VOLUME 282 (2007) PAGES 37738–37746

Poly(ADP-ribose) polymerase-2 controls adipocyte differentiation and adipose tissue function through the regulation of the activity of the retinoid X receptor/peroxisome proliferator-activated receptor-γ heterodimer.

Péter Bai, Sander M. Houten, Aline Huber, Valérie Schreiber, Mitsuhiro Watanabe, Borbála Kiss, Gilbert de Murcia, Johan Auwerx, and Josiane Ménissier-de Murcia

There was an error in the title of the article. The correct title is shown above.

VOLUME 283 (2008) PAGES 1653–1659

Novel binding site for Src Homology 2-containing protein-tyrosine phosphatase-1 in CD22 activated by B lymphocyte stimulation with antigen.

Chenghua Zhu, Motohiko Sato, Teruhiko Yanagisawa, Manabu Fujimoto, Takahiro Adachi, and Takeshi Tsubata

Dr. Adachi was inadvertently omitted as an author of this article. The correct authors are listed above. Dr. Adachi’s affiliation is the Laboratory of Immunology, School of Biomedical Science, and the Department of Immunology, Medical Research Institute, Tokyo Medical and Dental University and Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, 113-8510 Tokyo, Japan.


The localization and activity of sphingosine kinase 1 are coordinately regulated with actin cytoskeletal dynamics in macrophages.

David J. Kusner, Christopher R. Thompson, Natalie A. Melrose, Stuart M. Pitson, Lina M. Obeid, and Shankar S. Iyer

On Page 23157, the final sentence of the legend to Fig. 8 should read as follows: Data represent the mean ± S.D. of duplicate determinations from a single representative experiment of a total of four identical experiments. On Page 23158, there is an error in the data in Fig. 9 (A–C), and these three panels should be retracted.
Peroxisome Proliferator-activated Receptor (PPAR)-2 Controls Adipocyte Differentiation and Adipose Tissue Function through the Regulation of the Activity of the Retinoid X Receptor/PPARγ Heterodimer

Péter Bai1,§, Sander M. Houten3, Aline Huber1, Valérie Schreiber1, Mitsuhiro Watanabe4, Borbála Kiss1, Gilbert de Murcia5, Johan Auwerx2,*, and Josiane Ménissier-de Murcia1

From the 1Département Intégrité du Génome, UMR 7175, CNRS, Ecole Supérieure de Biotechnologie de Strasbourg, BP 10413, Illkirch 67412, France, the 2Institut de Génétique et Biologie Moléculaire et Cellulaire, 1 Rue Laurent Fries, BP 10142, Illkirch 67404, France, the 3Department of Medical Chemistry, Medical and Health Science Center, University of Debrecen, Nagyerdei krt. 98, Pf. 7., Debrecen 4032, Hungary, the 4Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam 1105 AZ, The Netherlands, and the 5Institut Clinique de la Souris, 1 Rue Laurent Fries, BP 10142, Illkirch 67404, France

The peroxisome proliferator-activated receptor-γ (PPARγ, NR1C3) in complex with the retinoid X receptor (RXR) plays a central role in white adipose tissue (WAT) differentiation and function, regulating the expression of key WAT proteins. In this report we show that poly(ADP-ribose) polymerase-2 (PARP-2), also known as an enzyme participating in the surveillance of the genome integrity, is a member of the PPARγ/RXR transcription machinery. PARP-2−/− mice accumulate less WAT, characterized by smaller adipocytes. In the WAT of PARP-2−/− mice the expression of a number of PPARγ target genes is reduced despite the fact that PPARγ1 and -γ2 are expressed at normal levels. Consistent with this, PARP-2−/− mouse embryonic fibroblasts fail to differentiate to adipocytes. In transient transfection assays, PARP-2 small interference RNA decreases basal activity and ligand-dependent activation of PPARγ, whereas PARP-2 overexpression enhances the basal activity of PPARγ, although it does not change the maximal ligand-dependent activation. In addition, we show a DNA-dependent interaction of PARP-2 and PPARγ/RXR heterodimer by chromatin immunoprecipitation. In combination, our results suggest that PARP-2 is a novel cofactor of PPARγ activity.

