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# Conservation of Promoter Sequence but Not Complex Intron Splicing Pattern in Human and Hamster Genes for 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase

KENNETH L. LUSKEY

*Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235*

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**Regulation of the expression of 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase is a critical step in controlling cholesterol synthesis. Previous studies in cultured Chinese hamster ovary cells have shown that HMG-CoA reductase is transcribed from a cholesterol-regulated promoter to yield a heterogeneous collection of mRNAs with 5′ untranslated regions of 68 to 670 nucleotides in length. Synthesis of these molecules is initiated at multiple sites, and multiple donor sites are used to excise an intron in the 5′ untranslated region. In the current paper, I report that the human HMG-CoA reductase gene resembles the Chinese hamster gene in having multiple sites of transcription initiation that are subject to suppression by cholesterol. The human gene differs from the hamster gene in that a single donor splice site is used to excise the intron in the 5′ untranslated region. All of the resulting RNAs have short 5′ untranslated regions of 68 to 100 nucleotides. This difference in the splicing pattern of the first intron is species specific and not a peculiarity of cultured cells in that HMG-CoA reductase mRNAs from Syrian hamster livers resemble those of the cultured Chinese hamster ovary cells. Comparison of the DNA sequences of the HMG-CoA reductase promoters from three different species—humans, Syrian hamsters, and Chinese hamsters—shows a highly conserved region of 179 nucleotides that extends from 220 to 42 nucleotides upstream of the transcription initiation sites. This region is 88% identical between the human and Chinese hamster promoter. When fused to the coding region of the *Escherichia coli* chloramphenicol acetyltransferase gene, this highly conserved region of the reductase gene directs the cholesterol-regulated expression of chloramphenicol acetyltransferase in transfected hamster cells, further indicating the interspecies conservation of the regulatory elements.**

De novo synthesis is one of two main routes by which mammalian cells obtain the cholesterol they need to manufacture plasma membranes, bile acids, steroid hormones, and lipoproteins. This pathway involves more than 20 enzymatic reactions that allow cells to make cholesterol from acetyl coenzyme A (acetyl-CoA) (5). The amount of cholesterol produced is controlled by regulating the activities of several key enzymes in this pathway. In particular, several enzymes at the beginning of the pathway that are responsible for the synthesis and phosphorylation of mevalonate are susceptible to negative-feedback regulation by cholesterol (7). Among these reactions, a major rate-limiting step involves the formation of mevalonate from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (5). This reaction is catalyzed by the microsomal enzyme HMG-CoA reductase.

In rapidly dividing cells such as cultured fibroblasts, cholesterol obtained from the receptor-mediated endocytosis of lipoproteins is the primary source of cellular cholesterol (5). The cholesterol from the lipoproteins suppresses the synthesis of cellular cholesterol by inhibiting the expression of several regulated enzymes, including HMG-CoA reductase. Recent studies have provided considerable insight into the mechanisms responsible for the regulation of HMG-CoA reductase. In cultured cells, reductase mRNA levels are regulated by the delivery of cholesterol (11, 12, 23, 30). These changes in mRNA levels have been shown to be associated with decreased transcriptional rates of the reductase gene (30). In addition to the regulation of RNA levels, changes in the degradation of the reductase protein also contribute to suppression of enzyme activity (9, 20, 31). In the presence of cholesterol, preformed enzyme is degraded approximately threefold more rapidly than when the cell is deprived of an external source of cholesterol. Decreased transcription from the reductase gene and increased turnover of the protein work in concert to suppress HMG-CoA reductase activity in response to cholesterol (14, 16).

The mechanism by which transcription of the HMG-CoA reductase gene is regulated by sterols has been studied primarily in UT-1 cells (11, 16, 30), a Chinese hamster ovary (CHO)-derived cell line that is resistant to a competitive inhibitor of HMG-CoA reductase, compactin (15). These cells have amplified the reductase gene 15-fold and express high levels of reductase mRNA and protein (30). In these cells, a complex array of reductase RNA transcripts has been described by Reynolds et al. (39, 40). This complexity is due to multiple sites of transcription initiation, multiple donor splice sites for an intron in the 5′ untranslated region, and multiple polyadenylation sites at the 3′ end of the gene. Two major classes of transcripts were defined by differences in the 5′ untranslated region of the mRNA. The majority of the transcripts (class 1) are initiated at various points within a span of 25 nucleotides and spliced at a single site 45 to 70 nucleotides downstream. The class 1 mRNAs therefore has a 5′ untranslated region of only 68 to 93 nucleotides. The class 2 transcripts are initiated near the site that class 1 transcripts are spliced. The initiation sites span a region of 30 nucleotides. These transcripts extend more than 300 nucleotides downstream before being spliced at one of three sites to the same acceptor site that is used for the class 1 transcripts. The class 2 mRNAs have 5′ untranslated regions that range from 354 to 670 nucleotides and contain up to eight ATG codons upstream of the ATG used to initiate

translation of the reductase protein. (For a schematic illustration of this complex transcription unit, see Fig. 4 in the article by Reynolds et al. [40]). Both classes of reductase RNA transcripts are subject to feedback regulation by cholesterol in UT-1 cells (40). The role that this variation in the 5′ untranslated region might play was uncertain.

The region upstream of the complex transcription initiation sites contains the reductase promoter, yet it lacks a classic TATA box or CCAAT box (36, 39). However, five repeats of the sequence 5′-GGGCGG-3′ or its complement are located within 300 nucleotides of the transcription initiation sites. This sequence is part of the recognition sequence for Sp1, a cellular transcriptional factor that has been characterized by Tjian and co-workers (4, 13, 24). This factor appears to be involved in the transcription of many cellular genes that contain such GC-rich sequences (24). The ability of this GC-rich region in the reductase gene to function as a promoter that is susceptible to inhibition by sterols has been demonstrated by Osborne et al. (36). In the studies by Osborne, a DNA fragment extending 280 nucleotides upstream of transcription initiation and including 229 nucleotides of the 5′ untranslated region was able to direct the sterol-regulated expression of chloramphenicol acetyltransferase (CAT) when a chimeric reductase promoter-CAT plasmid was transfected into mouse L cells. High-level expression required the presence of at least 180 nucleotides upstream of the site of transcription initiation.

In this paper, I characterize the 5′ end of the HMG-CoA reductase gene in humans and Syrian hamsters. The results show that the human reductase transcripts do not exhibit the complexity of splicing that was observed in UT-1 cells. However, both classes of reductase transcripts are observed in the liver of Syrian hamsters, suggesting that the splicing pattern is species specific and found in normal hamster cells as well as in UT-1 cells. The promoter region of the human HMG-CoA reductase gene can be expressed and regulated in the same manner as the Chinese hamster. Comparison of the promoters for this gene among these different species has indicated regions that are highly conserved and may therefore play a critical role in regulating the transcription of this gene.

## MATERIALS AND METHODS

**Materials.** A human genomic DNA library (27) was kindly provided by Tom Maniatis of the Department of Biology, Harvard University, Cambridge, Mass. A Syrian hamster genomic DNA library was kindly provided by Philip Tucker of the Department of Microbiology, University of Texas Health Science Center at Dallas. Compactin (15) was a gift from Akira Endo (Tokyo Noko University, Tokyo, Japan). The plasmid pRedCAT-1 (36) was provided by Tim Osborne of the Department of Molecular Genetics, University of Texas Health Science Center at Dallas, and pSVO-CAT (22) was provided by Bruce Howard, National Cancer Institute, Bethesda, Md. Restriction enzymes were purchased from New England BioLabs, Inc., Beverly, Mass.; the Klenow fragment of *Escherichia coli* DNA polymerase was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind.; S1 nuclease was purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md.; and avian myeloblastosis virus reverse transcriptase was purchased from Life Sciences, Inc., St. Petersburg, Fla. [$^{14}$C]chloramphenicol (50 to 60 Ci/mmol) and [$\alpha$-$^{32}$P]dCTP (3,000 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. [$\gamma$-$^{32}$P]ATP (>5,000 Ci/mmol) was obtained from ICN Pharmaceuticals Inc., Irvine, Calif. Newborn calf lipoprotein-deficient serum (density, >1.215 g/ml) was prepared by ultracentrifugation (21). Cholesterol and 25-hydroxycholesterol were purchased from Alltech Associates, Inc., Applied Science Div., State College, Pa., and Steraloids, Inc., Deerfield, Ill., respectively. Other materials were obtained from previously described sources (36, 39).

