nature structural & molecular biology

CtBP represses p300-mediated transcriptional activation by direct association with its bromodomain

Jae-Hwan Kim[1], Eun-Jung Cho[2], Seong-Tae Kim[3] & Hong-Duk Youn[1]

Histone acetyltransferase coactivators bind to acetylated histones through their bromodomains and catalyze the acetylation of histone H3 and H4 tails for transcriptional activation. C-terminal binding protein (CtBP) serves as a transcriptional corepressor by recruiting histone deacetylases. However, the precise mechanism by which CtBP represses transcription has not been determined. In this study we found that CtBP1 directly associates with p300 by binding to the PXDLS motif in the bromodomain of p300. Moreover, CtBP1 blocks the accessibility of p300 to histones in an NADH-sensitive manner and thus represses p300-mediated histone acetylation and transcriptional activation. In addition, an NADH-nonresponsive, monomeric mutant, CtBP1 (G183V), was found to strongly repress p300-mediated transcriptional activation. Thus, the dissociation of NADH from CtBP1 leads to the repression of p300-driven general transcriptional activity by CtBP1. These results suggest a novel mechanism whereby CtBP1 serves as an energy-sensing repressor of histone acetyltransferase(s) and thus affects general transcription.

© 2005 Nature Publishing Group http://www.nature.com/nsmb

CtBP was originally found to interact with an adenoviral E1A oncoprotein and to repress its transcriptional activity[1]. Structurally, CtBP shares substantial amino acid homology with NADH-dependent 2-hydroxyacid dehydrogenase and has dehydrogenase activity, although it is low[2–4]. In addition, it has been recently reported that CtBP has a ~100-fold higher affinity for NADH than NAD^+, and that nuclear NADH induces a conformational change in CtBP, which ultimately allows CtBP to act as a redox sensor[5,6].

CtBP primarily recognizes a conserved PXDLS (where X indicates any residue) motif and associates with a variety of DNA-specific transcriptional factors that have been implicated in development and tumorigenesis[7–12]. In addition, CtBP interacts with polycomb protein PC2, a general transcriptional repressor of homeotic gene expression, leading to the SUMOylation of CtBP. However, the role of PC2 in the functional modification of CtBP remains unclear[13,14]. The transcriptionally repressive activity of CtBP is thought to be mediated by its interaction with proteins related to transcriptional repression, such as histone deacetylases (HDACs)[15–17]. However, this interaction is unlikely to be the primary mechanism of CtBP1-mediated repression given several observations that CtBP1-mediated repression is relatively insensitive to HDAC[11,12,18]. These results raise the possibility that CtBP may achieve its repressive effects by controlling other types of chromatin modifiers. To obtain new insight into the repression mechanism of CtBP in general transcription, we searched for the PXDLS motif among proteins related to chromatin remodeling. In this study, we found that several histone acetyltransferases contain the PXDLS motif within their bromodomains. The bromodomain is known to mediate the binding of acetyltransferases to acetylated histones and to play a critical role in recognizing acetylated histones in chromatin for gene activation[19–23]. We further demonstrated that CtBP1 physically associates with the bromodomain of histone acetyltransferase of p300 and regulates the direct interaction between the bromodomain of p300 and acetylated histones in an NADH-dependent manner. Our results in this study showed that the energy level in living cells regulates the transcription and CtBP1 plays pivotal role in an energy-dependent transcription.

RESULTS

CtBP1 associates with acetyltransferases

While searching for PXDLS motif-containing chromatin remodeling proteins, we found that several bromodomain-containing proteins contain putative CtBP1-binding motifs (**Supplementary Fig. 1** online). In particular, acetyltransferase coactivators have a highly conserved PXDL(S/K) motif within the center of their bromodomains (**Fig. 1a**). In particular, the PXDL(S/K) motif in the bromodomain is located in helix A for the binding site of acetyl-histone (**Fig. 1b**). Moreover, the four-residue PXDL sequence of the bromodomain of TIF-1α has been reported to be sufficient to mediate direct DNA and nucleosome interactions[24]. Therefore, the possibility of CtBP1 binding to the bromodomain implies that CtBP1 regulates the accessibility of acetyltransferase coactivators to chromatin; this led us to examine whether CtBP1 associates with bromodomain-containing coactivators. In fact, GST-CtBP1 did pull down p300 and other bromodomain PXDL(S/K)-containing acetyltransferases (**Fig. 1c**). As the function of p300 in transcription is similar to those of CBP and pCAF, we further focused on the p300-CtBP1 interaction by coimmunoprecipitating HA-tagged p300 with anti-c-myc recognizing c-myc-CtBP1 (**Fig. 1d**).

[1]Department of Biochemistry and Molecular Biology, Cancer Research Institute, Interdisciplinary Program in Genetic Engineering, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea. [2]College of Pharmacy and [3]College of Medicine, Sungkyunkwan University, Suwon 440-746, Republic of Korea. Correspondence should be addressed to H.D.Y. (hdyoun@snu.ac.kr).

