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Lipoprotein Lipase Gene Expression in THP-1 Cells†

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ABSTRACT: Lipoprotein lipase (LPL) mRNA levels are under the control of signals that activate phospholipase C, resulting in activation of protein kinase C (PKC) and mobilization of intracellular Ca^{2+} in the human monocytic leukemia cell line THP-1. Induction of LPL in THP-1 cells appears to be mediated by PKC since it was affected by both phorbol 12-myristate 13-acetate (PMA) and a diacylglycerol analogue. This induction was blocked by the specific PKC inhibitor H-7. Although Ca^{2+} mobilization by the ionophore A23187 also induced LPL mRNA, the mechanism is most likely independent of activation of the Ca^{2+}/calmodulin protein kinase. Depletion of cells of PKC made them refractory to induction by A23187, suggesting that Ca^{2+} mobilization acts by activating PKC. Addition of cycloheximide (CHX) to undifferentiated THP-1 cells resulted in a transient increase in steady-state mRNA levels (3-fold). Sustained superinduction of LPL mRNA occurred when PMA and CHX were added simultaneously. These results suggest that the level of LPL mRNA is regulated either by a labile regulatory protein, which represses transcription of the LPL gene, or by a protein affecting mRNA stability.

Lipoprotein lipase (LPL)[1] is a glycoprotein that, after secretion, becomes bound to glycosaminoglycans on the luminal surface of capillary endothelial cells (Olivecrona & Bengtsson-Olivecrona, 1987). While attached to the endothelium, LPL binds its cofactor apoprotein CII, which is present on the surface of triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins), and hydrolyses the core triglycerides of these lipoproteins to glycerol and free fatty acids (Olivecrona & Bengtsson-Olivecrona, 1987). By this action LPL plays a major role in the maturation and processing of several different classes of lipoproteins. The free fatty acids generated during the hydrolysis of core triglycerides are utilized for energy by muscle tissues or can be stored in adipose tissue, making LPL a pivotal enzyme for energy utilization and storage (Eckel, 1987). The enzyme is synthesized by a number of different parenchymal cells including adipocytes, skeletal and cardiac muscle cells, and Kupffer's cells (Nilsson-Ehle et al., 1980). Macrophages and several macrophage-like cell lines, which secrete a variety of biologically active products (Nathan, 1987), also secrete LPL (Khoo et al., 1981; Chait et al., 1982; Ma-

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[1] Abbreviations: CHX, cycloheximide; di-C8, 1,2-dioctanoyl-*rac*-glycerol; H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride; HA-1004, *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride; IP_3, inositol 1,4,5-trisphosphate; LPL, lipoprotein lipase; PKC, protein kinase C; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate.

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honey et al., 1982; Tajima et al., 1985; Auwerx et al., 1988).

Treatment of several established cell lines with phorbol esters induces them to assume a more differentiated phenotype with morphologic, biologic, and antigenic characteristics of macrophages (Todd et al., 1985; Triesman, 1985; Tsuchiya et al., 1982). These cell lines, therefore, are useful models for the study of gene expression during phorbol ester induced macrophage differentiation. We earlier showed that differentiation of the human monocytic leukemia cell line THP-1 into macrophages is accompanied by induction of LPL mRNA levels and LPL secretion into the media (Auwerx et al., 1988). To gain more insight into the molecular mechanisms involved in the regulation of LPL gene expression in THP-1 cells, we investigated the roles of second messengers and protein synthesis inhibitors on synthesis, steady-state levels, and stability of LPL mRNA. The results show that the level of LPL mRNA in THP-1 cells is under the control of extracellular signals that activate phospholipase C (PLC), resulting in activation of protein kinase C (PKC) and mobilization of intracellular Ca^{2+}. Furthermore, LPL mRNA stability seems to be highly influenced by a labile protein or protein synthesis per se.

