Multiple Sterol Regulatory Elements in Promoter for Hamster 3-Hydroxy-3-methylglutaryl-coenzyme A Synthase*

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Through substitution mutagenesis and gene transfer experiments in cultured cells, we have identified three sequences in the 5' flanking region of the gene for hamster 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase that are required for sterol-mediated regulation of transcription. Point mutations in any one of these sequences largely prevented the increase in transcription that normally follows cellular sterol depletion. These mutations did not alter the low level of transcription that occurs in the presence of sterols. Two of the three sterol regulatory sequences contain an octanucleotide that shows a 7/8-base pair match with a sequence that was previously identified as a sterol regulatory element in the genes for HMG-CoA reductase and the low density lipoprotein receptor, both of which are induced by sterol deprivation. The third sterol regulatory region in the HMG-CoA synthase promoter shows only a low-level match with the other sterol regulatory elements. The current data suggest that the sterol regulatory elements in the HMG-CoA synthase promoter operate by a conditional positive mechanism: in the absence of sterols, regulatory proteins bind to these elements and stimulate transcription; in the presence of sterols, the regulatory proteins are inactivated and transcription decreases to the basal rate.

Animal cells achieve cholesterol homeostasis in part through feedback repression of transcription of genes encoding enzymes in the cholesterol biosynthetic pathway. Most extensively studied are the genes for two early sequential enzymes in the pathway, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase (1, 2) and HMG-CoA reductase (3, 4). When sterols accumulate within cells, the amounts of mRNA produced by both genes are reduced. Conversely, when sterols are depleted, the amounts of mRNA rise, and cholesterol synthesis increases. In parallel with these changes in enzymes of endogenous cholesterol synthesis, cells also regulate the uptake of exogenous cholesterol by regulating transcription of the gene for the low density lipoprotein (LDL) receptor (5). This gene is also transcribed at a high rate when cells are deprived of sterols, and it is repressed when sterols accumulate.

In the genes for HMG-CoA reductase and the LDL receptor, sensitivity to sterol-mediated repression is dependent upon an octamer, 5'-CACCACAC-3', in the 5' flanking region (6, 7). Mutations in this octamer abolish sterol-dependent regulation. In the LDL receptor gene, the sterol regulatory octamer is located adjacent to a binding site for a positive transcription factor, Sp1 (7, 8). In the HMG-CoA reductase gene, this sequence is located within a cluster of binding sites for proteins that resemble nuclear factor 1 (NF-1), a positive transcriptional activator. 2 When the sterol regulatory sequence from the LDL receptor or HMG-CoA reductase genes was inserted into the promoter for the herpes simplex virus (HSV) thymidine kinase gene, transcription of the transfected fusion gene became sensitive to repression by sterols (5–8).

The 5' flanking region of the HMG-CoA synthase gene contains two copies of a sequence that is similar to the sterol regulatory octamer in the HMG-CoA reductase gene and the LDL receptor gene. In the current studies we have sought to determine whether these sequences or other sequences in the 5' flanking region are responsible for sterol-dependent regulation of transcription of the HMG-CoA synthase gene.

**Experimental Procedures**

Materials—[γ-32P]ATP (>5000 Ci/mmol) was obtained from ICN. Polynucleotide kinase was obtained from Pharmacia LKB Biotechnology Inc. Enzymes used in plasmid constructions were obtained from New England Biolabs, Boehringer Mannheim, and Bethesda Research Laboratories. Reverse transcriptase was purchased from Life Sciences (Catalog number AMV 007). DNase I was obtained from Worthington (Catalog number LS 0633). G418 sulfate (Geneticin) was purchased from Gibco. Cholesterol and 25-hydroxycholesterol were purchased from Altech Associates and Steraloids, Inc., respectively. Fetal calf serum was purchased from Gibco. Newborn calf lipoprotein-deficient serum (d > 1.215 g/ml) was prepared by ultracentrifugation (9). Plasmid pSV3-Neo, which contains a bacterial gene that confers resistance to G418 (10), was obtained from Bethesda Research Laboratories. Plasmid pSV0-CAT (11) was kindly provided by Bruce Howard (National Institutes of Health). Plasmid pTK CAT-1 contains the HSV-thymidine kinase promoter (6) extending 109 base pairs upstream of the mRNA cap site fused to the chloramphenicol acetyltransferase gene and was a gift of Thomas Sudhof (University of Texas Southwestern Medical Center at Dallas). Materials for construction of HMG-CoA synthase-chloramphenicol acetyltransferase plasmids were obtained from previously reported sources (5–8). Oligonucleotides were synthesized on an Applied Biosystems model 380A DNA Synthesizer. Male golden Syrian hamsters

1 The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; CHO, Chinese hamster ovary; HSV, herpes simplex virus; LDL, low density lipoprotein; neo, gene encoding neomycin phosphotransferase.

