Transcriptional activity relies on coregulators that modify chromatin structure or serve as bridging factors between transcription factors and the basal transcription machinery. We identified a new coregulator of peroxisome proliferator-activated receptor γ (PPARγ), BRG1/Brm-associated factor of 60 kDa, subunit c2 (BAF60c2), in a yeast two-hybrid screen of a human adipose tissue cDNA library. BAF60c2 represents a new isoform of BAF60c, a component of the SWI/SNF (mating type switching/sucrose non-fermenting) chromatin remodeling complex. This new isoform as well as the previously identified protein, renamed BAF60c1, is localized primarily in the cell nucleus and is expressed in a wide variety of tissues. Both BAF60c isoforms bind to several nuclear receptors and transcription factors of various families. BAF60c proteins interact in a ligand-independent manner with peroxisome proliferator-activated receptor γ and enhance its transcriptional activity. Both isoforms are enriched in the central nervous system and also modulate the transcriptional activity of retinoic acid-related orphan receptor α1. In conclusion, BAF60c represents a new coregulator that constitutes an important anchoring point by which the SWI/SNF complex is recruited to nuclear receptors and other transcription factors.

In eukaryotic cells the structure of chromatin has been shown to repress gene activation, and both remodeling and repositioning of nucleosomes are necessary to allow binding of transcription factors and the formation of the transcriptional preinitiation complex (1). Chromatin modifying proteins and complexes have been classified based on their catalytic core ATPase subunit (9). ATP-dependent chromatin remodeling complexes have been classified based on their catalytic core ATPase subunit (9). They are involved in transcriptional control as well as in DNA replication, DNA repair, and recombination. The large multi-protein complexes are one subfamily of these remodeling factors where it acts as a master controller of the “thrifty gene response” (16, 17), but it has many additional functions (18). PPARγ heterodimerizes with the retinoid X receptors (RXRs) and is activated by naturally occurring fatty acids or fatty acid derivatives (19). In addition to these natural PPARγ ligands, several classes of synthetic PPARγ agonists have been described including the thiazolidinediones, which are potent insulin sensitizers used in the treatment of type 2 diabetes mellitus (20, 21). To activate transcription, PPARs, like most transcription factors, rely on coregulators that modify chromatin (22, 23). Coregulators partially determine the specificity of action of nuclear receptors and integrate their different activities to orchestrate a specific cellular response. It has long been known that the SWI/SNF complex can cooperate with nuclear receptors to modulate transcription (24–27). SWI/SNF complexes have been shown to be involved in the regulation of transcription triggered by the estrogen receptor (ER) (28–31), the glucocorticoid receptor (22, 32–37), the retinoic acid receptor (38), PPARγ (39), and the androgen receptor (40). Several mechanisms through which nuclear receptors recruit SWI/SNF complexes to their target promoters have been described and include direct interaction with hBrm

The abbreviations used are: BAF, BRG1/Brm-associated factor; BAF60c, BRG1/Brm-associated factor of 60 kDa, subunit c; FXX, farnesoid X receptor; BOR1, retinoic acid-related orphan receptor α1; SREBP1a, sterol regulatory element-binding protein 1a; PBX1, pre-B cell leukemia transcription factor 1; PPARγ, peroxisome proliferator-activated receptor γ; RXX, retinoid X receptor; ER, estrogen receptor; GFP, green fluorescent protein; EGF, enhanced GFP; TK, thymidine kinase; aa, amino acids; RNAi, RNA interference; GST, glutathione S-transferase; BrdUrd, 5-bromo-2-deoxyuridine; PDX-1, pancreas/duodenum homeobox-1; h-, human.
The local ethics committee of the CHU in Lille approved the project. Going endoscopic cholecystectomy after informed consent was obtained. Pose tissue was obtained from a female non-obese adult subject under-

using a LightCycler and the DNA double-strand specific SYBR Green I
gtc gtc tg-3

pSG5-hPPAR
Luc is a gift from V. Giguere. pCMV-p300HA was a gift from R. Eckner. pREP4-Luc was given by K. Zhao (43). The pREP4-J3-TK-Luc
tation. pREP4-Luc was given by K. Zhao (43). The pREP4-J3-TK-Luc construct contains three tandem repeats of the J site of apolipoprotein A-II promoter coupled upstream of the herpes simplex virus thymidine kinase (TK) promoter and the Luc reporter gene. pGL3-ROB63-TK-Luc
is a gift from V. Giguere. pCMV-p300HA was a gift from R. Eckner. pSG5-hPPAR2, pcDNA-BD64a-hPPAR-DE (amino acids (aa) 181–507), and pGL3-(GAL5)-TK-Luc reporter plasmids were described elsewhere (44).

