PARP-2 Interacts with TTF-1 and Regulates Expression of Surfactant Protein-B*

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Thyroid transcription factor 1 (TTF-1/Nkx-2.1) plays a critical role in lung morphogenesis and regulates the expression of lung-specific genes, including the surfactant proteins required for pulmonary function after birth. The activity of TTF-1 is influenced by its interactions with other transcription factors and coactivators, including CBP/p300 and SRC-1. In this study, we have identified poly(ADP-ribose) polymerases (PARP-2 and PARP-1) as TTF-1 interacting proteins that influence its transcriptional activity. Endogenous PARP-2 was coimmunoprecipitated from transformed mouse lung epithelial cell (MLE15) extracts with TTF-1 and was identified by mass spectrometry. PARP-1 and Ku70/Ku80 were also coimmunoprecipitated from the cell extracts with TTF-1. The E-domain of PARP-2 interacted via the C-terminal domain of TTF-1. Both PARP-1 and PARP-2 enhanced the activity of the promoter of surfactant protein-B (Sftpb gene) but not other surfactant proteins in vitro. PARP-2 was selectively expressed in epithelial cells of the conducting and peripheral lung tubules of the fetal mouse lung from embryonic day 12.5 and was detected in bronchial epithelial cells in the adult lung at cellular sites consistent with that of surfactant protein B. PARP-2 and PARP-1 interact with TTF-1 and regulate the expression of surfactant protein B, a protein required for lung function.

TRANSCRIPTIONAL COACTIVATORS

Transcriptional coactivators are transcription factor-associated proteins that promote transcription (1). cAMP-response element-binding protein (CREB)2-binding protein (CBP) and its structural homologue p300, SRC-1, ACTR, and TIF-2 have been reported to interact with transcription factors expressed in the lung and to affect gene expression of the genes encoding surfactant proteins (2–7).

Thyroid transcription factor 1 (TTF-1) plays a critical role in lung morphogenesis, respiratory epithelial cell differentiation, and gene expression (8, 9). TTF-1 is a homeodomain-containing transcription factor of the Nkx-2 family. TTF-1 regulates the expression of a number of genes selectively expressed in respiratory epithelial cells, including Sftpa, Sftpb, Sfkb, and Sftpd (10, 11). Recent microarray data from a transgenic mouse in which TTF-1 phosphorylation sites were mutated (Tff-1TM) reveals that TTF-1 regulates groups of genes regulating surfactant homeostasis, vasculogenesis, host defense, fluid homeostasis, and inflammation prior to birth (12). TTF-1 binds to a number of regulatory proteins and transcription factors. For example, TTF-1 interacts with the transcriptional coactivator-containing PDZ-binding motif (TAZ) (13) and coactivator-like factor BR22 (14, 15) as well as the histone acetyltransferase-related proteins CBP/p300, SRC-1, ACTR, and TIF-2. TTF-1 interacts with coactivators and often transcription factors, including retinoid acid receptor (16), GATA-6 (17, 18), NFκB (19), STAT3 (20), Runx1 (21), SMAD3 (22), Pax8 (23), nuclear factor I (24), and NFAT (25), to cooperatively activate target genes.

Recent biochemical assays have shown that transcription factors form protein complexes that contain histone modifiers and mediators of DNA polymerase II (25). Poly(ADP-ribose) polymerase (PARP-1) was identified as a component of the protein complex that affects transcription by NFκB (26–28), TFE-1 (29), AP-2 (30), Tax (31), B-MYB (32), E2F1 (33), HES1 (34), estrogen receptor (35), retinoid acid receptor (36), androgen receptor (37), and TCF-4 (38). PARPs include a family of 18 distinct proteins (39). PARP-1 and PARP-2 catalyze base repair excision following DNA damage and mediate chromosome stability. They are also essential during early embryogenesis in the mouse (39). PARP-2 heterodimerizes with PARP-1 and forms a DNA repair complex with DNA ligase III, x-ray cross-complementing factor 1 (XRCCl), DNA polymerase β (40), the telomeric protein TRF2 (41), and the nucleolar factor nucleophosmin/B23 (42). Interactions of PARP-2 with transcription factors have not been reported to date.