Experimental Procedures

Materials. Phorbol 12-myristate 13-acetate (PMA) was obtained from LC Services Corp. (Woburn, MA), 1,2-dioctanoyl-*rac*-glycerol and A23187 were from Sigma (St. Louis, MO), and the protein kinase inhibitors 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) and *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride (HA-1004) were from Seikagaku America (St. Petersburg, FL). Hybond N nylon membrane for blotting of RNA was from Amersham (Amersham, U.K.), and the random prime labeling kit was from Boehringer Mannheim (Indianapolis, IN).

Cell Culture. THP-1 cells (a human monocytic leukemia cell line) were purchased from the American Type Culture Collection and grown in RPMI 1640 medium supplemented with 10% fetal calf serum (low in endotoxin) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. PMA (1.6×10^{-7} M) was used to differentiate THP-1 cells into cells with macrophage-like appearance, as described earlier (Auwerx et al., 1988).

Analysis of RNA. Total RNA was isolated by the guanidine isothiocyanate method (Chirgwin et al., 1979) and analyzed either by electrophoresis through 1.2% agarose–formaldehyde gels (Lehrach et al., 1977), followed by capillary transfer to nylon membranes, or by dot blot hybridization as described previously (Auwerx et al., 1988). Transcripts were quantitated by densitometric tracing of autoradiographs following hybridization to ^{32}P-labeled cDNA probes (specific activity of 1×10^9 cpm/μg) (Auwerx et al., 1988). A 1.36-kb *Eco*RI fragment of the human lipoprotein lipase cDNA clone (HLPL-26) was used to detect LPL-specific mRNA (Auwerx et al., 1988). A 0.75-kb *Eco*RI-*Hin*dIII fragment of pA1, a chicken β-actin cDNA clone (Cleveland et al., 1980), and a 0.45-kb *Taq*I–*Alu*I fragment of the superoxide dismutase cDNA clone pS61-10 (Sherman et al., 1983; ATCC 57231) were used as control probes. All values of LPL mRNA were corrected for variations in actin mRNA levels. Each experiment was performed at least twice.

Results

Induction of LPL mRNA Is Primarily the Result of Transcriptional Activation of the LPL Gene. In THP-1 cells treated with PMA, LPL mRNA steady-state levels increased (20–30-fold) 6 h after addition of PMA, thereafter decreasing over the next 3 days (Auwerx et al., 1988) (Figure 1). Since we (Auwerx et al., 1988) and others (Enerback et al., 1988) reported a difference in relative abundance of various LPL mRNA species in different tissues, we further investigated if PMA treatment changed the relative proportion of LPL mRNA species. Northern blot analysis of RNA isolated from THP-1 cells at different times after addition of PMA showed that the relative proportion of the different mRNA species as determined by densitometric tracing was approximately constant (Figure 1).

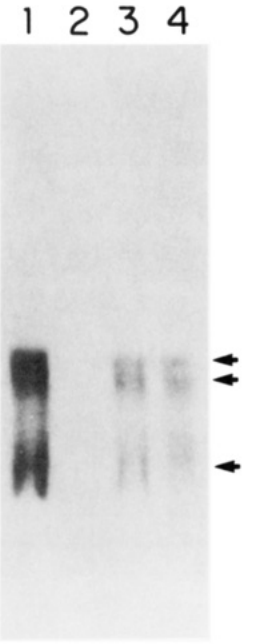

FIGURE 1: RNA gel blot analysis of LPL mRNA after addition of PMA to THP-1 cells. THP-1 cells were grown for the indicated time in the presence or absence of 1.6×10^{-7} M PMA. Total RNA was isolated from 60×10^6 to 100×10^6 cells as described under Experimental Procedures. Twenty micrograms of total RNA was electrophoresed, blotted, and probed according to the procedure described previously. Lane 1, 6 h after PMA addition; lane 2, untreated cells; lane 3, 3 h after PMA addition; lane 4, 18 h after PMA addition.