Adipose tissue is composed of adipocytes that store energy in the form of triglycerides. Excessive accumulation of white adipose tissue (WAT)2 leads to obesity, whereas its absence leads to lipodystrophic syndromes. The peroxisome proliferator-activated receptor-γ (PPARγ, NR1C3) is the main protein orchestrating the differentiation and function of WAT, as evidenced by the combination of in vitro studies, the analysis of mouse models, and the characterization of patients with mutations in the human PPARγ gene (1, 2). PPARγ acts as heterodimer with the retinoid X receptor (RXR) (3). The PPARγ/RXR receptor dimer is involved in the transcriptional control of energy, lipid, and glucose homeostasis (4, 5). The actions of PPARγ are mediated by two protein isoforms, the widely expressed PPARγ1 and adipose tissue-restricted PPARγ2, both produced from a single gene by alternative splicing and differing only by an additional 28 amino acids in the N terminus of PPARγ2 (3, 6).

PPARγ is activated by binding of small lipophilic ligands, mainly fatty acids, derived from nutrition or metabolic pathways, or synthetic agonists, like the anti-diabetic thiazolidinediones (2, 7, 8). Docking of these ligands in the ligand binding pocket alters the conformation of PPARγ, resulting in transcriptional activation subsequent to the release of corepressors and the recruitment of coactivators. Many corepressors and coactivators have been described such as the nuclear receptor corepressor and the steroid receptor coactivator, also known as p160 proteins (9–11). These corepressors and coactivators determine transcriptional activity by altering chromatin structure via enzyme such as histone deacetylases and histone acetyltransferases (CREB-binding protein/p300). Other mechanisms include DNA methylation, ATP-dependent remodeling, protein phosphorylation, sumoylation, ubiquitylation, and poly(ADP-ribose)ylation.

2 The abbreviations used are: WAT, white adipose tissue; PPAR, peroxisome proliferator-activated receptor; PARP-1 and -2, poly(ADP-ribose) polymerase-1 and -2; TTF1, thyroid transcription factor-1; WT, wild type; RT-qPCR, reverse transcription-coupled quantitative PCR; apoP, adipocyte fatty acid-binding protein 2; ERβ, estrogen receptor β; K19, keratin-19; ChIP, chromatin immunoprecipitation; RXR, retinoid X receptor; HEK293, human embryonic kidney 293; TNFα, tumor necrosis factor α; CREB, cAMP-response element-binding protein; DMEM, Dulbecco’s modified Eagle’s medium; MEF, mouse embryonic fibroblast; siRNA, small interference RNA; BES, 2-bis(2-hydroxyethyl)aminoterephthalic acid.

Received for publication, February 2, 2007, and in revised form, September 25, 2007. Published, JBC Papers in Press, October 19, 2007, DOI 10.1074/jbc.M701021200

The Journal of Biological Chemistry

© 2007 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in the U.S.A.
Poly(ADP-ribose) polymerase-2 (PARP-2) was described by Ame et al. (12) in 1999 as a 66.2-kDa nuclear protein with poly(ADP-ribose)ylating activity. Through its DNA-binding domain in the N terminus (amino acids 1–62), PARP-2 can bind to DNase I-treated DNA and to aberrant DNA forms, and its subsequent activation results in poly(ADP-ribose) polymer formation (12). According to the general scheme of PARP activation, the active enzyme catalyzes the polymerization of poly(ADP-ribosyl)ating activity. Through its DNA-binding domain (amino acids 1–62), PARP-2 can bind to DNA and to different acceptor proteins and itself using NAD$^+$ as a substrate (13). PARP-2 shares a similar catalytic domain (amino acid 202–593) as poly(ADP-ribose) polymerase-1 (PARP-1) (14), the founding member of the PARP family, though PARP-2 has a smaller reaction velocity compared with PARP-1 (12).

