Collaborator of Stat6 (CoaSt6)-associated Poly(ADP-ribose) Polymerase Activity Modulates Stat6-dependent Gene Transcription*

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The transcription factor Stat6 plays a critical role in interleukin-4-dependent gene activation. To mediate this function, Stat6 recruits canonical transcriptional co-activators including the histone acetyl transferases CREB-binding protein and NCoA-1 and other proteins such as a p100 co-factor. However, much remains unknown regarding the constituents of Stat6 enhancer complexes, and the exact molecular events that modulate Stat6-dependent gene activation are not fully understood. Recently, we identified a novel co-factor, CoaSt6 (collaborator of Stat6), which associates with Stat6 and enhances its transcriptional activity. Sequence homologies place CoaSt6 in a superfamily of poly(ADP-ribosyl)polymerase (PARP)-like proteins. We have demonstrated here that PARP enzymatic activity is associated with CoaSt6, and this function of CoaSt6 can append ADP-ribose to itself and p100. Further, we show that a catalytically inactive mutant of CoaSt6 was unable to enhance Stat6-mediated transcription of a test promoter. Consistent with these findings, chemical inhibition of PARP activity blocked interleukin-4-dependent transcription from target promoters in vivo. Taken together, we have identified a CoaSt6-associated PARP activity and provided evidence for a role of poly(ADP ribosylation) in Stat-mediated transcriptional responses involving a novel PARP.

Covalent protein modification by poly(ADP-ribose) (PAR) appears to be involved in a wide array of biological processes (1–3), and sustained inhibition of poly(ADP-ribosylation) is highly toxic to cells (2). A major source of poly(ADP-ribose) polymerase (PARP) activity is encoded by PARP-1 (3), which can engage in heterotypic interactions with transcription factors (4), components of the DNA repair complexes (2, 3), and at least one other PARP, PARP-2 (5). Analyses of PARP-1-deficient cells indicate that this protein constitutes a major portion of the global PARP activity in some cell types (3, 6). However, it is clear that PARP-1 selectively interacts with some proteins but not others (2, 3), that a substantial fraction of poly(ADP-ribosyl)ation is maintained in PARP-1 knock-out cells, and that a complete lack of PARP-1 is compatible with normal mouse development (6). Moreover, most cellular functions are maintained in PARP-1-deficient cells (6), which grow normally in the absence of genotoxic stress (3). These findings indicate that functions of other PARP enzymatic activities likely compensate for the absence of PARP-1.

Analyses of genomic sequences indicate that 15 mammalian proteins contain a segment with significant homology to the PARP catalytic domain of PARP-1 (reviewed in Ref. 2). These proteins in a PARP-like superfamily have been divided into subgroups based on analyses of their primary structures in silico. Prior cloning work and enzymatic analyses have indicated that several other members of the PARP superfamily are enzymatically active, but relatively little is known about their function (2). Members of one of the least-characterized branches of the PARP superfamily are termed the macro-PARP proteins (2). In these polypeptides, the portion exhibiting homology to the PARP catalytic region is preceded by one or several iterations of a domain homologous to a unique component of an atypical histone, macroH2a (7). Various functions have been ascribed to macrodomains taken from among the set of different proteins containing them, including binding of PAR polymers (8) or phosphoesterase activity (8, 9). The macro-PARP subfamily is of particular interest, because one of its members, termed BAL (B aggressive lymphoma), was highlighted in a differential display analysis of mRNAs in comparisons of diffuse large B cell lymphomas of greater versus less clinical aggressiveness (10). However, it has been noted that, despite the homology of its PARP-like domain, this enzymatic activity could not be detected in association with BAL1 (11).

Another member of the macro-PARP subfamily was identified recently by a search for additional proteins that could serve as co-factors for or modifiers of Stat6-mediated transcriptional regulation in cellular responses to the immunoregulatory cytokine interleukin (IL)-4 (12). A cytosolic 2-hybrid screen in yeast seeking Stat6-interacting proteins isolated a cDNA from mouse splenocytes that encodes a macro-PARP (12). Additional studies validated the interaction between this polypeptide, which was termed CoaSt6 (collaborator of Stat6) and Stat6. Functional analyses provided evidence that CoaSt6 can amplify the
function of Stat6 and thereby modulate the induction of target genes by IL-4. However, it is not known whether the PARP sequence homology within CoaSt6 would, similar to BAL, fail to encode a functional ribosyl transferase or whether it instead is a functional PARP. Furthermore, it is not clear whether the PARP domain is important for any of the transcriptional functions of CoaSt6.

