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ABSENCE OF THE PROXIMAL 3′-UNTRANSLATED REGION CAUSES TRANSLATIONAL UPREGULATION*

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Lipoprotein lipase (LPL) is a key enzyme in lipoprotein and adipocyte metabolism. Defects in LPL can lead to hypertriglyceridemia and the subsequent development of atherosclerosis. The mechanisms of regulation of this enzyme are complex and may occur at multiple levels of gene expression. Because the 3′-untranslated region (UTR) is involved in LPL translational regulation, transgenic mice were generated with adipose tissue expression of an LPL construct either with or without the proximal 3′-UTR and driven by the *aP2* promoter. Both transgenic mouse colonies were viable and expressed the transgene, resulting in a 2-fold increase in LPL activity in white adipose tissue. Neither mouse colony exhibited any obvious phenotype in terms of body weight, plasma lipids, glucose, and non-esterified fatty acid levels. In the mice expressing hLPL with an intact 3′-UTR, hLPL mRNA expression approximately paralleled hLPL activity. However in the mice without the proximal 3′-UTR, hLPL mRNA was low in the setting of large amounts of hLPL protein and LPL activity. In previous studies, the 3′-UTR of LPL was critical for the inhibitory effects of constitutively expressed hormones, such as thyroid hormone and catecholamines. Therefore, these data suggest that the absence of the 3′-UTR results in a translationally unrepressed LPL, resulting in a moderate overexpression of adipose LPL activity.

Lipoprotein lipase (LPL)[1] is a central enzyme in lipid metabolism. The enzyme is synthesized and secreted by adipocytes and muscle cells, and transported to the capillary endothelium, where hydrolysis of the triglyceride core of circulating VLDL and chylomicrons takes place. Although the changes in LPL activity with different physiologic states have been well described (1), the mechanism of LPL regulation is complex and occurs at levels of transcription (2–6), translation (7–11), and post-translational processing (12–15) in response to both cell type and regulatory factors. Previous studies have demonstrated translational regulation of LPL. In response to glucose (7), thyroid hormone (8), and catecholamines (9), there were significant changes in LPL protein synthesis, with no changes in adipocyte LPL mRNA levels. In addition, the decrease in LPL activity in both diabetic patients, and rats is due predominantly to decreased LPL translation (10, 16).

LPL is an important marker of adipocyte differentiation, and LPL expression increases in parallel with cellular triglyceride accumulation in preadipocytes (17, 18). Although adipose tissue can synthesize non-esterified fatty acids (NEFA) *de novo*, NEFA for lipid storage are preferentially obtained from LPL-mediated hydrolysis of plasma lipoproteins (19). Hence, LPL has been called "the gatekeeper of the adipocyte" (20), and has been implicated in the development of obesity.

Based on LPL's putative role as an adipocyte "gatekeeper," one might expect that transgenic mice would become obese if LPL were overexpressed in adipose tissue, and lean if LPL were overexpressed in muscle. Several recent studies have taken advantage of LPL knockout techniques combined with muscle-specific overexpression. LPL homozygous knockout mice had severe, fatal hypertriglyceridemia and little adipose tissue lipid, while the heterozygotes developed a milder form of hypertriglyceridemia (21, 22). Initial reports of muscle-specific LPL transgenics described very high muscle LPL overexpression, such that the mice developed weight loss, increased NEFA uptake by muscle, and a fatal myopathy (23). In subsequent studies, however, more moderate muscle specific overexpressors had less carcass lipid, lower triglycerides, and a resistance to high fat diet-induced obesity (24, 25). By breeding these mice onto the knockout background, mice were developed with LPL expression only in skeletal or cardiac muscle (22, 25, 26). The muscle-driven LPL transgene resulted in a normalization of the lipid profile, thus demonstrating the importance of muscle LPL in triglyceride clearance. Other studies have produced "liver-only" LPL expression, which resulted in rescue from the LPL deficiency state along with hypertriglyceridemia and hyperketonemia due to the elevated liver triglyceride uptake (27). LPL has not been overexpressed in adipose tissue previously.

In this study, we generated transgenic mice that overexpress LPL in adipose tissue. It was of interest to investigate the effects of the lack of hormone responsive 3′-untranslated regions in hLPL-overexpressing mice. Therefore, two different adipocyte-specific hLPL transgene constructs were made. One construct contained the full-length 3′-UTR of the LPL mRNA, whereas the other transgenic construct lacked the proximal 3′-UTR. These mice did not become obese, and the increase in

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[1] The abbreviations used are: LPL, lipoprotein lipase; NEFA, non-esterified fatty acids; UTR, untranslated region; HA, hemagglutinin; WAT, white adipose tissue; BAT, brown adipose tissue; FPLC, fast protein liquid chromatography.

adipose LPL activity was modest due to the down-regulation of the endogenous LPL (mLPL) protein by the hLPL transgenic protein. However, in mice that expressed hLPL without the proximal 3′-UTR, the levels of transgenic protein was far higher than one would predict based on the levels of transgenic mRNA. Because the 3′-UTR is important for inhibitory influences by hormones that are constitutively present, these data are consistent with the expression of a translationally unrepressed LPL due to the lack of most of the 3′-UTR.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Generation of Transgenic Mice—Two different human LPL transgenes, with or without the proximal 3′-UTR of the human *LPL* promoter were constructed. Both transgenes were under the transcriptional control of the mouse *aP2* promoter. For the construction of a human *LPL* transgene containing the complete 3′-UTR, the 5.4-kb mouse *aP2* promoter (a generous gift from Dr. Bruce Spiegelman, Boston, MA) was fused to a human *LPL* minigene described previously (23), containing the complete cDNA including 174 bp of the 5′-UTR, the entire coding region, a single intron (intron 3) and 1948 bp of the 3′-UTR with the polyadenylation signal. Additionally, 339 bp of the 3′-flanking region were ligated to the last exon for the proper termination of translation. The minigene (*Kpn*I-, *Sal*I-digested) was introduced behind the 5.4 kb sequence of the mouse *aP2* promoter (*Sma*I, *Hin*cII digest) into the pSK ± vector and the whole 10.4 kb aP2-hLPL minigene construct, further referred to as aP2-hLPL3′, was excised using *Kpn*I and *Sma*I. After purification and sequencing the DNA was used for microinjection.

