Regulation of Low Density Lipoprotein Receptor Gene Expression in Human Lymphocytes*

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Cholesterol homeostasis is maintained by coordinate regulation of endogenous synthesis and exogenous uptake of lipoprotein cholesterol by low density lipoprotein (LDL) receptors. In the lymphocyte, limiting the availability of exogenous cholesterol is known to increase the rate of endogenous sterol biosynthesis. However, the effect of cholesterol deprivation on the expression and regulation of the LDL receptor gene has not been delineated in lymphocytes. Here, LDL receptor mRNA was detected in freshly isolated human peripheral mononuclear cells. LDL receptor mRNA levels increased by 3-fold during a one-h in vitro culture in lipoprotein-deficient medium and by 6-fold during a 2-h incubation. Actinomycin D blocked the synthesis of LDL receptor mRNA in these cultures. However, neither cycloheximide nor LDL or oxygenated sterols suppressed the increase in LDL receptor mRNA levels observed after a 2-h incubation. The increase in LDL receptor mRNA was maintained for 24 h of culture in the absence of LDL. Ongoing gene transcription and not mRNA stabilization accounted for this expression. Inhibition of protein synthesis with cycloheximide completely prevented the sustained increase in LDL receptor mRNA levels measured after 24 h. Low concentrations of LDL (5 μg of cholesterol/ml) and oxygenated sterols also suppressed the level of LDL receptor mRNA measured after a 24-h incubation. These data show that the initial upregulation of LDL receptor gene expression is independent of protein synthesis and not suppressed by either LDL or oxygenated sterols. In contrast, the continued transcription necessary for the maintenance of steady-state levels of LDL receptor mRNA requires synthesis of new protein and is regulated by LDL and oxygenated sterols.

Cholesterol is required for cellular membrane synthesis and may be obtained by cells via endogenous synthesis or by exogenous uptake of cholesterol in lipoproteins (1–3). Cholesterol is obtained from exogenous sources through receptor-mediated endocytosis and lysosomal hydrolysis of plasma lipoproteins containing apolipoprotein B or E (1–3). When endogenous lipoproteins are not present, there is a compensatory increase in endogenous sterol synthesis and a coordinate increase in low density lipoprotein (LDL) receptor activity in cultured cells (1–3). The increase in LDL receptor activity results from an increase in the synthesis of new LDL receptors (4). Conversely, LDL or exogenously added sterols have been shown to decrease LDL receptor activity by suppressing synthesis of receptors (4).

More recent studies using cultured hamster cells transfected with human LDL receptor genomic sequences have shown that regulation of receptor expression is mediated at the level of transcription by sequences in the 5'-flanking region of the gene (5–7). These studies revealed that three imperfect direct repeats of 16 base pairs and a TATA-like sequence were necessary for LDL receptor gene transcription. Of the repeat sequences, repeats 1 and 3 have been shown to interact with the general positive transcription factor Sp1, whereas repeat 2 is thought to interact in a sterol-dependent manner with another regulatory protein that influences the action of Sp1 (7). Together, these cis-acting transcriptional elements appear to underlie the negative feedback regulation of LDL receptor gene expression by sterols.

Regulation of LDL receptor activity in man cannot be determined directly in vivo. Moreover, studies examining transcriptional regulation of LDL receptor gene expression by sterols in human cells have thus far been limited. LDL receptor mRNA has been detected in human A-431 epidermoid carcinoma cells and fibroblasts, and its down-regulation in these cells by sterols, the oxygenated sterol 25-hydroxycholesterol together with cholesterol, has been documented (8, 9). However, additional details concerning the regulation of LDL receptor gene expression in human cells are lacking. The studies reported here were undertaken to examine the control of LDL receptor mRNA levels in intact human cells. In previous studies, human peripheral blood mononuclear cells (PBM) have been shown to be a useful model system to examine sterol metabolism and LDL receptor function (3, 10–13). The current experiments utilized freshly isolated PBM to assess regulation of LDL receptor gene expression. The data demonstrate that increases in LDL receptor mRNA occur rapidly upon in vitro culture of cells. Although initial increases in LDL receptor mRNA do not require protein synthesis and are not regulated by either LDL or oxygenated sterols, subsequent maintenance of increased LDL receptor mRNA levels involves the action of newly synthesized protein and is subject to regulation of LDL and oxygenated sterols. These results provide a model to begin to dissect the complex nature of the regulation of LDL receptor gene expression in human cells.

