PARP-14 Functions as a Transcriptional Switch for Stat6-dependent Gene Activation*

Received for publication, June 23, 2010, and in revised form, November 1, 2010. Published, JBC Papers in Press, November 16, 2010, DOI 10.1074/jbc.M110.157768

Purvi Mehrotra, Jonathan P. Riley, Ravi Patel, Fang Li, Le’erin Voss, and Shreevrat Goenka

From the Department of Pediatrics, HB Wells Center for Pediatric Research and Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana 46202

A subset of poly ADP-ribose polymerases (PARP) that also contain macro domains regulate transcription. One such macro PARP, PARP-14 alters interleukin 4 (IL-4) and Stat6-dependent transcription. Stat6, activated by IL-4 plays an important role in T helper cell immunity and B cell responses. Here we define the mechanism by which PARP-14 regulates Stat6-activated transcription. Under non-stimulating conditions, PARP-14 recruits HDAC 2 and 3 to IL-4 responsive promoters. In the presence of IL-4, PARP-14 promotes efficient binding of Stat6 to its target genes. Moreover, HDAC 2 and 3 are released from the promoter with an IL-4 signal, this is aided by the ADP-ribosylation of the HDACs by PARP-14. The HDACs and PARP-14 get replaced by coactivators containing HAT activity. Based on these observations we put forth a mechanism in which PARP-14 functions as a transcriptional switch for Stat6-dependent gene induction. Thus, in the absence of a signal PARP-14 acts as a transcriptional repressor by recruiting HDACs. In contrast, in the presence of IL-4 the catalytic activity of PARP-14 facilitates Stat6 binding to the promoter, and release of HDACs so as to activate transcription.
PARP-14 Regulates Transcription

repressors rather than activators. It is possible that the macro domains found in PARP-14 may also function to repress transcription. However, we have shown that PARP-14 enhances Stat6-dependent transcription instead of repressing it (6). To reconcile this paradox here we present evidence that PARP-14 does function as a repressor first by recruiting HDAC 2 and 3. However, in the presence of IL-4 the ADP-ribosyl transferase activity of PARP-14 is activated, which results in relieving its repressive function.

EXPERIMENTAL PROCEDURES

Cell Lines and Mice—293T and M12 B cells were cultured in DMEM and RPMI, respectively, supplemented by 10% FBS as previously described (6), Parp1−/− mice on a C57BL/6 background were a kind gift from Dr. Mark Boothby. 6–8-week-old mice were used for the entire study. Mice were maintained in a pathogen-free condition, and all studies were approved by Indiana University School of Medicine and the Institutional Animal Care and Use Committee. B cells were isolated from spleens using the MACS system (Miltenyi Biotech), according to the manufacturer’s protocol and cultured in IMDM-10% FBS. The cells were treated with 5–10 ng/ml of IL-4 (PeproTech), 10 or 50 μM PJ34 (Alexis Biochemicals) and 5 nM TSA (Sigma-Aldrich) as indicated in the figures. For the ChiP and expression analysis of the Ie gene, the cells were treated with 2 μg/ml anti-CD40 (BD Biosciences).

Chromatin Immunoprecipitation—The cells were fixed with 1% formaldehyde for 10 min and quenched by adding 0.125 M glycine. The chromatin was sonicated to average length of 200–1000 bp. Samples were precleared with protein A beads and immunoprecipitated with anti-Stat6 (Santa Cruz Biotech), anti-PARP-14 (6), anti-HDAC2 (Santa Cruz Biotech), anti-HDAC3 (Santa Cruz Biotech), anti-acetyl H3 (Upstate Biotech), anti-acetyl H4 (Upstate Biotech), anti-p300 (Santa Cruz Biotech), anti-NCoA1 (Santa Cruz Biotech), anti-NCoA3 (Santa Cruz Biotech), or isotype control (Upstate Biotech). The immune complexes were collected on protein A beads, and the beads were washed three times in immunoprecipitation buffer. Samples were treated with proteinase K and cross-linking was reversed by heating at 65 °C for 4 h. DNA was extracted using phenol-chloroform and ethanol and analyzed on a PARP reaction buffer (20 μl) containing 10 μCi of 32P-labeled NAD (800 Ci/mmol) (Perkin Elmer), 50 μM cold NAD, 50 mM Tris-Cl, pH 8.0, 4 mM MgCl2, and 0.2 mM dithiothreitol for 30 min. The reaction was stopped by washing the beads with PBS and adding loading dye. To evaluate ADP-ribosylation, the proteins were then resolved on SDS-PAGE and autoradiographed.

