Role of the POZ Zinc Finger Transcription Factor FBI-1 in Human and Murine Adipogenesis*

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Matthias Laudes¶, Constantinos Christodoulides§, Ciaran Sewter¶, Justin J. Rochford¶, Robert V. Considine¶, Jaswinder K. Sethi**, Antonio Vidal-Puig§, and Stephen O'Rahilly¶¶

From the Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QO, United Kingdom and the Division of Endocrinology and Metabolism, Indiana University School of Medicine, Indianapolis, Indiana 46202

Poxvirus zinc finger (POZ) zinc finger domain transcription factors have been shown to play a role in the control of growth arrest and differentiation in several types of mesenchymal cells but not, as yet, adipocytes. We found that a POZ domain protein, factor that binds to inducer of short transcripts-1 (FBI-1), was induced during both murine and human preadipocyte differentiation with maximal expression levels seen at days 2–4. FBI-1 mRNA was expressed in human adipose tissue with the highest levels found in samples from morbidly obese subjects. Murine cell lines constitutively expressing FBI-1 showed evidence for accelerated adipogenesis with earlier induction of markers of differentiation and enhanced lipid accumulation, suggesting that FBI-1 may be an active participant in the differentiation process. Consistent with the properties of this family of proteins in other cell systems, 3T3L1 cells stably overexpressing FBI-1 showed reduced DNA synthesis and reduced expression of cyclin A, cyclin-dependent kinase 2, and p107, proteins known to be involved in the regulation of mitotic clonal expansion. In addition, FBI-1 reduced the transcriptional activity of the cyclin A promoter. Thus, FBI-1, a POZ zinc finger transcription factor, is induced during the early phases of human and murine preadipocyte differentiation where it may contribute to adipogenesis through influencing the switch from cellular proliferation to terminal differentiation.

Rather than being viewed as a simple storage depot for excess energy, adipose tissue is now seen as playing an active role in the control of energy homeostasis and in the mediation of the adverse consequences of obesity (1). The molecular mechanisms governing adipocyte function and differentiation are therefore the subject of increasingly intensive research. As is the case with other mesenchymal cells, adipocyte differentiation involves a two-step process. During the initial proliferation phase, growth-arrested preadipocytes re-enter the cell cycle and complete two rounds of cell division, a process known as mitotic clonal expansion. This is followed by the terminal differentiation phase in which the specific genes that define the adipocyte phenotype are induced (2).

In the present study, we detected the poxvirus zinc finger (POZ) zinc finger transcription factor factor that binds to inducer of short transcripts-1 (FBI-1) in an oligonucleotide microarray experiment designed to identify novel genes involved in early human adipogenesis. FBI-1 was initially cloned as a cellular factor binding to a specific sequence within the human immunodeficiency virus, type 1 promoter (3–5). FBI-1 is the human homologue of rat osteoclast-derived zinc finger protein (6) and the mouse lymphoma-related factor (LRF) (7) with an overall homology of 85% and a perfect match in the functional domains, the amino-terminal POZ complex, the four carboxyl-terminal zinc finger domains, and the nuclear localization signal (8). A common feature of POZ zinc finger proteins is their ability to repress transcription via the amino-terminal POZ domain. This domain enables the protein to interact with corepressors like the silencing mediator of retinoic acid and thyroid hormone receptors resulting in activation of a histone deacetylase complex and thereby gene silencing (9).

Members of the POZ zinc finger transcription factors have been implicated in promoting growth arrest and terminal differentiation in several tissues. In hematopoiesis, the promyelocytic leukemia zinc finger protein is induced during normal megakaryocytic development and promotes differentiation of these cells (10). Another POZ zinc finger protein, the B-cell lymphoma-6 protein, was found to be up-regulated during myogenesis (11, 12), and experimental data suggest that this factor facilitates the differentiation of proliferating myoblasts into mature skeletal muscle cells (11). Finally the osteoclast-derived zinc finger protein was identified in osteoclastogenesis where it is involved in the fusion of mononuclear precursor cells into multinuclear mature cells (6). Members of the POZ zinc finger family of transcription factors have not, to our knowledge, been previously implicated in adipogenesis.