Published online 17 April 2005; doi:10.1038/nsmb924

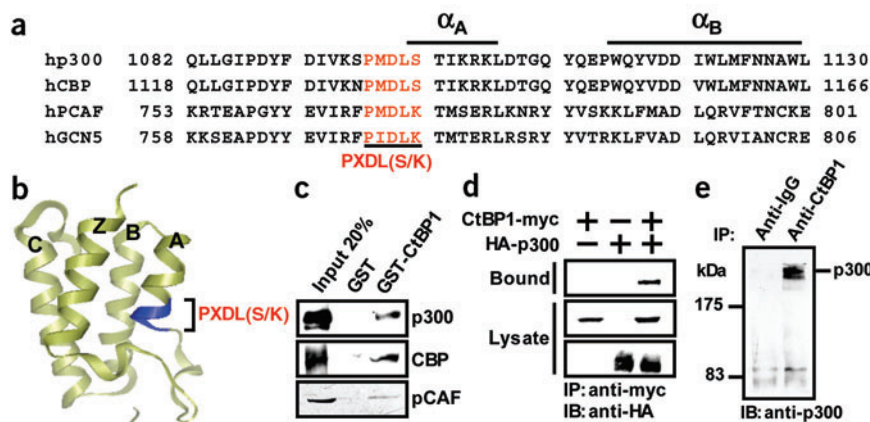

Figure 1 The binding of CtBP1 to histone acetyltransferases (HATs) through its PXDL (S/K) motif. (**a**) The alignment of PXDL(S/K) motifs in the bromodomains of HATs. (**b**) Crystal structure of bromodomain (PDB entry 1E6I)[19]. The bromodomain is composed of four α-helical domains: A, B, C and Z. The PXDL(S/K) motif is located in the acetylated histone-binding site. (**c**) CtBP1 binding to HATs. GST-CtBP1 pulled down each of the overexpressed HAT coactivators (p300, CBP and pCAF). (**d**) *In vivo* coimmunoprecipitation (IP) of CtBP1 with full-length p300. Myc-tagged CtBP1 and HA-tagged p300 were cotransfected into HEK-293T cells. Complexes were isolated with c-myc antibody (Covance) recognizing myc-CtBP1 in the presence of protein (A/G) agarose. Western blots (IB) were probed with anti-HA (Covance). (**e**) Endogenous coimmunoprecipitation between CtBP1 and p300. HeLa cell nuclear extracts were precleared using protein-A/G beads, and then precipitated with anti-CtBP1. Immunoprecipitates were probed with anti-p300.

In addition, we found that CtBP1 interacts with p300 at the endogenous level (**Fig. 1e**).

CtBP1 directly associates with the bromodomain of p300

To confirm the direct interaction between CtBP1 and the bromodomain of p300, we purified these two $(His)_6$-tagged recombinant proteins from *Escherichia coli* (**Fig. 2a**). When purified recombinant CtBP1 protein was eluted through a Superdex-75 gel filtration column, it was found to exist mainly in a monomeric form (**Fig. 2b**, panel i). In addition, recombinant p300-bromodomain protein was eluted at the expected molecular mass (**Fig. 2b**, panel ii). When CtBP1 and p300-bromodomain were mixed for 4 h and then eluted through the same column, p300-bromodomain was coeluted with CtBP1 (**Fig. 2b**, panel iii). Moreover, this procedure resulted in the elution of a molecular mass larger than CtBP1 (**Fig. 2b**, dotted red line), indicating that CtBP1 directly associates with p300-bromodomain. To address whether the PXDLS motif in the p300-bromodomain is required for interaction with CtBP1, we generated a p300-bromodomain double mutant by converting two amino acids (Asp1098 and Leu1099) to alanines. Initially we examined whether this mutation of the PXDLS motif affects protein folding. During the protein purification of p300-bromodomain and mutant p300-bromodomain, the expression of the mutant p300-bromodomain was found to be at the wild-type level. In addition, both proteins were soluble, implying that these mutations in the PXDLS motif do not severely disrupt protein folding. When the protein folding of bacterially purified mutant and wild-type p300-bromodomain were examined by fluorescence spectroscopy, their emission peaks shapes were similar, but the mutant p300-bromodomain showed a slight shift (346 to 341 nm) (**Fig. 2c**), indicating that these mutations do not severely disrupt protein folding. Then we examined the interactions between the wild-type or mutant p300-bromodomain with of CtBP1. Recombinant GST-CtBP1 did not interact with recombinant $(His)_6$-bromodomain (D1098A L1099A) mutant nor with full-length p300 (D1098A L1099A) mutant in cell extracts (**Fig. 2d**,**e**). Similarly, recombinant GST-bromodomain (D1098A L1099A) did not interact with CtBP1 (**Fig. 2e**), indicating that the PXDLS motif in the p300-bromodomain is necessary for CtBP1 binding.

