The Amino and Carboxyl Termini of Perilipin A Facilitate the Storage of Triacylglycerols*

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Perilipin A is the most abundant lipid droplet-associated protein in adipocytes and serves important functions in regulating triacylglycerol levels by reducing rates of basal lipolysis and facilitating hormonally stimulated lipolysis. We have previously shown that the central region of perilipin A targets and anchors it to lipid droplets, at least in part via three moderately hydrophobic sequences that embed the protein into the hydrophobic core of the droplet. The current study examines the roles of the amino and carboxyl termini of perilipin A in facilitating triacylglycerol storage. Amino- and carboxyl-terminal truncation mutations of mouse perilipin A were stably expressed in 3T3-L1 preadipocytes, which lack perilipins. Triacylglycerol content of the cells was quantified as a measure of perilipin function and was compared with that of cells expressing full-length perilipin A or control cells lacking perilipins. The amino-terminal sequence between amino acids 122 and 222, including four 10–11-amino acid sequences predicted to form amphipathic β-strands and a consensus site for cAMP-dependent protein kinase, and the carboxyl terminus of 112 amino acids that is unique to perilipin A were critical to facilitate triacylglycerol storage. The precocious expression of full-length perilipin A in 3T3-L1 preadipocytes aided more rapid storage of triacylglycerol during adipocyte differentiation. By contrast, the expression of highly truncated amino- or carboxyl-terminal mutations of perilipin failed to serve a dominant negative function in lowering triacylglycerol storage during adipocyte differentiation. We conclude that the amino and carboxyl termini are critical to the function of perilipin A in facilitating triacylglycerol storage.

Triacylglycerols packaged within the lipid droplets of adipocytes provide the most abundant form of stored energy in the body. Lipid droplets are spherical structures composed of a core of neutral lipids coated by a phospholipid monolayer into which specific proteins are embedded. The study of lipid droplet-associated proteins is an emerging field of inquiry. Only a few of the component proteins have been identified, and little is known about the structural properties that define lipid droplet-associated proteins and facilitate their functions in lipid metabolism. The aim of the current study is to begin to define functional domains of perilipins, the first identified mammalian lipid droplet-associated proteins.

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1 The abbreviations used are: PKA, cAMP-dependent protein kinase; ATG, perilipin, adipophilin, and TIP47 family of structurally related lipid droplet-associated proteins; HSL, hormone-sensitive lipase; AFABP, adipocyte fatty acid binding protein; TAG, triacylglycerol.
presence of perilipins on the surfaces of lipid droplets is required to dock HSL onto lipid droplets (11), thus suggesting that perilipins may be HSL-binding proteins. Furthermore, mutagenesis of the three amino-terminal PKA site serines of perilipin A to alanines interfered with the docking of HSL on perilipin-coated lipid droplets (11), implying a role for perilipin phosphorylation in lipase docking. Therefore, the structure of perilipin A likely includes a sequence or domain that binds lipases, as well as sequences or domains that shield stored triacylglycerols from lipases.

The structure of lipid droplets with a hydrophobic rather than aqueous core surrounded by a phospholipid monolayer necessitates that perilipins and related proteins possess structural motifs and properties that have not yet been characterized in proteins integral to membranous compartments of the cell. We have recently demonstrated that the sequence contained within the central 25% of the primary amino acid sequence of perilipin A, which includes three moderately hydrophobic stretches surrounding an acidic region, is required to target and anchor perilipins to lipid droplets (15). Therefore, the hydrophobic amino acid sequences within the central domain of perilipin A are most likely embedded into the lipid droplet core, while the more highly charged amino and carboxyl termini are most likely oriented away from the surface of the lipid droplet and folded into functional domains. We hypothesize that the amino and carboxyl termini are critical to perilipin function in modulating rates of both basal and stimulated lipolysis. In the current study, we have tested this hypothesis by stably expressing amino- and carboxyl-terminal truncation mutations of perilipin A in 3T3-L1 fibroblasts and quantifying triacylglycerol content of the cells as a test of perilipin function under basal, or non-stimulated, conditions.

