Cholesterol homeostasis is modulated by amphiphiles at transcriptional and post-transcriptional loci

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Abstract A variety of amphiphiles inhibit plasma membrane cholesterol esterification and induce 3-hydroxy-3-methylglutaryl-coenzyme A reductase accumulation in cultured cells; among these are steroids, hydrophobic amines, phenothiazines, ionophores, colchicine, and lysophosphatides. It has been proposed that these amphiphiles signal a sterol deficiency to regulatory sites by blocking the movement of plasma membrane cholesterol into the cell (Lange, Y., and Steck, T. L. 1994. *J. Biol. Chem.* **269:** 29371–29374). If this were the case, these agents also should enhance transcription of sterol responsive genes and stabilize 3-hydroxy-3-methylglutaryl-coenzyme A reductase. As a test of this hypothesis, the effect of the amphiphiles on such transcriptional and post-transcriptional events was assessed. A mouse embryo cell line was transfected with a construct containing the promoter for the human low density lipoprotein receptor upstream of the DNA sequence coding for chloramphenicol acyltransferase (CAT). Incubation of these cells for 7–18 h with the aforementioned agents caused the level of expression of the promoter/CAT construct to increase 2- to 9-fold. We showed further that the amphiphiles stimulated 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity by increasing gene transcription as well as by decreasing degradation of the enzyme. These are the predicted homeostatic responses to cell cholesterol deficiency. These findings support the hypothesis that certain amphiphiles falsely signal a cholesterol deficiency to the intracellular sites regulating cholesterol homeostasis.—**Lange, Y., H. Duan, and T. Mazzone.** Cholesterol homeostasis is modulated by amphiphiles at transcriptional and post-transcriptional loci. *J. Lipid Res.* 1996. **37:** 534–539

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Cell cholesterol is tightly regulated at multiple levels, including its biosynthesis and esterification and the receptor-mediated endocytosis of low density lipoproteins (1). In particular, the biosynthesis of HMG-CoA synthase, HMG-CoA reductase, and LDL receptors all increase as cell cholesterol declines in various cell types (1). Expression of the genes for HMG-CoA synthase and the LDL receptor are under the control of a 10 bp sequence in its 5′ flanking region, termed the sterol regulatory element-1 (2). This regulatory element promotes LDL receptor gene expression when liganded by the sterol regulatory element-1 binding protein. The latter is released from anchorage in the endoplasmic reticulum and outer nuclear membranes by proteolysis stimulated by cholesterol deficiency (3).

In addition to regulating transcription and translation of the HMG-CoA reductase gene, cell sterols can affect the stability of HMG-CoA reductase itself (1). As a result, levels of HMG-CoA reductase activity can vary widely and acutely.

As most of the cholesterol in the cell is in the plasma membrane (4), how is its abundance sensed by these internal regulatory sites? A clue to this unknown mechanism comes from the recent finding that a broad class of amphiphiles inhibited esterification of cholesterol and stimulated HMG-CoA reductase activity and cholesterol biosynthesis (5). These agents included progesterone, monensin, chloroquine, imipramine, trifluoperazine, nigericin, colchicine, and lysolecithin. Regardless of their various other actions, this class of agents acted as if signalling a deficiency of cholesterol within the cell (5). Their action was reversed by oxysterols, conceivably at the same control point. These and related data led to a general model of cholesterol homeostasis (5) with the following features: *a)* While almost all of the cholesterol resides in the plasma membrane, the metabolic processes that regulate this pool are located in intracellular membranes. *b)* Homeostatic activities are governed by the local level of cholesterol

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; CAT, chloramphenicol acyltransferase; CHO, Chinese hamster ovary; LDL, low density lipoprotein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; LPDS, lipoprotein-deficient serum.