**Isolation and sequencing of genomic HMG-CoA reductase clones.** Recombinant bacteriophage from a human genomic library (27) or a Syrian hamster genomic library were screened by the method of Benton and Davis (2). Hybridization was performed in 0.75 M sodium chloride–75 mM sodium citrate–50 mM sodium phosphate (pH 7.0)–0.2% bovine serum albumin–0.2% Ficoll (Pharmacia Fine Chemicals, Piscataway, N.J.)–0.2% polyvinylpyrrolidone–200 μg of denatured salmon sperm DNA per ml–0.1% sodium dodecyl sulfate–30% formamide with approximately $2 \times 10^5$ cpm of $^{32}$P-labeled DNA probe per ml at 42°C for 16 h. $^{32}$P-labeled DNA probes were prepared by random hexanucleotide-primed labeling (17). Filters were washed in 0.3 M sodium chloride–30 mM sodium citrate (pH 7.0)–0.1% sodium dodecyl sulfate at 55°C. Hybridization-positive clones were isolated, and phage DNA was prepared. Restriction fragments containing the HMG-CoA reductase promoter were identified by restriction enzyme mapping and hybridization with $^{32}$P-labeled B1 probe (39) from the Chinese hamster HMG-CoA reductase promoter. Restriction enzyme fragments were subcloned into appropriate plasmid vectors or bacteriophage M13 vectors. DNA sequencing was performed by the dideoxy chain termination method (41) or, when indicated, the chemical degradation method of Maxam and Gilbert (33). Specific oligonucleotide primers were synthesized by the phosphoramidite method (6) by using an oligonucleotide synthesizer (model 380A; Applied Biosystems, Foster City, Calif.). DNA sequences were analyzed with a DNA analysis program (Microgenie; Beckman Instruments, Inc., Fullerton, Calif.) for the IBM personal computer (38).

**Growth of cells for preparation of mRNA.** All cells were grown in monolayer cultures at 37°C in an atmosphere of 5 to 7% $CO_2$. Stock cultures of simian virus 40 (SV40)-transformed human fibroblasts were grown in medium A (Dulbecco modified Eagle medium containing 100 U of penicillin per ml and 100 μg of streptomycin per ml) supplemented with 10% (vol/vol) fetal calf serum. For preparations of RNA, $8 \times 10^6$ cells were initially seeded in roller bottles containing 75 ml of medium A supplemented with 10% fetal calf serum. These cultures were refed every 48 h with the above medium. On the day before harvest, the cells were washed two times with phosphate-buffered saline and fed with either induction medium or suppression medium. Induction medium consisted of medium A supplemented with 10% lipoprotein-deficient serum. In some cases, 10 μM compactin was also added to the induction medium, as indicated in the figures. Suppression medium consisted of medium A containing 10% fetal calf serum supplemented with 10 μg of cholesterol per ml and 1 μg of 25-hydroxycholesterol per ml. After 24 h of incubation under these conditions, the cells were washed two times with phosphate-buffered saline, and total cellular RNA was prepared by extraction with guanidinium isothiocyanate and centrifugation through a cesium chloride cushion, as previously described (39).

RNA was also prepared, as previously described (32), from the livers of Syrian hamsters that had been fed a diet

containing cholestyramine, a bile-acid binding resin, and mevinolin, an HMG-CoA reductase inhibitor, to induce the expression of HMG-CoA reductase.

**5′-End mapping of HMG-CoA reductase mRNA transcripts.** Uniformly labeled $^{32}$P-labeled single-stranded probes ($5 \times 10^5$ cpm of [$^{32}$P]dCTP incorporated per pmol) and 5′-$^{32}$P-end-labeled single-stranded probes ($10^7$ cpm/pmol) complementary to HMG-CoA reductase mRNA were prepared from M13 templates, as described by Reynolds et al. (40). The radioactively labeled DNA probes were hybridized with total cellular RNA (10 to 100 μg) and analyzed by S1 nuclease mapping as described by Berk and Sharp (3). The products were analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography. The 5′ termini of the mRNA transcripts were also analyzed by primer extension (39). Total cellular RNA was hybridized at 55°C for 1 h with 0.2 pmol of synthetic oligonucleotides complementary to the HMG-CoA reductase mRNA that was 5′ $^{32}$P end labeled ($10^7$ cpm/pmol) with [γ-$^{32}$P]ATP and T4 polynucleotide kinase. Primer-extended products were then synthesized in the presence of 0.33 mM deoxynucleoside triphosphate–10 μg of actinomycin D per ml–8 U of avian myeloblastosis virus reverse transcriptase at 37°C for 1 h. The products were analyzed by denaturing gel electrophoresis and autoradiography.

**Assay of HMG-CoA reductase promoter-CAT fusion genes.** Dihydrofolate reductase (DHFR)-deficient CHO cells (DX-B11) (46) were obtained from L. Chasin of Columbia University, New York, N.Y. These cells were maintained in medium B (Ham F-12 medium containing 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM glutamine) supplemented with 10% (vol/vol) fetal calf serum. The plasmid pHRedCAT-3 was introduced into DHFR-deficient CHO cells by cotransfection with pFD11 (42), a plasmid that contains the mouse DHFR cDNA driven by the SV40 promoter (kindly provided by A. Levinson of Genentech, San Francisco, Calif.). The Chinese hamster HMG-CoA reductase promoter-CAT construct, pRedCAT-1, was introduced into DHFR-deficient CHO cells in a similar manner. For transfection, on day 0, $5 \times 10^5$ DHFR-deficient CHO cells were seeded into each petri dish (100 by 15 mm) containing 12 ml of medium B with 10% fetal calf serum. On day 1, a calcium phosphate coprecipitate (47) of either pHRedCAT-3 or pRedCAT-1 (9.5 μg) and pFD11 (0.5 μg) was added to the medium of each dish. After a 5-h incubation with the DNA, the medium was removed, and medium B supplemented with 10% (vol/vol) glycerol was added to the cells for 4 min at 37°C. The cells were washed three times with phosphate-buffered saline and then cultured with medium B. The next day, selection was begun with medium C (Ham F-12 medium lacking hypoxanthine, glycine, and thymidine and containing 25 mM HEPES, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM glutamine, 1 nM methotrexate, and 10% fetal calf serum). The cells were refed medium C every 3 days until visible colonies were evident. At this time, all of the colonies in each dish were pooled and maintained as stock cultures for assays of reductase promoter function.

To analyze the promoter, cells from the pooled stock cultures were seeded (100,000 cells per 60-mm petri dish or 300,000 cells per 100-mm petri dish) in medium C. On days 1 and 2, the cells were refed medium B containing 10% lipoprotein-deficient serum containing the following additions. To suppress HMG-CoA reductase activity, 10 μg of cholesterol per ml and 0.1 μg of 25-hydroxycholesterol per ml were added to the medium on days 1 and 2. To induce HMG-CoA reductase activity, no addition was made on day 1, and 1 μM compactin was added on day 2. On day 3, cells from each treatment protocol were harvested to analyze CAT enzymatic activity (22, 36), and in certain experiments, RNA was prepared from the cells as described above.

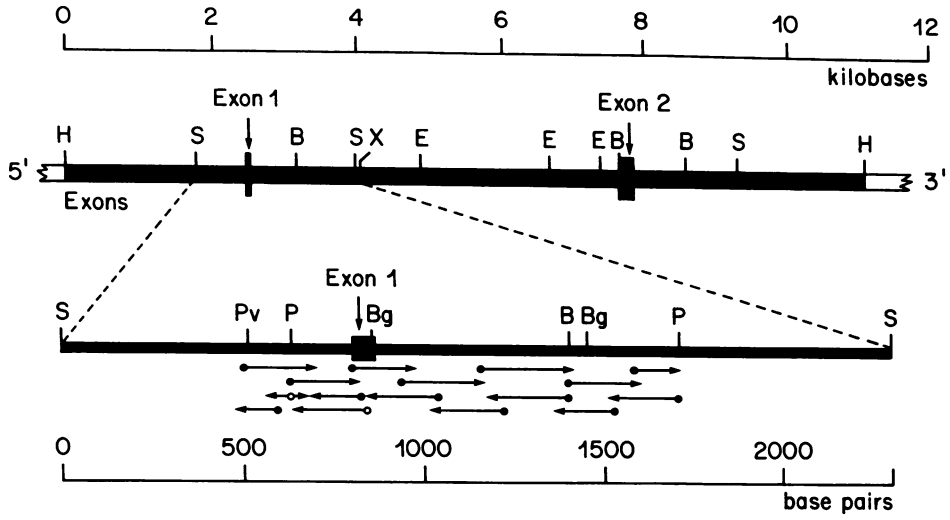
**Other assays.** CAT activity was measured as previously described (36). Protein concentration was determined by the method of Lowry et al. (29).

## RESULTS

**Characterization of the 5′ flanking region of the human HMG-CoA reductase gene.** Genomic clones for the human HMG-CoA reductase gene that had been isolated previously (31) did not extend far enough 5′ to include the first exon of the gene. To obtain this region of the gene, recombinant bacteriophage clones were selected from a human genomic library (27) by hybridization to a 430-base-pair *Eco*RI-*Bgl*II genomic fragment that includes 280 base pairs of the first intron and 150 base pairs of the second exon of the human HMG-CoA reductase gene. One of these genomic clones, λHRed-5, contained an 11-kilobase (kb) *Hin*dIII fragment (Fig. 1) that cross-hybridized with the 5′ flanking region of the Chinese hamster HMG-CoA reductase gene (data not shown). This 11-kb *Hin*dIII human restriction fragment corresponded to a similar-sized fragment that had been detected in *Hin*dIII digests of human genomic DNA (28). This *Hin*dIII fragment was subcloned into a plasmid vector, and the region that cross-hybridized with the Chinese hamster reductase promoter was identified and subjected to DNA sequencing (Fig. 1). As will be described, this sequence spans the promoter and sterol regulatory elements, multiple sites of transcription initiation, the 5′ untranslated region, and the 5′ part of the first intron of the human reductase gene.

