3'-Untranslated sequences mediate post-transcriptional regulation of 3-hydroxy-3-methylglutaryl-CoA reductase mRNA by 25-hydroxycholesterol

Jae Won CHOI* and Dennis M. PEFFLEY†

Department of Pharmacology and Molecular Biology, University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064, U.S.A.

In an earlier study [Choi, Lundquist and Peffley (1993) *Biochem. J.* **296**, 859–866], we determined that 25-hydroxycholesterol regulates 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase mRNA through a post-transcriptional mechanism that requires protein synthesis. To investigate whether 3'-untranslated sequences play a role in 25-hydroxycholesterol-mediated post-transcriptional control, we ligated approx. 1400 bp of the 3'-untranslated region of HMG-CoA reductase cDNA to the coding region of human β -globin DNA. β -Globin-3'-untranslated reductase fusion constructs were then transiently expressed in Chinese hamster ovary fibroblasts under conditions known to regulate reductase mRNA. There were no differences in β -globin RNA levels in transfected cells incubated with or without lovastatin, a competitive inhibitor of reductase. However, in the

presence of lovastatin and an oxysterol, 25-hydroxycholesterol, β -globin RNA levels were decreased approx. 2-fold. Inhibition of protein synthesis with cycloheximide blocked the effects of 25-hydroxycholesterol on β -globin RNA. Moreover, replacing the 3'-untranslated sequences with 1367 bp of the simian virus 40 enhancer region eliminated the regulatory effect of 25-hydroxycholesterol. Because the fusion construct has no sterol regulatory elements necessary for transcription, our results indicate that the change in β -globin RNA occurred at a post-transcriptional level. In addition, we have shown that the 3'-untranslated region of HMG-CoA reductase cDNA imparted oxysterol-mediated post-transcriptional regulation to β -globin RNA, an effect that required protein synthesis.

INTRODUCTION

Cholesterol biosynthesis in mammalian cells is regulated by the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase [1]. An endoplasmic-reticulum-membrane-associated protein with a monomeric weight of 97 kDa [2,3], HMG-CoA reductase is responsible for the production of mevalonate, a precursor of cholesterol as well as non-sterol isoprenoids such as ubiquinone, dolichol and isopentenyl-tRNA [4]. Both cholesterol and mevalonate are required for the inhibition of reductase in mammalian cells; inhibition occurs at the transcriptional [2,3,5–10], post-transcriptional [11–15] and post-translational levels [16–18]. When both cholesterol (or an oxysterol such as 25-hydroxycholesterol) and mevalonate are provided exogenously to mammalian cells, reductase levels decrease rapidly. This is due to a 2–4-fold reduction in HMG-CoA reductase mRNA levels, a 30–90-fold reduction in translation and a 2–3-fold increase in degradation of the enzyme [6,7,11,13,14,19].

We recently found that inhibition of protein synthesis with cycloheximide blocked oxysterol-mediated reduction of reductase mRNA in baby hamster kidney (BHK) cells. When 25-hydroxycholesterol and cycloheximide were added simultaneously to cells treated with lovastatin, a competitive inhibitor of HMG-CoA reductase, reductase mRNA levels were not reduced relative to cells treated only with lovastatin [20]. In addition, when cycloheximide was added to cells grown in medium supplemented with serum, conditions under which reductase mRNA levels are equal to those in cells treated with 25-hydroxycholesterol, suppression of reductase mRNA was blocked, and mRNA levels increased approx. 10-fold within 5 h. As determined by run-on assays, in neither case did we find that cycloheximide treatment

blocked oxysterol-mediated control of HMG-CoA reductase transcription. These results indicated that inhibition of protein synthesis stabilized reductase mRNA and prevented normal oxysterol control of this mRNA at a post-transcriptional level.

To determine whether oxysterol-dependent degradation of HMG-CoA reductase mRNA is mediated by an instability element, a fusion gene containing both the human β -globin gene and the 3'-untranslated region of hamster HMG-CoA reductase cDNA was expressed transiently in Chinese hamster ovary (CHO) cells. Expression of this fusion construct was under the control of the simian virus 40 (SV40) promoter, which is not influenced by oxysterol levels in the cell [19,21]. Results from this study indicate that 3'-untranslated sequences of reductase are indeed responsible for mediating the post-transcriptional effect of 25-hydroxycholesterol-induced suppression of HMG-CoA reductase mRNA.