Role of NADH in CtBP1 binding to the bromodomain of p300

The occupation of NADH in the central dehydrogenase domain of CtBP1 (GXGXXG) induces a conformational change in CtBP1 to trigger its dimerization, and consequently an increase in its binding to proteins such as E1A and ZEB[5]. Upon confirming that the CtBP1-

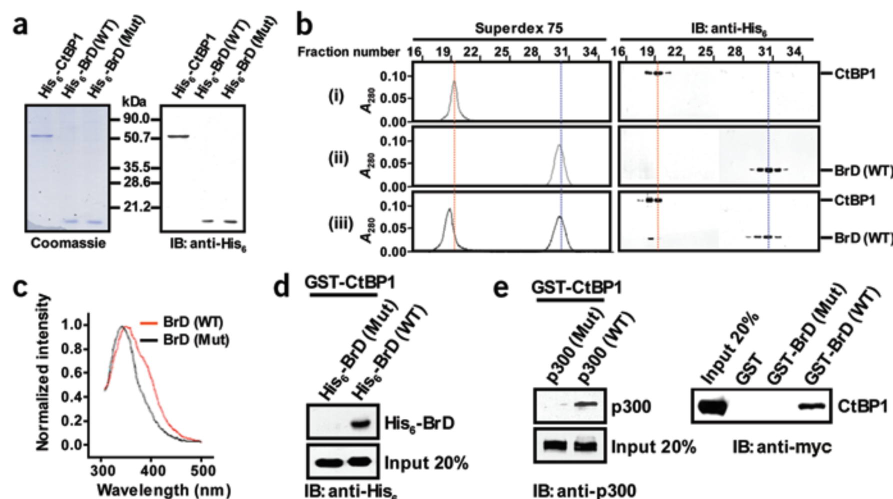

Figure 2 CtBP1 directly associates with the p300-bromodomain. (**a**) Purification of recombinant $(His)_6$-tagged CtBP1 and wild-type (WT) or mutant p300-bromodomain. Purities were confirmed by Coomassie staining. BrD, bromodomain; $(His)_6$-BrD (Mut), $(His)_6$-tagged bromodomain mutated at both D1098A and L1099A. (**b**) p300-bromodomain was coeluted with CtBP1. Recombinant CtBP1(i), BrD(ii), or both (iii) were eluted through a Superdex-75 10/300 GL prepacked column at a flow rate of 0.5 ml min^{-1}. Each fraction was subjected to 12% (w/v) SDS-PAGE, and immunoblotted with anti-$(His)_6$ (Amersham). (**c**) Fluorescence emission spectra of both wild-type and mutant recombinant p300-bromodomains. (**d**,**e**) The D1098A L1099A mutation in the bromodomain abrogated its interaction with CtBP1. GST-CtBP1 with GSH-beads pulled down either recombinant wild-type or mutant p300-bromodomains, or cell lysates containing either wild-type full-length p300 or mutant. On the other hand, CtBP1-overexpressing cell lysates incubated with either wild-type or mutant recombinant GST-bromodomain were pulled down by GSH beads.

© 2005 Nature Publishing Group http://www.nature.com/nsmb

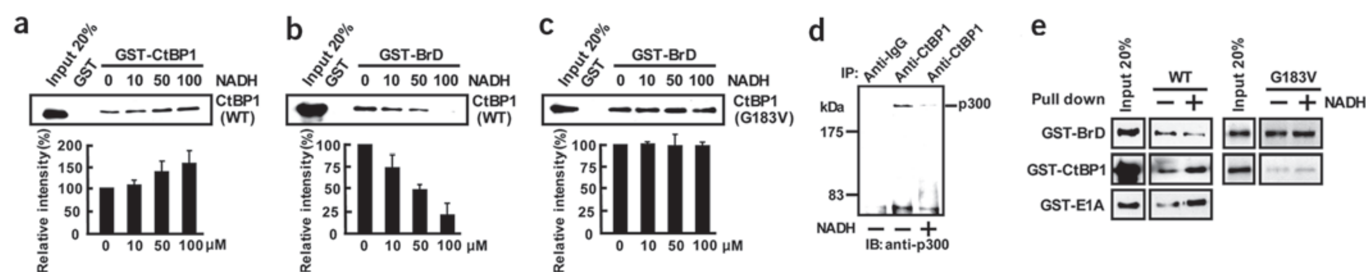

Figure 3 The effect of NADH on the interaction between CtBP1 and the p300-bromodomain. (**a**,**b**) A gradual increase in NADH augmented CtBP1 dimerization, and reduced bromodomain-CtBP1 interaction. (**c**) CtBP1 (G183V) constitutively binds to the bromodomain in an NADH-insensitive manner. Either GST-CtBP1 or the GST-bromodomain was precipitated from cell lysates containing either myc-CtBP1 (WT) or CtBP1 (G183V). (**d**) The abolishment of the endogenous CtBP1-p300 interaction in the presence of NADH. IB, immunoblot. CtBP1 was detected with anti-myc recognizing c-myc-CtBP1. (**e**) Bromodomain preferentially binds to CtBP1 in the absence of NADH, and to CtBP1 (G183V), an NADH-nonresponsive monomeric mutant. The GST-bromodomain was precipitated with bacterially purified His_6-CtBP1 (WT) or ^{35}S-labeled CtBP1 (G183V).