RNA Isolation, Reverse Transcription-PCR, and Quantitative PCR
Oligonucleotides—RNA was isolated from cell lines, murine tissues, and human adipose tissue by the acid guanidinium thiocyanate/phenol/ chloroform extraction method (45). RNA from other human tissues were pur-
achased at Clontech (human total RNA master panel II). Reverse trans-
scription of RNA was performed as described (46). Oligonucleotides
used for PCR were 5′-agt gtg acg gct cca cca-3′ and 5′-gag gag ttt gcc agg ctg ctc-3′ for hBAF60c1, 5′-atg gcc gcg gac gaa gtc gcg gcc gga-3′ and 5′-gag gcc ccc ctc gct gcc ggc gcc gga-3′ for hBAF60c2, 5′-agt gag gat ttt gcg gca gga gaa ggt cgg ggc gaa-3′ and 5′-cta gct ggt ccc ggc gaa gaa ggt cgg ggc gaa-3′ for mBAF60c, 5′-agt gag gat ttt gcg gca gga gaa ggt cgg ggc gaa-3′ and 5′-agt gag gat ttt gcg gca gga gaa ggt cgg ggc gaa-3′ for mBAF60c2, 5′-agt gag gat ttt gcg gca gga gaa ggt cgg ggc gaa-3′ and 5′-cat gcg gca gga gaa ggt cgg ggc gaa-3′ for hBAF60c C-terminal part.

For immunofluorescence cells were grown on cover slips and incubated with antibodies after fixation and permeabilization with metha-

Preparations were then incubated with Texas Red-conjugated anti-

mouse IgG and fluorescein isothiocyanate-conjugated anti-rabbit IgG.

Cell Counting and 5-Bromo-2′-deoxyuridine (BrdUrd) Incorporation
Assay—Cells (2000) were plated in 6-well plates and counted in trip-
licates the day after 4 days of culture. BrdUrd incorporation assay cells
were plated on cover slips. After 1 night cells were incubated 2 h with BrdUrd, and an immunofluorescence assay was performed using a mouse anti-BrdUrd antibody (DAKO A/S, Glostrup, Denmark). Nuclei were labeled by Hoechst staining. The percentage of cells incorporating BrdUrd was assessed by counting 5 different areas of about 100 cells. Cell counting was done in three independent experiments, and error bars indicate S.D.

RESULTS

Isolation of BAF60c2 and Genomic Organization—PPARγ is highly expressed in adipose tissue, where its function has been well characterized (16). To isolate new PPARγ coregulators we performed a yeast two-hybrid screen of a human adipose tissue cDNA library with the DE domain of PPARγ fused to the DNA binding domain of the yeast Gal4 activator as a bait. The C-terminal DE domain of PPARγ encompasses the ligand bind-

In Situ Hybridization Experiments—CD1 embryos from 11.5 to 16.5 days post coitum and C57Bl6 adult brains were directly embedded in cryomatrix (Shandon, Pittsburgh, PA). In situ hybridization were performed as described (47). A portion of mouse BAF60c cDNA in pBS was linearized by XhoI and EcoRI. Antisense mBAF60c mRNA was synthesized using T7 polymerase (Promega, Madison, WI). A sense RNA probe for mBAF60c (synthesized using T3 polymerase) was used as negative control (data not shown).
One of the constructs isolated in our yeast two-hybrid screen (clone 7) was a cDNA containing a 1452-bp open reading frame encoding a polypeptide of 484 residues. This cDNA was identical to the one coding for the BAF60c protein except that the 39 bp, coding for the 13 first amino acids of this protein, were replaced by 78 bp coding for 26 residues (Fig. 1A). To determine whether the isolated clone could represent an alternatively spliced form of BAF60c, we first reconstituted the genomic structure of BAF60c by comparing its cDNA sequence to human genome sequences available in databases (AC005486 and NM_003078) and identified the intron/exon boundaries in that gene (Fig. 1C).