MATERIALS AND METHODS

Materials

CHO K1 cells were obtained from American Type Culture Collection. Cells were maintained in minimal essential medium (MEM) containing 5% fetal bovine serum (FBS). For experimental purposes, cells were cultured in MEM supplemented with either 5% whole FBS (FBS/MEM) or 5% delipidated FBS (DFBS/MEM). Lovastatin was a gift from Dr. A. Alberts (Merck, Sharp and Dohme Laboratories) and was prepared as described by Endo et al. [22]. Cycloheximide was from Sigma, and 25-hydroxycholesterol from Steraloids. Cycloheximide was

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; MEM, minimum essential medium; FBS, fetal bovine serum; DFBS, delipidated fetal bovine serum; CHO K1, Chinese hamster ovary fibroblasts; BHK, baby hamster kidney cells; SV40, simian virus 40; CAT, chloramphenicol acetyltransferase; GM-CSF, granulocyte-monocyte colony-stimulating factor.

* Present address: Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea.

† To whom correspondence should be addressed.

dissolved in water to a final concentration of 10 mg/ml, and 25-hydroxycholesterol in 100% ethanol to a final concentration of 1 mg/ml. Concentrations of ethanol in media did not exceed 0.01% (v/v). *Escherichia coli* strain JM109, pGEM vectors and restriction enzymes were from Promega.

Construction of plasmids

Standard procedures for plasmid DNA preparation, restriction enzyme digestion, bacterial cell transformation, DNA ligation and selection for ampicillin resistance were used [23]. A fusion gene construct containing both β -globin and reductase 3'-untranslated sequences was made as follows.

Plasmid pTAG-1, containing the entire sequence for the human β -globin gene [24,25], was kindly provided by Dr. Judith Fridovich-Keil (Emory University, Atlanta, GA, U.S.A.). The *Hind*III–*Bam*HI fragment of pTAG-1, which contained 50 bp of 5'-untranslated region, 95 bp of the first exon, 129 bp of intervening sequence and 196 bp of the second exon of human β -globin gene, was inserted into the *Hind*III–*Bam*HI restriction sites of pGEM3Z to create p β Glo.

We then excised the 4600 bp *Bam*HI–*Hinc*II fragment containing full-length cDNA for hamster reductase from pRed227 [26] and cloned it into the *Bam*HI–*Sma*I restriction sites of p β Glo to create p β Glo/Red-1. Except for 1416 bp of the untranslated region, all reductase sequences were removed from p β Glo/Red-1 by *Bam*HI–*Bgl*II digestion, and the plasmid was religated to create p β Glo/Red-2. The remaining reductase untranslated region contained two polyadenylation sites used endogenously in hamster cells [27].

The *Bgl*II–*Xba*I fragment of vector pCAT-Control (Promega) was isolated and cloned into the *Bam*HI–*Xba*I cloning site of pGEM7Z+ to create pGEM/CAT. This fragment contained the SV40 promoter, bacterial chloramphenicol acetyltransferase (CAT) gene, and SV40 enhancer sequences. CAT and SV40 enhancer sequences were removed from pGEM/CAT with a *Hind*III–*Hinc*II digestion.

In the final step, p β Glo/Red-2 was linearized with *Eco*RI and the restriction site blunt-ended with Klenow fragment. The 1883 bp fragment of p β Glo/Red-2 was subsequently removed with *Hind*III and cloned into the *Hind*III–*Hinc*II restriction site of pGEM/CAT. This plasmid, p β glo/3'UT, contained 1416 bp of the 3'-untranslated region of HMG-CoA reductase cDNA, 467 bp of the human β -globin gene and the SV40 promoter (Figure 1a).

As a control vector that lacked reductase 3'-untranslated sequences, we used a plasmid, p β Glo/SV40, that contained the *Hind*III–*Bam*HI fragment of pTAG-1 and SV40 enhancer sequences. This plasmid was constructed by replacing the *Nde*I–*Xba*I fragment of p β Glo/3'UT with a 1367 bp *Hae*III–*Xba*I fragment of pCAT-Control vector (Figure 1b).

