



University of Kentucky
UKnowledge

Clinical and Translational Science Faculty
Publications

Center for Clinical and Translational Science

1997

Translational Regulation of Lipoprotein Lipase by Epinephrine Involves a Trans-Acting Binding Protein Interacting with the 3' Untranslated Region

Gouri Ranganathan
University of Arkansas for Medical Sciences

Diane Vu
University of Arkansas for Medical Sciences

Philip A. Kern
University of Kentucky

Follow this and additional works at: https://uknowledge.uky.edu/ccts_facpub



Part of the [Translational Medical Research Commons](#)

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Repository Citation

Ranganathan, Gouri; Vu, Diane; and Kern, Philip A., "Translational Regulation of Lipoprotein Lipase by Epinephrine Involves a Trans-Acting Binding Protein Interacting with the 3' Untranslated Region" (1997). *Clinical and Translational Science Faculty Publications*. 57.
https://uknowledge.uky.edu/ccts_facpub/57

This Article is brought to you for free and open access by the Center for Clinical and Translational Science at UKnowledge. It has been accepted for inclusion in Clinical and Translational Science Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Translational Regulation of Lipoprotein Lipase by Epinephrine Involves a Trans-Acting Binding Protein Interacting with the 3' Untranslated Region

Digital Object Identifier (DOI)

10.1074/jbc.272.4.2515

Translational Regulation of Lipoprotein Lipase by Epinephrine Involves a Trans-acting Binding Protein Interacting with the 3' Untranslated Region*

(Received for publication, June 26, 1996, and in revised form, October 28, 1996)

Gouri Ranganathan, Diane Vu, and Philip A. Kern‡

From the Department of Medicine, University of Arkansas for Medical Sciences, and the John L. McClellan Memorial Veterans Hospital, Little Rock, Arkansas 72205

To better characterize the translational regulation of lipoprotein lipase (LPL) by epinephrine, cytoplasmic extracts were prepared from 3T3-L1 adipocytes, 3T3-F442A adipocytes, and other nonadipocyte cell lines (C2 cells, 3T3 fibroblasts, and Chinese hamster ovary cells). After treatment with epinephrine, cell extracts from the adipocytes inhibited LPL translation in an *in vitro* translation assay, whereas extracts from the C2 cells and 3T3 fibroblasts did not affect LPL translation. To identify the region on the LPL mRNA that controlled translation, *in vitro* translation was carried out using constructs containing different LPL sequences. Specific deletion of the first 50 (1601–1650) nucleotides of the 3' untranslated region (UTR) resulted in a loss of translation inhibition. The addition of LPL 3' UTR to a heterologous reporter gene construct resulted in an inhibition of translation. Inhibition of the reporter LPL 3' UTR translation was demonstrated by the addition of epinephrine-treated cell extracts to an *in vitro* translation assay, as well as by transfection of this construct into 3T3-F442A cells, followed by treatment of the cells with epinephrine. Competition for a trans-acting binding protein was demonstrated by the addition of sense mRNA strands corresponding to the proximal 135 nucleotides of the 3' UTR of LPL. To identify a RNA-binding protein, adipocyte extracts were incubated with ³²P-labeled RNA sequences followed by RNase treatment. The epinephrine-treated cell extract protected a fragment of RNA when the RNA included sequences on the proximal 3' UTR of LPL. Cross-linking of this protected fragment and analysis by SDS-polyacrylamide gel electrophoresis revealed a protein that migrated at about 30 kDa.

Thus, the addition of epinephrine to 3T3 adipocytes results in an inhibition of translation through the production of a RNA-binding protein that binds to a region on the proximal 3' UTR of the LPL mRNA.

lipoproteins and is subject to regulation by a number of different hormones (1). Catecholamines are among the physiological regulators of LPL and inhibit adipose lipid accumulation during periods of active lipolysis (e.g. fasting). Previous studies have demonstrated a decrease in LPL activity after the addition of epinephrine or other catecholamines to adipocytes *in vitro* (2, 3).

A number of studies have demonstrated that the regulation of LPL is complex. Under some conditions, the levels of LPL mRNA are regulated, whereas other times there is regulation of LPL translation or posttranslational processing (1). Regulation of LPL translation has been demonstrated in response to several conditions, including elevated glucose (4) and thyroid hormone absence (5). When epinephrine was added to adipocytes, there was a rapid decrease in LPL synthesis using [³⁵S]methionine pulse labeling, in spite of no change in adipocyte LPL mRNA levels (2). In a recent study, we partially characterized this translational regulation (6). A cytoplasmic extract from epinephrine-treated 3T3-L1 cells inhibited LPL translation in an *in vitro* translation system. Furthermore, when constructs were prepared that lacked the 3' untranslated region (UTR), the extract failed to inhibit translation, indicating that the cytoplasmic extract contained a factor that interacted with the 3' UTR of the LPL mRNA.