Increased steady-state levels of LPL mRNA as observed in PMA-treated THP-1 cells (Auwerx et al., 1988) could be the result of enhanced LPL transcription and/or increased stability of the mRNA. Treatment of THP-1 cells with actinomycin D (5 μg/mL) for 90 min prior to the addition of PMA blocked induction of LPL mRNA (Figure 2A, left panel: compare lanes 2 and 4), while only a minimal effect was seen on the level of actin mRNA (Figure 2A, right panel: compare lanes 2 and 4), suggesting that the increase in LPL mRNA required transcription.

To assess the contribution of RNA stability to the increase in LPL mRNA detected after PMA treatment, its rate of disappearance was measured at 1, 3, 6, and 72 h after induction with PMA. Actinomycin D was added to block further transcription at each of these time points, after which samples were collected at 0.5, 1, 2, 3, and 6 h for measurement of LPL mRNA. Insufficient LPL mRNA was present after 1-h exposure to PMA to perform analysis of mRNA stability. LPL mRNA was very stable throughout the 72-h period (Figure 2B), and therefore altered stability is not likely to have contributed significantly to the PMA-induced increase in LPL mRNA levels.

LPL Gene Expression Is Regulated by Protein Kinase C. Receptor-mediated activation of PLC results in the formation of DAG and IP_3, which activate PKC and mobilize Ca^{2+},