Plasmid pCS17-3, which contained full-length cDNA for Chinese hamster S17 ribosomal protein [28], was generously provided by Dr. Donald J. Roufa (Kansas State University Center for Basic Cancer Research, Manhattan, KS, U.S.A.). A 369 bp *Pst*I fragment from pCS17-3 was inserted into the *Pst*I cloning site of pGEM3Z to create pRPS17, which was used as a control for RNA-loading effects in RNAase-protection assays.

Transient transfection experiments

CHO K1 cells were plated at a density of 5×10^6 /150 mm culture dish in FBS/MEM. Cellular transfection was performed by the calcium phosphate/DNA precipitate method, as described by Sambrook et al. [23]. Supercoiled plasmid DNA was purified by

Figure 1 Schematic diagram of plasmid constructs used to transfect CHO K1 cells

Details of the construction of each plasmid are found in the Materials and methods section.

CsCl gradient centrifugation, as described previously [23]. Medium was removed from each plate, 3 ml of calcium phosphate/DNA suspension was added, and the incubation continued for 15 min at room temperature. Cells were subsequently refed with fresh FBS/MEM, incubated for 24 h, then treated with trypsin and divided into four equal portions on 90 mm plates. They were maintained in FBS/MEM for 24 h, after which the medium was changed to DFBS/MEM and 25 μ M lovastatin. After 16 h of incubation, either 25-hydroxycholesterol alone (1.2 μ M) or 25-hydroxycholesterol (1.2 μ M) plus cycloheximide (10 mg/ml) was added to the medium; cells were incubated for an additional 4 h and lysed in 4 M guanidinium isothiocyanate. Both β -globin and S17 ribosomal protein RNA levels were then measured by the RNAase-protection assay.

Preparation of riboprobes

Riboprobes used to measure β -globin RNA in transfected cells were generated from plasmid p β Glo/Red-1 linearized with *Pvu*II. Riboprobes for S17 ribosomal RNA protein were synthesized from pRPS17 digested with *Ava*II. Both antisense probes were

synthesized using T7 RNA polymerase according to the conditions of Melton et al. [29].

Lysate RNAase-protection assay

RNAase-protection assays were performed as described by Haines and Gillespie [30] with minor modifications. Cells (2×10^7) were dissolved in 5 M guanidinium isothiocyanate/0.1 M EDTA. To control for RNA-loading differences, levels of hamster ribosomal protein S17 RNA were also determined. S17 is a constitutively expressed protein, and its mRNA has been shown to remain unchanged in other studies using similar conditions [19]. Cell lysate (20 μ l) was hybridized with 5×10^5 c.p.m. of riboprobe (either β -globin or S17) at 37 °C for 8–16 h. After hybridization, 500 μ l of RNAase ONE digestion buffer, 3 units of RNAase ONE (Promega), 10 μ g of RNAase-free DNAase (Boehringer-Mannheim) and 5 μ l of 1 M $MgCl_2$ were added. After digestion at 37 °C for 45 min, 10 μ l of 20 % SDS, 10 μ l of proteinase K (20 mg/ml) and 2 μ l of 10 mg/ml carrier yeast tRNA were added, and the incubation was continued at 37 °C for 30 min. The mixture was then extracted with phenol/chloroform (1:1, v/v), and undigested riboprobes were precipitated with 2 vol. of 100 % ethanol at –70 °C for 1 h. Precipitated riboprobes were dissolved in RNA sample loading buffer and electrophoresed on 6–8 % polyacrylamide (acrylamide/bisacrylamide, 38:2)/7 M urea gels at 250 V. The resultant gel was exposed to X-ray film (Kodak) for an appropriate period at –70 °C.

RESULTS

To determine whether the oxysterol-regulated degradation of HMG-CoA reductase mRNA reported previously [20] involves an instability element, p β Glo/3'UT, a fusion gene that has both the human β -globin gene and the 3'-untranslated region of hamster HMG-CoA reductase cDNA (Figure 1a), was transfected into CHO cells. Although the cycloheximide-mediated block to oxysterol-dependent suppression of HMG-CoA reductase mRNA was originally observed in BHK C100 cells, we chose to transfect CHO cells with β -globin fusion constructs because the transfection frequencies were consistently higher in these cells than in BHK cells. In addition, regulation of HMG-CoA reductase in the two cell lines has been demonstrated to be identical [3,5].