This study was intended to further characterize this translational regulation of LPL by epinephrine. In addition to further defining the important region of the LPL mRNA, we have identified a RNA-binding protein and demonstrated that the inhibition of LPL expression can be transferred to a heterologous reporter.

MATERIALS AND METHODS

Cell Culture and Differentiation—3T3-L1 cells and 3T3-F442A cells were obtained from Dr. Howard Green (Harvard Medical School, Boston, MA). 3T3-L1 cells were grown on 75-cm² culture flasks (Costar) in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Inc.) supplemented with 10% fetal calf serum. Cells were grown to confluence and differentiated by incubation in DMEM with 10% fetal calf serum containing 1 μg/ml insulin, 0.5 mM isobutylmethylxanthine, and 0.25 μM dexamethasone for 72 h. Cells were then maintained in DMEM containing 10% serum and 1 μg/ml insulin for 5–7 days. Medium was then changed to DMEM containing 10% serum. 3T3-F442A cells were treated similarly, except differentiation was accomplished with 1 μg/ml insulin alone. 3T3 fibroblasts and Chinese hamster ovary cells were obtained from the American Type Culture Collection and grown in DMEM containing 10% fetal calf serum. Mouse myocyte cells, C2 cells (7), were maintained in DMEM with 5% fetal bovine serum and 15% calf serum. For differentiation, cells were switched to DMEM containing 2% horse serum.

Preparation of Cytoplasmic Cell Extract—A cytosolic fraction was prepared as described previously (6). Cells were lysed using an isotonic lysis buffer, and the postnuclear extract was used to prepare a high-

Lipoprotein lipase (LPL)¹ hydrolyzes the triglyceride core of

* Supported by Grant DK 39176 from the National Institutes of Health and a grant from the American Diabetes Association. This work was done during the tenure of an Established Investigatorship from the American Heart Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: ACOS-Research, 598/151, John L. McClellan Memorial Veterans Hospital, 4300 W. 7th St., Little Rock, AR 72205. Tel.: 501-660-2035; Fax: 501-671-2599; E-mail: PAKern@AOL.com.

¹ The abbreviations used are: LPL, lipoprotein lipase; UTR, untranslated region; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco's modified Eagle's medium; PAGE,

polyacrylamide gel electrophoresis; kb, kilobase(s); bp, base pairs.

speed supernatant fraction (S-100) by centrifugation at $100,000 \times g$ for 2 h at 4 °C. Solid ammonium sulfate was added to the cytosolic fraction to 60% saturation and precipitated for 0.5 h on ice. Precipitated proteins were collected by centrifugation at $6,000 \times g$ for 10 min at 0°C, redissolved, and dialyzed against Buffer A (20 mM Tris-HCl, pH 7.4, 20 mM KCl, 7 mM β -mercaptoethanol, 0.1 mM EDTA, and 10% glycerol). Protein concentration in the cell extract was determined with a Bio-Rad protein assay, using bovine serum albumin as a standard. Equal quantities of the cell extract (0.1 μ g) were used in the rabbit reticulocyte lysate.

In Vitro Translation—For *in vitro* translation, RNA transcripts from a variety of human LPL cDNA constructs were used (described below). Template DNA was linearized with a suitable restriction enzyme to obtain a complete transcript of the cloned DNA. 1 μ g of linearized DNA was transcribed with either SP6 or T7 polymerase using the SP6/T7 transcription kit (Boehringer Mannheim). Equal quantities of RNA transcripts (0.1 μ g) were translated in a rabbit reticulocyte lysate system (Promega) in the presence of [³⁵S]methionine, and the translation products were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography.

Preparation of Constructs for In Vitro Translation—The construct used in Fig. 1 was LPL35, described by Wion *et al.* (8). It contained 174 nucleotides of the 5' untranslated sequence, the complete coding sequence (1428 nucleotides), and 822 nucleotides of the 1950-nucleotide 3' UTR cloned in transcription vector pGEM-2.

The 3.2-kb LPL construct (clone A in Fig. 2) was prepared using overlapping clones of human LPL cDNA in pGEM4Z (8), as described previously (9).