**FIG. 1.** Panel A, promoter activity of chimeric genes containing varying lengths of the HMG-CoA synthase promoter. The DNA fragment from the 5' end of the hamster synthase gene used to construct pSynCAT-1, -2, and -4 is represented at the top by the solid and open bar and bounded by the indicated restriction endonuclease sites. Nucleotide positions -527 to +39 are numbered in relation to the transcription initiation site, indicated by the arrow at position +1. A putative TATA box is represented as a stippled box. The open bar represents a portion of the first exon in the 5' untranslated region of the synthase gene. The chloramphenicol acetyltransferase (CAT) coding sequence is denoted by the cross-hatched bar. Relative transcription was determined by measuring the amount of mRNA produced in transfected CHO cells incubated in the absence or presence of sterols (see Panel B). The data are expressed as a fraction of the level obtained in the absence of sterols with pSynCAT-1, the plasmid with the largest amount of synthase gene sequence. Panel B, primer extension analysis of mRNA produced by chimeric genes containing varying lengths of the HMG-CoA synthase promoter. Each chimeric plasmid (pSynCAT-1, -2, and -4) was cotransfected into CHO cells with pSV3-Neo. Following selection for G418 resistance, 300-600 stably transfected colonies were pooled and propagated in mass culture. Cells were set up for experiments according to the standard protocol described under "Experimental Procedures." After incubation in the presence or absence of 10 µg/ml cholesterol plus 0.5 µg/ml 25-hydroxycholesterol for 24 h, total RNA was isolated. Ten micrograms of total RNA were then assayed for chloramphenicol acetyltransferase mRNA by primer extension with 32P-labeled oligonucleotide primer complementary to the chloramphenicol acetyltransferase coding region (primer 2, see "Experimental Procedures"), generating a 293-nucleotide extension product from the pSynCAT-1 chimeric genes. As a control for endogenous synthase regulation, an additional primer (primer 4) was used to detect the two transcripts produced by the endogenous HMG-CoA synthase gene, generating extension products with 32P-labeled oligonucleotide primer complementary to the chloramphenicol acetyltransferase coding region or untranslated region of the synthase gene. The chloramphenicol acetyltransferase structural gene; it has no defined eukaryotic promoter or enhancer sequences. To construct pSynCAT-1, a 366-base pair EcoRI-SmaI fragment of clone M53K-44 (2) containing the synthase promoter was blunt-ended with deoxynucleotides and reverse transcriptase and ligated to HindIII linkers for insertion into the HindIII site of pSV0-CAT. Two deleted versions of the synthase promoter were made from the HindIII-HindIII fragment of pSynCAT-1. A 423-base pair Sau3A-HindIII fragment and a 324-base pair AvaI-HindIII fragment containing the 3' portions of the promoter were each blunt-ended and ligated to HindIII linkers for insertion into the HindIII site of pSV0-CAT to create pSynCAT-2 and pSynCAT-4, respectively.

To construct mutants A-R, oligonucleotide-directed mutagenesis
FIG. 2. Sequence of the hamster HMG-CoA synthase promoter showing positions of footprints (FP1–FP6) and sequences that were subjected to substitution mutagenesis (A–R). Coding strand footprints are denoted by solid brackets; noncoding strand footprints are denoted by dashed brackets. The asterisk indicates the site of transcription initiation (2, 14). The double overline in region R denotes the potential TATA box. The boxed sequences in regions D, E, and F denote potential sterol regulatory sequences (see Fig. 5). Four “GC-box” sequences, potential Sp1 binding sites, are underlined.

