Alternatively Spliced Lipin Isoforms Exhibit Distinct Expression Pattern, Subcellular Localization, and Role in Adipogenesis*

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We recently identified mutations in the Lpin1 (lipin) gene to be responsible for lipodystrophy in the fatty liver dystrophy (fld) mouse strain. Previous studies revealed that lipin plays a critical role in adipogenesis, explaining the adipose-deficient phenotype of the fld mouse. In the current study, we demonstrate that alternative mRNA splicing generates two lipin isoforms, lipin-α and lipin-β, which are differentially expressed during adipocyte differentiation. Lipin-α expression peaks at day 2 of 3T3-L1 cell differentiation, after which its levels gradually decrease. In contrast, lipin-β expression is transiently elevated at 10 h, followed by a drop to background levels at 20 h and a gradual increase between days 2 and 6 of differentiation. The two lipin isoforms also exhibit differences in subcellular localization. Lipin-α is predominantly nuclear, whereas lipin-β is primarily located in the cytoplasm of 3T3-L1 adipocytes, suggesting distinct cellular functions. Using primary mouse embryonic fibroblasts expressing either lipin-α or lipin-β, we demonstrate functional differences between the two isoforms. Whereas lipin-α is required for adipocyte differentiation, the predominant effect of lipin-β expression is the induction of lipogenic genes. In vivo, overexpression of lipin-β specifically in mature adipocytes leads to elevated expression of lipogenic genes and adipocyte hypertrophy, confirming a role of lipin-β in the regulation of lipogenesis. In conclusion, our data suggest that the two lipin isoforms have distinct, but complementary, functions in adipogenesis, with lipin-α playing a primary role in differentiation and lipin-β being predominantly involved in lipogenesis.

Lipodystrophies are a group of disorders characterized by loss of adipose tissue and the ensuing development of metabolic abnormalities of lipid and glucose metabolism (1). We previously isolated a novel gene, Lpin1 (lipin), which is responsible for lipodystrophy in a naturally occurring mouse model of this disease, the fatty liver dystrophy (fld) mouse strain (2). Lpin1 mutations in fld mice lead to dramatically reduced body fat, insulin resistance, and increased susceptibility to diet-induced atherosclerosis (3). Lipin is a novel protein without any known protein domains other than a nuclear localization signal. However, it contains two evolutionarily conserved regions (NLIP and CLIP domains), which identify a novel gene family including two additional members in mammals, Lpin2 and Lpin3, and several orthologous genes in invertebrates, plants, and fungi (2). Recent studies in Schizosaccharomyces pombe revealed interactions between the NLIP domain and several nuclear proteins (4). Although the molecular function of lipin remains to be identified, studies in yeast suggest a role in the maintenance of nuclear envelope structure and nuclearcytoplasmic transport.

Consistent with the effects of lipin mutations on adipose tissue function, lipin is prominently expressed in adipose tissue (2). Lipin is phosphorylated in response to insulin stimulation, which suggests an important role for this protein in adipose tissue (5). However, unlike other markers of adipocyte differentiation, lipin exhibits a unique biphasic expression pattern during the conversion of 3T3-L1 fibroblasts into adipocytes (6). First, coincident with the expression patterns of the early adipogenic transcription factors CCAAT enhancer-binding protein (C/EBP)β and C/EBPδ, lipin expression transiently peaks at 10 h into the differentiation program before returning to a low level by 20 h. In a second wave of expression, lipin expression gradually increases during days 2–6 of differentiation and reaches highest levels in mature adipocytes. Using lipin-deficient cells, we demonstrated that early lipin expression is required prior to peroxisome proliferator-activated receptor (PPAR)γ for adipocyte differentiation to occur (6). Using a transgenic mouse model, we have recently shown that lipin overexpression in mature adipocytes leads to increased lipogenic gene expression and fat accumulation (7). Collectively, these studies suggest distinct roles for lipin in adipocyte differentiation and lipogenesis (8).