During the differentiation of cultured 3T3-L1 adipocytes, the expression of perilipin A begins between 48 and 72 h after the initiation of differentiation by the addition of dexamethasone, isobutylmethylxanthine, and insulin to the culture media of confluent monolayers of 3T3-L1 preadipocytes (16). The expression of HSL is initiated at approximately the same time (17), leading to an increase in cytosolic lipase activity concomitant with increased expression of the proteins that facilitate glucose and fatty acid uptake, transport, and metabolism and the enzymes that catalyze triacylglycerol synthesis (17). Thus, the timing of the initiation of perilipin expression is critical to facilitate triacylglycerol storage in an increasingly lipolytic environment. To study the effects of precocious expression of full-length perilipin A on triacylglycerol accumulation during adipocyte differentiation, confluent monolayers of 3T3-L1 preadipocytes stably expressing ectopic perilipin A were induced to differentiate, and the triacylglycerol content of the cells was compared with that of control cells lacking ectopic perilipins.

Finally, a comparable experiment tested potential dominant negative effects of perilipins truncated at either the amino or carboxyl terminus on triacylglycerol accumulation during adipocyte differentiation.

EXPERIMENTAL PROCEDURES

Materials—Fetal bovine serum, fatty acid-free bovine serum albumin, triolein, oleic acid, isobutylmethylxanthine, insulin, dexamethasone, and horse radish peroxidase-conjugated goat anti-rabbit IgG or goat anti-guinea pig IgG were purchased from Sigma. Geneticin was purchased from Mediatech (Herndon, VA). Ammonium-sulfate-impregnated silica gel H thin layer chromatography plates were purchased from Analtech (Newark, DE). Pfu DNA polymerase was purchased from Stratagene. BCA Protein Assay Reagents were purchased from Pierce.

Cell Cultures—3T3-L1 preadipocytes and 293T cells were cultured as described previously (4). Confluent monolayers of 3T3-L1 cells were induced to differentiate into adipocytes by the daily addition of culture medium containing 8 μg/ml biotin, 0.5 mM isobutylmethylxanthine, 10 μg/ml insulin, and 10 μM dexamethasone for 72 h followed by daily addition of culture medium containing biotin but without the other additives for up to 5 more days (16).

Expression of Full-length Perilipin A and Mutated Perilipins in Cells—The pSRAmSVKneo retroviral expression vector containing the coding cDNA sequence of mouse perilipin A (4) and various amino- and carboxyl-terminal truncation mutations of perilipin A (15) were prepared previously. Additional amino-terminal truncation mutations of mouse perilipin A were prepared by the previously reported procedures (15); briefly, the coding sequence of perilipin A was amplified by PCR using Pfu DNA polymerase and oligonucleotide primers corresponding to the sequences for a HindIII restriction site and an ATG start codon prior to the nucleotide sequences encoding the first amino acid of the truncated amino terminus for the 5’ primer, and the nucleotide sequence encoding the final amino acids of the 3’ primer, and the sequence for a HindIII site for the 3’ primer. The amplified cDNA sequences were ligated unto the unique HindIII site of the pSRAmSVKneo retroviral expression vector (18). All constructs were sequenced to verify the fidelity of the PCR reaction. Retroviral stocks were prepared from transfected 293T cells and used to infect 3T3-L1 preadipocytes, as described previously (4, 15). Cells stably expressing perilipin A and mutated perilipins were selected for by resistance to gentamicin at a concentration of 0.6 mg/ml culture medium. Analysis of Perilipin A and Mutated Perilins in Lipid Droplet Fractions—Confluent monolayers of 3T3-L1 preadipocytes stably expressing full-length perilipin A or mutated perilipins were incubated with 400 μM oleic acid complexed to fatty acid-free bovine serum albumin at a 6:1 molar ratio for 24 h to increase triacylglycerol synthesis and storage in lipid droplets prior to harvest of cells to isolate lipid droplet fractions, as described previously (4, 15, 19). Lipid droplet-containing fractions were delipidated by precipitation with cold acetone overnight at −20 °C (15, 19), followed by solubilization of the precipitated proteins in 2× Laemmli’s sample buffer (20). Lipid droplet proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred electrophoretically to nitrocellulose membranes. Immunoblots were probed with polyclonal antibodies raised against either a recombinant amino-terminal peptide of perilipin A or a recombinant carboxyl-terminal peptide of perilipin A (16), followed by horseradish peroxidase-conjugated secondary antibody; enhanced chemiluminescence reagents (Amersham Biosciences) were used for signal detection.