FIG. 3. Expression of mutant HMG-CoA synthase promoters in transfected CHO cells incubated in the absence and presence of sterols. Each of the mutant synthase promoters A–R of Fig. 2 was fused to the chloramphenicol acetyltransferase (CAT) coding sequence and cotransfected into CHO cells with pSV3-Neo and a control plasmid containing the HSV-thymidine kinase (TK) promoter fused to the chloramphenicol acetyltransferase coding sequence (pTK CAT-1). Stably transfected colonies were selected, and RNA was harvested from pooled colonies after growth in the presence and absence of sterols as described in the legend to Fig. 1. Thirty µg of total RNA was then assayed for chloramphenicol acetyltransferase mRNA by primer extension employing a 32P-labeled oligonucleotide primer complementary to the chloramphenicol acetyltransferase coding region. This primer hybridized to the transcripts from the pSyn-CAT and pTK-CAT genes. Two extension products are generated from the pTK-CAT gene due to two different transcription initiation sites produced by the HSV-thymidine kinase promoter (18). The 5′ extended product from the pSyn-CAT genes is shorter than the product of the thymidine kinase-chloramphenicol acetyltransferase gene because the 5′ untranslated region of the former transcript is shorter. Primer extensions shown in Panels A, B, and D employed primer 1 (see “Experimental Procedures”), generating extension products of approximately 148 nucleotides from pTK CAT-1 and 133 nucleotides from pSynCAT. The primer extension shown in Panel C employed primer 2, generating extension products of approximately 308 nucleotides from pTK CAT-1 and 293 nucleotides from pSynCAT. After fixation and drying, the gels shown in Panels A, B, C, and D were exposed to x-ray film at −70 °C with an intensifying screen for 49, 48, 17, and 75 h, respectively. After densitometry, the quantity of synthase-chloramphenicol acetyltransferase mRNA extension product (x) was normalized to the quantity of thymidine kinase-chloramphenicol acetyltransferase mRNA extension product (y) in each lane by taking the ratio (x/y). Fold induction was calculated as described in the legend to Fig. 1.

(13) was performed with oligonucleotides of 50 nucleotides in length that hybridized to 15 nucleotides of the wild-type sequence on either side of a 20-nucleotide target sequence. For each mutant the same 20-nucleotide sequence 5′-TGGACAGTCGACAAGGTCAA-3′ was substituted for 20 nucleotides of the wild-type target sequence. The sequence that was introduced contains a SalI site. All mutant promoters were reinserted into pSV0-CAT. The position of each mutation is diagramed in Fig. 2. Mutants S, T, U, and V were made by oligonucleotide-directed mutagenesis using three 20-mers, each containing the indicated mismatches (Fig. 5). The oligonucleotide used to make mutant W was a 34-mer with the indicated mismatches (Fig. 5).

DNA Transfection—Chinese hamster ovary (CHO-K1) cells were grown in monolayer culture and transfected with 5 µg of the test
plasmid, 0.5 μg of pSV3-Neo, and 10 μg of pTK CAT-1 by the calcium phosphate coprecipitation technique as described previously (5, 8). (Plasmid pTK CAT-1 was used as an internal control for efficiency of transfection and quantification of mRNA.) In the experiment of Fig. 1, the pTK CAT-1 plasmid was not used. After two to three weeks, colonies were counted in each well, and resistant colonies were pooled (300–500 colonies/transfection), expanded in suspension culture in the presence of 1 mg/ml G418, incubated for 24 h in medium containing lipoprotein-deficient serum in the absence or presence of sterols (10 μg/ml cholesterol plus 0.5 μg/ml 25-hydroxycholesterol), and harvested for RNA analysis as previously described (5, 8).

**Primer Extension Assays**—Primer extension assays for measurement of mRNA were carried out as previously described with minor modification (8, 14). Primers were hybridized to total cellular RNA for 25 min at 68°C, and the extension reaction was carried out for 25 min at 40°C. To detect transcripts containing chloramphenicol acetyltransferase sequences (derived from pTK CAT-1 or from a pSynCAT test plasmid), an oligonucleotide primer of 40 nucleotides complementary to bases 240–279 (primer 1) or the bases 400–439 (primer 2) of the chloramphenolic acetyltransferase mRNA was used (15). Neo transcripts (derived from pSV3-Neo) were detected with an oligonucleotide primer of 40 nucleotides (primer 3) complementary to bases +261 to +300 of the mRNA (16). Endogenous HMG-CoA synthase transcripts were detected with a 60-nucleotide primer (primer 4) complementary to nucleotides +41 to +100 of the hamster HMG-CoA synthase cDNA (1). MspI restriction fragments of 32P-labeled pBR322 were used as size standards. Quantitative densitometry was performed with a Hoefer scanning densitometer (Model GS 300).