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TABLE 1. Effect of mevinolin on LDL receptor gene expression

Treatment	Inc	CAT Activity	Relative Activity
	h	*%/mg/h*	
Control	6	38 ± 8.5	1.0
Mevinolin		66 ± 4.4	1.7 ± 0.1
Control	24	27 ± 6.	1.0
Mevinolin		74 ± 4.1	2.7 ± 0.2

Cells transfected with the LDLR-CAT gene construct were incubated for 48 h in medium containing 1% human plasma-derived serum. Thirty μM mevinolin was added to half of the dishes and the cells were incubated for an additional 6 or 24 h prior to assay of their CAT activity. Triplicate dishes were used for each condition. Values are expressed relative to that of the control (mean ± SD).

in intracellular membranes. *c)* These local levels of cholesterol are controlled by the regulated circulation of sterol between cell surface and intracellular membranes. *d)* By partitioning into the plasma membrane, the amphiphiles affect its sterol "sensor" and thereby reduce the delivery of its cholesterol to the cytoplasmic membranes. Conversely, oxysterols would increase the level of cholesterol at the regulatory intracellular sites (5).

TABLE 2. Effect of amphipaths on LDL receptor gene expression

Expt.	Agent	Conc	Inc	Relative Activity
		μM	*h*	
1	None	0	18	1.0
	Progesterone	10		3.8 ± 0.6
	Monensin	1.5		3.9 ± 0.2
	Chloroquine	75		9.2 ± 1.7
2	None	0	18	1.0
	Imipramine	80		7.1 ± 1.5
	Nigericin	3		1.8 ± 0.4
	Lysolecithin	45		7.1 ± 0.1
	Colchicine	80		1.7 ± 0.1
3	None	0	7	1.0
	Trifluoperazine	20		3.6 ± 0.7

The medium in replicate dishes of cells transfected with the LDLR-CAT gene construct was replaced with medium containing 1% human plasma-derived serum. After 48 h incubation, the agents were added in ethanol or Me_2SO (< 1% final), and the cells were incubated for a further 7 or 18 h before extraction and assay of CAT activity as described under Experimental Procedures. Control dishes received solvent alone. Triplicate flasks were used for each condition. Values are expressed relative to that of the control (mean ± SD).

If, as postulated, these amphiphiles enhance cholesterol biosynthesis and inhibit cholesterol esterification by sending a false signal of cell cholesterol depletion, they should activate the transcription of LDL receptor and HMG-CoA reductase genes and post-translationally stabilize HMG-CoA reductase. We now have tested this prediction.

EXPERIMENTAL PROCEDURES

Materials

[^{14}C]HMG-CoA (57.7 mCi/mmol) was from DuPont-New England Nuclear. [1,2-^{3}H]cholesterol (40 mCi/mmol) was purchased from Amersham Corp. Chemicals and human serum were purchased from Sigma. Fetal bovine serum was from GIBCO. Mevinolin was a generous gift from Caroline Staemmler of Merck, Sharp and Dohme.

Cell culture and transfection

NIH 3T3 cells were obtained from the American Type Culture Collection. For transfection, 5×10^5 cells were plated in 100-mm dishes in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. After 24 h, 9 μg of a human LDL receptor genomic-CAT construct 6.5 LDLR-CAT (6), containing sequences from -6407 bp to +36 bp of the human LDL receptor gene 5′ flanking sequence (numbered with reference to

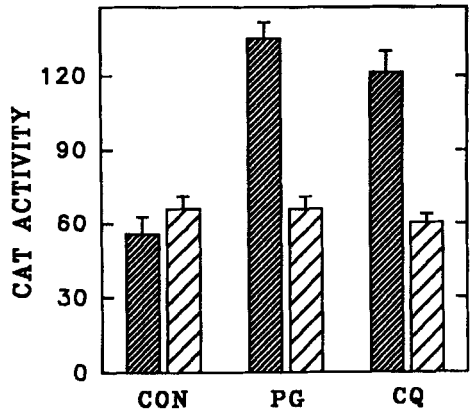

Fig. 1. The effect of 25-hydroxycholesterol on the stimulation of LDL receptor gene expression by progesterone and chloroquine. The medium in replicate dishes of cells was replaced with medium containing 1% human plasma-derived serum. After 48 h incubation, the progesterone or chloroquine minus (left bar) or plus 12 μM 25-hydroxycholesterol (right bar) was added in ethanol (< 1% final). The same amount of ethanol was present in all samples. The cells were incubated for 18 h and then CAT activity was assayed as described under Experimental Procedures. Triplicate dishes were used for each condition. Values (mean ± SD) are given as % conversion/mg protein per h. CON, ethanol control; PG, 10 μM progesterone; CQ, 75 μM chloroquine.