Flow Cytometry—Cells were fixed with 1.5% formaldehyde for 10 min and permeabilized with 100% cold methanol for 10 min. The cells were then stained with PE-conjugated anti-pStat6 and FITC-conjugated anti-B220 (BD Biosciences) and analyzed by flow cytometry.

RESULTS

PARP-14 and Its Enzymatic Activity Promote the Binding of Stat6 to Target Genes—It has been previously demonstrated that Stat6-dependent transcription is enhanced by PARP-14 and the enzymatic activity associated with it (6, 17). To further investigate the exact molecular mechanism by which ADP-ribosylation promotes Stat6 transcription, we tested the binding of Stat6 to IL-4-responsive promoters in the presence of a PARP inhibitor. The M12 B cell line was pretreated with PJ34, a PARP inhibitor for 30 min before incubation with IL-4. The binding of Stat6 to two IL-4 responsive promoters (Fcer2a and Ie) was then determined by chromatin immunoprecipitation (ChiP) at different intervals after IL-4 treatment.
Stat6 binding to the Fcer2a promoter region was observed as early as 5-min post-IL-4 treatment. The maximum response was observed 30 min after the addition of IL-4. Importantly, pretreatment of cells with PJ34 resulted in reduced Stat6 binding to the Fcer2a promoter (Fig. 1A). A similar trend was observed for the le promoter (Fig. 1A). It is possible that the reduced binding of Stat6 to its promoter elements in the presence of PJ34 was due to decreased Stat6 phosphorylation. To test the effect of inhibition of PARP activity on Stat6 phosphorylation, we treated M12 cells similar to the ChIP experiments outlined above, and performed flow cytometric analysis with an antibody specific for phospho-Stat6. We did not observe any changes in the phosphorylation of Stat6 when the cells were treated with PJ34 (supplemental Fig. S1a). Taken together, these data indicate that PARP activity promotes the binding of Stat6 to targets genes dependent of the proximal signaling events that activate Stat6. To evaluate if reduced Stat6 binding to the Fcer2a and le promoters in the presence of PJ34 resulted in a corresponding decrease in the expression of these genes, we performed quantitative RT-PCR for the Fcer2a and le transcripts. When cells were treated with IL-4, with increasing amounts of PJ34, we observed a dose-dependent inhibition of Fcer2a and le induction (Fig. 1B). These data indicate that the maximal binding of Stat6 to the promoters it activates requires PARP activity, and this correlates to the level of transcription induced by Stat6.

We have previously shown that ectopic expression of PARP-14 in M12 B cells results in the increased expression of CD23 (6). To test whether the increase in CD23 expression in these M12 cell lines was due to increased Stat6 binding to its promoter element, we performed Stat6 ChIP experiments in the absence and presence of IL-4. Consistent with the increase in CD23 expression (6) we observed that there was increased binding of Stat6 to both Fcer2a and le promoters in cell lines overexpressing PARP-14 (C6-1 and C6-2) as compared with the controls (Fig. 1C and Ref. 6). Taken together, these data indicate that the expression level of PARP-14 and the enzyme activity associated with it play an important role in the binding efficiency of Stat6 to its cognate promoter elements.

PJ34 is a broad spectrum inhibitor of PARP catalytic activity and, thus, from the above experiments we are unable to determine the role PARP-14 specifically plays in the binding of Stat6 to the promoters it activates. Therefore, to determine if PARP-14 affects Stat6 binding to its cognate promoters, we performed ChIP experiments on B cells isolated from PARP-14 deficient animals (21) and littermate controls with intact PARP-14 expression. As expected the binding of Stat6 to both the Fcer2a and le promoter region was observed after IL-4 treatment in B cells isolated from wild type mice (Fig. 1D). Similar to our observation with PJ34 treatment, lack of PARP-14 expression resulted in a significant decrease in the binding of Stat6 to both the Fcer2a and le promoters (Fig. 1D). Particularly, the binding of Stat6 to the le promoter was completely lost in the absence of PARP-14 expression (Fig. 1D, right panel). The IL-4-dependent phosphorylation of Stat6 in B cells with and without PARP-14 expression was compared by flow cytometric analysis and no differences were observed (supplemental Fig. S1b). These data are consistent with our previous results with PJ34 indicating that, neither PARP activity nor PARP-14 influences Stat6 phosphorylation. We further tested the expression of Fcer2a and le transcripts in WT and Parp14-/- mice that were left untreated or treated with the PARP inhibitor. As expected the expression of both transcripts were decreased in PARP-14-deficient animals as compared with WT (supplemental Fig. S2). Consistent with our Stat6 binding data, the le gene expression was more dependent on PARP-14. Fcer2a expression was more affected by the presence of PJ34 than the absence of PARP-14 suggesting that other PARP enzymes may also play a role in regulating Fcer2a. However, it seems unlikely that other PARPs are involved in regulating le, as PJ34 was unable to significantly reduce the expression of le in Parp14-/- B cells.