Using single-point substitutions as well as assay of purified bacterially produced recombinant protein, we have shown here that CoaSt6 is associated with PARP catalytic activity that can ADP-ribosylate itself as well as p100, a co-activator recruited by Stat6. Many transcriptional functions of PARP-1 appear not to require its PARP enzymatic activity. For instance, PARP-1 serves as a co-factor for NF-κB transcription factors in a manner independent from its PARP catalytic activity (13). The role of PARP-1 instead is to promote multiprotein interactions (14). Our data indicate that a catalytically inactive point mutant of CoaSt6 failed to potentiate the Stat6-dependent activation of a reporter. Moreover, pharmacological inhibition of PARP activity blocked IL-4 induction of endogenous genes as well as a Stat6 and CoaSt6 responsive reporter. We conclude that CoaSt6-associated PARP function plays a role in IL-4-induced, Stat6-mediated gene regulation.

EXPERIMENTAL PROCEDURES

Cell Lines and Recombinant DNAs—Cell lines 293T (derived from human embryonic kidney), FNX (derived from 293), HepG2 (human hepatocellular carcinoma), and M12 (mouse B lymphoma) and their culture in standard media (Dulbecco’s modified Eagle’s and RPMI 1640 media) containing 10% fetal bovine serum were as described previously (12, 15, 16). Wild-type CoaSt6 in the expression vectors pcMV-Tag2 and pcDNA3 were generated as described previously (12). Mutated cDNAs encoding portions of CoaSt6 (N-terminal, middle, C-terminal, and CoaSt6-(1216–1817)—were generated by PCR with Ffu polymerase and cloned into the pCMV-Tag2 vector in-frame with the FLAG epitope tag. CoaSt6-(1216–1817) was also cloned in-frame with a His tag in the bacterial expression vector pET28. Point mutations were engineered in the PARP domain of CoaSt6 using the QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA). PCR primers were designed containing the point mutation and were used in PCR reactions with wild type CoaSt6 in pcMV-Tag2 as a template. The methylated template DNA containing wild type CoaSt6 sequences was fragmented using DpnI. After bacterial transformation, clones containing the desired point mutations were verified by DNA sequencing. Inserts in pCMV-Tag2 were excised and subcloned into pcDNA3. FLAG epitope-tagged p100 in pSG5. Lysates were then subjected to immunoprecipitation with anti-FLAG. In each case, the immune complexes were collected using protein G beads (Santa Cruz Biotechnology), rinsed, and divided equally. One portion was eluted and used for Western blotting. The other fraction was incubated for 30 min at room temperature in PARP assay buffer (20 μl) containing 5 μCi (0.25 μm) 32P-labeled NAD+ (1000 Ci/mmol) (GE Healthcare), 12.5 μM cold NAD+, 50 mM Tris-Cl (pH 8.0), 4 mM MgCl2, and 0.2 mM dithiothreitol (20). Where indicated, PARP activity was inhibited with 1 mM 3-aminobenzamide (3-ABA) (Sigma-Aldrich). After completion of the reaction, the beads were washed twice with phosphate-buffered saline to remove excess substrate, and the bound proteins were eluted, resolved on SDS-PAGE, and autoradiographed. PARP assays were also performed with cold NAD+ only and probed with the monoclonal anti-PAR antibody 10H (Alexis Biochemicals, San Diego, CA). For detection of PARP-modified CoaSt6 in cell lysates, extracts were prepared and analyzed by Western blotting using 10H anti-PAR antibody as above. His-tagged CoaSt6-(1216–1817) was expressed in Escherichia coli, and proteins expressed in inclusion bodies were extracted under denaturing conditions and refolded by sequential dialysis against buffer containing decreasing amounts of urea. Refolded CoaSt6-(1216–1817) was purified using Talon beads (Clontech, Mountain View, CA) and used in PARP assays and Western blotting.

Northern Blotting—M12 B cells were incubated with increasing concentrations of 3-ABA and a combination of IL-4 (5 ng/ml) and lipopolysaccharide (5 μg/ml). Northern blots of total RNA isolated from these cultured cells were probed with cDNAs for CD23, Ge, and GAPDH, subjected to autoradiography, and quantified using a phosphorimaging device (Fuji FLA-2000).