To generate transgenic mice expressing a human *LPL* gene deficient in the proximal 3′-untranslated region in adipose tissue, we utilized elements of two existing h*LPL* sequence constructs. The N-terminal region of *hLPL* coding sequence, nucleotides 27–810, with intron 3 was amplified from the *LPL* minigene described above (23) using Vent Polymerase (New England Biolabs) and cloned into PCR2 (Invitrogen). The C-terminal region of *hLPL* between nucleotides 791 and the stop codon tagged with influenza hemagglutinin epitope (a gift from Dr. Robert Eckel, University of Colorado Health Sciences Center, Denver, CO) was cut out and shuttled through pGEM2 as a *Acc*I/*Spe*I fragment. It was ligated into the PCR2 construct 3′ to the N-terminal sequences as a *Acc*I/*Spe*I (blunted) fragment. The 3′-UTR sequence of *hLPL*, starting at nucleotide 2614 and including 300 bases of 3′-flanking sequence, was amplified from the *LPL* original minigene using PCR with Vent polymerase. *Bam*HI and *Sac*I sites were included in the primers. The gel-purified DNA was cut with *Bam*HI and *Sac*I and cloned 3′ to the epitope tag on *hLPL* coding sequence in pGEM2. The entire sequence of the *hLPL* construct was then excised from PCR2 using *Xho*I and *Kpn*I and cloned 3′ to the *aP2* promoter in Bluescript vector. The entire aP2-hLPLe transgene with the *aP2* promoter was excised using *Kpn*I and *Sac*I, and this 9.8-kb fragment was gel purified and quantitated. Each step in the cloning was verified by automated sequencing. These mice were designated hLPLe, to indicate the presence of the epitope on the human *LPL* transgene.

Animals—Transgenic mice were created at the National Institutes of Health facility at the University of Alabama, Birmingham (hLPLe) and the animal facility of the Rockefeller University (hLPL3′). Linearized and purified DNA was injected into the fertilized pronuclei of (B6SJL) females that had been mated to males of the same genetic background. Founder animals were bred to B6SJL/J F_1 animals, and four transgenic lines were established. Animal rooms were illuminated between 6 a.m. and 6 p.m. Animals were weaned at 21 days of age. Blood was drawn using either a tail bleed or orbital bleed, and fasting was for 4 h, commencing at 0800. Mice were fed Harlan Teklad rodent chow (product number 7012). To study high fat feeding, mice were fed a high fat diet from Bioserv (product number F3282), which contained 35.5% fat and 32.7% carbohydrates.

Animal Genotyping—Genotypes were determined by PCR analysis of genomic DNA isolated from tail tips according to manufacturer's recommendations (Puregene). To detect the *hLPLe* transgene, the upstream primer corresponded to bp 818–840 of the *hLPL* coding sequence (5′-GTC CCC TGG TCG AAG CAT TGG-3′) and the downstream primer corresponded to sequences in the influenza hemagglutinin epitope (5′-GTA GTC AGG CAC ATC GTA AGG-3′), leading to an 800 bp product. To detect the *hLPL3′* transgene, the following primers, amplifying a 5′ 410 bp sequence of the hLPL cDNA, were used: 5′-GTT ACC GTC CAG CCA TGG ATC ACC A-3′ and 5′-CCT CAA GGG AAA GCT GCC CAC-3′. PCR conditions were as described previously (25).

Southern Blotting—For Southern blotting of hLPLe mice, 10–15 μg of tail tip DNA, isolated according to manufacturer's recommendations (Puregene), was digested with *Eco*RI, fractionated by agarose gel electrophoresis, and blotted on nylon membranes. DNA corresponding to the *hLPLe* minigene was detected with a radiolabeled 0.8-kb fragment amplified by PCR from aP2-*hLPLe* as described above. For Southern blotting of hLPL3′ mice, 10–15 μg DNA were digested with *Pvu*II, and after electrophoresis and blotting the hLPL was detected with a radiolabeled 1.1-kb *Eco*RI fragment of the hLPL cDNA (exon 10). Since *Pvu*II cuts in exon 10, two bands were detected (Fig. 2).

RNA Analysis: Northern Blotting of hLPL3′ Mice—Total RNA was isolated from tissues of fed mice, 10 μg were separated on a formaldehyde-agarose gel, blotted onto Hybond™-XL membrane (Amersham Biosciences) and hybridized with a radioactively labeled 1.1-kb *Eco*RI cDNA fragment of exon 10 of the hLPL. The human LPL signal was detected with a PhosphorImager Screen (Apbiotech, Freiburg, Germany). The blot was stripped, checked on the PhosphorImager screen, and reprobed with a 700-bp *Pst*I cDNA fragment from the last *mLPL* exon.

Quantitative RT-PCR of hLPLe Mice—RNA was extracted from adipocytes as previously described (28). cDNA was synthesized from 5 μg of RNA using 250 ng of oligo(dT) primer (Invitrogen) and Superscript II (Invitrogen) according to manufacturer's recommendations. To detect the endogenous *mLPL* gene, the upstream primer, 5′mLPL, corresponded to nucleotides 1158–1177 of the mLPL coding sequence (5′-ACA GGA GGT GGA CAT CGG-3′), and the downstream primer, 3′mLPL, corresponded to nucleotides 1369–1389 of the mLPL coding sequence (5′-TCA GAG ACT TGT CAT GGC A-3′) (29). For detection of the aP2-hLPLe transgene, the PCR conditions described above were used. 10 μl of the PCR products were run on a 2% ethidium bromide agarose gel, and an 800- or 200-bp product was observed in tissues expressing the aP2-hLPLe transgene or the endogenous mLPL, respectively. Ethidium bromide signal densities were integrated using Eagle Eye software. Values from varying cycle numbers (15, 17, 20, 25, 30, 35, 40, and 45 cycles) were obtained. Products were normalized for size with markers of known DNA quantities (GeneChoice DNA Ladder I, PGC Scientifics), and normalized densities were plotted with PCR cycle number to establish linearity.

Western Blotting—White adipose tissue (WAT) extracts were resolved by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Epitope-tagged proteins were detected with anti-HA monoclonal antibody (clone 16B12, Covance) at a concentration of 1:1000 and visualized with peroxidase-conjugated anti-mouse antibody at a dilution of 1:5000, followed by enhanced chemiluminescence (Santa Cruz Biotechnology). Western blotting with anti-LPL antibodies was performed using an affinity-purified anti-LPL antibody described previously (30).