FIGURE 1. PARP-14 and the enzyme activity associated with it dictate the DNA binding ability of Stat6. A, M12 cells were either untreated or pretreated with PJ34 (10 µM) for 30 min, after which IL-4 was added for the indicated times. Nuclear extracts from these cells were then used for ChIP with anti-Stat6 and evaluated for the presence of either the Fcer2a or germ-line epsilon (le) promoter by qPCR. B, total RNA was extracted from M12 cells that were treated as indicated. Transcripts for either Fcer2a or le were quantified using qRT-PCR. The data plotted is the relative expression of Fcer2a normalized to Gapdh and compared with untreated samples, relative expression of le is normalized to Gapdh and compared with untreated controls. C, parental M12 cells (P), two M12 cell lines overexpressing PARP-14 (C6-1 and C6-2) and an empty vector control (E-1) that were generated and published earlier (6) were treated with IL-4 and Stat6 ChIPs were performed similar to A. D, B cells were isolated from either Parp14+/- or wild-type littermate controls and were treated with IL-4 for the indicated time points. ChIP experiments similar to A were performed on nuclear extracts obtained from these cells. The data plotted for each panel is the mean (± S.E.) of three independent experiments.
PARP-14 Regulates Transcription

(supplemental Fig. S2). Taken together, these data indicate that the efficient binding of Stat6 to its promoter elements and therefore, the transcription competence is dependent on both the expression of PARP-14 and the enzyme activity associated with it.

PARP-14 Is Associated with Stat6-responsive Promoters in the Absence of IL-4 and Disassociates upon IL-4 Treatment—We have shown thus far that PARP-14 and the catalytic activity associated with it dictates the efficiency by which Stat6 binds to the promoter element it activates. However, it is not known whether PARP-14 regulates Stat6 mediated transcription indirectly or directly by binding to the same promoter DNA sequence as Stat6. To test the DNA binding capacity of PARP-14 we first performed DNA affinity precipitation assays (DAPA) with 50 bp biotinylated double-stranded oligonucleotides corresponding to the Fcer2a and Ie promoter region flanking the Stat6 binding site. Cell extracts from M12s treated in the presence or absence of IL-4 were incubated with the oligonucleotides immobilized on beads. After extensive washing of the beads, proteins bound to the oligonucleotides were probed with anti-Stat6. Treatment with IL-4 increased binding of Stat6 to both oligonucleotides (supplemental Fig. S3a). We performed similar experiments with 293T cell extracts transfected with PARP-14 or a vector control. A robust association of PARP-14 to both the promoter fragments was observed (supplemental Fig. S3b). These data indicate that PARP-14 associates with DNA that contains a Stat6 binding element. To investigate whether PARP-14 associates with native Stat6 responsive promoters we performed ChIP experiments with an antibody directed against PARP-14. M12 cells were treated with IL-4 for varying lengths of time and ChIPs were performed using anti-PARP-14 antibody, followed by qPCR to quantify DNA sequences corresponding to either, the Fcer2a and Ie promoters, or the coding sequences of these genes. We observed that under non-stimulating conditions PARP-14 associated with both of the Stat6 responsive promoters tested. Moreover, this association was lost when the cells were treated with IL-4 (Fig. 2A, black bars). PARP-14 was bound only to the promoter region of Fcer2a and Ie and not the coding region of these two genes (Fig. 2A, gray bars). These results indicate that PARP-14 regulates Stat6-mediated transcription by using a mechanism in which it is bound to the promoter in the absence of IL-4 and is no more required at the promoter in the presence of IL-4. To validate the specificity of the anti-PARP-14 antibody for our ChIP experiments, we isolated B cells from PARP-14-deficient animals and their wild-type littermates and performed similar ChIP experiments. Our data are consistent with our M12 studies where we observed that PARP-14 was associated with DNA in the absence of IL-4 and this association was lost upon IL-4 treatment (Fig. 2B, gray bars). Importantly, we observed no positive ChIP signal in PARP-14-deficient samples (Fig. 2B, black bars).

