Ataxin-3 Is a Histone-binding Protein with Two Independent Transcriptional Corepressor Activities*

Received for publication, May 28, 2002, and in revised form, September 22, 2002
Published, JBC Papers in Press, September 23, 2002, DOI 10.1074/jbc.M205259200

Fusheng Li, Todd Macfarlan, Randall N. Pittman‡, and Debabrata Chakravarti§
From the Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084

The mechanisms of pathology for the family of polyglutamine disease proteins are unknown; however, recently it was shown that several of these proteins inhibit transcription suggesting that transcriptional repression may be a potential mechanism for pathology. In the present study we use cell transfections, in vitro binding, co-immunoprecipitations, and reporter assays to show that the polyglutamine disease protein, ataxin-3, interacts with the major histone acetyltransferases cAMP-response-element binding protein (CREB)-binding protein, p300, and p300/CREB-binding protein-associated factor and inhibits transcription by these coactivators. Importantly, endogenous ataxin-3 is co-immunoprecipitated with each of these coactivators in non-transfected cells. The C-terminal polyglutamine-containing domain of ataxin-3 inhibits coactivator-dependent transcription and is required for binding coactivators. The N-terminal domain of ataxin-3 inhibits histone acetylation by p300 in vitro and inhibits transcription in vivo. Histone binding and blocking access of coactivators to acetylation sites on histones appears to be the mechanism of inhibition. Together, our data provide a novel mechanism of transcriptional regulation by ataxin-3 that involves targeting histones, coactivators, and an independent mode of direct repression of transcription, and suggests that its physiological function and possibly pathological effects are linked to its interactions with these proteins.

Ataxin-3 (AT3) is the polyglutamine-containing protein mutated in spinocerebellar ataxia type 3 (1). It is a member of a growing family of polyglutamine neurodegenerative diseases including Huntington’s disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxia types 1, 2, 3, 6, and 7 (2, 3). All of these diseases result from an expansion of a CAG repeat that codes for a polyglutamine domain in the proteins. Except for the polyglutamine domain, the proteins have little sequence similarity. However, it was shown recently that several of these disease proteins repress transcription (4–12). Convergence of these widely divergent proteins onto a common cellular function raises the possibility that transcriptional repression may be a key aspect of disease. Most studies investigating transcriptional regulation by polyglutamine disease proteins have used truncated proteins with polyglutamine-rich domains rather than full-length proteins; therefore, the impact of the full-length protein or the role of other domains on transcriptional regulation remains unclear. This is particularly relevant in light of recent studies showing that (a) pathology in Huntington disease is most consistent with effects of the full-length mutant protein rather than a fragment containing the expanded polyglutamine domain (13) and (b) the specificity of transcriptional regulators sequestered by nuclear inclusions of AT3 depends on whether inclusions are composed of full-length AT3 or just the C-terminal polyglutamine-containing fragment (4). This raises the critical issue of the nature of the pathological agent(s) in polyglutamine diseases as the full-length protein with its repertoire of normal or modified interaction domains or a polyglutamine-containing fragment. AT3 is an excellent candidate to study transcriptional regulation because its reasonably small size allows the function of other domains of the protein to be investigated in the context of a normal or pathological polyglutamine domain.

Three mechanisms for transcriptional repression have been proposed for polyglutamine disease proteins including sequestration of transcriptional activators/coactivators by inclusions of polyglutamine-containing protein (4, 6–9), inhibition of histone acetyltransferase activity of coactivators such as CBP/p300 (10), and direct corepressor activity (11, 12). The most commonly proposed mechanism of transcriptional repression is sequestration of transcriptional activators by inclusions formed by pathological polyglutamine proteins; the androgen receptor, huntingtin, atrophin, and ataxin-7 are suggested to work, at least in part, through this mechanism. Sequestration of transcription factors is an attractive mechanism for repression because of the tendency of pathological proteins with expanded polyglutamine domains to form nuclear inclusions (14–16) and the presence of transcriptional regulators within these inclusions (4, 7–9, 11, 12, 17, 18). Sequestration may be particularly relevant for transcriptional coactivators such as CBP that are present in limiting quantities in the cell (7). In addition to sequestering transcriptional regulators, pathological polyglutamine proteins may have a direct role in transcriptional repression. Exon 1 of huntingtin that contains the polyglutamine-rich domain represses transcription by inhibiting histone acetylase activity of CBP/p300 coactivators (5, 10), and atrophin may function as a direct repressor and is part of a corepressor complex in developing Drosophila (11, 12).