Measurement of LPL Activity—Heparin releasable and extractable LPL activities were determined in 4-h fasted mice (31) and combined to give total LPL activity. To measure heparin releasable LPL, 100 mg of minced adipose tissue was incubated in 1 ml of Dulbecco's modified Eagle's medium containing 10 units/ml heparin for 45 min at 37 °C. After collecting the heparin-released fraction, tissue LPL was extracted in 50 mM phosphate-buffered saline, pH 7.4, containing 0.2% Triton X-114, as described previously (32). The LPL assay used an emulsified triolein substrate as described previously (33). To differentiate between mLPL and hLPL activity, the 5D2 mouse anti-human monoclonal antibody was used (34). 100 μl of the medium containing heparin-extractable LPL from tissues were incubated with 2 μg of 5D2 monoclonal antibody for 1 h on ice before addition of the radiolabeled triolein substrate.

Plasma Parameters—Blood was taken from control and transgenic mice by eye bleeds using heparinized capillary tubes following a 4-h fasting period, and plasma was prepared immediately. Triglycerides (Sigma Diagnostics), total plasma cholesterol (Sigma Diagnostics), and NEFA (WACO) levels were measured enzymatically with commercially available kits, and all manufacturer's recommendations were followed. Postheparin plasma was drawn from the tail 15 min after the intraperitoneal injection of heparin (100 units/kg).

Lipoprotein Analysis—Samples from several animals were pooled and equal volumes were injected into the sample loop of a fast protein liquid chromatography (FPLC) apparatus as described (21). Fifty fractions (0.5 ml each) were collected and assayed enzymatically for individual triglyceride and cholesterol content.

LPL Synthetic Rate—The synthetic rate of LPL was measured in adipocytes using a 30-min pulse with [^{35}S] methionine (100 μCi/ml), as described previously (35). The unincorporated label was aspirated, and the total cellular proteins were extracted in lysis buffer containing 50 mM phosphate buffer, pH 7.4, 2% deoxycholate, 1% SDS, 20 mM phen-

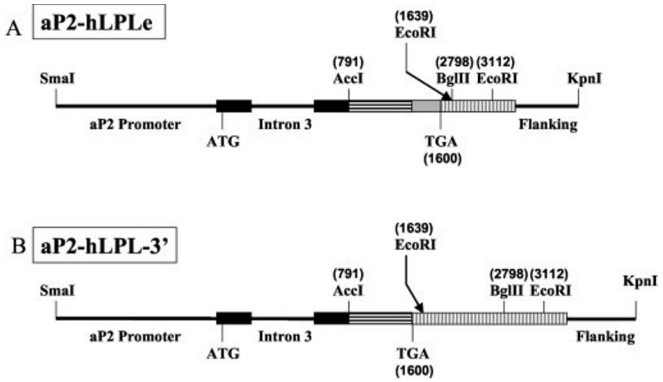

FIG. 1. **The hLPL transgenic constructs.** Schematic representation of the transgenic constructs used to make transgenic mice. The first construct (aP2-hLPLe) contains the *aP2* promoter, an influenza hemagglutinin epitope (*e*), and lacks the proximal 3′-UTR. The second construct (aP2-hLPL3′) is similar, except there is no epitope, and it contains the full-length 3′-UTR. Base pair numbers are given in *parentheses*, and relevant restriction enzyme sites are indicated. *Solid black boxes* show coding sequence regions of *hLPL*. The *gray bar* in the *A box* indicates the influenza hemagglutinin epitope. The *vertically striped* region indicates the 3′-UTR.

ylmethylsulfonyl fluoride, 2 mM leupeptin, and 2 mM EDTA. The extracts were immunoprecipitated using the monoclonal anti-HA antibody (Covance) according to manufacturer's recommendations, followed by immunoprecipitation with a polyclonal affinity purified anti-LPL antibody as previously described (36). Immunoprecipitated samples were analyzed on 10% SDS-PAGE followed by autoradiography. In some experiments, the immunoprecipitation was performed without prior [^{35}S]methionine labeling, and the samples were then analyzed by SDS-PAGE, and Western blotted, as described above, using the polyclonal anti-LPL antibody.

Statistical Analysis—All data were expressed as the mean ± S.D. Statistical significance was set at the $p < 0.05$ level, and all comparisons used the Student's *t* test.

RESULTS

Transgenic Mice Were Generated That Expressed LPL in Adipose Tissue—The constructs used to generate transgenic mice are shown in Fig. 1. In both constructs, the transgene contained the *aP2* promoter for adipose-specific expression, and the entire coding sequence of human *LPL*. The construct in Fig. 1*A* contained only the distal 3′-untranslated region beginning with nucleotide 2798, and also contained an influenza hemagglutinin epitope such that transgenic LPL could be easily distinguished from endogenous mLPL. This construct was designated aP2-hLPLe to indicate the human origin and the presence of an epitope. Mice were also generated using a different construct (termed aP2-hLPL3′), which contained the full-length 3′-UTR, and designated hLPL3′ mice (Fig. 1*B*).

Mice were initially identified as transgenic or non-transgenic based on a PCR assay from tail-clipped DNA (data not shown). Southern blotting of hLPLe mice with a labeled hLPL probe, which hybridized to both mouse and human LPL sequences showed differing levels of transgene copy number in the founders (Fig. 2*A*). Based on the Southern blot, mice were designated as a high or low expressor. For example, in Fig. 2*A*, the mouse represented in *lane 2* was a low expressor, and the mice in *lanes 3*, *4*, and *5* were designated high expressors. Similar differences in gene copy number were observed in the hLPL3′ mice probed with a hLPL-specific cDNA fragment (Fig. 2*B*).