Here we present evidence for a role of FBI-1 in human and murine preadipocyte differentiation. Our data suggest that this transcription factor facilitates adipogenesis and might be involved in termination of the initial mitotic clonal expansion phase and subsequent induction of terminal differentiation.
FBI-1 in Human and Murine Adipogenesis

MATERIALS AND METHODS
Preparation, Culture, and Differentiation of Human Preadipocytes— Adipose tissue samples were obtained from metabolically healthy subjects by cell-sparing open abdominal surgery. All subjects fasted for 6 h prior to the operation, and all underwent general anesthesia. Cambridge Research Ethics Committee approval was obtained. All patients gave their informed consent. Adipose tissue biopsies were taken under sterile conditions and were transported into the laboratory in normal saline. After dicing the tissue into 1–2-mm pieces, samples were digested in collagenase solution-balanced salt solution, 3 mg/ml collagenase (type II, Sigma), and 1.5% bovine serum albumin at 37 °C for 1 h. Subsequently the digest was filtered through a 260-μm stainless steel mesh and centrifuged at 400 × g for 5 min. The cell pellet containing the stromavascular fraction was treated with red cell lysis buffer (0.154 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA) for 5 min at room temperature. After centrifugation the pellet was resuspended in minimum essential medium (Dulbecco’s modified Eagle’s medium; Ham’s F-12 (1:1), 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin), and isolated preadipocytes were inoculated into 75-cm² flasks. Medium was changed every 2 days. Cells were passaged four times before they were cultured to confluence. 3 days postconfluence, differentiation was induced by adding serum-free differentiation medium (Dulbecco’s modified Eagle’s medium: Ham’s F-12 (1:1), 2 mM L-glutamine, 1% penicillin-streptomycin, 0.1% L-glutamine, 17 mM pantothenic acid, 10 μg/ml human apotransferrin, 0.2 mM triiodothyronine, 100 mM cortisol, 500 mM insulin, 100 mM BRL49653 (rosiglitazone), 100 mM LG100268). For the first 3 days of culture, 0.25 mM [3H]thymidine was added in the microarray experiment. Expression of LPS was assessed according to the instructions of the manufacturer: anti-p38 mitogen-activated protein (MAP) kinase (Dako, Denmark). The anti-aP2 antibody was kindly provided by D. A. Lockwood (14). FBI-1-overexpressing and control 3T3-L1 cells were induced to differentiate, and at the given time points, medium was replaced by medium containing 0.5% bovine serum albumin and 2 μCi/ml [3H]thymidine. After incubation for 1 h, cells were washed twice with PBS (pH 7.4) followed by an incubation for 30 min in 10% trichloroacetic acid at 4 °C. Subsequently cells were washed with 5% trichloroacetic acid and solubilized with 0.2 mM NaOH. After neutralization using 0.4 M HCl, activity was measured by scintillation counting.

Preparation, Culture, and Differentiation of 3T3-L1 Preadipocytes— Cells were washed twice with cold PBS (pH 7.4) and scrapped into lysis buffer (50 mM HEPES, 150 mM NaCl, 10 mM EDTA, 10 mM Na₂HPO₄, 100 mM NaF, 200 mM phenylmethylsulfonyl fluoride, 800 μM benzamide, 100 mM NaVO₃, 1% Nonidet P-40, 1 mM dithiothreitol). After centrifugation at 4 °C at 10,000 × g for 10 min, equal amounts of protein were solved in Laemmli buffer, heated to 100 °C, and separated by SDS-PAGE. Proteins were transferred to polyvinylidene fluoride membranes (Millipore). The following antibodies were used: anti-aP2 (sc-317, Santa Cruz Biotechnology), anti-cyclin A (sc-751, Santa Cruz Biotechnology), anti-cyclin-dependent kinase (Cdk) 2 (sc-163, Santa Cruz Biotechnology), anti-E2F-4 (sc-866, Santa Cruz Biotechnology), anti-p107 (sc-318, Santa Cruz Biotechnology), anti-p130 (sc-269, Santa Cruz Biotechnology), anti-peroxisome proliferator-activated receptor (PPAR) γ (sc-7273, Santa Cruz Biotechnology), anti-CCAAT/enhancer-binding protein α (sc-61, Santa Cruz Biotechnology), anti-phospho-PPAR γ (sc-109, Santa Cruz Biotechnology), anti-phospho-Akt (sc-297, Santa Cruz Biotechnology), anti-phospho-Erk1/2 (sc-104, Santa Cruz Biotechnology), and anti-phospho-p38 MAPK (sc-133, Santa Cruz Biotechnology). Secondary antibodies were purchased from Dako and used at a 1:500 dilution in PBS + 1% milk.