Previously, we have shown that PARP-14 specifically enhances Stat6 but not Stat1-dependent gene induction (6). Therefore, we next tested whether the association of PARP-14 under non-stimulating conditions was specific for Stat6-responsive promoters. Similar PARP-14 ChIP experiments were performed and the immunoprecipitated DNA was analyzed for the presence of DNA elements corresponding to the Stat1-responsive Irf1 promoter and Stat4-responsive Il18r1 promoter (22, 23). Consistent with our previous finding the association of PARP-14 was specific for Stat6-responsive genes and no association was observed for either, Stat1 or Stat4 responsive promoters (supplemental Fig. S3). Taken together, these results suggest that PARP-14 specifically participates in regulating Stat6 but not Stat1- or Stat4-responsive promoters. PARP-14 is bound to the promoter in the absence of a signal, and then in the presence of an activating signal, PARP-14 exits the promoter before transcription initiates.

In the previous section we have shown that the PARP enzyme activity plays an important role in Stat6-dependent gene expression and, now we present evidence that PARP-14 dissociates from Stat6-responsive promoters upon IL-4 stimulation. Therefore, next we wanted to determine if the disassociation of PARP-14 from DNA was dependent on the PARP catalytic activity. To test this, cells were treated with a combination of IL-4 and PJ34 as indicated (Fig. 2C) and PARP-14 ChIPs were performed as before. PARP-14 was unable to dissociate from both the Fcer2a and Ie promoters upon IL-4 treatment in the presence of PJ34 (Fig. 2C). These data indi-
cate that to regulate Stat6-dependent transcription PARP-14 uses a mechanism in which it leaves the promoter with a cytokine signal, and this function requires PARP enzymatic activity.

Histone Deacetylases Are Recruited by PARP-14 and Regulate Stat6-dependent Transcription—Our finding that PARP-14 is associated with Stat6-responsive promoters in the absence of IL-4, and is dissociated following stimulation, suggests that PARP-14 may initially act as a repressor or recruit repressors to prevent transcription under non-stimulating conditions. However, in the presence of an appropriate signal the repressive function of PARP-14 may no longer be required and thus, it may need to dissociate from the promoter for transcription to be activated. As outlined in the introduction, PARP-14 contains three iterations of the macro domains, and similar macro domains found in macroH2A are known to associate with HDACs (20). Thus, we evaluated if the class I HDACs, that are predominantly nuclear, associate with PARP-14. We co-expressed Flag-tagged HDAC and Myc-tagged PARP-14 in 293T cells and performed co-immunoprecipitation experiments. When the cell extracts were subjected to immunoprecipitation with anti-Flag or an isotype control antibody we observed positive signals for HDAC 2 and 3 only with anti-Flag. Importantly, when the same immunoprecipitates were probed with anti-Myc we observed positive signals corresponding to the molecular weight of PARP-14 only in the anti-FLAG IPs (Fig. 3A). These results indicate that PARP-14 associates with HDAC 2 and 3. To test which regions of PARP-14 associated with HDAC 2 and 3, we performed pull-down experiments with the N (aa 1–813), middle (aa 800–1395), or C (aa 1389–1817) portion of PARP-14 and GST and HDAC 2 and 3. Bacterially expressed regions of PARP-14 tagged with GST or GST alone were immobilized on glutathione-agarose beads and incubated with cell extracts transfected with Flag-tagged HDAC 2 or 3. After extensive washing of the beads the bound proteins were subjected to Western blotting and probed with antibodies specific for the Flag epitope. GST by itself was unable to pull-down either HDAC 2 or 3. However, HDAC2 showed association with all three regions of PARP-14, and HDAC3 was brought down by the middle and C-terminal of PARP-14 (Fig. 3B). Taken together, these data indicate that PARP-14 associates with HDAC 2 and 3 and the N-terminal and middle portion of PARP-14 participate in its ability to bind the two HDACs.