Clone B consisted of nucleotides 1–3196, with a deletion of nucleotides 1600–1654. This cDNA was generated using the megaprimer polymerase chain reaction (PCR) method (10). The first segment, nucleotides 1–1599, was amplified using the *Eco*RI cut of LPL35, which cuts at nucleotide 1640. A *Pst*I site was attached to the upstream primer, which contained the first 17 nucleotides of the human LPL sequence (ATATCTGCAGCCCCTGTTCTCCTC). The downstream fusion primer contained the deletion of 54 bases and extended from nucleotides 1585–1600 followed by nucleotides 1654–1670 (GTTACTTCCTCCACTTCTCAGCCTGACTTCT). The second segment, nucleotides 1654–3196, was amplified from the full-length clone of LPL (9). A complement of the fusion primer described above was used as the upstream primer. The downstream primer used included a *Bam*HI site (ATATGGATCCCCAACAAACAATCATACT). These two fragments were used as megaprimers to amplify the 3.2-kb cDNA containing the 54-nucleotide deletion. To improve the yield and minimize errors, *Taq* Extender PCR additive (Stratagene Cloning Systems, La Jolla, CA) was included with *Taq* DNA polymerase. The PCR product was cloned into pGEM-2. The clone was confirmed to have a deletion of bases 1600–1654 using informative restriction fragment analysis.

Clones C and D were a generous gift of Dr. Robert H. Eckel (University of Colorado School of Medicine, Denver, CO). Clone C has the complete 5' UTR coding sequence (1428 nucleotides) and the first 25 nucleotides of LPL 3' UTR cloned in pGEM-2. Clone D has both the 3' and 5' UTRs eliminated and contains the complete LPL coding sequence. These sequences were confirmed by double-stranded DNA sequencing (U. S. Biochemical Corp.; sequenase sequencing kit).

Heterologous construct E was prepared that contained the chloramphenicol acetyltransferase (CAT) coding sequence followed by nucleotides 1512–2451 of human LPL cloned 3' to CAT. This construct was generated by inserting a polylinker after the CAT coding sequence using pBLCAT2 (11). The region between nucleotides 1512 and 2451 of LPL cDNA was amplified using PCR. Restriction sites were included on both ends of the PCR primers, and the resulting 939-bp fragment of LPL cDNA was cloned 3' of the CAT coding sequence. A control construct F was generated that contained nucleotides 1312–2212 of SV40 3' UTR cloned 3' of the CAT coding sequence. This insert was also generated by PCR using the pBLCAT2 plasmid as template. A T7 promoter sequence was introduced 5' of the CAT coding sequence. These constructs were confirmed by informative restriction cuts. *Taq* Extender PCR additive was included along with *Taq* polymerase to minimize errors and improve the yield of the PCR reaction. For expression, purified plasmid DNA was digested at a polylinker restriction site downstream of the LPL insert, and *in vitro* RNA transcription was carried out using the appropriate upstream promoter for viral RNA polymerase.

Constructs for Transient Transfection—The luciferase vector pcDNA3-luc was a generous gift from Dr. Roger Davis (San Diego State University, San Diego, CA). The region between nucleotides 1512 and 2451 of LPL cDNA was amplified using the appropriate primers. Re-

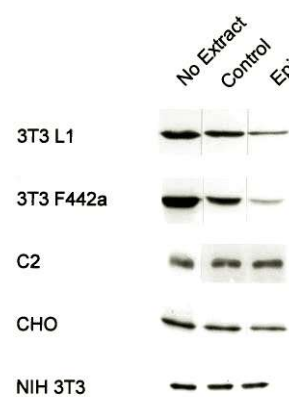


FIG. 1. Effect of cytoplasmic extracts on translation of LPL. S-100 extracts were prepared from control and epinephrine-treated 3T3-L1 and 3T3-F442A adipocytes, C2 myocyte cells, Chinese hamster ovary cells, and 3T3 fibroblast cells as described under "Materials and Methods." The cytoplasmic extract was added to a rabbit reticulocyte *in vitro* translation system in the presence of [³⁵S]methionine and the LPL35 transcript. The translation products were analyzed by SDS-PAGE and autoradiography. The gels are representations of at least three experiments.

striction sites were incorporated on either side of the fragment, which was then cloned into the pcDNA3-luc vector, which has a polylinker after the luciferase stop codon. Two control constructs were used, both of which were obtained by amplification using PCR. For a control construct, a 717-bp fragment of hormone-sensitive lipase sequence between nucleotides 1717 and 2434 was amplified using PCR and inserted into pcDNA3-luc.

Transient Transfection of 3T3-F442A Cells—Transient transfections of 3T3-F442A preadipocytes were performed by electroporation using a Bio-Rad gene pulser followed by butyrate treatment (12). 5×10^6 cells were cotransfected with luciferase-LPL (30 μ g) and β -galactosidase (5 μ g) plasmid DNA. The plasmids were resuspended in 0.5 ml of electroporation buffer containing 20 μ g of salmon sperm carrier DNA. The cells were transferred into growth medium containing 5 mM butyrate for 16 h and then transferred into differentiation medium for 72 h. Luciferase activity and β -galactosidase activity were measured using Promega assay systems.