Promoter-Reporter Gene Assay—HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin at 5% CO₂ and 37 °C. For transient transfection experiments using FuGENE 6 (Roche Applied Science), cells were seeded in 24-well plates at 80% confluence. A B-glucuronidase expression vector carrying the firefly luciferase under control of the mouse cyclin A promoter was kindly provided by M. Schorpp-Kistner (15). 0.1 μg of this plasmid was used, and 0–0.3 μg of pCDNA3.1-FBI-1 was used. To correct for transfection efficiency, 2 ng/well pRL-CMV (Promega) were co-transfected. Equal amounts of plasmid DNA per well were obtained by adding appropriate amounts of pCDNA3.1. 48 h after transfection, cells were lysed by passive lysis buffer (Promega), and luciferase activity was detected in a luminometer (EG Berthold) using the dual luciferase reporter assay (Promega).

Taqman® Quantitative Real Time Reverse Transcription-PCR—RNA preparation, reverse transcription, and conditions for Taqman real time reverse transcription PCR were performed as described previously (16). Expression levels of aP2 and PAR-2 in mouse 3T3-L1 cells were determined using the following primers and probes: aP2: forward 5′-CACCACGACAGACAGGAAG-3′; reverse 5′-GATGCCGGGGGGCCACACG-3′; PAR-2: forward 5′-TGAGAAGCCACATATAACCTAC-3′; reverse 5′-CCGAGCGGTGAGGAAG-3′. Taqman® dual-labeled probes were used: forward primer, 5′-GATGCCGGGGCCACACG-3′; reverse primer, 5′-AGAGTCCACAGACAGTGCTTAC-3′; probe, 5′-AGAGTCCACAGACAGTGCTTAC-3′. For FBI-1 expression in human adipose tissue samples, the following primers and probes were used: forward primer, 5′-AACGCCAAGGAGGCAATC-3′; reverse primer, 5′-CTCCGCGATGCGAATGAC-3′; probe, 5′-CAAGTCGTCGCGACAGCGG-3′.

Oil Red O Staining—Cells were washed with PBS and fixed in 0.5% glutaraldehyde (Sigma) for 5 min. After washing twice with PBS and once with 60% isopropanol, cells were incubated in oil red O (60% stock solution: 0.5 g of oil red O (Sigma O-0625), 200 ml of isopropanol) and 40% water, filtered before use) at room temperature. Finally cells were washed once with 60% isopropanol and twice with PBS.

Statistical Analysis—Statistical significance was tested using the Mann-Whitney test. *p < 0.05; **p < 0.01; ***p < 0.001.
RESULTS