* The abbreviations used are: LDL, low density lipoprotein; PBM, peripheral blood mononuclear cells.

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**Materials and Methods**

Cell Preparation and Culture—PBM were isolated from anticoagulated venous blood obtained from normal adults as described previously (3). For some experiments, PBM were separated into T cell-depleted populations (<50% rosette-positive) by rosetting with neuraminidase-treated sheep red blood cells and passing rosette-positive cells over a nylon wool column as detailed (3, 10). Cells were cultured in RPMI 1640 medium (Inland Laboratories, Austin, TX) containing L-glutamine (0.5 mg/ml), gentamicin (10 µg/ml), and penicillin (200 units/ml), and supplemented with 1% lipoprotein-poor plasma (density > 1.230 g/ml) prepared as described previously (10, 13). Where indicated, cultures were supplemented with 25-hydroxycholesterol (Steraloids, Inc., Wilton, NH), the sodium salt of mevalonate, prepared as described previously (3) or human LDL (density = 1.020-1.050 g/ml) isolated as described (3). Incubations were carried out in sterile tissue culture flasks for RNA isolation with 1–2 × 10^6 cells/ml initially cultured. For assays of rates of sterol synthesis, cells were incubated in 16 × 150-mm glass tubes with 5 × 10^6 cells/ml. In some experiments, the protein synthesis inhibitor cycloheximide or the RNA synthesis inhibitor actinomycin D (both purchased from Sigma) were added to the cultures.

Measurement of mRNA by Nuclease Protection—Total RNA was isolated from PBM in a solution of 5 M guanidinium thiocyanate (Fluka Chemical Corp., Ronkonkoma, NY), 30 mM sodium citrate, 0.5 M NaCl, and 100 mM DTT (dithiothreitol) followed by centrifugation through a 5 M cesium chloride (Bethesda Research Laboratories) cushion (14). Single-stranded probes for human β-actin and LDL receptor were prepared as follows. The β-actin clone pHFA-1 containing a cDNA insert for human fibroblast cytoplasmic β-actin (15) was kindly provided by Dr. P. Gunning and Dr. L. Kedes (St. Louis University School of Medicine, St. Louis, MO). A 382-nucleotide Smal-MspI fragment from the cDNA region encompassing nucleotides 124–505 (16), was subcloned into the Smal site of the M13mp19 vector (Pharmacia LKB Biotechnology Inc.) as described (17) after blunt ending the MspI site with the Klenow fragment of E. coli DNA polymerase (Boehringer Mannheim), and the resulting "P-labeled probe fragment of Escherichia coli DNA polymerase (Boehringer Mannheim) (14). A 264-nucleotide PstI fragment from the cDNA plasmid pLDLR2 (9) encoding parts of exons 8 and 9 of the human LDL receptor (14) was subcloned into the PstI site of the M13mp19 vector. Single-stranded "P-labeled probes were synthesized with an oligonucleotide primer in the presence of 0.5 µM (α"P)TTP (~3000 Ci/mmol, ICN Radiochemicals) and excess unlabeled dCTP (0.5 µM for LDL receptor and 250 µM for β-actin probes); 0.1 mM dATP, dTTP and dGTP; and the Klenow fragment of E. coli DNA polymerase as detailed (19). The extended product was digested with HindIII (Boehringer-Mannheim), and the resulting "P-labeled probe was purified by 7 M urea, 6% polyacrylamide gel electrophoresis, electroelution, and precipitation. The sizes of the probes (matching M13 sequences) were: β-actin = 457 nucleotides, and LDL receptor = 337 nucleotides.