Reporter Assays—Using a constant total DNA for each sample, CMV-β-galactosidase (100 ng), and the indicated expression constructs in pCMV-Tag2 or pcDNA3 (1 μg), along with a CD23b-(N3)5-Luc (21) or Ge-(N4)5-Luc (22) reporter construct (1 μg) were co-transfected into HepG2 cells using Transfectin reagent (Bio-Rad) according to the manufacturer’s protocol. After 24 h, the cells were divided equally and either left untreated or treated with 5 ng/ml of IL-4 for an additional 24 h. For assays after inhibition of PARP activity, the cells were divided 24 h after transfection, recultured for 12 h, and then treated with IL-4 along with increasing concentrations of 3-ABA for a final 12 h. Firefly luciferase and β-galactosidase assays were performed on extracts of the transfected cells as described (12).

RESULTS

Poly(ADP-ribosyl)ation of CoaSt6 in the Presence of NAD+—We recently identified a novel co-factor, CoaSt6, which interacts with Stat6 and amplifies its activity in mediating transcriptional responses to IL-4 (12). To understand better the mechanism(s) by which CoaSt6 modulates Stat6 function, we focused on a region toward the C terminus that exhibited sequence homology with the catalytic domain of poly(ADP-ribose) polymerases (PARP) (Fig. 1a). Because the prototypical PARP-1 and PARP-2 each can serve as a substrate for poly(ADP-ribosylation), we
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The observed poly(ADP-ribosyl)ation of CoaSt6 could arise because the protein participates in a heterotypic interaction with a PARP such as PARP-1, which is known to form various heterodimers. Alternatively, the PARP-like region of CoaSt6 could encode a bona fide poly(ADP-ribose) polymerase. To test whether CoaSt6 sequences were enzymatically active independent of other protein interactions, we expressed His-tagged CoaSt6-(1216–1817) in *E. coli*, purified the protein, and performed PARP assays. Purified CoaSt6-(1216–1817) catalyzed the addition of NAD$^+$ to the protein in *vivo* (Fig. 1h), a finding most consistent with intrinsic PARP activity residing in the PARP-like domain independent of other heterotypic interactions.

Role of PARP Domain Residues in CoaSt6-associated PARP Activity—To determine whether the PARP-like domain contained in CoaSt6 was crucial for poly(ADP-ribosyl)ation, we tested a variety of mutated cDNAs. Several deletion mutants of CoaSt6 were expressed in bacteria and purified. One fraction of the purified protein was subjected to PARP reaction conditions and resolved on SDS-PAGE, stained with Coomassie Blue, and autoradiographed. Another part was probed with antibodies raised against CoaSt6.