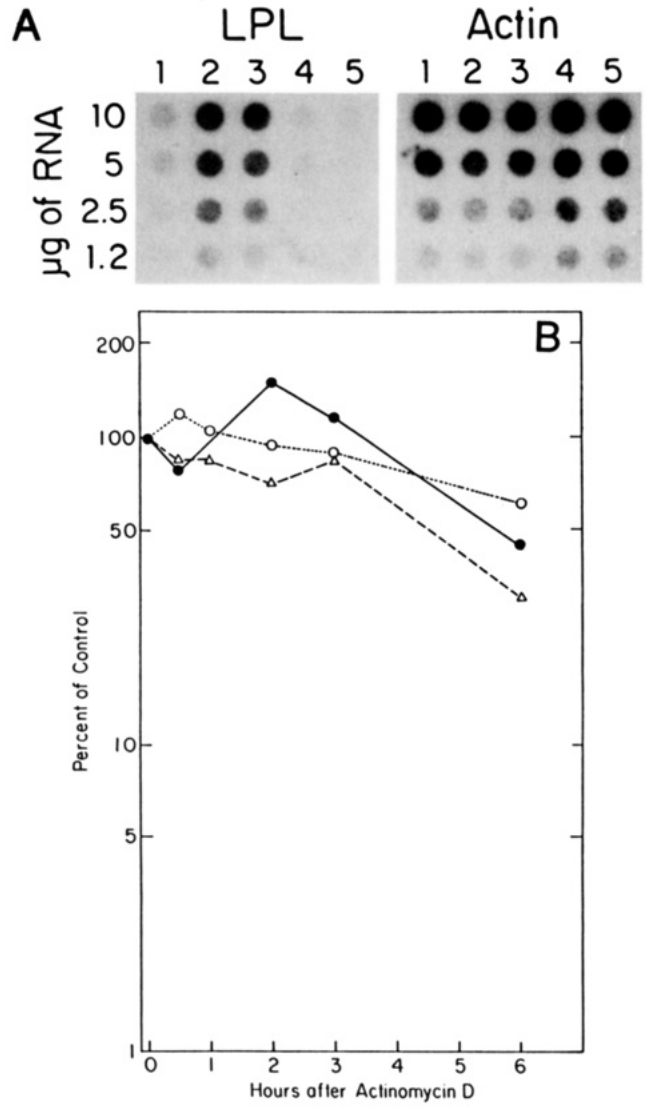

FIGURE 2: Analysis of transcriptional (A) and posttranscriptional (B) mechanisms in the regulation of LPL mRNA in THP-1 cells. (A) Effect of actinomycin D (5 μg/mL added 90 min prior to addition of PMA) on induction of LPL (left panel) and actin mRNA (right panel): lane 1, cells without any addition; lane 2, cells 6 h after addition of PMA; lane 3, cells after addition of A23187; lane 4, cells treated with actinomycin D and PMA; lane 5, cells treated with actinomycin D and A23187. RNA analysis was performed as described under Experimental Procedures. (B) Stability of LPL mRNA. THP-1 cells were treated with PMA for 3 (●), 6 (○), or 72 h (△), after which time actinomycin D (5 μg/mL) was added. Thereafter, total RNA was isolated at the indicated time points for quantification of LPL mRNA. The relative number of transcripts remaining is expressed as a percentage of the transcripts present before the addition of actinomycin D.

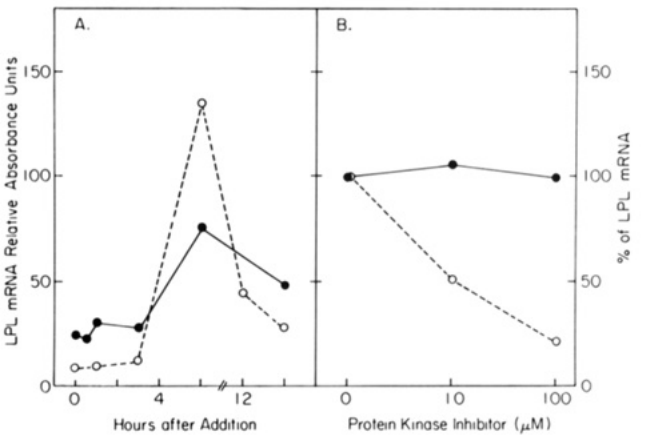

FIGURE 3: Induction of LPL mRNA mediated by protein kinase C. (A) Time course of LPL mRNA accumulation after treatment of THP-1 cells with either the diacylglycerol analogue, di-C8, or PMA. Cells (60×10^6) were grown for the indicated time periods in medium containing 20 μg/mL di-C8 (●) or 1.6×10^{-6} M PMA (○). Total RNA was isolated; serial dilutions of RNA were then dot blotted and hybridized to the labeled probe. (B) Accumulation of LPL mRNA inhibited by a PKC inhibitor. THP-1 cells were exposed to 1.6×10^{-7} M PMA for 6 h in the absence or presence of different concentrations of either H-7 (○) or HA-1004 (●). The amount of mRNA present with a given concentration of protein kinase inhibitor was expressed as a percentage of the amount of LPL mRNA in cells treated with PMA alone.

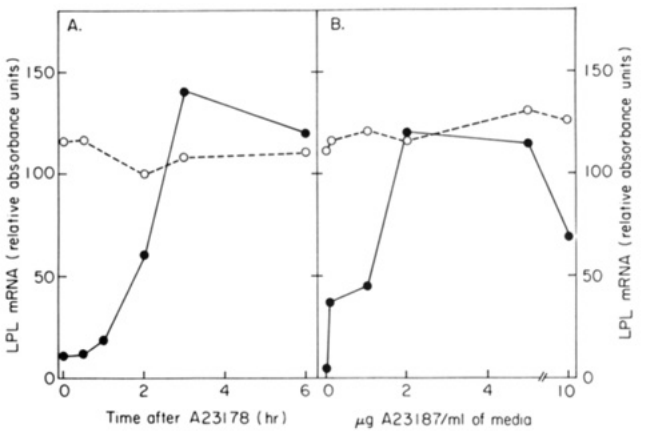

FIGURE 4: Induction of LPL mRNA steady-state levels by the calcium ionophore A23187. Time-course (A) and dose-response (B) curves of LPL (●) and actin (○) mRNAs after treatment of THP-1 cells with PMA are shown. Total RNA was isolated, blotted, probed, and quantified as described under Experimental Procedures. Cells were grown either at a constant concentration (5 μg of A23187/mL) and varying times (A) or during a fixed time period (3 h) with varying concentrations (B) of A23187.