Expression of hLPL mRNA—To study LPL mRNA expression, Northern blotting was performed. In hLPL3′ mice, adipose and muscle tissues were probed with cDNAs specific to either hLPL or mLPL (see "Experimental Procedures"). As shown in Fig. 3, the hLPL transcript was readily detected in transgenic mice, and was not present in non-transgenic control

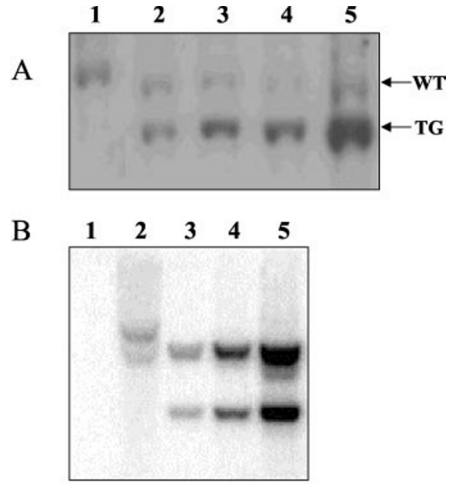

FIG. 2. **Expression of the LPL transgene.** Representative Southern blots to confirm integration of the transgene and determine the copy number of the transgenes. 10–15 μg of tail DNA were digested with *Eco*RI or *Pvu*II, Southern blotted, and detected with different *hLPL* probes as described under "Experimental Procedures." *A*, Southern blot of hLPLe mice. *Lane 1* contains DNA from a non-transgenic mouse. *Lane 2* represents DNA from Founder 2, a low expressor of the transgene. *Lanes 3 and 4* show DNA from Founder 4, and *lane 5* shows DNA from Founder 5, a high expressor of the transgene. The probe used for this blot recognized both mLPL and hLPL, and the *arrows* indicate the endogenous mLPL and the smaller hLPLe hybridizations. *B*, Southern blot of hLPL3′ mice. The blots were performed in a similar manner, but the probe was specific for hLPL. *Lane 1*, mouse control DNA; *lane 2*, human control DNA; *lanes 3–5*, aP2-hLPL3′ mouse DNA from different founders.

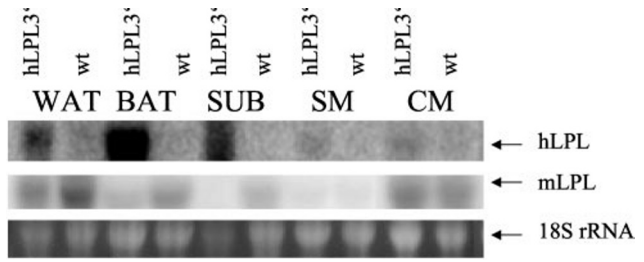

FIG. 3. **LPL mRNA expression in hLPL3′ mice.** Total RNA was isolated from adipose and muscle tissues of fed mice, and probed with specific cDNAs, as described under "Experimental Procedures." *A*, representative blot probed with a human-specific LPL cDNA. *B*, blot A was stripped and hybridized with an mLPL-specific cDNA probe. *C*, RNA loading on the 1% agarose gel (ethidium bromide staining). *SUB*, subcutanous adipose tissue; *SM*, skeletal muscle; *WAT*, abdominal white adipose tissue. *CM*, cardiac muscle; *BAT*, brown adipose tissue.

mice, whereas the mLPL mRNA transcript was detected in both control and transgenic mice.

In hLPLe mice, which lacked the proximal 3′-UTR, the level of hLPL mRNA expression was much lower, such that the hLPL mRNA could not be detected with Northern blotting (data not shown). Hence, RT-PCR was used to detect and quantitate the level of hLPL and mLPL mRNA. Using RT-PCR of different cycle numbers, mRNA expression of hLPLe and mLPL in WAT was quantitated as described in detail under "Experimental Procedures" and illustrated in Fig. 4*A*. The normalized densities of mLPL and hLPLe increased in parallel, and mLPL mRNA could be detected at approximately equivalent levels in both transgenic and wild-type mice. However, the transgene could be detected only in transgenic mice and at much lower levels compared with the endogenous *mLPL* gene (Fig. 4*B*). As shown in Fig. 4*C*, the amount of mLPL mRNA was similar between control and transgenic mice, but the total amount of LPL mRNA was higher in the transgenics due to the presence of the hLPLe transgenic mRNA ($p = 0.054$).

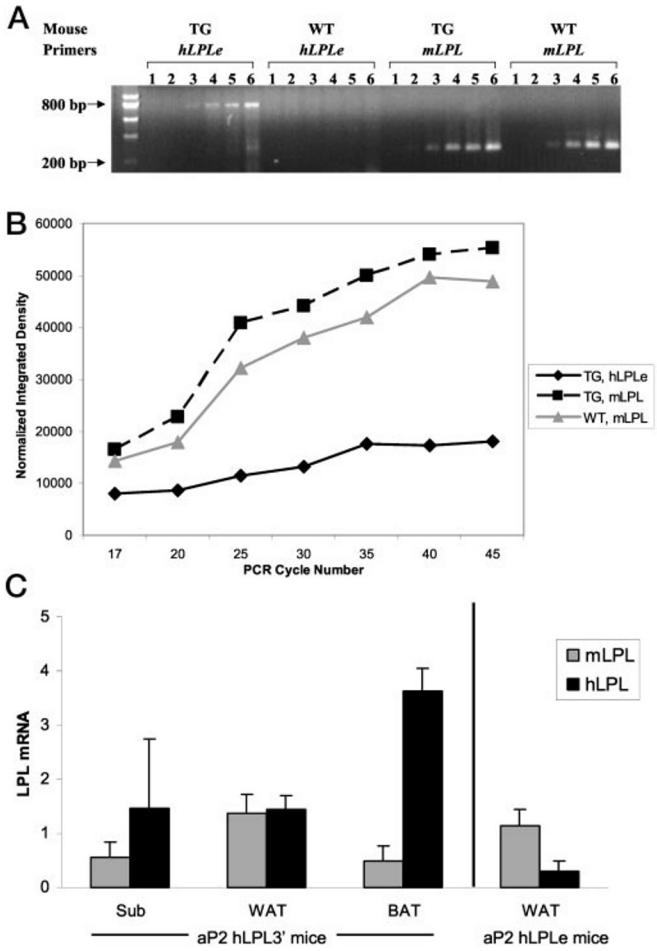

FIG. 4. **LPL mRNA expression in hLPLe mice.** *A*, quantitative RT-PCR for the aP2-hLPLe transgene and mLPL in transgenic (*TG*) and non-transgenic (*WT*) mice. RNA from WAT of transgenic and non-transgenic mice was isolated and 5 μg was used to synthesize cDNA. PCR was performed at different cycle numbers to ensure linearity of the product. This gel is representative and shows 15, 17, 20, 25, 30, and 35 cycles of PCR, *lanes 1–6* respectively, for each group. *B*, integrated densities of quantitative PCR products were normalized to markers of known quantities of DNA and values were plotted. Graph is representative of two independent experiments with a minimum of 5 different PCR cycle numbers. *C*, relative mRNA levels of total LPL mRNA in transgenic and wild-type mice. Normalized integrated densities of quantitative PCR products for hLPLe and mLPL were added together to give total LPL mRNA levels in transgenic mice, and mLPL levels represent total LPL for wild-type mice. Values shown represent the average of six independent values from pooled mouse adipocytes. Total LPL mRNA was significantly higher ($p = 0.054$) in aP2-hLPLe mice.