**FIGURE 1. Poly(ADP-ribose) modification of CoaSt6.** a, diagram of CoaSt6 and CoaSt6-(1216–1817) indicating the region that shares homology to the PARP catalytic domain and other conserved areas (macrodous, cross-hatched; WWE motif containing two conserved tryptophans and a conserved glutamic acid residue). An asterisk denotes the location of the peptide epitope recognized by the anti-CoaSt6 antibodies. b, 293T cells were transiently transfected with either an empty pcMV-Tag2 vector or pcMV-Tag2 encoding FLAG-CoaSt6. Proteins were immunoprecipitated from extracts of these transfected cells using anti-FLAG and protein G beads. Bean-bound immune complexes were assayed for transfer of $^{32}$P-labeled ADP-ribose from NAD$^+$ onto proteins (20) or probed with anti-FLAG. Shown are autoradiographs of the labeled proteins (upper panel) and immunoblots (lower panel) after separation by SDS-PAGE. c, PARP reactions were performed as described for b in the absence or presence of 1 mM PARP inhibitor 3-ABA. After resolving the proteins on SDS-PAGE, the gel was silver-stained (lower panel) and autoradiographed (upper panel). d, immunoprecipitated FLAG-tagged proteins were subjected to PARP reactions as described for b, except that $^{32}$P-NAD$^+$ was omitted so that only cold NAD$^+$ was available as a substrate. Immunoblots of the eluted proteins resolved by SDS-PAGE were then probed with antibody specific for polymeric poly(ADP-ribose) chains. e, PARP modification of CoaSt6 in cells. 293T cells were transfected as described above. Extracts prepared from these cells were subjected to SDS-PAGE, and immunoblots were probed with anti-PAR as described for d. f, CoaSt6 was immunoprecipitated from M12 B cells using anti-CoaSt6 antibodies. One portion was subjected to PARP reaction conditions as described for c in the absence or presence of 1 mM 3-ABA (upper panel). The other portion was probed with anti-CoaSt6 (lower panel). g, immunoprecipitates of CoaSt6 (as described for f) were probed with anti-PAR and anti-CoaSt6. h, His-tagged CoaSt6-(1216–1817) was expressed in bacteria and purified. One fraction of the purified protein was subjected to PARP reaction conditions and resolved on SDS-PAGE, stained with Coomassie Blue, and autoradiographed. Another part was probed with antibodies raised against CoaSt6.
CoaSt6 (Fig. 2a) were tested for their ability to self-poly(ADP-ribosyl)ate in immunoprecipitates incubated with radiolabeled NAD⁺. Each version of CoaSt6 that contained the PARP-like domain was poly(ADP-ribosyl)ated (Fig. 2b). These included the C-terminal only, a combination of the C and middle portion of CoaSt6, and CoaSt6-(1216–1817), which is the version of CoaSt6 that contains the C-terminal and one hismacro domain (Fig. 2b). In contrast, no labeling by [³²P]NAD⁺ was observed for the CoaSt6 N-terminal portion or the triplicate hismacro domains, which lack the PARP-like region. These results suggest that the PARP-like domain of CoaSt6 is responsible for adding the ADP-ribose moieties. To extend these results, we identified several amino acid residues within the PARP-like domain of CoaSt6 that are conserved among active PARPs, several of which are essential for catalytic activity in PARP-1 (Fig. 2c). When substitution mutants were tested for their ability to undergo poly(ADP-ribosylation) in immune precipitates (as described above), we observed a dramatic decrease in their capacity to self-poly(ADP-ribosyl)ate as compared with their wild type counterpart (Fig. 2d).

**Enhancement of the Poly(ADP-ribosyl)ating Function of CoaSt6 in the Presence of IL-4-activated Stat6**—CoaSt6 was originally identified as a co-factor that associated with Stat6 and enhanced its transcription function (12). To explore the interplay between the poly(ADP-ribosyl)ating activity of CoaSt6 and Stat6, FLAG-tagged CoaSt6 and Stat6 were co-expressed, Stat6 was induced by IL-4, and the resultant cell extracts were subjected to co-immunoprecipitation of Stat6 and CoaSt6. The resultant IPs were then assayed with radiolabeled NAD⁺. As above, CoaSt6 poly(ADP-ribosyl)ated itself in cells with little endogenous Stat6 and untreated with IL-4 (Fig. 3, lanes 5 and 6). Although there were abundant protein levels of Stat6 and CoaSt6 in the IPs, no positive signal for the poly(ADP-ribosyl)ation of Stat6 by CoaSt6 was observed (Fig. 3, lanes 7 and 8). These results suggest that, although Stat6 associates with CoaSt6, Stat6 is not an efficient substrate for the PARP catalytic activity of CoaSt6. However, the ability of CoaSt6 to poly(ADP-ribosyl)ate itself was substantially increased in the presence of Stat6 after induction by IL-4 (Fig. 3, lanes 7 and 8). These observations indicate that the co-association of CoaSt6 with IL-4-activated Stat6 enhances the auto-PARylation activity of CoaSt6.

**Allomodification of an Exogenous Protein Substrate by CoaSt6/PARP-14**—We wanted to determine whether CoaSt6 is able to target heterologous proteins for poly(ADP-ribosyl)ation, or if, instead, it is only efficient at automodification. The preceding data indicate that Stat6, despite its physical interaction with CoaSt6, is not an efficient substrate for CoaSt6-mediated poly(ADP-ribosyl)ation, but CoaSt6 might modify other proteins that are involved in enhancer complexes with Stat6. When CoaSt6 endogenous to B cells was immunoprecipitated using anti-CoaSt6 and used in PARP assays, a protein migrating at the 100-kDa position was detected in addition to the 200-kDa PARP-1 (Fig. 2b).
kDa CoaSt6 signal (Fig. 4a). A nuclear protein p100 is a Stat6-associated transcriptional co-factor thought to enhance interactions between Stat6 and RNA polymerase II as part of the basal transcriptional machinery (18). To test whether this p100 was a target for poly(ADP-ribosyl)ation, we immunoprecipitated endogenously expressed p100 and probed the precipitate with an anti-PAR antibody. Consistent with the in vitro PARP reaction, we observed a signal for PAR at the 100-kDa position which co-migrated with the signal observed with anti-p100 (Fig. 4b). Therefore, we evaluated whether CoaSt6 can target p100 for poly(ADP-ribosyl)ation. First, we tested whether p100 and CoaSt6 associate with one another independently of Stat6. Untagged CoaSt6 and epitope-tagged p100 were co-expressed and subjected to immunoprecipitation with anti-FLAG followed by immunoblotting with anti-CoaSt6 antibodies. A CoaSt6-specific signal was observed in anti-FLAG immune complexes only when both the proteins were expressed (Fig. 4c), indicating that CoaSt6 associated with p100. Similar results were obtained when cells were separately transfected with individual expression constructs (CoaSt6; p100) and cell-free extracts were mixed prior to IP (data not shown).