respectively (Nishizuka, 1986). To ascertain whether PMA exerted its effects through PKC, THP-1 cells were treated with 1,2-dioctanoyl-*rac*-glycerol (di-C8), a synthetic analogue of diacylglycerol, which resulted in a 3-fold induction of LPL mRNA. Induction of LPL mRNA with di-C8 and PMA followed a similar time course, although the level of LPL mRNA accumulation was lower with the di-C8 than after PMA (Figure 3A). In contrast to the differentiation that is observed after treatment of THP-1 cells with PMA, no macrophage differentiation was observed with the concentration of di-C8 (20 μg/mL) used.

The effect of the specific inhibitor of PKC, H-7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride], on induction of LPL by PMA was next investigated. H-7 has been shown to be a potent inhibitor of PKC with a K_i of 6 μM (Kawamoto & Hidaka, 1984; Matsui et al., 1986). A dose-dependent inhibition of the PMA effect on LPL mRNA by H-7 was observed (Figure 3B). A less potent and specific PKC inhibitor, HA-1004 [*N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride] (K_i = 60 μM), had no effect.

Mobilization of Intracellular Ca^{2+} Induces LPL mRNA. The involvement of the second pathway of signal transduction activated after membrane phospholipid hydrolysis, i.e., IP_3 and Ca^{2+}, in the control of intracellular LPL mRNA levels was next investigated. The Ca^{2+} ionophore A23187 was used to mobilize intracellular Ca^{2+} in THP-1 cells. Treatment of THP-1 cells with A23187 resulted in approximately a 15-fold increase in steady-state levels of LPL mRNA within 3 h (Figure 4A). The time course of activation was similar to that observed with PMA (Auwerx et al., 1988) and DAG (Figure 3). The effect of the ionophore was dose dependent

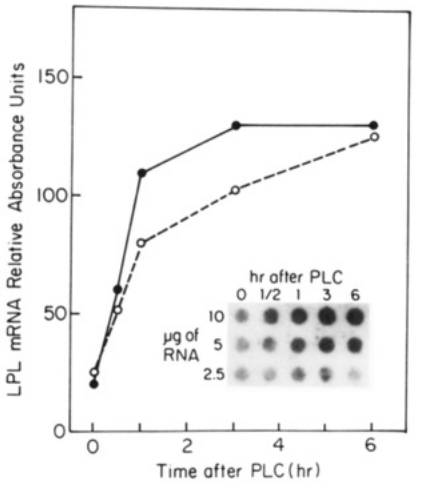

FIGURE 5: Induction of LPL mRNA steady-state levels by PLC. THP-1 cells (60×10^6) were exposed for the indicated times to PLC at 2 (○) or 5 units/mL (●) and analyzed for LPL mRNA. The inset shows an autoradiograph of a dot blot of cells exposed to 5 units of PLC for the indicated times.

up to 2 μg/mL of medium (Figure 4B). As in the case of PMA, induction with A23187 was blocked by actinomycin D (Figure 2A, lanes 3 and 5). No additive effect of PMA and A23187 was seen on LPL mRNA accumulation when both agents were added together (data not shown). Depletion of cellular PKC by pretreatment of THP-1 cells for 72 h with PMA also abolished the stimulatory effect of A23187 on LPL mRNA accumulation, suggesting that the increase of LPL mRNA by A23187-induced Ca^{2+} release is mediated via activation of PKC or another common intermediate along the pathway of signal transduction.

Exogenous Phospholipase C Induces LPL mRNA. In order to establish that hydrolysis of membrane phospholipids into DAG and IP_3 could induce LPL mRNA levels, THP-1 cells were treated with PLC. Addition of 2 and 5 units of PLC/mL of medium resulted in an approximately 2- and 5-fold increase in the steady-state levels of LPL mRNA within a few hours (Figure 5). These results provide further evidence for the role of phospholipid degradation in the induction of LPL mRNA levels.