The human BAF60c gene is located on chromosome 7 (7q35–36) and comprises 13 exons and 12 introns (Fig. 1B). The N-terminal domain of clone 7 is encoded by a so far unidentified exon located between exon 1 and 2 of the previously described hBAF60c gene (10) (Fig. 1B). Hence, we renamed the exon encoding the N-terminal part of the BAF60c gene described in the literature as "exon 1A" and the newly identified exon as "exon 1B." Sequencing of the cDNAs obtained by reverse transcription-PCR with two different primer sets confirmed the existence of two different mRNAs for BAF60c. Therefore, our yeast two-hybrid screen allowed us to isolate a splice variant of BAF60c, which we named BAF60c2, encoded by a cDNA of 2004 bp. The nucleotide sequence for the BAF60c2 gene has been deposited in the GenBankTM data base under GenBankTM accession number AY450430. The BAF60c protein previously identified (10) was renamed BAF60c1. Its nucleotide sequence has been deposited in the GenBankTM data base under GenBankTM accession number AY450431.

The new BAF60c2 isoform has not yet been described in human or mouse. A data base search revealed, however, the existence of a mouse clone (NM_025891) whose coding sequence is highly similar to hBAF60c2 (93% identity between DNA sequences, with a 100% identity for the first 78 bp; see Fig. 1A) and which might be the mouse counterpart of hBAF60c2. The genomic sequence of mouse chromosome 5 (NW_000225), carrying the mBAF60c gene, also contains a sequence with high homology to exon 1A, but the putative exon 1A in mouse contains 4 additional bases, disrupting the reading frame. The mBAF60c1 protein can, hence, not be produced in contrast to hBAF60c1 and mBAF60c2. The mouse genomic sequence furthermore demonstrates the perfect conservation of exon 1B coding for the N terminus of mBAF60c2 as compared with hBAF60c2 exon 1B.

BAF60c Is Abundantly Expressed and Enriched in the Central Nervous System—Both isoforms of BAF60c were broadly expressed, with the highest levels in brain, testis, and uterus (Fig. 1, D–E). Strikingly, hBAF60c2 is the predominant isoform in adipose tissue (from which it was cloned), skeletal muscle, lung, heart, and thyroid. hBAF60c1 showed only a higher expression than hBAF60c2 in brain, spleen, and trachea. These
expression data extend and refine the expression results previously reported for BAF60c (10).

We also performed in situ hybridization experiments to study the expression of BAF60c mRNA during mouse development (Fig. 2). In mouse embryos, BAF60c mRNA expression was detected by reverse transcription-PCR starting from 7 days post coitum (data not shown). In situ hybridization experiments indicated BAF60c mRNA expression in numerous organs in mouse embryos, such as brain (cortex, thalamus, cerebellum), spinal cord, muscle and heart, diaphragm, tongue, stomach epithelium, and olfactory epithelium (Fig. 2A).

In adult mice (Fig. 2B) mBAF60c mRNA is highly enriched in the brain and cerebellum. In the cerebellum, which is involved in the coordination and control of motor functions, BAF60c is highly expressed in Purkinje cells and in deep cerebellar nuclei, i.e. medial, posterior interposed, and lateral (or dentate) nuclei. Purkinje cells are the principal cells of the cerebellum, which transmit their motor signals via the deep nuclei. Consistent with this, the deep nuclei have been shown to play a role in motor functions as well as sensory-motor learning and memory. BAF60c mRNA is also expressed in cerebellar pedoncule, more particularly, in the vestibular nuclei, such as spinal and medial vestibular nuclei (Fig. 2B), which are involved in equilibrium and motricity. BAF60c mRNA is broadly expressed in the primary and secondary motor cortex. Furthermore, BAF60c mRNA is localized in the hippocampus. A high expression of BAF60c mRNA is also observed in piriform cortex as well as olfactory tubercle (data not shown) and the adjacent anterior olfactory nuclei, i.e. medial and ventral (Fig. 2B). Altogether these data indicate that BAF60c could be involved in various functions in the central nervous system and, in particular, motor and olfactory activities.

