Yin Yang 1 enhances cyclooxygenase-2 gene expression in macrophages

Myungsoo Joo,1 Jeffrey G. Wright,1 Ning Ning Hu,1 Ruxana T. Sadikot,2 Gye Young Park,2 Timothy S. Blackwell,1 and John W. Christman2

1Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; and 2Section of Pulmonary, Critical Care and Sleep Medicine, University of Illinois and the Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois

Submitted 8 December 2006; accepted in final form 9 January 2007

MACROPHAGES ARE ACTIVATED by exposure to gram-negative bacterial endotoxin (LPS) and play an important role in the pathogenesis of inflammatory diseases in the lung (9, 11, 27, 31, 41, 42, 51). The recognition and response to LPS by macrophages is mediated by Toll-like receptor 4 (TLR4), a receptor for LPS that is abundantly expressed on the cell surface of macrophages (30, 35, 43, 61). When engaged by LPS, TLR4 triggers Toll/IL-1 receptor-mediated signaling in MyD88-dependent and -independent manners, activating various transcription factors, including NF-κB and AP-1 (18). It was reported that the MyD88-dependent pathway regulates many classic markers and mediators of inflammation that include cyclooxygenase-2 (COX-2) (5), which along with the constitutively expressed isoenzyme COX-1 converts arachidonic acid to prostaglandin H2 (PGH2). PGH2 serves as a precursor for other prostanoids, including prostaglandin D2 (PGD2), via the prostaglandin D synthase pathway (12). PGD2 functions as pro- or anti-inflammatory lipid depending on the context of the inflammatory milieu (1, 14, 20, 29, 34, 49).

COX-2 gene expression is linked to the pathogenesis of colon and lung cancer and various inflammatory diseases, including severe sepsis and acute respiratory distress syndrome (37, 60). The magnitude of constitutive COX-2 gene expression is associated with the incidence and outcome of cancer of the colon (45). Diverse transcription factor binding motifs including CRE binding protein (CREB), NF-κB, and C/EBP-β are identified in a COX-2 promoter, and these transcription factors are known to be crucial in COX-2 gene expression (7, 10, 19, 22, 36, 52, 55, 56, 58). Despite a number of studies, transcriptional control of COX-2 expression is incompletely understood and appears to be regulated differently in individual cell types. For example, recent studies have indicated that in macrophages, but not in fibroblasts, C/EBP-β is required for the induction of COX-2 expression (7). In addition, PU.1, a macrophage-specific transcription factor, plays a role in COX-2 expression by promoting acetylation of C/EBP-β (24).

The COX-2 promoter also contains a Yin Yang 1 (YY1) consensus binding motif, but the role of YY1 in COX-2 gene expression has not been addressed. YY1 is a ubiquitously expressed transcription factor that also contributes to the pathogenesis of cancer and inflammation (2, 15). YY1 expression is essential for cell survival, because homozygous deletion of yy1 gene results in cell death (50). YY1 can activate or suppress gene transcription (39, 46, 47, 53) by interacting with regulatory partner proteins such as p300, a histone acetyltransferase, and histone deacetylases 1/2 (HDAC1/2) (15). It has been proposed that the balance of interactions among YY1, p300, and HDAC1/2 determine the acetylation status of YY1, which in turn controls whether YY1 functions as a transcriptional activator or repressor (59). Much work has been done in vitro to address the role of YY1 in gene transcription, but little is known about the interaction between YY1 and its partner proteins in regulating specific genes under physiological conditions, particularly in regard to the LPS signaling pathway and COX-2 gene expression in macrophages.

The purpose of this research was to investigate whether YY1 has a physiological role in COX-2 gene expression in macrophages. Our data show that YY1 avidly binds to the COX-2 promoter in LPS-treated macrophages and that this is associated with increased COX-2 protein production and functional enzymatic activity. Following treatment with LPS, there was a disruption in the functional interaction between YY1 and p300
without a change in the interaction between YY1 and HDAC1/2. Our results suggest that YY1 has a physiological role in COX-2 expression in macrophages and that disruption of the interaction between YY1 and p300 contributes to macrophage COX-2 gene expression. These studies may provide a link between YY1 and COX-2 in pathogenesis of inflammatory lung diseases.