In the current study, we demonstrate that the Lpin1 mRNA undergoes alternative splicing, which generates two lipin isoforms, lipin-α and lipin-β. We show that the two isoforms exhibit differences in expression, subcellular localization, and cellular function. Our results suggest that the lipin isoforms have complementary roles in adipogenesis, with lipin-α playing a primary role in differentiation, whereas lipin-β is predominantly involved in the regulation of lipogenesis in mature adipocytes.

MATERIALS AND METHODS

Cell Culture, Transfection, and Retroviral Infection—3T3-F442A and 3T3-L1 fibroblasts (kindly provided by Dr. Bruce Spiegelman) were propagated in Dulbecco’s modified Eagle’s medium containing 10% calf serum. Pre-adipocytes were differentiated into adipocytes as described (9). 3T3-L1 fibroblasts were transfected with FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer’s instructions. 3T3-L1 adipocytes were transfected by electroporation (10). Isolation of primary mouse embryonic fibroblasts (MEFs) and in vitro differentiation into adipocytes were performed as described (6).
Reconstruction of lipin-deficient MEFs was performed by retroviral infection with pBABE retroviral vectors containing no insert, or lipin-α or lipin-β inserts (constructs described below) (11). To ensure infection of all cells used for analyses, MEFs were selected with 2 µg/ml puromycin for 4 days.

Plasmid and Retroviral Constructs—To generate vectors for the expression of lipin with the V5 epitope tag, full-length lipin-α (GenBank™ accession number NM_172950) and lipin-β (NM_015763) cDNAs were PCR-amplified using the primers GCC TAG CCA CCA TGA AGT CAG GGC AGC TGG CC and GCC TAG CCA TGA GGC ATG CAT GTC, and TA-cloned into the pcDNA3.1-V5/His-TOPO expression vector (Invitrogen). To produce retroviral vectors for expression of lipin isoforms, lipin-α and -β cDNAs with 3′-FLAG epitope tag were subcloned into the pBABE retroviral vector, and expression in MEFs was performed as described (11). All constructs were verified by sequencing.

Reverse Transcription PCR and RNA Quantitation—Total RNA was isolated with RNeasy kit (Qiagen) and reverse-transcribed using SuperScript II reverse transcriptase (Invitrogen). Relative expression of lipin-mRNAs could be distinguished based on the distinct combinations of 5′-untranslated exons: 1A-3, 1B-2–3, and 1B-3 (Fig. 1a) (32884). The number of replicates for each study is indicated in the figure legends. Values are represented as means ± S.E. Two-tailed unpaired Student’s t test was used to calculate p values.

RESULTS

Alternative Splicing of Lipin—Our initial studies on lipin indicated the presence of Lipin1 mRNAs of different sizes (2). To identify the source of this heterogeneity, we surveyed EST sequences belonging to the Lipin1 UniGene cluster (Mm.153625). This analysis revealed that several mRNA variants are produced from this gene (Fig. 1a). Alignments with genomic sequences indicated alternative polyadenylation sites (data not shown) and the presence of several alternatively utilized exons flanked by consensus splicing motifs (Fig. 1b). Three types of Lipin1 mRNAs could be distinguished based on the distinct combinations of 5′-untranslated exons: 1A-3, 1B-2–3, and 1B-3 (Fig. 1a).

**TABLE ONE**

| Primer | Sequence |
|--------|----------|
| Lipin-p1 | GCA GCT CCC TAG CCA TGA |
| Lipin-p2 | GCC TGC TCG TGA ATC CTC |
| Lipin-p3 | CGA TGC ATC CCG ACA |
| Lipin-p4 | CCC TGC ATT TCA AGC CAC CTT |
| Lipin-p5 | GGT CCC CCA GCC CCA GTC CTT |
| Lipin-p6 | CAG CTT GGT AGA TTG CCA GA |
| Lipin-p7 | GCC GTG TGT GCC AAT TCA |
| Adipsin-f | GCA CAC TGC ATG GAT GGA GT |
| Adipsin-r | CTA GAG GGC TGC CGG AGT CT |
| Pref-1-f | GCC AGT GCT TCT GCC AGG AT |
| Pref-1-r | GCT CGT GCC CGG GCC ACT |
| FAS-f | CGT GTG GCC CAT CAC CCA GAG CT |
| FAS-r | CGT GGC AGC AGC GCC TCC AGC ACC TT |
| SCD1-f | GGT GAT GTT CCA GAG GAG GTA CT |
| SCD1-r | GGT GCT AAC GAA CAG GCT |