CBP/p300 are transcriptional coactivators with histone acetyltransferase activity (HAT) that are proposed to play an important role in transcription (19–21) and are regulated by polyglutamine proteins (7, 8, 10). CBP/p300 mediate transcriptional activation by a large number of signal-dependent transcription factors including the cAMP response element (CRE) binding protein, CREB (22, 23). In response to cAMP, CREB
bound to the CRE enhancer is phosphorylated, which results in recruitment of CBP/p300 and eventually transcriptional activation of CAMP-responsive genes. CBP/p300 contribute to transcription by forming complexes with other HATs/coactivators and by acetylating histones (20, 22, 24). Because histone acetylation represents a transcriptionally active state of chromatin, and CBP/p300 are transcriptional coactivators with HAT activity, it is reasonable to expect that cellular proteins like polyglutamine disease proteins that interact with CBP/p300 will play an important regulatory role in modulating transcription. Conversely, proteins that interact with histones and alter their acetylation would also be expected to modulate transcription (25–27).

Several polyglutamine disease proteins repress transcription; therefore, characterizing mechanisms of transcriptional repression is critical for understanding cellular pathology as well as for identifying potential targets for therapeutic intervention. Whereas the major focus of previous studies has been on describing the role of the polyglutamine domain in transcription, little is known about the functional contributions, if any, of the rest of the protein. Therefore, we wished to determine whether full-length AT3 regulated transcription and the contribution of non-polyglutamine domains of AT3 in the process as well as to identify mechanisms of transcriptional regulation by AT3. We show that endogenous AT3 binds coactivators CBP, p300, and PCAF. Furthermore, AT3 represses transcription through at least two distinct mechanisms. One mechanism of repression involves the polyglutamine-containing C terminus. We also identified a novel mechanism of repression by the N terminus of AT3 consistent with histone binding and masking being responsible for this mode of repression. Together, our data indicate that endogenous AT3 interacts with key regulators of transcription, that it has multiple mechanisms for repressing transcription, and that regions outside the polyglutamine domain can regulate transcription.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Proteins—**Eukaryotic expression constructs AT3–27Q, AT3–78Q, C-27Q, and C-78Q were described previously (18). AT3-QF and Gal4-AT3-QF, Gal4-AT3–27Q, Gal4-AT3–78Q were generated by cloning appropriate PCR-amplified fragments into pAG-NLS vector and CMXGal4-N vector, respectively. Bacterial expression constructs MBP-AT3–27Q and MBP-AT3–78Q were described previously (28). MBP-AT3-QF and MBP-C-27Q were generated by cloning appropriate PCR-amplified fragments into MBP vector. Recombinant proteins were expressed in BL21 Escherichia coli (Novagen), MBP fusion proteins were purified by amylase beads (PerkinElmer Life Sciences) as described (29). Baculovirus-expressed FLAG-tagged p300 was purified as previously described (29).

**In Vitro Pull-down Assays—**In vitro, [35S]methionine-labeled CBP, PCAF, and CREB were synthesized using TNT coupled transcription-translation system (Promega). The labeled proteins were incubated with MBP, MBP-AT3–27Q, or MBP-AT3–78Q bound to amylase beads in binding buffer containing 15 mM HEPES-KOH, pH 7.9, 10% glycerol, 200 mM KCl, 2 mM MgCl₂, 1% Nonidet P-40, 1 mM dithiothreitol, 20 μg/ml BSA, and protease inhibitor mixture (Roche Molecular Biochemicals). Beads were washed extensively, and bound proteins were separated by SDS-PAGE and analyzed by PhosphorImager. For interaction of AT3 with AT3, purified p300 was incubated with MBP, MBP-AT3–27Q, or MBP-AT3–78Q bound to amylase beads. Beads were washed extensively, and bound proteins were separated by SDS-PAGE, transferred to polyvinylidene difluoride membrane and analyzed by Western blot for p300. For interaction assays of histones with AT3, purified core histones were incubated with MBP, MBP-AT3–27Q, MBP-AT3–78Q, MBP-AT3-QF, or MBP-C-27Q bound to amylase beads. Beads were washed extensively, and bound proteins were separated by SDS-PAGE and analyzed by Coomassie Blue staining.