E1A interaction and CtBP1 dimerization are increased in an NADH-dependent manner (**Fig. 3a**,**e**), we examined the effect of NADH on the bromodomain-CtBP1 interaction by GSH-Sepharose pull-down assays of either mixtures of cell lysates overexpressing c-myc-CtBP1 or mixtures of recombinant His_6-CtBP1 proteins (**Fig. 3b**,**e**). In both cases, the CtBP1-bromodomain interaction became weaker in the presence of NADH. Moreover, the addition of NADH abolished endogenous interactions between CtBP1 and p300 (**Fig. 3d**). Notably, the bromodomain-CtBP1 interaction by NADH seems to occur in the opposite manner to the E1A-CtBP1 interaction by NADH.

It has been shown that the mutation of a glycine residue in the GXGXXG motif (G183V) in CtBP1 abolishes NADH responsiveness[2], thus we tested its effect on CtBP1-bromodomain interactions and on CtBP1 dimerization. Even though the CtBP1 (G183V) mutant substantially lost its ability to dimerize (**Fig. 3e**), it still interacted with the GST-bromodomain in the presence of up to 100 μM of NADH, at which level wild-type CtBP1 completely lost its ability to interact with the GST-bromodomain (**Fig. 3c**,**e**).

CtBP competes with histone for binding to the p300-bromodomain

Because the CtBP1-binding site overlaps with the acetylated histone-binding site within bromodomain of p300 (**Fig. 1b**), we examined whether Arg1098 and Leu1099 in the bromodomain are also critical for acetylated histone binding to the bromodomain by binding assays between purified histones and either the wild type or the (D1098A L1099A) mutant of the GST-bromodomain. We found that the mutation of aspartate and leucine residues in the bromodomain weakened the interaction between the bromodomain and acetylated histones (Ac-H3 and Ac-H4) (**Fig. 4a**). In fact, this mutant of p300 worked as a dominant-negative competitor against endogenous p300 for VP16-mediated activation, and was no longer able to activate transcription (**Supplementary Fig. 2** online), indicating that the PXDLS motif is critical for p300-mediated transactivation. As this mutation diminished the binding of the bromodomain to CtBP1 and to histones, we speculated that CtBP1 might compete against acetylated histones for binding to the p300-bromodomain. When purified recombinant His_6-CtBP1 was added to a mixture of purified histones containing recombinant GST-bromodomain, CtBP1 effectively blocked the interaction between the GST-bromodomain and acetylated histones (**Fig. 4b**). As the interaction between CtBP1 and bromodomain weakened in the presence of NADH (**Fig. 3b**,**e**), we investigated how CtBP1 influences the binding between bromodomain and histones in the presence of NADH. When the GST-bromodomain was incubated with purified histones in the absence of CtBP1, the GST-bromodomain interacted with histones in an

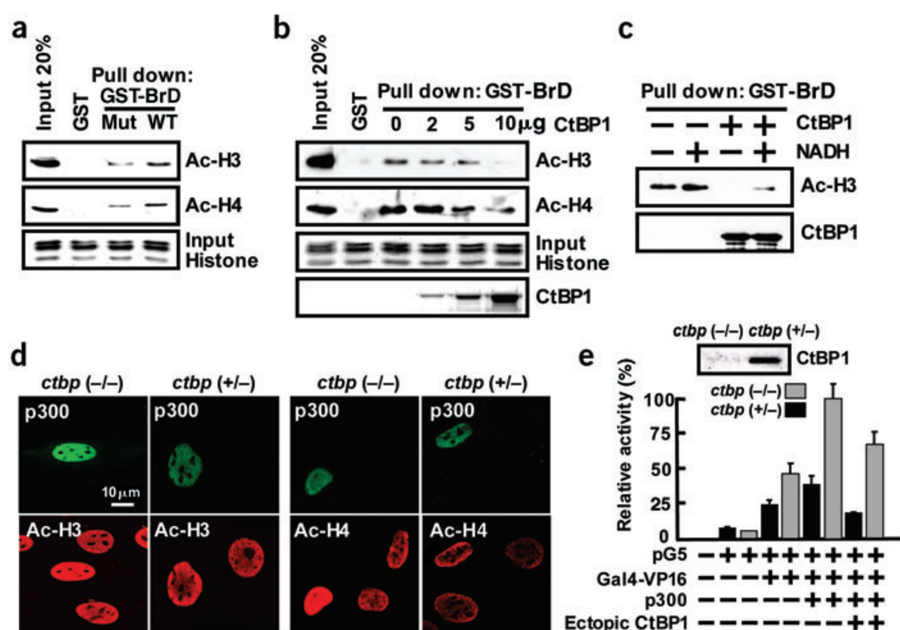

Figure 4 CtBP1 controls the p300-mediated acetylation of histones. (**a**) The PXDLS motif is critical for the binding of the bromodomain to acetylated histones. (**b**) CtBP1 competes against acetylated histones for binding to the bromodomain. (**c**) The addition of NADH restored the interaction between the bromodomain and acetylated histones, which was repressed by CtBP1. (**d**) The ectopic expression of p300 in *ctbp*(–/–) MEF cells upregulated histone acetylation; however, it increased histone acetylation only slightly in *ctbp*(+/–) cells. (**e**) p300-mediated transactivation in *ctbp*(–/–) was higher than in *ctbp*(+/–) cells. Both cells were transiently transfected with different combinations of Gal4-VP16 and/or p300 along with pG5(5×Gal4)-luciferase reporter gene.