Owing to the complexity of transcription initiation in this promoter, the nucleotide corresponding to the most 5′ site of transcription initiation is designated +1. Sequences downstream are numbered in a positive fashion. Sequences upstream of transcription initiation begin at −1, and the numbers increase in a negative fashion as one moves in a 5′ direction farther away from the sites of transcription initiation. The sequence from positions +56 to +82 is identical to the most 5′ 27 nucleotides in the human reductase cDNA, pHRed-102 (31).

The splicing pattern of the first intron of the human HMG-CoA reductase gene was examined by S1 nuclease analysis by using a uniformly labeled probe derived from the cDNA, pHRed-102 (31) (Fig. 2). This 367-nucleotide probe is complementary to 92 nucleotides from the 5′ end of the coding region of HMG-CoA reductase as well as the 50 nucleotides of the 5′ untranslated region that are present in pHRed-102. Additional sequences in the probe are derived from the SV40 promoter and GC tail constructed in the course of cloning the cDNA. This probe spans the splice site for the first intron at position −23 in the 5′ untranslated region of the cDNA, the location where heterogeneous splicing of the Chinese hamster reductase mRNA was observed (40). After hybridization with total cellular RNA from SV40-transformed human fibroblasts and digestion with S1 nuclease, a single protected fragment of 142 nucleotides was detected (Fig. 2, lane D). This band was decreased when the RNA was obtained from cells that had been treated with sterols to suppress HMG-CoA reductase activity (Fig. 2, lane C). The amount of protected fragment was increased

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-300  GCCTGGTCCC CTATCGCCTC CGCCTAGCAG CTGCCATCGG TGCGCCCCCA CAGCTCTAGG ACCAATAGG  CAGGCCCTAG TGCTGGGACT CGAACGGCTA  -201
-200  TTGGTTGGCC GAGCCGTGGT GAGAGATGGT GCGGTGCCTG TTCTTGGCCC TGCAGAGAGC TGTGGGCGGT TGTTAAGGCG ACCGTTCGTG ACGTAGCGCC  -101
-100  GTCAGGCCGA GCAGCCCCCA GGCGATTGGC TAGACAATCG AACGATCCTC TCTTATTGGT CGAAGGCTCG TCCAGCTCCG AGCGTGCGTA AGGTGAGGGC    -1

 +1   TCCTTCCGCT CCGCGACTGC GTTAACTGGA GCCAGGCTGA GCGTCGGCGC CGGGGTTCGG TGGCCTCTAG TGAGATCTGG AGgtgaggcg ggcggtgacc  +100

+101  gagaagaggg gcaggggcgg cggggagcgg ggcgagatgg gtgggagcgg ggtttgggct gtgttggtgg caattctgga gcttccctcg gccctgggaa  +200
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+901  gatacaaata tagaacagtt aatttctgca g                                                                          +931
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FIG. 1. Restriction map and DNA sequence of the 5′ end of the human HMG-CoA reductase gene. At the top, the restriction map of an 11-kb *Hin*dIII DNA fragment that spans the 5′ end of the human reductase gene is shown. A more detailed restriction map of a 2.3-kb *Sph*I fragment is shown in an expanded format. The strategy for sequencing this region of the human reductase gene is indicated. The arrows indicate the direction and extent of each sequencing reaction, with sequencing performed by the dideoxy chain termination method (●) or by the chemical degradation method of Maxam and Gilbert (○) (33). The positions of exons 1 and 2 are indicated by the closed boxes. Additional downstream exons are not indicated in this diagram. Restriction enzymes were abbreviated as follows: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; S, *Sph*I. At the bottom, the DNA sequence of the first exon as well as its 5′ and 3′ flanking regions are shown. This DNA sequence is numbered relative to the most 5′ initiation site being designated +1. Sequences upstream of transcription initiation are numbered from −1 to −300 in a 5′ direction. As described in the text, the sites of transcription initiation determined by S1 nuclease analysis (○) and by primer extension analysis (●) are indicated. The splice site at the 5′ end of the first intron (▲) is indicated. The single copy of the GC box, GGGCGG, is enclosed in a box.

when the RNA was obtained from cells that had been treated with compactin to induce HMG-CoA reductase activity (Fig. 2, lane E). No additional protected fragments were detected under any of these conditions. In particular, we did not observe a 115-nucleotide fragment that would have been generated if the sequence upstream of position −23 differed from the sequence of the cDNA probe. This splicing pattern was also observed with RNA from human fetal liver (Fig. 2, lane I) or fetal adrenal (Fig. 2, lane J). This indicates that in a variety of human cells all of the reductase transcripts were spliced to the 5′ untranslated sequence upstream of −23 that was present in pHRed102. The 5′ splice site at +82 of the first exon of the human reductase gene (Fig. 1) is the only sequence used to excise the first intron in human cells. This splicing pattern is in contrast to the four splice donor sites that were described for the first intron of HMG-CoA reductase from Chinese hamster UT-1 cells (40).

The sites for transcription initiation of the human reductase gene were determined by two methods. S1 nuclease analysis was performed with a single-stranded end-labeled probe that is complementary to the 5′ end and extends beyond the start sites of human reductase mRNA (Fig. 3A). Multiple protected fragments ranging from 45 to 77 nucleotides in length were visualized when this probe was hybridized to RNA derived from cells grown in the absence of sterols (Fig. 3A, lane C). The initiation sites that gave rise

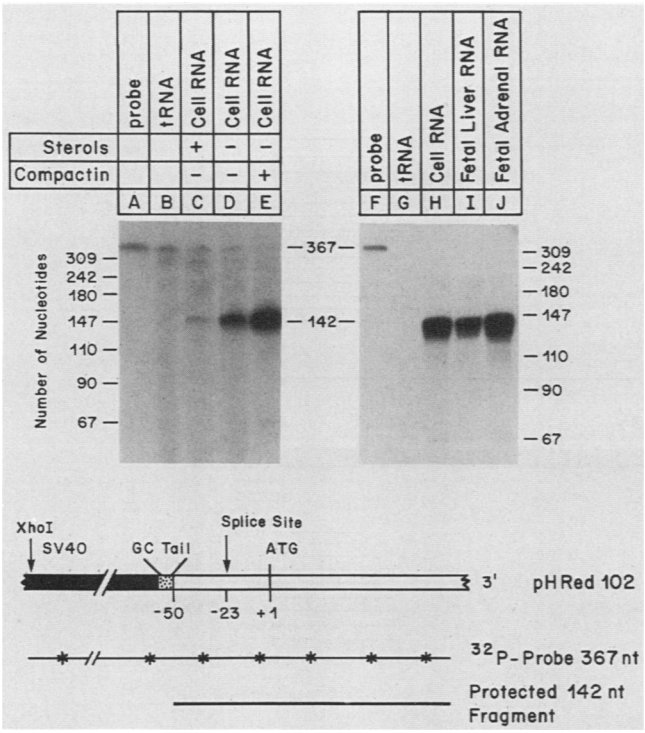



FIG. 2. S1 nuclease analysis of intron 1 in the human reductase gene mapped with a cDNA probe. Total RNA from SV40-transformed human fibroblasts (10 μg) was annealed at 55°C for 16 h to a single-stranded uniformly labeled $^{32}$P-probe from pHRed-102 ($2 \times 10^4$ cpm). Cellular RNA was obtained from fibroblasts grown in the presence of lipoprotein-deficient serum (lane D) or after the addition of sterols (lane C) or compactin (lane E). A control hybridization with tRNA (10 μg) is shown in lane B. The RNA-DNA hybrids were digested with 200 U of S1 nuclease for 1 h at room temperature and subjected to electrophoresis and autoradiography for 16 h at −70°C with an intensifying screen. The position of the 367-nucleotide undigested probe and the 142-nucleotide protected fragment are indicated to the right of the gel. Another experiment is shown on the right in which human fetal liver RNA (lane I) or human fetal adrenal RNA (lane J) was hybridized to the cDNA probe. Control hybridizations with tRNA (lane G) or cellular RNA from fibroblasts after the addition of compactin (lane H) are shown. The hybridizations, S1 nuclease digestions, and electrophoresis were done as described above. In the diagram at the bottom, the structure of pHRed-102 is indicated. Symbols: ■, portion of the plasmid derived from SV40 DNA; ▩, GC tail at the cloning site; □, portion of pHRed-102 that encodes HMG-CoA reductase. In this figure, nucleotides are numbered relative to the cDNA sequence. Position +1 corresponds to the A of the ATG codon for the first methionine of the reductase protein. The 5′ untranslated region of the cDNA is numbered −1 to −50. The 5′ boundary of the second exon is located at position −23 and is labeled splice site. Nucleotides −50 to −24 in the cDNA correspond to +56 to +82 in the first exon of the human HMG-CoA reductase gene (Fig. 1). Beneath the diagram of pHRed-102, the locations of the uniformly labeled $^{32}$P-probe and the observed protected fragment are indicated. nt, Nucleotides.

to the major protected bands are shown in the right-hand part of Fig. 3 and are indicated in the sequence in Fig. 1. No protected fragments were seen when RNA was obtained from cells grown in the presence of sterols to suppress HMG-CoA reductase activity (Fig. 3, lane B), indicating that transcription from all of the initiation sites was susceptible to feedback suppression. A complex pattern of transcription initiation was also detected when the 5′ ends of the mRNA were analyzed by primer extension (Fig. 3B). The primer consisted of a 5′-end-labeled oligonucleotide that was labeled at the same position as that of the S1 nuclease probe (position +77). When this oligonucleotide was hybridized to RNA obtained from cells induced for HMG-CoA reductase, primer extension with reverse transcriptase yielded products of 45 to 77 nucleotides (Fig. 3, lane F). These sites of initiation are indicated in the sequence in Fig. 1. Although there were minor differences in the pattern of bands obtained by S1 nuclease and primer extension analyses, they both indicate a similar pattern for the main sites of transcription initiation. Just as with S1 nuclease analysis, the mRNAs detected by primer extension were all suppressed when RNA was obtained from cells grown in the presence of sterols (Fig. 3, lane E).