Total RNA (10 µg) was hybridized with both "P-labeled probes simultaneously at 48 °C overnight as described (20), and then digested with 5 units of mung bean nuclease (Bethesda Research Laboratories) in modified buffer (50 mM sodium chloride, 10 mM sodium acetate, pH 4.6, 1 mM zinc chloride, 1 mM β-mercaptoethanol, and 0.001% Triton X-100). After precipitation with salmon sperm DNA carrier, samples were analyzed on 7 M urea, 6% polyacrylamide gels with single-stranded "P-labeled MspI fragments of pBR322 (New England Biolabs) as size standards.

After electrophoresis, gels were fixed and dried before being exposed to Kodak XAR-5 film for 12–24 h at room temperature with intensifying screens. By varying the specific activity of ["P]dCTP, a similar intensity of "P-labeled bands corresponding to mRNAs having vastly different abundances was achieved. Following exposure, radiolabeled bands were identified, excised, and ["P]cDNA content was quantified by liquid scintillation spectrometry. The ["P]cDNA content of identically sized excised bands from samples containing no RNA (<50 cpm) was used as a measurement of nonspecific background and was subtracted from all results. The proportion of ["P]labeled cDNA protected from endonuclease digestion by hybridization with mRNA was 0.1–5% for LDL receptor and 10–30% for β-actin. The proportion incorporated into each probe varied between different experiments. Consequently, the absolute amount of protected ["P]cDNA cannot be compared between experiments. However, comparisons within an experiment using the same ["P]cDNA probe can be used to determine changes in relative abundance of mRNA. The relative content of LDL receptor mRNA under different conditions, within each individual experiment was calculated by normalizing for the amount of ["P]cDNA incorporated into the β-actin band of the same sample. The relative abundance of LDL receptor mRNA in each sample was determined by comparing the relative content of LDL receptor mRNA to that observed in PBM incubated for 2–24 h in lipoprotein-deficient medium.

Measurement of Rates of Sterol Synthesis—Sterol synthesis was determined by measuring the rate of incorporation of [1-14C]acetate into digitonin-precipitable sterols using previously described techniques (3). Briefly, freshly isolated cells were incubated for 2 or 4 h at 37 °C with 1 µCi [1-14C]acetate, after which they were saponified, and the sterols were extracted and precipitated with digitonin. Data are expressed as the rate of sterol synthesis (picomoles of acetate incorporated into digitonin-precipitable sterols/h/10^6 cells).

**Results**

Detection of LDL Receptor mRNA in Circulating Human PBM—Small amounts of LDL receptor mRNA were detected in total RNA obtained from freshly isolated PBM (Fig. 1). When the mixed cell population was separated, LDL receptor mRNA was detected in both T cell-depleted and T cell-enriched populations. The levels of both LDL receptor mRNA and actin mRNA were 2-fold higher in RNA isolated from the T cell-depleted preparations when compared to the T cell-enriched preparations. Thus, circulating mononuclear cells, both T lymphocytes and T cell-depleted populations, contain small but detectable amounts of mRNA for the LDL receptor.

Induction of LDL Receptor mRNA by Culture in Lipoprotein-deficient Medium—The capacity of PBM to increase LDL receptor mRNA levels during in vitro culture in lipoprotein-deficient medium was examined next. As can be seen in Fig. 1, LDL receptor mRNA increased under these conditions. The relative amount of LDL receptor mRNA, when compared with β-actin mRNA, increased in both the T cell-enriched and T cell-depleted populations, although the degree of increase was much greater in the former. Similar results were obtained with PBM obtained from 20 different normal volunteers (data not shown).