© 2005 Nature Publishing Group http://www.nature.com/nsmb

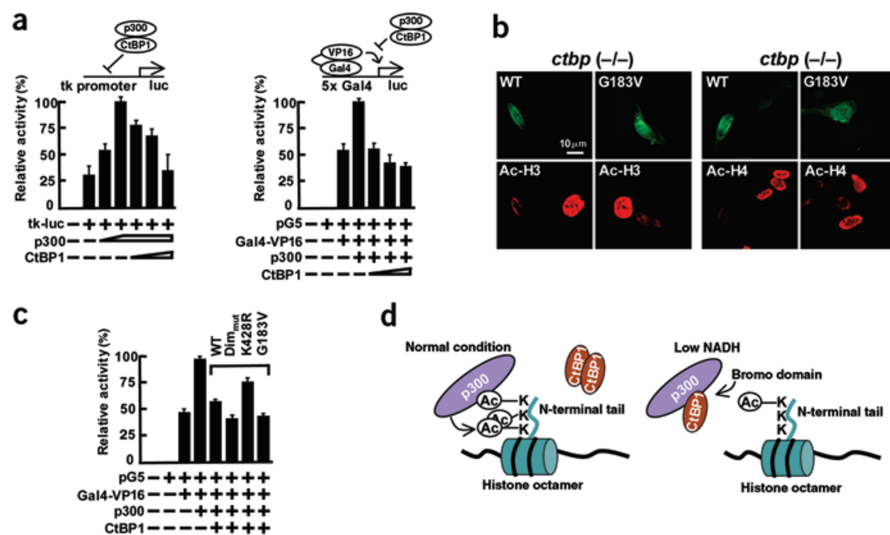

Figure 5 The repression of p300-mediated transactivation by CtBP1. (**a**) CtBP1 represses the p300-mediated transactivation in two different general transcription reporter systems (tk-driven and 5× Gal4–driven luciferase). (**b**) The overexpression of CtBP1 (WT) and CtBP1 (G183V) reduced the p300-mediated acetylation of histones (H3 and H4). (**c**) NADH-nonresponsive, monomeric mutant forms of CtBP1 (G183V) and CtBP1 (Dim_{mut}) were found to strongly inhibit p300-mediated transcriptional activity using Gal4-VP16 activating Gal4-luciferase reporter gene. The mutants of CtBP1 used were CtBP1 (Dim_{mut}), composed of mutations R141A, R142A, R163A and R171A to disrupt critical salt links and hydrogen bonding across the interface[2]; and CtBP1 (K428R); K428R disrupts the SUMOylation site of CtBP1 (refs. 14,26). (**d**) NADH-sensitive repression mode of CtBP1 to p300 in general transactivation.

© 2005 Nature Publishing Group http://www.nature.com/nsmb

NADH-independent manner. But when CtBP1 was added to these reaction mixtures in the absence of NADH, the GST-bromodomain lost its ability to bind to histones. Notably, the interaction between the GST-bromodomain and histones was recovered by adding NADH (**Fig. 4c**). Thus, we concluded that CtBP1 inhibits the physical accessibility of the p300-bromodomain to histones by direct binding to the bromodomain in an NADH-sensitive manner. These findings raised the possibility that CtBP1 might repress the p300-mediated *in vivo* acetylation of histones in chromatin. To address this possibility, we transiently transfected *ctbp*(–/–) and *ctbp*(+/–) MEF cells[25] with HA-tagged p300, and then investigated histone acetylation (**Fig. 4d**). Notably, p300-transfected *ctbp*(–/–) cells showed marked acetylation upregulation of H3 and H4 versus p300-untransfected *ctbp*(–/–) cells, whereas p300-transfected heterozygous *ctbp*(+/–) cells showed slight histone acetylation upregulation (**Fig. 4d**). We next tested the effect of endogenous CtBP1 on p300-mediated general transcriptional activity under Gal4-VP16-activating Gal4-luciferase reporter genes in both *ctbp*(–/–) and *ctbp*(+/–) MEF cells (**Fig. 4e**). As expected, p300-mediated transactivation in *ctbp*(–/–) was higher than that in *ctbp*(+/–), and the ectopic overexpression of CtBP1 in both cells repressed p300-mediated transactivation, supporting the notion that CtBP1 represses histone acetylation mediated by p300.