PARP-2 has multiple in vivo functions comprising DNA surveillance and DNA repair processes (reviewed in Ref. 15), spermatogenesis (16, 17), inflammation, and oxidative injury (18–20). Most of these functions are accomplished through protein-protein interactions. In PARP-2, the interaction platforms can be mapped to the DNA-binding domain and to the domain E (amino acids 63–202) (21–25). A role for PARP-2 in the regulation of transcription has already been described. In lung epithelial cells PARP-2 interacts with thyroid transcription factor-1 (TTF1). TTF1 is a homeodomain-containing transcription factor of the Nkx-2 family. In these cells, PARP-2 regulates the expression of the surfactant protein-B by affecting TTF1 activity (25). In this study we show that PARP-2 affects the transcriptional activity of PPARY both in vitro and in vivo.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were from Sigma-Aldrich unless stated otherwise.

**Animals**—PARP-2$^{-/-}$ mice and their wild-type (WT) littermates (26) coming from heterozygous crossings were used. Mice were housed separately, had ad libitum access to water and chow, and were kept under a 12-h dark-light cycle. The animals were killed at the age of 7 months by cervical dislocation after 4 h of fasting, and tissues were collected.

**Cell Culture**—3T3-L1 cells were maintained in DMEM (Invitrogen), 10% newborn calf serum (Invitrogen), Gentamicin (Invitrogen), and HEK, and mouse embryonic fibroblasts (MEFs) were maintained in DMEM, 10% fetal calf serum (Adgenix, Voisins le Bretonneux, France), and Gentamicin (Invitrogen). The 3T3-L1 cells were maintained subconfluent.

**MEF Preparation and Differentiation**—MEFs were prepared from embryos as described elsewhere (26). For the differentiation studies 4 $\times 10^5$ MEFs were seeded in 12-well plates and maintained in DMEM, 10% fetal calf serum. The medium was changed every 2 days until confluence. The cells were maintained at confluence for 2 days. Cells were then differentiated in DMEM, 10% newborn calf serum, 5 $\mu$M troglitazone, 5 $\mu$M dexamethasone, 500 $\mu$M isobutylmethylxanthine, and 10 $\mu$g/ml insulin (later defined as differentiation mix), while the control cells received DMEM, 10% fetal calf serum, and Me$_2$SO as vehicle. The medium with the differentiation mix was replaced every 2 days, and the cells were differentiated for 8 days. Control cells after confluence were cultured in DMEM plus 10% fetal calf serum containing only vehicle (Me$_2$SO, 0.2%).

**DNA Constructs**—To create an siRNA-expressing construct, double stranded oligonucleotides were cloned into the pSuper vector (for sequences see Table 1) (27). The oligonucleotides siPARP-2-sense and siPARP-2-antisense (containing the siRNA sequence), as well as the control scrPARP-2-sense and scrPARP-2-antisense (scrambled version of the siRNA sequence), respectively, were annealed in annealing buffer (150 mM NaCl, 1 mM EDTA, 50 mM Hepes, pH 8.0). The resulting duplexes carried BglII and HindIII sites and were cloned into the pSuper using these sites.

**TABLE 1**

Oligonucleotides used to generate pSuper-siPARP-2 and pSuper-scrPARP-2.
The interfering sequences are in bold.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5'–3')</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>siPARP-2 sense</td>
<td>GACTTAAATATGGCCAGGATCTTCAAGAGAAGTTCTCCTGTGGCAGATCATCTTTTA</td>
<td>BglII/sense/loop/antisense/T(5)/HindIII</td>
</tr>
<tr>
<td>siPARP-2 antisense</td>
<td>AGCCATTTTCGCCGGGAAAACACGTGGACCTTACCAAGAGMTTGCGACGTTTGTTCCCCCGA</td>
<td>BglII/sense/loop/antisense/T(5)/HindIII</td>
</tr>
<tr>
<td>scrPARP-2 sense</td>
<td>GACTTTTTCGCCGGGAAAACACGTGGACCTTACCAAGAGMTTGCGACGTTTGTTCCCCCGA</td>
<td>HindIII/T(5)/antisense/loop/sense/BglII</td>
</tr>
<tr>
<td>scrPARP-2 antisense</td>
<td>AGCTTTAAGAATCCGGGGAACAAACGTGGACCTTACCAAGAGMTTGCGACGTTTGTTCCCCCGA</td>
<td>HindIII/T(5)/antisense/loop/sense/BglII</td>
</tr>
</tbody>
</table>

**Transfections**—Transfections were preformed either by the BES-buffered saline method (26) or by JetPei (Polyplus Transfections, Illkirch, France).