**DNase I Footprinting**—Hamster liver nuclear extracts were prepared and used for DNase I footprinting as previously described (4). DNA fragments containing the HMG-CoA synthase promoter were end-labeled by taking advantage of the SalI site created in the mutant promoters P, H, Q, and in an additional mutant in which the 20-nucleotide-substituted region was immediately 5’ to region A (Fig. 2). This SalI site was 5’ end-labeled with [γ-32P]ATP and T4 polynucleotide kinase. We then performed a second restriction endonuclease digestion with HindIII and isolated the appropriate fragment by gel purification (12).

**RESULTS**

To determine whether the 5’ flanking region of the HMG-CoA synthase gene contains a sterol regulatory element, we prepared fusion genes containing various portions of the 5’ flanking region fused to the coding region of chloramphenicol acetyltransferase. The fusion genes were introduced into Chinese hamster (CHO) cells by transfection, pooled colonies of stable transfectants were selected, and the amount of mRNA produced by the chimeric gene was measured in cells grown in the absence and presence of sterols (Fig. 1). A construct containing 527 base pairs of 5’ flanking sequence (pSynCAT-1) showed a relatively low level of transcription when cells were grown in the presence of sterols, and this was increased by nearly 6-fold when sterols were removed. A somewhat shorter segment of 5’ flanking region, extending to position −378 (pSynCAT-2), showed slightly higher transcription in the presence of sterols, and there was an 8.5-fold increase when sterols were omitted. When the promoter sequence was further shortened to position −279 (pSynCAT-4), low-level transcription in the presence of sterols was still observed, but there was no significant induction when sterols were removed. These studies indicate that signals responsible for sterol-dependent regulation of transcription are present in the first 378 base pairs of the 5’ flanking region and that the 5’ boundary of a particularly important element or elements is found between position −279 and −378. As controls for these experiments, we measured: 1) the amount of mRNA produced by the endogenous HMG-CoA synthase gene (Fig. 1B); and 2) the amount of mRNA produced from the neomycin phosphotransferase (neo) gene that was introduced into the CHO cells on a cotransfected plasmid under control of the SV40 promoter. Whereas the transcription of the endogenous HMG-CoA synthase gene was increased upon sterol deprivation, transcription of the transfected neo gene was not influenced (Fig. 1B).

Fig. 2 shows the sequence of the HMG-CoA synthase promoter fragment extending to position −368. The position of six protein-binding regions, designated footprints 1–6 (FP1–6), are indicated (see below). To determine the location of functional sequences within this region, we prepared a series of mutants (A–R) in which sequential 20-base pair segments of the promoter gene were systematically replaced by unrelated sequence. A plasmid containing each mutant promoter fragment adjacent to the chloramphenicol acetyltransferase gene was introduced into CHO cells, and the amount of mRNA produced in the absence or presence of sterols was measured by primer extension. Fig. 3 shows the raw data from...
several representative experiments, and Fig. 4 shows a quantitative summary of the data obtained from multiple experiments with each mutant promoter. Seven regions appeared to be most important for transcription: these were disrupted by mutations D, E, F, K, N, Q, and R.

Mutations D, E, and F did not affect the level of transcription when cells were grown in the presence of sterols. However, each of these mutations limited the degree of induction when sterols were removed. Whereas the wild-type synthase gene (pSynCAT-1) showed an average induction of 5.7-fold, the induction with the D, E, and F mutants was less than 2-fold (Fig. 4B). We interpret these data to indicate that the regions encompassed by D, E, and F mutations contain elements that allow an increase in transcription when sterols are depleted.

The results with the K, N, and Q mutations were less cut than the results with mutants D, E, and F. The K, N, and Q mutations reduced transcription when the cells were grown either in the absence or presence of sterols. Promoters bearing these mutations consistently showed an induction of greater than 2-fold when sterols were absent. We interpret these data to indicate that regions K, N, and Q probably represent sites of action of transcription factors that function constitutively and independently of sterol regulation. Mutant R abolished transcription in the absence or presence of sterols. This mutation disrupts the sequence TATAAA located 23 base pairs upstream of the mRNA initiation site. This sequence is presumably functioning as a TATA box.

Two of the apparent sterol regulatory regions in the HMG-CoA synthase promoter (regions E and F) each contain a sequence that shows a 7/8-base pair match with the octamer sequence, 5'-CACCCGAC-3', previously identified as a sterol regulatory element in the LDL receptor and HMG-CoA reductase genes (6, 7). The sequence is oriented in the same direction as its orientation in the LDL receptor gene, but opposite to its orientation in the HMG-CoA reductase gene. The core of this sequence is a cluster of 3 or 4 cytosine residues. Region D contains an 8-base pair sequence with a core of three cytosines that loosely resembles the sterol regulatory sequence, but differs at three positions. These three potential sterol regulatory sequences are boxed in Fig. 2, and are shown in more detail in Fig. 5.