Lipid Analysis—Confluent monolayers of 3T3-L1 preadipocytes stably expressing perilipin A or mutated perilipins were incubated with fresh culture medium for 24 h prior to harvesting cells for lipid analysis. Cellular triacylglycerol content was determined following solvent extraction of cells, as described previously (19). Triacylglycerol content was expressed either on a per 100-mm dish basis or relative to total cellular protein content measured by the bicinchoninic acid method (21).

Northern Blot Analysis—Total RNA was extracted from 3T3-L1 preadipocytes and differentiating 3T3-L1 adipocytes using RNeasy minicolumns (Qiagen) according to the protocols of the manufacturer. RNA was separated electrophoretically in 1% agarose gels using NorthernMax™. Gly reagents (Ambion, Inc.) RNA was transferred electrophoretically to MagnaCharge nylon membranes (Osmomics), and the membranes were probed with 32P-labeled cDNA probes for perilipins, HSL, adipose fatty acid-binding protein (AFABP), and 18 S. RNA.

RESULTS

Expression of Truncation Mutations of Perilipin A in Cells—The schematic diagram of perilipin A depicted in Fig. 1 indicates the putative structural motifs encoded by the amino acid sequence and the sites of truncation relative to those motifs (summarized in Table I). A conserved sequence of 105 amino acids at the amino terminus of perilipin A (amino acids 17–121) is highly similar to amino-terminal sequences in the related lipid droplet-associated proteins adipophilin (32% identical, 65% similar) and TIP47 (38% identical, 60% similar) (1, 15, 22) and thus predicts a structural domain that may serve a common function in all three proteins. To test whether this conserved domain plays a role in the protection of stored triacylglycerols from soluble lipases, we prepared mutated perilipins with truncations at five sites within this region by removing the sequences encoding the first 33, 54, 81, 91, and 121 amino acids corresponding to mutations named N1–N5; removal of the first 81 amino acids (mutation N3) deletes the first consensus
PKA site, while removal of the first 121 amino acids (N5) deletes the entire highly conserved domain. An additional truncation mutation, N6, removed the first 182 amino acids including all 5 of the 10–11 amino acid sequences predicted to form amphipathic β-strands (23), and mutation N7 removed the first 222 amino acids including the second PKA consensus site.

Truncation mutation N8 lacks an additional 10 amino acids. The names of these truncation mutations do not correspond to our earlier report that described sequences involved in targeting perilipin A to lipid droplets (15); mutation N3 from the current study corresponds to N1 of the previous study, while current mutations N5, N6, N7, and N8 correspond to mutations N2, N3, N4, and N5 of the previous study, respectively.

Carboxyl-terminal truncation mutations removed the final 28 amino acids including PKA consensus sites 5 and 6 (C1); the last 88 amino acids including PKA consensus sites 4, 5, and 6 (C2); the last 112 amino acids including the entire perilipin A-specific carboxyl terminus (C3); and the last 153 amino acids including all of the carboxyl terminus following the third hydrophobic sequence (C4) (Table I and Fig. 1) that embeds perilipins into lipid droplets2 (15). Carboxyl-terminal truncation mutation C5 removes the entire 193 amino acid carboxyl terminus following the central acidic motif. The names of the first four of these carboxyl-terminal truncation mutations correspond to the names of perilipin mutations in our earlier report on perilipin sequences that serve as lipid droplet-targeting motifs; however, current perilipin mutation C5 was named mutation C6 in that study (15).