β -Globin mRNA is a convenient reporter gene because it is not expressed in this fibroblastic cell type (Figure 2a, control lane) and does not contain any of the sequence elements required for sterol-mediated regulation [10,19]. Initially, transfected cells were treated with either FBS/MEM alone or DFBS/MEM plus lovastatin, a competitive inhibitor of reductase [31] that increases cellular reductase mRNA levels through transcriptional mechanisms [19]. When 32 P-labelled antisense β -globin RNA was hybridized to lysates prepared from these cells, 196- and 145-base RNA fragments were observed (Figure 2a, lanes 1 and 2). The 145-base fragment represents exon 1 sequences (50 bases of untranslated sequence and 95 bases of coding sequence) whereas the 196-base fragment represents exon 2 sequences [25]. Two fragments were expected because there is one intervening sequence between the two exons of the β -globin *HindIII*–*BamHI* fragment in the fusion gene construct [25] that is not represented in the mature transcript. Although the 196-base fragment was observed consistently, detection of the 145-base fragment was variable possibly because the 145 bp hybrid relative was less stable than the larger hybrid under the RNAase-digestion conditions used.

To quantify the relative amounts of β -globin RNA, auto-

Figure 2 Regulation of β -globin RNA by lovastatin, 25-hydroxycholesterol and cycloheximide in cells transfected with either p β Glo/3'UT (a) or p β Glo/SV40 (b)

Transfected CHO cells were prepared as described in the Materials and methods section. Cells were incubated for 16 h in DFBS/MEM plus 25 μ M lovastatin, then supplemented with either 25-hydroxycholesterol alone (1.2 μ M) or 1.2 μ M 25-hydroxycholesterol plus 10 μ g/ml cycloheximide. Incubations were continued for an additional 4 h before lysis of the cells. Levels of β -globin RNA were estimated by RNAase-protection assays using lysates prepared from non-transfected cells (control lane) or transfected cells treated with FBS/MEM (lane 1), DFBS/MEM plus lovastatin (lane 2), DFBS/MEM plus lovastatin and 25-hydroxycholesterol (lane 3), or DFBS/MEM plus lovastatin, 25-hydroxycholesterol and cycloheximide (lane 4). Two RNA species of 196 and 145 bases are visible on the autoradiograph. The relative amount of the upper 196-base RNA band was estimated by laser densitometric scanning and these values for transcript levels are listed in Table 1. As a control for RNA loading, levels of ribosomal S17 protein mRNA were determined by RNAase-protection assay in each lysate.

Table 1 Effects of lovastatin, 25-hydroxycholesterol and cycloheximide on β -globin RNA levels in cells transfected with p β Glo/3'UT or p β Glo/SV40

Relative changes in mRNA levels were estimated by laser densitometric scanning of autoradiographs presented in Figure 2. The relative amount of the upper 196-base band was used to estimate changes in β -globin RNA. Values for transcript levels were corrected for variation in RNA loading on the basis of the amount of ribosomal protein S17 mRNA in each lysate RNAase-protection assay. The level of β -globin RNA in cells treated with FBS/MEM was set at 100 %, and all other values are reported relative to this treatment. Values are means $\pm$ S.D. of three independent determinations.

	β -globin RNA			
	FBS	DFBS + lovastatin	DFBS + lovastatin + 25-hydroxycholesterol	DFBS + lovastatin + cycloheximide
p β Glo/3'UT	100 $\pm$ 6	108 $\pm$ 20	41 $\pm$ 10	200 $\pm$ 7
p β Glo/SV40	100 $\pm$ 10	103 $\pm$ 16	106 $\pm$ 21	117 $\pm$ 12

radiographs were prepared from the gels, and the 196-base RNA species was scanned by laser densitometry. These results are reported in Table 1. Compared with cells treated with FBS/MEM (Figure 2a, lane 1) the levels of β -globin RNA did not change markedly in cells cultured with DFBS/MEM plus lovastatin (Figure 2, lane 2). These results suggest that the steady-state level of the fusion gene construct was largely dependent on constitutive transcription mediated through the SV40 promoter. Furthermore, lovastatin treatment did not change the β -globin RNA level, a result we expected, as the SV40 promoter does not have a sterol-response element required for transcriptional control of reductase mRNA by cholesterol or oxysterols [19,21].