To further understand the mechanisms by which TTF-1 activates gene expression in the lung, we isolated TTF-1-interacting proteins by immunoprecipitation using anti-TTF-1 antibody with nuclear extracts from SV40 large T antigen immortalized mouse lung epithelial cells (MLE15 cells). We identified PARP-2 and PARP-1 as TTF-1-interacting proteins. PARP-2 and PARP-1 enhanced TTF-1-mediated activation of the Sftpb gene. PARP-2 was expressed in the respiratory epithelial cells of the developing and mature mouse lung. Thus PARP-2 interacts with the transcription factor TTF-1 and influences target gene expression in pulmonary cells.

EXPERIMENTAL PROCEDURES

Plasmids and Expression Constructs—Rat TTF-1 expression vector pRc-CMV-TTF-1 and truncated TTF-1 expression vector (aa 1–159) (Δ14) (43) were gifts from Dr. Roberto Di Lauro (Stazione Zoologica “A Dohrn,” Laboratory of Animal Genetics, Napoli, Italy). 3xFLAG-Δ3 truncated TTF-1 expression vector (aa 221–372) (Δ3) was produced previously (13). The expression vectors containing FLAG-tagged TTF-1 (TTF) (p3xFLAG-CMV-7.1.TTF1) and pCDNA3.1.TTF1 were made by inserting PCR products from pRC-CMV-TTF-1.
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pEGFP.mousePARP-2 (GFP-PARP-2), GST-tagged protein expressing pBC constructs containing full-length PARP-2 (GST-mPARP-2), truncated pAR-2 expressing aa 1–69 (GST-mPARP-21-69), aa 64–202 (GST-mPARP-264_202), and aa 203–559 (GST-mPARP-2203_559) have been previously described (40). A PstI/PstI fragment encoding human PARP-1 was taken from pBCh.PARP-1 (44) and subcloned into the PstI site of pEGFP-C3 (GFP-PARP-1). The luciferase reporter constructs containing the 5’-flanking region of the mouse SP-A promoter (pGL3.mSP-A, 1.1 kb) (45), the mouse SP-B promoter (pGL3.mSP-B, 1.7 kb) (45), the mouse SP-C promoter (pGL3.mSP-C, 4.8 kb) (45), and the mouse SP-D promoter (pGL3.mSP-D, 0.68 kb) (11) have been previously described.

**Immunoprecipitation Assays**—Transformed mouse lung epithelial cell line (MLE15) that expresses endogenous TTF-1 and surfactant proteins was cultured as described previously (13, 46). FLAG-tagged TTF-1 cell line (MLE15) that expresses endogenous TTF-1 and surfactant proteins was cultured as described previously (13, 46). FLAG-tagged TTF-1 was transfected into ~80% confluent MLE15 cells on five 150-mm culture dishes using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. After 24 h, the transfected cells and untransfected confluent MLE15 cells were harvested with lysis buffer (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 0.5% IGEPAL CA-630 (Sigma) containing protease inhibitor mixture (Complete tablet, Roche Diagnostics GmbH, Mannheim, Germany). The combined cell lysates were incubated for 16 h at 4 °C and then cleared by centrifugation at 12,000 revolutions/min for 20 min at 4 °C. The lysates were incubated with anti-FLAG M2 antibody (100 µl) attached to agarose beads (Sigma) for 16 h at 4 °C and eluted with 3xFLAG peptide (Sigma). The beads were washed five times with the lysis buffer according to the manufacturer’s instructions. The eluted proteins were then immunoprecipitated with anti-TTF-1 monoclonal antibody attached to magnetic Dynabeads (Dynal Biotech, Oslo, Norway), incubated for 16 h at 4 °C, and eluted with the lysis buffer containing N-lauroylsarcosine (0.5%) (Sigma). After washing five times with the lysis buffer, the eluted protein complexes were boiled in SDS sample-loading buffer, fractionated by SDS-PAGE, and detected with Coomassie staining (SimplyBlue SafeStain solution (Invitrogen).

**Protein Identification by Mass Spectrometry**—The stained protein bands from one-dimensional gels were excised and transferred to 96-well plates. The plates were transferred to a Massprep digestion robot (Micromass, Beverly, MA) for destaining (47) and in-gel digestion with trypsin (48). Following digestion, tryptic peptides were extracted from the gel slices with 5% formic acid/5% CH3CN using the Massprep robot. The extracted peptides were diluted to 100 µl/well with 0.1% formic acid.