The data of Fig. 3 indicate that the sites for transcription initiation span the region from +1 to +33. Inasmuch as all of these transcripts are spliced at position +82, they all generate relatively short 5′ untranslated sequences of 73 to 105 nucleotides (including the 23 untranslated nucleotides at the 5′ end of the second exon). None of these 5′ untranslated regions contains ATG codons upstream of the ATG codon used to initiate translation of HMG-CoA reductase. No human reductase mRNAs that contain a long 5′ untranslated sequence with ATG codons upstream of that used to initiate translation of the reductase protein were found. These findings contrast with those of Reynolds et al. (40) who found that such long transcripts constitute about 30% of all reductase mRNAs in Chinese hamster UT-1 cells.

**Assay of the human HMG-CoA reductase promoter.** In the Chinese hamster HMG-CoA reductase gene, the sequences responsible for promotion of transcription and its inhibition by sterols are contained within a region that extends from 300 base pairs upstream of the transcription initiation sites through the first 200 nucleotides of the 5′ untranslated region (36). To ascertain whether the 5′ end of the human HMG-CoA reductase gene behaves in a similar manner, a restriction fragment from the 5′ end of the human reductase gene was fused to the gene for *E. coli* CAT. The DNA fragment extends from a *Pvu*II restriction site at position −270 to a *Bgl*II site at position +77. The fragment includes the multiple sites of transcription initiation. The plasmid containing the fusion gene, pHRedCAT-3, was transfected into DHFR-deficient CHO cells together with a plasmid that confers DHFR activity. Pooled colonies of cells expressing DHFR were selected and analyzed for expression of CAT activity. For purposes of comparison, a plasmid containing the corresponding fragment of the Chinese hamster HMG-CoA reductase promoter fused to the CAT gene (pRedCAT-1) was introduced into DHFR-deficient CHO cells and analyzed in a similar manner. Two independent pools of cells transfected with either pHRedCAT-3 or pRedCAT-1 were analyzed for CAT activity (Table 1). In cells in which the human promoter was linked to the CAT gene, CAT activity was 1.09 to 7.13 nM/min per mg of protein when these cells were grown in the presence of compactin to induce HMG-CoA reductase activity. When these cells were grown in the absence of compactin and in the presence of sterols to suppress HMG-CoA reductase activity, CAT activity was suppressed by 65 to 79%. The extent of suppression of the human reductase promoter was similar to that of the Chinese hamster promoter (Table 1).

The amount of mRNA produced by the human reductase promoter-CAT chimeric gene was assessed by quantitative primer extension analysis (Fig. 4) with RNA isolated from cells transfected with pHRedCAT-3. Primer extension yielded a collection of products from 45 to 77 nucleotides in

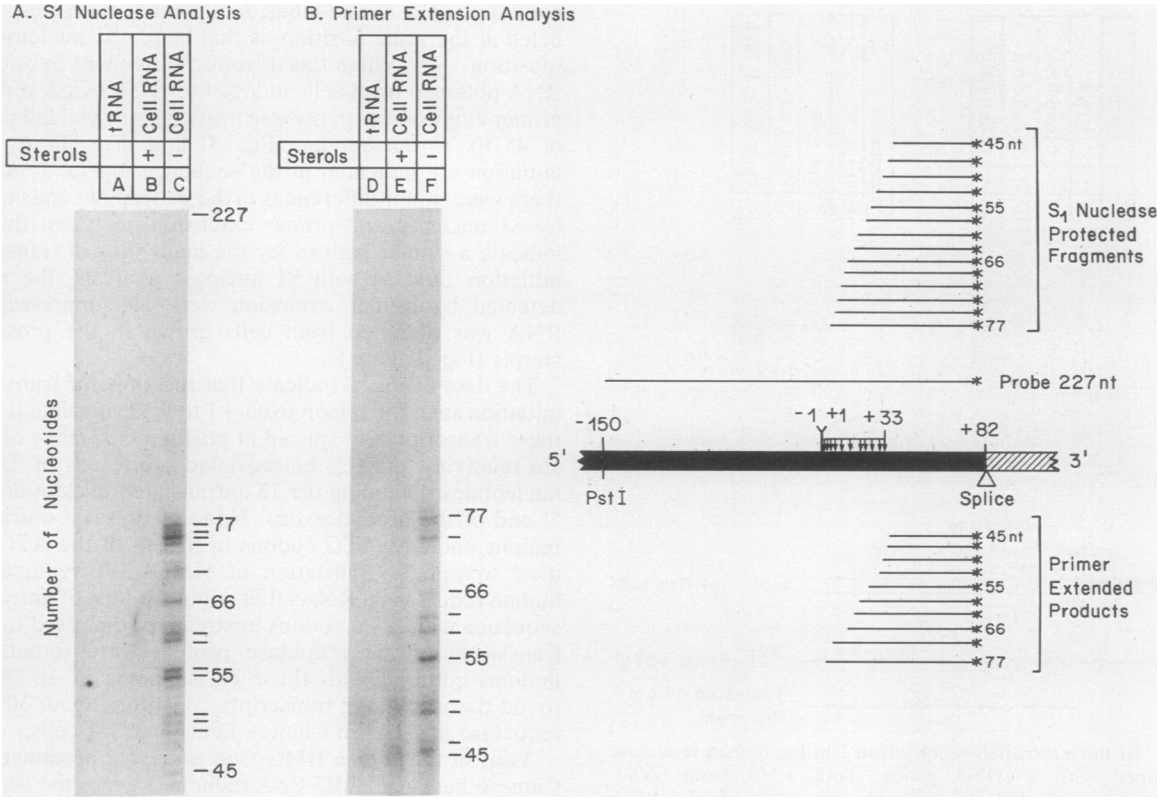



FIG. 3. Analysis of the 5′ end of human HMG-CoA reductase transcripts. (A) S1 nuclease analysis. Total RNA (100 μg) from SV40-transformed human fibroblasts was annealed at 55°C for 16 h to a single-stranded, 5′-end-labeled $^{32}$P-probe ($5 \times 10^3$ cpm) complementary to nucleotides +77 to −150 (Fig. 1) in the human HMG-CoA reductase gene. RNA from cells grown in 10% lipoprotein-deficient serum either with (lane B) or without (lane C) sterols was analyzed. A control hybridization was done with tRNA (100 μg; lane A). The RNA-DNA hybrids were digested with 800 U of S1 nuclease for 1 h at room temperature and subjected to electrophoresis and autoradiography for 16 h at −70°C. The positions of the undigested probe and the protected fragments are shown at the right of the autoradiogram. (B) Primer extension analysis. Total RNA (20 μg) was hybridized with a 5′-$^{32}$P-end-labeled synthetic oligonucleotide complementary to positions +77 to +56. Complementary DNAs were synthesized at 37°C for 1 h with reverse transcriptase. Primer-extended products were analyzed by electrophoresis and autoradiography for 16 h at −70°C. RNA from cells grown in lipoprotein-deficient serum either with (lane E) or without (lane F) sterols was analyzed. Primer extension analysis with tRNA was also performed (lane D). The lengths of the primer extended products are indicated at the right side of the autoradiogram. At the far right, a schematic diagram of the 5′ end of the human HMG-CoA reductase gene is shown. The positions of the most abundant S1 nuclease-protected fragments and primer-extended products are indicated (arrows). From these analyses, the most 5′ site of transcription initiation is designated +1 (Fig. 1). The most 3′ site of transcription initiation that was observed appears at position +33. Splicing of the first intron occurs at position +82. This position corresponds to position −24 in the human reductase cDNA shown in Fig. 2. nt, Nucleotides.

length (Fig. 4, lanes B and E). When these cells were grown in the presence of sterols to suppress HMG-CoA reductase activity, none of these products were detected (Fig. 4, lanes C and F). Primer extension of RNA derived from a DHFR-deficient CHO cell line did not reveal any detectable HMG-CoA reductase transcripts (Fig. 4, lane D). This is because the Chinese hamster HMG-CoA reductase gene does not share sufficient homology with the oligonucleotide primer to allow extension of cDNA products. Thus, all of the products detected in the transfected cells were derived from the human reductase promoter. The lengths of the primer-extended products in Fig. 4 revealed that the human promoter-CAT chimeric gene used a cluster of initiation sites that were identical to those of the native HMG-CoA reductase gene in human fibroblasts. Thus, the primer-extended products from SV40-transformed human fibroblasts grown in the absence of sterols (Fig. 4, lane G) or in the presence of compactin (Fig. 4, lane A) are the same as that detected from the pHRedCAT-3-transfected hamster cells.