LPL mRNA Is Induced by Cycloheximide. It has been demonstrated previously that the transcription of several genes is influenced by labile negative regulatory proteins. To test whether inhibition of protein synthesis influenced LPL gene expression, CHX was added to THP-1 cells. The addition of CHX alone resulted in a 10-fold induction of LPL mRNA. However, when THP-1 cells were treated with CHX in the presence of PMA or A23187, the LPL gene was "superinduced", and LPL mRNA accumulated to levels at least 100-fold higher than seen in untreated cells (Figure 6). The increase in LPL mRNA occurred later in those cells treated with CHX than in cells treated with either PMA (Figure 6) or A23187 (Figure 4) alone. When CHX was present together with PMA, LPL mRNA also remained elevated for a longer time (Figure 6). The effects of CHX could be exerted through an enhancement of the transcription rate and/or through stabilization of the mRNA (Kelly et al., 1983).

Discussion

Addition of phorbol esters to undifferentiated THP-1 cells results in a rapid and transient increase in LPL mRNA steady-state levels and LPL enzyme secretion into tissue culture medium (Auwerx et al., 1988), without any change in the relative amounts of the various LPL mRNA species.

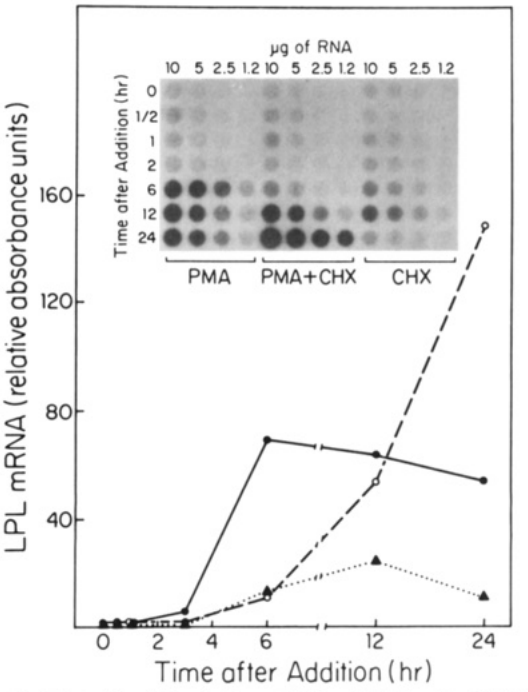

FIGURE 6: Effect of cycloheximide on LPL mRNA levels. Total RNA was isolated from 100×10^6 cells as described under Experimental Procedures. Serial dilutions of RNA isolated at various times after addition of either CHX, PMA, or CHX and PMA were dot blotted, hybridized to labeled probe, autoradiographed, and scanned as described under Experimental Procedures. Relative LPL mRNA concentrations after addition of PMA (●), CHX (△), and CHX + PMA (○) are illustrated. The inset shows the dot blot.

Actinomycin D completely blocked the PMA-induced increase in LPL mRNA levels, indicating that increased transcription of the LPL gene was the cause of mRNA accumulation. The similarity in decay rates of LPL mRNA at different times after PMA addition lends further support to this hypothesis.