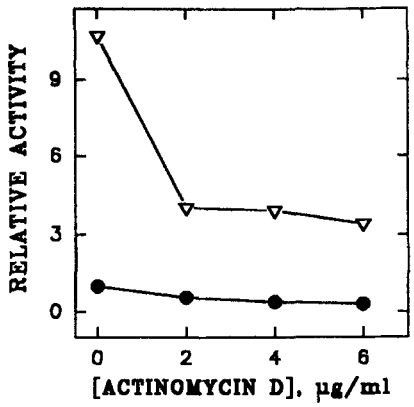

Fig. 2. The effect of actinomycin D on stimulation of HMG-CoA reductase activity by imipramine. Subconfluent flasks of human foreskin fibroblasts were given fresh growth medium containing 10% fetal bovine serum with (▽) or without (●) 80 µM imipramine plus the indicated concentrations of actinomycin D and incubated for 16 h at 37°C. The concentration of ethanol (< 1%) was the same in all samples. The cells then were extracted and HMG-CoA reductase activity was determined. Assays were performed in duplicate; duplicates agreed to 3%. Values are expressed relative to those for cells incubated without either imipramine or actinomycin D. In the absence of added agents, the value was 760 dpm/h per 100 µg protein.

the major transcription start site) was added together with 1 µg of pSV_2neo. Transfection was carried out in duplicate dishes using the Lipofectin reagent (Life Technologies Inc.) according to the manufacturer's instructions. After 7 h, fresh DMEM containing 10% fetal bovine serum was added to the dishes. Seventy-two hours later, stable transfectants were selected by adding 850 µg/ml Geneticin. After 6 weeks, > 50 individual colonies were pooled and expanded for use in the experiments.

Transfected cells were seeded at 3.5×10^4 cells/cm^2 and grown to near confluence in DMEM containing 10% fetal bovine serum. The monolayers then were washed and overlayered with DMEM containing 1% human plasma-derived serum. Forty-eight hours later, the agents or solvent alone were added to the dishes. The cells were incubated for the times indicated in the legends, washed twice with ice-cold phosphate-buffered saline, and scraped into tubes in this same buffer. The cells were pelleted, resuspended in 0.25 M Tris (pH 7.8), and quick frozen in liquid nitrogen for storage at -70°C.

Human foreskin fibroblasts were obtained and cultured as described (7). Chinese hamster ovary (CHO-K1) cells transfected with the plasmid pSV2-HMGal (CHO-HMGal cells, see reference 8) were a generous gift from Robert D. Simoni, Stanford University. The cells were grown as monolayers in minimum essential medium (MEM) supplemented with 250 µg/ml geneticin and 5% fetal bovine serum.

CAT assay

Frozen samples were subjected to three freeze-thaw cycles and then heated to 65°C for 10 min. CAT enzymatic assay was as described (9). Protein in the thawed samples was assayed by the Lowry procedure (10), using bovine serum albumin as a standard. The CAT activity in controls ranged from 26 to 112% conversion/mg protein per h, depending on the duration of cell growth prior to start of the experimental incubations.

Northern blot analysis

To isolate RNA, washed cells were solubilized in guanidine isothiocyanate and the extract was sedimented through cesium chloride as previously described (11). For Northern blot analysis, formaldehyde-treated RNA samples were fractionated in 1% agarose gels, transferred to Nytran, and hybridized with probes labeled by random-primed synthesis. The HMG-CoA reductase probe was a 1.1 kb Hind III fragment of the full length cDNA. The beta-actin probe contained a 0.7 kb Pst I fragment of the beta-actin cDNA. Signals on autoradiographs were analyzed by laser scanning densitometry.