A microbore HPLC system (Surveyor, ThermoFinnigan, San Jose, CA) was modified to operate at capillary flow rates using a T-piece flow splitter. Columns (10 cm × 180 µm inner diameter) were prepared by packing 100 Å, 5 µm of Zorbax C18 resin at 500 pounds/square inch pressure. Peptides were eluted with a gradient using buffer A (5% acetonitrile, 0.1% formic acid) and buffer B (90% acetonitrile, 0.1% formic acid) at a flow rate of 700 nanoliters/min. Following an initial wash with buffer A for 10 min, peptides were eluted with a linear gradient of 0–100% buffer B over 30 min. The samples were introduced to the column using a Surveyor auto sampler (Surveyor, ThermoFinnigan), which first transferred peptides onto a (300 mm × 5 µm) C18 trapping column (LC Packings, San Francisco, CA) and then used a switching valve to transfer the eluted peptides onto the analytical column. The HPLC column eluent was eluted directly into the electrospray ionization source of the LCQ-DECA ion trap mass spectrometer. Spectra were acquired over the range of 400–1400 mass units. Automated peak recognition, dynamic exclusion, and daughter ion scanning of the top three most intense ions were performed using the Xcalibur software (49).

Tandem mass spectrometry data were analyzed using SEQUEST. SEQUEST allows the correlation of experimental data with theoretical spectra generated from known protein sequences (50). In this work, the criteria for a positive peptide identification for a doubly charged peptide were a correlation factor ($X_{corr}$) >2.5, and a Δ cross-correlation factor ($ΔX_{corr}$) >0.1 (indicative of a significant difference between the best match and the next best match), a minimum of one tryptic peptide terminus, and a high preliminary scoring. For triply charged peptides, the correlation factor was set at 3.5. All matched peptides were confirmed by visual evaluation of the spectra. All spectra were searched against a composite data base containing the latest version of the non-redundant protein data base.

**Coimmunoprecipitation, GST Pull-down, and Immunoblot Assays**—FLAG-tagged proteins were immunoprecipitated with anti-FLAG M2 antibody with agarose beads (Sigma). GST-tagged proteins were coprecipitated using glutathione-agarose beads (Sigma) (40). Endogenous TTF-1 in MLE15 cell extracts was immunoprecipitated with anti-TTF-1 antibody (17) and Dynabeads. Proteins were fractionated by SDS-PAGE and transferred to Trans-Blot nitrocellulose membrane (Bio-Rad). Immunoblot analysis was performed as previously described (51), except that membranes were incubated with anti-FLAG (Sigma), anti-PARP-2 (Alexis, Lausen, Switzerland) (52), anti-PARP-1 (Pharmingen, San Jose, CA), anti-GFP, anti-Ku80, anti-GST (Abcam, Cambridge, UK), anti-Ku80 (anti-Ku86, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-TTF-1 (17) and probed with horseradish peroxidase-coupled secondary antibodies. Immunoblots were developed by ECL Western blotting detection reagents (Amersham Biosciences) according to the manufacturers’ instructions.

**Transient Transfection Assays**—HeLa cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with penicillin-streptomycin and 10% fetal calf serum at 37 °C under 5% CO2. One day before transfection, the cells were plated in six-well plates at 0.4×106 cells/well. DNA mixtures containing expression vectors, 1 µg each of luciferase reporter constructs and CMV, β-galactosidase were added to each well using Lipofectin (Invitrogen). After 24 h, the transfected cells were harvested, and luciferase and β-galactosidase activities were determined as previously described (53). Relative light units were obtained by the mean ± S.D. of the luciferase activity normalized to β-galactosidase activity from triplicate wells. The results from one of at least three representative experiments are given as fold activation (relative light units relative to transfections lacking expression vectors).

**In Situ Hybridization**—The detection of PARP-1 and PARP-2 transcripts was performed with antisense mRNA probes as described previously (40) on serial sections (10 µm) of frozen lungs dissected from 16-week-old CD1 mice. Sense mRNA probes for both PARP-1 and PARP-2 were used as controls. Exposure was for 4 weeks.

**Immunohistochemistry**—Mouse lungs were dissected from the pups of adult pregnant FVBn mice at various developmental stages. Tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Immunostaining of PARP-2 was performed as previously described (54) using rabbit polyclonal antibody purchased from Alexis (Lausen, Switzerland) as in Ref. 52. For verification of the antibody specificity, the antibody was preincubated with the recombinant mouse PARP-2 (Alexis) at a final concentration of 0.2 mg/ml overnight, and the absorbed antibody was then used for the immunostaining as the negative control.
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FIGURE 1. TTF-1 Interacts with PARP-2. MLE15 cells were transfected with FLAG-TTF-1. Twenty-four hours after transfection, total cell lysates were immunoprecipitated with anti-FLAG affinity gel. FLAG-TTF-1 and its associated proteins were eluted by FLAG antibody, and the eluted proteins were again immunoprecipitated with mouse IgG control (lane 1) or mouse monoclonal TTF-1 antibody (lane 2). Arrows indicate Ku80, Ku70, PARP-2, and FLAG-TTF-1. Lane 1, protein marker (A). Amino acid sequences of Ku80, Ku70, and PARP-2 were identified by mass spectrometry (8).

