Inhibition of 3T3-L1 Adipocyte Differentiation by Expression of Acyl-CoA-binding Protein Antisense RNA*

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Several lines of evidence have underscored the significance of fatty acids or fatty acid-derived metabolites as signaling molecules in adipocyte differentiation. The acyl-CoA-binding protein (ACBP), which functions as an intracellular acyl-CoA pool former and transporter, is induced during adipocyte differentiation. In this report we describe the effects of expression of high levels of ACBP antisense RNA on the differentiation of 3T3-L1 cells. Pools of 3T3-L1 cells transfected with vectors expressing ACBP antisense RNA showed significantly less lipid accumulation as compared with cells transfected with the control vector. When individual clones were analyzed the degree of differentiation at day 10 was inversely correlated with the level of ACBP antisense RNA expression at day 0. Furthermore, in the clones with the highest levels of ACBP antisense expression, the induction of expression of the adipogenic transcription factors peroxisome proliferator-activated receptor γ and CCAAT/enhancer-binding protein α as well as several adipocyte-specific genes was significantly delayed and reduced. The adipogenic potential of antisense-expressing cells was partially restored by transfection with a vector expressing high levels of ACBP. Taken together, these results are strong evidence that inhibition of differentiation is causally related to the decreased expression of ACBP, indicating that ACBP plays an important role during adipocyte differentiation.

A number of established adipoblast cell lines undergo a highly regulated adipose conversion when they are treated with an appropriate combination of adipogenic factors. This differentiation process is accompanied by sequential expression and activation of a set of transcription factors governing expression of adipocyte-specific markers. Members of the CCAAT/enhancer-binding protein (C/EBP)1 and peroxisome proliferator-activated receptor (PPAR) families, in particular C/EBPα and PPARγ, have been demonstrated to be of crucial importance. C/EBPα and PPARγ appear to act synergistically in adipocyte differentiation by reciprocally activating the expression of one another and by cooperatively activating the expression of adipocyte genes (reviewed in Ref. 1).

Several reports have shown that fatty acids play a critical role in the regulation of adipocyte differentiation in vivo (2, 3) as well as in vitro (4–7). Whereas fatty acids or derivatives thereof may stimulate adipogenesis by combined effects on different signal transduction pathways, several results suggest that an important role of fatty acids or fatty acid derivatives in adipocyte differentiation is to activate members of the PPAR family (8, 9), most notably PPARγ. The PPAR family belongs to the nuclear hormone receptor superfamily of ligand-activated transcription factors. Numerous reports have shown that activators of PPARγ, such as the antidiabetic thiazolidinediones and certain prostaglandin J2 derivatives, are very potent inducers of adipocyte differentiation (10–12), and it has been shown that these activators bind directly to PPARγ with KD values that are comparable with the concentrations that stimulate adipocyte differentiation (13–16). It has been debated whether fatty acids activate members of the PPAR family directly as ligands or indirectly by inducing the synthesis of ligands. However, although the identity of the physiologically most relevant ligands remains unclear, it was recently shown that fatty acids and fatty acid analogs are indeed true ligands of the PPARs (16, 17).

The transport and biological functions of fatty acids and their metabolites are at least to some extent dependent on carrier proteins (18–20). The acyl-CoA-binding protein (ACBP) is a highly conserved 10-kDa protein that binds medium to long chain acyl-CoA esters (but not fatty acids) with high affinity (KD ~ 1 nM) (18) and functions as an intracellular acyl-CoA pool former and transporter (21–23). Despite its tight binding of acyl-CoA esters, ACBP is able to mediate acyl-CoA transport and donate acyl-CoA esters to acyl-CoA-dependent biological systems (24, 25). ACBP is expressed in virtually all cell types but at very different levels (reviewed in Ref. 26). Adipocyte differentiation is accompanied by a marked increase in ACBP abundance reflecting transcriptional activation of the ACBP gene (27, 28). In this report we show that expression of high levels of ACBP antisense RNA is able to significantly inhibit accumulation of lipid as well as induction of adipocyte-specific genes.