Thus far, our data are consistent with a hypothesis that HDAC 2 and 3 through PARP-14 may repress Stat6-dependent transcription under non-stimulating conditions. If this was truly the case then we should observe the regulation of IL-4- and Stat6-dependent transcription by HDAC 2 and 3. To test this, M12 cells were co-transfected with a Stat6-responsive reporter construct and either an empty vector, or one encoding HDAC or one encoding HDAC 2 or 3. The transfected cells were treated with IL-4 or nothing and the reporter activity was determined. In the presence of both HDAC 2 and 3, the IL-4-mediated induction of the reporter was decreased by at least 50% (supplemental Fig. S4a). To further evaluate if HDACs play a role in IL-4-dependent gene induction we used an alternate approach.
PARP-14 Regulates Transcription

approach. M12 cells were treated with a HDAC inhibitor, TSA (trichostatin A), the expression of Fcer2a and Ie transcripts was evaluated by qRT-PCR after IL-4 treatment. Both the Fcer2a and Ie transcripts were significantly increased in the presence of TSA (supplemental Fig. S4b). Our reporter assays along with the experiments with TSA strongly indicate that HDAC 2 and 3 regulate IL-4- and Stat6-dependent transcription.

If HDAC 2 and 3 repress basal transcription from an IL-4 responsive promoter then these molecules should be present at the promoter under non-stimulating conditions. To test this we performed ChIP experiments with the HDAC 2 and 3 antibodies and examined the presence of Fcer2a and Ie promoter elements in the immunoprecipitates. As expected we observed that both HDAC 2 and 3 were bound to the Fcer2a and the Ie promoters in the absence of an IL-4 signal (Fig. 3C, gray bars). This association was lost when the cells were incubated with IL-4 (Fig. 3C, black bars). Thus far, our biochemical and functional data with HDAC 2 and 3 are highly suggestive that these molecules participate in regulation of transcription mediated by Stat6 and this may occur via the mechanism by which the HDACs are binding to the Stat6-responsive promoters via HDAC 2 and 3 in these lanes (Fig. 4B). Other higher molecular weight bands were also observed in these lanes. Importantly, these signals were absent in the PARP-14 NM sample, which lacks the PARP catalytic domain and, the samples that were treated with the PARP inhibitor (Fig. 4B). To verify that indeed the higher molecular weight bands were modified HDAC 2 and 3, we performed similar experiments with PARP-14 and HDAC 2 and 3. However, instead of performing radioactive PARP assays we performed PARP assays with cold NAD and probed the resultant blots with HDAC 2 and 3 specific antibodies (Fig. 4C). Consistent with our assay with PARP NAD, we observed higher molecular weight species of HDAC 2 and 3 (Fig. 4C). Taken together these data strongly indicate that PARP-14 is able to ADP-ribosylate both HDAC 2 and 3.

Association between HDACs and PARP-14 Is Lost in the Presence of NAD—We showed earlier that PARP-14 associates with HDAC 2 and 3. Further, we have demonstrated that PARP-14 is able to ADP-ribosylate itself at the N terminus and, both HDAC 2 and 3. It is possible that once both PARP-14 and the HDACs are ADP-ribosylated they are unable to interact due to the presence of negatively charged ADP-ribose residues on both these molecules. To test this hypothesis we coexpressed FLAG-tagged HDACs with c-Myc-tagged PARP-14, the NM or MC version in 293T cells. The resultant extracts were used for immunoprecipitation with anti-c-Myc and the immunoprecipitate was divided equally. One portion was treated with cold NAD for the PARP reaction to proceed and the other was left untreated. After the PARP reaction was completed, the immunoprecipitates were washed thoroughly to remove all unbound proteins. The immunoprecipitated proteins were then probed with anti-HDAC 2 and 3, respectively, to determine the amount of HDACs that coimmunoprecipitated with PARP-14 (Fig. 5A). Consistent with our previous data we observed strong association between PARP-14 and the HDACs in the absence of NAD (Fig. 5A). Notably, this association was significantly reduced when the immunoprecipitates were treated with NAD. When the NM version of PARP-14 lacking the PARP catalytic domain was used for a similar assay, there was very little difference in the extent of association between PARP-14 and HDACs in the absence or presence of NAD (Fig. 5A). However, the MC version of PARP-14 that contains the PARP catalytic domain showed a similar profile like full-length PARP-
The MC version of PARP-14 was unable to coimmunoprecipitate the HDACs to the same extent as full-length PARP-14 or NM suggesting that the N-terminal of PARP-14 is more important to mediate the association between the two proteins (Fig. 5A). With this data and the data presented in the previous sections we conclude that PARP-14 recruits HDAC 2 and 3 to Stat6-responsive promoters. Once the enzyme activity of PARP-14 is induced by IL-4-activated Stat6, PARP-14 ADP-ribosylates itself as well as the HDACs. The ADP-ribosylated HDACs are no longer able to associate with PARP-14 and this allows for the HDACs to exit the promoter region.