Cellular Localization of BAF60c—The presence of a putative nuclear localization signal in BAF60c isoforms (hatched boxes, Fig. 1A) suggested that it functions as a nuclear protein. To evaluate the intracellular localization of BAF60c2, 293T cells were transfected with expression vectors for GFP, GFP-hBAF60c1, or GFP-hBAF60c2 fusion proteins and visualized by fluorescence microscopy (Fig. 3A). GFP-BAF60c1 and GFP-BAF60c2 proteins were detected exclusively in the nucleus of 293T cells (middle and bottom panels), whereas the GFP protein was evenly distributed between the nucleus and the cytoplasm (top panel). This nuclear localization was further supported by immunofluorescence studies in transfected cells. We raised polyclonal antibodies directed against specific domains of hBAF60c1 (aa 1–13) or hBAF60c2 (aa 4–20) or against their common region (aa 292–310) (see the asterisks for localization of the epitope, Fig. 1A). COS-1 cells were transfected with expression vectors for hBAF60c1 or hBAF60c2. The three antibodies are highly specific for their respective targets. The overexpressed BAF60c proteins were invariably detected in the cell nucleus (Fig. 3B). Despite the fact that some endogenous BAF60c mRNA was present in these COS-1 cells (data not shown), no endogenous BAF60c protein could be detected with
our antibodies. Altogether, these results suggest that both BAF60c isoforms are primarily nuclear proteins.

BAF60c Establishes Multiple Contacts with PPARγ and Anchors the SWI/SNF Complex to PPARγ—To confirm the interaction between BAF60c and PPARγ detected in the yeast two-hybrid screen, we produced GST fusion proteins consisting of full-length BAF60c or BAF60c2 or regions of BAF60c (Fig. 4A). The quality and quantity of the GST fusion proteins used for these experiments were first verified by Coomassie staining of these proteins separated on denaturating gels (data not shown). In vitro interactions between these various GST fusion proteins and a His-tagged version of the E domain of PPARγ, His-PPARγE, were assayed in pull-down experiments. BAF60c1 and BAF60c2 full-length as well as the N-terminal part of BAF60c2 (BAF60c2Ct) interacted with His-PPARγE (Fig. 4B, top panel, lanes 3–8). The interaction occurred both in the absence as well as in the presence of 1 μM rosiglitazone, which was in sharp contrast to the ligand-dependent interaction between the GST-p300 protein and His-PPARγE (Fig. 4B, top panel, lanes 11–12). Both isoforms of BAF60c contain in their common C-terminal domain two LXXL motifs (see Figs. 1A and 4A), which are consensus motifs for the ligand-dependent interaction of some coregulators with nuclear receptors. However, a fusion protein containing the C-terminal domain of BAF60c with only one of these LXXL motifs (BAF60c2Ct) does not interact with His-PPARγE in our assay (Fig. 4B, top panel, lanes 9–10), whereas the N-terminal fragment of BAF60c that lacks these LXXL motifs still interacts with His-PPARγE (lanes 7–8).

We then tested in a pull-down experiment the interaction between in vitro-translated full-length 35S-labeled PPARγ2 and BAF60c. When fused to GST both the full-length BAF60c proteins (Fig. 4B, bottom panel, lanes 3–6) as well as their N-terminal domains (Fig. 4B, bottom panel, lanes 7–8) interacted with PPARγ in a ligand-independent manner, confirming the results described above with the E domain. The GST-p300 protein interacted in a ligand-dependent manner with PPARγ (Fig. 4B, bottom panel, lanes 11–12), as described previously (44). It was noteworthy that, although the C-terminal domain of BAF60c did not interact with His-PPARγE (Fig. 4B, top panel, lanes 9–10), it showed a weak but consistent interaction with PPARγ (Fig. 4B, bottom panel, lanes 9–10). This result suggests that several interaction domains exist between BAF60c and PPARγ.

To further consolidate the interaction pattern between BAF60c and PPARγ we performed pull-down experiments with various GST-PPARγ fusion proteins (Fig. 4C) and in vitro translated BAF60c proteins. The N-terminal part of PPARγ2 (bAB domain) strongly interacted with both isoforms of BAF60c (Fig. 4D, lane 2). The interaction of the PPARγDE fusion protein with BAF60c was again ligand-independent (Fig. 4D, lanes 3–4) and was weaker than the interaction with the bAB domain. This result confirms the existence of multiple contact domains between PPARγ and BAF60c.

