fractions derived from heparin agarose (Bio-Rad) chromatography of hamster liver nuclear protein (72 mg of protein applied to an 18-ml column). The sample was applied to the column in buffer A and the flow-through was collected (lane C). The column was washed with buffer A containing 0.1 M KCl (lanes D and E), and eluted with a 140-ml linear gradient of 0.1–0.5 M KCl in buffer A (fractions 25–43).

To define regions of the promoter that bind proteins that are important for *in vitro* transcription, we performed DNase I footprinting assays with the reductase promoter (Fig. 2). In the presence of a hamster liver nuclear extract, large regions of the promoter were protected from DNase I; these regions were arbitrarily divided into six general footprint regions (lane B). When the nuclear extract was applied to a heparin-agarose column, the proteins that gave rise to the footprints were adsorbed to the column, and very little footprinting activity emerged in the flow-through or in low-salt washes (lanes C–E). The proteins giving rise to the footprints were eluted from the column with a KCl gradient. The proteins that produced footprints 1, 2, 5, and 6 eluted earliest from the column, followed by the proteins that produced footprints 3 and 4. Footprints 4–6 were visualized more discretely when we used a different fragment of the promoter in which the position of labeling was closer to the DNase I-protected sites (Fig. 3).

To search for a functional role for these footprinted regions of DNA, we prepared a series of "scrambled" substitution mutations in which the sequence of short regions at various locations was altered. The sequence of the promoter region, the positions of the footprints, and the location of each scrambled mutation is shown in Fig. 4. Each of these plasmids was tested for transcription activity in the HeLa extract (Fig. 5). Scrambling the sequence corresponding to the 3′ end of footprint 2 (plasmid D) produced a consistent increase in transcription. Scrambling short segments within footprint 3, which contains 2 GC boxes, did not have a major effect on transcription. Alteration of footprint 4 nearly abolished transcription (plasmids H and I). Scrambling base pairs between footprints 4 and 5 or within footprint 5 had no major effect. On the other hand, scrambling base pairs within footprint 6, which is in the position that is normally occupied by a TATA box, strongly reduced transcription.

All of the above transcription reactions were carried out in the presence of the MLP, which was used as an internal

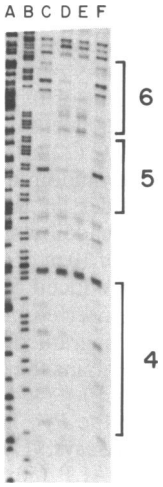

FIG. 3. Footprints 4, 5, and 6 of HMG-CoA reductase promoter generated by DNase I digestion in the presence of nuclear proteins from hamster liver. To prepare an appropriately labeled fragment, we took advantage of the *Sal* I site that was introduced at position −126 of mutant E of the reductase promoter. The fragment spanning nt −126 to +277 was 5′-end-labeled with ^{32}P at position −126 and subjected to DNase I footprinting analysis. To position the protected regions, Maxam and Gilbert sequencing reactions (C+T and A+G) (14) were performed on the same DNA fragment (lanes A and B). The reactions in lanes C and F were performed in the absence of nuclear proteins. The reactions in lanes D and E were performed with 25 and 75 μg of crude nuclear proteins, respectively.

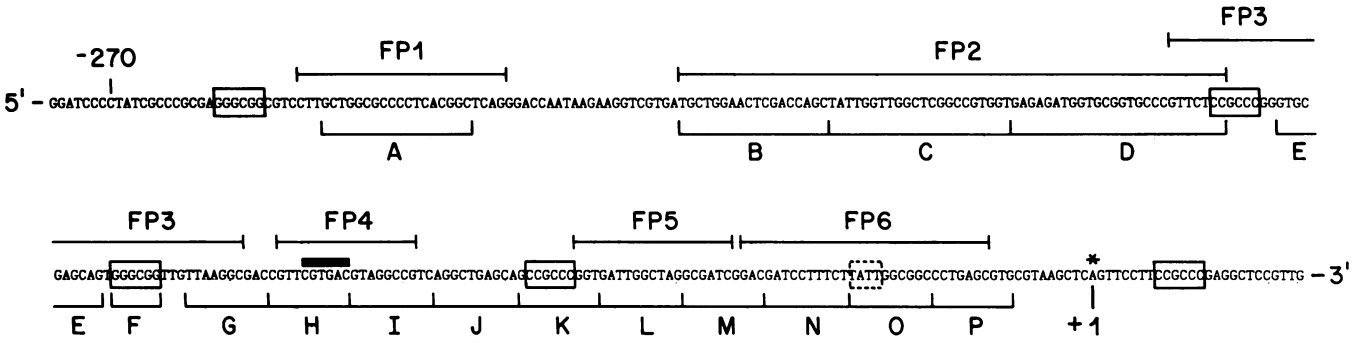

FIG. 4. Sequence of the HMG-CoA reductase promoter region showing positions of footprints (FP1–FP6) and sequences that were subjected to mutation (A–P). The five G+C-rich sequences are boxed. Asterisk indicates the site of transcription initiation; dashed box denotes a possible TATA box; 6-bp homology with the DNA binding site for the MLP transcription factor is denoted by the heavy overline in FP4.

standard. To rule out possible artifacts arising from competition for common transcriptional factors, we performed another series of transcription reactions in which the MLP was omitted. The results exactly paralleled those that were obtained in the presence of the MLP except that plasmids K and L, which contain scrambled sequences in footprint 5, were now transcribed at only 12–22% of the wild-type level (data not shown).