**Co-immunoprecipitations—**293T cells were cotransfected with myc-tagged AT3 constructs and either CMX-CBP, CMX-PCAF or CMV-p300. 48 h after transfection, cells were harvested using lysis buffer containing 25 mM Tris-HCl, pH 8.0, 10% glycerol, 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 1 mM dithiothreitol, and protease inhibitor mixture. Cleared cell lysates were incubated with anti-CBP (Upstate Biotechnology), anti-PCAF (Santa Cruz), or anti-p300 (Santa Cruz) antibodies bound to Dynabeads protein G (DYNALE). The beads were washed extensively, and bound proteins were separated by SDS-PAGE and analyzed by immunoblot with anti-myc antibody (Calbiochem). For co-immunoprecipitation of CREB and AT3, 293T cells were cotransfected with myc-tagged AT3 constructs, CMX-CREB, and CMX-CPB. 48 h after transfection, cells were harvested after a 30-min treatment with 10 μM forskolin. Cleared cell lysates were incubated with anti-CREB (Santa Cruz) antibody bound to protein G Dynabeads. The beads were washed extensively, and bound proteins were separated by SDS-PAGE and analyzed by immunoblot with anti-myc antibody. For endogenous proteins, 293T nuclear extracts were prepared as previously described (30), and the nuclear extracts were incubated with anti-ataxin-3 (31) or anti-histone (Roche Molecular Biochemicals) antibodies bound to protein G Dynabeads. The beads were washed extensively, and bound proteins were separated by SDS-PAGE and analyzed by immunoblot with anti-myc antibody. For endogenous proteins, 293T nuclear extracts were prepared as previously described (30), and the nuclear extracts were incubated with anti-ataxin-3 (31) or anti-histone (Roche Molecular Biochemicals) antibodies bound to protein G Dynabeads. The beads were washed extensively, and bound proteins were separated by SDS-PAGE and analyzed by immunoblot with anti-myc antibody.
Phosphorylation of CREB by Purified PKA

In vitro phosphorylation buffer (40 mM Tris-HCl, pH 7.5, 20 mM MgCl2, 1 mM NaF, 20 mM ATP, 100 nM okadaic acid, and proteinase inhibitor mixture) for 1 h at 37 °C bound to amylase beads were incubated with in vitro-translated, 32P-labeled CBP (upper panel) or PCAF (lower panel), or with baculovirus-expressed and purified p300 (middle panel). Bound proteins were analyzed by PhosphorImager (CBP and PCAF) or Western blot (p300). Both normal (27Q) and pathological (78Q) AT3 interact with CBP, p300, and PCAF. AT3–78Q bound each coactivator more effectively than AT3–27Q. C, co-immunoprecipitation of full-length AT3 with CBP, p300, and PCAF. Lysates from 293T cells transfected with full-length myc-tagged AT3 (AT3–27Q or AT3–78Q) and either CBP, p300, or PCAF were immunoprecipitated with anti-CBP (lane 3), anti-p300 (lane 6), or anti-PCAF (lane 9) antibodies. Immunoprecipitated AT3 was detected by immunoblotting with anti-myc antibody. Untransfected cell lysates were used as negative controls. CBP, p300, and PCAF were co-immunoprecipitated with both AT3–27Q (middle panel) and AT3–78Q (lower panel). D, co-immunoprecipitation of the polyglutamine-containing C terminus of AT3 with CBP, p300, and PCAF. Lysates from 293T cells transfected with truncated AT3 either lacking (AT3-QF) or containing (C-27Q, and C-78Q) the polyglutamine domain and either CBP, p300, or PCAF were immunoprecipitated with anti-CBP (lane 3), anti-p300 (lane 6), or anti-PCAF (lane 9) antibodies. Immunoprecipitated AT3 was detected by immunoblotting with anti-myc antibody. CBP, p300, and PCAF interacted with the C-terminal domain of AT3 (C-27Q, middle panels; C-78Q, lower panels) but not with the N-terminal portion of AT3 (AT3-QF, upper panels), indicating the interaction required the polyglutamine-containing C terminus of AT3. The immunoglobulin band (IgG) migrating close to AT3-QF is indicated by an asterisk.

2000 (Invitrogen). The total DNA for each transfection was kept constant by addition of empty pCDNA3 vector. Alternatively, cells were transfected with internal control pRL-null, GAL-TK-Luc reporter, and CMX-Gal4-vector, CMX-Gal4-AT3-QF, CMX-Gal4-AT3–27Q, CMX-Gal4-AT3–78Q, AT3–27Q, AT3–78Q, or AT3–QF. Culture media were replaced 6 h after transfection, cells were harvested 48 h after transfection, and luciferase assays were performed using a dual-luciferase reporter assay system (Promega). 293T cells were seeded at 20,000 cells per well in 24-well plates and transfected with internal control pRL-null, pCRE-Luc reporter (Stratagene), pCDNA3-PKA, in the absence or presence of AT3–27Q, AT3–78Q, C-27Q, C-78Q, or AT3–QF using Fugene 6 (Roche Molecular Biochemicals). The total DNA for each transfection was kept constant by addition of empty pCDNA3 vector. Culture media were replaced 24 h after transfection and cells were treated with 10 µM forskolin for another 24 h, and luciferase assays performed. Most AT3 constructs used for transfection studies contained a single SV40 NLS; initial experiments performed in this study without the added NLS gave similar results to those with the NLS; however, adding the NLS resulted in less data scatter and improved reproducibility between experiments. Normally, AT3 is present in both the cytoplasm and nucleus and its distribution is dynamically regulated by mechanisms that are only partially defined. The purpose of adding the non-regulated NLS to AT3 was to bypass this complex regulation associated with its nucleocytoplasmic shuttling and the problems in reproducibility between experiments that this created. Adding a single non-regulated SV40 NLS to AT3 increases nuclear AT3 3- to 4-fold (from 10% to 20% to 50–60% of total cellular AT3; Ref. 40) but does not alter the basic findings in the study. All assays were performed in duplicate, and data in all figures represent the average of three independent experiments.

