Transcription factor PU.1 is expressed in white adipose and inhibits adipocyte differentiation

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Wang F, Tong Q. Transcription factor PU.1 is expressed in white adipose and inhibits adipocyte differentiation. Am J Physiol Cell Physiol 295: C213–C220, 2008. First published May 7, 2008; doi:10.1152/ajpcell.00422.2007.—PU.1 transcription factor is a critical regulator of hematopoiesis and leukemogenesis. Because PU.1 interacts with transcription factors GATA-2 and C/EBPα, and both are involved in the regulation of adipogenesis, we investigated whether PU.1 plays a role in the regulation of adipocyte differentiation. Our data indicate that PU.1 is expressed in white adipose tissue. PU.1 protein can also be detected in cultured 3T3-L1 adipocytes. Forced expression of PU.1 in 3T3-L1 cells inhibits adipocyte differentiation, whereas deletion of the transactivation domain of PU.1 abolishes this effect. The inhibition of adipocyte differentiation by PU.1 is achieved, at least in part, through repression of the transcriptional activity of C/EBPα and C/EBPβ. Furthermore, GATA-2 and PU.1 have an additive inhibitory effect on C/EBP transactivation and adipogenesis. Finally, the expression of PU.1 is increased in white adipose of obese mice.

PU.1 TRANSCRIPTION FACTOR belongs to the ETS family of DNA binding proteins (15, 18). It was originally identified as the oncogene Sp1-1 (8) and was thought to be specifically expressed in hematopoietic and lymphatic cells, such as granulocytes, macrophages, and B lymphocytes (14). PU.1 is essential for hematopoiesis and leukemogenesis (10). Mice lacking PU.1 are defective in producing mature macrophages, granulocytes, or B cells and die early (19, 35).

PU.1 protein contains three functional domains: the NH2-terminal transactivation domain, the COOH-terminal DNA-binding domain, and a PEST domain in the middle (12). PU.1 recognizes the consensus sequence GAGGAA with its DNA binding domain (36), which is also called Ets domain, because it is conserved in the Ets family of transcription factors (3). The PEST domain controls the degradation of PU.1 and its interaction with other proteins. PU.1 was reported to interact with other transcription factors, such as GATA-1, GATA-2 (48), C/EBPα (32), and c-Jun (1). Some of these proteins, such as GATA-2 and C/EBPα, are important regulators of adipocyte differentiation. This led us to hypothesize that PU.1 may also play a role in adipogenesis.

Adipose tissue is essential for energy homeostasis and metabolic regulatory functions (34). Although it is still not clear how adipogenic progenitor cells are derived from mesenchymal stem cells, extensive research based on in vitro adipocyte differentiation using cultured cell lines, such as 3T3-L1 or 3T3-F442A, has uncovered the main transcriptional network controlling the terminal differentiation of mature adipocytes from committed preadipocytes (33, 41). The nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ and C/EBP family of transcription factors are key proadipogenic regulators (26). The expression of C/EBPα and C/EBPβ increases dramatically in the first two days after the induction of adipocyte differentiation (2). They subsequently activate the expression of C/EBPα and PPAR-γ, which in turn drive the expression of the genes involved in adipocyte function (31).

The GATA family of transcription factors plays a critical role in cell lineage determination (46). GATA-2 determines peripheral blood cell lineage development from hematopoietic stem cells (24), whereas GATA-3 is essential for the differentiation of type 2 T-helper lymphocytes (21, 49). GATA-2 and GATA-3 also negatively regulate adipocyte differentiation (40, 42). Constitutive expression of either GATA factor results in an inhibition of adipogenesis. The inhibition is mediated by GATA’s repression on the promoters of PPARγ and C/EBPα genes and by an inhibitory protein-protein interaction between GATA and C/EBP transcription factors (40, 42).

In this study, we found PU.1 is expressed in white adipose tissue. Constitutive expression of PU.1 inhibits adipocyte differentiation, and this is mediated by the suppression of C/EBP transactivation by PU.1. Whether there is a combinatorial effect between PU.1 and GATA-2 on the suppression of adipogenesis has also been investigated.