EXPERIMENTAL PROCEDURES

Plasmids—The vector pUBI was constructed by replacing the Rous sarcoma virus enhancer and the murine mammary tumor virus LTsu promoter of the pMAMneo vector (CLONTECH, Palo Alto, CA) with the human ubiquitin C promoter (position –1464 to –15) (28). To construct the ACBP antisense expression vector pUBI-ASmACBP, the murine ACBP cDNA fragment (29) was inserted into the pUBI vector in the antisense orientation. Similarly, pCEP4-ASmACBP was constructed by inserting the murine ACBP cDNA in the pCEP4 vector (Invitrogen, San Diego, CA) in the antisense orientation. pCMV-ACBP was constructed

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1 The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; ACBP, acyl-CoA-binding protein; ALBP, adipocyte lipid-binding protein; CMV, cytomegalovirus; MIX, 3-isobutyl-1-methylxanthine; MDI, MIX, dexamethasone, and insulin; DI, dexamethasone and insulin only; PPAR, peroxisome proliferator-activated receptor.
by inserting the rat ACBP cDNA (27) in pcDNA I/Amp in the sense orientation. For convenience, the pcDNA I/Amp vector was named pCMV. The pHYG vector conferring resistance to hygromycin was constructed from pTPS (30) by digestion with HindIII and religation of the 7.5-kilobase pair fragment.

Cell Culture and Transfections—The cells were propagated in Dulbecco’s modified Eagle’s medium containing 10% (v/v) calf serum (HyClone or Sigma), 62.5 μg/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 10% CO₂ at 37 °C. Medium was renewed every second day. For differentiation, the cells were grown to confluence, and differentiation was induced 2 days post-confluence (designated day 0) by changing the medium to Dulbecco’s modified Eagle’s medium containing 10% (v/v) fetal calf serum (Life Technologies, Inc.) supplemented with adipogenic inducers. For the 3-isobutyl-1-methylxanthine (MIX), dexamethasone, and insulin (MDI) differentiation protocol (31), the medium was supplemented with 1 μM dexamethasone (Sigma), 0.5 mM MIX (Aldrich), and 1 μM insulin (Boehringer) from day 0 to day 2 and with 1 μM insulin from day 2 to day 4. For the dexamethasone and insulin only (DI) differentiation protocol, the medium was supplemented with 1 μM dexamethasone from day 0 to day 2 and with 10 μg/ml insulin from day 2 and on.

Stable transfections were carried out according to a modified Chen and Okayama phosphate transfection procedure (32). Cells stably transfected with pCEP4-ASmACBP or pCEP4 were selected for 10 days in growth medium containing 150 μg/ml hygromycin (Sigma), trypsinized, pooled, and subjected to adipogenic inducers according to the DI and the MDI differentiation protocols, respectively. Hygromycin was maintained in the medium until confluence. Cells stably transfected with the pUBI-ASmACBP were selected 2–3 weeks in growth medium containing 400 μg/ml G418 (Life Technologies, Inc.), and individual clones were isolated. The isolated clones were analyzed for integration of pUBI-ASmACBP by polymerase chain reaction and Southern blotting and for expression of ACBP sense and antisense transcripts by Northern blotting. The ability of the individual clones to differentiate after being subjected to the DI and the MDI differentiation protocols was determined by staining the cells with oil red-O 10 days after addition of adipogenic inducers as well by Northern and Western blotting and for differentiation experiments. Two days after confluence (day 0), the cells were subjected to treatment according to the MDI differentiation protocol. Accumulation of lipid droplets was determined by oil red-O staining (33).

Analysis of RNA—Total RNA was purified (34) from the clones at various time points after addition of differentiation inducers and analyzed by Northern blotting (32). Filters were stained with methylene blue to confirm equal loading and hybridized to DNA probes labeled with 32P by random primer extension (35). Signals were quantitated by phosphor imaging using the ImageQuant™ software (Molecular Dynamics).

Western Blotting—Cells from day 0, 2, 4, and 10, respectively, were lysed in 0.5 ml of 2.5% SDS-sample buffer/10-cm dish. Lysates were subjected to SDS-polyacrylamide gel electrophoresis (16%) for detection of proteins below 15 kDa and 12.5% for detection of proteins larger than 15 kDa). Approximately 20 μg of cellular protein were loaded per lane. The separated proteins were transferred to a polyvinylidene difluoride membrane and stained with Ponceau S for control of equal load. The membranes were blocked in 5% nonfat dry milk, incubated with the appropriate primary antibodies (affinity-purified rabbit anti-rat ACBP, rabbit anti-mouse C/EBPα (M. D. Lane), rabbit anti-mouse C/EBPβ (M. D. Lane), rabbit anti-mouse PPARγ (M. A. Lazar), or anti-mouse ALBP (D. A. Bernlohr)) for 1 h and horseradish peroxidase-conjugated secondary antibody (DAKO A/S, Denmark) for another hour. Immunoreactive protein bands were detected by ECL (Amersham Pharmacia Biotech).