**RESULTS**

*Ataxin-3 Represses Transcription when Targeted to DNA—* Studies on transcriptional effects of polyglutamine disease proteins have focused on the role of glutamine-rich domains; however, less information is available on transcriptional regulation by full-length polyglutamine-containing proteins and the contribution of non-polyglutamine domains in the overall activity of the protein. To determine whether full-length AT3 repressed transcription when targeted to DNA, a Gal4 DNA-binding domain was fused to full-length AT3 containing either a wild-type (27Q) or pathological (78Q) glutamine domain. Both proteins repressed transcription from a thymidine kinase promoter containing an upstream activating sequence (GAL-TK-LUC) element (Fig. 1A). Transcription was not repressed, however, when the N-terminal domain of AT3 lacking the polyglutamine domain was targeted to the promoter with a Gal4 DNA-binding domain. To determine whether AT3 could function as a non-specific transcriptional repressor, full-length proteins without the Gal4 DNA-binding domain were transfected into cells;
transcription from the TK promoter was not altered by these constructs (Fig. 1B). These data indicate that full-length AT3 is not a nonspecific transcriptional repressor but rather can function as a repressor when targeted to chromatin and that this repression requires the polyglutamine-containing C terminus of the protein.

**Ataxin-3 Binds to CBP, p300, and PCAF**—AT3 actively repressed transcription when recruited to promoters as a Gal4 DNA binding domain fusion protein (Fig. 1); therefore, the possibility exists that endogenous AT3 might repress transcription if recruited to chromatin by interacting with transcriptional regulators bound to DNA. CBP, p300, and PCAF are transcriptional coactivators with intrinsic HAT activity that are recruited to promoters by interacting with DNA-binding transcription factors such as CREB. To determine whether binding to CBP/p300 and PCAF could serve as a potential mechanism for targeting AT3 to chromatin, we performed both in vitro binding and in vivo association assays. In vitro binding experiments using equimolar amounts of different MBP-fusions of AT3 and radiolabeled coactivators indicated that AT3 bound to CBP as previously shown (4) as well as the other major HATs, p300 and PCAF (Figs. 2, A and B). Interestingly, pathological AT3 containing 78 glutamines bound somewhat more efficiently than wild-type AT3 containing 27 glutamines. We next determined whether AT3 interacted with CBP, p300, and PCAF in intact cells. For that purpose, co-immunoprecipitation experiments were performed on cells transfected with wild-type or pathological AT3 and either CBP, p300, or PCAF. Both normal and pathological AT3 were efficiently co-immunoprecipitated with each of these transcriptional regulators (Fig. 2C, lanes 3, 6, and 9). The domain of AT3 responsible for interacting with CBP, p300, and PCAF was determined by co-immunoprecipitation experiments performed on cells transfected with either the N or C terminus of AT3. The C terminus containing either a normal or pathological polyglutamine domain co-immunoprecipitated with CBP, p300, and PCAF, whereas the N terminus did not (Fig. 2D, lanes 3, 6, and 9). The C terminus containing the expanded pathological glutamine
domain bound coactivators better than the normal length polyglutamine domain. These data indicated that both wild-type and pathological AT3 bound to CBP, p300, and PCAF through the polyglutamine-containing C terminus.