Epidermal adipose tissue samples were harvested from male aP2-lipin transgenic and non-transgenic littermates after 6 weeks on a high fat diet (35% fat and 33% carbohydrate; Diet F3282, Bio-Serve, Frenchtown, NJ). Adipose tissue was fixed in formalin, sectioned at 8 µm, and stained with hematoxylin-eosin. Sections were examined by light microscopy and photographed at a final magnification of ×320. Adipose tissue triglyceride content was determined as described above. Adipose tissue cellularity was determined by fluorometric DNA quantitation using Hoechst dye 33258 from preweighed gonadal adipose tissue depots (14).

Statistical Analyses—The number of replicates for each study is indicated in the figure legends. Values are represented as means ± S.E. Two-tailed unpaired Student’s t test was used to calculate p values.

- Adipocyte and Adipose Tissue Measurements—Six days after the initiation of differentiation, MEFs were stained with Oil Red O to visualize accumulated lipid. Triglyceride content was determined using the GPO-Trinder (Sigma-Aldrich) or L-Type TG H (Wako Chemicals USA, Richmond, VA) kits. Protein content was determined using the Micro BCA Protein Assay kit (Pierce).

- Mice and Adipose Tissue—The aP2-lipin transgenic mouse strain has been described previously (7). To generate these mice, lipin-β cDNA was cloned downstream of the 5.4-kb adipocyte fatty acid-binding protein (aP2) promoter/enhancer (12) and upstream of an SV40 intron/poly(A) sequence (13). The aP2-lipin-β construct was injected into C57BL/6 oocytes and a line of mice expressing 2–3-fold elevated lipin mRNA in adipose tissue was established (7). For experiments, transgenic mice were compared with age- and sex-matched non-transgenic C57BL/6 littermate controls.
Alternative Splicing of Lipin

**FIGURE 1. Alternative splicing of Lpin1 mRNA.** a, the upper part of the figure shows a schematic representation of the exon-intron organization of a portion of the Lpin1 gene. Boxes represent exons and gray shading indicates coding regions. Names and orientations of primers used for RT-PCR are shown above the exons. The lower part of the figure shows alternative splicing patterns of the Lpin1 message as deduced from the analysis of RT-PCR products. Solid lines indicate exon sequences retained in mature Lpin1 mRNAs. Pre, preadipocyte; Ad, adipocyte; Tes, testis. b, exon-intron boundaries of alternatively spliced Lpin1 exons. Intron and exon sequences are shown in lower and uppercase, respectively. Canonical splice signals are underlined. Exons 1A and 1B do not exhibit splice acceptor sequences as they are likely alternative first exons of the gene. c, equivalent amounts of cDNA derived from various mouse tissues were analyzed by PCR using primers p4 and p7. d, domain structure of lipin-α and -β isoforms. Boxed with diagonal lines indicates the 33-amino acid insertion in lipin-β, which results from alternative splicing of exon 7. The subtable illustrates the putative distal promoter is inactive in adipose tissue. The abundance of Lpin1 splice variants suggests complex post-transcriptional regulation of this gene. To test the potential functional significance of alternative splicing events, we analyzed lipin mRNA species in adipose tissue, one of the primary tissues of lipin expression. We reasoned that functionally important splicing patterns would be recognized as those that change during the conversion of pre-adipocytes into mature adipocytes. Therefore, we compared Lpin1 splicing in 3T3-F442A cells and after in vitro differentiation into adipocytes. The 3T3-F442A cell line was chosen for these studies, because, unlike 3T3-L1 fibroblasts, these cells express significant amounts of Lpin1 mRNA even in the undifferentiated state. Using RT-PCR, exon 1A-containing Lpin1 messages could not be detected either before or after differentiation in 3T3-F442A cells, but were present in testis (Fig. 1a) suggesting that the putative distal promoter is inactive in adipose tissue. Transcripts beginning with exon 1B were readily detectable in 3T3-F442A cells and showed an overall increase in abundance during adipocyte differentiation. A similar induction during differentiation was seen regardless of whether exon 2 was also present (compare levels of 1B-2-3 and 1B-3). In contrast, lipin mRNA containing exon 7 showed a dramatic increase in relative abundance during differentiation. Whereas pre-adipocytes expressed similar amounts of mRNAs with and without exon 7, mature adipocytes predominantly expressed the longer splice variant containing exon 7. Analysis of cDNAs obtained from a panel of mouse tissues revealed differential expression of mRNAs with and without exon 7 in tissues such as heart and brain, suggesting that Lpin1 mRNA splicing may be regulated in these tissues as well (Fig. 1c). Thus, of the alternatively spliced exons examined, only exon 7 appears to be differentially utilized in pre-adipocytes compared with mature adipocytes. Importantly, inclusion of the 99-nucleotide-long exon 7 produces an in-frame addition of 33 amino acids in the mature lipin protein, which may have consequences for protein function (Fig. 1d). The subsequent studies described here focus on the functional characterization of these two splice variants (Lpin1α and Lpin1β) and their corresponding protein isoforms (lipin-α and lipin-β).