RESULTS

Stable Transfection of 3T3-L1 Cells with ACBP Antisense Constructs Inhibits Lipid Accumulation—To investigate the role of ACBP in adipocyte differentiation, 3T3-L1 preadipocytes were stably transfected with a vector expressing high levels of ACBP antisense RNA (pCEP4-ASmACBP) or a control vector (pCEP4), respectively. The transfectedants were pooled, replated, and induced to differentiate, and the degree of differentiation by day 10 was assessed by oil red-O staining. Two different differentiation protocols were used: one where the cells were treated with MDI and one where the cells were given DI (see “Experimental Procedures”). The DI protocol gives a slower and less synchronous differentiation; however, by day 10 3T3-L1 cells differentiated by the DI protocol are morphologically almost indistinguishable from those differentiated by the MDI protocol. In the growth phase there was no clear morphological difference between the pools of cells transfected with the antisense construct and pools of control cell, but when treated with differentiation inducers, the cells transfected with pCEP4-ASmAACBP had a significantly decreased ability to differentiate compared with that of control cells. The effect was most pronounced when the DI protocol was used for differentiation. Fig. 1 shows the result from one of two independent experiments in which the DI protocol was used for differentiation. Similar results were obtained in both experiments.

ACBP Antisense RNA Expression Down-regulates Endogenous ACBP Sense Transcripts and Inhibits Lipid Accumulation in Individual Clones—In parallel experiments 3T3-L1 cells were stably transfected with the pUBI-ASmACBP vector in which the ubiquitin promoter controls the expression of ACBP antisense RNA. Individual clones were analyzed by polymerase chain reaction, and eight clones that had integrated pUBI-ASmACBP were selected for further analyses. Southern blotting revealed that 10–20 copies of the integrated plasmid were present in the genome of the individual clones (results not shown). Some investigators using the antisense approach for abrogation of gene expression have reported that although the antisense transcript seemed to decrease the level of the endogenous sense transcript, the antisense transcript was undetectable by Northern blotting (Ref. 36 and references therein).
However, all of the selected clones expressed detectable levels of the ACBP antisense transcript at day 0 (Fig. 2A) as well as day 10 (results not shown). In seven of the eight clones the level of antisense transcript was at least five to ten times higher than the level of endogenous ACBP sense transcript at day 0 and equal to or twice the level of sense transcript at day 10. ACBP sense transcript levels (Fig. 2A) as well as ACBP protein levels (as quantified by Western (Fig. 2B) and enzyme-linked immunosorbent assay (data not shown)) at day 0 were significantly lower in the antisense-expressing clones than in untransfected 3T3-L1 cells. Thus, the antisense construct was efficiently transcribed, and endogenous ACBP expression decreased in all the clones. Because of the inherent instability of preadipocyte cell lines some variance in the phenotype of selected stably transfected clones will be expected whether the cells are genetically manipulated or not. However, when the eight ACBP antisense RNA-expressing clones were analyzed for their ability to undergo adipocyte differentiation, all clones showed a markedly decreased percentage of cells with lipid accumulation on day 10 when the DI protocol as well as when the MDI protocol was used (Fig. 3). As with the pools of pCEP4-ASmACBP transfected cells, the most pronounced difference in differentiation potential was observed with the DI protocol. The result in Fig. 3 is representative of four independent experiments. It should be noted that the percentage of differentiation of the different clones, especially using the MDI protocol, was subject to some variance. However, the GP2-4-1 clone, which had the lowest ACBP antisense RNA level, was always among the clones that differentiated the best.

**FIG. 2. The expression of ACBP mRNA and protein is decreased in all ACBP antisense RNA-expressing clones.** A, Northern blot showing the expression of ACBP and ACBP antisense transcripts at day 0 in the clones transfected with pUBI-ASmACBP. 10 μg of RNA was loaded per lane, and the blot was hybridized to a rat ACBP cDNA probe (27). RNA from untransfected 3T3-L1 cells at day 10 is included for comparison. B, Western blot showing expression of ACBP on day 0 in the clones transfected with pUBI-ASmACBP. Recombinant rat ACBP expressed in *Escherichia coli* as well as protein extracts from untransfected 3T3-L1 cells at day 0 and day 10 are included for comparison.