Other assays

For the assay of HMG-CoA reductase activity, cells were extracted from the flasks with 1% Kyro EOB (12) in a buffer containing 50 mM sodium fluoride. Enzyme activity was determined essentially as described (13). Beta galactosidase activity was measured using a chemiluminescence assay (Galactolight reporter kit) obtained from Tropix (Bedford, MA). Values were expressed as relative light units per mg protein in the cell extract.

TABLE 3. Effect of actinomycin D on the stimulation of HMG-CoA reductase activity by amphiphiles

Agent	Actinomycin D	Relative HMG-CoA Reductase Activity
None	-	1.0
None	+	0.5
78 µM Imipramine	-	46
78 µM Imipramine	+	10.5
78 µM Chloroquine	-	16.9
78 µM Chloroquine	+	5.3
20 µM Trifluoperazine	-	50
20 µM Trifluoperazine	+	5.0

Human fibroblasts were given fresh growth medium containing 10% fetal bovine serum plus agents and either actinomycin D (2 µg/ml) in ethanol or ethanol alone (< 1% final). The cells were preincubated for 16 h at 37°C prior to extraction and the activity of HMG-CoA reductase was determined. The values, expressed per mg protein and then normalized to the control, are means of duplicates that agreed to within 5%. In the absence of agents, the value was 100 dpm/h per 100 µg protein.

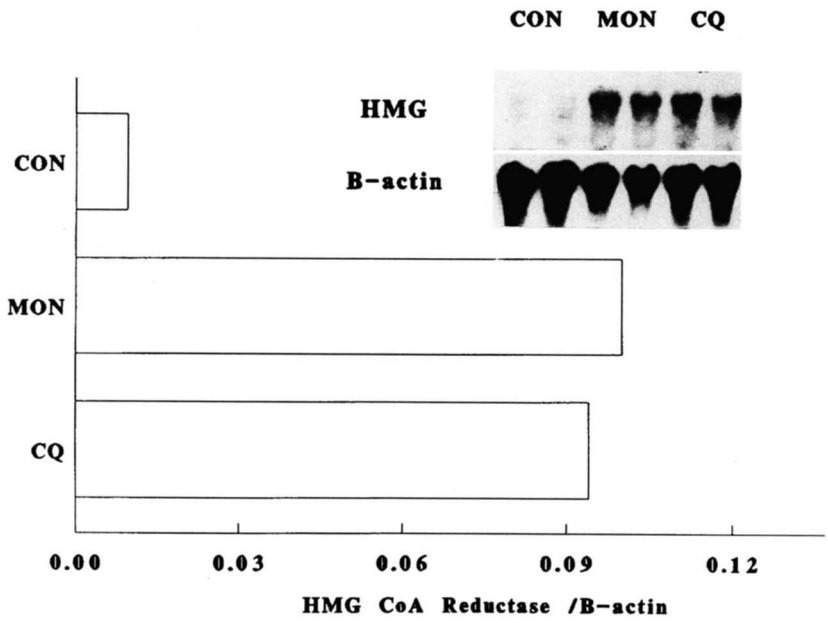

Fig. 3. The effect of chloroquine and monensin on HMG-CoA reductase mRNA abundance. Subconfluent flasks of human foreskin fibroblasts were given fresh growth medium containing 10% fetal bovine serum and 0.4 μM monensin (MON), 77 μM chloroquine (CQ), or ethanol (CON). The same amount of ethanol (0.04%) was present in all samples. After 17 h incubation, the cells were harvested for Northern blot analysis as described in Experimental Procedures. Ratios of signals for HMG-CoA reductase and beta-actin, determined by laser densitometry, are plotted.