The 13 amino- and carboxyl-terminal truncation mutations of perilipin A described in the current study all targeted to lipid droplets when expressed in cultured 3T3-L1 preadipocytes; a previous study showed data for ten of these constructs (15). The three forms of mutated perilipin that were not included in the previous report include N1 and N2, which have deletions in shorter amino-terminal sequences than those previously reported, and N4, which has a truncation of an intermediate length to those previously reported. The targeting of these additional forms of mutated perilipin to lipid droplets was confirmed by immunofluorescence microscopy experiments (not shown) and immunoblotting of proteins from lipid droplet fractions. Fig. 2 depicts immunoblots of the lipid droplet fractions from 3T3-L1 preadipocytes stably expressing the eleven forms of mutated perilipins that are further characterized in Fig. 3; all 11 forms of truncated perilipins were detected in lipid droplet fractions. Prior to harvesting lipid droplets from cells stably expressing the mutated perilipins, the cells were incubated for 24 h with oleic acid complexed to albumin to increase triacylglycerol synthesis and storage and concomitantly stabilize the ectopic perilipins (19), thus increasing perilipin content. Although lipid loading of cells increased the cellular content of the most highly truncated forms of perilipins (N6, N7, C3, C4) to a modest extent, neither the protein levels of these mutated perilipins nor the triacylglycerol content increased during lipid

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2 V. Subramanian, A. Garcia, A. Sekowski, and D. L. Brasaemle, unpublished results.
loading to the extent observed in cells expressing longer forms of perilipins. Nonetheless, our previous studies have shown that truncated forms of perilipins are efficiently translated (15) and that the protein levels of ectopic perilipins are driven by the capacity of cells to synthesize triacylglycerols that stabilize perilipins (19) and hence are not limited by efficiency of the retroviral expression system. Thus, these highly truncated perilipins may lack the structural motifs required for triacylglycerol-mediated stabilization of the protein.

**Amino- and Carboxyl-terminal Domains of Perilipin A Facilitate the Storage of Triacylglycerol—**The ectopic expression of full-length perilipin A in 3T3-L1 preadipocytes leads to increased storage of triacylglycerol relative to control cells lacking perilipins (Fig. 3 and Re. 4), due to decreased rates of basal lipolysis in the cells (4). Thus, perilipin A plays a protective role in shielding stored triacylglycerols from cytosolic lipases. The triacylglycerol content of 3T3-L1 preadipocytes was expressed relative to total cell protein; data represent means ± S.D. of data pooled from 4 to 14 experiments, each containing triplicate or quadruplicate samples. Triacylglycerol content of control cells and of cells stably expressing truncation mutations of perilipin A was compared with that of cells expressing full-length perilipin A using Student’s paired t test; *, triacylglycerol content differed from perilipin A-expressing cells at p < 0.05; **, triacylglycerol content differed from perilipin A-expressing cells at p < 0.001.

perilipin A (Fig. 3A). While minor decreases in triacylglycerol content were observed following the additional deletion of amino acids up to and including the first 121 amino acids (N5), significant decreases in the ability of cells to store triacylglycerol accompanied the deletion of the first 182 amino acids (N6) including both the conserved amino-terminal domain and sequences predicted to form amphipathic β-strands. Further deletion of the amino terminus to amino acid 222 (N7) to remove the second PKA consensus sequence restored cellular triacylglycerol content to the low levels found in control cells, thus predicting a more rapid rate of triacylglycerol turnover in cells expressing the N7 perilipin mutation, comparable to that of cells lacking perilipins.

The sequential deletion of portions of the carboxyl terminus
of perilipin A also reduced triacylglycerol storage in 3T3-L1 preadipocytes expressing the mutated perilipins relative to that of cells expressing full-length perilipin A. Removal of the final 28 amino acids from perilipin A including two PKA consensus sites had no effect on the ability of the mutated perilipin to protect stored triacylglycerols from hydrolysis (perilipin mutation C1; Fig. 3B). Removal of 88 amino acids from the carboxyl terminus of perilipin A including all three carboxyl-terminal PKA consensus sites (C2) significantly reduced the triacylglycerol content of cells stably expressing the mutated perilipin relative to cells expressing full-length perilipin A (Fig. 3B). The removal of additional amino acids to delete the entire carboxyl-terminal 112 amino acid sequence that is unique to perilipin A (C3) restored cellular triacylglycerol content to the low levels found in control cells lacking perilipins.