MATERIALS AND METHODS

Cell culture. We maintained the murine macrophage cell line RAW 264.7 (ATCC, Rockville, MD) in DMEM (Cellgro) containing 10% fetal bovine serum (Hyclone) and penicillin/streptomycin (GIBCO-BRL). The stable cell line harboring the 815-nl-long mouse COX-2 promoter sequence fused with firefly luciferase reporter gene was described previously (38) and was maintained similarly in DMEM containing 100 μM G418 (Sigma, St. Louis, MO).

Bacteria. Pseudomonas aeruginosa 103 (PA 103) was obtained from ATCC. Bacteria from frozen stock were streaked onto trypticase soy agar plates and grown in a deferrated dialysis of trypticase soy broth supplemented with 10 mM nitrilotriacetic acid (Sigma), 1% glycerol, and 100 mM monosodium glutamate at 33°C for 1–3 h in a shaking incubator. Cultures were centrifuged at 8,500 g for 5 min, and the bacterial pellet was washed twice in Ringer lactate and diluted into the appropriate number of colony-forming units (CFU) per milliliter in Ringer lactate solution as determined by spectrophotometer. We measured the bacterial concentration by diluting all samples and plating out the known dilution on sheep blood agar plates. For bacteria treatment, PA 103 (5 × 10^6 CFU) was inoculated on a cell plate (5 × 10^4 cells) for 5 h before harvest for luciferase assay.

Reagents and YY1 plasmid. TLR4-specific Escherichia coli LPS (Alexis Biochemical, San Diego, CA) was added to the cell culture medium with 1 μg/ml LPS at final concentration. Antibodies for YY1, COX-1, COX-2, p300, HDAC1/2, tubulin, and actin were obtained from Santa Cruz Biotechnology, and antibody for acetyl-histone 3 was kindly provided by Dr. Y. Shi (Harvard Medical School, Boston, MA). Plasmids were prepared in an endotoxin-free condition by using endotoxin-free plasmid MaxiPrep kit (Qiagen). Transfection of macrophages with plasmids was performed with GenePORTER II (Gene Therapy Systems, San Diego, CA) using the manufacturer’s suggested protocol. Each transfection was normalized with pCMV plasmid to 4 μg.

Electrophoretic mobility shift assay. Electrophoretic mobility shift assay (EMSA) was performed as described previously (6). A short hairpin-structured oligonucleotide was designed: 5′-TTTCTTGTAAG-CatggACAGAAGTTTTTCTTTTGTcatGrrTACAGAAA-3′. The complementary sequences included a potential YY1 binding site are underlined and separated by a guanidine, four repeats of thymidine, and a cystidine, which form a loop structure separated by six nucleotides, shown in bold. The putative YY1 binding site is shown in italics, and the consensus sequence of the YY1 binding motif is shown in lowercase (48). Competitor oligonucleotides were designed similarly and contain 5′-TTCTTGTAAG-CctgccACAGAAGTTTTTTCT-TTTCTTGTAAG-CatggACAGAAGTTTTTCTTTTGTcatGrrTACAGAAA-3′. The competitor oligonucleotides have five nucleotide substitutions in the YY1 consensus sequence with the least probable nucleic acids, as referenced to analysis results of the TFSSEARCH program (version 1.3; Tokyo University, Tokyo, Japan) and published literature (48).