**Temporal Changes in Lpin1α/β Splicing during 3T3-L1 Differentiation**—To confirm the results obtained in 3T3-F442A cells and to characterize temporal changes in Lpin1α/β splicing in more detail, we analyzed the adipogenic conversion of the 3T3-L1 cell line. Post-confluent cultures of 3T3-L1 pre-adipocytes were stimulated to differentiate with a hormone mixture containing insulin (9), and RT-PCR was performed at various time points afterward. Differentiation was followed by analyzing the expression of adipsin and Pref-1, markers of mature adipocytes and pre-adipocytes, respectively. As expected, adipin expression was undetectable in pre-adipocytes and gradually increased during differentiation, whereas Pref-1 expression was strongest in pre-adipocytes and decreased during the course of this process (Fig. 2a, lower panels).
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As demonstrated previously, overall Lipin1 expression increased significantly during adipogenesis (2). The most dramatic elevation in expression occurred within the first 2 days of differentiation, after which lipin mRNA levels increased moderately. Consistent with observations in pre-adipocytes (0 h) showed a preponderance of lipin-α-type splicing (Fig. 2a, upper panels). In contrast, Lipin1β was the predominant splice variant present at 10 h, when overall lipin expression peaks (6). At 20 h, expression of Lipin1β dropped dramatically, followed by a gradual increase. To quantify changes in Lipin1 splicing during early differentiation, we performed real-time RT-PCR using primers specific to each of the two splice variants (Fig. 2b). The results indicate that the Lipin1β/Lipin1α ratio increases more than 10-fold between 0 and 10 h, and then decreases by a similar magnitude between 10 and 20 h.

In summary, our data demonstrate dramatic shifts in Lipin1 splicing coincident with changes in Lipin1 expression during adipocyte differentiation. Whereas Lipin1α is the principal splice variant in undifferentiated cells, Lipin1β is the predominant isoform during the transient induction at 10 h as well as in mature adipocytes. The distinct expression dynamics and protein sequence differences between lipin-α and lipin-β suggest independent roles in adipocyte biology.

Lipin Isoforms Exhibit Different Subcellular Localization—As described earlier, the lipin-α and -β isoforms differ in the absence or presence of a stretch of 33 amino acids. Our previous studies demonstrated that lipin-α is a nuclear protein (2). To test whether the additional amino acid sequence present in lipin-β affects its intracellular distribution, we compared the subcellular localization of exogenous lipin-α and lipin-β using fluorescence microscopy. Consistent with previous results, lipin-α exhibited predominantly nuclear localization in mature 3T3-L1 adipocytes (Fig. 3a). In contrast, even though lipin-β also contains the nuclear localization signal, this isoform was predominantly cytoplasmic in most cells examined. Quantitative analyses indicated a ~10-fold difference in the percentage of cells exhibiting cytoplasmic lipin-α versus lipin-β (Fig. 3b).

