CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector
Current Biology, Vol. 15, 1434–1438, August 9, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.06.057

The PHD Finger/Bromodomain of NoRC Interacts with Acetylated Histone H4K16 and Is Sufficient for rDNA Silencing

Yonggang Zhou and Ingrid Grummt*
Division of Molecular Biology of the Cell II
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
D-69120 Heidelberg, Germany

Summary

The SNF2h-containing chromatin-remodeling complex NoRC is responsible for silencing a fraction of mammalian rRNA genes (rDNA). NoRC silences transcription by establishing heterochromatic features—including DNA methylation, hypoacetylation of histone H4, and methylation of H3K9—at the rDNA promoter [1, 2]. We have investigated the mechanism of NoRC-mediated rDNA silencing and show that binding of the bromodomain of TIP5, the large subunit of NoRC, to acetylated nucleosomes is a prerequisite for NoRC function. A point mutation within the bromodomain impairs the association of NoRC with chromatin, prevents heterochromatin formation, and abolishes transcriptional repression. Moreover, the association of NoRC with chromatin requires acetylation of histone H4 at lysine 16 (acH4K16), and binding to acH4K16 is required for subsequent deacetylation of H4K5, H4K8, and H4K12, indicating that acetylation of H4K16 plays an active role in NoRC-mediated heterochromatin formation. The bromodomain cooperates with an adjacent PHD finger to recruit HDAC1, DNMT1, DNMT3, and SNF2h to rDNA. If specifically targeted to the rDNA promoter, the PHD finger/bromodomain is capable of establishing heterochromatic features and rDNA silencing. Thus, the PHD finger/bromodomain represents an autonomous unit that binds to acH4K16 and coordinates the chain of events that establish the repressed state of rDNA.

Results and Discussion

NoRC Function Requires Binding of TIP5 to Acetylated Histone H4

NoRC, which is the key determinant in epigenetic memory and maintains rDNA arrays in a “closed” chromatin configuration [1–3], consists of two subunits, TIP5 (*T*TF-I *i*nteracting *p*rotein *5*) and the ATPase SNF2h [4]. The C-terminal part of TIP5 contains a bromodomain, a sequence element that is found in many chromatin-associated proteins and has been shown to bind to acetylated histone tails [5–9]. Mutation of a conserved tyrosine residue in the bromodomain of yeast Bdf1 impairs the interaction of Bdf1 with acetylated histones [6]. To determine the role of the bromodomain in NoRC function, we assayed silencing of a Pol I reporter plasmid after overexpression of wild-type TIP5 and TIP5/Y1775F, a mutant in which the conserved tyrosine in the bromodomain has been replaced by phenylalanine. TIP5, but not TIP5/Y1775F, repressed Pol I transcription (Figure 1A), indicating that the interaction of the bromodomain with acetylated histone tails is a key event in NoRC-mediated rDNA silencing. Chromatin immunoprecipitation (ChIP) experiments revealed that wild-type TIP5 has occupied the rDNA promoter and triggered histone H4 hypoacetylation and H3K9 dimethylation, whereas TIP5/Y1775F did not associate with rDNA and did not promote modification of histones (Figure 1B, lanes 1–9). Likewise, wild-type but not mutant TIP5 recruited HDAC1, DNMT1, and SNF2h to rDNA (lanes 10–15). Finally, overexpression of TIP5 triggered de novo methylation of the Pol I reporter plasmid, whereas TIP5/Y1775F did not (Figure 1C). Thus, the integrity of the bromodomain is essential for anchoring NoRC to chromatin, for heterochromatin formation, and for transcriptional silencing.

*Correspondence: i.grummt@dkfz-heidelberg.de

The Bromodomain of TIP5 Interacts with Acetylated H4K16

NoRC-mediated nucleosome remodeling and transcriptional silencing requires the tail of histone H4 [4, 10]. This, together with our observation that TIP5/Y1775F binding to chromatin was severely impaired, suggested that the bromodomain recognizes specific lysine residues at the tail of histone H4. To test this, we used immobilized GST-TIP5/BD, a fusion protein containing the bromodomain of wild-type TIP5, to capture histones (Figure 2A). Whereas GST-TIP5/BD bound to all four forms of acetylated histone H4, no binding was observed with GST alone and GST-TIP5/BD^{Y1775F}. Immunoblots of the different histone H4 isoforms with residue-specific antibodies revealed that all captured histone H4 moieties were acetylated at K16 (Figure 2B). Di-, tri-, and tetra-acetylated histone H4 was modified at K16/12, K16/12/8, and K16/12/8/5, respectively. This indicates that retention of NoRC on chromatin requires the interaction of the bromodomain of TIP5 with acetylated histone H4K16 and supports the hypothesis of a “zip” model whereby acetylation of histone H4 starts at K16 and continues in the N-terminal direction until all four sites are acetylated [11].