Expression of the Transgene Increased LPL Activity in WAT—To determine whether there were increased levels of LPL expression, we examined LPL activity (LPLa) in different tissues of transgenic and control mice. In hLPL3′ mice containing the complete *hLPL* 3′-UTR, there was a significant increase in LPLa in WAT of about 2-fold control levels, and also an increase in LPLa in brown adipose tissue (BAT) ($p < 0.05$)(Fig. 5*A*). By using a human-specific antibody, we found that the increase in LPLa above that of control mice was entirely due to the transgene expression of *hLPL*. In the 3′-UTR-deficient hLPLe mice, we also detected a 2-fold increase in LPL activity, however this increase was found only in WAT (Fig. 5*B*). Using the same antibody, this increase in LPLa was also due to the hLPL expression (Fig. 5*B*). To determine if this increase in LPLa correlated with expression level of the transgene in hLPLe mice, mice were separated into low expressor or high expressor groups, based on Southern blot data, as described in Fig. 2, and LPL activity in adipose tissue was again assayed. No significant difference was seen between controls and low expressors (Fig. 5*C*). However, a significant 2-fold increase ($p = 0.001$) was seen with the high expressor group as compared with controls (Fig. 5*C*). This increase in LPLa was accompanied by higher levels of hLPLe levels when adipose tissues from these mice were Western blotted with antibodies to hLPLe (Fig. 5*D*). Therefore, higher expression levels of hLPLe correlated with increased LPL activity in WAT.

To examine total LPL, we measured postheparin plasma LPL. Mice were injected intravenously through the tail vein with 100 μg/kg heparin, and were bled 15 min later. Although adipose tissue LPL activity was increased, as described above, there was no significant change in postheparin plasma LPL, as shown in Table I. In addition, there was no change in plasma triglycerides, cholesterol, or NEFA (data not shown). To determine whether there were any subtle changes in lipoproteins, fasting blood was obtained from four high expressor male hLPLe mice, along with age/gender-matched controls, and subjected to FPLC lipoprotein analysis. As shown in Fig. 6, there were no differences in the migration pattern of the VLDL, LDL, or HDL fractions, and no differences in lipid composition.

Despite Increased LPL Activity in WAT, No Difference in Body Weight Was Observed between Transgenic and Wild-type Mice—With higher levels of LPL activity in WAT, one might expect an increase in total body weight or fat pad weight in transgenic mice, which may be exacerbated by high fat feeding. Hence, weight was determined in mice fed either a normal chow diet or a high fat diet over a 3-month period following weaning. No significant difference in total body weight gain was observed in either hLPL3′ mice (data not shown) or in hLPLe mice on a chow diet. When fed a high fat diet following weaning, both transgenic and control mice gained more weight than chow-fed mice, but no significant difference in total weight gain between transgenic mice and controls was noted (Fig. 7*A*). Similarly, there were no significant differences in WAT fat pad weights between age- and sex-matched transgenic and wild-type mice (Fig. 7*B*).

In previous studies, mLPL translation was inhibited by catecholamines and thyroid hormone (8, 9). Because the aP2-hLPLe construct did not contain the 3′-UTR response element for this translation inhibition, we wished to determine the efficiency of LPL translation of mLPL and hLPLe. To demonstrate the presence of the hLPLe protein in these mice, Western blotting was performed using the anti-epitope antibody. As shown in Fig. 5*D*, the anti-epitope antibody recognized the hLPLe protein in aP2-hLPLe adipocytes, and not in control mouse adipocytes.

To better demonstrate the proportional expression of the hLPLe and mLPL proteins, adipocytes were isolated from WAT from high expressor hLPLe mice, and Western blotting was performed with either anti-LPL, or anti-epitope antibodies. As shown in Fig. 8*A*, hLPLe mice demonstrated more total LPL, and this increase in total LPL was essentially entirely explainable by the increase in hLPLe, demonstrated using the anti-epitope antibody. To further evaluate LPL synthesis, we performed immunoprecipitation of LPL after pulse labeling adipocytes with [^{35}S]methionine. Adipocytes from hLPLe mice were labeled and immunoprecipitated with anti-LPL antibodies, followed by SDS-PAGE and autoradiography. As shown in Fig. 8*B*, hLPLe mice (*lane 1*) synthesized more total LPL than control mice (*lane 2*). To demonstrate that this increase in LPL synthesis was due to the hLPLe protein, sequential immunoprecipitation following [^{35}S]methionine labeling was performed. Adipocytes from hLPLe and control mice were labeled with [^{35}S]methionine as described under "Experimental Procedures," and immunoprecipitated first with anti-epitope anti-