CtBP1 represses p300-mediated transactivation

We then examined whether CtBP1 represses p300-mediated transcription using two different reporter systems. An increase in the ectopic overexpression of CtBP1 decreased p300-mediated transcriptional activation (**Fig. 5a**). Also, wild-type CtBP1 markedly reduced histone acetylation when it CtBP1 was expressed in *ctbp*(–/–) cells (**Fig. 5b**). Moreover, as we found that CtBP1 (G183V) is an NADH-nonresponsive and monomeric mutant and constitutively binds to the bromodomain independent of NADH (**Fig. 3c**,**e**), we examined the effect of CtBP1 (G183V) on the p300-mediated histone acetylation and transcriptional activation. When *ctbp*(–/–) cells were transiently transfected with CtBP1 (G183V) mutant along with several CtBP1 mutants, CtBP1 (G183V) substantially inhibited, more so than wild-type CtBP1, p300-enhanced histone acetylation and transcriptional activation (**Fig. 5b**,**c**). To clarify the repressive effect of CtBP1 on the p300-mediated transcriptional activation, we used a CtBP1 (K428R) mutant that cannot be SUMOylated[14,26], and consequently cannot enter into nucleus nor interact with p300 *in vivo*. We found that this CtBP1 (K428R) mutant could not repress p300-mediated transcriptional activation, suggesting that direct interaction of CtBP1 with p300 is required for the CtBP1-mediated repression of p300-driven transcriptional activation (**Fig. 5c**). Notably, CtBP1 (Dim_{mut}), another mutant of CtBP1 that cannot dimerize[2], strongly inhibited p300-driven transcriptional activity, just like CtBP1 (G183V) (**Fig. 5c**). We also confirmed that this CtBP1 (Dim_{mut}) mutant and CtBP1 (G183V) bind to p300 more stably than wild-type CtBP1 (**Supplementary Fig. 3** online). Basically, these two mutants have a low repressive effect on E1A transcriptional activity because they cannot dimerize[2,5] (**Fig. 3e**). Nonetheless, the fact that they effectively inhibited p300-mediated transcriptional activation suggests that the NADH-dependent equilibrium of CtBP1 between dimer and monomer determines its binding partners. Thus, the monomeric form of CtBP1 preferentially binds to the bromodomain and inhibits p300-mediated histone acetylation at low NADH concentrations (**Fig. 5d**).

DISCUSSION

The histone acetyltransferase family of transcriptional coactivators is essentially required for gene activation by acetylating histones in the promoter regions of targeted genes. A member of this family, p300, is known to be brought into a specific promoter region by DNA-binding transcriptional activators and catalyzes histone acetylation in the promoter region[27]. In addition, p300 has an activator-independent binding ability to chromatin through its bromodomain[21]. In this study, we found that specific mutation of the PXDLS motif in the bromodomain of p300 results in a substantial reduction of transactivation, indicating that the association of bromodomain of p300 with chromatin is also critical for its transactivation (**Supplementary Fig. 2**).

We also found that CtBP1 specifically binds to bromodomain through the same core PXDLS motif that plays a pivotal role in recognizing acetylated histones, a process that ultimately leads to the repression of p300-mediated general transactivation. As the direct interaction between CtBP1 and p300 does not primarily require HDACs to repress p300-mediated transactivation, this interaction offers an explanation for the previous finding that CtBP1-mediated repression is independent of HDAC[11,12,18]. However, we cannot exclude the possibility that HDAC is also involved in CtBP-mediated repression, because the large CtBP1 complex does contain HDACs[17]. Thus, we surmise that two independent repression mechanisms synergistically affect CtBP1-mediated transcriptional repression.

In addition to p300, we observed that CtBP1 interacts with other histone acetyltransferases, including pCAF and CBP (**Fig. 1c**). In addition, we found that many bromodomain-containing proteins contain putative CtBP1-binding motifs, which strongly suggests that CtBP1 exerts its effect on a wider range of target proteins (**Supplementary Fig. 1**). In particular, each of the PXDL(Q/E) motifs was found in the double bromodomains of TAF1/TAF_{II}250 (refs. 20,22), suggesting that CtBP1 may also affect TAF1-mediated general transcription in an NADH or cellular energy–dependent fashion. Moreover, Sir2 is known to catalyze the deacetylation of histone in an NAD^+-dependent manner[28]; therefore, it seems likely that CtBP1, in cooperation with Sir2, serves as an energy sensor for regulating the general transcription on chromatin in a cellular energy–dependent manner. Recently, some NAD(P)H-related metabolic enzymes have been shown to represent a growing family of transcriptional regulators[29], therefore, studies of the NADH-mediated repression by CtBP1 should pave the way toward a better understanding of energy-dependent transcriptional regulation.

METHODS

Cell culture and transient transfections. HEK-293T cells and HeLa cells were grown in DMEM containing 10% (v/v) FBS and penicillin-streptomycin. Both *ctbp*(–/–) and *ctbp*(+/–) MEF cells were grown in DMEM containing 10% (v/v) FBS, penicillin-streptomycin and 2 mM L-glutamine (Life Technologies). Transfections were done using lipofectamine reagent (Invitrogen).

Plasmids. The full-length open reading frame of CtBP1 was obtained by PCR using pGEX-CtBP1 as a template and inserted into pcDNA3.1-myc expression vector (Invitrogen). For bacterially expressed His-tagged CtBP1, the PCR product of full-length CtBP1 was inserted into pRSET vector (Invitrogen). The mutants of CtBP1 (Dim_{mut}, K428R and G183V) were generated using the QuikChange site-directed mutagenesis kit (Stratagene). pGEX-bromodomain was generated by inserting PCR fragments spanning amino acids 1067–1139 of human p300 into pGEX-5X-1 (Amersham Pharmacia Biotech). pGEX-CtBP1 and pcDNA3-E1A were provided by G. Chinnadurai (Saint Louis University School of Medicine).