To further demonstrate acetylation-specific binding, we incubated [^{35}S]-labeled TIP5 with immobilized H4 peptides that were either unmodified or acetylated at K12 or K16. Clearly, the acH4K16 peptide, but not the unmodified acH4K12 peptide, retained TIP5 (Figure 2C). Given that acetylation of H4K16 is required for NoRC binding to chromatin, one would expect that NoRC induces hypoacetylation of histone H4 at lysines 5, 8, and 12, whereas lysine 16 should remain acetylated. Indeed, ChIP assays with antibodies against acetylated isoforms of histone H4 demonstrate that overexpression of TIP5 leads to deacetylation of H4K5, H4K8, and H4K12 at the rDNA promoter, whereas no deacetylation of H4K16 was observed (Figure 2D). Thus, NoRC-mediated hypoacetylation of histone H4 is restricted to H4K5/K8/K12.

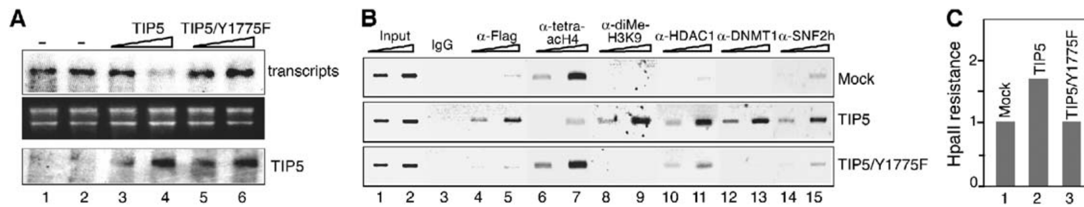

Figure 1. Binding of the Bromodomain to Acetylated Histones Is Required for NoRC Function

(A) Overexpression of TIP5/Y1775F does not repress Pol I transcription. NIH3T3 cells were cotransfected with 2.5 μg pMr1930-BH and 4 or 8 μg pcDNA-Flag-TIP5 or pcDNA-Flag-TIP5/Y1775F, and transcripts from the reporter plasmid were monitored on Northern blots. A Western blot showing similar expression levels of wild-type and mutant TIP5 is shown below.

(B) A point mutation in the bromodomain impairs NoRC association with rDNA, histone modification, and recruitment of chromatin-modifying enzymes. Crosslinked chromatin from NIH3T3 cells that were cotransfected with the reporter pMr1930-BH and pcDNA-Flag-TIP5 or pcDNA-Flag-TIP5/Y1775F was precipitated with the indicated antibodies, and coprecipitated reporter plasmid was analyzed by PCR. One percent and three percent of input DNA and 5% and 15% of coprecipitated DNA were amplified.

(C) TIP5/Y1775F fails to mediate de novo methylation of rDNA. NIH3T3 cells were cotransfected with 1 μg reporter plasmid pMr131 and 4 μg pcDNA-Flag-TIP5 or pcDNA-Flag-TIP5/Y1775F. Purified DNA was digested with HpaII or MspI and analyzed by real-time PCR with primers that map upstream or downstream of the HpaII site at −143. Data represent the difference of HpaII resistance in cells overexpressing TIP5 compared to mock-transfected cells from two independent experiments.