**Ectopic Expression of Perilipin A in 3T3-L1 Preadipocytes Facilitates More Rapid Accumulation of Triacylglycerols during Adipose Differentiation**—During adipocyte differentiation, the initiation of the expression of perilipin A coincides with the expression of HSL; thus, we hypothesize that the expression of perilipin A is critical to facilitate triacylglycerol storage in an increasingly lipolytic environment. To study the effects of premature expression of perilipin A on triacylglycerol accumulation during adipocyte differentiation, confluent monolayers of 3T3-L1 cells stably expressing ectopic perilipin A were induced to differentiate, and the triacylglycerol content of the cells was compared over time to that of control cells stably expressing the retroviral expression vector without the perilipin cDNA. Cells expressing ectopic perilipin A accumulated triacylglycerol more rapidly during the first 8 days of adipocyte differentiation (Fig. 4A).

Since mutated perilipins lacking either the amino or carboxyl terminus are ineffective at reducing basal levels of lipolysis and facilitating triacylglycerol storage when stably expressed in 3T3-L1 preadipocytes, we tested whether these mutated perilipins would have dominant negative function in preventing efficient triacylglycerol accumulation during adipose differentiation of the 3T3-L1 cells stably expressing the truncated perilipins. If truncated perilipins serve a dominant negative function, then a mixed coating of endogenous full-length perilipin A and ectopic truncated perilipins should produce an incomplete or leaky surface barrier to lipolysis and reduce triacylglycerol accumulation during adipocyte differentiation. Cells stably expressing either an amino-terminal truncation mutation of perilipin A consisting of amino acids 233–517 (perilipin mutation N8), or a carboxyl-terminal truncation mutation consisting of amino acids 1–324 (perilipin mutation C5), were induced to differentiate into adipocytes, and the triacylglycerol content of the cells was assessed through 8 days of differentiation; these two additional truncation mutations of perilipin A were the shortest forms of mutated perilipins that targeted to lipid droplets and anchored into the droplet well
enough to confer resistance to removal by alkaline carbonate solutions in our previous study (15). The triacylglycerol content of the cells expressing perilipin truncated at the carboxyl terminus (C5) was comparable to that of control cells lacking ectopic perilipin A throughout differentiation (Fig. 4B), but not reduced, as might be expected if a surface layer of mixed perilipin content including both full-length endogenous perilipin A and truncated ectopic perilipin would facilitate increased rates of basal lipolysis. Surprisingly, the triacylglycerol content of cells stably expressing perilipin (N8) truncated at the amino terminus (N8) was significantly increased over that of control cells throughout differentiation, suggesting that this mutated perilipin was able to confer partial reduction of basal lipolysis during adipose differentiation.

While the premature expression of perilipin A or perilipin truncated at the amino terminus facilitated more rapid accumulation of triacylglycerol during subsequent adipocyte differentiation, the cells did not differentiate more rapidly, as evidenced by similar timing and levels of expression of mRNAs of endogenous perilipins, HSL, and AFABP in ectopic perilipin A-expressing cells relative to control cells (Fig. 5, for control and perilipin A-expressing cells; data not shown for N8). While cells expressing ectopic perilipin A showed slightly higher levels of expression of mRNAs of endogenous perilipin A and HSL than control cells by 8 days of differentiation, these differences were not significant, and levels of AFABP mRNA were lower in perilipin-expressing cells than in control cells at day 8. Additionally, the mRNA level of endogenous perilipin A was equivalent to that of ectopic perilipin A by day 5 after the initiation of differentiation.

