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*

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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) 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 coactivators, 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.

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This work is dedicated to the memory of Josiane Ménissier-de Murcia, who passed away (July 15, 2007).

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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-quantitative PCR; p2, adipocyte fatty acid-binding protein 2; E2, 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)amino)ethanesulfonic acid.
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-ribosyl)ating 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) polymerase activity. Through its DNA-binding domain, PARP-2 can bind to DNA and to aberrant DNA forms, and its subsequent activation results in poly(ADP-ribose) polymerase activity. 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 PPARγ 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. Animals were killed at the age of 7 months by cervical dislocation 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 (Adegenx, 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 × 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 μM troglitazone, 5 μM dexamethasone, 500 μM isobutylmethylxanthine, and 10 μg/ml insulin (later defined as differentiation mix), while the control cells received DMEM, 10% fetal calf serum, and MeSO 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 (MeSO, 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-2sense and siPARP-2antisense (containing the siRNA sequence), as well as the control scrPARP-2sense and scrPARP-2antisense (scrambled version of the siRNA sequence), respectively, were annealed in annealing buffer (150 mM NaCl, 1 mM EDTA, 50 mM Heps, pH 8.0). The resulting duplexes carried BglII and HindIII sites and were cloned into the SnaBI site of the pBC-mPARP-2 (23) and inserted into the SnaBI site of pBABE-puro (Addgene, Cambridge, MA), giving the pBABE-pPARP-2 vector. All other constructs pGL3-(Jw1)TKluc reporter construct (28), pSG-PPARγ2 (3), pSG5-PPARα (29), pSG5-PPARβ (30), pCMX-ERβ, and vitellogeninA2-ERE-TKLuc (ER-luc) (31) were described before. The pCMV-βGal construct was used to control the transfection efficiency.

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

**Luciferase Activity Measurement**—3 × 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 μg of pSuper-siPARP-2/pSuper-scrPARP-2/pBabe/PBabe-PARP-2, 0.4 μg of β-galactosidase expression plasmid, 1 μg of pSG-PPARα/pSG-PPARβ/pSG-PPARγ2/pCMX-ERβ expression vector, and 1 μg of PPAR-ER-responsive construct. Six hours after transfection, cells were scrapped, 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 μM), monoethylyxyl phthalate (100 μM), troglitazone (5 μM), and β-estradiol (10 μM). After 6 h of transfection, cells were washed with phosphate-buffered saline, scraped, and stored at −80°C.

**TABLE 1**

| Name            | Sequence (5’-3’)                                                                 | Structure               |
|-----------------|---------------------------------------------------------------------------------|-------------------------|
| siPARP-2 sense  | GAGTCTAAGATGAAGCAGGAAGATTCTAACAGAAGGTCTCTGTGGAATCATTCATTTCCTCTCCTGGAGAAGGACTCTTGTGGTCCTTGGAGATATTTA | HindIII/sense/loop/antisense/T(5)/HindIII |
| siPARP-2 antisense | AGCTTTCCCGGGGAAACACGTCGACTTCAAAGAGTTGCGACCTTGTGGTCCTTGGAGATATTTA | HindIII/sense/loop/antisense/T(5)/HindIII |
| scrPARP-2 sense | GAGTCTAAGATGAAGCAGGAAGATTCTAACAGAAGGTCTCTGTGGAATCATTCATTTCCTCTCCTGGAGAAGGACTCTTGTGGTCCTTGGAGATATTTA | HindIII/sense/loop/antisense/T(5)/HindIII |
| scrPARP-2 antisense | AGCTTTCCCGGGGAAACACGTCGACTTCAAAGAGTTGCGACCTTGTGGTCCTTGGAGATATTTA | HindIII/sense/loop/antisense/T(5)/HindIII |
rried 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, cytosolic 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 by flow cytometry using a FACSCalibur machine (BD Bio
determining Nile red uptake (modified from Ref. 32) followed
differentiation, cytosolic triglyceride content was assessed by

**Cycler system (Roche Applied Science) and a qPCR Supermix
reactions. The qPCR reactions were performed using the Light-
cia, CA). 50
was purified on QIAquick PCR cleanup columns (Qiagen, Valen-