Oligonucleotide Microarray Analysis—Human subcutaneous preadipocytes were isolated by collagenase digestion and differentiated in vitro for 2 days using the standard hormonal induction medium with (treatment group) and without (control group) the receptor PPARγ agonist rosiglitazone. At 48 h, RNA was extracted and subjected to expression profiling using U95A Affymetrix microarrays (12,627 genes). The microarray experiment was primarily designed to compare gene expression of differentiating human preadipocytes in the presence versus absence of a PPARγ agonist. However, as rosiglitazone-treated human preadipocytes differentiate much more rapidly and completely than cells exposed only to a conventional differentiation mixture, any differences seen between the two treatments could reflect either a specific effect of the PPARγ agonist or a general effect of this agent on extent and pace of the differentiating process. Only 1.1% of the genes present on the chip showed significantly different expression levels between the treatment and control conditions (Fig. 1A). A list of all the genes detected to be increased or decreased more than 2-fold in the presence of rosiglitazone is shown in Tables I and II, respectively. Semiquantitative RT-PCR was performed on a selection of the identified genes (Fig. 1B), and in all cases the microarray results were confirmed.

The largest effect (18.2-fold) was found for FABP-4, the human homologue of mouse aP2. This gene has been previously reported to be a target for PPARγ and be up-regulated during differentiation (17). In addition to FABP-4, several other genes known to play a role in triglyceride synthesis and lipid transport were found to be increased in preadipocytes differentiated with rosiglitazone. These include glyceral-3-phosphate dehydrogenase (18), perilipin (19), and phospholipid transfer protein (20). Another group of up-regulated genes were reported to be involved in regulation of cell proliferation (insulin-like growth factor II (21)) and differentiation processes (M6 antigen (22) and epidermal type fatty acid-binding protein (23)). FBI-1 (AF097916) was identified as a transcript second only to FABP-4 in its -fold difference between the groups. FBI-1 is a cellular POZ zinc finger domain protein able to bind to the human immunodeficiency virus, type 1 promoter (3–5) and is also known as human TTF-1-interacting peptide 21 (TIP-21, GenBank™ accession number AF000561).²

Expression Analysis of FBI-1—Human preadipocytes were isolated and grown to confluence under cell culture conditions before inducing them to differentiate by adding hormonal induction medium. RNA was then extracted at different time points during the differentiation process, and FBI-1 expression was measured by semiquantitative RT-PCR (Fig. 2A). During this time course, FBI-1 was found to be regulated in a biphasic manner with an increase in the first 48 h of differentiation followed by a decline in later stages of adipogenesis.

To ensure that the expression of FBI-1 in cultured preadipocytes was not simply an artifact of cell culture we examined the expression of this gene in human adipose tissue biopsies. Al-

### Table I

| Fold change | GenBank™ accession number | Gene | Putative function |
|-------------|--------------------------|------|-----------------|
| 18.2        | AA128249                 | FABP-4 | Fatty acid transport |
| 5.0         | AF000561                 | FBI-1/TTF-1-interacting peptide 21 | Transcriptional repressor |
| 4.4         | AB005293                 | Perilipin | Lipolysis |
| 4.1         | J03242                   | Insulin-like growth factor II | Cell growth |
| 3.7         | M94856                   | FABP (epidermal) | Fatty acid transport |
| 3.4         | W28170                   | cDNA clone | |
| 3.3         | U54778                   | 14-3-3 e | Scaffolding protein |
| 3.0         | X56681                   | Jun D | Transcription factor |
| 3.0         | Z54367                   | Plectin | Cytoskeletal protein |
| 3.0         | AF032108                 | Integrin α, | Extracellular matrix |
| 2.9         | U44385                   | Metalloproteinase inhibitor 2 | |
| 2.8         | L25879                   | p53/HEI epoxide hydrolase | Lipoprotein metabolism |
| 2.6         | L26232                   | Phospholipid transfer protein | Cell surface glycoprotein |
| 2.4         | AC006128                 | Chromosome 19, cosmId F 20900 | |
| 2.3         | X64364                   | M6 antigen | |
| 2.2         | U79287                   | cDNA clone | |
| 2.1         | L35240                   | Enigma | Endocytosis |

² I. Grummet, personal communication.
though FBI-1 has been shown to exhibit widespread expression (8) no studies of adipose tissue have been reported so far. Human adipose tissue biopsies were collagenase-digested and separated into stromovascular and adipocyte fractions before measuring FBI-1 mRNA using real time RT-PCR (Taqman) analysis. FBI-1 mRNA was detectable in both fractions but was approximately 4-fold more abundant in the stromovascular fraction (Fig. 2B). Additionally we compared FBI-1 mRNA levels between visceral and subcutaneous adipose tissue and found it to be expressed at similar levels in these two depots. Finally adipose tissue from morbidly obese subjects expressed higher levels of FBI-1 than that from normal weight subjects (Fig. 2C).