**FIG. 3.** ACBP antisense RNA expression decreases the differentiation potential of individual clones. Individual clones stably transfected with pUBI-ASmACBP were propagated and treated with differentiation inducers according to the DI differentiation protocol (A) or the MDI differentiation protocol (B). At day 10, cells were fixed and stained with oil red-O.

ACBP Antisense RNA Expression Inhibits Adipocyte Differentiation at a Stage Prior to Induction of PPARγ and C/EBPα—To analyze how expression of genes encoding members of the C/EBP and PPAR families were affected by the reduced ACBP expression, expression levels were quantitated by Northern blotting of RNA isolated from seven ACBP antisense-expressing clones and control 3T3-L1 untransfected cells. The DI protocol was used for differentiation because the
inhibitory effect of ACBP antisense RNA expression on lipid accumulation had been shown to be most pronounced with this protocol, and RNA was isolated at days 0, 2, 4, and 10, respectively.

Expressions of C/EBPβ and C/EBPδ, which are thought to play a role early during the differentiation process (reviewed in Ref. 1), were slightly increased throughout the differentiation in all clones expressing ACBP antisense RNA compared with untransfected 3T3-L1 cells (Fig. 4). The expression of PPARδ, which has also been suggested to play a role in the initiation of adipogenesis (37), was not affected by the ACBP antisense RNA expression. In contrast, induction of PPARγ and C/EBPa transcripts was significantly delayed and decreased in the antisense-expressing clones (Fig. 4). By day 2 the PPARγ transcript was detected solely in untransfected 3T3-L1 cells, and even by day 4 the transcript was still only detectable in untransfected cells and in the clone that expressed ACBP antisense RNA at a significantly lower level than the other clones.

Surprisingly, however, by day 10 the levels of PPARγ transcript were only significantly reduced compared with the level in control cells in the three clones (clone GP2-5-1, GP2-1-1, and GP2-4-2) with the highest levels of ACBP antisense RNA expression and the lowest differentiation potential. The level of PPARγ transcript in the remaining clones were comparable with that in 3T3-L1 cells. Western blotting, however, revealed that PPARγ protein levels were significantly reduced in all clones that express ACBP antisense RNA (Fig. 5), suggesting that PPARγ expression is subject to translational/post-translational regulation.

The expression of C/EBPa was induced at day 2 in the untransfected control cells, but no expression was detected in the antisense-expressing clones either at day 2 or at day 4. By day 10, the clones with a high level of PPARγ transcript had levels of C/EBPa transcript up to 50% of the level in control cells. In the clones with the lowest levels of PPARγ transcript and the highest level of ACBP antisense RNA expression, the levels of C/EBPa transcript were below 20% of that in control cells (Fig. 4). Results obtained by Western blotting showed that C/EBPa protein levels were barely detectable in these clones (Fig. 5). Thus, not only lipid accumulation but also the induction of the two adipogenic transcription factors PPARγ and C/EBPa are inhibited in the clones expressing high levels of ACBP antisense RNA.

The levels of expression of genes encoding lipoprotein lipase, adipocyte lipid-binding protein (ALBPA/P2) and glycerol-3-phosphate dehydrogenase, all of which are up-regulated during adipocyte differentiation, were also assessed by Northern blotting (data not shown). The induction of expression of these genes was delayed in all clones (i.e. expression was not induced by day 4). By day 10, lipoprotein lipase, ALBP, and glycerol-3-phosphate dehydrogenase transcripts were expressed at significant levels in all clones as well as control cells; however, the levels were lowest in the clones with the highest ACBP antisense expression at day 0. Thus, the induction of adipogenic transcription factors and other adipocyte-specific genes is delayed and to some degree abrogated in the clones expressing ACBP antisense RNA compared with control cells.