Several lines of evidence support the conclusion that LPL gene expression is controlled by degradation of membrane phospholipids resulting in activation of PKC and release of intracellular Ca^{2+}. First, exposure of cells to PLC, the enzyme responsible for the initial hydrolysis of membrane phosphoinositides and subsequent activation of PKC and release of intracellular Ca^{2+} (Nishizuka, 1986; Bell, 1986), induced LPL mRNA levels. Second, transcriptional activation of the LPL gene was affected by activators of PKC (PMA and DAG). The DAG analogue di-C8 induced LPL mRNA to a lesser extent than PMA, probably since di-C8 causes a short-lived stimulation of PKC (Yamamoto et al., 1985; Kreutter et al., 1985; Calabretta, 1987). Third, treatment of THP-1 cells with PMA in the presence of varying concentrations of H-7, an inhibitor of PKC (Kawamoto & Hidaka, 1984; Matsui et al., 1986), resulted in a dose-dependent decrease in LPL mRNA induction. HA-1004, an inhibitor of other protein kinases, had no effect on LPL mRNA levels. Several other genes such as human metallothionein IIA (Imbra & Karin, 1987; Lee et al., 1987), c-fos (Mitchell et al., 1985), collagenase (Angel et al., 1987a,b), and the low density lipoprotein receptor (Auwerx et al., 1989) also were reported to be transcriptionally activated by phorbol esters. It has been shown that for some of these genes activation involves interaction of a transcription factor, AP-1, with a specific 5′ upstream phorbol ester responsive enhancer motif (Lee et al., 1987; Angel et al., 1987a,b). Whether the activation of the LPL gene is mediated through the same conserved enhancer sequence awaits further study.

Extracellular signals bind to membrane receptors and stimulate PLC, resulting in both PKC activation and Ca^{2+}

mobilization that transduce these signals into short- and long-term cellular responses (Nishizuka, 1986; Bell, 1986). Studies using the ionophore A23187 demonstrated that in THP-1 cells LPL gene expression is influenced not only by PKC activation but also by Ca^{2+} mobilization. At present it is not clear whether the effects of Ca^{2+} mobilization are independent of PKC activation, since high concentrations of intracellular Ca^{2+} per se have been reported to activate PKC (Nishizuka, 1986; Bell, 1986). The absence of additive effects of PKC activation and A23187 as well as the inability of Ca^{2+} mobilization to induce LPL mRNA in PKC-depleted cells suggest a common intermediate along the pathway of signal transduction controlling LPL gene expression.

Inhibition of protein synthesis with cycloheximide also induces LPL mRNA levels. LPL mRNA reached its maximum level at a later time after CHX (12 h) than after PMA (6 h) treatment. Addition of CHX together with PMA resulted in a massive superinduction (>100-fold) of LPL mRNA levels. Unlike the transient induction observed with PMA, accumulation of LPL mRNA continued for at least 24 h after addition of PMA and CHX. Thus, the LPL gene also belongs to a class of genes that are superinducible by inhibitors of protein synthesis. Other genes in this class include the immunoglobulin light chain (Wall et al., 1986), several oncogenes (Mitchell et al., 1985; Greenberg et al., 1986), β-interferon (Dinter & Hauser, 1987; Goodbourn et al., 1986), and the low density lipoprotein receptor (Auwerx et al., 1989). CHX could either enhance mRNA transcription due to inactivation of a labile repressor protein or increase mRNA stability (Kelly et al., 1983). The slower accumulation seen after CHX as well as the nontransient and synergistic (as opposed to additive) effect of PMA and CHX argues in favor of enhanced LPL mRNA stability.

We conclude the LPL mRNA steady-state levels are regulated by membrane inositol phospholipid turnover and subsequent PKC activation and Ca^{2+} mobilization. We further suggest that a labile protein is involved in regulation of stability of LPL mRNA or that protein synthesis per se is required for degradation of this mRNA (Linial et al., 1985). Precedents for this type of posttranscriptional regulation have been described previously for the granulocyte–monocyte colony stimulating factor, tumor necrosis factor, the c-fos, c-sis, c-myc, and c-myb oncogenes, and interleukin 1 and interleukin 2 genes [reviewed in Shaw and Kamen (1986)] in which AU-rich domains in the 3′ untranslated region play an important role in mRNA stability.

Registry No. LPL, 9004-02-8; PLC, 9001-86-9; Ca, 7440-70-2; protein kinase, 9026-43-1.

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