If indeed this were the case then inhibiting PARP activity during IL-4 stimulation would preclude HDACs to dissociate from the promoter. To address this scenario we carried out ChIP experiments with anti-HDAC 2 and 3-like before and included samples in which the cells were also treated with PJ34 to inhibit PARP catalytic activity (Fig. 5B). Inhibiting PARP activity resulted in HDAC 2 and 3 to remain on the promoter in the presence of IL-4 (Fig. 5B). These data are consistent with the idea that PARP-14 ADP-ribosylates itself and the HDACs, preventing them from dissociating from the promoter.

FIGURE 4. PARP-14 ADP-ribosylates itself on the N-terminal, as well as, HDAC 2 and 3 get modified by PARP-14. A, indicated c-Myc-tagged PARP-14 variants were coexpressed with FLAG-tagged N-terminal, middle region, and C-terminal of PARP-14 in 293T cells. The resultant extracts were subjected to immunoprecipitation with anti-c-Myc and anti-FLAG and the immunoprecipitate was divided into three portions. One set of samples was incubated with 32P NAD (PARP assay) and autoradiographed after resolving the proteins on SDS-PAGE. The other fractions of the immunoprecipitate were probed with the indicated antibodies. B, disassociation of HDAC 2 and 3 from Stat6-responsive promoters upon IL-4 stimulation requires PARP catalytic activity. M12 cells were treated with PJ34 and IL-4 as indicated, and HDAC 2 and 3 ChIPs were performed as indicated in Fig. 3C.
PARP-14 Regulates Transcription

FIGURE 6. Molecular events involved in the transcription from the Fcer2a and Jc promoters. M12 cells were treated with IL-4 for the indicated time intervals. ChIP studies were performed using the indicated antibodies. The pulled-down DNA was quantified for the presence of Stat6-responsive elements found in the Fcer2a and Jc genes by qPCR. The results are plotted as a mean (± S.E.) from three independent experiments.

consistent with the molecular mechanism that we have proposed above.

Sequence of Molecular Events at Stat6-responsive Promoters upon IL-4 Stimulation—Previously, it has been demonstrated that histone acetyl transferases such as CBP/p300, NCoA-1, and NCoA-3 promote Stat6-dependent transcription (11, 14, 15). However, the exact sequence of events by which IL-4- and Stat6-responsive genes are activated is not known. Therefore, to delineate the exact molecular mechanism that occurs at the Stat6-responsive promoter, we performed a series of ChIPs at various time points after IL-4 stimulation with antibodies raised against the indicated molecules for two IL-4-responsive genes (Fig. 6). As expected, under non-stimulating conditions Stat6 was not bound to the promoters. Consistent with our previous result, PARP-14, HDAC 2 and 3 were bound to DNA in the absence of IL-4. Fifteen minutes after IL-4 treatment we observed the association of Stat6 to both the promoters tested and the loss of association of PARP-14 and the HDACs. These events coincided with the association of the p300, NCoA-1 and 3 to the promoters and the acetylation of histone H3 and H4. For the most part, both promoters showed a similar pattern for protein-DNA interaction, however, some differences between Fcer2a and Jc were observed. For example, for the Fcer2a promoter less of Stat6 was bound at 4-h post IL-4 treatment, and this overlapped with the re-association of PARP-14. However, for the Jc promoter Stat6 did not dissociate from the promoter nor was there significant re-association of PARP-14 at the 4 h time point. For both Fcer2a and Jc, once the HDACs disassociated from the DNA they did not re-associate even after 4 h of IL-4 treatment. These data indicate that there is a reciprocal association of Stat6 and PARP-14 at these promoters, and confirms previous data that HDACs are present at the promoter only when PARP-14 is present. The association of the HATs with the Fcer2a and Jc promoters was also distinct for each of the genes. NCoA-1 associated transiently with the Fcer2a promoter for 30 min after IL-4 treatment and was no more bound at the 1 h time point. In contrast, for the Jc promoter, NCoA-1 showed a more sustained binding which was lost after 4 h of IL-4 treatment. NCoA-3 on the other hand, bound quickly to the Fcer2a promoter after IL-4 treatment and the binding persisted for 4 h. The Jc promoter showed a different pattern, as NCoA-3 bound to DNA only after 30 min of IL-4 induction and started to fall off the promoter starting at 2 h. The maximum binding of p300 to the DNA was observed at 1 h IL-4 treatment after which it waned for the Fcer2a gene. On the other hand, for the Jc gene there was a gradual increase in the association of p300 that increased until the last time point tested. The acetylation status of histone H3 and H4 mirrored the association of the HATs with the promoter, though H4 demonstrated a greater increase in acetylation. Taken together, these data define the dynamic molecular events that occur at a Stat6-responsive promoter upon IL-4 treatment.