As shown in Fig. 2a, expression of the Lipin1α splice variant decreased, whereas that of Lipin1β increased during the differentiation process indicating a gradual shift from α-type to β-type splicing (Fig. 2a, upper panels). RNA samples obtained at various times after the addition of differentiation medium were analyzed by RT-PCR using primer pairs specific for lipin, adipsin, and Pref-1. As demonstrated previously, overall Lipin1 expression increased significantly during adipogenesis (2). The most dramatic elevation in expression occurred within the first 2 days of differentiation, after which lipin mRNA levels increased moderately. Consistent with observations in 3T3-F442A cells, expression of the Lipin1α splice variant decreased, whereas that of Lipin1β increased during the differentiation process indicating a gradual shift from α-type to β-type splicing (Fig. 2a, upper panels). RNA samples obtained at various times after the addition of differentiation medium were analyzed by RT-PCR using primer pairs specific for lipin, adipsin, and Pref-1. To determine the splicing pattern of early lipin expression, we analyzed RNA samples collected at 10-h intervals during the first 2 days of differentiation, after which lipin expression peaks (6). At 20 h, expression of Lipin1β dropped dramatically, followed by a gradual increase. To quantify changes in Lipin1 splicing during early differentiation, we performed real-time RT-PCR using primers specific to each of the two splice variants (Fig. 2b). The results indicate that the Lipin1β/Lipin1α ratio increases more than 10-fold between 0 and 10 h, and then decreases by a similar magnitude between 10 and 20 h.

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observed in pre-adipocytes, although the magnitude of the difference (~1.8-fold) was considerably less than in mature adipocytes. These results demonstrate that alternative splicing of exon 7 has a major effect on the subcellular localization of lipin and suggest that the two isoforms may have different cellular functions.

**Lipin-α and Lipin-β Play Distinct Roles in Adipogenesis**—Differences in temporal expression pattern and subcellular localization between lipin-α and lipin-β raised the possibility that the two isoforms may have different functions in adipogenesis. To test this hypothesis, we generated MEFs expressing only one or the other lipin isoform. Lipin-deficient MEFs prepared from *fld* mouse embryos were infected with retrovirus containing *Lpin1* (α) or *Lpin1B* (β), or with empty retrovirus (−) as a control. Infected cells were selected in antibiotic, and lipin isoform expression levels determined by Western blot analysis performed using equivalent amounts of cell extract and probing with anti-FLAG antibody. Triglyceride accumulation in reconstituted MEF cultures (*n* = 3; *, *p* < 0.005 versus vector), #, *p* < 0.001 (lipin-α versus lipin-β). C, oil Red O staining of 6-day differentiated MEF cultures reconstituted with vector, lipin-α, or lipin-β. Images in top and bottom rows were taken at low (×50) and high (×250) magnification, respectively. D, real-time RT-PCR analysis of genes involved in adipocyte differentiation and lipid metabolism in reconstituted MEFs (*n* = 3; *, *p* < 0.05 versus vector).