Overexpression of ACBP Partly Rescues the Ability of the Antisense-expressing Clones to Differentiate—To show that the decreased level of ACBP in the antisense-expressing clones was causally related to the inhibition of differentiation, we attempted to overcome the high level of antisense expression by introducing vectors for high level ACBP sense RNA expression. Thus, the clone GP2-4-2 was stably transfected with pCMV-rACBP and pCMV, respectively. When the selected clones were pooled and treated according to the MDI differentiation protocol, the pool transfected with pCMV-rACBP differentiated significantly better than the pool transfected with pCMV (Fig. 6). In comparison with standard 3T3-L1 cells, differentiation of the rescued antisense-expressing cells appeared to be more patchy. This behavior was observed in three independent experiments. Although all pooled cells were resistant to hygromycin, analysis of ACBP expression by immunostaining revealed that the number of cells expressing detectable levels of ACBP at day 0 varied from experiment to experiment, and only made up 30–50% of the total population of transfected cells (results not shown). Thus the patchy appearance of differenti-
Cells of the GP2-4-2 clone were stably transfected with pCMV-rACBP and pHYG (A and B) or with pCMV and pHYG (C and D), respectively. The bars represent 50 μm. Hygromycin-resistant clones were pooled, replated, and treated with adipogenic inducers according to the MDI differentiation protocol. 10 days later, cells were fixed and stained with oil red-O to assess lipid accumulation.

The combination of BRL49653 and dexamethasone rescues the ability of ACBP antisense-expressing clones to differentiate—The thiazolidinedione BRL49653 has been shown to be a ligand of PPARγ and a very potent adipogenic inducer (15). To investigate whether the thiazolidinedione BRL49653 alone or in combination with other adipogenic inducers could rescue the ability of ACBP antisense-expressing clones to undergo adipocyte differentiation, we subjected the clone GP2-4-2 and control 3T3-L1 cells to various combinations of adipogenic stimuli. 10 days after addition of inducers, the cells were fixed and stained with oil red-O (Fig. 7). In 3T3-L1 cells neither insulin nor MIX had any effect on adipogenesis when administered alone; however, adipogenesis was stimulated by 2 days of treatment with dexamethasone as well as by treatment with BRL49653. The most robust adipocyte differentiation was observed by MDI treatment alone or in combination with BRL49653.

In the GP2-4-2 clone, dexamethasone or BRL49653 alone had very little effect on adipogenesis; however, when administered in combination, these two agents resulted in lipid accumulation in almost 100% of the cells. Similarly, the combination of MDI and BRL49653 resulted in almost 100% differentiation. Thus, the combination of dexamethasone and BRL49653 is able to rescue the ability of the antisense-expressing clone GP2-4-2 to differentiate. Interestingly, when the GP2-4-2 cells were left in Dulbecco’s modified Eagle’s medium and fetal calf serum or in this medium plus insulin or MIX, they survived significantly better than 3T3-L1 control cells (Fig. 7).

Western blot analyses of 3T3-L1 control cells and GP2-4-2 cells subjected to MDI or MDI + BRL49653 differentiation inducers, respectively, confirmed the results obtained by oil red-O staining. Although MDI treatment failed to efficiently induce expression of the adipogenic transcription factors PPARγ and C/EBPs in GP2-4-2 cells, these transcription factors were readily detected in extracts from GP2-4-2 cells treated with MDI + BRL49653 (Fig. 8). Thus, in the ACBP antisense-expressing clone GP2-4-2 the combination of MDI and BRL49653 induced adipocyte differentiation as assessed by lipid accumulation as well as by induction of adipocyte-specific genes. In keeping with previous observations (39), we find that PPARγ is the predominant PPAR isoform induced during adipocyte differentiation whether or not BRL49653 is present. Of interest, we consistently see a significant decrease in the abundance of PPARγ1 by day 10 so that the ratio between PPARγ1 and PPARγ2 at this time point approaches 1. The presence of BRL49653 considerably accelerated the induction and enhanced the expression of C/EBPα in 3T3-L1 cells. Thus, in BRL49653-treated cells the expression of C/EBPα was significantly increased, and maximal expression of C/EBPα was observed already at day 2, a time by which 3T3-L1 cells stimulated by the MDI treatment have just finished their first round of post-confluent mitoses. Given the well documented strong antimitotic action of p42 C/EBPα, this finding suggests that clonal expansion may be partially curtailed in the BRL49653-treated cells.