The induction of LDL receptor mRNA expression occurred through an unknown mechanism, as shown in Fig. 1. Detection of LDL receptor mRNA in circulating human mononuclear cells. PBM (lanes 2 and 5) were prepared from one individual and paired samples of T cell-depleted (lanes 3 and 6) and T cell-enriched (lanes 4 and 7) populations were prepared from each of two different individuals (lanes 3 and 4 from one individual and lanes 6 and 7 from another individual). Total RNA was isolated immediately (length of incubation, Nil) or after incubation for 24 h in lipoprotein-deficient medium. RNA (Nil, lane 1; 10 µg, lanes 2–7) was hybridized with ["P]labeled probes as described under “Materials and Methods.” The RNA-DNA hybrids were digested with mung bean nuclelease, and the resistant products were separated by electrophoresis, electroelution, and precipitation. The sizes of the probes (matching M13 sequences) were: β-actin = 457 nucleotides, and LDL receptor = 337 nucleotides; LDL receptor protected band = 271 nucleotides.)
rapidly following in vitro incubation (Fig. 2, lanes 4 and 12). A 3-fold increase in LDL receptor mRNA was apparent after a 1-h incubation at 37 °C in lipoprotein-depleted medium, and by 2 h LDL receptor mRNA levels were 6-fold above those in freshly isolated cells (Fig. 2, lanes 4, 5, and 12). No increase in LDL receptor mRNA levels could be detected after a 30-min incubation at 37 °C, nor when the cells were incubated for 2 h at 4 °C (Fig. 2, lanes 3 and 11). Since there was no change in actin mRNA expression during a 2-h incubation, the difference in steady-state LDL receptor mRNA cannot be explained by a nonspecific increase in all mRNA during cell isolation and incubation. Moreover, there was no increase in mRNA levels for the transferrin receptor or for interleukin-2 receptor during this period of time (data not shown), indicating the levels of all mRNAs encoding receptors or activation antigens were not augmented. That de novo synthesis of RNA was required was indicated by the finding that actinomycin D completely blocked the increase in LDL receptor mRNA observed during the 2-h incubation and resulted in mRNA levels less than observed in freshly isolated cells (Fig. 2, lane 6). When added after the initial 2-h incubation, actinomycin D decreased LDL receptor mRNA levels by ~50% over the subsequent 2 h (Fig. 2, lane 13). In contrast, a protein synthesis inhibitor, cycloheximide, had no effect on the increased levels of mRNA induced by in vitro incubation for 2 h in two separate experiments using RNA isolated from PBMs obtained from different individuals (Fig. 2; experiment 1, lanes 5 and 6; experiment 2, lanes 8 and 9).

Exogenous sterols have been shown to regulate LDL receptor gene expression at the transcriptional level (8, 9). The effect of LDL on the induction of LDL receptor mRNA was therefore examined (Fig. 3). The presence of LDL did not inhibit the increase in LDL receptor mRNA observed after a 2-h incubation in vitro (lanes 2 and 3). Similar results were obtained in three additional experiments with both PBMs and T cell-enriched and T cell-depleted populations (data not shown). It should be noted that although mRNA levels for the LDL receptor were not altered by exogenous LDL during a 2-h incubation, LDL (50 µg of cholesterol/ml) suppressed endogenous sterol synthesis in freshly isolated PBMs by 28 ± 4% (mean ± S.E., n = 3) during a similar period of time (Table I). Mevalonate, the product of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme in endogenous sterol synthesis, also had no effect on the increase in LDL receptor mRNA observed after a 2-h incubation in lipoprotein-deficient medium (Fig. 3, lane 4). Whereas mevalonate did not alter LDL receptor mRNA levels, this concentration of mevalonate (10 mM) suppressed endogenous sterol synthesis by 79 ± 3% (n = 3) during a 2- or 4-h incubation in lipoprotein-deficient medium (Table I). Finally, the addition