BAF60c has been described as a subunit of the SWI/SNF complex whose core subunit is either BRG1 or hBrm. We performed co-immunoprecipitations from nuclear extracts to demonstrate the existence of endogenous complexes composed of PPARγ, BAF60c, and BRG1 in HeLa cells. Nuclear extracts of HeLa cells were prepared, and immunoprecipitations with antibodies directed against BAF60c1, BAF60c2, the C terminus domain of BAF60c, and PPARγ were performed. In each case the endogenous BRG1 protein was detected in the immunoprecipitate by Western blot (Fig. 4E, bottom). Immunoprecipitations performed with preimmune sera did not reveal any significant presence of BRG1 (Fig. 4E, top). This confirms the in vivo association of BAF60c, BRG1, and PPARγ and indicates that BAF60c anchors the SWI/SNF complex to PPARγ.

BAF60c Interacts with Other Nuclear Receptors and Transcription Factors—We next tested whether RXRα (or NR2B1), the heterodimeric partner of PPARγ, also interacted with BAF60c. A GST-RXRα fusion protein was incubated with various parts of in vitro translated BAF60c (Fig. 5A, BAF60c1Nt, aa 1–728; BAF60c2Nt, aa 1–767) or with the N-terminal part of p300 (p300Nt). GST-RXRα binds p300Nt in a strictly ligand-dependent manner (Fig. 5B, panel e, lanes 2–3). In contrast, the binding between GST-RXRα and the BAF60c1 and -c2 full-length proteins is again ligand-independent (Fig. 5B, panels a and b, lanes 2–3). Similarly to PPARγ, RXRα interacts also with the N-terminal part of BAF60c (Fig. 5B, panels c and d, lanes 2–3). We also tested the interaction between BAF60c and various other nuclear receptors. ERe (or NR3A1), a nuclear receptor that binds DNA as a homodimer, and the bile acid receptor farnesoid X receptor (FXR or NR1H4) also interacted with GST-BAF60c full-length proteins in a ligand-independent manner (Fig. 5C). A GST-BAF60c fusion protein binds to the retinoic acid-related orphan receptor a1 (RORα1 or NR1F1), the liver receptor homolog 1 (LRH-1 or NR5A2), and the steroidogenic factor 1 (SF1 or NR5A1) (Fig. 5D, lanes 2–3), whereas the GST protein alone does not interact with these receptors (Fig. 5D, lane 1). To determine whether BAF60c only
interacted with nuclear receptors or also with other transcription factors, we performed pull-down experiments with several transcription factors belonging to distinct transcription factor families. We tested the interaction between BAF60c isoforms and the sterol regulatory element-binding protein 1a (SREBP1a), a helix-loop-helix transcription factor, c-Jun, a bZIP (basic region and leucine zipper domain) transcription factor and two homeobox proteins, the pre-B cell leukemia transcription factor 1 (PBX1) and the pancreas/duodenum homeobox-1/insulin promoter factor 1 (PDX-1).

In vitro-translated SREBP1a interacted specifically with the two GST-BAF60c fusion proteins but not with the GST protein alone (Fig. 5E, panel a). This was also the case for the proto-oncogene c-Jun and the homeobox protein pre-B cell leukemia transcription factor 1 (Fig. 5E, panels b and c). However, we found no interaction between GST-BAF60c proteins and PDX-1, another homeobox protein (Fig. 5E, panel d). We hence conclude that BAF60c interacts promiscuously with various nuclear receptors and a diverse array of transcription factors. It is, however, important to note that although BAF60c binds to many transcription factors, it does not bind to all of them.

BAF60c Coactivates Nuclear Receptor-mediated Transcription—To test the possibility that BAF60c acts as a coactivator, CV-1 cells were co-transfected with the proliferative-responsive element driven-promoter construct pREP4-J3-TK-Luc together with expression vectors for PPARγ/H9253 and BAF60c1 or -c2 or the corresponding empty vector in the presence or absence of rosiglitazone (0.1 μM) (Fig. 6A, top panel). PPARγ transcriptional activity is stimulated in the presence of ligand. BAF60c1 and BAF60c2 increase this activation of PPARγ in the presence of ligand by 3.4- and 3.9-fold, respectively (Fig. 6A).