MATERIALS AND METHODS

Animals. C57BL/6 mice or various obese mice (Leptor, Leptoth, tubby, Cpefat, and KKα) were purchased from Jackson Laboratory. For high-fat-diet experiments, 6-wk-old male C57BL/6 were fed on standard chow diet (Lab Diet S053; Purina Mills) or high-fat diet (no. 112734; Dyets) for 5 wk. Epididymal fat tissues of 3-mo-old Leptor mice and 6-mo-old Agouti (A+)(45) mice were also used.

Plasmids. pMEX PU.1 and pMEX PU.1ΔTAD were kindly provided by Dr. Stephanie S. Watowich. PU.1 and PU.1ΔTAD fragments were excised with EcoRI and ligated into the EcoRI site of pbabe-puro vector to generate pbabe-puro-PU.1 and pbabe-puro-PU.1ΔTAD.

Adipose tissue fractionation. Epididymal fat pads from three 1-yr-old C57BL/6 male mice were minced in Krebs-Ringer phosphate buffer and digested with 1 mg/ml collagenase type I (Worthington Biochemical) at 37°C for 1 h as described in the literature (11).

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Digested tissue was filtered through a nylon mesh and centrifuged at 500 rpm for 10 min. The top layer (adipocyte fraction) was collected. The remaining was centrifuged again at 1,500 rpm for 10 min, and the pellet (stromal-vascular fraction) was collected. Protein and RNA were extracted from both fractions.

Cell culture, transfection, and infection. 3T3-L1 and 3T3-F442A fibroblasts were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% calf serum. For differentiation of 3T3-L1 cells, 2 days after confluence, cells were switched to DMEM containing 10% FBS, 1 μM dexamethasone, 0.5 mM isobutyl methylxanthine, and 5 μg/ml insulin for 3 days. The cells were then maintained in DMEM with FBS and insulin for another 3–6 days. Medium was replaced every 2 days. 3T3-F442A cells were stimulated and maintained in DMEM containing 10% FBS and 5 μg/ml insulin for 12 days.

For retroviral induction, Bosc23 cells were transfected with 5 μg pBabe-puro plasmids carrying PU.1 or mutant PU.1ΔTAD by the calcium phosphate method in T25 flasks. After transfection (2 days), packaged retroviral particles in the supernatant were filtrated with 0.45-μm filters. Virus suspension (3 ml) was then mixed with 1 ml medium containing 16 μg polyanhydride. The mixture was used to infect 3T3-L1 cells in T25 flasks. After infection (48 h), 2 μg/ml puromycin were added to the medium to select for positive cells for 5–7 days. The pooled positive cells were used for further experiments. For double infection, pBabe-hydro-GATA2 was transfected into Bosc23 cells to produce retrovirus to infect 3T3-L1 cells expressing PU.1 or PU.1ΔTAD. The double-infected cells were selected with 50 μg/ml hygromycin for 2 wk.

Northern blot analysis. Total RNA was isolated from tissue or cell samples using TRIzol Reagent (Invitrogen) following the manufacturer’s instructions. RNA (10 μg) of each sample was separated on 1% agarose gels and then transferred to nylon membranes. Probes for PU.1, aP2, PPARγ, and adipin were labeled with [α-32P]dCTP to detect the expression of corresponding genes.

Real-time PCR. Real-time PCR was done by using FastStart SYBR Green Master reagent (Roche) or Power SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer’s instruction. PU.1 was amplified by a pair of primers: mPU.1-qF1, CTCGCACTCCAGGTTTCCCTACA; mPU.1-qR1, CTCCTACCCCTTCCTCACTTG. 18S rRNA was amplified as an endogenous control with primers: m18sRNA-qF1, AAGAGACTCTGGCAGTGCATTAG; m18sRNA-qR1, CGCCACTTGTCTCTAAGAG. Primers for PPARγ and adipin amplification are as follows: mPPARγ-qF1, GCTGTATGGGTGAACTCT; mPPARγ-qR1, TGGCATCTCCTGTGCAAACCA; mAdipin-qF1, AATGATGGAGTGACGGATGAC; mAdipin-qR1, ATACATCCTGCTGTAGGTTTCAG.