To determine whether these sequences play a role in sterol-mediated regulation, we prepared a series of plasmids containing point mutations in one or more of the potential sterol regulatory elements (Fig. 5). In each case we replaced 2 residues that flank the core cytosines. Mutations of each of the three potential sterol regulatory elements (plasmids S, T, and U) did not affect transcription in the presence of sterols. However, each of these mutations blunted the rise in transcription when sterols were removed. We also made pair-wise combinations of mutations in the first and second sterol regulatory elements (plasmid V) and in the second and third elements (plasmid W). When two of the three elements were mutated, the results were not significantly different from the results when only a single element was mutated. In each case transcription in the presence of sterols was unaffected, but induction in the absence of sterols was largely prevented.

To determine whether the sterol regulatory elements could be correlated with protein binding sites, we performed a series of DNase I footprint assays using a crude nuclear extract from hamster liver (Fig. 6). Six distinct protein binding regions, or footprints, were observed on the coding and noncoding strands of the DNA. These six footprint regions are positioned on the sequence in Fig. 2. Footprint 2 covered the region encoded by the most upstream of the putative sterol regulatory regions, and footprint 3 encompassed the middle sterol regulatory region. The third sterol regulatory region is immediately adjacent to footprint 3, but it was not protected from DNase I by the proteins in the nuclear extract under the conditions employed. The addition of 25-hydroxycholesterol in ethanol at a concentration of 0.5 µg/ml had no effect on this footprint pattern (data not shown).

**DISCUSSION**

The current studies identify three segments of the 5' flanking region of the HMG-CoA synthase gene that are necessary for sterol-regulated transcription. Disruption of any of these regions, either by gross 20-base pair substitutions or by point mutations, attenuates the increase in transcription that normally occurs when sterols are removed from the culture medium.

Two of the three sterol regulatory regions, defined by mutations E and F (Fig. 2), contain octanucleotide sequences that show a 7/8-base pair match with the consensus sequence for the sterol regulatory element in the HMG-CoA reductase promoter (6) and the LDL receptor promoter (7). This sequence, designated SRE1, is illustrated in Fig. 7. A single copy of this sequence is present in the LDL receptor promoter and the HMG-CoA reductase promoter, but it is located on opposite strands of the DNA. In the HMG-CoA synthase promoter, both copies of SRE1 are located on the coding strand as is the single copy within the LDL receptor promoter.
The third sterol regulatory region in the HMG-CoA synthase promoter, defined by mutant D, contains an octanucleotide sequence that shows only a low-level similarity to SRE1. Nevertheless, this octanucleotide appears to be a true sterol regulatory element since substitution of 2 out of the 8 base pairs nearly abolished sterol-mediated regulation of transcription (Fig. 5). We have designated this element as SRE2 in Fig. 7. The SRE2 sequence is part of a protein binding region that is defined by footprint 2 (Figs. 2 and 6). One of the copies of SRE1 is also a protein binding site (footprint 3), and the other copy of SRE1 is located immediately adjacent to footprint 3.

All three of these sterol regulatory elements appeared to be required in order to achieve high rates of transcription from the HMG-CoA synthase promoter in the absence of sterols. Disruption of any one of the three elements markedly attenuated this induction without affecting basal transcription in the presence of sterols (Fig. 5). Transcription in the presence of sterols was not reduced even when two of the three elements were disrupted (Fig. 5), reinforcing the notion that these sequences play a role in transcription only when sterols are absent.

The simplest interpretation of these data is that sterols influence transcription of hamster HMG-CoA synthase by inactivating a conditional-positive transcriptional factor. This factor binds to the sterol regulatory elements and stimulates transcription only in the absence of sterols. In the presence of sterols this factor is inactive, and the synthase is transcribed only at basal levels. These results are similar to the results obtained when the sterol regulatory element in the