RESULTS

TTF-1 Binds to PARP-2 in Immortalized Lung Epithelial Cells—To isolate TTF-1-interacting proteins, a FLAG-tagged TTF-1 (FLAG-TTF-1) expression vector was transiently transfected into SV40 large T antigen immortalized lung epithelial cells (MLE15 cells). FLAG-TTF-1 was immunoprecipitated by double immunoprecipitation using FLAG and TTF-1 antibodies. Several proteins coimmunoprecipitated with FLAG-TTF-1 as assessed by Coomassie staining after SDS-PAGE (Fig. 2, A).

PARP-2 and PARP-1 Bind to TTF-1—To further verify that TTF-1 interacted with PARP-2, Ku70 and Ku80, the complex obtained by immunoprecipitation with FLAG-TTF-1, was immunoblotted with antibodies of PARP-2, Ku70, and Ku80. The protein complex immunoprecipitated with FLAG-TTF-1 contained endogenous PARP-2, Ku70, and Ku80 that were detected by Western blot with antibodies of PARP-2, Ku70, and Ku80 (Fig. 2A). Moreover, endogenous PARP-2 coimmunoprecipitated with endogenous TTF-1 in extracts prepared from MLE15 cells (Fig. 2B). Because PARP-1 has been established as a coactivator for several transcription factors and also associates with PARP-2 (40), we performed coimmunoprecipitation using PARP-1 antibody to test whether TTF-1 also interacts with PARP-1. As Fig. 2, A and B show, PARP-1 bound TTF-1, indicating that TTF-1 forms a protein complex that includes both PARP-2 and PARP-1.

PARP-2 and PARP-1 Coactivate TTF-1-Mediated Activation of the SftpB Promoter in HeLa Cells—To test whether PARP-2 and PARP-1 influence the transcriptional activity of TTF-1, transient transfection reporter assays were performed by cotransfecting vectors expressing TTF-1 and/or PARP-2 and PARP-1 fused to GFP with an SftpB promoter-luciferase construct. GFP-tagged PARP-2 and PARP-1 did not influence SftpB promoter activity but significantly enhanced TTF-1-dependent activity in HeLa cells (Fig. 3A). The stimulatory effect of the PARPs on the SftpB promoter were not altered by 3-aminobenzamide, an inhibitor of poly(ADP-ribose)ylation, indicating that activation was independent of enzymatic activities of the PARPs (data not shown). FLAG-TTF-1 was coimmunoprecipitated with GFP-tagged PARP-2 and PARP-1 using GFP-specific antibody to test whether TTF-1 interacted with the PARPs in HeLa cells (Fig. 3B). TTF-1 coimmunoprecipitated with both GFP-tagged PARP-2 and PARP-1 in HeLa cells, confirming the findings in MLE15 cells.

The E Domain of PARP-2 Binds to TTF-1—To identify the domain of PARP-2 that interacted with TTF-1, GST pull-down assays were performed using cell extracts from HeLa cells transfected with GST-tagged full-length PARP-2 (GST-mPARP-2), a series of truncated PARP-2 constructs GST-mPARP-21–69 (aa 1–69), GST-mPARP-263–202 (aa 63–202), or GST-mPARP-2203–559 (aa 203–559). Precipitations were performed with the extracts from the cells transfected above with the extracts from cells transfected with TTF-1 expression construct. The E domain of PARP-2 consisting of aa 63–202 interacted with TTF-1 (Fig. 4).

PARP-2 Mediates TTF-1 Transcriptional Activation through the C Terminus of TTF-1—TTF-1 contains transcriptional activation domains in both the N terminus (aa 51–123) and C terminus (aa 295–372) (43). To identify the domain of TTF-1 that interacted with PARP-2, GST pull-down assays were performed using deletion mutant TTF-1 proteins Δ14 and Δ3 (13, 43). Both deletion mutant TTF-1 proteins bound to PARP-2 in coimmunoprecipitation assays (Fig. 5B). In contrast, in the transient transfection reporter assay with the SftpB luciferase construct, TTF-1 Δ3 (containing the C terminus of TTF-1) was more active than TTF-1 Δ14 (containing the N terminus of TTF-1) (5.9-versus 2.2-fold in the presence of PARP-2) (Fig. 5C), suggesting that PARP-2 mediates transcription primarily by interacting with the C-terminal domain of TTF-1.