![Fig. 2. Induction of LDL receptor mRNA by culture in lipoprotein-deficient medium. PBMs were prepared from three different individuals (lanes 1–7, 8, and 9, 10–13) and RNA isolated after varying lengths of incubation (Nil to 4 h) in lipoprotein-deficient medium at 37 °C (lanes 3–9, 12, and 13) or 4 °C (lane 11). In experiment 1 (lanes 1–7) and experiment 2 (lanes 8 and 9) cycloheximide (10 µg/ml, lanes 6 and 9) or actinomycin D (10 µg/ml, lane 7) was present during the 2-h incubation in lipoprotein-deficient medium. In experiment 3 (lanes 10–13), actinomycin D was added after an initial 2-h incubation and RNA isolated after a total 4-h incubation in lipoprotein-deficient medium (2-h incubation with actinomycin D). Total RNA (Nil, lanes 1 and 10; 10 µg, lanes 2–9, 11–13) was hybridized with 32P-labeled probes, hybrids were digested with mung bean nuclease and resistant products detected by autoradiography after electrophoresis. Actin and LDL receptor bands were identified by comparison to standards as described in legend to Fig. 1 and "Materials and Methods." The relative abundance of LDL receptor mRNA was calculated after quantification of 32P-labeled cDNA content by liquid scintillation spectroscopy as described under "Materials and Methods." nt, nucleotides.]
of the oxygenated sterol 25-hydroxycholesterol had no effect on LDL receptor mRNA levels in PBM incubated for 2 h in lipoprotein-deficient medium (lane 7) and had only a modest effect (21% inhibition) when cells were cultured for 6 h (data not shown). By contrast, this concentration of 25-hydroxycholesterol inhibited endogenous sterol synthesis by 50 ± 1% \((n = 3)\) after a 2-h incubation (Table I). These findings indicate that there is a very rapid increase in transcription of the LDL receptor gene following \textit{in vitro} incubation, and that this increase is independent of protein synthesis and not regulated by sterols.

**Maintenance of LDL Receptor mRNA Levels in Human PBM**—Levels of LDL receptor mRNA in PBM did not further increase when \textit{in vitro} incubations were prolonged to 24 h (Fig. 4). Although the absolute amount of LDL receptor mRNA did not change between 2 and 24 h, there was a dramatic difference in the effect of cycloheximide. During shorter incubations (2-4 h), cycloheximide had no effect on LDL receptor mRNA levels (Fig. 2). In contrast, when cells were incubated for 12 h or longer, cycloheximide markedly reduced LDL receptor mRNA levels (Fig. 4). In these cultures, cycloheximide had no consistent effect on levels of actin mRNA. The data indicate that protein synthesis is required for the maintenance of LDL receptor gene expression.

The maintenance of LDL receptor mRNA levels with prolonged incubation was dependent on continued transcription. Thus, the addition of actinomycin D for the last 2 h of a 24- or 26-h incubation decreased LDL receptor mRNA levels by ~50% (Fig. 5). The decrease was similar to that observed when actinomycin D was added for the last 2 h of a 4-h incubation in the absence of sterols (Fig. 2). Thus, the half-life of LDL receptor mRNA is approximately 2 h and is unchanged by the length of incubation.

The next series of experiments examined whether regulation of LDL receptor gene expression by sterols was observed in longer cultures. The ability of LDL to modulate expression of the LDL receptor gene was examined by incubating PBM from three different donors for 24 h in medium supplemented with various amounts of LDL. Low concentrations of LDL (5 \(\mu\)g of cholesterol/ml) decreased the amount of LDL receptor mRNA by 41 ± 10% \((\text{mean} ± \text{S.E., } n = 3)\) compared to that detected in the absence of LDL (Fig. 6, lanes 1-3). Higher concentrations of LDL (50 \(\mu\)g of cholesterol/ml) decreased the amount of LDL receptor mRNA by 57 ± 16% \((n = 3)\).