DISCUSSION

The major conclusions of the current study are as follows: (*i*) the 5′-flanking region of the HMG-CoA reductase gene contains promoter elements that drive transcription by RNA polymerase II *in vitro* in the presence of a HeLa cell extract; and (*ii*) at least two upstream elements are absolutely required for this transcription. These elements correspond to the regions identified as footprints 4 and 6.

The footprint 4 sequence is ≈18 bp long, and it is located ≈85 bp upstream of the major *in vitro* transcription initiation site (Figs. 4 and 5). Alteration of the base-pair sequence in this region, as in mutants H and I, nearly abolished *in vitro* transcription. Alteration of the sequences immediately surrounding this region (mutants G and J) had comparatively little effect. The footprint 4 sequence contains a match in 6 of 6 bp with a sequence (CGTGAC) in the MLP that is known to be required for transcription and is part of the binding site for a transcription factor (12, 16). We do not yet know whether this factor is the same as the protein that binds to footprint 4.

The footprint 6 sequence important for reductase transcription is located ≈30 nt upstream of the transcription initiation site and contains the sequence TTATT, which is likely to be serving as a TATA box *in vitro*. Disruption of this sequence, as in mutant O, abolishes *in vitro* transcription. In previous studies of the histone H2B gene, *in vitro* transcription has been shown to be dependent on the presence of a TATA box (17). On the other hand, the dihydrofolate reductase gene, which lacks a TATA box, is transcribed efficiently *in vitro*, but multiple transcription initiation sites are observed (18).

It is of interest that the TATA box-equivalent in the HMG-CoA reductase gene seems to be much more significant *in vitro* than it is *in vivo*. In the *in vitro* assay, we found only one site of transcription initiation, which appeared to be