**Ataxin-3 Represses CBP-, p300-, and PCAF-mediated Transcription**—Because AT3 and in particular the glutamine-rich C-terminal domain of AT3 bound to CBP, p300, and PCAF, we determined the functional consequences of these interactions using reporter-gene assays and constructs of the coactivators fused to a Gal4 DNA-binding domain. As observed previously, a Gal4 fusion of CBP or p300 activated transcription of a luciferase reporter gene (Fig. 3, A and B, lanes 2) (25, 26, 32). Like CBP and p300, PCAF also activated transcription, although the level of activation was relatively low compared with CBP/p300 (Fig. 3C). Full-length normal or pathological AT3, as well as the polyglutamine-containing C terminus repressed CRE-mediated transcription. Data represent the average of three independent experiments. C, AT3 does not bind CREB directly in vitro. Amylose beads bound with MBP (lane 2), MBP-AT3-27Q (lane 3), or MBP-AT3-78Q (lane 4) were incubated with in vitro translated, ^35^S-labeled CREB, which was unphosphorylated (upper panel) or phosphorylated (lower panel) with PKA. Bound proteins were analyzed by SDS-PAGE and PhosphorImager. D, AT3 is present in a complex with CREB in vivo. Lysates from forskolin-treated 293T cells transfected with full-length AT3 (AT3–27Q or AT3–78Q), CBP, and CREB were immunoprecipitated with anti-CREB antibody (lane 3) then blotted with anti-myc antibody to detect transfected AT3. Lysate from untransfected cells was used as a control. CREB was co-immunoprecipitated with both the normal AT3 (AT3–27Q, middle panel) and pathological AT3 (AT3–78Q, lower panel).

**Endogenous Ataxin-3 Binds to CBP, p300, and PCAF**—Data presented in Figs. 2 and 3 indicated that transfected AT3 interacted with CBP, p300, and PCAF and inhibited transcription by these coactivators. However, these experiments did not address the important issue of whether endogenous AT3 may have similar functions. Our previous results demonstrate that AT3 is localized both in the cytoplasm and nucleus (33), whereas transcriptional coactivators such as CBP/p300 and PCAF are predominantly nuclear. Therefore, to determine whether endogenous AT3 interacts with CBP, p300, and PCAF, co-immunoprecipitation experiments were performed on nuclear extracts from non-transfected cells. Endogenous AT3 was efficiently immunoprecipitated with all three endogenous coactivators (Fig. 4A, lane 3). However, as expected, no binding was observed when cytoplasmic extracts were used (data not shown). This suggests that interactions between endogenous AT3 and these coactivators may be a normal (and possibly pathological) aspect of transcriptional regulation.

Our data indicated that nuclear AT3 interacted with coactivators CBP and p300, which play important roles in CRE/CREB-mediated transcription, and repressed these coactivator-mediated transcription. Therefore, AT3 should repress
transcription of a reporter gene driven by a CRE enhancer. As expected, both the full-length and the polyglutamine-containing C terminus inhibited transcription from a CRE reporter (Fig. 4B). A possible mechanism for such inhibition may involve direct interaction between AT3 and CREB. Alternatively, AT3 may indirectly target CREB and CREB-mediated transcription by associating with CREB-interacting proteins such as CBP and p300. To address this issue, in vitro binding assays and co-immunoprecipitation were performed. In vitro binding assays indicated that AT3 did not directly bind CREB or phosphorylated CREB (Fig. 4C) but AT3 could be co-immunoprecipitated with CREB from transfected cells (Fig. 4D, lane 3). These results suggest that AT3 may regulate CRE-dependent transcription by binding to coactivators or other regulators rather than directly interacting with CREB. These data show that endogenous AT3 associates with coactivators, and when overexpressed it represses CRE-dependent transcription.

**The N Terminus of Ataxin-3 Binds to Histones and Regulates HAT Activity and Transcription**—Unlike full-length AT3, the N terminus lacking the glutamine domain did not actively repress transcription when targeted to a promoter with the Gal4 DNA-binding domain (Fig. 1A). To determine whether the N terminus of AT3 could regulate Gal4-CBP and CRE/CBP-dependent transcription, cells were transfected with increasing amounts of cDNA for the N terminus of AT3 along with reporter constructs with UAS or CRE enhancers, and reporter activity was measured. The N terminus of AT3 repressed transcription from both UAS- and CRE-driven reporter genes (Fig. 5, A and B). Transcriptional activation by the Gal4-CBP requires its HAT activity (25, 26, 32); therefore, one possible mechanism for repression would be that the N terminus of AT3 inhibits HAT activity of CBP/p300 coactivators. We tested this possibility by determining whether full-length AT3, or the N or C terminus inhibited acetylation of histone H4 by purified p300. Acetylation was inhibited by the N terminus of AT3 as well as by full-length AT3 containing a normal or pathological polyglutamine domain (Fig. 6, A and B, panels I–III). Surprisingly, however, the C terminus containing the polyglutamine domain and p300 binding site (Fig. 2D) was much less effective at inhibiting histone acetylation (Fig. 6B, panel IV and Fig. 6C). This result suggested that inhibition of histone acetylation was independent of binding to p300 and primarily caused by the N terminus of AT3.