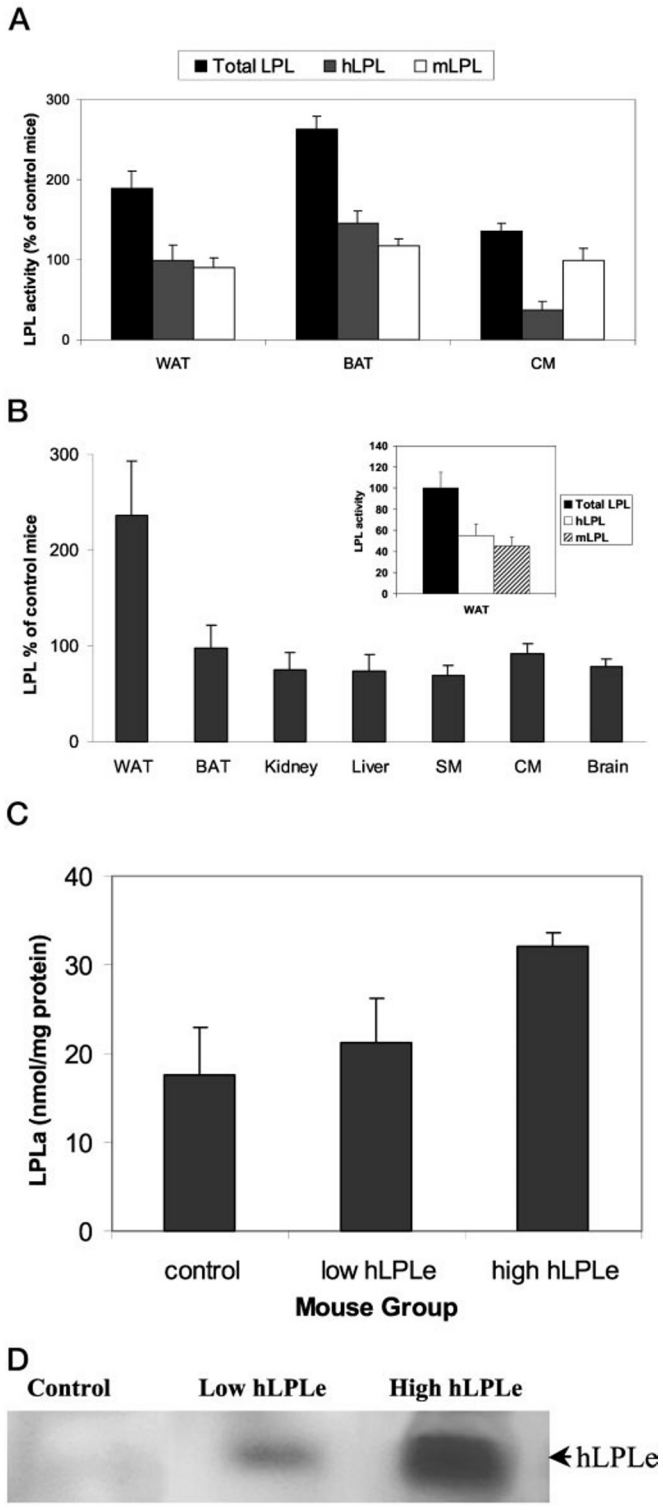

FIG. 5. **LPL activity in transgenic or non-transgenic mice.** Total LPL activity (LPLa) was measured in tissue extracts of hLPL transgenic (and control) mice. *A*, LPLa in hLPL3′ mice. In WAT, BAT, and cardiac muscle, LPLa was measured in the presence and absence of an anti-hLPL antibody, as described under "Experimental Procedures." LPL activity that was not inhibited with the antibody was designated mLPL. All values represent mean ± S.D. of 8 mice. *B*, LPLa in hLPLe mice. LPL activity in a variety of tissues was compared with wild-type mice. *Inset*, total LPLa, hLPLa, and mLPLa were measured in WAT as described under "Experimental Procedures." All values represent mean + S.D. of 14 mice. *C*, increase in LPLa corresponds with expression levels of aP2-hLPLe in *hLPLe* transgenic mice. Total LPLa was measured in WAT from control, low expressor (*low hLPLe*), and high expressor (*high hLPLe*) transgenic mice. LPLa was 17.6 ± 9, 21.1 ± 9, and 32.0 ± 3 nmol/mg of protein in the control, low expressor, and high expressor mice, respectively. All values represent the average ± S.D. of 6 mice. *D*, hLPLe protein in WAT of hLPLe mice. Adipose tissue from 2 mice in each groups were pooled and blotted with antibodies to the influenza hemagglutinin epitope.

TABLE I

Plasma lipase and lipid concentrations in transgenic and control mice

Blood was drawn from fasted transgenic and control mice (8-12 weeks old), and plasma was immediately prepared. Postheparin plasma LPL activity was determined, and the concentrations of triglycerides, cholesterol, and NEFA were measured.

	PHP LPLa	Triglyceride	Cholesterol
	μmol FFA/ml/h	*mg/100ml*	*mg/100ml*
Control	7.7 ± 1.8	89 ± 4	67 ± 5
hLPLe	10.3 ± 2.5[a]	85 ± 4	65 ± 4
hLPL3	9.43 ± 2.8[b]	79 ± 21	65 ± 11

[a] Four-hour fasted.
[b] Overnight fasted.

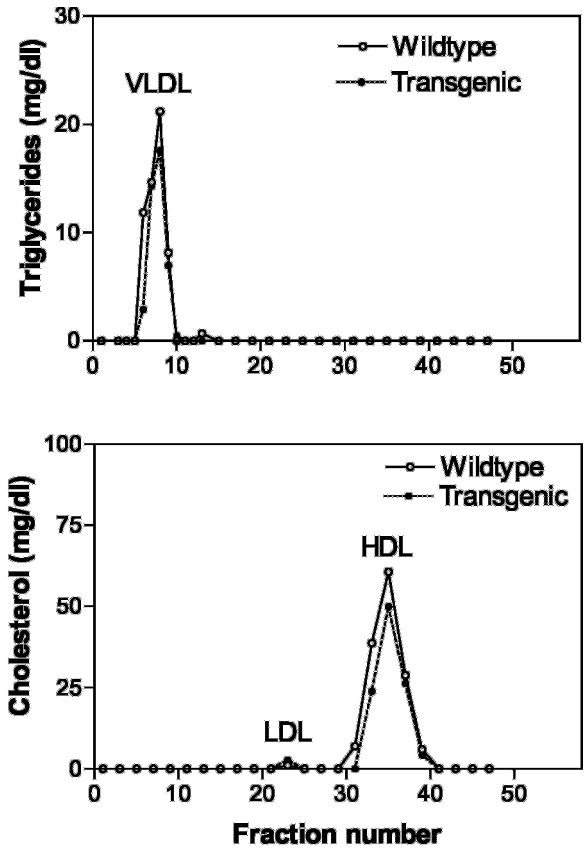

FIG. 6. **Lipoprotein profiles of hLPLe transgenic mice.** Pooled serum samples from transgenic mice (*solid symbols*) and control wild-type mice (*open symbols*) were separated by gel filtration chromatography. Individual fractions were assayed for triglyceride (*top panel*) and cholesterol (*bottom panel*) content using enzymatic techniques. VLDL peaks spanned fractions 6–10, LDL fractions 20–25, and HDL 30–40.

body, and then with anti-LPL to bind all remaining LPL species. This material was then analyzed by SDS-PAGE and autoradiography. As shown in Fig. 9, control mice demonstrated no immunoprecipitable material with anti-epitope antibody, as expected. In contrast, the hLPLe mice demonstrated approximately equal quantities of LPL immunoprecipitated with anti-epitope, and subsequently with anti-LPL.