**Luciferase Activity Measurement**—3 $\times 10^5$ HEK cells were seeded in 6-well plates and were transfected with pSuper-siPARP-2, pSuper-scrPARP-2, pBabe, or pBabe-PARP-2 using the BES-buffered saline method. Two days later the cells were once more transfected with the constructs mentioned above. Cells were transfected 24 h later with 0.6 $\mu$g of pSuper-siPARP-2/pSuper-scrPARP-2/pBabe/pBabe-PARP-2, 0.4 $\mu$g of $\beta$-galactosidase expression plasmid, 1 $\mu$g of pSG-PPAR$\alpha$/pSG-PARR$\beta$/pSG-PPAR$\gamma$/pCMX-ER$\beta$, and 1 $\mu$g of PPAR-ER-responsive construct. Six hours after transfection, cells were scraped, and luciferase activity was determined. For the determination of PPAR activity, just before transfection, cells were washed in serum-free DMEM medium, and the transfection was carried out in DMEM plus 10% fat-free serum. As ligand we used, fenofibrate (50 $\mu$M), monoethylhexyl phthalate (100 $\mu$M), troglitazone (5 $\mu$M), and $\beta$-estradiol (10 $\mu$M). After 6 h of transfection, cells were washed with phosphate-buffered saline, scraped, and stored at 80°C. Luciferase assay was car-
teried out by standard procedures. Luciferase activity was expressed as luciferase activity/β-galactosidase activity.

* Nile Red Flow Cytometry*—To assess the extent of MEF differentiation, cytotoxic triglyceride content was assessed by determining Nile red uptake (modified from Ref. 32) followed by flow cytometry using a FACSCalibur machine (BD Biosciences). Cells were harvested by adding trypsin/EDTA, and the detached cells were stained with Nile red (20 μg/ml, 5 min). Cells were subjected to flow cytometric analysis with 10,000 events collected for each sample; each measurement point was repeated in 4 parallel replicates. Samples for each cell line were normalized against the non-differentiated cells of the same line. The rate of differentiation was expressed as the percentage of the differentiated cells *versus* total number of cells.

*SDS-PAGE and Western Blotting*—Cells were lysed in lysis buffer (50 mM Tris, 500 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, protease inhibitor mixture, pH 8.0). Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. For the detection of PARP-2, a polyclonal rabbit antibody was used 1:2,000, followed by IgG-peroxidase conjugate (Sigma, 1:10,000). Reactions were developed by enhanced chemiluminescence (Amer-}

### TABLE 2

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’-3’)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>F 5’-AAAG AAC GCC AGC GCC GTT CTC TT-3’ (652–674)</td>
<td>NM_003003.1</td>
</tr>
<tr>
<td>aP2</td>
<td>R 5’-GGT TGG GGT GAT CCT CTT CAG TGG T3-3’ (875–893)</td>
<td>BC054426</td>
</tr>
<tr>
<td>CD36</td>
<td>F 5’-GAT GTG GAA CCC ACA ACT GCA TG3-3’ (1378–1403)</td>
<td>NM_001039507.1/NM_010719.5</td>
</tr>
<tr>
<td>Cyclophilin B</td>
<td>F 5’-TGG AGA CCA CCA AGA CAG ACA-3’ (561–581)</td>
<td>M60456</td>
</tr>
<tr>
<td>LPL</td>
<td>R 5’-TCC GGG AGT CAG CAA GTA T-3’ (626–628)</td>
<td>NM_001039507.1/NM_010719.5</td>
</tr>
<tr>
<td>FAS</td>
<td>F 5’-GCT GCG GAA ACT TCA GAA AAT-3’ (6612–6632)</td>
<td>BC046513</td>
</tr>
<tr>
<td>Leptin</td>
<td>R 5’-AGG ACC CCT GGA ACC AC-3’ (147–163)</td>
<td>NM_008493</td>
</tr>
<tr>
<td>Perilipin</td>
<td>R 5’-AGG CTT CTT GCC GCA GCT-3’ (1511–1527)</td>
<td>NM_175640</td>
</tr>
<tr>
<td>PPARγ1</td>
<td>F 5’-CCA CCA CCT GCA GTA GTG-3’ (158–178)</td>
<td>NM_001116</td>
</tr>
<tr>
<td>PPARγ2</td>
<td>R 5’-CTT GGG ACA GCC ATT TGT A-3’ (436–456)</td>
<td>NM_001039507.1/NM_010719.5</td>
</tr>
<tr>
<td>HSL</td>
<td>F 5’-CCT CTT TCA TCA ACT CC-3’ (1633/2075–1649/2091)</td>
<td>NM_001116</td>
</tr>
<tr>
<td>TNFα</td>
<td>R 5’-GGT TGT GAG GAG GAG CA-3’ (672–671)</td>
<td>NM_00136932</td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>Chip primers</th>
<th>Name</th>
<th>Sequence</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>aP2</td>
<td>F 5’-CCC AGC AAC GCC AGC GCC GTT CTC TT-3’</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>CD36</td>
<td>F 5’-TTT GCT GCG ACA CAG CAA ACA TCA TCA-3’</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>K19</td>
<td>F 5’-AGG CTT TCA TCA TCA ACT CC-3’</td>
<td>AF237661</td>
<td></td>
</tr>
</tbody>
</table>