Western blot analysis. Cells were lysed in lysis buffer (50 mM Tris, 50 mM KCl, 20 mM NaF, 1 mM Na3VO4, 10 mM EDTA, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 5 μg/ml leupeptin, pH 8.0). Protein concentration was determined with the bicinchoninic acid protein assay kit (Pierce). Protein (5–50 μg) was separated in 10% SDS-PAGE and electrotransferred to nitrocellulose membranes. The following antibodies were used: anti-PU.1 (sc-352, 1:500; Santa Cruz), anti-PPARγ (sc-61, 1:1,000; Santa Cruz), anti-C/EBPα (sc-61, 1:500; Santa Cruz), anti-C/EBPβ (sc-150, 1:500; Santa Cruz), Anti-PPARγ (sc-7196, 1:1,000; Santa Cruz).

Luciferase assay. A luciferase reporter construct under the direction of a 200-bp fragment from human complement component C3 promoter that has a high-affinity C/EBP-binding site (39) was provided by Nikolai A. Timchenko (Baylor College of Medicine, Houston, TX). Reporter construct (1 μg) was transfected into HEK293T cells along with plasmids expressing C/EBPα, C/EBPβ, PU.1, PU.1ΔTAD, or GATA-2 using the calcium phosphate method. Renilla luciferase reporter plasmid was included as an internal control for transfection efficiency. After transfection (24 h), cells were lysed, and luciferase activity was measured with the dual luciferase kit (Promega) according to the manufacturer’s instruction. Briefly, 20 μl of cell lysate was mixed with 90 μl of stop and glow buffer was added, and the luciferase activity was recorded as firefly luciferase activity (representing the transcription activity of C/EBPs), and then 75 μl of stop and glow buffer was added.

Fig. 1. PU.1 expression in adipose. A: RNA expression of PU.1 in mouse tissues. Total RNA was isolated from the indicated tissues of 9-mo-old male mice. RNA (10 μg) was used for Northern blot analysis for PU.1. Ethidium Bromide (EtBr) staining of the RNA is shown for the loading and the integrity of the RNA samples. B: quantitative PCR of PU.1 mRNA expression in tissues from 1-yr-old male mice. PU.1 mRNA level is normalized by 18S rRNA. C: quantitative PCR of PU.1 mRNA expression in mature adipocytes fractions (Adi) and stromal-vascular fraction (SV) isolated from a pool of epididymal fat pads from three male 1-yr-old mice. Peroxisome proliferator-activated receptor (PPARγ) was assayed as an adipocyte marker to show the efficiency of SV fractionation. D: Western blot analysis of PU.1 expression in the bone marrow (BM), white adipose tissue (WAT), Adi, and SV fraction. Total protein (20 μg) of each sample except 5 μg for bone marrow was used in this assay. Actin was used as a loading control. PPARγ protein levels were determined and shown as an endogenous control for the efficiency of SV fractionation.
PU.1 inhibits adipocyte differentiation

RESULTS

PU.1 is expressed in adipose tissue and adipocyte. PU.1 was thought to be expressed only in hematopoietic and lymphatic systems. To our knowledge, it has not been investigated whether PU.1 is expressed in the adipose tissue or not. We performed Northern blot analysis to detect PU.1 gene expression in various mouse solid tissues. As shown in Fig. 1A, PU.1 mRNA can be detected by Northern blot analysis in the white adipose tissue and the lung. Using the real-time PCR method, we compared the mRNA levels in white adipose with bone marrow and other tissues. PU.1 expression in bone marrow is five times higher than in white adipose, whereas PU.1 expression in testis, brain, kidney, and liver can still be detected, but the levels are >20 times lower than that in the bone marrow (Fig. 1B). To test if PU.1 mRNA is expressed in preadipocytes or mature adipocytes, we fractionated mouse epididymal fat into a stromal-vascular fraction (containing preadipocytes) and adipocytes. PU.1 mRNA was 12 times higher in preadipocytes than in the mature adipocytes, as detected by quantitative PCR (Fig. 1C). To our surprise, the PU.1 protein distribution pattern is opposite to that of the mRNA (Fig. 1D). PU.1 protein level is much higher in adipocytes than in the stromal-vascular fraction when detected by Western blot analysis with a widely used anti-PU.1 antibody (5, 17). Interestingly, the PU.1 protein levels in adipose tissue and adipocytes are equivalent if not higher than that in the bone marrow.