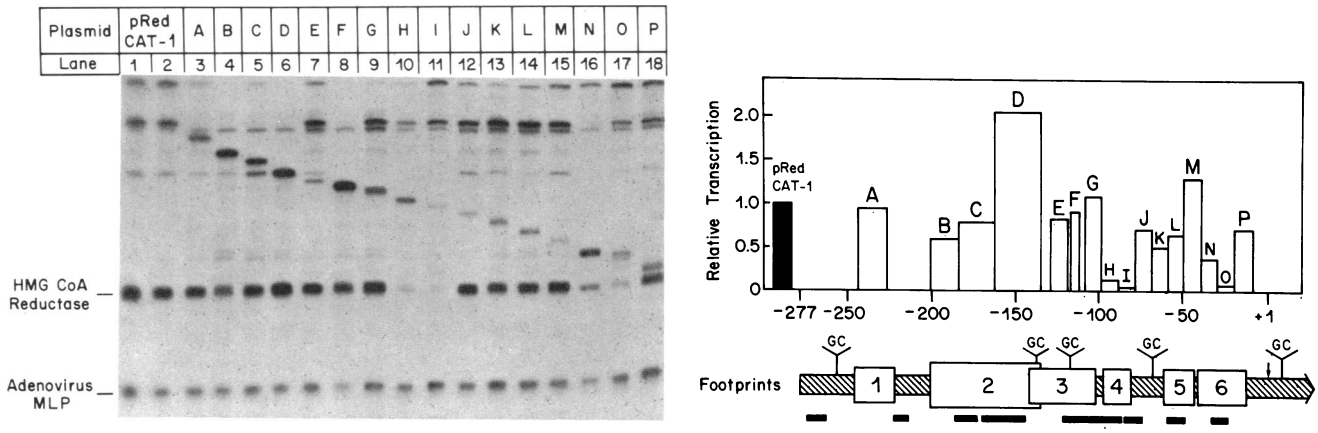

FIG. 5. *In vitro* transcription of mutant HMG-CoA reductase promoter plasmids. (*Left*) pRedCAT-1 (lanes 1 and 2) and mutant plasmids A–P (lanes 3–18) were transcribed with a HeLa whole-cell extract as described in *Methods*. The adenovirus MLP construct was included in all reactions as an internal control. The positions of the S1 nuclease-resistant transcription products specific for reductase and MLP RNAs are indicated. The band that moves in stair-step fashion down the gel from left to right results from S1 nuclease cleavage at the mismatch region in the hybrid between the wild-type DNA probe and RNA transcripts that initiate upstream of the reductase promoter insert in each mutant plasmid. (*Right*) Relative transcription of plasmids driven by wild-type and mutant reductase promoters *in vitro*. The horizontal axis indicates the location of each mutant in the promoter sequence. Position +1 corresponds to the major *in vitro* RNA initiation site; it is located at position −744 relative to nucleotide A of the ATG codon that initiates translation of reductase protein (4). The width of each mutant bar is a relative indication of the size of the substitution mutation. The height of each bar is proportional to the amount of correctly initiated mRNA produced from each plasmid in relation to the amount of mRNA produced from MLP in the same tube. Autoradiograms were scanned with a densitometer (Hoefer GS300), and the ratio of the reductase signal to the MLP was set at 1 for pRedCAT-1; all values are plotted relative to this value. Data represent the average of two to five independent experiments for each plasmid. A schematic representation of the reductase promoter and the locations of the footprint regions is shown below. Small thick horizontal lines at the bottom denote regions of sequence identity between the hamster and human reductase promoters (15). Each line represents at least a 9-bp perfect match between the two promoters; the width of each line is proportional to the length of sequence identity.

controlled by the TTATT sequence at position −29. On the other hand, in human liver and adrenal (15), hamster liver (4), and cultured Chinese hamster ovary cells (3, 4, 6), reductase transcripts originate at multiple sites. The one that predominates *in vitro* is the most upstream of these sites.

Mutations that scramble the sequence of various GC boxes (mutants D, F, and K) did not reduce *in vitro* transcription of HMG-CoA reductase by >50%. In other genes that contain multiple GC boxes, transcription does not cease until all of them are eliminated (18, 19). Thus, our failure to find a single crucial GC box is consistent with the possible redundancy of GC box functions.

Mutants that altered the sequence of the 3′ end of footprint 2 (plasmid D) showed a 2-fold increased transcription when compared with the wild-type promoter. This was true whether transcription was studied in the presence of the MLP (Fig. 5) or in its absence (data not shown). These data raise the possibility that the 3′ end of footprint 2 contains a negative sequence that inhibits transcription. Whether this sequence plays any role in the normal sterol-mediated feedback regulation of reductase remains to be determined.

There is extensive sequence conservation in the hamster and human HMG-CoA reductase promoters (15), as indicated by the underlined regions in Fig. 5 (*Right*). Several of these conserved regions, such as those in footprints 3 and 5, did not appear crucial for transcription in the HeLa cell system. This may be attributable to a redundancy of function, as suggested above for the GC boxes, or it might be due to a more subtle role that these sequences play in regulatory or tissue-specific events rather than in basal transcription.

The current studies delineate the protein-binding regions of the HMG-CoA reductase promoter and reveal at least two regions that are crucial for *in vitro* transcription and one region that may be inhibitory. These studies lay the groundwork for a more detailed analysis of the proteins that control transcription of this negatively regulated housekeeping gene.

We thank Michael Briggs and Robert Tjian for helpful advice on DNase I footprinting. Amy Chen and Anna Delaloye provided excellent technical assistance, Lavon Saunders provided invaluable help in growing cultured cells, and James Cali synthesized all of the oligonucleotides. This research was supported by a research grant from the National Institutes of Health (HL 20948) and the Lucille P. Markey Charitable Trust. T.F.O. is a Lucille P. Markey Scholar.

1. Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21,** 505–517.
2. Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S. & Goldstein, J. L. (1983) *J. Biol. Chem.* **258,** 8462–8469.
3. Osborne, T. F., Goldstein, J. L. & Brown, M. S. (1985) *Cell* **42,** 203–212.
4. Reynolds, G. A., Basu, S. J., Osborne, T. F., Chin, D. J., Gil, G., Brown, M. S., Goldstein, J. L. & Luskey, K. L. (1984) *Cell* **38,** 275–286.
5. McKnight, S. & Tjian, R. (1986) *Cell* **46,** 795–805.
6. Reynolds, G. A., Goldstein, J. L. & Brown, M. S. (1985) *J. Biol. Chem.* **260,** 10369–10377.
7. Kadonaga, J. T., Jones, K. A. & Tjian, R. (1986) *Trends Biochem. Sci.* **11,** 20–23.
8. Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3855–3859.
9. Dynan, W. S. & Tjian, R. (1985) *Nature (London)* **316,** 774–778.
10. Hansen, U. & Sharp, P. A. (1984) *Compr. Virol.* **19,** 65–97.
11. Zoller, M. J. & Smith, M. (1983) *Methods Enzymol.* **100,** 468–500.
12. Carthew, R. W., Chodosh, L. A. & Sharp, P. A. (1985) *Cell* **43,** 439–448.
13. Dynan, W. S. & Tjian, R. (1983) *Cell* **35,** 79–87.
14. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
15. Luskey, K. L. (1987) *Mol. Cell. Biol.*, in press.
16. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43,** 165–175.
17. Sive, H. L., Heintz, N. & Roeder, R. G. (1986) *Mol. Cell. Biol.* **6,** 3329–3340.
18. Farnham, P. J. & Schimke, R. T. (1986) *Mol. Cell. Biol.* **6,** 2392–2401.
19. Jones, K. A. & Tjian, R. (1984) *Nature (London)* **317,** 179–182.