**DISCUSSION**

In this report we present evidence that ACBP appears to play an important role during activation of the adipogenic differentiation program in 3T3-L1 cells. First we showed that pools of 3T3-L1 cells stably transfected with vectors expressing high levels of ACBP antisense RNA differentiated significantly less when subjected to two different differentiation protocols than did cells stably transfected with the control vector. In this experiment differentiation was monitored solely as morphological differentiation (i.e. cells were stained with oil red-O). To investigate in more detail how antisense expression affected the ability of individual clones to differentiate, eight clones were isolated. Seven of these expressed high levels of ACBP antisense RNA, and one expressed low levels of ACBP antisense RNA. All of these clones showed a significantly reduced ability to undergo morphological differentiation. Further evidence for a causal relation between the ACBP antisense RNA expression and the lack of morphological differentiation was obtained by stably transfecting the differentiation deficient clone GP2-4-2 with a vector expressing rat ACBP under the control of the strong CMV promoter. Transfection with this expression vector partially restored the ability to undergo adipocyte differentiation, whereas transfection with the control vector had no effect. Immunofluorescence analysis of the transfected cells revealed a clear correlation between the number of cells expressing detectable levels of ACBP at the start of the differentiation program, and the number of cells that accumu-

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3 J. B. Hansen and K. Kristiansen, unpublished results.
lated fat during the course of differentiation.

To investigate whether not only lipid accumulation but also adipocyte-specific gene expression was abrogated by high levels of ACBP antisense RNA expression, RNA was purified from the cells at different time points after addition of differentiation inducers. Northern blot analysis showed that in the clones with the highest levels of ACBP antisense RNA expression, the induction of several adipocyte-specific transcripts was significantly delayed and reduced. Thus, the induction of the two transcription factors, C/EBPα and PPARγ, known to be of decisive importance in terminal differentiation, was delayed in all clones that express ACBP antisense RNA. At day 10, levels of C/EBPα and PPARγ transcripts as well as proteins were significantly reduced in the clones with expression of ACBP antisense RNA. The fact that PPARγ protein levels appeared to be significantly more reduced than PPARγ transcript levels in these clones suggests that the expression of PPARγ is subject to translational/post-translational control. Transcript levels of C/EBPβ and C/EBPδ, which are thought to play a role in the initiation of differentiation, were slightly elevated. Thus, ACBP antisense RNA expression appears to interfere not only with lipid accumulation but also with induction of adipocyte-specific transcripts at a stage where differentiation can also be inhibited by retinoic acid (39), i.e. prior to induction of PPARγ but after induction of early adipocyte markers.

Clonal expansion has generally been regarded as a prerequisite for terminal differentiation of preadipocyte cell cultures (40–43). Clonal expansion was not abrogated in the clones expressing ACBP antisense RNA and comparison of growth rates for the clone GP2-4-2 and untransfected 3T3-L1 cells did not reveal significant differences (results not shown), indicating that expression of ACBP antisense RNA interferes neither with preconfluent cell growth nor with the reinitiation of cell cycling induced by the addition of differentiation inducers.

It is conceivable that the effect of ACBP antisense expression is related to perturbations of metabolic pathways involving handling of acyl-CoA esters. The reduction in ACBP expression may interfere with synthesis of triglycerides; however, the results presented here indicate that proper handling of acyl-CoA esters is also of importance for signal transduction pathways leading to adipocyte differentiation.

Long chain acyl-CoA esters play a decisive role in regulation of gene expression in bacteria via binding to the FadR transcription factor (44). Similarly, we have recently demonstrated
that perturbation of ACBP expression in yeast, which leads to changes in the intracellular levels of acyl-CoA esters (23), results in transcriptional deregulation of the \( \Delta 9 \) desaturase gene (OLE1), suggesting that ACBP/acyl-CoA esters may also be involved in transcriptional regulation in eukaryotes.

The well documented stimulatory effect of fatty acids and fatty acid derivatives on adipocyte differentiation may to a large extent depend on the activation of members of the PPAR family (8, 9). It is not clear what role acyl-CoA esters play in this activation; however, cotransfection of vectors expressing acyl-CoA synthetase was shown to inhibit PPAR-mediated transactivation (38), suggesting that the fatty acids or fatty acid analogs rather than their CoA derivatives are the activators of the PPARs. Recent results from our laboratory indicate that acyl-CoA esters antagonize the effects of activating ligands on the PPAR/RXR complex that acyl-CoA esters antagonize the effects of activating ligands necessary for PPAR activation. This view is compatible with our finding that administration of a very potent ligand BRL49653, is able to by-pass the blockade of adipocyte differentiation imposed by the down-regulation of ACBP expression.

Taken together, the results presented in this report strongly support the idea that proper intracellular handling of acyl-CoA esters is of crucial importance for adipose conversion and that perturbation of the normal expression of the acyl-CoA handling protein ACBP exerts a profound influence on the differentiation process.

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