Chromatin immunoprecipitation assay. Reagents were obtained from Upstate Biotechnology (Lake Placid, NY), and chromatin immunoprecipitation (ChIP) assay was performed as described previously (38). Briefly, we grew cells to 90% confluence with 1–2 × 10^7 cells for each experiment. After being treated with 1% formaldehyde for 5 min, cells were harvested, suspended in SDS-lysis buffer (50 mM Tris·HCl, pH 8.1, 10 mM EDTA, 1% SDS, and protease inhibitors), and underwent sonication (4 times for 12 s each at one-fifth of the maximum potency). Following centrifugation at 4°C for 10 min, supernatants were diluted 1:10 with dilution buffer (16.7 mM Tris·HCl, pH 8.1, 1.2 mM EDTA, 167 mM NaCl, 0.01% SDS, and 1.1% Triton X-100) and added with salmon sperm saturated protein A (Zymed, San Francisco, CA) for 2 h at 4°C to remove nonspecific immunoglobulin. Immunoprecipitation was performed by adding 1 μg of specific antibodies to the cell lysate overnight at 4°C. Immune complexes captured with 40 μl of salmon sperm DNA-saturated protein A were washed twice (5 min each at 4°C) with low-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl, pH 8.1, and 150 mM NaCl), once with high-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl, pH 8.1, and 500 mM NaCl), once with LiCl buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris·HCl, pH 8.1), and twice with Tris-EDTA buffer for 5 min each. The immune complex was extracted three times with 200 μl of elution buffer (1% SDS, 0.1 M NaHCO3). Elutes were heated at 65°C for at least 4 h to reverse formaldehyde cross-linking. The samples were treated with 10 μg of proteinase K at 45°C for 1 h. The recovered DNA was purified with a DNA clean-up kit (Qiagen), and samples of input DNA were also prepared in the same way. PCR conditions were as follows: 94°C for 240 s; 30–32 cycles at 94°C for 40 s, 54°C for 40 s, and 72°C for 60 s; and final elongation at 72°C for 10 min. PCR for the input was performed with 100 ng of genomic DNA. One set of primers was used: 5′-ATCAAAACAGTGTTCATGAA-3′ and 5′-TGATACGACACACCTTTGTT-3′. This primer set covers the murine COX-2 promoter segment from -906 to -705 nt, which contains the putative YY1 binding site. The PCR products ran on either 1% agarose or 8% polyacrylamide gel. To ensure the fidelity of PCR, some PCR products were cloned and sequenced.

Immuno precipitation and Western blotting. Total cell lysate was prepared with radioimmunoprecipitation assay (RIPA) cell lysis buffer (50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% sodium orthovanadate, 1% Triton X-100, 0.5% deoxycholate, and 0.1% sodium dodecyl sulfate) supplemented with protease inhibitors (Roche). For immunoprecipitation, 1–2 μg of antibodies were added to precleared cell lysate that were normalized by protein contents. After incubation overnight at 4°C, immune complexes were captured by adding 30 μl of protein A-Sepharose (Zymed) and incubating for 30 min at 4°C. Captured immune complexes were washed with RIPA buffer. Membrane proteins were prepared as described elsewhere (23). Nuclear extract was prepared after treating cells with hypotonic buffer (6). The amount of proteins was quantified by Bradford assay (Bio-Rad), which was incubated with appropriate antibodies, and a specific immune complex was revealed by enhanced chemiluminescence (ECL Plus; Amersham).

Reporter assay. Luciferase activity was measured with a luciferase assay kit (Promega) per the manufacturer’s protocol. NF-κB-mediated luciferase activity was normalized with the protein quantity of the cell lysate used for each measurement.

Prostanoid measurement. PGD2 was measured by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) as previously described (25). Liquid chromatographic separation was performed isocratically on a Phenomenex Luna 3-mC18 5.0 × 0.2 cm column. The mass spectrometer was operated in positive-ion ESI mode. Detection of the analytes was accomplished by selected reaction monitoring (SRM), employing the following reactions: 370 → 317 (PGE2 and PGD2), 374 → 321 (PGD2-d3). The Quantum mass spectrometer was set to the following parameters: capillary voltage = 35 V, spray voltage = 4.3 kV, capillary temperature = 300°C, tube lens voltage = 137 V, sheath gas pressure = 49 lb/in.2, auxiliary gas = 25 (no units), and CID pressure = 1.0 mTorr. These values were observed to maximize the response of the SRM.
A putative YY1 site in the COX-2 promoter is shown, along with PU.1 and NF-κB binding sites. Individual transcription factor binding sites are underlined, and the first nucleotides of these sites are denoted. Asterisks indicate the consensus nucleotide sequences of YY1. B: EMSA were performed. From RAW 264.7 cells treated with LPS (1 μg/ml) for 30 min, total cell lysate were prepared and incubated with the 32P-labeled YY1 probe (lane 1). Cell extract similarly prepared was incubated with either isotypic IgG (lane 2) or α-YY1 antibody (lane 3). The cell extract was further incubated with a series of different ratios of radiolabeled and cold probes (lanes 5–7) or with 100 molar excess mutated probes (lane 4). Similar experiments were performed 3 times independently, and representative results are shown.