PARP-2 Activates the SftpB Promoter in MLE15 Cells—MLE15 cells express several lung-specific genes and transcription factors, including surfactant proteins and TTF-1 (46). TTF-1 is known to activate the expression of all surfactant protein genes Sftpa, SftpB, SftpD, and SftpD (8, 11). PARP-2 and PARP-1 activated the SftpB gene promoter in MLE15 cells (Fig. 6). PARP-2 and PARP-1 activated the SftpB promoter activity in HeLa cells only in the presence of TTF-1 (Fig. 3). Neither PARP-1 nor PARP-2 activated SftpA, SftpC, or SftpD in MLE15 cells (Fig. 6).
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**FIGURE 3.** PARP-2 and PARP-1 enhance effects of TTF-1 on the Sftbp gene promoter in HeLa cells. In A, HeLa cells were transfected with 1 μg of the Sftbp luciferase construct, 1 μg of pCMV β-galactosidase, 0.2 μg of TTF-1 expression vector (pcDNA3.1/TTF-1), 0.5 μg of GFP-PARP-2, or GFP-PARP-1 expression vector as indicated. Results are presented as fold activation of light units normalized to β-galactosidase activity relative to control constructs. *, p < 0.05. PARP-1 and PARP-2 activated the Sftbp-luciferase in the presence of TTF-1. In B, immunoprecipitates of HeLa cells were assessed by Western blot after transfection with GFP-PARP-2 (left panel) and GFP-PARP-1 (right panel), and FLAG-TTF-1 (lane 2) or FLAG control vector (lane 1). Cell lysates and immunoprecipitates were obtained with anti-FLAG antibody (IP-FLAG) and blotted with anti-GFP to identify GFP-PARPs. Both GFP-PARP-2 and GFP-PARP-1 bound to TTF-1.

**FIGURE 4.** TTF-1 interacts with the E domain of PARP-2. PARP-2 is shown in A. DBD is the DNA binding domain. Numbers indicate amino acids in PARP-2. In B, GST pull-down assays were performed by incubating extracts from HeLa cells expressing GST (lane 1) and GST-PARP-2 (lane 2) or truncated PARP-2 (lanes 3–5) with extracts from HeLa cells expressing TTF-1 (lanes 1–5). Interacting proteins were analyzed by GST pull-down and Western blot with anti-TTF-1 (8, top). The expressed GST was analyzed by Western blot assay with anti-GST (8, bottom).

PARP-2 Is Expressed in Respiratory Epithelial Cells and Alveolar Macrophages in Mouse Lung—In situ hybridization experiments were performed to compare the expression pattern of PARP-1 and PARP-2 genes in the lungs of adult mice. PARP-1 and PARP-2 gene expression was detected in the epithelial cells of lung tubules (Fig. 7). Immunostaining of PARP-2 was detected in the respiratory epithelial cells of the developing mouse lung. PARP-2 staining at embryonic day 12.5 with the PARP-2 antibody was depleted by preabsorption with recombinant PARP-2 protein, showing the specificity of the antibody (Fig. 8). At embryonic days 12.5, 16, and 18.5, PARP-2 was detected in the respiratory epithelial cells of both proximal and peripheral lung tubules at sites consistent with Sftbp gene expression. Immunostaining was detected in both epithelial and mesenchymal cells at embryonic day 12.5. PARP-2 was readily detected in the adult lung, where it was observed primarily in epithelial cells in the bronchioles and in alveolar macrophages (Fig. 9).