DISCUSSION

PARP-14 Functions as a Molecular Switch—Based on the observations presented here we propose a mechanism by which PARP-14 regulates Stat6- and IL-4-mediated transcription. Our data are consistent with a model wherein PARP-14 functions as a transcriptional switch, first functioning to keep transcription off under non-stimulating conditions by binding to the promoter and recruiting HDAC 2 and 3 to the promoter (Fig. 7). In the presence of an IL-4 signal and activated Stat6, it is likely that the enzymatic activity of PARP-14 is activated, and this converts it from a repressor to an activator (Fig. 7). Our previous data indicating higher PARP-14 enzymatic activity in the presence of IL-4 activated Stat6 supports this conclusion (17). We have also previously demonstrated that the enzymatic activity associated with PARP-14 is required for its enhancement function (17). Moreover, here we provide evidence that this enzymatic activity is required for efficient binding of Stat6 to its cognate DNA element (Fig. 1). All of these observations indicate that the ADP ribosyl transferase function of PARP-14 is extremely important for its transcriptional enhancement function. It is intriguing to speculate that once the enzymatic activity of PARP-14 is activated this is a signal for PARP-14 and HDACs to exit the promoter making the promoter accessible to the histone acetyl transferases. Indeed, we provide data here that PARP-14 ADP ribosylates its N terminus and the HDACs (Fig. 4), these modifications most likely cause PARP-14 and HDACs to dissociate from each other and the promoter (Figs. 2 and 5). Based on our model PARP-14 acts as a repressor first, therefore, the prediction would be that loss of PARP-14 expression
PARP-14 Regulates Transcription

should result in hyperexpression of Stat6-responsive genes under non-stimulating conditions. But our data do not show this result, we observe no differences in the expression of Fcer2a and 1e under non-stimulating conditions in the presence or absence of PARP-14 expression (supplemental Fig. S2). We speculate that PARP-9 which has the same domain architecture as PARP-14 but does not contain PARP catalytic activity (26), compensates for the repressive but not the activation potential of PARP-14 in PARP-14-deficient cells. Consistent with the mechanism we are proposing, Ouatathni et al. (25) have shown that for the inducible Hsp70.1 and Hsp70.2 promoters, PARP-1 that is bound to these promoters is inactive under basal conditions. In the presence of a heat shock signal PARP-1 gets auto-modified and this leads to the release of both PARP-1 and mH2A from the promoter (25). This group also demonstrated that mH2A is associated with HDAC1. As PARP-14 contains both the macro domain and the PARP catalytic domain it may utilize a similar mechanism as observed with PARP-1 and mH2A for the Hsp70 promoters. Nevertheless, here we provide evidence for a unique mechanism in which a single molecule, PARP-14 functions first to switch off transcription under basal conditions, however, in the presence of a signal the catalytic activity associated with PARP-14 allows for the transcription to be activated.

DNA Binding Ability of PARP-14—Our ChIP analysis with anti-PARP-14 indicates that it has the ability to associate with DNA. We have shown that PARP-14 associates with promoter elements rather than the coding sequence of the gene (Fig. 2). Moreover, PARP-14 is bound to IL-4- and Stat6-responsive promoters but not Stat1- or Stat4-responsive genes (supplemental Fig. S3). These data indicate that PARP-14 exhibits specificity to the DNA it associates with. The specific ability of PARP-14 to associate with DNA may be dictated by the direct binding of PARP-14 to a particular DNA sequence. Or, other factors associated with a specific DNA sequences may recruit PARP-14 to a particular region of a gene. A search for conserved domains in PARP-14 did not reveal a known DNA binding motif. The only conserved domains that were found in PARP-14 were the macro domains, the WWE domain and the PARP catalytic domain (6). Neither of these domains has been reported to have any DNA binding ability. The N-terminal of PARP-14 is rather large and it is possible that this region of PARP-14 contains a unique DNA binding domain. This will need to be further investigated experimentally or by structural analysis. The other alternative that other DNA binding factors recruit PARP-14 to Stat6-responsive genes is an intriguing thought. It is known that both C/EBP and NF-kB collaborate with Stat6 to induce transcription (10). It is possible that these factors are already present at the promoter and they are the ones that associate with PARP-14 to bring it to the promoter under non-stimulating conditions. Further investigations need to be conducted to better understand the mechanism by which PARP-14 associates with DNA. However, we provide strong evidence that there is a dynamic association and dissociation of PARP-14 with Stat6 promoters and, that this interaction is required for PARP-14 to regulate IL-4-dependent transcription.