Sense Strand Competition—The sense RNA strands described in Fig. 4 were generated by PCR of clone A using the appropriate primers, except for the addition of T7 polymerase sequences on the upstream primer.

RNase Protection and UV Cross-Linking—For RNase protection analysis, ³²P-labeled RNA transcripts to specific regions of the LPL 3' UTR were generated from the PCR fragments also used in the sense strand competition experiments. The labeled transcripts were purified by phenol extraction and ethanol precipitation, and the integrity was checked on a 5% nondenaturing PAGE. Binding reactions were carried out with S-100 cytoplasmic extracts (25 μ g) and 25,000 cpm (0.5 ng) of ³²P-labeled transcript in Buffer A. After incubation at 25° for 30 min, 2.5–5 units of RNase T1 (Ambion, Inc.) were added for 20 min, followed by the addition of 5 mg/ml heparin in Buffer A. The RNA-protein complex was then analyzed on a 5% nondenaturing PAGE (13). For the cross-linking assay, after the addition of heparin the reactions were exposed to UV light. UV cross-linking was done for 10 min on ice at a distance of 5–6 cm from an 8-W UV bulb. The samples were analyzed on 10% SDS-PAGE followed by autoradiography.

Statistics—Luciferase activity in transfected cells was analyzed using Student's *t* test.

RESULTS

Previous studies have demonstrated an inhibition of LPL translation by a cytoplasmic extract from epinephrine-treated 3T3-L1 adipocytes (6). To determine the specificity of this translation inhibition, cytoplasmic extracts were prepared from other cell types in the presence and absence of epinephrine and added to an *in vitro* translation system. The transcript for the reticulocyte lysate system was the *in vitro*-transcribed human LPL mRNA. As shown in Fig. 1, a cytoplasmic extract from epinephrine-treated 3T3-F442A adipocytes inhibited LPL

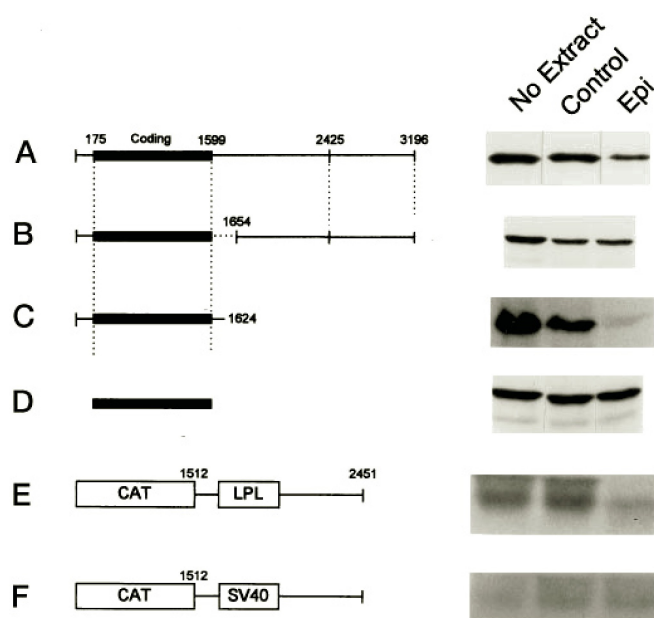


FIG. 2. Analysis of translational regulation in response to control or epinephrine-treated S-100 extract from 3T3-F442A adipocytes. The constructs used were generated as described under "Materials and Methods." Cytoplasmic extract from control or epinephrine-treated adipocytes was added to a reticulocyte *in vitro* translation system in the presence of each specific transcript. Autoradiograms are representative of three experiments with each construct.

translation in the reticulocyte lysate system. On the other hand, there was no inhibition of translation by cytoplasmic extracts from other cells, including 3T3 fibroblasts or C2 cells. Extracts prepared from Chinese hamster ovary cells inhibited LPL translation consistently, although the degree of inhibition was not as great as with the adipocyte cell lines.