RESULTS

YY1 binds to the murine COX-2 promoter in vitro. Sequence analysis of the murine COX-2 promoter by the TFSEARCH program revealed a potential YY1 binding site from –796 to –780 nt from a transcription initiation site of cox-2 gene (Fig. 1A). To examine whether YY1 recognizes this site in vitro, we performed EMSA with probes containing the putative YY1 binding site. RAW 264.7 cells were treated with LPS (1 μg/ml) for 30 min, and total cell lysate of the treated cells was prepared and incubated with the radiolabeled probe. As shown in Fig. 1B, the probe yielded a protein-DNA complex (lane 1). To determine whether this complex contains YY1, RAW 264.7 cell lysate mixed with the probe was incubated with either isotypic IgG (lane 2) or α-YY1 antibody (lane 3). When the YY1 antibody was added, the immune complex was further shifted toward the top of the gel, which indicates that the protein-DNA complex contains YY1. To demonstrate a specific interaction with YY1 and the probe, we incubated RAW 264.7 cell lysate with differential ratios of radiolabeled and unlabeled or cold probes. As the ratio of the cold probe was increased, the shifted band disappeared (lanes 5–7). To exclude the possibility that this disappearance of the band results from dilution of the specific probe, RAW 264.7 cell lysate was mixed with the cold probe in which YY1 consensus sequences were mutated. As shown in lane 4, 100 molar excess of the mutated probe did not compete with the radiolabeled probe, indicating that the shifted band is a resultant of YY1 binding to the cognate site in the COX-2 promoter. Together, these results demonstrated that YY1 is capable of binding to the putative site in the murine COX-2 promoter in vitro.

YY1 binds to the murine COX-2 promoter in vivo. Gene transcription results from a complex interplay of transcription factors, regulatory elements on DNA, and chromatin structural modifications (3). Therefore, it is possible that despite in vitro interactions between YY1 and the DNA probe, YY1 does not bind to the endogenous COX-2 promoter in cells activated to produce COX-2. To investigate this possibility, we performed ChIP assay to measure YY1 binding to the endogenous promoter. RAW 264.7 cells were treated with purified LPS to selectively activate TLR4 signaling. At selected time points, cells were fixed with formaldehyde, and the nuclear protein fraction was collected, sonicated, and immunoprecipitated with α-YY1 antibody. DNA fragments that coprecipitated with an immune complex of YY1 and YY1 antibody were amplified by PCR with a specific set of primers flanking the putative YY1 binding site of the murine COX-2 promoter. As shown in Fig. 2A, YY1 was induced to bind to the putative binding site in the COX-2 promoter by 15 min after LPS treatment. Since acety-
lation of histone 3 (H3) and other histones is associated with transcriptional activation (17, 38), we performed a similar experiment to examine the acetylation status of H3 in the YY1 binding site. As shown in Fig. 2B, H3 bound to the region of the YY1 binding site was acetylated following LPS treatment.

To examine whether the inducible YY1 binding in macrophages results from an increase in total YY1 protein in response to LPS treatment, we measured the level of YY1 by Western blot analysis of total cell lysate. As shown in Fig. 3A, LPS treatment did not change the level of YY1. Next, to test whether LPS treatment changes partition of YY1 between nuclear and cytoplasmic fractions, we treated RAW 264.7 cells with LPS, isolated cytoplasmic and nuclear fractions, and analyzed by Western blotting for YY1. As shown in Fig. 3B, all detected YY1 remained in the nuclear fraction at baseline and after LPS treatment. Together, these results suggest that YY1 is a nuclear protein and is induced to bind to the COX-2 promoter in vivo in response to LPS treatment.

YY1 enhances the transcriptional activity of COX-2 promoter. Next, to examine whether YY1 is functionally involved in COX-2 transcription, we performed a luciferase reporter assay using a RAW 264.7 derived cell line stably transfected with a reporter construct containing the proximal 815 bp of the murine COX-2 promoter sequence fused with the firefly luciferase gene (Fig. 4A) (38). This reporter cell line was transfected with a plasmid encoding human YY1 and treated with LPS for 5 h before harvest for measurement of luciferase activity. As shown in Fig. 4B, although YY1 alone was not sufficient for COX-2 transcription (columns 5 and 6), overexpression of YY1 greatly enhanced luciferase activity in the presence of LPS (columns 2–4).