To see the influence of BAF60c on different domains of PPARγ, modified mammalian two-hybrid experiments were performed. CV-1 cells were co-transfected with expression vectors coding for hPPARγDE domain fused to the binding domain of the Gal4 yeast transcription factor (BDGal4-PPARγDE), increasing amounts of hBAF60c expressing vectors and a Gal4-responsive reporter construct. In the absence of any coregula-
Fig. 5. BAF60c interacts directly with nuclear receptors and other transcription factors in vitro. A, scheme of the various BAF60c isoforms and mutants used. B, GST-RXRα was incubated with in vitro translated 35S-radio-labeled hBAF60c1 (panel a), hBAF60c2 (panel b), the respective BAF60c N-terminal regions (aa 1–726 or 1–766, panels c and d), or p300Nt (panel e) in the presence or absence of 10 μM LG1069, an RXR agonist. C–E, in vitro translated ERα and FXR (C), RORα1, liver receptor homolog 1 (LHR-1), and steroidogenic factor 1 (SF1) (D), SREBP1a, c-Jun, PBX1, and PDX-1 (E) were incubated with GST, GST-BAF60c1, or GST-BAF60c2 fusion proteins. The presence or absence of ligand is indicated in panel C (17β-estradiol (10 μM) and GW4064 (10 μM) for ER and FXR, respectively).

Fig. 6. BAF60c transactivates PPARγ and RORα1. A, CV-1 cells were cotransfected in 6-well plates with expression vectors for hBAF60c (200 ng/well) and PPARγ (100 ng/well) and with the pREP4-J3-TK-Luc reporter (1 μg/well). Cells were then grown for 24 h in the presence or absence of 10−7 M rosiglitazone. The numbers above the shaded bars indicate the fold induction of the normalized luciferase activity compared with control (Ctrl). RLU, relative luciferase units; β-gal, β-galactosidase activity; DMSO, dimethyl sulfoxide. The experimental design is schematized on the top of each panel. B, cells were cotransfected with different amounts of expression vectors for BAF60c (0–1200 ng/well), an expression vector for BDGal4-PPARγDE (100 ng/well), and with the pGL3-(GAL5)-TK-Luc reporter (500 ng/well). Cells were then grown for 24 h in the presence or absence of 10−6 M rosiglitazone. Results are presented as described under A. C, cells were cotransfected with different combinations of expression vectors for BAF60c (500 ng/well), an expression vector for BDGal4-PPARγDE (100 ng/well), an expression vector for p300 (500 ng/well), and with the pGL3-(GAL5)-TK-Luc reporter construct (500 ng/well). Cells were then grown and analyzed as described under B. D, COS-1 cells were cotransfected in 24-well plates with expression vectors for BAF60c1 or -c2 (0–40 ng/well) and RORα1 (50 ng/well) and with the RORE3-TK-Luc reporter (200 ng/well). The numbers above the shaded bars indicate the fold induction of the luciferase activity compared with control.
BAF60c Does Not Influence Adipogenesis, Cell Proliferation, or DNA Synthesis in vitro.

Fig. 7. BAF60c does not induce adipogenesis, cell proliferation, or DNA synthesis in vitro. A, real-time Q-PCR analysis of BAF60c mRNA expression in 3T3-L1 cells either infected with either an empty retroviral vector (white bar) or with a retroviral vector expressing hBAF60c2 (black bar) or transfected with a RNAi vector targeting mBAF60c (black bar) or the corresponding empty control vector (white bar). Data are normalized for the expression of 36B4 as a control gene. B, oil-red-O staining of 3T3-L1 cells with increased BAF60c2 level or with decreased BAF60c expression and their respective controls. Confluent stably infected cells (overexpressing) or stably transfected (RNAi) were induced to differentiate into adipocytes and stained with oil-red-O after 6 days to visualize neutral lipid accumulation. C, real-time Q-PCR analysis of aP2 and LPL mRNA expression in 3T3-L1 from the experiments shown in panels A and B, D, cell counting in NIH3T3 with increased BAF60c2 level (left) or with a decrease of BAF60c expression (right) was followed for 4 days. Three independent experiments were performed, and error bars represent the S.D.CTR, control. E, analysis of BrdUrd incorporation in the same NIH3T3 cell lines than in panel D. The percentage of cells incorporating BrdUrd after incubation with BrdUrd was assessed in two independent experiments. Data are given by comparison with the corresponding control cell line.