Protein purifications. GST-fusion proteins were expressed in the DH5α strain of *E. coli*, and $(His)_6$-tagged proteins were expressed in the BL21(DE3)pLysS strain of *E. coli*. In both cases, proteins were induced by adding IPTG (1 mM) for 4 h. GST-fusion proteins were purified using glutathione Sepharose (Amersham) with 10 mM of reduced glutathione. For the purification of $(His)_6$-tagged CtBP1 and wild-type or mutant p300-bromodomain, each cDNA was subcloned into the pRSET vector (Invitrogen). Bacterial lysates were eluted through TALON metal affinity resin (Clontech) and washed with 100 mM imidazole. Recombinant proteins were eluted with a gradual increase of imidazole concentration from 150 to 250 mM. Fractions containing proteins were concentrated and further purified using a Superdex-75 10/300 GL prepacked column (Amersham) at a flow rate of 0.5 ml min^{-1}. Molecular masses of proteins were calibrated using an LMW gel filtration calibration kit (Amersham).

Fluorescence spectroscopy. To monitor the protein folding of recombinant p300-bromodomains, purified proteins (1.25 μM) were excited at 295 nm and emission spectra were scanned from 300 to 500 nm using an Aminco Bowman Series2 Luminosense Spectrometer.

Purification of the acetylated free histones. HeLa cells were treated with 10 mM sodium butyrate for 24 h, and harvested in PBS containing 10 mM sodium butyrate. Cells were lysed with lysis buffer (10 mM Tris-Cl, pH 7.4, 1% (v/v) Nonidet P-40, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM sodium butyrate, 1 mM phenylmethyl fluoride) and centrifuged at 500*g* for 5 min. Pellets were then washed twice with lysis buffer and once with lysis buffer containing 100 mM NaCl. Pellets were resuspended in washing buffer (10 mM Tris-Cl, pH 7.4, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM sodium butyrate, 1 mM phenylmethyl fluoride, 400 mM NaCl) and centrifuged at 15,000*g* for 5 min. Nuclear pellets were extracted twice with ten pellet volumes of 0.2 M H_2SO_4 on ice for 30 min. After centrifugation at 15,000*g* for 25 min, the supernatants were dialyzed extensively with 100 mM acetic acid at 4 °C overnight.

GST-pull down assay. Transiently transfected HEK-293T cells were lysed with lysis buffer (20 mM Tris-Cl, pH 7.4, 0.5% (v/v) Nonidet P-40, 150 mM NaCl, 1 mM phenylmethyl fluoride, and protease inhibitors (Roche)). Cell lysates were first precleared with Sepharose bead and then incubated with GST-fusion proteins for 4 h at 4 °C in the presence of BSA-precoated glutathione Sepharose (Amersham). Precipitated proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with appropriate antibodies. Proteins were visualized by chemiluminescence (Pierce).

Immunoprecipitation assay. For immunoprecipitation experiments, tagged proteins were immunoprecipitated from cell lysates using appropriate antibodies and protein A/G PLUS-agarose (Santa Cruz Biotechnology) for 4 h at 4 °C. Beads were collected and washed (three times for 10 min). Precipitated proteins were resolved by SDS-PAGE and analyzed by western blotting. For the endogenous immunoprecipitation of p300 with anti-CtBP1, nuclear extracts from HeLa cells were precleared with 100 μl of protein A/G-agarose for 1 h. These precleared nuclear extracts were then mixed with polyclonal anti-CtBP1 (Santa Cruz Biotechnology) in the presence of 50 μl of BSA-precoated protein A/G-agarose, and incubated overnight at 4 °C. Immunoprecipitates were then probed with polyclonal anti-p300 (Santa Cruz Biotechnology).

Immunofluorescent staining. Both *ctbp*(–/–) and *ctbp*(+/–) MEF cells were grown on coverslips. After 24 h of transfection, cells were fixed with 4% (w/v) paraformaldehyde, and blocked with 2% (w/v) BSA in PBS. The cells were then stained with anti-HA, monoclonal anti-myc (Covance) and polyclonal anti-Ac-H3 and H4 (Upstate Biotechnology); fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody and rhodamine red-X-conjugated anti-rabbit antibody (Jackson Immunoresearch) were then added and immunofluoresence was observed under a Zeiss LSM 510 laser scanning microscope.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

We thank J.O. Liu (Johns Hopkins School of Medicine) for critical reading of the manuscript; G. Chinnadurai (Saint Louis University) for pGEX-CtBP1 and pcDNA3-E1A plasmids; and J.D. Hildebrand (University of Pittsburgh) for *ctbp*(–/–) and *ctbp*(+/–) MEF cells. This work was supported by Research for Pure Basic Science, Korea Research Foundation (2002-KRF-C00070, to H.-D.Y.), and in part by Korea Science & Engineering Foundation (KOSEF) through the Center for Aging and Apoptosis Research at Seoul National University.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 27 September 2004; accepted 14 March 2005
Published online at http://www.nature.com/nsmb/