3T3-L1 cells using α-PARP-2, α-PPARγ2 (Alexis), and α-matrix metalloproteinase-9 (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. We used also a no antibody control. The chromatin fragments collected upon precipitation with the above antibodies were amplified using promoter-specific primers by qPCR. For the analysis of the coding sequence the same qPCR primer set was used as the one for the quantitation of the given gene. The respective primers are listed in Tables 2 and 3. The results were normalized for the signal of the input and were expressed as a percentage of the aP2 signal with the PARP-2 antibody.

For the testing of the K19 primer set we used non-confluent 3T3-L1 cells transfected with pCMX-ERβ. Chromatin immunoprecipitation was performed using the α-ERβ (Santa Cruz Biotechnology), and as controls we used an α-MRE11 (Santa Cruz Biotechnology) and a no antibody control. The chromatin fragments collected upon precipitation with the above antibodies were amplified using K19 promoter-specific primers by qPCR.

*Microscopy*—Formaldehyde-fixed, paraffin-embedded sections (7 μm) were made from WAT samples and were stained with hematoxylin and eosine. The same sections were stained with a biotin-conjugated F4/80 antibody (Sero-tec, Raleigh, NC, 1:100 dilution), and the bound primary antibodies were detected using streptavidin-peroxidase (Vector ABC kit) and diaminobenzidine as chromogenic substrate. Terminally differentiated MEFs were stained by Oil red O as described elsewhere.
Triglyceride Measurement—The triglyceride content of the MEFs was determined using a commercially available Sigma kit according to the manufacturer’s instructions.

Statistical Analysis—Significance was analyzed by Student’s t test. Error bars represent ± S.E., unless noted otherwise.

RESULTS

In Vivo Dysfunction of the PPARγ/RXR Heterodimer in the WAT of PARP-2−/− Mice—The different fat depots (epididymal, mesenteric, and inguinal) and the interscapular brown adipose tissue-associated WAT were measured in 7-month-old PARP-2−/− mice and their wild-type littermates. A proportional loss of the weight of all adipose tissue depots was observed in the PARP-2−/− mice (Fig. 1A). The epididymal WAT stained with H&E (100× magnification), C, the arrow points toward a dilated capillary in the PARP-2−/− epididymal WAT (100× magnification, H&E). Staining with the F4/80 antibody detects macrophages (marked by #) in the vicinity of the dilated capillaries (*).

To identify the molecular changes that contribute to the decreased fat accumulation and abnormal adipocyte morphology, we determined the expression of the PPARγ target genes, TNFα, and hormone-sensitive lipase by RT-qPCR in the epididymal WAT.