**Fig. 4. Maintenance of LDL receptor mRNA levels: requirement for protein synthesis.** PBM were prepared from five different individuals (experiments 1-5) and RNA isolated after varying lengths of incubation (Nil to 24 h) in lipoprotein-deficient medium at 37 °C. In experiments 2 (lanes 4 and 5), 3 (lanes 6 and 7), and 4 (lanes 8 and 9), cycloheximide (10 \(\mu\)g/ml) was present during the incubation (lanes 5, 7, and 9). In experiment 5 (lanes 10 and 11), cycloheximide was added after 18 h (lane 11) and the incubation was continued for an additional 6 h. RNA (10 \(\mu\)g) was hybridized with \(\text{\textsuperscript{32P}}\)-labeled probes and actin and LDL receptor bands resistant to mung bean nuclease digestion were identified and relative abundance calculated as described in legend to Fig. 2 and "Materials and Methods." Hybridizations for each experiment (1-5) were carried out on different days with probes having different absolute counts/min for each experiment, precluding direct comparison of amounts of LDL receptor mRNA between experiments. nt, nucleotides.

**Fig. 5. Suppression of LDL receptor mRNA by actinomycin D.** PBM from two different individuals were prepared and RNA isolated after 24 or 26 h incubation in lipoprotein-deficient medium at 37 °C. In experiment 1 (lanes 1 and 2), actinomycin D (10 \(\mu\)g/ml) was added after 22 h (lane 2) and RNA was isolated from both samples after 24 h incubation (2 h with actinomycin D). In experiment 2 (lanes 3 and 4), RNA was isolated after 24 h (lane 3) or actinomycin D was added after 24 h (lane 4) and RNA was isolated after 26 h incubation (2 h with actinomycin D). RNA (10 \(\mu\)g) was hybridized with \(\text{\textsuperscript{32P}}\)-labeled probes and actin and LDL receptor bands resistant to mung bean nuclease digestion were identified and quantified as described in legend to Fig. 2 and "Materials and Methods."

**Fig. 6. LDL regulation of LDL receptor mRNA in PBM.** PBM (one individual) and T cell-depleted and T cell-enriched populations (both from one individual) were prepared and RNA isolated after a 24-h incubation in lipoprotein-deficient medium. LDL (5 \(\mu\)g of cholesterol/ml, lanes 2, 5, and 8; 50 \(\mu\)g of cholesterol/ml, lanes 3, 6, and 9) was present during the 24-h incubation as indicated. RNA (10 \(\mu\)g) was hybridized with \(\text{\textsuperscript{32P}}\)-labeled probes, and actin and LDL receptor bands resistant to mung bean nuclease digestion were identified and total abundance calculated as described in legend to Fig. 2 and "Materials and Methods." nt, nucleotides.
cell-enriched population (75% inhibition) versus the T cell-depleted populations (28% inhibition). The results in the two populations were more equivalent at the higher LDL levels (Fig. 6, lanes 6 and 9). Thus, in both T lymphocytes and T cell-depleted populations incubated for 24 h in lipoprotein-deficient medium, levels of LDL receptor mRNA were effectively down-regulated by LDL concentrations corresponding to <5% of the circulating plasma concentration.

LDL receptor mRNA levels in PBM incubated in vitro for 24 h were also modestly suppressed by the addition of mevalonate, and markedly inhibited by 25-hydroxycholesterol (Fig. 7). Inhibition by mevalonate in cells isolated from four different individuals was 22 ± 7%, whereas in two experiments 25-hydroxycholesterol decreased LDL receptor mRNA levels by 78 and 82%. The capacity of LDL and 25-hydroxycholesterol to regulate LDL receptor mRNA levels could also be detected in cells incubated for 12 h in lipoprotein-deficient medium (Fig. 7), although as noted above, exogenous sterols did not regulate LDL receptor mRNA in cells cultured for less than 12 h. These results demonstrate that maintenance of LDL receptor mRNA levels in PBM is regulated by LDL, oxygenated sterols, and other metabolites that modulate cellular sterol metabolism.

**DISCUSSION**

The current studies demonstrate that LDL receptor gene expression in PBM responds to changes in cholesterol homeostasis in two distinct ways. Upon *in vitro*, culture, there was an increase in steady-state mRNA levels resulting from increased gene transcription that was detectable after 1 h. The increase in LDL receptor mRNA levels was independent of protein synthesis and not regulated by exogenous sterols. In contrast, continued LDL receptor gene expression detected after longer incubations in the absence of sterols was dependent on protein synthesis as well as continued transcription and was regulated by exogenous sterols. These results suggest that the induction of LDL receptor gene expression is rapid, whereas the development of regulation by sterols is considerably slower.