We then tested whether CoaSt6 was able to poly(ADP-ribosyl)ate p100. FLAG-tagged versions of both of these proteins were co-expressed, immunoprecipitated, and subjected to PARP assays as done previously. A positive signal indicative of a poly(ADP-ribosyl)ated protein and corresponding to the migration pattern of p100 was observed only when CoaSt6 was expressed along with p100 (Fig. 4d, lanes 3 and 4). The modification of p100 by CoaSt6 was blocked by 3-ABA (Fig. 4d, lanes 5 and 6). Moreover, a catalytically inactive mutant of CoaSt6 was unable to lead to poly(ADP-ribosyl)ation of p100 (Fig. 4d, lanes 7 and 8). Taken together, these data indicate that the p100 co-factor for Stat6 interacts with CoaSt6 and is a target for poly(ADP-ribosyl)ation.
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Role of the PARP Catalytic Activity in Transcriptional Induction of a Stat6- and IL-4-responsive Gene—The preceding data suggest that CoaSt6 can mediate PARylation of at least one Stat6-associated constituent of a presumptive enhancement complex. In the case of PARP-1, there are instances where the co-factor function of PARP-1 has been found not to require the ability to ADP ribosylate targets. Thus, co-activation was fully maintained either after deletion of the PARP domain or a catalytically inactivating substitution mutation of PARP-1 analogous to CoaSt6 (E1810K) (13, 23). We sought to determine whether poly(ADP-ribose)ylation plays any role in IL-4-induced, Stat6-mediated gene expression. By analogy to earlier studies for Stat-independent genes, the well characterized cell-permeable PARP inhibitor 3-ABA was used. Thus, B cells were incubated with increasing amounts of 3-ABA in the presence or absence of IL-4, and induction of the CD23 gene was evaluated. The potent and rapid induction of CD23 mRNA by IL-4 was abrogated by increasing concentrations of 3-ABA (Fig. 5a). Of note, under these conditions, no cellular cytotoxicity was observed (not shown), and GAPDH mRNA levels relative to rRNA were unaffected. This finding indicates that PARP enzymatic activity plays an important role in the induction of CD23 by IL-4. Similar results were observed for the germ line IgH Cε (Ge) transcripts (Fig. 5b). To evaluate whether the inhibitory effect of 3-ABA was targeting CoaSt6, we performed assays in which HepG2 cells were transfected with Stat6-responsive reporters along with an empty vector or one containing CoaSt6. Transfected cells were then incubated with increasing amounts of 3-ABA, and the CoaSt6-dependent enhancement of the reporter was determined. Consistent with the Northern blot data (Fig. 5, a and b), increasing amounts of 3-ABA inhibited the CoaSt6-enhanced reporter activity. These results suggest that 3-ABA, in part, targets the PARP activity of CoaSt6 to inhibit the IL-4 and Stat6-mediated transcriptional activation (Fig. 5, c and d).

To test more directly whether or not the PARP activity encoded by CoaSt6/PARP-14 plays a role in Stat6-dependent transcription, we utilized the E1810K point mutant of CoaSt6 that has dramatically reduced PARP catalytic activity (Fig. 2d) and is analogous to the enzymatically inactive mutants of other members of the PARP superfamily (11, 24). IL-4 induction of a CD23 promoter-driven luciferase reporter was assayed in the presence of wild type or a PARP domain point mutant of CoaSt6. These experiments were performed with lesser amounts of Stat6 so as to minimize the cellular toxicity from higher amounts of DNA while maintaining CoaSt6 enhancement of the reporter. As shown previously, CoaSt6 enhanced the IL-4- and Stat6-dependent trans-activation of the reporter (Fig. 5e). Importantly, the CoaSt6 E1810K mutant, which could not poly(ADP-ribosyl)ate itself or p100, did not augment the Stat6-dependent, IL-4-induced transcriptional activity (Fig. 5e). Taken together, these data provide evidence of a role for poly(ADP-ribosyl)ation in Stat-induced gene expression and indicate that the PARP catalytic activity encoded by CoaSt6/PARP14 participates in the transcriptional induction of a Stat6-responsive promotor.