RESULTS AND DISCUSSION

LDL receptor gene expression

Mevinolin, a potent inhibitor of HMG-CoA reductase, has been shown to increase LDL receptor transcription in liver, presumably by depriving the cells of endogenous cholesterol (14). The expression of the LDL receptor/CAT transgene responded similarly to mevinolin in NIH 3T3 cells. That is, cellular CAT activity increased by 1.8- and 2.7-fold, respectively, after 6 and 24 h of mevinolin treatment (**Table 1**).

Next, we tested the effect on CAT activity of the amphiphiles previously found to inhibit cholesterol esterification and stimulate its biosynthesis (5). As shown in **Table 2,** these agents increased CAT activity at the concentrations used before (5). This stimulation ranged from 1.7- to 9.2-fold after 18 h of treatment. Overnight treatment with trifluoperazine led to significant changes in cell morphology; therefore, incubation with this agent was only for 7 h, at which time CAT expression was stimulated 3.6-fold (Table 2). The varied degree of stimulation of CAT gene expression by the different amphiphiles was reminiscent of that found for HMG-CoA reductase activity under similar conditions (5).

Our hypothesis also predicts that 25-hydroxycholesterol should oppose the effect of the amphiphiles on LDL receptor gene transcription. The oxysterol had little effect on LDL receptor gene expression in control cells (**Fig. 1**). After incubation in 1% plasma-derived serum, cells became quiescent and the resultant low expression of LDL receptor gene-CAT constructs was not further suppressed by oxysterols (T. Mazzone, unpublished data). As expected, CAT gene expression was stimulated by progesterone and chloroquine. However, this stimulation was completely reversed by 25-hydroxycholesterol (Fig. 1).

HMG-CoA-reductase activity

The previous observation that amphiphiles stimulated this enzyme activity (5) did not distinguish between transcriptional and post-transcriptional mechanisms. The data reported above suggest that the transcription of genes regulating sterol homeostasis can be modulated by the amphiphiles. However, HMG-CoA reductase also is modulated at a post-transcriptional step by sterols (3). We now have dissected the relative contributions of these two kinds of regulation using several approaches. In the first, we examined the effect of the RNA polymerase inhibitor, actinomycin D, on the stimulation of HMG-CoA reductase activity by amphiphiles.

Fibroblasts were incubated for 15 h with or without the hydrophobic amine imipramine, plus actinomycin

TABLE 4. Effect of amphiphiles and cholesterol depletion on degradation of HMGal

Agent	Incubation Time	Beta-Galactosidase Activity
	h	*10^{-6} × relative light units/mg protein*
None	17	7.1 ± 0.4
2 μM Monensin	17	16.7 ± 2
80 μM Chloroquine	17	13.8 ± 1
LPDS	23	22.1 ± 0.2

CHO-HMGal cells were grown in 6-well plates in MEM supplemented with 5% FBS plus 250 μg/ml geneticin. Forty-eight hours prior to assay, the medium was replaced with MEM plus 5% FBS. Twenty-three hours prior to assay, the medium was replaced with fresh medium of the same composition or MEM plus 5% lipoprotein-deficient serum (LPDS). After 6 h, the agents or solvent alone were added and the cells were incubated for 17 h at 37°C. Beta-galactosidase specific activity was determined in triplicate.

TABLE 5. Effect of estradiol on HMG-CoA reductase activity and on esterification of plasma membrane cholesterol

Expt	Addition	[^{3}H]Cholesterol Esterification	HMG-CoA Reductase Activity
		%/h	*dpm/h/100 μg protein*
1	Ethanol	0.18	
	Estradiol	0.08	
2	Ethanol		365
	Estradiol		6530

Esterification: Cultured fibroblasts were labeled in suspension with [^{3}H]cholesterol (5). Aliquots were incubated for 30 min at 37°C with 37 μM beta-estradiol in ethanol, or the same amount of ethanol (< 1%) as a solvent control. The incorporation of label into cholesteryl esters was measured and expressed as a percent of total ^{3}H incorporated per h (5). HMG-CoA reductase activity: Duplicate flasks of fibroblasts were given fresh growth medium containing 10% fetal bovine serum plus 37 μM estradiol in ethanol or the same amount of ethanol (< 1%) as a solvent control. The cells were preincubated for 15 h at 37°C prior to extraction for determination of HMG-CoA reductase activity. Values are means of duplicate determinations that agreed to within 5%. Each data set is representative of three experiments that gave similar results.