Because adipose tissue contains extensive vasculature infiltrated with immune cells and PU.1 is known to be expressed in macrophages and neutrophils, there is a possibility that, in adipose tissue, PU.1 is expressed in cells other than preadipocytes or adipocytes. To confirm our findings on the expression of PU.1 in preadipocytes and adipocytes, we used the 3T3-L1 preadipocyte cell line that can be differentiated into adipocytes in vitro (9). Total RNA was collected from 3T3-L1 cells at different time points of adipocyte differentiation. PU.1 mRNA was detected by quantitative PCR. As shown in Fig. 2A, PU.1 mRNA level is higher in undifferentiated preadipocytes cells (day 0) than in the confluent 3T3-L1 cells (day 0). Upon hormonal stimulation for adipocyte differentiation, PU.1 expression reaches its peak in the confluent 3T3-L1 cells (day 0). The expression of adipocyte differentiation markers, as well as exogenous PU.1, was also assessed by Northern blot analysis (Fig. 2B). Adipsin, PPARγ, and C/EBPα are all downregulated in PU.1-overexpressing cells. Similar phenomenon has been observed in 3T3-F442A cells, another preadipocyte cell line. Forced expression of PU.1 in 3T3-F442A cells also inhibited lipid accumulation (Fig. 2C) and adipocyte marker expression (Fig. 2D) during adipocyte differentiation. At first glance, the inhibitory role of PU.1 on adipogenesis seems at odds with the presence of PU.1 protein in the mature adipocytes. Given the fact that PU.1 RNA level is high in preadipocytes but protein level is below detection, its protein only starts to accumulate after 3 days into the differentiation. Because the retroviral driven PU.1 expression is constitutive, it is possible that a high level of PU.1 during the early phase of adipocyte differentiation hinders the differentiation process.

PU.1 inhibits C/EBPα and C/EBPβ transcriptional activity. To explore the molecular mechanism underlying PU.1’s inhibitory action on adipogenesis, we focus on the effect of PU.1 on

expression of PU.1 suppresses adipocyte differentiation substantially, whereas deletion of PU.1’s transactivation domain abolishes this effect. Multiple batches of retroviral infection and differentiation were performed, and the representative results were presented in Fig. 3A. The expression of adipocyte differentiation markers, as well as exogenous PU.1, was also assessed by Northern blot analysis (Fig. 3B). Adipsin, PPARγ, and C/EBPα are all downregulated in PU.1-overexpressing cells.

![Image](http://ajpcell.physiology.org/)

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the C/EBP family of proteins, since C/EBP transcription factors play important roles in promoting adipocyte differentiation, especially at the early stage of the process. In addition, it was previously reported that PU.1 physically interacts with C/EBP family members, such as C/EBP\textsubscript{ε} and C/EBP\textsubscript{β} (32). To test whether PU.1 inhibits adipocyte differentiation through inhibiting C/EBP transcription factors, a luciferase reporter construct containing the C/EBP binding site from human complement component C3 promoter (39) was used. C/EBP\textsubscript{β} robustly activated this reporter construct, as shown in Fig. 4A; however, with the coexpression of PU.1, C/EBP\textsubscript{β} transactivation activity was significantly reduced. Deletion of the transactivation domain of PU.1 abolishes this repression. Furthermore, PU.1 has a similar inhibitory effect on transactivation driven by C/EBP\textsubscript{β} (Fig. 4B). In addition, the repression of C/EBP transactivation by PU.1 is dose dependent (Fig. 4C). As for PPAR\textsubscript{γ}, another key proadipogenic factor, we failed to identify any effect of PU.1 on its transactivation using a PPAR\textsubscript{γ} responsive element-containing luciferase reporter (data not shown). In addition, we found no noticeable inhibition of PU.1 on the luciferase reporter constructs containing 5.4 kb or 1.575 bp C/EBP\textsubscript{α} gene promoters (38) or a 0.6-kb PPAR\textsubscript{γ} gene promoter (40) (data not shown), suggesting that PU.1 does not directly inhibit the expression of C/EBP\textsubscript{α} or PPAR\textsubscript{γ}.