**The HMG-CoA reductase gene in Syrian hamster liver.** The data presented above indicate that the 5′ untranslated region of the human HMG-CoA reductase mRNA is much less complex than the 5′ untranslated region of the HMG-CoA reductase mRNA produced by UT-1 cells. As discussed above, the latter cells produce two different classes of transcripts, designated class 1 and class 2, that vary at their sites of transcription initiation and at the 5′ donor sites used to remove the first intron.

To determine whether normal hamster tissues produce complex HMG-CoA reductase mRNAs like those of the UT-1 cells, we isolated the 5′ end of the HMG-CoA reductase gene from Syrian hamsters. The cDNA for the Syrian hamster HMG-CoA reductase is highly homologous to the Chinese hamster cDNA (44). To isolate a portion of the Syrian hamster gene a genomic library derived from Syrian hamster DNA was screened with a radiolabeled probe from the Chinese hamster HMG-CoA reductase promoter. A genomic clone was selected, and the region homologous to the Chinese hamster promoter was subcloned as a 2.2-kb *Eco*RI fragment into a plasmid vector. The DNA

TABLE 1. Sterol regulation of CAT activity in CHO cells transfected with HMG-CoA reductase promoter-CAT chimeric genes[a]

| Promoter (plasmid) | CAT activity (nmol/min per mg of protein) | | % Suppression by sterols |
|---|---|---|---|
| | Induced | Suppressed | |
| Human, −270 to +77 (pHRedCAT-3) | | | |
| Expt. 1, pool A | 1.09 | 0.74 | 65 |
| Expt. 1, pool B | 7.13 | 2.04 | 71 |
| Expt. 2, pool B | 4.32 | 0.92 | 79 |
| Chinese hamster, −280 to +229 (pRedCAT-1) | | | |
| Expt. 1, pool A | 1.08 | 0.12 | 89 |
| Expt. 1, pool B | 1.74 | 0.54 | 69 |
| Expt. 2, pool B | 1.48 | 0.18 | 88 |

[a] Transfected cells were grown as described in Materials and Methods. Before harvest, monolayers were grown in medium B containing 10% lipoprotein-deficient serum and either treated with 1 μM compactin to induce the HMG-CoA reductase promoter or treated with 10 μg of cholesterol per ml and 0.1 μg of 25-hydroxycholesterol per ml in the absence of compactin to suppress the HMG-CoA reductase promoter. Cells were harvested and analyzed for their expression of CAT. Each value represents the mean of duplicate determinations.

sequence was then determined in the region spanning the HMG-CoA reductase promoter and the first exon of the Syrian hamster HMG-CoA reductase gene (Fig. 5). (The nucleotide that corresponds to the +1 position of the human HMG-CoA reductase gene has been numbered +1.)

To map the 5′ and 3′ boundaries of the first exon in the Syrian hamster gene, we performed a series of S1 nuclease experiments with total cellular RNA from Syrian hamster liver. Figure 6 shows the results of experiments with three different complementary single-stranded probes. These overlapping probes were either end labeled or uniformly labeled in a fashion that allowed the identification of both class 1 and 2 transcripts. Probe 1 was an end-labeled probe that extended from position +68 to −58. After hybridization with RNA and digestion with S1 nuclease, protected fragments of 43 to 50 nucleotides were observed. When DNA sequencing gels were used to analyze these class 1 fragments, they resolved into three doublets whose termini are marked in Fig. 5. Probe 2 was an end-labeled probe that extended from position +227 to −60. When hybridized to hamster liver RNA, probe 2 gave rise to a set of protected fragments that were 140 to 209 nucleotides in length. The termini of these fragments corresponded to the class 2 transcription initiation sites marked in Fig. 5. The largest of these protected fragments (209 nucleotides) corresponds to mRNAs that initiate at the sites that were detected with probe 1. The smaller fragments represent transcription initiation sites that were 3′ of those detected with probe 1. The finding of protected fragments with a probe labeled at position +227 indicated that some of the mRNA species extended past the splice donor site at position +73 (class 1 transcripts). These transcripts appeared to correspond to the class 2 transcripts previously detected in UT-1 cells. To further characterize the class 1 and 2 transcripts, I used probe 3, a uniformly labeled probe complemetary to nucleotides +476 to −58. After hybridization of probe 3 to RNA and S1 nuclease digestion, several groups of fragments were observed. The first group is labeled A and consists of fragments of 48 to 55 nucleotides in length, 5 nucleotides longer than the fragments detected with probe 1. These transcripts are initiated at +19 to +27 and use the 5′ donor splice site at position +73. Additional fragments that were detected with probe 3 are labeled B and C. These represent reductase mRNA molecules that are initiated at the sites detected with probe 2 and are spliced downstream of the splice site at +73. Because the sites of initiation and splicing both varied, the exact positions of initiation and splicing could not be accurately determined in this experiment. However, the approximate locations that would account for the observed fragments are shown on the schematic diagram at the right of Fig. 6. These experiments show that in the livers of Syrian hamsters treated with a drug regimen to induce expression of HMG-CoA reductase, mRNA molecules analogous to the class 1 and 2 transcripts observed in UT-1 cells were present. Additional experiments with RNA from the livers of Syrian hamsters that had not been treated with drugs to induce reductase activity show that all species of reductase mRNA transcripts were present but reduced in abundance (data not shown).

**Conservation of the 5′ end of the HMG-CoA reductase gene.** A comparison of the DNA sequence of the 5′ end of the human HMG-CoA reductase gene with that of the Chinese hamster shows that those regions involved in promoting transcription and regulating transcription by sterols, as well as the portion of the 5′ untranslated region that is present in mRNAs in both species, were conserved (Fig. 7). However, beyond the splice site for the class 1 transcripts these genes diverge. To compare these homologous regions in more detail, the human and Chinese hamster sequences were optimally aligned from nucleotide −297 to +119 in the human sequence. The result of this comparison is illustrated in Fig. 8 with insertions, deletions, and mismatches in the human sequence relative to the Chinese hamster sequence indicated.

Within this homologous region, it can be seen that the differences are not evenly distributed. The most highly conserved region is found between nucleotides −220 to −42. Over this stretch of 179 nucleotides, 88% are identical. Several extended stretches of complete identity between the human and Chinese hamster sequences are found. These include a perfect match of 26 nucleotides from −188 to −163 and a perfect match of 35 nucleotides from −139 to −105. Just 29 nucleotides upstream of transcription initiation, a 13-nucleotide insertion is present in the human gene. Several more small insertions and substitutions are noted throughout the 5′ untranslated region of the gene. After the consensus sequence for the 5′ donor site of the intron, the sequences of these two species diverge greatly and cannot be aligned.

A comparison of the 5′ ends of the Chinese hamster and Syrian hamster HMG-CoA reductase genes shows that they are almost identical (Fig. 9). The region that is highly conserved in the human gene was also highly conserved in the Syrian hamster. In the Syrian hamster an insertion of nine nucleotides was found between −31 and −23. A 13-nucleotide insertion is found at the same position in the human sequence. Within the 5′ untranslated region of the class 2 transcripts in Chinese hamster cells, Reynolds et al. noted the occurrence of several ATG codons followed by open reading frames of various lengths (39, 40). In the Syrian hamster sequence, the first four of these ATG codons were not conserved. The four downstream ATG codons were present. However, the peptides encoded by the Chinese hamster 5′ untranslated region would differ greatly from those encoded in the Syrian hamster owing to nucleotide changes and a frameshift. In particular, the 106-amino-acid open reading frame described by Chin et al. (10), which