Reconstitution of lipin expression with the lipin-α isoform resulted in an ~80-fold increase in triglyceride accumulation indicating that lipin-α is required for adipocyte differentiation. Lipin-β had a substantially smaller effect on triglyceride accumulation than lipin-α indicating that the two isoforms are not functionally equivalent. We investigated two possible reasons for the higher triglyceride content in lipin-α compared with lipin-β expressing cells: increased number of cells induced to differentiate and accumulate triglyceride, or increased lipid content per cell. To discriminate between these two possibilities, we stained cultures of differentiated cells with oil red O and examined the distribution of lipid in the cells. We found that a greater proportion of cells stained in lipin-α than lipin-β expressing cultures (Fig. 4c, upper panel). This suggested that lipin-α is more effective at inducing differentiation, thus leading to the observed higher triglyceride content in cultures expressing this lipin isoform. We noticed, however, that the differentiated adipocytes that were present in lipin-β-expressing cultures contained larger lipid droplets than those in lipin-α cultures (Fig. 4c, lower panel). This observation raised the possibility that, in contrast to the involve-
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To characterize the effects of lipin isoforms on adipogenic and lipogenic gene expression, we determined the relative mRNA levels for several genes involved in these processes by real-time RT-PCR. Lipin-α expression led to highly elevated mRNA levels of the adipogenic transcription factors PPARγ and C/EBPα as well as αP2, a marker of mature adipocytes (Fig. 4d). In sharp contrast, lipin-β had no (PPARγ, C/EBPα) or only minor (αP2) effect on the expression of these genes. The analysis of genes involved in lipogenesis and triglyceride synthesis revealed a strikingly different pattern of expression. Lipin-β strongly stimulated the expression of mRNAs encoding critical enzymes of fatty acid (acyl-CoA carboxylase 1 (ACCI), fatty acid synthase (FAS), stearoyl CoA desaturase 1 (SCD1)), glycerol (phosphoenolpyruvate carboxykinase (PEPCK)), and triglyceride (diacylglycerol acyltransferase (DGAT)) synthesis. On this set of genes, lipin-α expression had less pronounced effects than lipin-β. mRNA levels of three genes involved in triglyceride droplet structure or lipolysis (hormone-sensitive lipase, perilipin, and adipocyte differentiation-related protein) were indistinguishable between MEFs expressing lipin-α or lipin-β (data not shown). In summary, these data indicate that the two lipin isoforms exert distinct effects on the expression of adipogenic and lipogenic genes.

Overexpression of Lipin-β Leads to Adipocyte Hypertrophy in Vivo—To confirm the role of lipin-β in lipogenesis in vivo, we characterized aP2-lipin-β transgenic (Tg) mice. These mice have enhanced lipin-β expression under the control of aP2 gene regulatory elements, which direct expression specifically in mature adipocytes (12). This model therefore provides a valuable tool with which to investigate the effect of lipin-β on mature adipocyte functions, such as lipogenesis, without any effect on earlier events in adipocyte differentiation. Adipose tissue from these mice exhibit a 2–3-fold elevation in lipin-β mRNA levels with no alteration in lipin-α mRNA levels (7). Consistent with the in vitro results presented above, we previously showed that adipose tissue from the lipin-β Tg mice exhibits elevated expression of lipogenic genes such as DGAT, but not adipogenic genes such as PPARγ and C/EBPα (7). Examination of gonadal adipose tissue in lipin-β Tg animals revealed that the adipocytes were larger (Fig. 5a) and contained more triglyceride per cell than non-Tg littermates (Fig. 5b). There was no difference in cellularity of the gonadal fat pad (Fig. 5c), which suggests that increased adiposity in the Tg animals is a consequence of elevated lipid accumulation per cell, and not hyperplasia. Thus, our in vitro and in vivo results establish that lipin-β is a positive regulator of lipid accumulation in mature adipocytes.

DISCUSSION

Alternative splicing gives rise to multiple Lipin1 mRNA variants. Here, we characterize splice variants, which give rise to two distinct protein isoforms, lipin-α and -β. Lipin-α and -β exhibit distinct expression profiles during adipocyte differentiation. In pre-adipocytes, lipin-α is the predominant mRNA splice variant, whereas lipin-β is the principal splice form in mature adipocytes. The contrasting expression patterns suggest distinct roles for the lipin isoforms in adipocyte biology.

To investigate the cellular functions of the two lipin isoforms independently, we took advantage of MEFs harboring a null mutation (fld) in the lipin gene (2). Retroviral expression of lipin-α and -β in these cells demonstrates that the two isoforms are functionally non-equivalent. Although both isoforms stimulate overall lipid accumulation, the underlying mechanisms seem to be different. Lipin-α expression dramatically increases the number of cells undergoing differentiation concomitant with elevated levels of PPARγ and C/EBPα, two critical transcriptional regulators of adipogenesis. This result is consistent with our previous studies showing that lipin-α acts upstream of PPARγ in the