D and then HMG-CoA-reductase activity was assayed (**Fig. 2**). As before (5), 80 μM imipramine greatly stimulated enzyme activity. The presence of 6 μg/ml actinomycin D inhibited HMG-CoA-reductase activity approximately 3-fold both in the control cells (from 1.0 to 0.3), and in those treated with imipramine (from 10.7 to 3.4). Nonetheless, at concentrations of actinomycin D up to 6 μg/ml, the highest tested, the enzyme activity in the imipramine-treated cells was significantly and consistently higher than in the control cells (Fig. 2). Actinomycin in this concentration range had no effect on cholesterol esterification (data not shown).

We selected two additional test compounds from the menu of amphiphiles previously shown to stimulate HMG-CoA reductase activity (5): chloroquine and trifluoperazine. Both had the anticipated effect on this enzyme activity (**Table 3**). In each case, the stimulation was only partially reversed by actinomycin D (Table 3). Judged by the incorporation of [^{3}H]uridine into mRNA, we found that under the conditions of the experiment shown in Table 3, 2 μg/ml of actinomycin D inhibited RNA synthesis by greater than 97% (data not shown). These results, therefore, provided evidence that regulation of HMG-CoA reductase activity by these amphiphiles operated at transcriptional (i.e., actinomycin sensitive) and post-transcriptional (i.e., actinomycin insensitive) loci.

This interpretation was tested further in experiments in which we measured directly the effect of two of these agents on HMG-CoA reductase mRNA abundance. As shown in **Fig. 3,** incubation with 0.4 μM monensin or 77 μM chloroquine for 17 h resulted in a 10-fold increase in HMG-CoA reductase mRNA levels in fibroblasts.

To investigate the effect of the amphiphiles on post-transcriptional regulation of HMG-CoA reductase, we used CHO cells transfected with a chimeric gene, HMGal, composed of the membrane domain of HMG-CoA reductase fused to beta-galactosidase (8). The constitutive transcription of the fused gene is driven by the SV40 early promoter and is not affected by sterols. That the cells were responsive to sterols (15) was confirmed in serum-starved cells by showing a 55% reduction in beta-galactosidase activity upon incubation for 19 h with 15 μg/ml cholesterol plus 1.5 μg/ml 25-hydroxycholesterol (data not shown). Treatment of these cells with two of the sterol active amphipaths, monensin and chloroquine, significantly increased beta-galactosidase activity (**Table 4**). As a positive control, we incubated cells in parallel in medium lacking serum lipoproteins and found comparable stimulation of beta galactosidase activity (Table 4). These data suggest that monensin and chloroquine decrease the degradation of HMG-CoA reductase.

Estradiol

This compound was of interest because it has been shown to increase the expression of LDL receptors in the livers of rats by an unknown mechanism (16). We wondered whether it might affect cholesterol homeostasis in the same fashion as other amphiphiles such as progesterone. Therefore we tested beta estradiol in human fibroblasts exactly as described for progesterone in reference 5. Estradiol at a concentration of 37 μM reduced the esterification of plasma membrane cholesterol approximately 2-fold within minutes, and stimulated HMG-CoA reductase activity 18-fold after 15 h of preincubation (**Table 5**). We therefore add estradiol to the list developed in reference 5.

Concluding comments

We have shown that all the various amphiphiles previously found to inhibit plasma membrane cholesterol esterification and increase HMG-CoA reductase activity (5) also stimulate the expression of the LDL receptor gene. The stimulation of expression of the LDL/CAT construct was less than that seen previously for cholesterol biosynthesis and HMG-CoA reductase activity. However, as we now have shown, the activity of the latter apparently reflected not only gene expression but the turnover of the enzyme. In that regard, the LDL/CAT assay provides unambiguous evidence that the amphiphiles act at the transcriptional level.