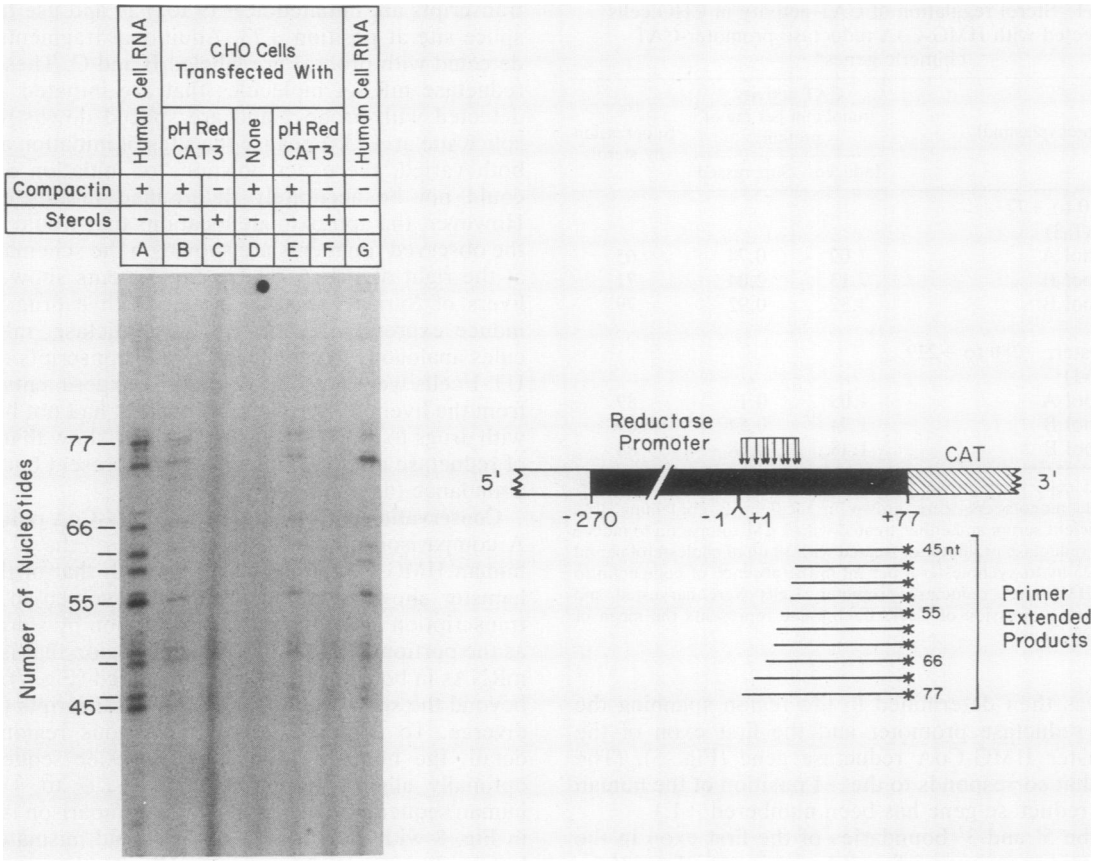


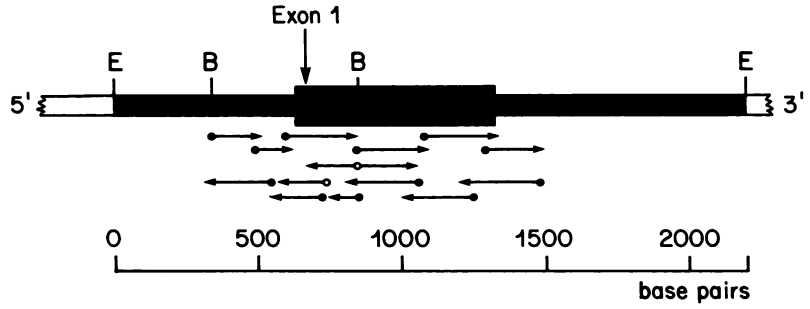

FIG. 4. Primer extension analysis of RNA transcripts from CHO cells transfected with a human reductase promoter-CAT chimeric gene. The human reductase promoter from −270 to +77 was fused to the coding region of CAT in the plasmid pHRedCAT-3. This plasmid was cotransfected with pFD11, a plasmid that can confer DHFR activity, into DHFR-deficient CHO cells. Cells were selected that expressed DHFR, and a population of pooled transformants was analyzed for expression of the 5′ end of the human reductase mRNA. Total RNA (20 μg) from cells was hybridized with a 5′-$^{32}$P-end-labeled oligonucleotide complementary to positions +77 to +56. Before RNA isolation, these cells had been grown in lipoprotein-deficient serum with either compactin (lanes B and E) or sterols (lanes C and F). Control hybridizations were done with total RNA from nontransfected CHO DHFR-deficient cells (lane D) or RNA isolated from SV40-transformed human fibroblasts grown in lipoprotein-deficient serum in the presence (lane A) or absence (lane G) of 1 μM compactin. Primer-extended products were synthesized with reverse transcriptase and analyzed by electrophoresis and autoradiography. The gel was exposed for 48 h at −70°C. The length of the primer-extended products is indicated. On the right, the schematic diagram shows the relation of the observed primer-extended products to the human reductase promoter-CAT chimeric gene. The arrows indicate the observed sites of transcription initiation from the transfected gene, as well as the normal reductase gene in human cells. nt, Nucleotides.

overlaps the reading frame of the HMG-CoA reductase protein, was terminated after only 27-amino-acid residues in Syrian hamsters. These potential peptides are unlikely to be involved in regulating the expression of HMG-CoA reductase since their structure has not been conserved.

## DISCUSSION

The current studies reveal important differences as well as similarities between the 5′ end of the human HMG-CoA reductase gene and the previously described HMG-CoA reductase gene for Chinese hamsters. The most striking difference lies in the observation that the human gene uses only a single splice donor site for the intron in the 5′ untranslated region and therefore produces only a single class of mRNAs with short 5′ untranslated regions. The earlier studies of the Chinese hamster gene were performed with UT-1 cells, which were selected for overexpression and amplification of the reductase gene. To ensure that the findings in the UT-1 cells reflected true species differences, in the current studies I analyzed the promoter region and mRNA transcripts of HMG-CoA reductase in Syrian hamster liver. In this normal organ I found two classes of reductase mRNA transcripts containing either short or long 5′ untranslated regions. Thus the complex pattern of splicing of the first intron at the 5′ donor site is not a consequence of gene amplification, but rather represents species-specific differences in the expression of HMG-CoA reductase.

The heterogeneity of reductase RNA transcripts in UT-1 cells results from the use of multiple sites of transcription initiation as well as multiple sites for splicing of the first intron (39, 40). In the current studies, I observed heterogeneous transcription initiation in human fibroblasts and in Syrian hamster liver. In human cells, at least nine sites between positions +1 and +33 were used. In the liver of Syrian hamsters, most of the transcripts initiated at multiple sites between +19 and +27. These positions for transcription initiation are similar to the sites used for class 1 transcripts in UT-1 cells (between +4 and +29). In Chinese hamster and Syrian hamster, but not in human cells, a minority of transcripts initiated downstream between +40 and +100 (class 2 transcripts).

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-290          GGATCCCCTA TCGCCCGCGA GGGTGGCGTC CTTGCTGGCG CCCCGCACGG CTCAGGGAC CAATAAGAAGG CCGCGATGCT GGACCTCGAC  -201
-200 CAGCTATTGG TTGGCTCGGC CGTGGTGAGA GATGGTGCGG TGCCCGTTCT CCGCCCGGGG TGCGAGCAG TGGGCGGTTGT TAAGGCGACC GTTCGTGACG -101
-100 TAGGCCGTCA GGCCGAGCAG CCGCCCGGCG ATTGGCTAGG CGATCGGACG ATCCTTTCTG ATTGGCGGC CGCTGGCGGCC GGAGCGTGCG TAAGCGCAGT   -1
  +1 TCCTTCCGCC CGAGGCTCCG TTGACTGGAG ACGGCGGCTG GGCCGGCTTG GGGACCTCCA TTGAGATCCG GAGGTGAGCG GGCAGACTCT GGGGACCGGC  +100
+101 CGGGCAGTGG CCGCGGGGCT GGCGGGCGGC GGAGCCCGCG CTCCGCCAGG GCTCACGAGG TGGCTGGGGA CCGAGGGGAC CGCGAGACGT CCTTGAGGCC  +200
+201 CGGCGGCAGG GCGGGGGCCT GGGGATCCGG GGGCCGTCTA GGCCGCTGAG CCAGTGCAGC ATCGGGACGT CCGTGGGTCC AGTTCAGAGG GCTGTGTGCC  +300
+301 GACCGGGCGG TCACGTCCTT ACCGGTAGGA CGAGTGTCAG GCAGGGGGGA CCGCGGGAGC GGATATTCGT GGAGCCCTGG GGGACAAGTG TTCACGCCCA  +400
+401 GGCGTTGCCT TGAAGCAGGA CCTCTTTATA GTGATAGAGA AGGTCCCGGT GTAGGCTGCA GATTGACCCA AAGCCAGAAT CCGCGTGGAA ACGGCAGGTT  +500
+501 ATGCCTCTCC TAGTGGGCCG GGAGGATGCG AGGTCAGTGC AGGCAAAGCC AGCCCTCAAA AGTCTGTCTC GCTGCCTGTA TGTCTTGTCT TTCTCCGAAG  +600
+601 GGGCGTAGGC TCATTGATAC TCATGTCCTT ACTTTGCACT CCTTTTGGAA TTACTTGGTT TGAGTGAGGA AGACCGGACC TTGGAGGTTC GCAAGTTAAA  +700
+701 CAATAGACTT CTGAGgtagg aggaggttaa tgtatcccat cttaagtagt catatggaat attaacttac ttgaggatgt gccttttcgg gctaggttct  +800
+801 gtaaagccat aggaaaacctt tgcttctggt ctttactgct aagtcttcgt gtacattaga                                          +860
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FIG. 5. Restriction map and DNA sequence of the Syrian hamster HMG-CoA reductase promoter. On the top, the restriction map of a 2.2-kb *Eco*RI fragment that spans the 5′ end of the Syrian hamster HMG-CoA reductase gene is shown. The arrows indicate the direction and extent of DNA sequencing by the dideoxy chain termination method (●) or by the chemical and degradation method of Maxam and Gilbert (○) (33). Abbreviations for restriction enzymes are the following: B, *Bam*HI; E, *Eco*RI. On the bottom, the DNA sequence of this region of the Syrian hamster HMG-CoA reductase gene is shown. The sites of transcription initiation, as determined by S1 nuclease analysis as described in the text, are indicated by circles. The 5′ ends of class 1 (●) and class 2 (○) transcripts are shown. The site at which class 1 transcripts are spliced is indicated (▲). The GC boxes within the promoter are enclosed in boxes. The sequence is numbered such that the nucleotide homologous to +1 of the human reductase gene is numbered +1.