Since LPS is a gram-negative bacterial component, we tested whether YY1 enhances COX-2 transcription using live

![Fig. 3. LPS treatment does not change the amount and distribution of YY1 between cytoplasmic and nuclear compartments. A: after RAW 264.7 cells were treated with LPS (1 μg/ml) for indicated periods, total cell lysate was prepared for Western blot (WB) analysis for YY1. The amount of protein was quantified, and 30 μg of proteins were fractionated by SDS-PAGE and transferred to the polyvinylidene difluoride membrane, which was subsequently incubated with α-YY1 antibody (top). To ensure equal loading, the membrane was stripped and probed again with α-actin antibody (bottom). B: cytoplasmic (C) and nuclear (N) fractions were prepared from RAW 264.7 cells treated with LPS (1 μg/ml) for indicated periods. Protein quantity was measured, and equal amounts of proteins were loaded for SDS-PAGE and subsequent Western blotting for YY1. Similar experiments were performed 3 times independently, and representative results are shown.

![Fig. 4. YY1 promotes COX-2 gene transcription. A: the schematic indicates the murine COX-2 luciferase reporter construct. Some of the key transcription factor binding sites including YY1 are shown. B: RAW 264.7 cells stably transfected with the reporter construct were transfected with either pCMV (columns 1 and 2) or a plasmid encoding human YY1 (columns 3–6). Transfection with different amounts of YY1 was normalized with pCMV to 4 μg. At 48 h after transfection, cells were treated with LPS (1 μg/ml) for 5 h. Total cell lysate was harvested at 5 h after LPS treatment. After the amount of proteins in each experimental group was measured, an equal quantity of proteins was used to measure luciferase activity. Results are means ± SE of 5 independent sets of experiment (n = 5). *P < 0.05; **P < 0.01; ***P < 0.005 compared with control value. C: the stable cell line was transfected with either pCMV or the YY1-encoding plasmid for 48 h. Transfected cells were incubated with Pseudomonas PA 103 in a multiplicity of infection of 1 for 5 h. Total cell lysate was prepared, quantified, and analyzed for luciferase activity, as described in B. Results are means ± SE of 5 independent sets of experiment (n = 5). *P < 0.05; **P < 0.01 compared with control value.

P. aeruginosa, a common cause of ventilator-associated nosocomial gram-negative bacterial pneumonia and the most serious respiratory pathogen in cystic fibrosis patients (44). The stable COX-2 promoter reporter cells were transfected with the YY1-encoding plasmid, and the transfected cells were incu-
bated with PA 103 for 5 h before harvest for luciferase assay. As shown in Fig. 4C, transfection with YY1 augmented COX-2 transcription in response to PA 103 treatment. These studies show that YY1 enhances transcriptional activity of the COX-2 promoter in macrophages treated with either soluble LPS or live P. aeruginosa.

YY1 increases productions of COX-2 protein and PGD2. To examine whether YY1 contributes to functional COX-2 enzyme production, we transfected RAW 264.7 cells with the YY1-encoding plasmid, and the transfected cells were treated with LPS (1 μg/ml). At 2 h after LPS treatment, total cell lysate was prepared and analyzed by Western blot for COX-2 expression. As shown in Fig. 5, top, LPS treatment induced COX-2 protein expression (lanes 1 and 2). Although overexpression of YY1 was insufficient for supporting COX-2 expression (lane 3), LPS-induced COX-2 expression was greatly enhanced by overexpression of YY1 (lanes 2 and 4). On the other hand, as shown in Fig. 5, middle, expression of COX-1 was affected by neither LPS treatment nor overexpression of YY1.

Since PGD2 is derived from PGH2, the final product of COX-1 and COX-2, and the level of produced PGD2 is directly affected by COX-2 expression in RAW 264.7 cells (Joo M, Christman JW, unpublished observations), we tested whether YY1 enhances PGD2 production. We transfected RAW 264.7 cells with either an empty plasmid or the YY1-encoding plasmid, and the transfected cells were treated with LPS (1 μg/ml). At 2 h after LPS treatment, total cell lysate was collected for PGD2 measurement. As shown in Fig. 6, transfaction with YY1 greatly enhanced PGD2 production by LPS-treated macrophages, indicating that YY1 contributes to production of functional COX-2 protein.