We wished to establish whether the murine adipocyte cell line 3T3-L1 might be a suitable model for the further study of the putative role of FBI-1 in adipogenesis. Expression of the mouse homologue of FBI-1 (LRF) in these cells was measured during differentiation (Fig. 3, A and B). These experiments revealed that the expression in 3T3-L1 preadipocytes during differentiation follows a pattern very similar to human cells with an increase in early adipogenesis and a second decline in later stages of the differentiation process. Marked FBI-1 induction was found only when 3T3-L1 cells were treated with the full differentiation mixture, whereas treatment with the single components alone increased expression only marginally (Fig. 3C). These findings suggest that FBI-1 induction is related to

![Fig. 2. FBI-1 expression in human adipose tissue and adipogenesis. A, after induction of differentiation of human preadipocytes in vitro, RNA was extracted at the given time points, and semiquantitative RT-PCR for FBI-1 was performed. A 2% agarose gel with ethidium bromide staining is shown. Data were normalized using the GAPDH control. B and C, real time PCR for FBI-1 expression in the stromovascular fraction versus mature adipocytes (B) and in whole adipose tissue of subjects with different body mass index (BMI) (C). Data are mean ± S.E. *, p < 0.05; **, p < 0.01; ***, p < 0.001.](http://www.jbc.org/)

![Fig. 3. FBI-1 expression in 3T3-L1 adipogenesis. A and B, semiquantitative RT-PCR for the mouse FBI-1 homologue LRF at different time points during differentiation of 3T3-L1 preadipocytes. Data were normalized by GAPDH expression. A, representative image of a 2% agarose gel stained with ethidium bromide. B, densitometry of n = 3 independent experiments (mean ± S.E.). The effect of single components of the differentiation mixture (C) and of rosiglitazone (D) on FBI-1 expression during 3T3-L1 adipogenesis is shown. Results of the semiquantitative RT-PCR are shown on a 2% agarose gel with ethidium bromide staining. Data were normalized by GAPDH expression. *, p < 0.05. FCS, fetal calf serum; Ins, insulin; Dex, dexamethasone; IBMX, 1-methyl-3-isobutylxanthine; MDI, 1-methyl-3-isobutylxanthine + dexamethasone + insulin (full differentiation mixture); rel., relative.)
the differentiation process itself rather than this protein being a specific target of one of the components of the differentiation mixture. Although FBI-1 was induced during differentiation of 3T3-L1 preadipocytes, in contrast to human preadipocytes its expression was not enhanced by the addition of rosiglitazone to these cells (Fig. 3D).

**FBI-1 Facilitates 3T3-L1 Adipogenesis**—To investigate whether FBI-1 is an active participant in the preadipocyte differentiation process, a 3T3-L1 cell line stably overexpressing the cDNA of human FBI-1 was generated using a retroviral vector system. FBI-1 overexpression was confirmed in preconfluent cells by semiquantitative RT-PCR (Fig. 4A). Levels of overexpression in the FBI-1 cell line were within the physiological range of FBI-1 up-regulation observed during differentiation of normal 3T3-L1 cells. We then induced control cells and FBI-1-overexpressing cells to differentiate by using the standard hormonal induction mixture. At 48-h intervals during differentiation, RNA was extracted, and expression of molecular markers of terminal differentiation was measured by Taqman real time RT-PCR. In the early stages of adipogenesis (day 2), PPARγ2 and aP2 mRNA expression was found to be significantly increased in FBI-1-overexpressing cells compared with control cells (Fig. 4B). We further examined expression of molecular markers of terminal differentiation at the protein level by Western blotting; higher levels of PPARγ2, aP2, and CCAAT/enhancer-binding protein α were seen in cells overexpressing FBI-1 with maximal differences seen at day 4 (Fig. 4C). Finally FBI-1-overexpressing cells exhibited accelerated lipid accumulation as shown by oil red O staining with maximal difference between FBI-1-overexpressing and control cells being seen 6 days after induction of differentiation (Fig. 4D). Taken together, these results suggest that FBI-1 is capable of facilitating terminal differentiation and therefore may act as an active participant in adipogenesis.