The heterogeneous pattern of transcription initiation is not peculiar to HMG-CoA reductase as several other mRNA species have been shown to initiate at multiple sites. These include hypoxanthine phosphoribosyltransferase (34, 37), phosphoglycerate kinase (43), PrP (the prion gene) (1), and the late promoter of SV40 (18). All of these promoters lack a TATA box, the sequence 20 to 30 nucleotides upstream of transcription initiation that is thought to position the start of transcription. The HMG-CoA reductase gene also seems to lack a functional TATA box. Although the sequence 5′-TTATT-3′ is located upstream of the transcription initiation sites in the human and Chinese hamster gene, this sequence does not position the transcription start site. In the Chinese hamster gene, this sequence is 28 nucleotides upstream of the most 5′ RNA start site, whereas in the human gene this sequence is 43 nucleotides upstream of the most 5′ start site. Thus, there is no fixed distance after the 5′-TTATT-3′ at which RNA synthesis starts. Moreover, this sequence is not conserved in the Syrian hamster gene, in which it is 5′-TGATT-3′ and located 54 nucleotides upstream of the most 5′ start site. Immediately upstream of the RNA start sites, a 13-nucleotide identical match is found among the human, Syrian hamster, and Chinese hamster sequences (residues −21 to −9 in the human sequence). Upstream of this sequence there is a 13-nucleotide insertion in the human gene and a 9-nucleotide insertion in the Syrian hamster gene compared with the Chinese hamster gene. If a sequence that positioned transcription initiation were on the 5′ side of this insertion, one would expect to see different initiation sites in the three species. Since the initiation sites are similar, it is likely that the signals responsible for positioning the beginning of RNA synthesis are located downstream of the insertion, most probably in the totally conserved 13-nucleotide sequence.

The single 5′ donor splice site for the first intron in human reductase is located at position +82, which is analogous to the donor site at +73 in the class 1 transcripts produced by the Chinese hamster gene. Because of the heterogeneity of initiation, the human reductase RNAs vary somewhat in length at their 5′ end. However, all of the transcripts have relatively short 5′ untranslated regions, and none of them contain an ATG codon upstream of the initiation codon for HMG-CoA reductase.

In the Chinese hamster reductase gene, the class 2 transcripts are spliced at sites 3′ of position +73. I did not find class 2 transcripts in human cells, but they were found in the Syrian hamster liver. I do not know the functional significance of the class 2 transcripts. The multiple ATG codons upstream of the ATG for the reductase protein might prevent translation of this mRNA (26). Although there are open

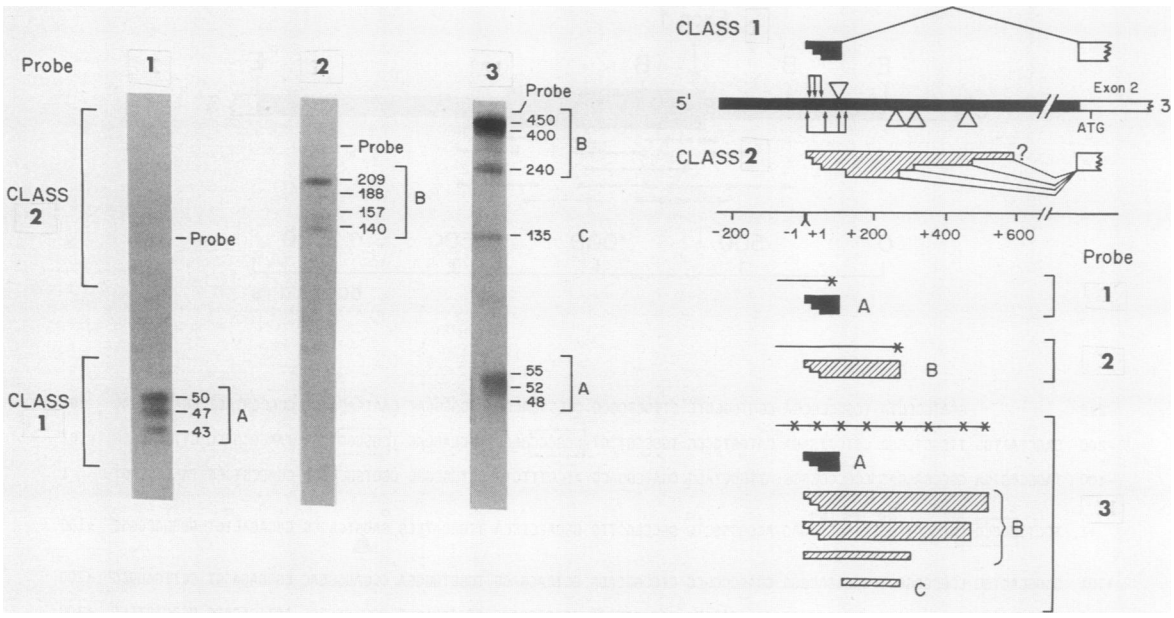
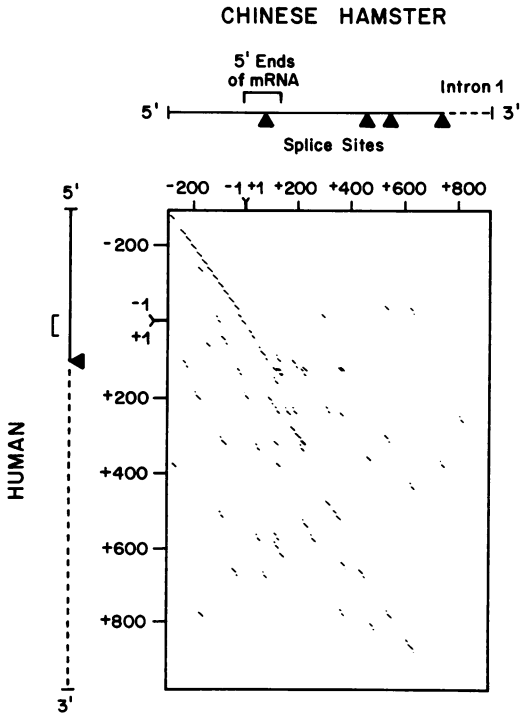



FIG. 6. S1 nuclease analysis of the 5′ end of the Syrian hamster HMG-CoA reductase gene. Total RNA (100 μg) from the livers of Syrian hamsters in which HMG-CoA reductase activity had been induced by feeding the hamsters a diet containing cholestyramine and mevinolin (32) was hybridized to single-stranded DNA probes from the 5′ end of the Syrian hamster reductase gene. The hybridizations were performed at 55°C for 16 h. The RNA-DNA hybrids were digested with 800 U of S1 nuclease at 37°C for 1 h. The protected fragments were analyzed by electrophoresis and autoradiography for 16 h at −70°C. Probe 1 consisted of a 5′-end-labeled fragment ($5 \times 10^3$ cpm) complementary to nucleotides +68 to −58 of the 5′ end of the Syrian hamster reductase gene (Fig. 5). S1 nuclease-protected fragments detected with this probe are shown in the left lane and are labeled A. Probe 2 consisted of a 5′-end-labeled DNA fragment ($5 \times 10^3$ cpm) complementary to nucleotides +227 to −58. Protected fragments detected with this probe are shown in the center lane and labeled B. Probe 3 consisted of a uniformly labeled single-stranded DNA fragment ($3 \times 10^4$ cpm) complementary to nucleotides +476 to −58. Protected fragments observed with this probe are shown in the right-hand lane. A group of small fragments just 5 nucleotides longer than the fragments detected with probe 1 is observed. This group is labeled A and corresponds to class 1 transcripts. A group of larger fragments from 240 to 450 nucleotides in length is observed (group B) that would correspond to the class 2 transcripts detected with probe 2. An additional fragment of 135 nucleotides (labeled C) was also found. On the right, a diagram is shown which schematically outlines the fragments detected with each of the radiolabeled probes. Fragments corresponding to the class 1 transcripts are indicated by the solid blocks. Fragments corresponding to class 2 transcripts are indicated by the striped blocks. The splicing pattern for class 1 and class 2 transcripts in the Syrian hamster reductase gene is illustrated at the top of the diagram with initiation sites (arrows) and the splice sites (△) indicated. The most 3′ splice sites for class 2 transcripts were not determined and are indicated by a question mark.




reading frames after the ATG codons in the untranslated region of the Chinese hamster and Syrian hamster mRNAs, the proteins predicted from these reading frames are not conserved. Therefore, it is unlikely that the 5′ region encodes a functionally important peptide.