Whether the changes in LDL receptor gene expression are reflected in changes in LDL receptor protein has not been addressed in the current studies. Previous investigators have reported that the number of LDL receptors on human lymphocytes, the major cell population in PBM, is very low (21). Therefore, an accurate correlation between LDL receptor gene expression and LDL receptor protein number may not be possible. In addition, cultured human fibroblasts, the half-life of the receptor protein is ∼24 h (22). Consequently, rapid changes in LDL receptor gene expression may not be reflected by easily measurable changes in receptor number until additional prolonged incubation has occurred. Furthermore, translational modulation of LDL receptors may additionally complicate the correlation of gene expression with receptor number. The difference in half-life of mRNA (2 h) and protein (24 h) may also contribute to differences between the results of the present experiments and those of other investigators measuring LDL receptor activity of human peripheral blood cells (21, 23, 24). Lymphocytes have been reported to gradually acquire increased numbers of LDL receptors during a 72-h incubation in lipoprotein-deficient medium (21). In contrast, LDL receptor mRNA levels did not increase further between 2 and 24 h. One possible explanation for the length of time required to reach steady-state levels of LDL receptor binding activity is that protein synthesis and expression of membrane LDL receptors may be relatively slow when compared with gene transcription.

In the studies reported here, we were unable to detect an increase in the level of LDL receptor mRNA when PBM were maintained for 2 h at 4 °C. Furthermore, there was no increase in LDL receptor mRNA during an initial 30-min incubation at 37 °C. The increase in mRNA levels at 2 h for the LDL receptor could not be ascribed to a generalized increase in mRNA levels for cellular receptors since mRNA for the transferrin and interleukin-2 receptors were not increased. These findings indicate that induction of LDL receptor gene expression in PBM during a 2-h incubation at 37 °C is an active and specific process. When the transcription inhibitor actinomycin D was added during the 2-h incubation, there was no increase in LDL receptor mRNA levels, indicating that stabilization of mRNA alone cannot account for the finding. In addition, actinomycin D decreased the level of LDL receptor mRNA by ∼50% in 2 h when added after the initial 2-h incubation, suggesting that the half-life of LDL receptor mRNA was approximately 2 h. The half-life of the LDL receptor mRNA appeared to remain constant over the 24-h incubation period used in these studies.

Regulation of LDL receptor gene expression has been shown to depend on the presence of three imperfect direct repeats in the 5'-flanking region of the gene (5–7). Two of these, referred to as repeats 1 and 3, are able to bind the positive protein transcription factor Sp1, whereas repeat 2 is thought to bind a repressor protein in a sterol-dependent manner (7). Modulation of the synthesis or activity of these protein transcription factors is one possible mechanism of gene regulation of LDL receptor activity. The results presented here indicate that de novo protein synthesis is not required for the initial increase in LDL receptor mRNA levels, suggesting that transcription factors required for induction are present within the isolated PBM. We do not at present know whether these factors are activated by a post-transcriptional mechanism as has been described in other systems (25). Alternatively, the derepression of LDL receptor gene expression upon culture of PBM may be caused by the loss of a short-lived repeat 2-binding repressor protein. This protein may be irreversibly inactivated or degraded during the isolation of the PBM. If de novo synthesis or activation of the
sterol repressor protein was a slow process, the failure of sterols to repress gene expression during the initial 2-h incubation (Fig. 3) would be explained.