Because the N terminus of AT3 did not bind CBP/p300, we examined the possibility that AT3 interacted with histones and blocked access of p300 to acetylation sites. In vitro binding assays indicated that H3 and H4 histones bound to normal and pathological full-length AT3; binding was almost exclusively through the N terminus of AT3 (Fig. 6D). The C terminus of AT3 did not bind histones effectively, which correlated well with the inability of the C terminus of AT3 to block histone acetylation. These observations, combined with data presented above suggested that AT3, through its N terminus, decreased histone acetylation by binding and masking histones; this may also account for the transcriptional repression seen with the N terminus of AT3. Transcriptional repression through this novel mechanism would therefore require that AT3 and histones interact in intact cells. To determine whether endogenous AT3 also interacts with histones, we performed co-immunoprecipitation experiments on nuclear extracts from non-transfected cells. Endogenous AT3 and histones were co-immunoprecipitated (Fig. 6E, lane 3). Together these data provide a novel mechanism of transcriptional regulation by AT3 that involves targeting histones, coactivators, and an independent mode of direct repression of transcription, suggesting that its physiological function and possibly its pathological effects might be linked to its interactions with these proteins.

**DISCUSSION**

The mechanism of pathology in polyglutamine diseases is unknown; however, current data support the view that acute dysregulation of gene expression in response to pathological polyglutamine proteins (34) initiates a sequence of events eventually resulting in accumulated cellular insults, dysfunction, and death (34–37). Mechanisms for altering gene expression have not been identified, although one possibility is that polyglutamine disease proteins repress transcription. Currently, three mechanisms are proposed to account for transcriptional repression by polyglutamine disease proteins: (a) nuclear inclusions/aggregates of mutant proteins sequester transcription factors (4, 6–9); (b) proline- and polyglutamine-rich fragments inhibit histone acetylase activity of CBP, p300, and/or PCAF.
(10); and (c) the full-length protein functions as a corepressor (11, 12). We initiated the current study to determine whether AT3 repressed transcription and to characterize the mechanism of repression with particular emphasis on determining whether other domains in addition to the polyglutamine domain regulate transcription.

Our data support the role of AT3 in transcriptional repression through multiple mechanisms. First, endogenous AT3 binds to CBP, p300, and PCAF coactivators and when overexpressed regulates CBP/p300- and PCAF-dependent transcription, indicating that AT3 normally interacts with key transcriptional regulators and therefore is in a position to regulate transcription. Second, both normal and pathological AT3 repress CBP-, p300- and PCAF-mediated transcription as well as transcription controlled by a CRE enhancer. Third, AT3 represses transcription when targeted directly to an enhancer by fusing it to a Gal4 DNA-binding domain. Fourth, a novel mechanism for transcriptional repression was identified for the N terminus of AT3, which was shown to bind to histones and inhibit their acetylation by transcriptional coactivators/HATs. Based on these results, we propose that AT3 may utilize multiple transcriptional regulatory domains and mechanisms to promote transcriptional repression.

Although both the N and C termini of AT3 repress transcription, the mechanisms of transcriptional repression are distinctly different. The polyglutamine containing C terminus binds CBP, p300, and PCAF coactivators and represses their transcriptional activity. Therefore, the C terminus may target full-length AT3 to DNA regulatory elements and function similar to typical corepressors. Interestingly, the C terminus containing a pathological polyglutamine stretch binds coactivators to a greater extent than the C terminus with a normal polyglutamine stretch. Whether the polyglutamine domain alone is responsible for binding coactivators and repressing transcription or the additional 43–59 amino acids at the C terminus are also involved is unknown. It will be interesting to determine whether the alternative splice variants of the C terminus of AT3 (38) alter its binding and transcriptional repression of histones and inhibit their acetylation by transcriptional coactivators/HATs. Based on these results, we propose that AT3 may utilize multiple transcriptional regulatory domains and mechanisms to promote transcriptional repression.
CBP, p300, and PCAF. The N terminus of AT3 represses CBP- and CRE-mediated transcription; however, the N terminus fused to a Gal4 DNA-binding domain does not repress transcription. The mechanism underlying this difference in transcriptional regulation by the N terminus is unknown; however, it may be that the N terminus indirectly contributes to transcriptional repression by regulating HAT-dependent transcriptional activation through histone binding, which is more prominent in CBP- and CRE-mediated transcription and less prominent when Gal4-AT3 directly binds DNA. In this sense, the N terminus of AT3 would function in a manner similar to the proposed mechanism of action of INHAT (see below; Ref. 25) and the C terminus would function like classical corepressors such as SMRT/NCoR (22).