1. Schaeper, U. *et al.* Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation. *Proc. Natl. Acad. Sci. USA* **92**, 10467–10471 (1995).
2. Kumar, V. *et al.* Transcription corepressor CtBP is NAD^+-regulated dehydrogenase. *Mol. Cell* **10**, 857–869 (2002).
3. Chinnadurai, G. CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Mol. Cell* **9**, 213–224 (2002).
4. Marmorstein, R. Dehydrogenases, NAD, and transcription—what's the connection? *Structure* **10**, 1465–1466 (2002).
5. Zhang, Q, Piston, D.W. & Goodman, R.H. Regulation of corepressor function by nuclear NADH. *Science* **295**, 1895–1897 (2002).
6. Fjeld, C.C., Birdsong, W.T., & Goodman, R.H. Differential binding of NAD^+ and NADH allows the transcriptional corepressor carboxy-terminal binding protein to serve as a metabolic sensor. *Proc. Natl. Acad. Sci. USA* **100**, 9202–9207 (2003).
7. Mannervik, M., Nibu, Y., Zhang, H. & Levine, M. Transcriptional coregulators in development. *Science* **284**, 606–609 (1999).
8. Turner, J. & Crossley, M. The CtBP family: enigmatic and enzymatic transcriptional co-repressors. *Bioessays* **23**, 683–690 (2001).
9. Sewalt, R.G.A.B., Gunster, M.J., Van der Vlag, J., Satjin, D.P.E. & Otte, A.P. C-terminal binding protein is a transcriptional repressor that interacts with a specific class of vertebrate polycomb proteins. *Mol. Cell. Biol.* **19**, 777–787 (1999).
10. Yu, X. & Baer, R. Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. *J. Biol. Chem.* **275**, 18541–18549 (2000).

© 2005 Nature Publishing Group http://www.nature.com/nsmb

11. Meloni, A.R., Smith, E.J. & Nevins, J.R. A mechanism for Rb/p130-mediated transcription repression involving recruitment of the CtBP corepressor. *Proc. Natl. Acad. Sci. USA* **96**, 9574–9579 (1999).
12. Koipally, J. & Georgopoulos, K. Ikaros interactions with CtBP reveal a repression mechanism that is independent of histone deacetylase activity. *J. Biol. Chem.* **275**, 19594–19602 (2000).
13. Levine, S.S. *et al.* The core of the polycomb repressive complex is compositionally and functionally conserved in flies and humans. *Mol. Cell. Biol.* **22**, 6070–6078 (2002).
14. Kagey, M.H., Melhuish, T.A. & Wotton, D. The polycomb protein Pc2 is a SUMO E3. *Cell* **113**, 127–137 (2003).
15. Sundqvist, A., Sollerbrant, K. & Svensson, C. The carboxy-terminal region of adenovirus E1A activates transcription through targeting of a C-terminal binding protein-histone deacetylase complex. *FEBS Lett.* **429**, 183–188 (1998).
16. Subramanian, T. & Chinnadurai, G. Association of class I histone deacetylases with transcriptional corepressor CtBP. *FEBS Lett.* **540**, 255–258 (2003).
17. Shi, Y. *et al.* Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* **422**, 735–738 (2003).
18. Dahiya, A. *et al.* Linking the Rb and polycomb pathways. *Mol. Cell* **8**, 557–568 (2001).
19. Dhalluin, C. *et al.* Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491–496 (1999).
20. Jacobson, R.H., Ladurner, A.G., King, D.S. & Tjian, R. Structure and function of a human TAF_{II}250 double bromodomain module. *Science* **288**, 1422–1425 (2000).
21. Manning, E.T. *et al.* p300 forms a stable, template-committed complex with chromatin: role for the bromodomain. *Mol. Cell. Biol.* **21**, 3876–3887 (2001).
22. Matangkasombut, O. & Buratowski, S. Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. *Mol. Cell* **11**, 353–363 (2003).
23. Kanno, T. *et al.* Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. *Mol. Cell* **13**, 33–43 (2004).
24. Remboutsika, E., Yamamoto, K., Harbers, M. & Schmutz, M. The bromodomain mediates transcriptional intermediary factor 1α-nucleosome interactions. *J. Biol. Chem.* **277**, 50318–50325 (2002).
25. Hildebrand, J.D. & Soriano, P. Overlapping and unique roles for C-terminal binding protein 1 (CtBP1) and CtBP2 during mouse development. *Mol. Cell. Biol.* **22**, 5296–5307 (2002).
26. Lin, X. *et al.* Opposed regulation of corepressor CtBP by SUMOylation and PDZ binding. *Mol Cell* **11**, 1389–1396 (2003).
27. Kundu, T.K. *et al.* Activator-dependent transcription from chromatin in vitro involving targeted histone acetylation by p300. *Mol. Cell* **6**, 551–561 (2000).
28. Blander, G. & Guarente, L. The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.* **73**, 417–435 (2004).
29. McKnight, S. Gene switching by metabolic enzymes – How did you get on the invitation list? *Cell* **114**, 150–152 (2003).

© 2005 Nature Publishing Group http://www.nature.com/nsmb

npg