TNFα expression was undetectable in 8 of the 22 mice used for this study (4 out of 14 PARP-2+/+ and 4 out of 8 PARP-2−/−). In the TNFα-positive mice, expression levels were not different, ruling out a major role for inflammation in the adipose tissue dysfunction in PARP-2−/− mice. The expression level of hormone-sensitive lipase, which is responsible for lipolysis, was also not different between the two genotypes. The expression of several PPARγ target genes, however, was markedly decreased. These include genes involved in chylomicron and very low density lipoprotein triglyceride hydrolysis (lipoprotein lipase), free fatty acid uptake (CD36), de novo fatty acid synthesis, and endocrine signaling (leptin and adiponectin) (Fig. 1D). Interestingly,
no difference was detected in PPARγ₁ and PPARγ₂ mRNA levels between the different genotypes.

MEF Differentiation Is Affected by PARP-2 Ablation—We next aimed to determine whether MEFs differentiation toward adipocytes was affected by the PARP-2 deletion. Differentiation of PARP-2⁻/⁻ MEFs into adipocytes was decreased as judged by Oil red O staining, determination of lipid content, and Nile red staining followed by fluorescence-activated cell sorting analysis (Fig. 2A).

The expression of genes involved in adipocyte differentiation and function such as PPARγ₁ and PPARγ₂ were decreased in the PARP-2⁻/⁻ MEFs (34). Because the PPARγ transcripts are primarily present in the differentiated cells, these data confirm that PARP-2⁻/⁻ cells differentiate less into adipocytes. The expression of PPARγ target genes, such as lipoprotein lipase, fatty acid synthase, leptin, adiponectin, and adipocyte fatty acid-binding protein 2 (aP2), were decreased in parallel (Fig. 2B).

PARP-2 Expression Modulates Transactivation of PPARs—To measure whether changes in PARP-2 expression affect PPAR transactivation, we used HEK 293 cells transfected with a PPARγ₂ expression vector and a PPARγ-responsive luciferase construct. In these experiments we modulated the expression of PARP-2 expression by overexpression and siRNA depletion. For the siRNA depletion of PARP-2 we used the pSuper-siPARP-2 construct, whereas for PARP-2 overexpression we used the pBabe-PARP-2. The pSuper-scrPARP-2 and the empty pBabe vector served as the respective controls. PARP-2 levels were assessed by Western blotting using a PARP-2-specific antibody. For both constructs, the cells were transfected twice, on day 0 and on day 2. On day 3, the specific siRNA decreased PARP-2 protein levels significantly, whereas the scrambled PARP-2 siRNA did not alter the PARP-2 levels. A strong increase in PARP-2 protein was observed on day 3 of the overexpression experiment (Fig. 3).

PARP-2 depletion diminished the basal PPARγ activity and abrogated receptor activation by its synthetic ligand, troglitazone. Conversely, PARP-2 overexpression induced by 3-fold the basal PPARγ activity, although it does not significantly change the ligand-dependent activation by troglitazone (Fig. 4A). To verify whether this effect of PARP-2 was specific for PPARγ, we performed similar experiments for the related nuclear receptors PPARα (NR1C1) and PPARβ (NR1C2), and
the unrelated estrogen receptor β (ERβ, NR3A2). Interestingly, siRNA depletion of PARP-2 increased the basal activity of both PPARα and -β (Fig. 4, B and C). PARP-2 overexpression did not affect PPARβ but increased PPARα activity. The activation of PPARα and -β with fenofibrate and monoethylhexyl phthalate, respectively, was not modified by the modulation of PARP-2 expression. In addition, neither PARP-2 depletion, nor PARP-2 overexpression had an effect on the basal or ligand-induced activity of ERβ (Fig. 4D). Combined these results indicate specificity of the PARP-2-dependent effect on PPARγ.

**PARP-2 is the Member of the RXR/PPARγ Transcription Complex**—To demonstrate an interaction between PPARγ and PARP-2 we used ChIP assays. To precipitate chromatin from undifferentiated 3T3-L1 cells we used antibodies against PARP-2 and PPARγ. An anti-matrix metalloproteinase-9 antibody and a sample without antibody served as negative controls. We used qPCR to amplify the promoters of the aP2 (6) and CD36 (35) as promoters driven by PPARγ and keratin-19 (K19), as a non-related, ERβ-regulated promoter (36). PARP-2 and PPARγ gave a strong signal on PPARγ-regulated promoters. These signals were significantly higher compared with the signal from the K19 promoter (Fig. 5A). We also performed qPCR reactions to cover the coding sequences of aP2 using the chromatin fragments obtained in the ChIP experiments. The signal of PARP-2 and PPARγ coding sequences in the immunoprecipitates was strongly decreased compared with the signal of the corresponding promoter. Apparently, both PARP-2 and PPARγ are present on the PPARγ-driven promoters but not in the coding sequence (Fig. 5B). In addition, our results suggest that PARP-2 possesses specificity toward the PPARγ-driven promoters, because the signal from ERβ-driven K19 promoter was significantly lower than that from PPARγ-driven promoters.