Expression of β -globin RNA in the presence of both lovastatin

Figure 3 Regulation of β -globin RNA by DFBS, 25-hydroxycholesterol, lovastatin and cycloheximide in cells transfected with either p β Glo/3'UT (a) or p β Glo/SV40 (b)

Transfected CHO cells were prepared as described in the Materials and methods section. Cells were incubated for 16 h in either DFBS/MEM or DFBS/MEM plus 25 μ M lovastatin. Either 25-hydroxycholesterol (1.2 μ M) or cycloheximide (10 μ g/ml) was added and cells were incubated for an additional 4 h. β -Globin RNA levels were estimated by RNAase-protection assays. Lysates were prepared from transfected cells treated with FBS/MEM (lane 1), DFBS/MEM (lane 2), DFBS/MEM plus 25-hydroxycholesterol (lane 3), DFBS/MEM plus lovastatin and cycloheximide (lane 4) and DFBS plus lovastatin (lane 5). The relative amount of the 196-base RNA species was estimated by laser densitometric scanning and these values are reported in Table 2. Ribosomal protein S-17 mRNA levels were determined for each lysate and these values were used to correct for variation in RNA loading (results not shown).

and 25-hydroxycholesterol was also measured (Figure 2a, lane 3). The oxysterol, 25-hydroxycholesterol, is a potent suppressor of reductase mRNA in lovastatin-treated cells [11,20,32] and has been shown to mediate its effects at the transcriptional level through the sterol-response element in the 5' promoter region of the reductase gene [10,19]. In the presence of 25-hydroxycholesterol, β -globin RNA decreased approx. 2-fold compared with that in transfected cells treated with DFBS/MEM plus lovastatin (Figure 2a, lane 2; Table 1). Again, because there is no sterol-response element in the SV40 promoter to mediate suppression of transcription by this oxysterol, these results indicate that 25-hydroxycholesterol regulates expression of the fusion construct through a post-transcriptional mechanism that involves the 3'-untranslated region of reductase.

Cells were also treated with 25-hydroxycholesterol in the absence of lovastatin and these results are summarized in Figure 3 and Table 2. In cells treated with DFBS/MEM plus 25-hydroxycholesterol (lane 3), we saw no decline in β -globin RNA levels compared with RNA levels in cells grown in DFBS/MEM (lane 2), FBS/MEM (lane 1) or DFBS/MEM plus lovastatin (lane 5). These results indicate that inhibition of HMG-CoA reductase by lovastatin and subsequent reductions in non-sterol intermediates of cholesterol biosynthesis are required to set-up

permissive conditions for oxysterol-dependent suppression of β -globin RNA levels.

We then investigated the role of protein synthesis in oxysterol-mediated suppression of β -globin mRNA by exposing transfected cells to both 25-hydroxycholesterol and the protein synthesis inhibitor cycloheximide (Figure 2a, lane 4). Under such conditions, β -globin RNA levels were not suppressed compared with those in cells treated with 25-hydroxycholesterol (lane 3); rather they were elevated 5-fold (Table 1). In addition, β -globin RNA levels were increased by approx. 2-fold compared with lovastatin-only treatment (Figure 2a, lane 2; Table 1). An increase in β -globin RNA levels of similar magnitude was observed in cells treated with lovastatin in the absence of 25-hydroxycholesterol (Figure 3a, lane 4; Table 2). These results are analogous to those reported previously in which cycloheximide stabilized cellular reductase transcripts and blocked oxysterol-mediated suppression of HMG-CoA reductase mRNA [20]. Apparently, the 3'-untranslated region of HMG-CoA reductase mRNA increases the stability of the β -globin transcript when protein synthesis is inhibited with cycloheximide.