**PU.1 and GATA-2 has an additive effect on the inhibition of adipocyte differentiation.** Both PU.1 and GATA-2 transcription factors inhibit adipocyte differentiation, and it is known that PU.1 and GATA-2 interact with each other (44). We tested if they act in an additive fashion on inhibiting adipocyte differentiation. For this purpose, 3T3-L1 cells were double-infected with pBabe-hygro-GATA2 together with pBabe-puro-PU.1 or pBabe-puro-PU.1\textsubscript{TAD. After selection with hygromycin and puromycin, stable cell lines were stimulated for adipocyte differentiation. The expression of adipocyte differentiation markers was detected by Northern blot analysis (Fig. 5A). Constitutive GATA2 expression effectively inhibits adipocyte differentiation (40, 42). Coexpression of GATA-2 and PU.1 further inhibits adipogenesis. Again, the transactivation domain of PU.1 is critical for its action. This combinatory effect of PU.1 and GATA-2 has also been demonstrated on the suppression of C/EBP transactivation. Although PU.1 or GATA-2 represses C/EBP\textsubscript{α} individually, coexpression of PU.1 and GATA-2 inhibits C/EBP\textsubscript{α} activity further, as shown in Fig. 5B.

**PU.1 expression in adipose tissue is upregulated in obese mice.** To investigate PU.1 level changes in the adipose tissue of obese mice, we examined PU.1 mRNA levels in five different mouse models of genetic obesity (Lep\textsuperscript{ob}, Lepr\textsuperscript{db}, tubby, Cpe\textsuperscript{fat}, and KKA\textsuperscript{Y}). We found that the expression of PU.1 mRNA level is upregulated in the white adipose tissue of obese mice compared with their lean wild-type controls (Fig. 6A). Because PU.1 mRNA level may not correlate with its protein level, we also examined PU.1 protein expression in two mouse models of genetic obesity (Lep\textsuperscript{ob} and Agouti A\textsuperscript{Y}). We found that PU.1 protein levels are dramatically upregulated in the white adipose tissue of obese mice compared with their lean wild-type strain and sex-matched controls (Fig. 6, B and C). The diet-induced
obesity mouse model was also used by feeding C57BL/6 mice on high-fat diet for 5 wk. PU.1 protein level in the white adipose tissue is elevated in high-fat-induced obese mice, as shown in Fig. 6D.

DISCUSSION

PU.1 was thought to be expressed exclusively in hematopoietic and lymphoid cells. However, in this study, we have found that PU.1 is also expressed in the white adipose tissue. This finding was further supported by the detection of PU.1 mRNA and protein expression in differentiated 3T3-L1 adipocytes. A recent study also reported that PU.1 is expressed in testis (25), supporting a broader expression pattern of PU.1 in nonhematopoietic tissues.

In addition, the PU.1 level in the white adipose tissue is elevated in several mouse models of obesity, suggesting that adipocyte hypertrophy and hyperplasia during obesity might be responsible for the elevation of PU.1 in the obese fat. The presence of PU.1 in adipocytes may provide an explanation for the expression of proinflammation factor interleukin (IL)-18 in adipocytes (37), since PU.1 regulates IL-18 gene expression.

Fig. 4. The suppression of C/EBPα and C/EBPβ transactivation activity by PU.1. A and B: HEK293T cells were transfected with a C/EBP responsive luciferase reporter (pC3-Luc) and a Renilla luciferase construct (pRL-TK), together with a C/EBPα- or C/EBPβ-expressing construct. Relative luciferase activity is presented to demonstrate the effect of PU.1 or PU.1ΔTAD on C/EBPα (A) or C/EBPβ (B) transactivation. C: experiment was set up similar to B. Various amounts of PU.1-expressing plasmid (1–5 μg) were used to test the dosage effect of PU.1 on C/EBPβ transactivation. The results are presented as means ± SD of 3 independent experiments. *P < 0.05 and **P < 0.01.

Fig. 5. The additive effect of PU.1 and GATA-2. A: 3T3-L1 cells with double expression of GATA-2 (G2), as well as PU.1 or PU.1ΔTAD, were tested for adipocyte differentiation. The expression of PU.1 and adipocyte differentiation markers was detected by Northern blot analysis. B: HEK293T cells were transfected with a C/EBP responsive luciferase reporter (pC3-Luc) and a Renilla luciferase construct (pRL-TK), together with expressing constructs for C/EBPβ, GATA-2, and PU.1. Relative luciferase activity is presented as means ± SD of 3 independent experiments. *P < 0.05 and **P < 0.01.
in myeloid cells (16, 30). By increasing the secretion of proinflammatory cytokines, such as IL-18, PU.1 may promote inflammation in obese adipose, which may lead to insulin resistance and metabolic syndrome (13).