DISCUSSION

Poly(ADP-ribose) polymerases are proteins conserved across a wide evolutionary spectrum and were originally analyzed as global modifiers of general chromatin structure (25). However, increasing evidence indicates that PARP-1, the root member of the mammalian PARP gene superfamily, plays specific roles as a local gene-specific transcription co-factor that can enhance or inhibit gene expression (4). In addition to PARP-1, sequence analyses of mammalian genomes suggest that there are 15 other proteins that belong to a PARP gene superfamily defined by homology with the catalytic domain sequence of PARP-1. The enzymatic function of most members of this superfamily remains to be established, very little is known about their biologic functions, and it is not known which of these other proteins participate in gene-specific regulation of transcription.
We recently identified a Stat6-interacting protein, CoaSt6, which contains a domain showing significant sequence homologies with the PARP family, leading to its designation as PARP-14. In addition to its interaction with Stat6, CoaSt6/PARP-14 can enhance transcription that is dependent on Stat6, a transcription factor activated by the cytokine IL-4. However, CoaSt6 does not bind to the related transcription factor Stat1 or affect gene induction mediated by Stat1 after interferon-γ stimulation (12). Along with several other mammalian proteins, CoaSt6/PARP-14 encodes three iterations of a macrodomain homologous to the non-histone portion of the atypical histone macroH2a (7, 12), which are situated upstream from the PARP-like domain. Based on this domain architecture, CoaSt6/PARP-14 belongs to the macro-PARP subfamily within the PARP superfamily (2). Two lines of evidence we present here point to a conclusion that CoaSt6 encodes an intrinsic PARP catalytic activity and is extremely unlikely to be compatible with the alternative model in which CoaSt6-associated polymerase function arises only because of heterotypic interaction with a different PARP. First, recombinant protein expressed in E. coli and purified after denaturation was able to catalyze ADP-ribosylation. Second, the CoaSt6-associated PARP activity was disrupted by each of three independent point substitutions chosen based on sequence homology, structural data, and catalytic function of other PARPs. Further, our data indicate that, in addition to its autopoly(ADP-ribosyl)ation capacity, CoaSt6 can bind to p100, a known Stat6-interacting co-factor (18) and target it for PARP-mediated modification. Finally, the findings provide evidence that this enzymatic function can participate in enhancing Stat6-mediated transcription.

To date, the specific functional mechanisms of macro-PARPs (other than CoaSt6/PARP-14) are unknown, including whether or not they operate as transcriptional co-activators. When transfected into a lymphoma cell line, BAL1/PARP-9 led indirectly or directly to the induction of an interferon-like gene expression profile (26). In addition to its correlation with the clinical aggressiveness of diffuse large B cell lymphomas, BAL1/PARP-9 transfection led to increased responsiveness to the chemotactic SDF-1 protein (10). However, there is no information regarding the mechanism(s) for these effects and assays of BAL1 for PARP activity did not reveal this enzymatic function (11). In light of the association of CoaSt6 with the Stat6 signaling pathway, it is intriguing that many diffuse large B cell lymphoma tumors exhibit increased expression of IL-4-inducible genes that are Stat6-dependent (27, 28), whereas other samples of this tumor type display a gene expression profile more characteristic of interferon-regulated signaling, which tends to be antagonistic to Stat6-mediated IL-4 gene induction (26). Together, these findings raise the possibility that different members of the macro-PARP family play distinct roles in influencing the gene expression patterns of various diffuse large B cell lymphomas.

Our analyses provide evidence that poly(ADP-ribose) polymerization may be vital for an IL-4 response and that the intrinsic PARP catalytic capacity of CoaSt6/PARP-14 mediates its ability to amplify promoter activity dependent on Stat6. Furthermore, this capacity can include the PARP modification of p100, a Stat6- and CoaSt6-associated transcriptional co-factor, at least under conditions of exogenous co-expression. It is of interest to compare these findings to the diversity of fundamentally distinct mechanisms by which PARP-1 has been reported to impact transcription regulation in recent studies (13, 23, 29–33). In the majority of instances, this function occurs in a manner independent from the enzymatic activity.