To confirm that HMG-CoA reductase 3'-untranslated sequences of p β Glo/3'UT were specifically required for post-transcriptional regulation by 25-hydroxycholesterol, we transfected cells with p β Glo/SV40 (Figure 1b) in which reductase sequences were replaced with SV40 enhancer sequences. When corrected for loading differences by scanning the S17 ribosomal protein RNA band, no appreciable difference in β -globin levels was observed under the same conditions as used above (Figure 2b; Table 1). In cells transfected with the control vector, 25-hydroxycholesterol did not suppress β -globin RNA compared with cells treated with lovastatin alone (compare lanes 2 and 3). Moreover, when cycloheximide plus 25-hydroxycholesterol was added (lane 4), there was no change in β -globin mRNA levels compared with those in cells treated with FBS/MEM alone (lane 1), with lovastatin (lane 2) or with lovastatin plus 25-hydroxycholesterol (lane 3). Clearly, the 3'-untranslated region of HMG-CoA reductase is required to mediate the effects of both 25-hydroxycholesterol and cycloheximide on β -globin RNA levels. Similarly, as shown in Figure 3(b) and Table 2, β -globin RNA levels did not change in cells treated with DFBS/MEM (lane 2) or DFBS/MEM plus 25-hydroxycholesterol (lane 3) compared with RNA levels in cells treated with either FBS/MEM (lane 1) or DFBS/MEM plus lovastatin (lane 4) and 25-hydroxycholesterol.

DISCUSSION

Although previous studies have shown that oxysterols such as 25-hydroxycholesterol regulate transcription of HMG-CoA reductase mRNA through sterol regulatory elements in the

Table 2 Effects of DFBS, 25-hydroxycholesterol, lovastatin and cycloheximide on β -globin RNA levels in cells transfected with p β Glo/3'UT or p β Glo/SV40

The relative amount of β -globin RNA was estimated by laser densitometric scanning of the upper 196-base band in autoradiographs presented in Figure 3. Values for transcript levels were corrected for variation in RNA loading on the basis of the amount of ribosomal S17 protein mRNA in each lysate RNAase-protection assay. Corrected values were normalized to β -globin RNA in cells grown in FBS/MEM which was set at 100%, and all other values are reported relative to this treatment. Values are means $\pm$ S.D. of three independent determinations.

	β -globin RNA				
	FBS	DFBS	DFBS + 25-hydroxy cholesterol	Lovastatin + cycloheximide	Lovastatin
p β Glo/3'UT	100 $\pm$ 9	118 $\pm$ 14	107 $\pm$ 28	231 $\pm$ 11	110 $\pm$ 16
p β Glo/SV40	100 $\pm$ 2	89 $\pm$ 3	88 $\pm$ 9	72 $\pm$ 11	Not determined

5'-promoter region of the gene [10,19], this study is the first to demonstrate the role of reductase 3'-untranslated sequences in mediating post-transcriptional effects of 25-hydroxycholesterol. Our results are significant because they describe a second mechanism by which oxysterols, a group of key regulators of HMG-CoA reductase produced via the cholesterol biosynthetic pathway, regulate steady-state levels of this enzyme's mRNA. Moreover, this study has shown that the cycloheximide-induced block to oxysterol-mediated suppression of reductase mRNA, which was described in an earlier study from this laboratory [20], is also dependent on 3'-untranslated sequences. It is of interest that the magnitude of change for β -globin RNA expressed in cells transfected with p β Glo/3'UT agrees closely with changes in cellular reductase mRNA after oxysterol treatment in both the presence and absence of cycloheximide [11,14,20,32]. This supports the hypothesis that 3' untranslated reductase sequences play a physiological role in regulating levels of reductase mRNA.

The observation that oxysterol-mediated suppression of β -globin RNA in cells transfected with p β Glo/3'UT occurs only in cells treated with lovastatin suggests that mevalonate may either (1) modify proteins associated with reductase sequences in such a manner that they are less susceptible to oxysterol-mediated degradation or (2) inactivate RNAses involved in the degradation of reductase mRNA. In support of the first hypothesis, we have recently demonstrated that mevalonate regulates reductase synthesis at the translational level by suppressing the rate of initiation on reductase mRNA (D. M. Peffley and A. K. Gayen, unpublished work). Because regulation of translation initiation involves the interaction of specific proteins with mRNA [33–36], we have proposed that mevalonate similarly mediates its effects on reductase synthesis through a protein binding to reductase mRNA. The interaction of such a protein with reductase mRNA untranslated sequences in cells transfected with p β Glo/3'UT may also attenuate post-transcriptional effects of 25-hydroxycholesterol on β -globin mRNA levels under conditions where mevalonate synthesis is not inhibited by lovastatin.