**DISCUSSION**

While previous studies have shown that perilipin A functions to facilitate triacylglycerol storage through the reduction of basal lipolysis, the major conclusion of this study is that both the amino and carboxyl termini of perilipin A serve important roles in this process. 3T3-L1 preadipocytes stably expressing mutated perilipins with truncations of portions of either the amino terminus or carboxyl terminus stored less triacylglycerol than cells expressing full-length perilipin A. Surprisingly, the amino-terminal domain of 105 amino acids that is conserved among all members of the PAT (perilipin, adipophilin, and TIP47) family of lipid droplet-associated proteins (1, 2, 24), appears to be dispensable; its removal has no effect on the ability of the truncated perilipin to facilitate triacylglycerol storage. These observations suggest that this conserved domain does not wrap around the surface of the lipid droplet to serve a shielding function but instead folds into an uncharacterized structure (as depicted in Fig. 6) that plays an as yet unknown role in perilipin function. Further truncation of the proximal sequences that are predicted to fold into amphipathic β-strands (23), as well as neighboring residues including the second PKA consensus sequence at amino acids 219–222, is required to eliminate the protective function of perilipin A in
shielding stored triacylglycerols from cytosolic lipases. By contrast, removal of only 88 amino acids from the carboxyl terminus of perilipin A reduced triacylglycerol storage in cells stably expressing the truncated perilipin, and further truncation to remove the entire 112-amino acid carboxyl terminus that is unique to perilipin A reduced triacylglycerol content to that of control cells lacking perilipins. These observations suggest that the unique carboxyl terminus of perilipin A serves a shielding function against cytosolic lipases. A very recent report (27) has reported similar findings that the final 216 amino acid peptide of the carboxyl terminus of perilipin A serves an important role in reducing levels of basal lipolysis by non-HSL lipases; however, that study suggests that the amino terminus of perilipin A lacks protective function. We note that study of the larger amino-terminal truncations described in the recent report may have missed the effects that we have uncovered.

The loss of triacylglycerol content in cells expressing successively more highly truncated forms of perilipin A suggests that portions of perilipin A protect stored triacylglycerol by blanketing the surfaces of lipid droplets and sterically hindering lipase access. Alternatively, perilipin A may serve as a scaffold at the surface of lipid droplets to which additional protective coat components may bind to make up a protective shield; in this model, a discrete loss of protection would occur following the truncation of binding sites of the coat proteins. The most critical sequences for either of these potential shielding functions are the amino-terminal sequences between amino acids 121 and 222, from the end of the highly conserved domain through the second PKA consensus site, and the unique 112-amino acid carboxyl terminus of perilipin A. Sequences between amino acids 122 and 183 have been predicted to form amphipathic \( \beta \)-strands that might shallowly embed this portion of the perilipin amino terminus into the lipid droplet with charged residues facing outward, providing a potential shield, as depicted in the theoretical model for perilipin A positioning on an adipocyte lipid droplet shown in Fig. 6. As depicted in Fig. 6, the central region of perilipin A from amino acid 243 to 364 contains three sequences of moderate hydrophobicity that are most likely embedded into the hydrophobic core of the lipid droplet (15), with the highly acidic sequence from amino acid 291 to 319 looping away from the droplet surface (Fig. 6); we have previously shown that this central region is required to target and anchor perilipins to lipid droplets (15). By contrast, the protective carboxyl terminus of perilipin A contains numerous dispersed charged amino acids and seems ill suited for a role as a lipid-associated shield but may participate in protein-protein interactions. Furthermore, we have previously demonstrated that while perilipin A-coated lipid droplets normally form tight clusters of aggregated lipid droplets when perilipin A is stably expressed in fibroblasts (4, 15), truncation of the carboxyl terminus of perilipin A to amino acid 364 (perilipin mutation C4) causes the dispersion of lipid droplets throughout the cytoplasm (15). Thus, the carboxyl terminus of perilipin A contains amino acids that mediate the clustering of perilipin A-coated lipid droplets into tight aggregates of droplets. Therefore, part of the protective mechanism of perilipin A in facilitating the storage of triacylglycerols may be due to hindered lipase access to aggregated lipid droplets.