The PHD Finger/Bromodomain Is an Autonomous Unit that Mediates rDNA Silencing

Tandem PHD finger/bromodomains have been shown to serve as an interface that targets chromatin modifiers to gene promoters [12–14]. In support of this, we have previously shown that deletion of the PHD finger/bromodomain of TIP5 abolished NoRC-mediated transcriptional silencing and heterochromatin formation [2], demonstrating that an intact PHD finger/bromodomain is indispensable for rDNA silencing. To examine whether the PHD finger/bromodomain on its own was capable of repressing Pol I transcription, we assayed two chimeric proteins: TIP5/PB containing TIP5 amino acids (aa) 1579–1850 fused to a hemagglutinin (HA) tag, and TTF-TIP5/PB, in which the C terminus of TIP5 was fused to the DNA binding domain (DBD) of TTF-I. Because NoRC is recruited to rDNA by the interaction of TIP5 (aa 510–732) with TTF-I bound to the promoter-proximal terminator T_0 [4, 15], fusion with the DBD of TTF-I should target the PHD finger/bromodomain to rDNA. We verified the validity of the targeting approach by monitoring the nucleolar localization of GFP-tagged TTF-TIP5/PB (Figure 3B). Measurement of rDNA occupancy by ChIP revealed that TTF-TIP5/PB, the protein containing the DBD of TTF-I, bound to the Pol I promoter whereas TIP5/PB did not (Figure 3C). Next, we examined whether the PHD finger/bromodomain on its own was capable of silencing Pol I transcription if targeted to rDNA by TTF-DBD. Indeed, TTF-TIP5/PB repressed transcription in a dose-dependent manner,

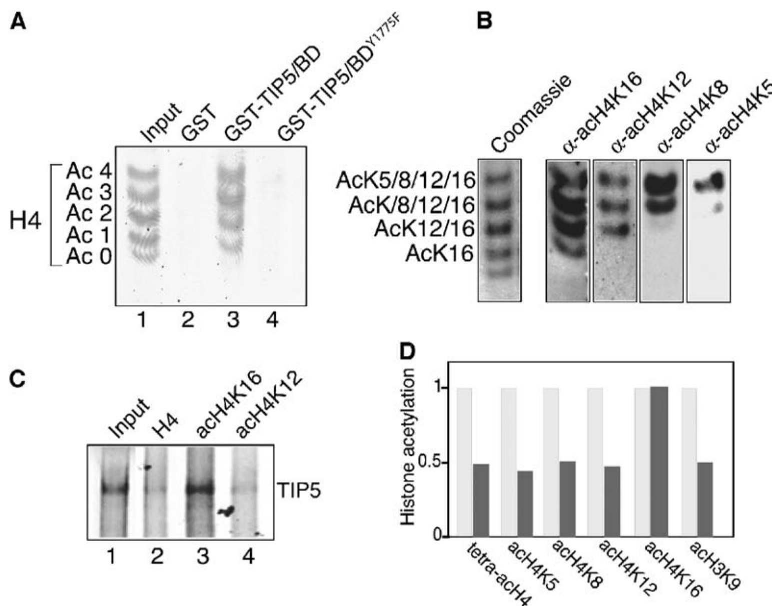

Figure 2. The Bromodomain of TIP5 Interacts with Acetylated H4K16

(A) TIP5 bromodomain binds to mono-, di-, tri-, and tetra-acetylated histone H4. Immobilized GST, GST-TIP5/BD, and GST-TIP5/BDY1775F were incubated with histones purified from butyrate-treated cells. Bound histones were analyzed by electrophoresis on triton-acetic acid-urea (TAU) gels. The area of the gel containing histone H4 is shown.

(B) Western blot of captured histone H4 moieties. Histones bound to GST-TIP5/BD were resolved on TAU gels, and histone H4 isoforms acetylated at specific lysines were identified on immunoblots with antibodies specific for acH4K16, acH4K12, acH4K8, and acH4K5. Input histone H4 stained with Coomassie brilliant blue is shown at the left.

(C) Peptide binding assay. In vitro-translated full-length TIP5 was incubated with immobilized peptides (aa 1–24 of histone H4) that were unmodified or acetylated at K16 or K12 as indicated. Ten percent of input peptides is shown on lane 1.

(D) TIP5 triggers lysine-specific deacetylation of histone H4. Crosslinked chromatin from NIH3T3 cells overexpressing Flag-TIP5 was precipitated with antibodies against tetra-acetylated histone H4, acH4K5, acH4K8, acH4K12, acH4K16, and acH3K9, and coprecipitated rDNA was analyzed by real-time PCR. The bar diagram shows the average change of histone acetylation in cells overexpressing TIP5 (dark bars) compared to mock-transfected cells (light bars) observed in two independent experiments.