Other studies have demonstrated the importance of 3'-untranslated sequences in regulating various genes [37–40]. When fused with β -globin-coding sequences and expressed in cells, 3'-untranslated sequences impart to β -globin RNA regulatory control that is characteristic of the heterologous mRNA. For example, transcripts for *c-fos* and lymphokine granulocyte-monocyte colony-stimulating factor (GM-CSF) are degraded more rapidly than β -globin mRNA [38–40]. However, when 3'-untranslated sequences of either *c-fos* [38,39] or GM-CSF cDNA [40] are fused to β -globin cDNA-coding sequences, mRNA from the β -globin fusion construct decays with kinetics characteristic of *c-fos* and GM-CSF mRNA, i.e. more rapidly than β -globin mRNA itself. The motif responsible for this rapid degradation is AUUA, located in the 3'-untranslated regions of *c-fos* and GM-CSF mRNA [40,41] as well as other mRNAs with short half-lives [37].

In the case of reductase mRNA, the 3'-untranslated region of the mRNA contains AUUUA motifs characteristic of unstable mRNAs [42]. However, their role in oxysterol-mediated post-transcriptional control of reductase mRNA, as well as their stabilization by cycloheximide, has not yet been established. Post-transcriptional control of reductase by oxysterols may function in one of two ways. Oxysterols may enhance the activity of a specific nuclease or group of nucleases that recognize specific motifs or secondary structure in 3'-untranslated reductase sequences. Alternatively, they may reduce the affinity of a specific mRNA-binding protein that protects reductase mRNA from degradation. Proteins binding to AUUUA [43] and other sequence motifs [37,44] in the 3'-untranslated sequences of several

mRNAs have been identified. However, their role in degradation or stabilization of mRNA has not been demonstrated. Results from studies in this laboratory in which cycloheximide blocked oxysterol-mediated reduction in reductase mRNA [20] have suggested that a labile protein is involved in the post-transcriptional process. This was confirmed here with the β -globin fusion construct. Current experiments are directed toward identifying not only specific sequences in the 3'-untranslated 1416 bp region that control oxysterol regulation of reductase but also proteins that interact with these regions.

This work was supported by Public Health Service Grant HL44006 (to D.M.P.). We thank Dr. Patricia Hentosh for critical reading of the manuscript.