**Effect of FBI-1 on Mitotic Clonal Expansion**—We next investigated possible mechanisms whereby FBI-1 might facilitate the early steps of preadipocyte differentiation. FBI-1 expression peaks at days 2–4 (Figs. 2A and 3, A and B) during adipogenesis. During the initial 4 days of differentiation, 3T3-L1 preadipocytes undergo two cycles of synchronized cell division, a process known as mitotic clonal expansion. We therefore speculated that FBI-1 might be involved in regulation of this initial proliferation step.

Control cells and FBI-1-overexpressing 3T3-L1 preadipocytes were grown to confluence and induced to differentiate 2 days later. We then measured DNA synthesis within the first 26 h of differentiation by [3H]thymidine incorporation. Previous studies indicate that [3H]thymidine incorporation in this time frame shows a parabolic shaped curve with a peak at 20 h (24) reflecting mitotic clonal expansion. Control cells and FBI-1-overexpressing cells followed this pattern, indicating that mitotic clonal expansion occurs in both groups (Fig. 5A). FBI-1-overexpressing cells, however, exhibited lower levels of [3H]thymidine incorporation for all of the time points measured with a maximal reduction of 34.38 ± 4.68% at 20 h (p < 0.05).

To further elucidate the inhibitory action of FBI-1 on mitotic clonal expansion we investigated whether FBI-1 affects the expression of proteins involved in the regulation of this initial

![Image](http://www.jbc.org/)

**Fig. 4. Effect of FBI-1 on terminal differentiation.** A, generation of FBI-1-overexpressing 3T3-L1 preadipocytes. Semiquantitative RT-PCR for FBI-1 expression in preconfluent 3T3-L1 preadipocytes (2% agarose gel, etidium bromide staining) is shown. B, expression of molecular markers of differentiation on RNA level. Control cells and FBI-1-overexpressing 3T3-L1 preadipocytes were differentiated, and RNA was isolated before and 2 days after induction. Subsequently expression of the two molecular markers of adipogenesis aP2 and PPARγ2 was analyzed by real time PCR (Taqman). Results are shown as -fold difference compared with control cells of five independent experiments for PPARγ2 and three independent experiments for aP2. Gray columns, control cells; black columns, FBI-1 cells (mean ± S.E.). C, expression of molecular markers of differentiation on the protein level. Control cells and FBI-1-overexpressing 3T3-L1 preadipocytes were differentiated and lysed at the given time points, and whole cell proteins were subjected to Western blotting. D, oil red O staining. Control cells and FBI-1-overexpressing cells were differentiated and stained for lipids 6 days after induction of differentiation. *, p < 0.05. EV, empty vector control; rel., relative; C-EBPα, CCAAT/enhancer-binding protein α.
expression of proteins involved in cell cycle control (26). In the present study we examined E2F-4 expression and found it to be decreased in early stages of differentiation in 3T3-L1 cells overexpressing FBI-1.

Finally the membrane was stripped and reprobed with an antibody for PPARγ. Due to the lower expression at early stages of differentiation, the exposure time was prolonged compared with the Western blots shown in Fig. 4C. This experiment revealed an earlier induction of PPARγ2 in cells overexpressing FBI-1 with detectable levels at 12 and 24 h, while in control cells a signal was not obtained before 32 h of differentiation.