In a previous study, we determined that the region that regulated LPL translation was the proximal portion of the 3' UTR of the LPL mRNA. To further define the region of regulation and to determine whether other downstream elements were important in translational regulation, additional experiments were performed using different mRNA constructs. Cytoplasmic extracts were prepared from control and epinephrine-treated 3T3-F442A cells and added to the *in vitro* translation system containing the constructs illustrated in Fig. 2. To determine the importance of the proximal 3' UTR, a construct was prepared that contained the complete coding sequence (nucleotides 1–1599) and the full-length 3' UTR, except for nucleotides 1600–1654. As shown in Fig. 2, the deletion of nucleotides 1600–1654 on the 3' UTR resulted in a loss of translation inhibition by epinephrine. To further isolate the region on the 3' UTR that controlled translation, a construct was prepared that contained the complete coding sequence and the first 24 nucleotides of the 3' UTR. The translation of this construct was inhibited by the addition of epinephrine-treated cell extract from 3T3-F442A cells. On the other hand, a construct that lacked the 5' and 3' UTRs did not respond to the epinephrine-treated cell extract. To determine whether the coding sequence of LPL was involved in translational regulation, a heterologous construct was prepared that contained the coding sequence for CAT, followed by nucleotides 1512–2451 of LPL, cloned 3' to the CAT coding sequence. As shown in Fig. 2, the addition of the epinephrine-treated 3T3-F442A cell extract resulted in an inhibition of translation of this heterologous transcript, whereas no inhibition of translation occurred when the control extract was used and when the same construct was used with an irrelevant sequence cloned 3' to CAT.

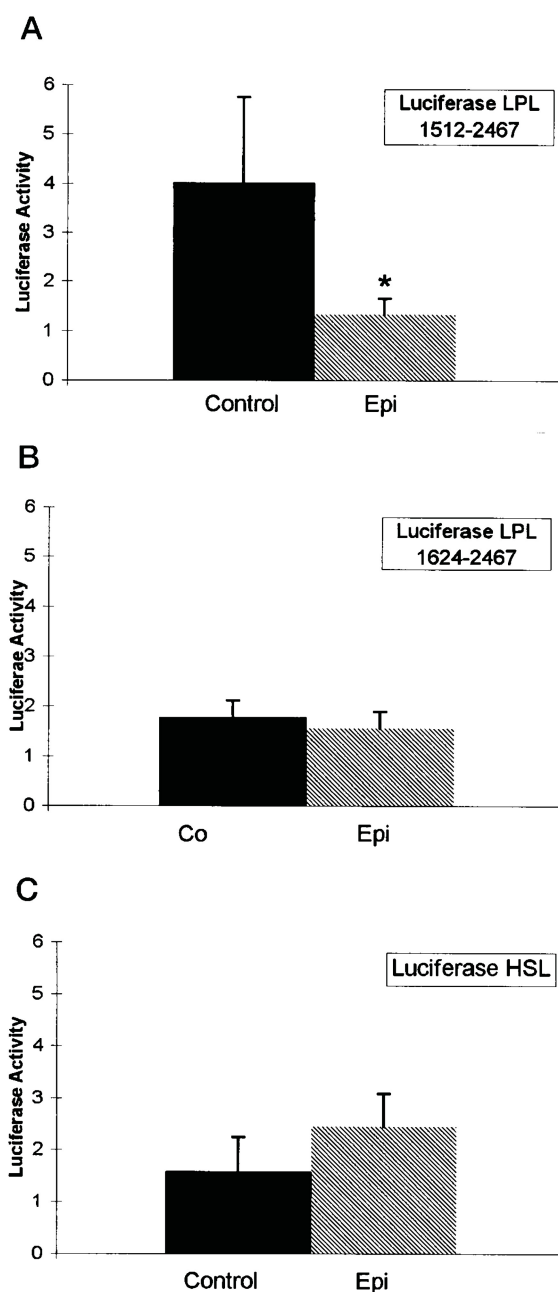


FIG. 3. Expression of luciferase in 3T3-F442A cells transfected with luciferase constructs containing different 3' UTR sequences. 3T3-F442A cells were transfected with constructs containing: A, luciferase-LPL (nucleotides 1512–2467); B, luciferase-LPL (nucleotides 1624–2467); or C, luciferase-hormone-sensitive lipase as described under "Materials and Methods." Cells were then treated with epinephrine, as described under "Materials and Methods", followed by the measurement of luciferase activity. Four separate transfection experiments were performed for each construct. In each experiment triplicate transfection was performed for control and epinephrine treatment. *, $p < 0.05$ versus control cells.

Although we have demonstrated that the proximal 3' UTR of LPL mediated the translational regulation of LPL using *in vitro* translation, we wished to determine whether the addition of the LPL 3' UTR to a heterologous construct could confer inhibition of translation in cells. A luciferase construct was prepared that contained the 939 nucleotides of the LPL 3' UTR (nucleotides 1512–2451) cloned 3' to a luciferase reporter and driven by the cytomegalovirus promoter. This construct was expressed in 3T3-F442A cells (as described under "Materials and Methods"), which were then treated with epinephrine. As

shown in Fig. 3, epinephrine inhibited luciferase expression in 3T3-F442A cells transfected with the luciferase-LPL 3' UTR construct. To confirm that the inhibition of luciferase expression was due to the proximal region of the LPL 3' UTR, two additional constructs were prepared; one contained the same sequence except that it was missing the proximal 24 nucleotides of the 3' UTR, and the other contained 717 nucleotides (nucleotides 1717-2434) of irrelevant sequence (hormone-sensitive lipase coding sequence) cloned 3' to luciferase. As shown in Fig. 3, epinephrine had no inhibitory effect on luciferase expression using these last two constructs.