Initial attention focused on a global ability of PARP-1 to alter chromatin structure by modifying histones, thereby leading to nucleosome destabilization and chromatin decondensation (34) or the release of histones from destabilized nucleosomes (35, 36). These processes are thought to be important in the repair response to DNA damage (3) and perhaps to increase access of the transcription machinery to DNA (4), but whether this mechanism acts in a gene-specific manner is not known. Furthermore, it is not evident that PARP-1-deficient cells exhibit a global decrease in rates or efficiency of overall gene transcription. Among the mechanistic studies directed to gene-specific regulation, the muscle-specific MCAT-1 promoter (33) appears to provide the antecedent most similar to the present findings with CoaSt6/PARP-14. PARP-1 is recruited to the MCAT1 promoter element and poly(ADP-ribose)lates the DNA-binding transcription factor TEF-1. As with IL-4 induction and CoaSt6, inhibition of the PARP-1 catalytic activity by 3-ABA resulted in decreased reporter activity driven by the MCAT1 element (33), but the function of a catalytically inactive PARP-1 in MCAT1-driven gene expression was not established. Based on these data, it was proposed that the recruitment of PARP-1 at a specific promoter element may allow local chromatin modification involving poly(ADP-ribosylation)ation which may promote transcription activation. In principle, this is an attractive model for CoaSt6 function, but its ability to target p100 for PAR modification raises the possibility that the modification of the Stat6 co-factor p100 is an active part of the mechanism.

Several alternative mechanisms have been supported for PARP-1, because it can regulate the expression of specific gene targets by heterotypic interaction with the multimolecular complex of transcription factors and co-factors that assembles at promoters or enhancers. Thus, PARP-1 interacts with transcription factors that bind DNA directly (e.g. NF-κB, B-MYB, RAR, and HTLV Tax-1) and somehow increases promoter activity as a consequence of this interaction (23, 31, 32, 37). This role of PARP-1 would be quite similar to the capacity of CoaSt6 to bind to both p100 and Stat6. Of note, this mechanism appears to be independent of the poly(ADP-ribose) polymerase activity in PARP-1 in most instances analyzed to date (13, 23, 31), which differs from the present findings with CoaSt6/PARP-14. In examples of another type of mechanism (29, 30, 32, 38), PARP-1 appears to be present at a promoter in the basal state, either through an intrinsic DNA-binding capacity (29, 38) or as a part of a co-repressor complex (30, 32). Activating signals then result in the dissociation of PARP-1 (29) or the switch from (co)repressor to (co)activator (30, 32). This type of mechanism can be independent of the PARP enzymatic activity (as it is for a retinoic acid receptor-based ejection of cdk8 from the Mediator complex (32)) or can require polymerase activity (as in the case of platelet-derived growth factor induction of the MASH-1 promoter (30)). However, each of these mechanisms...
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appears somewhat different from the present findings on the ability of CoaSt6/PARP-14 to amplify the Stat6 effect at an IL-4-activated promoter. Unlike these mechanisms, CoaSt6 associates with and provides co-factor function to a DNA binding transcription activator that does not appear in the nucleus until after receptor stimulation (12). The apparent lack of repressive effect in our initial studies and the present findings suggest that CoaSt6/PARP-14 can act by means differing from these modes of PARP-1 function. Nonetheless, the failure of the available anti-CoaSt6/PARP-14 antibodies to immunoprecipitate chromatin means that this model cannot as yet be rigorously excluded, and it is still possible that this type of mechanism will apply to some aspect of CoaSt6 transcriptional function.

As a member of the macro-PARP family, CoaSt6/PARP-14 contains copies of the macrodomain N-terminal to the polymerase domain. The conservation of this structure raises questions as to its potential significance given the evidence establishing that CoaSt6/PARP-14 encodes a PARP activity within its primary sequence and can positively regulate interactions and cross-talk among macro-PARPs. Notwithstanding these speculative possibilities, the data presented herein indicate that the macro-PARP CoaSt6/PARP-14 encodes a PARP activity within its primary sequence and can positively regulate IL-4-induced promoter activity in a PARP-dependent manner.

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