**FBI-1 Acts as a Transcriptional Repressor**—Since FBI-1 is known to be a transcriptional repressor, we wished to investigate whether some of the proteins being expressed at lower levels in FBI-1-overexpressing cells might be direct FBI-1 targets. The POZ zinc finger transcription factor promyelocytic leukemia zinc finger protein has been shown to reduce cell proliferation by inhibiting cyclin A expression (27). Furthermore FBI-1 binding sites have recently been identified, and a consensus sequence has been established (28). As this sequence is present within the mouse cyclin A promoter, we elected to use this promoter as a model system in which to test for direct transcriptional effects of FBI-1 during preadipocyte differentiation. Thus, promoter-reporter gene experiments revealed that co-transfection of increasing amounts of FBI-1 resulted in a significant (p < 0.01) inhibition of cyclin A promoter activity by $29.6 \pm 5.3\%$ (Fig. 6).

**DISCUSSION**

Although members of the POZ zinc finger domain transcription factors have been implicated in differentiation of several mesenchymal tissues (6, 10–12, 29, 30), a role in adipogenesis has, to our knowledge, not been reported so far. In the present report we describe the identification of FBI-1 in human and murine adipogenesis. Our data suggest that this POZ zinc finger transcription factor is an active participant of the preadipocyte differentiation process, and its proposed mechanism is concordant with the function of other members of this protein family in different mesenchymal tissues (11).

FBI-1 was detected in an oligonucleotide microarray experiment originally designed to identify novel rosiglitazone target genes in early human preadipocyte differentiation. This compound belongs to the thiazolidinediones, a class of antidiabetic
drugs known to promote adipogenesis via stimulation of the PPARy (31, 32). A similar experiment using mouse 3T3-L1 preadipocytes has recently been published suggesting that only a small number of genes are regulated by thiazolidinediones in early stages of differentiation (33). Consistent with these findings, our gene expression profiling revealed that only 1.1% of 12,627 genes were significantly altered by rosiglitazone in early human adipogenesis. FBI-1 was found to be markedly induced with a -fold change second only to the known PPARy target aP2. However, since rosiglitazone-treated human preadipocytes differentiate much more efficiently than cells incubated only with the standard differentiation mixture we further investigated whether the induction seen for FBI-1 was related to a higher degree of differentiation or whether this transcription factor is a thiazolidinedione target. An experiment with conditions similar to the microarray expression analysis was performed using the mouse cell line 3T3-L1. These cells are able to differentiate in the absence of thiazolidinediones. While aP2, a known PPARy target (17), was induced by rosiglitazone treatment in these cells, no difference in FBI-1 expression was found compared with vehicle-treated cells. Thus, induction of FBI-1 by rosiglitazone in human preadipocytes appears to be related to the differentiation process rather than this transcription factor being a specific PPARy target.

Expression analysis revealed that FBI-1 is regulated similarly in primary human preadipocytes in vitro and the murine cell line 3T3-L1 with a marked induction during differentiation and a maximum expression at days 2–4. In human adipose tissue biopsies, FBI-1 expression was found to be higher in the stromovascular fraction than in mature adipocytes, which is consistent with a peak in expression in early stages of differentiation. Thus, the extent of its induction and the consistency between human and murine cells regarding the expression pattern as well as the presence in adipose tissue biopsies suggest a biological relevance for FBI-1 in adipogenesis.