In preadipocytes and adipocytes, PU.1 mRNA level is not correlated with its protein level. This phenomenon is found in both white adipose tissue and in the cultured 3T3-L1 cells, with a high RNA level in preadipocyte and high protein level in mature adipocyte, respectively. The reason for this is not clear at this moment. Similar phenomenon has been reported for other genes. For an example, Conrads et al. (4) investigated RNA and protein correlation by using large-scale combined proteomic and microarray methods and found that there is little correlation between RNA and protein abundance levels. When gene expression changes in preosteoblast cells after inorganic phosphate induction were analyzed, the overall correlation is low among 1,900 genes identified by both proteomic and mRNA microarray analysis. Although many genes are positively correlated, there are also a number of genes that showed reverse correlation (4). In a recent report, microRNA-155 was shown to be able to regulate the protein level of PU.1 in B lymphocytes (43). Whether microRNA-155 is present in the adipose tissue and whether microRNA-155 is responsible for the different pattern of PU.1 mRNA and protein expression need to be explored in the future.

Constitutive expression of PU.1 in 3T3-L1 and 3T3 F442A cells inhibits adipocyte differentiation, suggesting PU.1 serves as an adipogenesis inhibitor while its protein level is increased in the differentiated adipocytes. Several adipogenesis inhibitors exhibit a similar pattern of expression. For example, the expression of adipogenesis inhibitors FOXO1 (23), p27 (20, 22, 23), p21 (20, 22, 23), and SIRT1 (29) are upregulated along with the progression of adipogenesis. Another example is that C/EBPζ, which acts as a dominant-negative regulator by forming heterodimers with C/EBPα and C/EBPβ to block their DNA binding (6), is only expressed at the late stage of 3T3-L1 cell differentiation. PU.1 shares a similar expression pattern and function mechanism. Because endogenous PU.1 protein level is only elevated after 3 days of differentiation, perhaps forced expression of exogenous PU.1 in the early stages (first 2 days) of the differentiation process hinders the initiation of the differentiation process. Our finding that PU.1 suppresses the action of C/EBPβ is consistent with this notion, since C/EBPβ and C/EBPδ drive the early events of adipocyte differentiation (2, 41). In addition, the expression of adipogenesis inhibitors in later stages of adipocyte differentiation suggests the possible existence of a negative feedback mechanism to control the adipocyte size or function.

Previously, C/EBPα was shown to be able to inhibit PU.1 transcription activity. C/EBPα binds to PU.1 through its leucine zipper in the DNA-binding domain to interfere with the action of the transactivation domain of PU.1 (32). Here we have found that PU.1 also inhibits the transactivation of C/EBPα and C/EBPβ, and this suppression was greatly compromised by the deletion of the transactivation domain of PU.1. We need to point out that HEK293T cells were used for these experiments, since 3T3-L1 cells are quite difficult for transfection. The relevance of this finding to adipocytes needs to be explored in the future. Because C/EBP proteins not only promote adipocyte differentiation but also regulate adipocyte function, such as adipokine secretion and insulin sensitivity (47), PU.1 may also regulate adipocyte function in addition to its effect on adipocyte differentiation. Further investigation is needed to elucidate the function of PU.1 in mature adipocytes.

It was known that PU.1 and GATA factors antagonize each other in deciding the commitment of hematopoietic stem cells to either myeloid or erythroid lineages (48) by mutually inhibiting each other’s transactivation function (48). PU.1 also inhibits the expression of GATA-2 (44), however; both PU.1 and GATA-2 are required for mast cell differentiation (44). Here we have found that PU.1 and GATA-2 act in a cooperative fashion in the suppression of adipocyte differentiation and C/EBP transactivation, suggesting the outcome of PU.1 and GATA interaction varies depending on the cellular context.

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**Fig. 6.** PU.1 expression is elevated in obese mice. A: total RNA was isolated from the white adipose tissues of indicated mice. Northern blot analysis for PU.1 was performed. Wt, wild type control; Mu, mutant. B–D: Western blot analysis of PU.1 protein levels in the epididymal fat tissues of Lepob mice (B), Agouti (A+/+) mice (C) and C57BL/6J mice (D) fed with regular or high-fat diet.
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