FIGURE 5. Enhanced lipid accumulation by lipin-β overexpression in vivo. a, gonadal adipose tissue sections prepared from aP2-Lipin-β transgenic (Tg) and non-transgenic (non-Tg) control mice, stained with hematoxylin-eosin and viewed at a magnification of ×320. b, triglyceride levels in fat pads from non-Tg and Tg adipocytes expressed as average triglyceride content per cell, as determined by normalization to DNA content (n = 5; *p < 0.05). c, adipose tissue cellularity determined in gonadal fat pads by DNA content per fat pad. Difference between means is not significant (n = 5).
adipogenic molecular cascade (6). Compared with lipin-α, lipin-β expression allows only a small number of cells to differentiate, and, consequently, its effect on PPARγ and C/EBPs expression was undetectable. However, we unexpectedly found that lipin-β stimulates the expression of lipogenic genes and triggers increased lipid accumulation per cell compared with lipin-α. Our results suggest that lipin-β is directly involved in the regulation of lipogenesis in adipocytes. To test this hypothesis, we overexpressed lipin-β specifically in mature adipocytes in ap2-lipin-β transgenic mice (7). Using these mice, we demonstrate that adipocytes with elevated levels of lipin-β accumulate more triglyceride. These results are consistent with the hypothesis that the cellular level of lipin-β is a modulator of lipid accumulation in adipocytes. In summary, our studies suggest distinct, but complementary, functions for lipin-α and lipin-β in adipocyte differentiation and lipid metabolism, respectively.

We demonstrated previously that lipin exhibits a biphasic expression pattern during adipogenesis with a first wave of expression in the very early stages of differentiation and a second wave in mature adipocytes (6, 8). Here we show that although lipin-α is expressed throughout the early differentiation period (day 0–2), it is lipin-β that predominates in a transient lipin expression peak at 10 h after induction of differentiation. Because lipin-α is the isoform required for stimulation of the adipogenic transcription factors PPARγ and C/EBPs, the functional significance of early lipin-β expression is presently unknown. The finding that MEFs expressing exclusively lipin-α have no major defects in their capacity to differentiate suggests that early expression of lipin-β may only have a subtle role in adipogenesis, at least in vitro. Also, it is likely that the function of early expressed lipin-β is distinct from its lipogenesis-stimulating role in mature adipocytes. Further studies will be required to elucidate the role of lipin-β during the early stages of adipogenesis.

The molecular mechanisms responsible for the effects of lipin-α and lipin-β on the expression of genes involved in differentiation and lipogenesis, respectively, are unknown. Lipin has a putative nuclear localization signal and can gain access to the nucleus (2). However, as it lacks a recognizable DNA binding domain, the effect of lipin on gene expression is likely to be indirect. Consistent with this possibility, a yeast lipin has been shown to interact with a component of the nuclear import signal and can gain access to the nucleus (2). However, as it lacks the latter possibility, however, seems unlikely, as we have been unable to induce nuclear translocation of lipin-β in adipocytes by treatment with a number of serum factors and hormones, including insulin.3

Alternative splicing of pre-mRNA is a versatile mechanism with the potential to regulate virtually all aspects of protein function (16). In line with results shown here, alternative splicing is a common mechanism in the regulation of subcellular distribution of a wide range of gene products (17–21). Developmentally regulated alternative splicing is also widespread in diverse tissues and developmental processes (22–24), including adipocyte differentiation (25, 26). What is the mechanism of splicing pattern changes in lipin1 mRNA during adipogenesis? A potential candidate for triggering shifts in splicing is insulin signaling. Insulin, an essential hormone for adipocyte differentiation, has been implicated in the modulation of insulin receptor (27) and protein kinase Cβ (28) mRNA splicing. Moreover, IGF-1, which activates a signaling cascade similar to that of insulin, regulates splicing of its own message (29). Likely mechanisms mediating the effect of insulin on splicing include the regulation of SR splicing factors, such as SRp80, at the transcriptional (29) and post-transcriptional (30) levels. Alternative splicing of the lipin message may involve similar mechanisms. However, lipin-β splicing first increases then decreases in the presence of insulin during early stages of 3T3-L1 differentiation indicating that the regulation of lipin splicing is complex. Ongoing studies in our laboratory are expected to shed light on the underlying mechanisms.

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3 M. Peterfy, unpublished observations.