**DISCUSSION**

TTF-1 is a transcription factor that plays a critical role in lung morphogenesis, respiratory epithelial differentiation, and gene expression (8, 9). To understand the mechanisms by which TTF-1 influenced transcription, TTF-1-interacting cofactors, including transcription factors and coactivators that modulate its transcriptional activation, have been sought. In this work, we identified TTF-1-interacting proteins in cell extracts from immortalized lung epithelial MLE15 cells. PARP-2 and PARP-1 were identified as TTF-1-interacting coactivators. The PARP family of proteins is known to play an important role in DNA repair (39). Recently, a role for PARP-1 as a transcriptional coactivator/mediator was identified (55). In an in vitro reconstituted transcription assay system, PARP-1 functioned as an indispensable mediator of retinoid acid receptor-dependent transcription (36). Although PARP-1 has been implicated in DNA repair and gene regulation, deletion of PARP-1 did not alter development and survival in PARP-1−/− mice (56, 57), supporting the concept that other PARP family members share overlapping functions. Consistent with this concept, deletion of both PARP-1 and PARP-2 caused embryonic lethality (58). In the present work, PARP-2 and PARP-1 were shown to have similar coactivation ability, interacting with TTF-1 to activate Sftpβ gene expression, demonstrating potential complementary roles for PARP-2 and PARP-1 on gene transcription in the respiratory epithelium.

PARP-2 consists of an N-terminal DNA binding domain, an E domain and a C-terminal F domain containing the catalytic site. Interactions between PARP-2 and TTF-1 were mediated by the E domain of PARP-2. The E domain of PARP-2 was previously shown to interact with PARP-1, DNA ligase III, XRCC1, and DNA polymerase β (40), suggesting a shared interaction site for PARP-2 protein-protein interactions with transcription factors and the base excision repair complex. Although the poly(ADP-ribosyl)ation by the catalytic site in the PARP is important for DNA repair (39), it is not required for the NF-kB coactivation function by PARP-1 (27). In this study, an inhibitor of poly(ADP-ribosyl)ation, 3-aminobenzamide, did not influence the TTF-1 coactivation function by PARP-2 (data not shown), suggesting the importance of PARPs as transcriptional mediators rather than serving an enzymatic role.

Ku70 and Ku80 were identified as TTF-1-interacting proteins by mass spectrometry and immunoblotting with antibodies of Ku70 and Ku80. Ku70 associates with Ku80 and DNA-protein kinase in a complex that interacts with various transcription factors containing homeodomains, including HoxC4, HoxD4, Dlx2, Oct-1, Oct-2, and PDX-1, suggesting that Ku70 and Ku80 may mediate transcription via the kinase activity of DNA-protein kinase (59, 60). These findings suggest the concept that TTF-1 may interact with Ku70 and Ku80 to influence its activity.
The homeodomain of TTF-1 binds to transcription factors retinoid acid receptor, GATA-6, Ref-1, and NFAT (11, 16, 17, 21). The N terminus of TTF-1 binds to coactivators CBP, SRC-1, and TAZ (5, 13) and transcription factors Ref-1, and Pax8 (21, 23). For optimal coactivation, Pax8 requires the N terminus of TTF-1 (23), whereas TAZ requires the entire TTF-1 (13). In the present study, the C terminus of TTF-1 was more active than the N terminus in coactivation of the Sftpb promoter with PARP-2. TTF-1 has redundant activation domains in the N and C termini (43). Potentially, both domains have protein-protein interactions with various coactivators. Although the N terminus of TTF-1 recruits coactivators with histone acetyltransferase activity, the C terminus of TTF-1 modulates TTF-1-mediated transcription through a complex including PARP-2 and PARP-1.

Activation of the Sftpb promoter activity by PARP-2 was TTF-1-dependent in HeLa cells. MLE15 cells are mouse lung epithelial cells that express endogenous lung genes, including TTF-1 and surfactant protein genes (46). Although PARP-2 was coprecipitated with TTF-1 in immunoprecipitation assays in MLE15 cell extracts, PARP-2 did not influence
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the promoter activities of the other surfactant protein genes Sftpa, Sftpc, and Sftpd. TTF-1 binding elements have been identified in each of these genes. Although the mechanisms underlying these observations are unknown at present, it is possible that interaction between TTF-1 and PARP-2 is influenced by the elements at distinct transcriptional targets or that interaction is modified by the presence of other proteins.

In a previous study (61), PARP-1 was identified in the rat lung on embryonic days 16–18 and then decreased postnatally. In the rat, PARP-1 was detected in the nuclei of lung cells in late gestation but in the cytoplasm of bronchial epithelial and smooth muscle cells in the postnatal period (61). In the present study, PARP-2 was detected in the nuclei of bronchiolar cells in the developing mouse lung, consistent with its potential role in Sftpb gene transcription.

In summary, PARP-2 and PARP-1 bind to TTF-1 and serve as coactivators with TTF-1 in the activation of the Sftpb gene in the lung. PARP-2 was coexpressed with SP-B, a protein required for surfactant homeostasis and lung function after birth (62, 63).

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