Despite the huge difference in the signal of the specific promoters and the non-specific regions (K19 promoter, coding sequence) we observed some background signal from the non-specific region. It is likely that this represents the real presence of PARP-2 in these regions, which is probably linked to the formaldehyde-induced DNA damage.

To provide proof that the interaction of ERβ with the K19 promoter is basically detectable we complemented 3T3-L1 cells with ERβ, and we performed ChIP probing with the K19 primer set. To precipitate chromatin from ERβ-complemented 3T3-L1 cells we used an antibody against ERβ, an anti-MRE11 antibody and a sample with no antibody served as negative controls. The precipitate of the ERβ-specific antibody gave significantly higher signal then the non-specific MRE11 (2.7-fold increase) as well as with the non-antibody control (6.1-fold increase) proving that the K19 primer pair is capable of detecting the K19 promoter if present in the precipitate (Fig. 5C).

**DISCUSSION**

PPARγ plays an important role in adipose tissue differentiation and function. In PARP-2 knock-out mice we have identified a defect of adipose tissue function and a decrease of adipocyte differentiation. In *vivo*, the adipose tissue depots had smaller weight and histologically showed an adipodegenerative phenotype.

We have detected a mild inflammation in the WAT of the PARP-2−/− mice. The capillaries were dilated, and we have detected F4/80-positive cells in the vicinity of the capillaries suggesting the presence of macrophages. The areas more distant from the capillaries are devoid of staining. Similar coloration was not observed in the WAT of the wild-type mice. Activated macrophages and adipocytes may secrete pro-inflammatory cytokines, such as TNFα that may induce adipocyte cell death (37). Because TNFα expression was not detectable in many mice and, if it was detected, its expression was not significantly increased by the absence of PARP-2, it is less likely that inflammation is a leading cause of the adipodegeneration in the PARP-2−/− mice. It is also unlikely that increased lipolysis may contribute to the phenotype in the PARP-2−/− mice, because there was no difference in the expression of hormone-sensitive lipase between the wild-type and PARP-2−/− mice.

We did observe decreased expression of multiple PPARγ target genes involved in adipocyte function. Expression of both PPARγ isoforms was normal, suggesting effects on PPARγ/RXR transactivation. *In vitro*, the differentiation of the PARP-2−/− MEFs into adipocytes was delayed when compared with the differentiation of wild-type MEFs. At the end of the differentiation the expression of both PPARγ1 and PPARγ2 was decreased in the PARP-2−/− cells indicating the lack of differentiation. Similarly, the expression of the PPARγ target genes was decreased.

In transfection assays, the ablation of PARP-2 results in the diminution, whereas PARP-2 overexpression raises transactivation by PPARγ. The effect of PARP-2 seems specific for PPARγ, because opposite or no effects were observed for the related PPARα and PPARβ, and the non-related ERβ.

PARP-2 achieves these activities, because it is part of the PPARγ/RXR transcription complex as shown by ChIP assays, suggesting that PARP-2 could act as a PPARγ/RXR receptor cofactor.

Both members of the PPARγ/RXR nuclear receptor dimer might be the effector behind the phenotype of the PARP-2−/− mice. If PARP-2 would directly influence RXR, all PPAR isoforms should respond the same way to the modulation of PARP-2 expression. PPARγ was differentially regulated when compared with PPARα and -β, suggesting that PARP-2 acts on
PARP-2 as a Cofactor of PPARγ

PARP-2 is a multidomain protein with multiple functions. These functions comprise DNA repair (reviewed in Ref. 15), spermatogenesis (16, 17), T-cell development (38), inflammation, and oxidative injury (18–20). Most of these functions are accomplished through protein-protein interactions. The N terminus, with the following domain E are apparently important protein-protein interaction domains, serving as an interaction platform for TRF-2 (21), B23 (22), PARP-1, XRCC1, and DNA polymerase β (23), and TTF1 (25). PARP-2 also homodimerizes with itself through its domain E (23).