YY1 interacts differentially with p300 and HDAC1/2. YY1 binds to various proteins, including p300 and HDAC1/2 (15), which affects acetylation status of YY1 and modes of transcriptional regulation by YY1 (59). Since hyperacetylation of YY1 is associated with transcriptional repression activity (59) and, as shown in Fig. 2, LPS treatment triggered YY1 binding to the promoter, we tested whether LPS treatment disrupts the interaction between YY1 and p300. We treated RAW 264.7...
cells with or without LPS (1 μg/ml) for 30 min and prepared total cell lysate for immunoprecipitation of YY1 and subsequent Western blotting for p300. In untreated cells, YY1 was physically associated with p300 (Fig. 7A, lane 3); however, LPS treatment resulted in disruption of this interaction (lane 4).

Next, we tested whether LPS treatment alters the interaction between YY1 and HDAC1/2. In experiments similar to those described above, we immunoprecipitated HDAC1/2 with a specific antibody and analyzed the precipitated immune complex by Western blotting for YY1. As shown in Fig. 7B, LPS treatment did not affect the interaction between YY1 and HDAC1/2 (lanes 3 and 4). Together, our results suggest that LPS treatment disrupts the intrinsic interaction between YY1 and p300 but not the interaction with HDAC1/2 in macrophages.

**DISCUSSION**

COX-2 expression is induced in multiple cell types, including epithelial, endothelial, and macrophages, and produces a variety of biologically active prostanoids that are involved in the pathogenesis of inflammation (54). The mechanism of COX-2 gene expression in various cell types is dependent on the exact stimulus and is related to cell-specific transcription factors in various cell types. COX-2 expression in macrophages, for example, results from a complex interplay between transcription factors, including CREB, NF-κB, PU.1, and C/EBP-β (7, 24). In addition, we identified a putative YY1 binding site located proximally to a transcription initiation site of the murine COX-2 promoter. YY1 is a ubiquitously expressed transcription factor that, like COX-2, is involved in the pathogenesis of cancer and inflammation (15), raising the possibility that YY1 is linked to the pathogenic role of COX-2. Our data show that YY1 binding to the endogenous COX-2 promoter occurred following treatment of macrophages with soluble LPS. We also showed that although insufficient for COX-2 gene expression in quiescent macrophages, overexpression of YY1 was associated with increased transcriptional activity of the murine COX-2 promoter elicited by either LPS or live *P. aeruginosa* treatment and with enhanced production of functional COX-2 protein following LPS treatment. Furthermore, we showed that LPS treatment of macrophages disrupts an intrinsic interaction between YY1 and p300. These results reveal that YY1 is involved in COX-2 gene and protein expressions in stimulated macrophages and suggest that interactions of YY1 with p300 and potentially other interacting proteins are regulated by stimuli.

COX-2 gene expression is regulated by transcription factors and chromatin structures that involve epigenetic modifications of transcription factors and histones, in part through acetylation (8, 33). Our results show that overexpression of YY1 was not sufficient for COX-2 expression. It is possible that this could be due to low transfection efficiency in RAW 264.7 cells, delivering insufficient amount of YY1 for supporting COX-2 expression. Yet, it also is likely that ectopic expression of YY1 by LPS or *Pseudomonas* treatment to exert its effect on COX-2 expression. Epigenetic modifications of YY1 have been well documented. For example, YY1 physically associates with p300 and HDAC1/2, which affects the acetylation status and transcriptional activity of YY1 (59). Therefore, ectopically expressed YY1 might need to be appropriately modified by LPS or *Pseudomonas* treatment to exert its transcriptional activity on the murine COX-2 promoter in macrophages.