AT3 through its N terminus inhibits histone acetylation by p300. Huntington also inhibits histone acetylation, but the mechanisms of inhibition by AT3 and huntingtin appear different. Exon 1 of huntingtin containing the polyglutamine domain directly inhibits catalytic HAT activity of CBP/p300 co-activators. An adjacent proline-rich domain in huntingtin is necessary for inhibiting HAT activity, suggesting that additional domains beyond the polyglutamine stretch play important roles in the function of the polyglutamine-containing proteins. Several polyglutamine disease proteins including the androgen receptor, atrophin-1, and ataxin-7 have proline-rich domains near their polyglutamine domain. It will be important to determine whether these other polyglutamine disease proteins also inhibit coactivator HAT activity. It will be equally important to determine whether other domains of huntingtin outside of exon 1 provide specificity through protein-protein interactions to more selectively inhibit coactivator HAT activity. It will be equally important to determine whether ataxin-1 modulates INHAT (ataxin-1).

N terminus of AT3 through histone masking. In cells, histone masking likely involves histones (25). Therefore, AT3 may function as an INHAT soror and histone-binding protein that regulates transcription. The present study also highlights the importance of analyzing transcriptional regulation in the context of the full-length polyglutamine disease protein as well as demonstrating that domains outside of the polyglutamine region provide specificity of molecular targets and transcriptional repression. The present study is consistent with transcriptional repression serving as a point of convergence for the family of polyglutamine disease proteins that have widely divergent sequences but a common neurodegenerative link.

Acknowledgments—We thank Anthony Bevivino and Pat Loll for MBR, MABP-AT3–27Q and MABP-AT3–78Q plasmids, J. Dawson and Yuki Mukai and Sara Kutney for purified p300, PCAF, and core histones. We thank Judy Meinloth for the wtPKA plasmid. We also thank Liselotte Jensen, Caroline Thorn, Steve Whitehead, and Julie Blendy for many helpful suggestions.

REFERENCES

1. Kawaguchi, Y., Okamoto, T., Tanikawa, M., Aizawa, M., Inoue, M., Katayama, S., Kawakami, H., Nakamura, S., Nishimura, M., Akiguchi, I., Kinbara, J., Narumiya, S., and Kikuzuku, A. (1994) Nat. Genet. 8, 221–226
2. Ross, C. A., Wood, J. D., Schilling, G., Peters, M. F., Nucifora, F. C., Jr., Cooper, J. K., Sharp, A. H., Margolis, R. L., and Borchelt, D. R. (1999) Philos. Trans. R. Soc. Lond. B Biol. Sci. 354, 1005–1011
3. Zoghbi, H. Y., and Orr, H. T. (2000) Annu. Rev. Neurosci. 23, 217–247
4. Chai, Y., Wu, L., Griffin, J. D., and Paulson, H. L. (2001) J. Biol. Chem. 276, 44889–44897
5. Haines, R. E., Lo, R. S., Davis, C., Strand, A. D., Neil, C. L., Olson, J. M., and Fields, S. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 13201–13206
6. La Spada, A. R., Fu, Y. H., Sopher, B. L., Libby, R. T., Wang, X. L., Li, L. Y., Einem, D. D., Huang, J., Puesen, D. E., Smith, A. C., Martinez, R. A., Koszul, K. L., Trettin, P. M., Ware, C. B., Hurley, J. B., Preece, L. J., and Chen, S. (2001) Neurology 51, 913–927
7. McCormick, A., Taylor, J. P., Taya, Y., Robertsek, J., Li, M., Walcott, J., Merry, D., Chai, Y., Pandele, H., Sobas, G., and Fischbeck, K. H. (2000) Hum. Mol. Genet. 9, 2193–2207
8. Nucifora, F. C., Jr., Sasaki, M., Peters, M. F., Huang, H., Cooper, J. K., Yamada, M., Takahashi, H., Tsuchiya, Y., Tomonou, J., Dawson, V. L., Dawson, T. M., and Ross, C. A. (2001) Science 291, 2423–2428
9. Steffen, J. S., Kazantsev, A., Spaic-Buskovic, O., Greenwald, M., Zha, Y. Z., Golshy, H., Wanker, E. E., Bates, G. P., Hausma, T. M., and Thompson, L. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6763–6768
10. Steffen, J. S., Bodai, L., Pallos, J., Poelmann, M., McCamplle, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M., Kurokawa, R., Housman, D. E., Jackson, G. R., Marsh, J. L., and Thompson, L. M. (2001) Nature 413, 739–743
11. Wood, J. D., Nucifora, F. C., Jr., Duan, K., Zhang, C., Wang, J., Kim, Y., Schilling, G., Saech, N., Liu, J. M., and Ross, C. A. (2000) J. Cell Biol. 150, 935–948
12. Zhang, S., Xu, L., Lee, J., and Xu, T. (2002) Cell 108, 45–56
13. Dyer, B. B., and McCormick, C. T. (2001) Nat. Genet. 29, 270–278
14. Chai, Y., Shao, J., Miller, V. M., Williams, A., and Paulson, H. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8310–8315
15. Paulson, H. L. (1999) Am. J. Hum. Genet. 64, 339–345
16. Ross, C. A. (1997) Neuron 19, 1147–1150
17. Boullet, J. M., Thomas, P., Neil, J. W., Weston, V. J., Duej, J., Harper, P. S., and Jones, A. L. (1999) Hum. Mol. Genet. 8, 1647–1655
18. Peretz, M. R., Paulson, H. L., Pandele, S. J., Sainoz, J., Bonni, N. M., and Pittman, R. N. (1998) J. Cell Biol. 143, 1457–1470
19. Bannister, A. J., and Kouzarides, T. (1996) Nature 384, 641–643
20. Chen, T. J., Kim, S., and Young, R. A. (2003) Curr. Opin. Cell Biol. 15, 218–224
21. Goygoylo, V. V., Shlitz, R. L., Rusanova, V. H., and Nakatani, Y. (1996) Cell 87, 853–859
22. Glass, C. K., and Rosenfeld, M. (2000) Genes Dev. 14, 121–141
23. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577
24. Nakatani, Y. (2001) Genes Cells 6, 79–86
25. Sol, S. B., McNamara, P., Housma, D. E., Turner, A., Lane, W. S., and Chakravarti, D. (2001) Cell 104, 119–136
26. See, S. B., Macfarlan, T., McNamara, P., Hong, R., Mukai, Y., Hoo, S., and Chakravarti, D. (2001) J. Biol. Chem. 277, 14055–14018
27. Bevivino, A. E., and Loll, P. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 11955–11960
Transcriptional Repression by Ataxin-3