When perilipin A becomes phosphorylated by PKA, it facilitates lipolysis (7–9, 11). One potential mechanism for this alteration in function is that the phosphorylation of key amino acids may induce a conformational change in perilipin A to disrupt the protective shield, driving domains of the protein away from the lipid droplet surface and allowing lipases to gain access to the lipid droplet. Several studies have shown that mutation of the three amino-terminal PKA site serines to alanines reduces the increase in lipolysis triggered by PKA, both when HSL is co-expressed in the cells expressing the mutated perilipins (8, 11), and when HSL is absent (9), and as yet unidentified neutral lipid lipases are present. A very recent study has demonstrated that HSL fails to translocate to the surfaces of lipid droplets in adipocytes from perilipin null mice or in Chinese hamster ovary cells stably expressing perilipin with 3 mutated amino-terminal PKA sites (11), thus suggesting that phosphorylated perilipins are required to promote the docking of HSL at the surfaces of lipid droplets. Thus, a second model for the role of perilipins in the promotion of lipolysis is that the phosphorylation of amino-terminal PKA site serines in perilipin acts as a switch to create a docking site for lipase binding at the lipid droplet surface. While our study does not rule out this second mechanism, it provides support for the idea that perilipin acts at least partially through a shielding mechanism and furthermore that the carboxyl terminus plays an important role in the shielding function of perilipin A.

Our observation that the unique carboxyl terminus of perilipin A is required to promote maximal storage of triacylglycerol suggests that perilipins B and C, which lack this domain, should be less effective at promoting triacylglycerol storage. A recent report (9) shows that triacylglycerol turnover in Chinese hamster ovary cells stably expressing perilipin B is more rapid than that of cells expressing perilipin A, and comparable to that of control cells lacking perilipins, in support of this hypothesis. Lipid droplets of cultured 3T3-L1 adipocytes (3, 16), and of adipocytes isolated from mouse (7) and human (25, 26) white fat, have much lower protein levels of perilipin B than of perilipin A. Furthermore, while protein levels of perilipin A are detectable by 2–3 days after the initiation of differentiation of 3T3-L1 adipocytes (16), protein levels of perilipin B are not detectable until sometime between days 5 and 8 (16), suggesting that the temporal regulation of expression of the perilipin B isoform is different from that of perilipin A. Thus, we speculate that the incorporation of low levels of perilipin B onto lipid droplets containing primarily perilipin A may be a way for the adipocyte to increase rates of basal lipolysis. To test the idea that truncated perilipins may have dominant negative effects in promoting basal lipolysis in the presence of perilipin A, we expressed perilipin truncated at either the amino or carboxyl terminus in 3T3-L1 preadipocytes prior to inducing adipose differentiation. While the premature expression of full-length perilipin A facilitated more rapid accumulation of triacylglycerol during adipocyte differentiation, the expression of truncated forms of perilipin failed to reduce triacylglycerol storage when the expression of endogenous perilipin A began. Thus, an early coating of perilipin A may assist triacylglycerol storage in lipid droplets as the intracellular environment of the differentiating adipocyte becomes increasingly lipolytic.

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REFERENCES

1. Greenberg, A. S., Egan, J. J., Wek, S. A., Moos, M. C., Jr., Londos, C., and Kimmel, A. R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 12035–12039
2. Xu, X., Gruia-Gray, J., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Londos, C., and Kimmel, A. R. (2001) Mamm. Genome 12, 741–749
3. Servetnick, D. A., Brassemle, D. L., Gruia-Gray, J., Kimmel, A. R., Wolff, J., and Londos, C. (1995) J. Biol. Chem. 270, 16970–16973
4. Brassemle, D. L., Rubin, B., Harten, T. A., Gruia-Gray, J., Kimmel, A. R., and Londos, C. (2000) J. Biol. Chem. 275, 38486–38493
5. Souza, S. C., de Vargas, L. M., Yamamoto, M. T., Lien, P., Franciosa, M. D., Moos, L. G., and Greenberg, A. S. (1998) J. Biol. Chem. 273, 24665–24669
6. Martinez-Botas, J., Anderson, J. B., Tessier, D., Lapillonne, A., Chang, B. H., Quast, M. J., Gorenstein, D., Chen, K. H., and Chan, L. (2000) Nat. Genet.