Two major acetylated domains in YY1 were identified, which are precisely overlapped with the domains associated with transcriptional activation and repression (46, 59). The degree of acetylation of the central region from 170 to 200 amino acids of YY1 is variable. Hyperacetylation of the region is associated with transcriptional repression. It has been proposed that acetylation in the region attracts HDAC1/2 physically associated with p300 (Fig. 7A, lane 3); however, LPS treatment resulted in disruption of this interaction (lane 4).
cally associated with YY1, and HDAC1/2 remove acetylated residues from YY1 (59). Another acetylated region is in the carboxyl terminus of YY1. Acetylation of the carboxyl-terminal region is constitutive and cannot be deacetylated. Thus acetylation of the region provides a point where HDAC1/2 bind constantly (59). These results suggest that the binding of YY1 to p300 is temporal, whereas the binding to HDAC1/2 is constitutive. Our data showing that LPS treatment disrupted the intrinsic interaction of YY1 with p300 but not with HDAC1/2 support the possibility that YY1 binds to p300 temporally but to HDAC1/2 constantly. In addition, our results suggest the possibility that physiological stimuli regulate a profile of factors that interact with YY1, highlighting a complexity of regulation of YY1 transcription activity and of COX-2 gene expression.

p300 is a transcriptional coactivator that enhances transcriptional activity, in part through acetylation of associated transcription factors and histones (28). However, outcomes of p300 effect on transcription are dependent on YY1 and involved gene. For example, YY1 cooperates with p300 to enhance the transcriptional activity of E1A-mediated transcription and Grp78/BiP promoter (4, 26). In contrast, the interaction between YY1 and p300 is associated with repressor activity of YY1(59) and inhibits p53-mediated gene transcription expression (16). It is not well understood how interaction of YY1 and p300 results in different outcomes in gene transcription. In the case of COX-2 gene expression, our results show that LPS treatment induced YY1 binding to the COX-2 promoter without p300 and concomitantly acetylated H3 in the YY1 binding site. These results suggest that p300 is involved in effective YY1-mediated transcriptional activation by preventing unnecessary binding of YY1 to the cognate site in the absence of stimulus and by acetylating H3 in the YY1 binding site in response to LPS treatment.

Several possible mechanisms are conceivable for how YY1 and p300 affect COX-2 gene expression. It is possible that in unstimulated conditions, YY1 interacted with p300 remains hyperacetylated and inaccessible to the COX-2 promoter. Alternatively, it is possible that p300, as a limiting factor, is involved in other gene transcription and physically tethers YY1 to the promoters of those genes, precluding YY1 binding to the COX-2 promoter. In response to LPS treatment, YY1 dissociates with p300, which presumably tips the balance between acetylation and deacetylation reactions, resulting in a deacetylation of YY1 by constantly associated HDACs. Now, YY1 binds to the COX-2 promoter, increasing COX-2 expression. In the meantime, dissociated p300 acetylates histones, changing chromatin structures in the YY1 site and the COX-2 promoter. In addition, given that p300 interacts with C/EBP-b (32), PU.1 (57), NF-kB (40), and RNA polymerase II (21), it also is conceivable that p300 interacts with those transcription factors, contributing further to COX-2 expression (Fig. 8). Therefore, we speculate that YY1 contributes to COX-2 expression directly as a transcription activator and indirectly by providing p300 for chromatin structural changes and transcriptional activities of C/EBP-b, PU.1, NF-kB, and RNA polymerase II.

In this study, we have provided evidence that links YY1 to COX-2 expression in an inflammatory milieu. COX-2 is associated with various diseases, including lung cancer and inflammation (13, 37, 60). Similarly, YY1 is related to tumorigenesis and inflammation (15). YY1 is expressed in most cell types and is essential for cell survival (50). Therefore, our results suggest the possibility that YY1 exerts its pathological effect, at least in part, through COX-2 gene expression in various cell types, and thus YY1 can be a therapeutic target of cancer and inflammation.

REFERENCES

L1226

28. Inoue H, Nanayama T, Hara S, Yokoyama C, Tanabe T. The cyclic
29. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kaba
33. Mink S, Haenig B, Klempnauer KH. Interaction and functional collabora
41. Park GY, Christman JW. Induction of cyclooxygenase-2 and prosta
43. Park K, Atchison ML. Isolation of a candidase repressor/activator, NF-E1 (YY1-1, b) that binds to the immunoglobulin κ 3C enhancer and the immunoglobulin heavy-chain μE1 site. Proc Natl Acad Sci USA 88: 9804–9808, 1991.