Modulation of Stat6 Activity in the Presence of PARP Inhibitors—Here we have demonstrated that in the presence of PARP inhibitors the efficiency at which Stat6 binds to the promoter it activates is compromised (Fig. 1A). Correspondingly, the expression of these genes is also reduced in the presence of PARP inhibitors (Fig. 1B). These data suggest that PARP inhibitors may be employed in disease states in which the pathogenesis is caused by Stat6 and IL-4. It is well established that IL-4 and Stat6 play a major role in the progression of asthma. IL-4 and Stat6 are responsible for the differentiation of naïve T helper (Th) cells to a Th2 phenotype that produces IL-4, IL-5, and IL-13 and promote an asthmatic response (7). Moreover, both IL-4 and Stat6 play a major role in immunoglobulin class switching in B cells. The IgE isotype is one of the major antibodies produced with an IL-4 and Stat6 signal. IgE is a central player in allergic hypersensitivity (7). As we have demonstrated that PARP-14 enhances IL-4 and Stat6 function (6), it is intriguing to speculate that PARP-14 may also be involved in Th2 differentiation and the pathogenesis of asthma. Indeed we have preliminary evidence that indicates that inhibiting PARP activity during differentiation of T helper cells reduces the ability of these cells to produce Th2

FIGURE 7. PARP-14 functions as a transcriptional switch for Stat6-dependent transcription. Conceptual model of how PARP-14 may regulate Stat6-mediated transcription. Under non-stimulating conditions PARP-14 is bound to Stat6-responsive promoters and recruits HDAC 2 and 3 and keeps the gene silent. Upon IL-4 stimulation, Stat6 is activated and binds to its promoter element and induces the PARP-14 enzymatic activity indicated by the star symbol. PARP-14 then modifies itself on the N terminus and HDAC 2 and 3 in the complex, again represented as stars. This results in the disassociation of PARP-14 and the HDACs from the promoter, which allows for association of PARP-14 and the HDACs to be recruited to the promoter and acetylation of the histones.
cytokines. Moreover, here we provide evidence that indicates that the germline transcription for the precursor of IgE (Ie) is reduced in the presence of PARP inhibitors (Fig. 1B), as well as, in the absence of PARP-14 expression (supplemental Fig. S2). Consistent with our Ie expression data, our experiments with Parp14-deficient cells indicates that the binding of Stat6 to the Ie promoter is significantly compromised when compared with cells with intact PARP-14 expression (Fig. 1C). Taken together these data indicate that targeting PARP activity may be a potential therapy for asthma and allergy. Nevertheless, here we provide insight into the molecular mechanism by which a unique macroPARP enzyme regulates transcription.

Acknowledgments—We thank Mark Kaplan for critical review of our manuscript, Mark Boothby for providing us with the Parp14−/− mice, Ed Seto for providing us with the HDAC 2 and 3 expression plasmids.

REFERENCES
1. Schreiber, V., Dantzer, F., Arne, J. C., and de Murcia, G. (2006) Nat. Rev. Mol. Cell Biol. 7, 517–528
2. Kim, M. Y., Zhang, T., and Kraus, W. L. (2005) Genes Dev. 19, 1951–1967
3. Hottiger, M. O., Hassa, P. O., Luscher, B., Schuler, H., and Koch-Nolte, F. (2010) Trends Biochem. Sci. 35, 208–219
4. Ladurner, A. G. (2003) Mol. Cell 12, 1–3
5. Pehrson, J. R., and Fried, V. A. (1992) Science 257, 1398–1400
6. Goenka, S., and Boothby, M. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 4210–4215
7. Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J., and Paul, W. E. (1999)
3 S. Goenka, unpublished observations.