The amphiphiles used have distinctly different molecular structures as well as diverse physiological and pharmacological effects on cells. While conceivable, it

seems implausible that all of these agents impact in the same way on the modes of regulation of cell cholesterol studied here. A more parsimonious hypothesis is that the amphiphiles, their other diverse effects notwithstanding, may act at a single site through their shared ability to intercalate into membranes. We have suggested (5) that their effect might be to inhibit the movement of plasma membrane cholesterol to the rough endoplasmic reticulum which, in the present instance, would lead to the observed stimulation of expression of LDL receptors through the sterol regulatory element binding protein (3). The stabilization of HMG-CoA reductase by treatment with the amphiphiles supports the hypothesis that their action mimics cholesterol depletion.

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REFERENCES

1. Goldstein, J. L., and M. S. Brown. 1990. Regulation of the mevalonate pathway. *Nature.* **343:** 425–430.
2. Briggs, M. R., C. Yokoyama, X. Wang, M. S. Brown, and J. L. Goldstein. 1993. Nuclear protein that binds sterol regulatory element of LDL receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. *J. Biol. Chem.* **268:** 14490–14496.
3. Wang, X., R. Sato, M. S. Brown, X. Hua, and J. L. Goldstein. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell.* **77:** 53–62.
4. Lange, Y. 1992. Tracking cholesterol with cholesterol oxidase. *J. Lipid Res.* **33:** 315–321.
5. Lange, Y., and T. L. Steck. 1994. Cholesterol homeostasis: modulation by amphiphiles. *J. Biol. Chem.* **269:** 29371–29374.
6. Sudhof, T. C., D. R. Van der Westhuyzen, J. L. Goldstein, M. S. Brown, and D. W. Russell. 1987. Three regulated repeats and a TATA-like sequence are required for regulated expression of the human low density lipoprotein receptor gene. *J. Biol. Chem.* **262:** 10773–10779.
7. Lange, Y., and H. J. G. Matthies. 1984. Transfer of cholesterol from its site of synthesis to the plasma membrane. *J. Biol. Chem.* **259:** 14624–14630.
8. Skalnik, D. G., H. Narita, C. Kent, and R. D. Simoni. 1988. The membrane domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase confers endoplasmic reticulum localization and sterol-regulated degradation onto beta-galactosidase. *J. Biol. Chem.* **263:** 6836–6841.
9. Mazzone, T., K. Basheeruddin, L. Ping, and C. Schick. 1990. Relation of growth- and sterol-related regulatory pathways for low density lipoprotein receptor gene expression. *J. Biol. Chem.* **265:** 5145–5149.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
11. Duan, H., Z. Li, and T. Mazzone. 1995. Tumor necrosis factor-alpha modulates monocyte/macrophage apoprotein E gene expression. *J. Clin. Invest.* **96:** 915–922.
12. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1973. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. *Proc. Natl. Acad. Sci. USA.* **70:** 2162–2166.
13. Drevon, C. A., D. B. Weinstein, and D. Steinberg. 1980. Regulation of cholesterol esterification and biosynthesis in monolayer cultures of normal adult rat hepatocytes. *J. Biol. Chem.* **255:** 9128–9137.
14. Ma, P. T. S., G. Gil, T. C. Sudhof, D. W. Bilheimer, J. L. Goldstein, and M. S. Brown. 1986. Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. *Proc. Soc. Acad. Sci. USA.* **83:** 8370–8374.
15. Roitelman, J., and R. D. Simoni. 1992. Distinct sterol and nonsterol signals for the regulated degradation of 3-hydroxy-3-methylglutaryl-CoA reductase. *J. Biol. Chem.* **267:** 25264–25273.
16. Kovanen, P. T., M. S. Brown, and J. L. Goldstein. 1979. Increased binding of low density lipoprotein to liver membranes from rats treated with 17 beta-ethinyl estradiol. *J. Biol. Chem.* **254:** 11367–11373.