If the inhibition of LPL translation was due to the production of a trans-acting binding protein, the addition of a sense RNA strand would be expected to compete for this binding protein and remove the translation inhibition. To examine this, a series of sense RNA strands were transcribed and added to the epinephrine-treated cell extract, followed by *in vitro* translation. As shown in Fig. 4, the addition of progressively shorter sense strands of 628, 230, and 130 bp resulted in an increase in LPL

translation, suggesting that these RNA strands were competing for a binding protein. The competitor RNA fragment had no effect on the control cell extract and had no effect on the *in vitro* translation reaction in the absence of any cell extract. Furthermore, the addition of a similar-sized sequence of irrelevant mRNA (neomycin phosphotransferase) had no effect on the epinephrine cell extract (data not shown). Thus, the sense RNA strand seemed to compete with the LPL mRNA for an inhibitory binding factor.

To demonstrate the presence of a trans-acting binding protein, a sequence of the LPL 3' UTR was labeled with [³²P]UTP, followed by the addition of control or epinephrine-treated cell extract and RNase T1. As shown in Fig. 5, the addition of the control cell extract did not result in any RNA protection. On the other hand, the addition of the epinephrine-treated cell extract resulted in the identification of a fragment protected from RNase. This protected fragment was found only in the epinephrine-treated cell extract and was present only when the LPL 3' UTR was used and included the proximal 24 nucleotides of the 3' UTR. As shown in Fig. 5, the use of an irrelevant sequence of RNA (human colipase) yielded no protected fragment. To obtain better information about this binding protein, RNase protection was performed in the same manner, except that the RNA/protein complex was cross-linked with UV irradiation. After this, the complex was analyzed on a SDS-polyacrylamide gel. As shown in Fig. 6, the ³²P-labeled protein complex migrated at 30 kDa. A weaker band was present upon longer exposure at 40 kDa, and smaller peptides were detected at the front, suggesting the possibility of either degradation or a subunit structure to the binding protein.

DISCUSSION

Previous studies have demonstrated that the regulation of LPL is complex and may occur at the level of transcription (14, 15), posttranslational processing (16, 17), or translation. Changes in LPL translation have been demonstrated in response to the addition of epinephrine (2) and glucose (4), and increased LPL translation has been demonstrated in response to hypothyroidism in rats (2) and in response to the treatment of humans and rats with diabetes (18, 19). In previous studies, we examined the inhibition of LPL translation by epinephrine and found that the important region of the LPL mRNA was on the 3' UTR (6).

In the studies described here, we further defined the effects of epinephrine on LPL translation. The factor mediating the translation inhibition was not ubiquitous because extracts

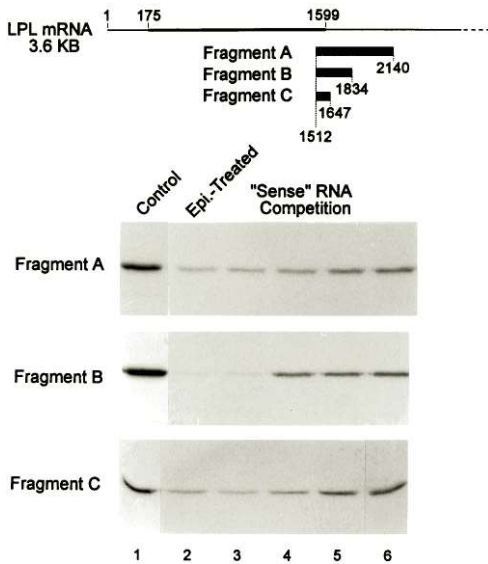


FIG. 4. Competition with LPL RNA sense strand. RNA sense strands corresponding to nucleotides 1512-2140, 1512-1834, or 1512-1647 were synthesized as described under "Materials and Methods" and added to the *in vitro* translation reaction along with the epinephrine-treated S-100 extract. Increasing quantities of sense RNA strand were added, up to a molar ratio of 8:1 (sense strand/LPL mRNA).