29. Chakravarti, D., Ogryzko, V., Kao, H. Y., Nash, A., Chen, H., Nakatani, Y., and Evans, R. M. (1999) *Cell* **96**, 393–403
30. Wang, S., and Pittman, R. N. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 10385–10389
31. Paulson, H. L., Perez, M. K., Trottier, Y., Trojanowski, J. Q., Subramony, S. H., Das, S. S., Vig, P., Mandel, J. L., Fischbeck, K. H., and Pittman, R. N. (1997) *Neuron* **19**, 333–344
32. Martinez-Balbas, M. A., Bannister, A. J., Martin, K., Haus-Steffert, P., Meisterernst, M., and Kouzarides, T. (1998) *EMBO J.* **17**, 2886–2893
33. Perez, M. K., Paulson, H. L., and Pittman, R. N. (1999) *Hum. Mol. Genet.* **8**, 2377–2385
34. Lin, X., Antalffy, B., Kang, D., Orr, H. T., and Zoghbi, H. Y. (2000) *Nat. Neurosci.* **3**, 157–163
35. Evert, B. O., Vogt, I. R., Kindermann, C., Oezmek, L., de Vos, R. A., Brunt, E. R., Schmitt, I., Klockgether, T., and Wullner, U. (2001) *J. Neurosci.* **21**, 5389–5396
36. Luthi-Carter, R., Strand, A., Peters, N. L., Solano, S. M., Hollingsworth, Z. R., Menon, A. S., Frey, A. S., Spektor, B. S., Penney, E. B., Schilling, G., Ross, C. A., Borchelt, D. R., Tapscott, S. J., Young, A. B., Cha, J. H., and Olson, J. M. (2000) *Hum. Mol. Genet.* **9**, 1259–1271
37. Wyttenbach, A., Swartz, J., Kita, H., Thykjaer, T., Carmichael, J., Bradley, J., Brown, R., Maxwell, M., Schapira, A., Orntoft, T. F., Kato, K., and Rubin, D. C. (2001) *Hum. Mol. Genet.* **10**, 1829–1845
38. Schmidt, T., Landwehrmeyer, G. B., Schmitt, I., Trottier, Y., Auburger, G., Laccone, F., Klockgether, T., Volpel, M., Epplen, J. T., Schols, L., and Riess, O. (1998) *Brain Pathol.* **8**, 669–679
39. Matilla, A., Koshy, B. T., Cummings, C. J., Isobe, T., Orr, H. T., and Zoghbi, H. Y. (1997) *Nature* **389**, 974–978
40. Perez, M. K., Paulson, H. L., and Pittman, R. N. (1999) *Hum. Mol. Genet.* **8**, 2377–2385
Ataxin-3 Is a Histone-binding Protein with Two Independent Transcriptional Corepressor Activities
Fusheng Li, Todd Macfarlan, Randall N. Pittman and Debabrata Chakravarti

*J. Biol. Chem.* 2002, 277:45004-45012.  
doi: 10.1074/jbc.M205259200 originally published online September 23, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205259200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 16 of which can be accessed free at http://www.jbc.org/content/277/47/45004.full.html#ref-list-1