REFERENCES

- Brown, M. S. and Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 505–517
- Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S. and Goldstein, J. L. (1983) *J. Biol. Chem.* **258**, 8462–8469
- Hardeman, E., Endo, A. and Simoni, R. (1984) *Arch. Biochem. Biophys.* **232**, 549–561
- Faust, J. R., Goldstein, J. L. and Brown, M. S. (1979) *Arch. Biochem. Biophys.* **192**, 86–89
- Reynolds, G. A., Basu, S. K., Osborne, T. F. et al. (1984) *Cell* **38**, 275–286
- Skalnik, D. G., Brown, D. A., Brown, P. C. et al. (1985) *J. Biol. Chem.* **260**, 1991–1994
- Clarke, C. F., Fogelman, A. M. and Edwards, P. A. (1984) *J. Biol. Chem.* **259**, 10439–10447
- Edwards, P. A., Lau, S. F. and Fogelman, A. M. (1983) *J. Biol. Chem.* **258**, 10219–10222
- Clarke, C. F., Edwards, P. A., Lau, S. F., Tanaka, R. D., Tanaka, A. M. and Fogelman, A. M. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3305–3308
- Osborne, T. F., Goldstein, J. L. and Brown, M. S. (1985) *Cell* **42**, 203–212
- Peffley, D. and Sinensky, M. (1985) *J. Biol. Chem.* **260**, 9949–9952
- Tanaka, R. D., Edwards, P. A., Lau, S. F. and Fogelman, A. M. (1980) *J. Biol. Chem.* **255**, 13331–13339
- Nakanishi, M., Goldstein, J. L. and Brown, M. S. (1988) *J. Biol. Chem.* **263**, 8929–8937
- Peffley, D., Miyake, J., Leonard, S., vonGunten, C. and Sinensky, M. (1988) *Somat. Cell Mol. Genet.* **14**, 527–539
- Simonet, W. S. and Ness, G. C. (1989) *J. Biol. Chem.* **264**, 569–573
- Liscum, L., Finer-Moore, J., Stroud, R. M., Luskey, K. L., Brown, M. S. and Goldstein, J. L. (1985) *J. Biol. Chem.* **260**, 522–530
- Edwards, P. A., Lan, S.-F., Tanaka, R. D. and Fogelman, A. M. (1983) *J. Biol. Chem.* **258**, 7272–7275
- Parker, R. A., Miller, S. J. and Gibson, D. M. (1989) *J. Biol. Chem.* **264**, 4877–4887
- Goldstein, J. L. and Brown, M. S. (1990) *Nature (London)* **343**, 425–430
- Choi, J. W., Lundquist, E. N. and Peffley, D. M. (1993) *Biochem. J.* **296**, 859–866
- Chin, D. J., Gil, G., Faust, J. R., Goldstein, J. L., Brown, M. S. and Luskey, K. L. (1985) *Mol. Cell. Biol.* **5**, 634–641
- Endo, A., Kuroda, M. and Tanzawa, K. (1976) *FEBS Lett.* **72**, 323–326
- Sambrook, J. T., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Fridovich-Keil, J. L., Gudas, J. M., Bryan, I. B. and Pardee, A. B. (1991) *Biotechniques* **11**, 572–579
- Lawn, R. M., Efstratiadis, A., O'Connell, C. and Maniatis, T. (1980) *Cell* **21**, 647–651
- Chin, D. J., Gil, G., Russel, D. W., Liscum, L. et al. (1984) *Nature (London)* **308**, 613–617
- Reynolds, G. A., Basu, S. K., Osborne, T. F. et al. (1984) *Cell* **38**, 275–285
- Chen, I., Dixit, A., Rhoads, D. D. and Roufa, D. J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6907–6911
- Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. and Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056
- Haines, D. S. and Gillespie, D. (1992) *Biotechniques* **12**, 736–740
- Alberts, A. W., Chen, J., Kuron, G. et al. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3957–3961
- Peffley, D. (1992) *Somat. Cell. Mol. Genet.* **18**, 19–32
- Hershey, J. W. B. (1991) *Annu. Rev. Biochem.* **60**, 717–755
- Kwon, Y. K. and Hecht, N. B. (1993) *Mol. Cell. Biol.* **13**, 6547–6557
- Richter, J. D. (1991) *BioEssays* **13**, 179–183
- Ranjan, M., Tafuri, S. R. and Wolffe, A. P. (1993) *Genes Dev.* **7**, 1725–1736
- Atwater, J. A., Wisdom, R. and Verma, I. M. (1990) *Annu. Rev. Genet.* **24**, 519–541
- Kabnick, K. S. and Housman, D. E. (1988) *Mol. Cell. Biol.* **8**, 3244–3250

-
- 39 Shyu, A.-B., Belasco, J. G. and Greenberg, M. E. (1991) *Genes Dev.* **5**, 221–231
- 40 Shaw, G. and Kamen, R. (1986) *Cell* **46**, 659–667
- 41 Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Shimer, S. and Cerami, A. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1670–1674
- 42 Ramharack, R., Tam, S.-P. and Deely, R. G. (1990) *DNA Cell. Biol.* **9**, 677–690
- 43 Bohjanen, P. R., Bronislawa, P., June, C. H., Thompson, C. B. and Lindsten, T. (1992) *J. Biol. Chem.* **267**, 6302–6309
- 44 Vakalopoulou, E., Schaack, J. and Shenk, T. (1991) *Mol. Cell. Biol.* **6**, 3355–3364
-

Received 22 September 1994/23 November 1994; accepted 5 December 1994