FIG. 5. RNase protection assay using S-100 extract from 3T3-F442A adipocytes. ³²P-labeled sense RNA encoding nucleotides 1512-2140, 1512-1834, and 1624-2140 of LPL (Fragments A, B, and C, respectively) or 430 nucleotides of RNA encoding human colipase (Fragment D) were incubated with equal amounts of control or epinephrine-treated cytoplasmic extracts. The reactions were then treated with RNase T1 and heparin and then analyzed on a 5% nondenaturing acrylamide gel followed by autoradiography.

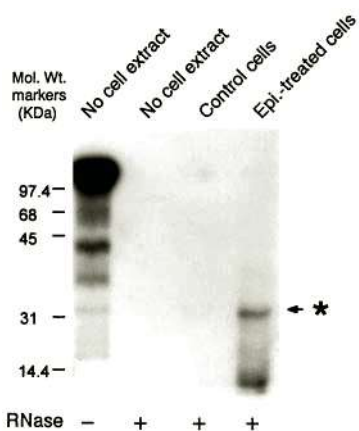


FIG. 6. UV cross-linking of sense RNA strands with cytoplasmic extracts from control or epinephrine-treated 3T3-F442A adipocytes. ^{32}P -labeled sense RNA encoding nucleotides 1512–1834 of LPL was incubated with equal amounts of control or epinephrine-treated cytoplasmic extract. After RNase T1 and heparin treatment, the reactions were UV cross-linked and analyzed on a 10% SDS-PAGE followed by autoradiography.

from C2 cells and fibroblasts did not affect LPL translation in the reticulocyte system, in contrast to extracts from adipocyte cell lines. Extracts from epinephrine-treated Chinese hamster ovary cells, which are known to express LPL (20), also inhibited LPL translation *in vitro*, although to a lesser extent than did the extracts from adipocyte cell lines.

To further characterize the region of the 3' UTR involved in this interaction, constructs were prepared that contained various deletions and added to the *in vitro* translation reaction. These studies suggested that the proximal 3' UTR was essential to translational inhibition by an epinephrine-treated cell extract. This was confirmed by adding the LPL 3' UTR to a heterologous mRNA followed by transient transfection into adipocytes. Epinephrine inhibited expression of the reporter gene only when the 3' UTR contained the initial 24 nucleotides of the LPL 3' UTR. Additional studies demonstrated that the coding region of LPL mRNA was not involved in translational regulation. Together, these studies demonstrated that the motif for translational inhibition of LPL is contained within the first 24 nucleotides of the 3' UTR and that upstream and downstream elements either were not involved in the translational regulation or were dependent on interactions with the proximal 3' UTR.

These studies with the epinephrine-treated cell extract, including the competition with sense RNA strands, suggested the presence of a trans-acting binding protein. To demonstrate the presence of such a protein, the cytoplasmic extract was permitted to interact with ^{32}P -labeled LPL 3' UTR sequence, followed by treatment with RNase. Using this technique, a protein with a subunit size of 30 kDa was protected from RNase. Because this protein was not found in control cells or in fibroblast extracts (which do not inhibit LPL translation *in vitro*), this 30-kDa protein is likely to be the protein responsible for the translation inhibition.

The regulation of translation has been well characterized in other systems (21). Ferritin is regulated at the level of initiation by a trans-acting protein that binds to a region of secondary structure on the 5' untranslated region of ferritin mRNA (22–24). Other studies have implicated changes in the 3' UTR of mRNA in the regulation of translation (25). The same protein that regulates ferritin mRNA initiation binds to an iron response element on the 3' UTR of transferrin receptor mRNA, resulting in the inhibition of mRNA degradation. The 3' UTR is involved in the inhibition of translation of γ -interferon in *Xe-*

nopus oocytes (26) and creatine kinase in the U937 cell line (27). Studies with ornithine decarboxylase, which is under the translational control of polyamines, have demonstrated coordinated regulation of translation by both the 5' and 3' UTRs (28, 29). Translational regulation of gene expression also plays an important role in development, in which many mRNAs are stored in an inactive state until they are utilized during specific stages (30). For example, protamine 2 mRNA translation is inhibited during germ cell development in the testes by the binding of a phosphoprotein to the 3' UTR of the mRNA (31).

In summary, translational regulation of LPL in 3T3-L1 and 3T3-F442A adipocytes is mediated by a trans-acting factor that is induced by epinephrine treatment. This factor is mainly expressed in adipocytes and binds to the 3' UTR of LPL between nucleotides 1600 and 1624. This sequence on the LPL 3' UTR can confer translational regulation to a reporter CAT construct *in vitro* and to a reporter luciferase construct transfected into 3T3-F442A adipocytes. RNase protection followed by UV cross-linking analysis using the LPL 3' UTR as probe detected the presence of a 30-kDa RNA-binding protein in S-100 extract isolated from epinephrine-treated 3T3-L1 adipocytes. Future studies are needed to characterize the RNA-binding protein and to elucidate its physiological role.