**TABLE 2**

| Name       | Sequence                  | Accession number |
|------------|---------------------------|------------------|
| Adiponectin| F 5′-AAC AGG GAC AGG GCC GTT CTT TT-3′ (652–674) | NM_009605.4 |
| aP2        | F 5′-CTG CAC AGA AAAT AGG GCAG AG-3′ (132–151) | BC054426 |
| CD36       | F 5′-GGT GAA CCA CCC ATC ACT GGA TT-3′ (1378–1403) | NM_007643 |
| Cyclophilin| F 5′-CTG AGA CCA CCC ATC ACT GGA AG-3′ (1527–1502) | M60456 |
| FAS        | F 5′-GGT GAA CCA CCC ATC ACT GGA TT-3′ (6695–6673) | BC046513 |
| LPL        | F 5′-AGG CAC CCT GAA GAC AC-3′ (317–333) | BC003305 |
| Leptin     | F 5′-GAC ACC AAA ACC ACT AT-3′ (147–163) | NM_008493 |
| Perilipin  | F 5′-GCT TCT TCC GGC CCA GC-3′ (1511–1527) | NM_175640 |
| PPARγ1     | F 5′-CCG CCA ACC ACT GCA TT-3′ (158–178) | NM_011146 |
| PPARγ2     | F 5′-CTG CTT GCC GCC CCA GTT A-3′ (2049/2491) | AY243585 |
| HSL        | F 5′-GTG CCT GCC TCA ACT CC-3′ (1633/2075–1649/2091) | NM_00103950.7 |
| TNFα       | F 5′-GAG ACG CTC GTC CAT CT-3′ (296–280) | NM_0136932 |

**TABLE 3**

| Name | Sequence                  | Reference |
|------|---------------------------|-----------|
| aP2  | F 5′-CCC AGG GAC AGG GCC GTT CTT TT-3′ (652–674) | 52        |
| CD36 | F 5′-CTG CAC AGA AAAT AGG GCAG AG-3′ | 39        |
| K19  | F 5′-GAG CGT GGA GCC ACC ACT GGA TT-3′ | AF273661  |

For the testing of the K19 primer set we used non-confluent 3T3-L1 cells transfected with pCMX-ERβ. Chromatin immuno
collections were amplified using promoter-specific primers by qPCR. For the analysis of the coding sequence the same
PCR 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.

**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. Terminal 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).

Histological examination of the PARP-2−/− epididymal WAT showed adipocytes with reduced and irregular size. This tissue contained dilated capillaries, indicative of inflammation, which was confirmed by a faint staining with the macrophage-specific F4/80 antibody in the PARP-2−/− (Fig. 1, B and C) and the macroscopic appearance of the WAT (Fig. 1A). The F4/80-positive cells were present in the vicinity of the blood vessels.

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 PPAR-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 PPAR-2−/− MEFs (34). Because the PPARγ transcripts are primarily present in the differentiated cells, these data confirm that PPAR-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 PPAR-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
activity of ER overexpression had an effect on the basal or ligand-induced expression. In addition, neither PARP-2 depletion, nor PARP-2 siRNA depletion of PARP-2 resulted on day 3 and day 4 in a robust induction of PARP-2 expression, the transfection with pBabe-PARP-2 resulted on day 3 and day 4 in a robust induction of PARP-2 expression, the transfection with pBabe alone did not modify PARP-2 expression.

The precipitate of the ER antibody and a sample with no antibody served as negative controls. The precipitate of the ER antibody and a sample with no antibody served as negative controls. PARP-2 was depleted by 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 PPAR-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 nonspecific regions (K19 promoter, coding sequence) we observed some background signal from the nonspecific 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 than 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 adipodegenerative phenotype 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

PPAR-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. This study has suggested that the interaction between PPAR-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
transcription initiation (48). Consistent with this line of thinking, there is molecular and in vivo evidence that the enzymatic activity of PARP-1 is necessary for efficient gene transcription, and inhibition of PARP activity impairs the transcription of a number of different genes, including different chemokines and inflammation-related genes (e.g. iNOS, TNFα, ICAM-1, IL-8, MIP-1α, and IL-12) (40, 49, 50).

PARP-2 specifically occupies the promoter of PPARγ target genes, because it bound efficiently to the regulatory sequence, whereas binding to the corresponding coding sequences was strongly decreased. Despite this rather specific binding, we observed a background signal rising most likely from non-coding regions or from non-PPARγ-dependent promoters, such as that of the K19 gene, which is under the control of ERβ. 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 PPARγ/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 PPARγ and PARP-2, show it is possible to modulate PPARγ 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.

extracts showed only a weak interaction between PARP-2 and PPARγ (data not shown), which was abrogated by low concentrations of Nonidet P-40 (>0.1%). Furthermore, like in the case for PARP-1, the N terminus of PARP-2, comprising its DNA-binding domain, seems also to play the most important role in the interaction with PPARγ. Consistent with this observation a nuclear receptor-binding consensus sequence (113L1QQL117) was present in the E domain of PARP-2.

Concerning the mode of action of PARP-2, it is possible that not only the physical presence but also the activity of PARP-2 is necessary for the nuclear receptor function. Poly(ADP-ribosyl)ation is reported to increase throughout the differentiation process of 3T3-L1 cells (46). Interestingly, this poly(ADP-ribosyl)ation activity is not completely inhibited by PARP-1 depletion (47), suggesting the involvement of other member(s) of the PARP family, such as PARP-2. Indeed both PARP-1 and PARP-2 are reported to poly(ADP-ribosyl)ate histones (13). Similarly to histone acetylation, poly(ADP-ribosyl)ation of the high mobility group of proteins and histones loosens chromatin structure enabling
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