PARP-1 has been described as a cofactor for numerous transcription factors (reviewed in 40 and 25), including for some members of the nuclear receptor family, such as the progesterone receptor (41), RXR (42, 43), androgen receptor (44), and the thyroid receptor (42). A recent study, based on in vitro results, suggested that PARP-2 acts as a cofactor of a homeodomain-containing transcription factor, TTF1, which belongs to the Nkx-2 family. Binding of PARP-2 through its E domain to the C terminus of TTF1 regulates the expression of the surfactant protein-B in lung epithelial cells. TTF1-mediated transcription encompasses similar mechanisms, including chromatin modification, and involves some of the same cofactors such as the steroid receptor coactivators as described for PPARγ-coupled transcription. This suggests that similar molecular mechanisms exist both in the case of PPARγ and TTF1-mediated transcription (25). Our results hence confirm the observation of Maeda and colleagues (25), that PARP-2 is a cofactor of some transcription factors, and extend these conclusions by showing that PARP-2 is involved in nuclear receptor-mediated transcriptional control in vivo.

Recent evidence has suggested that the interaction between PARP-1 and the promoter of target gene could be mediated via double strand breaks, which are produced by activation of a nuclear receptor followed by the unwinding of DNA by topoisomerase II (45). Our results do provide evidence that interaction with DNA is important for the interaction between PPARγ and PARP-2. ChIP assays that depend on DNA binding show strong interaction. In contrast, immunoprecipitation experiments performed on cell
sequence (113LIQLL117) was present in the E domain of PARP-2. This observation suggests that not only the physical presence but also the activity of PARP-2, including its enzymatic activity, is essential for efficient gene transcription and, as demonstrated in ChIP assays, the PARP-2 signal is increased in the PPAR\gamma-driven promoters compared with the non-related, ER\beta-driven K19 promoter. These data confirm that the K19 primer set is capable of detecting the presence of the ER\beta receptor on the K19 promoter.

Indeed both PARP-1 and PARP-2 are reported to poly(ADP-ribosyl)ate histones, and PARP-2 specifically occupies the promoter of PPAR\gamma target genes, because it binds efficiently to the regulatory sequence, whereas binding to the corresponding coding sequences was strongly decreased. This rather specific binding, we observed a background signal rising most likely from non-coding regions or from non-PPAR\gamma-dependent promoters, such as that of the K19 gene, which is under the control of ER\beta. When comparing the specific to the above mentioned nonspecific signal, it is at least 10- to 100-fold increased, which can be considered as a significant difference. It is likely that the nonspecific presence of PARP-2 on the K19 promoter and in the non-coding regions is explained by the fact that PARP-2 binds to the DNA-damage sites created by the formaldehyde treatment during the cross-linking of the cells. The cross-linking-related DNA damage is present throughout the entire genome, equally affecting coding regions and promoters, thus theoretically providing a background signal throughout the genome.

The present study indicates that PARP-2 modulates the activity of PPAR\gamma/RXR nuclear receptor complex, a key transcription factor involved in the pathogenesis of several important diseases such as obesity, insulin resistance, type II diabetes, atherosclerosis, and lipodystrophy. Because many of these diseases affect a large part of the population and have high costs to society, our data, linking the activation of PPAR\gamma and PARP-2, show it is possible to modulate PPAR\gamma activity via PARP-2. It is therefore tempting to speculate that the various PARP inhibitors that are currently being developed and tested in clinical trials (51) could also be useful in the metabolic disease arena.

Acknowledgments—We thank Dr. Maria Malanga and Dr. Felix Althaus (Institute of Pharmacology and Toxicology, University of Zurich-Tierspital) for the siRNA sequence against PARP-2. We also acknowledge the help of Dr. Máté Demény, Dr. László Tora, and Dr. Jean-Christophe Ame in the assays performed.

REFERENCES

PARP-2 as a Cofactor of PPARγ