**Fig. 6.** DNase I footprint analysis of HMG-CoA synthase promoter. Coding strand probes were endlabeled with $^{32}$P at position −381 or −261. The 3' end of both probes was at position +41. Noncoding strand probes were 5' end-labeled at position −218 or −38; the 3' end was at position −529. Probes were incubated in the absence (−) or presence (+) of 600 μg of crude nuclear extract from hamster liver (4) and subjected to partial DNase I digestion followed by denaturing polyacrylamide gel electrophoresis as described under "Experimental Procedures." The dried gel was exposed to x-ray film for 22 h at −70 °C with an intensifying screen. Portions of the autoradiogram corresponding to footprints 1–6 are shown. To assign the protected regions, Maxam and Gilbert (19) sequencing reactions (C + T and A + G) were performed on each of the probes. A schematic representation designating the positions of each footprint boundary is shown to the right of each footprint.
Fig. 7. Location of sterol regulatory elements (SRE1 and SRE2) in three promoters. In the gene for the LDL receptor a single copy of SRE1 is immediately adjacent to a sequence that binds transcription factor Sp1 (7). In the HMG-CoA reductase gene, a single copy of SRE1 is located on the opposite DNA strand in comparison with the element in the LDL receptor promoter, and it is located adjacent to a cluster of sites that bind a family of proteins that resemble nuclear factor 1 (NF-1). The HMG-CoA synthase promoter has three sterol regulatory elements, two of which resemble the elements in the LDL receptor and HMG-CoA reductase promoter (SRE1). The third sequence differs from SRE1 and is designated SRE2. These three sequences occur in a cluster that is upstream of the positive transcription region encompassed by mutants K, N, and Q.

LDL receptor promoter is mutated. Disruption of this element, which is contained within a sequence called repeat 2, prevented the induction of transcription that normally occurred when sterols were removed (7, 8).

Other experiments are not consistent with a simple conditional-positive mechanism for sterol-mediated regulation of transcription. First, when the sterol regulatory element from the LDL receptor promoter was inserted into a chimeric construct containing positive elements from the HSV-thymidine kinase promoter, the sterol regulatory element allowed sterols to repress transcription (5, 7). Repression occurred even when the positive driving force for transcription was derived from the thymidine kinase promoter. These findings suggest that the sterol regulatory element is a conditional-negative element that suppresses the activity of nearby positive elements when sterols are present.

The notion of a conditional-negative element is reinforced by observations of mutations of the sterol regulatory element in the HMG-CoA reductase promoter. When this element (designated footprint 2D) was disrupted, transcription was maintained at the fully induced level even in the presence of sterols (6). This result is opposite to the current results obtained with the HMG-CoA synthase promoter, in which disruption of the sterol regulatory element decreased transcription to the basal suppressed level even in the absence of sterols.

At present, the data can be unified most easily by the hypothesis that sterol regulatory elements can act both as conditional-positive and conditional-negative elements, depending on their location with respect to the binding sites of other transcription factors. In the absence of sterols, these elements may bind a positive transcription factor. In the presence of sterols, the elements may bind a negative transcription factor. The postulated positive and negative transcriptional regulators might be the same protein whose behavior is altered by sterols. Alternatively, different proteins could bind to these sequences in the absence and presence of sterols. In each promoter the balance between conditional-positive and conditional-negative effects would be determined by the location and strength of other positive elements.

Although the sterol regulatory elements in all three promoters have a similar sequence, they function in the presence of different positive promoter elements. In the LDL receptor promoter the major positive element appears to be nuclear factor Sp1 (7, 8). When the Sp1 binding sequence is mutated, transcription is abolished both in the presence and absence of sterols (8). Binding of Sp1 to this sequence has been demonstrated in vitro (7), and transcription in vitro has been shown to be dependent upon Sp1 (8). In the HMG-CoA reductase promoter the major positive activity comes from sequences designated footprint 3 and footprint 4, the latter of which resembles a sequence required for transcription driven by the adenovirus major late promoter (6). The HMG-CoA reductase promoter also contains multiple binding sites for a protein in the nuclear factor-1 (NF-1) family. One of these binding sites occurs over the footprint 2D region, raising the possibility that a sterol regulatory protein and NF-1 might compete for binding.

The positive elements responsible for basal transcription from the HMG-CoA synthase promoter are not yet well defined. None of the 20-base pair substitutions abolished transcription with the exception of the TATA-box mutation (mutant R, Fig. 4). Inasmuch as mutations in regions K, N, and Q reduced transcription partially in the absence and presence of sterols, these elements are potential sites for binding positive transcriptional factors. It is likely that multiple positive elements influence transcription of the HMG-CoA synthase gene and that multiple mutations (with the exception of the TATA-box element) would be necessary to abolish transcription.

A crucial step in the further elucidation of the mechanism for sterol-mediated regulation will be the identification of the putative DNA binding proteins that bind to the sterol regul

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ulatory elements in the HMG CoA synthase gene and the other sterol-regulated genes.

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REFERENCES