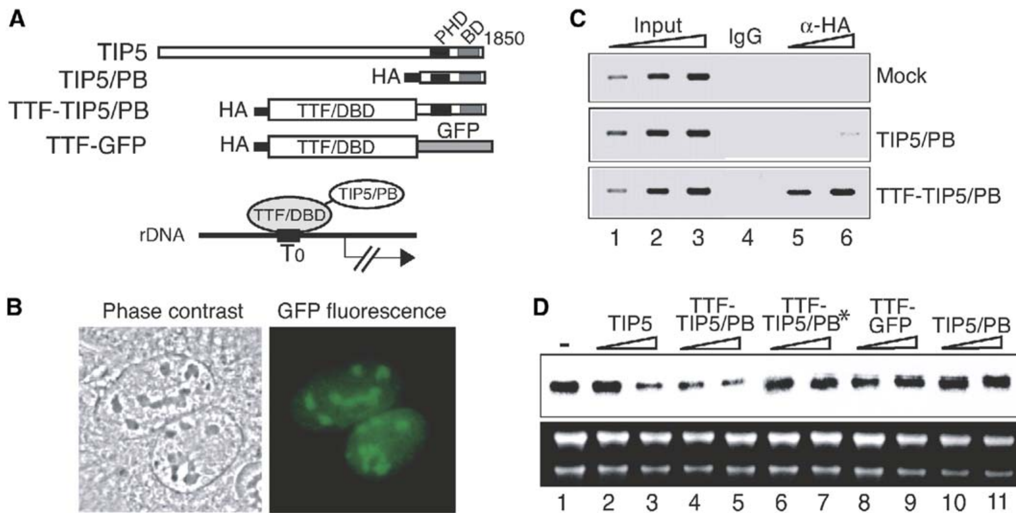

Figure 3. The PHD Finger/Bromodomain Is an Autonomous Unit that Mediates rDNA Silencing

(A) Diagram depicting the structure of recombinant proteins encoded by the respective expression vectors. The PHD finger (PHD), the bromodomain (BD) and the hemagglutinin tag (HA) are marked. The open box represents sequences harboring the DNA binding domain (DBD) of TTF-I. The scheme below illustrates that binding of TTF-TIP5/PB to the promoter-proximal terminator T_0 ultimately results in transcriptional repression.

(B) GFP-tagged TTF-TIP5/PB localizes within the nucleolus. Images of U2OS expressing GFP-TTF-TIP5/PB are shown.

(C) Fusion to the DNA binding domain (DBD) of TTF-I targets TIP5 to rDNA. Soluble chromatin from HEK293T cells overexpressing HA-tagged TIP5/PB or TTF-TIP5/PB was precipitated with anti-HA antibody, and association with the rDNA promoter was monitored by semiquantitative PCR. In lanes 1–3, 0.5%, 1%, and 3% of input DNA was amplified.

(D) Targeting the PHD finger/bromodomain of TIP5 to rDNA represses Pol I transcription. NIH3T3 cells were cotransfected with 2.5 μg pMr1930-BH and increasing amounts of the indicated expression vectors. TTF-TIP5/PB* represents TTF-TIP5/PBY1775F. Transcripts from the reporter plasmid were monitored on Northern blots.

whereas the point mutation within the bromodomain (TTF-TIP5/PB^{Y1775F}) had no effect (Figure 3D). TIP5/PB, the construct lacking the DBD of TTF-I, and TTF-GFP did not affect Pol I transcription either. These results demonstrate that the tandem PHD finger/bromodomain forms a cooperative unit that is capable of repressing Pol I transcription if specifically tethered to rDNA.

The PHD Finger/Bromodomain of TIP5 Interacts with HDAC1, DNMT1, DNMT3b, and SNF2h and Triggers Histone Deacetylation and rDNA Methylation

Repression of Pol I transcription by TTF-TIP5/PB was relieved by trichostatin A (TSA) or aza-deoxycytidine (aza-dC), inhibitors of histone deacetylases and DNA methyltransferases (Figure 4A). This indicates that the PHD finger/bromodomain provides an interface for specific protein-protein interactions that trigger histone deacetylation, DNA methylation, and chromatin remodeling, thereby establishing the repressed state of rDNA. In support of this, acetylation of histone H4 at the rDNA promoter was strongly decreased in cells overexpressing TTF-TIP5/PB (Figure 4B). It is noteworthy that TTF-TIP5/PB did not trigger hypoacetylation of histone H4 at a reporter plasmid that lacks the TTF-I binding site T_0 (Figure 4C). This is consistent with previous studies demonstrating that recruitment of NoRC to rDNA and transcriptional silencing depends on TTF-I bound to the promoter-proximal terminator T_0 [10, 16]. Finally, a significant fraction of rDNA from cells overexpressing TTF-TIP5/PB, but not TTF-GFP, became resistant to HpaII digestion, indicating that the C-terminal part of TIP5 on its own is sufficient to trigger rDNA methylation (Figure 4D). This demonstrates that the bipartite PHD finger/bromodomain of TIP5 alone is capable of establishing heterochromatic features, provided that it is specifically anchored to its target gene.