The fixation of BDGal4-PPARγDE to Gal4 response elements induces the transcription of the luciferase reporter gene in the presence of rosiglitazone (Fig. 6B). Increasing amounts of BAF60c induced the transcriptional activity of the chimeric BDGal4-PPARγDE protein in a BAF60c-dose-dependent manner (Fig. 6B). This observation supports the idea that the BAF60c protein coactivates PPARγ in vivo. The activation of the transcriptional activity of PPARγ by BAF60c was in the same range (2–3-fold) as the activation induced by p300, a known coactivator of PPARγ (Fig. 6C). No additional coactivation was observed when p300 was cotransfected with BAF60c proteins (Fig. 6C). Similar experiments were performed to study the influence of BAF60c on the N-terminal AF-1 function of PPARγ using the BDGal4-PPARγAB protein. BAF60c was, however, shown not to influence the AF-1 activation function contained within the hAB domain of PPARγ (data not shown).

We also verified whether BAF60c influenced ORα1 activity. COS-1 cells were co-transfected with a ROR-responsive element driven-promoter construct together with expression vectors for RORα1 and BAF60c or the corresponding empty vectors. BAF60c isoforms increase the transcriptional activity of RORα1 like that of PPARγ in a dose-dependent manner (Fig. 6D). A difference with PPARγ is the fact that BAF60c1 was a better coactivator than BAF60c2 for RORα.

BAF60c Does Not Influence Adipogenesis or Cell Proliferation—BAF60c2 was identified as a partner of PPARγ in adipose tissue. To study an eventual role of BAF60c in adipocyte differentiation and cell proliferation we changed the expression level of BAF60c using retroviral overexpression and the RNAi technique in the preadipocyte 3T3-L1 cell line. For overexpression of BAF60c we infected cells with retroviruses that encode hBAF60c2 or with an empty control retrovirus, whereas for down-modulation we used stable RNAi targeting BAF60c or the corresponding empty control vector. Stable cell lines were selected with neomycin (for the cells retrovirally infected) or puromycin (when BAF60c expression was down-modulated). Change in expression of the BAF60c mRNAs in the cell lines was confirmed by real-time PCR (Fig. 7A). Experiments were performed with a stable modulation of the expression level of BAF60c in view of the duration of the adipocyte differentiation process, which extends over a week. The different confluent 3T3-L1 stable cell lines were treated with the adipocyte differentiation mix for 2 days and then with insulin alone. After 6 days, all cells displayed a differentiated adipocyte-like phenotype, as evidenced by the lipid droplets in their cytoplasm revealed by oil-red-O staining (Fig. 7B). No significant difference could, however, be detected between the various cell lines (Fig. 7B, Control versus BAF60c). The mRNA expression of lipoprotein lipase (LPL) and aP2, two PPARγ target genes, analyzed by real-time PCR was also not significantly different between the different cell lines (Fig. 7C). Hence, stable modulation of the expression of BAF60c in 3T3-L1 fibroblasts does not seem to influence the differentiation of these cells into adipocytes.
To evaluate eventual effects of BAF60c on cell proliferation and DNA synthesis, we also modified BAF60c expression level in NIH3T3 fibroblastic murine cells by using the same techniques as previously described for 3T3-L1 cells. No significant differences in cell proliferation were found between the cell lines with either increased or decreased levels of BAF60c expression when cell number was followed for 4 days (Fig. 7D). Levels of DNA synthesis in these NIH3T3 cell lines, as measured by BrdUrd incorporation for 2 h were again not different between the distinct cell lines (Fig. 7E).

In combination, these studies suggest that changes in BAF60c expression level in 3T3-L1 do not influence adipocyte differentiation. Furthermore, changes in BAF60c expression levels in NIH3T3 cells do not influence their proliferation rate.

**DISCUSSION**

In an attempt to isolate new coregulators of PPARγ activity, we identified, cloned, and characterized a so far unidentified isoform of the BAF60c subunit of the SWI/SNF complex (10) that we named BAF60c2. BAF60c2 protein is also known as SMARCD3 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily d, member 3). We demonstrate that PPARγ, BAF60c2, and BRG1 are physically present in the same complex in vivo. The N-terminal part of BAF60c was shown to bind to the C-terminal part of PPARγ, whereas the C-terminal part of BAF60c interacts with the N-terminal part of PPARγ. Also other nuclear receptors and transcription factors interacted with the BAF60c subunit of the SWI/SNF-remodeling complex. This suggests that these transcription factors recruit the SWI/SNF complex and induce the remodeling of their target genes by contacting multiple SWI/SNF subunits. Consistent with this, BAF60c was shown to influence the transcriptional activity of PPARγ and RORα in a positive manner. Altogether, these results suggest that BAF60c1 and BAF60c2 are genuine coactivators involved in the modulation of the activity of several transcription factors.