The 5′ end of the human HMG-CoA reductase gene, like that of the hamster gene, promotes transcription and confers regulation by sterols. A reductase-CAT fusion gene that contains 347 nucleotides from the human reductase, including 270 nucleotides upstream of the sites of transcription initiation and 77 nucleotides from the 5′ untranslated region, produces RNA transcripts initiated at sites identical to those observed for the endogenous gene in normal human cells. Moreover, sterols suppress the synthesis of reductase-CAT RNA from this promoter. This expression is similar to that

FIG. 7. Dot matrix comparison of the 5′ end of the HMG-CoA reductase gene between Chinese hamsters and humans. The Chinese hamster sequence is displayed along the *x* axis. A schematic diagram indicates the locations of the multiple 5′ ends of the mRNA and the multiple splice sites above the numerical sequence. The human HMG-CoA reductase sequence is displayed along the *y* axis. The diagram along the *y* axis shows the location of the multiple 5′ ends of the mRNA and the single splice site used in the human reductase mRNA. The analysis required a 12 of 16 match to be shown on the comparison.

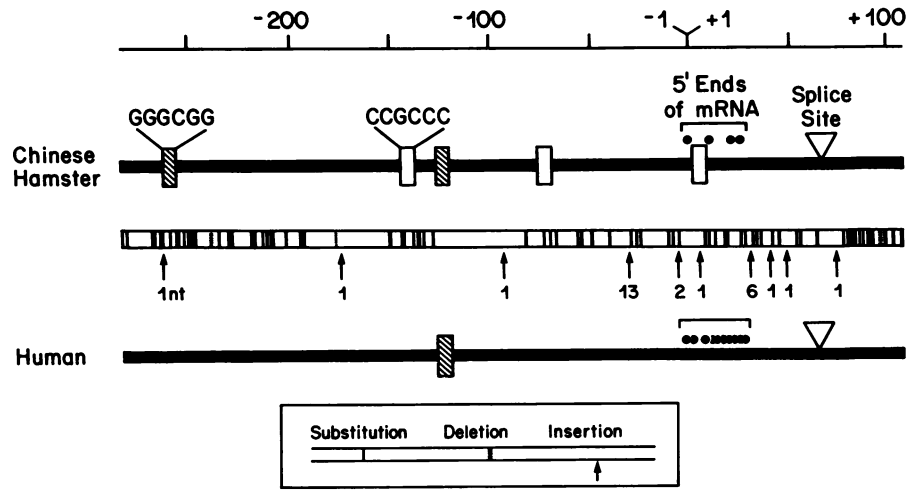



FIG. 8. Alignment of the 5' end of the Chinese hamster and human HMG-CoA reductase genes. The region of maximal homology between the Chinese hamster and human reductase promoters was aligned, and the results of this analysis are indicated schematically in this diagram. Landmarks in the Chinese hamster gene are indicated along the top. The GC boxes, the multiple 5' ends of class 1 transcripts, and the splice site of class 1 transcripts of the Chinese hamster gene are shown. Similar landmarks in the human reductase gene are shown at the bottom. The bar between these two sequences illustrates the observed differences. Substitutions are indicated by solid lines, and deletions in the human sequence relative to the Chinese hamster sequence are indicated by broken lines. The thickness of the lines corresponds to the number of nucleotides that differ. An insertion in the human sequence relative to the Chinese hamster sequence is indicated by an arrow with the number of inserted nucleotides listed. nt, Nucleotides.

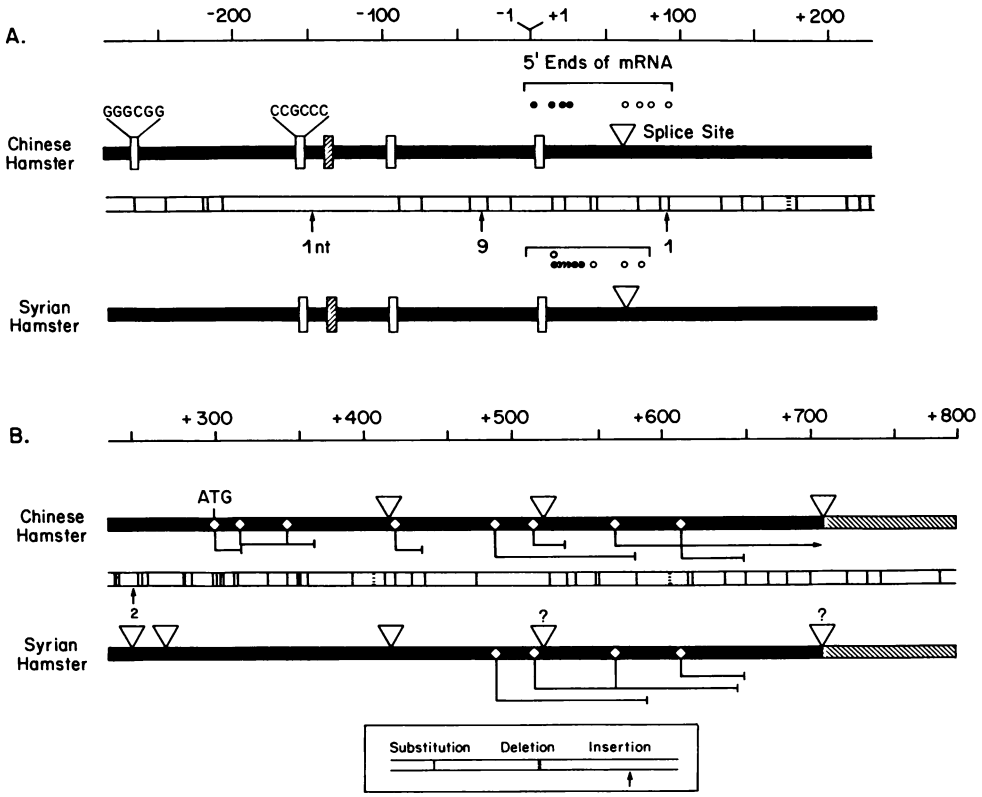



FIG. 9. Alignment of the 5' ends of the Chinese hamster and Syrian hamster HMG-CoA reductase genes. (A) Sequence from −280 to +240. (B) Sequence from +241 to +800. Landmarks including the GC boxes, the 5' ends of the mRNA, and the splice sites are indicated. The transcription initiation sites for class 1 (●) and class 2 (○) transcripts are indicated. ATG codons in the 5' untranslated region of class 2 transcripts are shown (◇). The lines extending from the ATG codons indicate potential open reading frames predicted from the DNA sequence. The line with the arrow below the Chinese hamster sequence indicates an open reading frame of 318 nucleotides that extends into the second exon (10). Substitutions, deletions, and insertions are indicated in the middle and are as described in the legend to Fig. 8. nt, Nucleotides.

observed by Osborne et al. for a chimeric gene that included nucleotides −280 to +229 of the Chinese hamster reductase gene (36). Since the human promoter-CAT construct contains only 77 nucleotides from the 5′ untranslated region, sequences downstream of +77 and beyond the 5′ donor splice site are not required for expression or regulation by sterols.

The highly conserved sequence upstream of transcription initiation is the most likely area that is responsible for regulating the expression of this gene. It is likely that regulation is mediated by proteins that bind to these conserved sequences. The presence of several long stretches of identity over a region greater than 150 nucleotides in length suggests that these interactions may involve several proteins that interact with different parts of the promoter. Despite this complexity, the high degree of conservation suggests that the mechanism for regulation is highly conserved between humans and hamsters. Indeed, the human reductase promoter is both expressed and regulated when introduced into hamster cells. Such a high degree of conservation is not seen in promoters for other "housekeeping" genes. For example, within the promoter of the hypoxanthine phosphoribosyltransferase gene, the longest perfect nucleotide match is only eight nucleotides between humans and mice (25, 34, 37). Within the promoter of the DHFR gene, the longest match is only 12 nucleotides between humans and Chinese hamsters (8, 35).

Somewhat surprisingly, the sequence of the reductase promoter shows little resemblance to promoters of other cholesterol-regulated genes. There are no identical matches of >10 nucleotides between the reductase promoter and the 5′ flanking sequences of either the Chinese hamster HMG-CoA synthase (19) or the human low-density lipoprotein receptor (45) genes. If the same cholesterol-dependent proteins interact with each of these promoters to suppress transcription, they must not recognize extended regions of sequence. Alternatively, the lack of conservation may indicate that different cholesterol-regulated promoters are regulated by different proteins.

The hexanucleotide 5′-GGGCGG-3′ or its complement is found five times in the promoter region of the Chinese hamster reductase gene but only once in the human promoter. This sequence is present, often in multiple copies, in the SV40 promoter and the promoters of several housekeeping genes (25). Tjian and his co-workers have purified a transcription factor, Sp1, that binds to many of these GC-rich sequences and promotes transcription (4, 13, 24). The consensus sequence for Sp1 binding is $^{G}_{T}$GGGCGG$^{GGC}_{AAT}$. In the GC box that is conserved between humans and hamsters in the reductase promoter, only 7 of 10 nucleotides match the Sp1 binding sequence; however, these 10 nucleotides are identical in the human and hamster reductase genes. Thus, if Sp1 promotes transcription of the HMG-CoA reductase gene, it may do so primarily by binding to the one GC box that is conserved.

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