Therefore, when comparing these two colonies of transgenic mice, the hLPL3′ mice demonstrated expression of hLPL in proportion to the increase in hLPL3′ mRNA, as shown in Fig. 10. On the other hand, the hLPLe mice demonstrated similar increases in LPLa, and parallel increases in LPL protein and

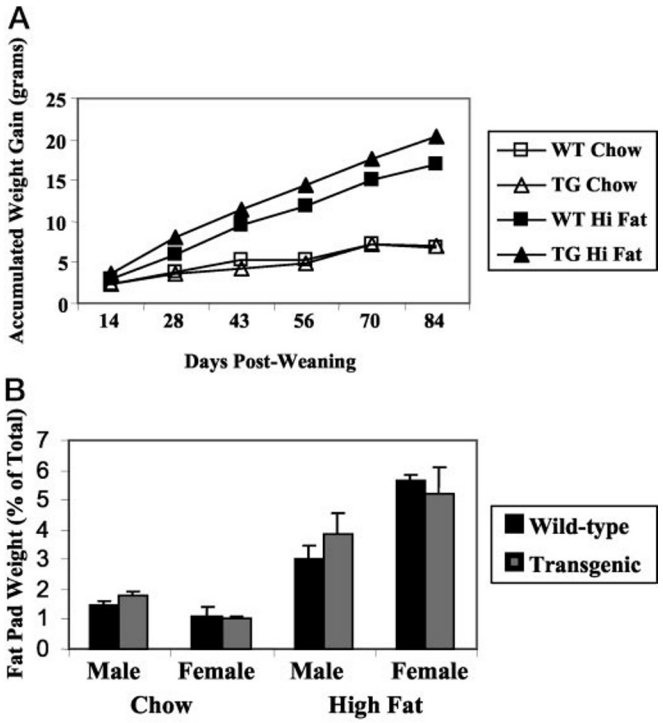

FIG. 7. **Body and fat pad weights for transgenic and wild-type mice.** *A*, cumulative weight gain for 3 months for transgenic and wild-type hLPLe mice fed regular chow or high fat diets after weaning. Total body weight (grams) of 20 animals of each group (wild-type mice fed chow diet, transgenic mice fed chow diet, wild-type mice fed high fat diet, and transgenic mice fed high fat diet) was determined every 14 days for a time course of 12 weeks. Values shown represent the average total weight gain. Standard deviations did not exceed 35% of the mean (data not shown). Weight gain in the hLPL3′ mice were similar. *B*, epididymal fat pad weights presented as the percentage of total body weight for hLPLe transgenic and wild-type mice fed either regular chow or high fat diets. Total body weights were acquired before sacrifice, and subsequently epididymal fat pads were removed and weighed. Values shown are the average percentages for 10 mice in each group + S.D. Fat pad weights were similar in the hLPL3′ mice.

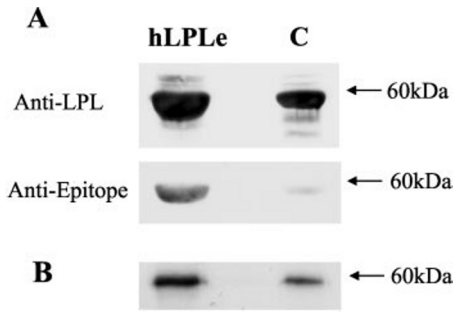

FIG. 8. **Low levels of hLPLe mRNA produce high levels of hLPLe protein.** *A*, representative Western blot to show protein expression of hLPLe in transgenic mice. Equivalent protein amounts of white adipose tissue lysate from either non-transgenic mice (*C*), or hLPLe mice were resolved by 10% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and detected with either an anti-LPL antibody, or a monoclonal antibody which recognized the influenza hemagglutinin epitope tag on the hLPLe protein. *B*, pulse-labeling and immunoprecipitation of LPL. Adipocytes from either control (*C*) or hLPLe mice were pulse-labeled with [^{35}S]methionine and immunoprecipitated with anti-LPL antibody and resolved on a gel as described above.

LPL synthesis, as shown above, but with only a very low level of hLPLe mRNA expression (Fig. 10). These data suggest that the hLPLe mRNA was preferentially translated, when compared with the mLPL transcript, such that the overall level of LPL activity was modestly increased in the transgenic adipocytes.

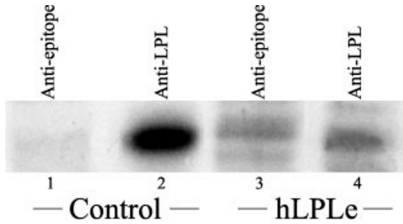

FIG. 9. **Sequential immunoprecipitation of hLPLe and mLPL.** Adipocytes from control and hLPLe mice were labeled with [^{35}S]methionine and first immunoprecipitated with antibodies to the influenza hemagglutinin epitope (anti-epitope, *lanes 1* and *3*). The supernatant of this reaction was subsequently immunoprecipitated with anti-LPL (*lanes 2* and *4*). Both the anti-epitope and the anti-LPL fractions were then resolved on a 10% SDS-PAGE.

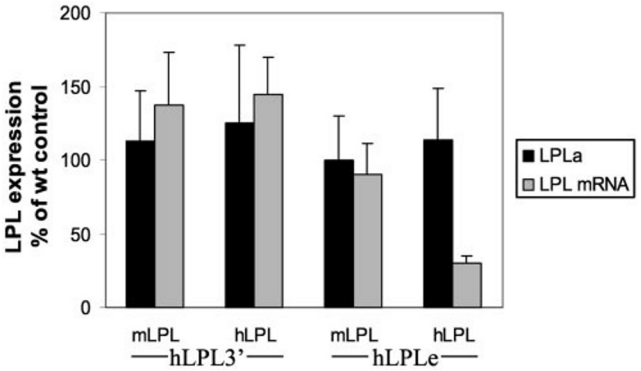

FIG. 10. **LPL mRNA and LPLa in hLPL3′ and hLPLe mice.** LPL mRNA and activity was measured in the WAT of transgenic and wild-type mice, as described under "Experimental Procedures," and normalized to mLPL mRNA or activity in wild-type mice.