Acknowledgments—We thank Richard Davis and John Ong for their valuable suggestions; Ada Yukht, Mehrnoosh Saghizadeh, and Rami Kaakaji for laboratory assistance; Dr. Robert H. Eckel for LPL constructs C and D used in Fig. 2; and Dr. Roger Davis for the luciferase vector. We also thank Charlotte Peterson and Mark Crew for the C2 cells and 3T3 fibroblasts.

REFERENCES

- Enerbäck, S., and Gimble, J. M. (1993) *Biochim. Biophys. Acta* **1169**, 107–125
- Ong, J. M., Saffari, B., Sinsolo, R. B., and Kern, P. A. (1992) *Mol. Endocrinol.* **6**, 61–69
- Eckel, R. H. (1987) in *Lipoprotein Lipase* (Borensztajn, J., ed), pp. 79–132, Evener, Chicago
- Ong, J. M., and Kern, P. A. (1989) *J. Biol. Chem.* **264**, 3177–3182
- Saffari, B., Ong, J. M., and Kern, P. A. (1992) *J. Lipid Res.* **33**, 241–249
- Yukht, A., Davis, R. C., Ong, J. M., Ranganathan, G., and Kern, P. A. (1995) *J. Clin. Invest.* **96**, 2438–2444
- Blau, H. M., Chiu, C.-P., and Webster, C. (1983) *Cell* **32**, 1171–1180
- Wion, K. L., Kirchgessner, T. G., Lusis, A. J., Schotz, M. C., and Lawn, R. M. (1987) *Science* **235**, 1638–1641
- Ranganathan, G., Ong, J. M., Yukht, A., Saghizadeh, M., Sinsolo, R. B., Pauer, A., and Kern, P. A. (1995) *J. Biol. Chem.* **270**, 7149–7155
- Sarkar, G., and Sommers, S. S. (1990) *BioTechniques* **8**, 404–407
- Gorman, C. M., Moffat, L. F., and Howard, B. H. (1982) *Mol. Cell. Biol.* **12**, 1044–1051
- Goldstein, S., Fordis, C. M., and Howard, B. H. (1989) *Nucleic Acids Res.* **17**, 3959–3971
- Leibold, E. A., and Munro, H. N. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 2171–2175
- Ong, J. M., Kirchgessner, T. G., Schotz, M. C., and Kern, P. A. (1988) *J. Biol. Chem.* **263**, 12933–12938
- Ong, J. M., Sinsolo, R. B., Saffari, B., and Kern, P. A. (1992) *Endocrinology* **130**, 2310–2316
- Ong, J. M., and Kern, P. A. (1989) *J. Clin. Invest.* **84**, 305–311
- Doolittle, M. H., Ben-Zeev, O., Elovson, J., Martin, D., and Kirchgessner, T. G. (1990) *J. Biol. Chem.* **265**, 4570–4577
- Sinsolo, R. B., Ong, J. M., Saffari, B., and Kern, P. A. (1992) *J. Lipid Res.* **33**, 89–95
- Tavangar, K., Murata, Y., Pedersen, M. E., Goers, J. F., Hoffman, A. R., and Kraemer, F. B. (1992) *J. Clin. Invest.* **90**, 1672–1678
- Rojas, C., Enerbäck, S., and Bengtsson-Olivercrona, G. (1990) *Biochem. J.* **271**, 11–15
- Kozak, M. (1992) *Annu. Rev. Cell Biol.* **8**, 197–225
- Klausner, R. D., and Harford, J. B. (1989) *Science* **246**, 870–872
- Harford, J. B., and Klausner, R. D. (1990) *Enzyme (Basel)* **44**, 28–41
- Munro, H. N. (1990) *J. Cell. Biochem.* **44**, 107–115
- Jackson, R. J., and Standart, N. (1990) *Cell* **62**, 15–24
- Kruys, V., Wathelket, M., Poupart, P., Contreras, R., Flers, W., Content, J., and Huez, G. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 6030–6034
- Ch'ng, J. L., Shoemaker, D. L., Schimmel, P., and Holmes, E. W. (1990) *Science* **245**, 1003–1006
- Kahana, C., and Nathans, D. (1985) *J. Biol. Chem.* **260**, 15390–15393
- Grens, A., and Scheffler, I. E. (1990) *J. Biol. Chem.* **265**, 11810–11816
- Sommerville, J. (1992) *BioEssays* **14**, 337–339
- Kwon, Y. K., and Hecht, N. B. (1993) *Mol. Cell. Biol.* **13**, 6547–6557