Having shown that FBI-1 is induced during adipogenesis, we tried to elucidate its involvement in the differentiation process. Using retroviral gene transfer, 3T3-L1 cell lines were generated expressing human FBI-1 under control of a constitutively active promoter. These cells differentiated more efficiently as shown by an earlier induction of markers of terminal differentiation and enhanced lipid accumulation, suggesting that FBI-1 facilitates adipogenesis. Moreover 3T3-L1 cells overexpressing FBI-1 showed lower levels of E2F-4 at base line and during early stages of differentiation compared with control cells. It has been shown that loss of this transcription factor in mouse embryonic fibroblasts allows the cells to undergo spontaneous adipogenesis (34) and that E2F-4 is capable of inhibiting PPARy expression (35). Therefore, repressing E2F-4 expression might be a mechanism through which FBI-1 facilitates terminal differentiation of preadipocytes. Further evidence that FBI-1 might be an active participant in preadipocyte differentiation was obtained from studies using whole adipose tissue from human subjects. These experiments revealed a higher expression of FBI-1 in morbidly obese individuals compared with lean controls. Under this perspective, it certainly would be intriguing to examine the effects of its loss of function on preadipocyte differentiation; however, there is no obvious dominant negative strategy, no FBI-1 knock-out cells are available, and RNA interference has not been reliably utilisable in 3T3-L1 cells so far.

In vitro differentiation of preadipocytes involves a sequential process. Upon reaching confluence, preadipocytes become contact-inhibited and stop proliferation at the G1/S phase boundary. Following hormonal induction, the cells complete two cycles of cell division known as mitotic clonal expansion (days 1–2). Finally, after a second growth arrest (days 3–4), preadipocytes undergo terminal differentiation (days 4–10) resulting in the expression of genes defining the adipocyte phenotype (36). At the present time the molecular mechanisms regulating the transition between cellular proliferation and differentiation of preadipocytes remain in part elusive. FBI-1 levels peak at the end of mitotic cell expansion, which is why we hypothesized that this molecule could be implicated in its termination. As in differentiation of other mesenchymal cells, the initial proliferation phase depends on the activation of G1 cyclins/Cdk and the retinoblastoma protein-E2F pathway (24, 25). In the present study we show that FBI-1-overexpressing 3T3-L1 cells exhibit decreased DNA synthesis and express lower levels of cyclin A, Cdk2, and p107 suggesting that these cells proliferate less than control cells treated under the same conditions. Furthermore the significant reduction of cyclin A promoter activity in response to increasing amounts of FBI-1 might indicate that some of the changes on protein levels might be due to direct repression of transcription. Future experiments will be performed to address this issue.

At the present time the degree to which mitotic clonal expansion is important in human adipogenesis remains a controversial issue. Earlier studies suggest that in vivo most preadipocytes have already undergone the initial proliferation step (37). Consistent with these results, FBI-1 expression in vitro was found to be much higher in the stromovascular fraction than in mature fat cells suggesting that most preadipocytes might already have terminated mitotic clonal expansion. In contrast, differentiation of human preadipocytes in vitro revealed low levels of FBI-1 expression before induction of differentiation followed by an increase similar to what was found in 3T3-L1 cells. In these experiments, however, we expanded the number of cells before differentiation by letting them proliferate and thereby possibly selected for cells that were originally at earlier stages of differentiation.

Numerous factors involved in differentiation processes have been shown to exhibit a “dual function” by promoting both growth arrest and terminal differentiation (for a review, see Ref. 38). The results obtained in the present study preliminarily suggest such a dual function for FBI-1 in human and 3T3-L1 adipogenesis. From this perspective, it is important to notice that FBI-1 is not simply inhibiting mitotic clonal expansion since it has been shown that this initial proliferation step is absolutely required for differentiation (24) and it is still occurring in FBI-1-overexpressing 3T3-L1 preadipocytes. In these cells, FBI-1 is rather shifting the transition from proliferation to differentiation at an earlier time point indicated by an earlier induction of molecular markers of terminal differentiation.

In the present study we present evidence for a possible role of the POZ zinc finger domain transcription factor FBI-1 in adipogenesis. Our preliminarily data suggest that FBI-1 is promoting growth arrest and terminal differentiation hereby exhibiting a dual function in differentiation of these mesenchymal cells. Furthermore the detection of FBI-1 in human adipose tissue samples suggests a biological relevance for this molecule in adipogenesis rather than being related to in vitro cell culture conditions. Finally increased levels of FBI-1 in adipose tissue of human subjects with morbid obesity might suggest a role for this transcription factor not only in normal physiology but also in the pathogenesis of this important metabolic disease.

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