The SWI/SNF complex refers to a large family of multisubunit complexes of varying composition (8, 14, 15). They all contain a core set of conserved components, including some BAF proteins and a DNA-dependent SWI2/SNF2-like ATPase (hBrm and BRG1), which accounts for their chromatin remodeling activity. Complexes containing both hBrm and BRG1 have been characterized as transcriptional activators as well as repressors of transcription. It was discovered more than 10 years ago that the SWI/SNF complex can cooperate with nuclear hormone receptors to activate transcription (24–27). The mechanisms through which nuclear receptors recruit SWI/SNF complexes to their target promoters are multiple and include a direct interaction of hBrm (29), BRG1 (29, 31), and BAF57 (30) with ER and an interaction between BAF250 (33) or BAF60a (41) and glucocorticoid receptor. Our current study adds BAF60c to the list of proteins of the SWI/SNF complex that interact with nuclear receptors.

PPARγ is one of the key factors triggering adipogenesis. Recent studies showed that PPARγ activity in adipocytes is highly influenced by a number of coregulators. More particularly, the coregulators cAMP-response element-binding protein (CREB)-binding protein (51), steroid receptor coactivator 1/transcriptional intermediary factor 2 (52), PPARγ coactivator 1 (53) and thyroid hormone receptor-associated protein (54) have been shown to impact adipocyte activity. Interestingly, a recent study suggests that recruitment of the SWI/SNF complex is important for adipogenesis (55). Hence, we hypothesized that the BAF60c protein, isolated in an adipose tissue DNA library, could act as a bridging factor between PPARγ and SWI/SNF and could represent a regulatory element in this process. This was also suggested to be relevant since it has been shown that one BRG1-containing complex called polybromo, BRG1-associated polybromo, BRG1-associated factors (PBAF) is necessary for PPARγ to induce transcription on chromatinized templates in vitro (39). However, 3T3-L1 cells in which BAF60c expression was altered differentiate in vitro at the same rate and to the same extent as cells where these coregulators are expressed at the normal level. This suggests either that the amount of endogenous BAF60c is adequate to achieve SWI/SNF recruitment by PPARγ or that other redundant proteins can anchor the SWI/SNF complex to PPARγ independent of the level of BAF60c in these cells. We also demonstrated that changes of BAF60c expression in NIH3T3 cells do not influence cell proliferation and DNA synthesis. It is possible that BAF60c assists PPARγ in other processes or other cell types but is not required for the processes of adipogenesis and cell proliferation. Although we were, hence, unable to identify a clear biological function for BAF60c using a well defined cell proliferation and differentiation system, an in vivo approach focusing on organs/tissues expressing high amounts of BAF60c might shed light on its action.

Expression studies on BAF60c suggest that its activity is not limited to coactivation of PPARγ. In fact, multiple tissues that express no or little PPARγ are highly enriched in BAF60c. This is consistent with our studies that demonstrate that BAF60c interacts with multiple nuclear receptors (including RXRα, RORα, RORγ, FXR, steroidogenic factor 1, and liver receptor homolog 1) and other transcription factors belonging to distinct families, such as certain homeobox, bZIP, and helix-loop-helix transcription factors. Especially interesting was the high level of BAF60c expression found in brain, skeletal muscle, and heart, suggesting that BAF60c may carry out a particular function in these tissues. It is tempting to speculate that BAF60c could in fact be relevant for higher brain functions coordinated by the cerebellum and cortex. In fact, in the adult cerebellum BAF60c is expressed in nuclei that are involved in motor functions as well as in sensory motor learning and memory. The cerebellar peduncle coordinates equilibrium and motricity, whereas the hippocampus has a primary role in cognition (memory and learning). Therefore, future work should focus on identifying those signaling pathways and transcription factors that crucially depend on BAF60c (and the SWI/SNF complex) for activity. This is a major challenge that will require a systematic approach combining studies in cellular systems, but more important is an in vivo approach using well defined animal model systems.

In summary, we report a functional interaction between several transcription factors and BAF60c isoforms of the SWI/SNF complex and demonstrate that BAF60c influences transcriptional activity of nuclear receptors, more particularly PPARγ and RORα, in a positive way. Our data also indicate that BAF60c isoforms do not influence adipocyte differentiation and cell proliferation.

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