The explanation that the concentration of sterols used was insufficient to lead to sterol-mediated repression is unlikely since identical concentrations were effective at suppressing LDL receptor mRNA levels with longer incubation. Furthermore, these same concentrations of sterols effectively inhibited endogenous sterol biosynthesis when present for a 2-h incubation. Similarly, a lack of sensitivity of the assay system is also an unlikely explanation for the unexpected inability of sterols to regulate in short term experiments. An increase in LDL receptor mRNA levels was readily detected after 1 h, although not apparent after 30 min. These latter data indicate that rapid changes in LDL receptor mRNA levels occur and can be detected by the current assay. Sterols were also unable to modulate LDL receptor gene expression when present for the last 2 h of a 24-h incubation, whereas sterol synthetic rates were down-regulated by either LDL or 25-hydroxycholesterol during the same time period, as with freshly isolated cells (data not shown). This observation suggests that incubation for longer than 2 h is necessary for negative feedback regulation regardless of the length of in vitro culture before RNA isolation. Thus, regulation of LDL receptor gene expression by exogenous sterols was similar after in vitro culture for 2 or 24 h, with no effect of a short (2 h) exposure to LDL or 25-hydroxycholesterol but modulation after a longer incubation. These findings suggest that the delay in the appearance of negative feedback regulation of LDL receptor gene expression by sterols is intrinsic and related to the mechanism of suppression rather than an artifact of the cell isolation or culture procedure.

The response to in vitro incubation was initially independent of protein synthesis but maintenance of increased levels of LDL receptor mRNA required de novo protein synthesis (Fig. 4). Thus, cycloheximide did not affect LDL receptor gene expression during a 4-h incubation, but decreased LDL receptor mRNA levels by 80% when added for the final 6 h of a 24-h incubation in lipoprotein-deficient medium. The effect of cycloheximide is very rapid, with complete suppression of protein synthesis occurring in minutes (data not shown), and, therefore, the lack of effect of cycloheximide on LDL receptor gene expression after 2-4 h is not caused by a delay in the onset of action of this inhibitor. These results suggest that incubation in the presence of cycloheximide for long periods of time (≥6 h) may deplete the PBM of a positive transcription factor required for LDL receptor gene expression.

The observations that neither cycloheximide nor sterols were able to regulate early increases in LDL receptor mRNA, whereas both suppressed LDL receptor mRNA levels with longer incubation, suggest the possibility that the mechanisms by which each modulates gene expression may be similar. Thus, sterols may regulate LDL receptor gene expression by a protein synthesis-dependent mechanism. Whether cycloheximide and sterols modulate LDL receptor mRNA levels by acting on the same protein is at present uncertain. Preliminary experiments indicate that, in the presence of cycloheximide, sterols do not further decrease LDL receptor mRNA levels (data not shown), suggesting that one mechanism whereby sterols may regulate LDL receptor gene transcription could be by inhibiting synthesis of a necessary protein. Thus, sterols may decrease the abundance of a positive transcription factor binding to repeat 2 and thereby suppress transcription rates. Another possibility is that sterols may decrease the synthesis of a specific protein that normally prevents the binding of a nonspecific negative transcription factor to repeat 2. Alternatively, sterol-mediated negative feedback regulation may depend on the synthesis of a repressor protein, as suggested above. Under these latter conditions, cycloheximide could block sterol-mediated regulation by preventing synthesis of the repressor protein. Whichever explanation is correct, the data suggest the possibility that sterols suppress LDL receptor gene expression by inhibiting the synthesis of a regulatory protein. The transcription factor Sp1 has been shown to bind to positive regulatory sequences (repeats 1 and 3) in the 5′-flanking region of the receptor gene (7). A decrease in the activity or concentration of Sp1 following inhibition of protein synthesis may also account for our findings. The recent cloning of a partial cDNA for the human Sp1 (26) should provide the tools necessary for a direct test of this hypothesis.

In summary, in human peripheral blood mononuclear cells, LDL receptor gene expression induced by in vitro culture is initially independent of protein synthesis and unregulated by exogenous sterols. Continued LDL receptor gene expression, however, requires ongoing protein synthesis and is subject to negative feedback regulation by sterols. The data suggest that complex interactions between protein transcription control factors and sterols regulate LDL receptor gene expression and that intrinsic properties of the proteins may be important in the kinetics of gene regulation.

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