DISCUSSION

To study the physiologic role of the translational regulation of LPL, we generated two transgenic mouse colonies. Both colonies used similar constructs that were driven by the *aP2* promoter and contained intron 3 and 3′-flanking sequence. These constructs differed primarily in the presence or absence of the proximal 3′-UTR up to nucleotide 2614. Although there have been numerous reports of transgenic mice with muscle- or heart-specific overexpression (24–26, 37), this is the first report of transgenic mice that specifically overexpress LPL in adipose tissue.

In these studies, mice with either construct demonstrated high expression levels of hLPL and ~2-fold higher LPL activity in WAT. With the hLPL3′ mice, there was also increased LPL expression in BAT, although this was not observed in the hLPLe mice. The reason for this apparent difference in BAT expression is not clear. In the hLPLe mice, mRNA expression was low overall, and perhaps the lack of the proximal 3′-UTR resulted in even lower expression of the LPL protein in BAT, which is regulated differently from WAT. With higher levels of LPL activity, we expected higher body weights or fat pad weights in these mice. However, no differences in body weight were found, either when the animals were fed either normal chow or high fat diets. There are several possible reasons for this lack of change in adiposity. In the transgenics, hLPL may have downregulated mLPL in adipocytes, or total body LPL may be unchanged, due to a down-regulation of LPL in other tissues. Other possibilities include an increase in energy expenditure, perhaps due to an increase in thermogenic activity in brown adipose tissue, or other homeostatic mechanisms, which may decrease caloric intake or otherwise limit weight gain.

To examine total LPL, we measured postheparin plasma LPL and adipose tissue LPL activities. Although adipose tissue LPL activity was increased, and no decrease in muscle LPL was

detectable, there was no significant change in postheparin plasma LPL, and no significant change in triglycerides. This lack of change in postheparin plasma LPL may simply reflect the modest increase in the adipose enzyme in relatively young and lean mice.

In adipocytes from transgenic mice, we examined the co-expression of mLPL and hLPL. In the hLPL3′ mice, which contain the 3′-UTR, the hLPL mRNA was readily detectable by Northern blotting, and was approximately as abundant as the mLPL mRNA. Similar observations were found with LPL activity in hLPL3′ mice, such that hLPL mRNA and activity were expressed at the same levels, relative to mLPL expression. However, in the hLPLe mice, which lacked the proximal 3′-UTR, there was a striking discordance between LPL mRNA and LPL protein expression. The hLPL mRNA was undetectable by Northern blotting, yet both forms of LPL mRNA were readily detectable by RT-PCR. We found that mLPL was expressed at similar levels in both control and transgenic mice whereas the hLPLe mRNA was present only at low levels. This low mRNA expression of hLPLe was high enough, however, to yield significantly more total LPL mRNA in transgenic mice as compared with controls. Importantly, the opposite phenomenon was observed when we compared co-expression of mLPL and hLPLe activity and protein. As demonstrated by the Western blotting, the immunoprecipitation of pulse-labeled proteins, and the sequential immunoprecipitations, hLPLe was as abundant as mLPL in hLPLe mice despite the low level of hLPLe mRNA. In low expressing transgenic mice, less hLPLe protein was detected, and there was a compensatory increase in the amount of mLPL protein present, suggesting that the level of the transgene regulated the level of endogenous LPL in an inversely proportional relationship.

Catecholamines inhibit LPL activity, and this is physiologically relevant in the suppression of lipogenesis and the mobilization of adipose tissue lipid during periods of fasting or exercise (1, 38). In *in vitro* studies, this regulation is dependent on the presence of the first 24 nucleotides of the 3′-UTR of *LPL* (39) and occurs at the level of translation. Thus, mice expressing LPL in adipose tissue with the hLPLe construct described herein would be predicted to have up-regulated LPL translation. This up-regulation of hLPLe translation is likely due to the absence of the 3′-UTR of the hLPLe construct. As described in previous studies, LPL translation is inhibited by epinephrine and thyroid hormone due to the stimulation of an RNA-binding protein that interacts with the first 24–40 nucleotides of the 3′-UTR (39, 40). Both epinephrine and thyroid hormone are constitutively present, and hypothyroid rats demonstrate a translational up-regulation of LPL (41). Therefore, the absence of the proximal 3′-UTR would be predicted to remove this constitutive, hormonally mediated, translational inhibition, resulting in an LPL mRNA that is more efficiently translated, as described in hLPLe mice.

The 3′-UTR is important for mRNA stability, but can also be involved in translational efficiency and initiation (42). For example, creatine kinase translation efficiency is inhibited by a 3′-UTR-binding protein (43, 44), and 15-lipoxygenase mRNA initiation is inhibited by the binding of a 48 kDa protein to a repeated pyrimidine-rich sequence on the 3′-UTR (45). In some instances there may be a complex involvement of the 3′-UTR with the coding sequence or the 5′-UTR, as has been demonstrated for β-interferon mRNA when expressed in *Xenopus* oocytes (46), and ornithine decarboxylase regulation by polyamines (47, 48). The poly(A) tail of the mRNA controls mRNA stability, but also is involved with translation regulation (49). It is possible that our transgenic mRNA may be less stable than endogenous mLPL mRNA because of the deletion of most of the 3′-UTR. However, this level of hLPLe mRNA was enough to give high levels of hLPLe protein because the 3′-UTR deletion removed sites where factors inhibitory to translation would normally bind.

In summary, we report two transgenic mouse colonies that express LPL in adipose tissue. These colonies differ primarily in the presence/absence of the proximal 3′-UTR of LPL. When compared with transgenic mice containing the 3′-UTR, the levels of hLPLe protein are much higher than one would expect based on the levels of corresponding mRNA. The 3′-UTR is important for inhibitory influences by hormones and catecholamines. Therefore, these data are consistent with the expression of a translationally unrepressed LPL due to the lack of most of the 3′-UTR, and with negative feedback to endogenous mLPL from the high levels of transgenic protein. Studies utilizing these mice may yield important information about LPL biology, translational regulation in general, and the potentially important role of the 3′-UTR of LPL in normal physiology.

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