To test whether the PHD finger/bromodomain of TIP5 is sufficient for recruiting chromatin-modifying enzymes, we incubated GST-TIP5 fusion proteins with nuclear extract and analyzed captured proteins on Western blots. GST-TIP5/PB, the protein harboring both the PHD finger and bromodomain, efficiently interacted with HDAC1, DNMT1, and DNMT3b, whereas the PHD finger (GST-TIP5/PHD) or the bromodomain (GST-TIP5/BD) alone did not pull down HDAC1 and DNMTs (Figure 4E).

Given that NoRC-mediated silencing requires the ATPase activity of SNF2h [16] and the C-terminal part of TIP5 is sufficient to repress Pol I transcription, SNF2h should interact with the PHD finger/bromodomain, too. Indeed, coimmunoprecipitation experiments with wild-type and mutant forms of TIP5 revealed that full-length TIP5 and TIP5ΔBD efficiently interact with SNF2h, whereas TIP5ΔPHD and TIP5ΔC failed to associate with SNF2h (Figure 4F). This demonstrates that the PHD finger mediates the interaction of TIP5 with SNF2h. With regard to SNF2h, we found that the SANT domain of SNF2h interacts with TIP5 (Figure S1A in the Supplemental Data available with this article online), and TIP5/PB binds to SNF2h with similar affinity as full-length TIP5 (Figure S1B). Together, our results demonstrate

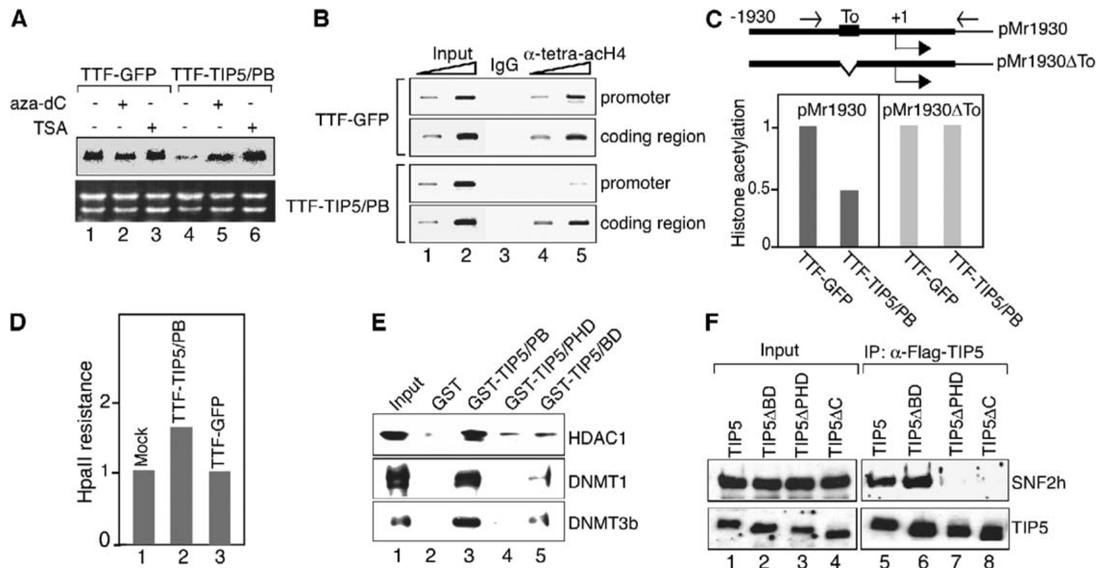

Figure 4. Targeted Recruitment of the PHD Finger/Bromodomain Triggers Histone Deacetylation and rDNA Methylation.

(A) TSA and aza-dC counteract repression of Pol I transcription. NIH3T3 cells were cotransfected with pMr1930-BH and either TTF-TIP5/PB or TTF-GFP, and transcripts were monitored on Northern blots. Where indicated, TSA (33 nM) and aza-dC (10 nM) were added 24 hr after transfection.

(B) Overexpression of TTF-TIP5/PB triggers hypoacetylation of histone H4. NIH3T3 cells were cotransfected with the reporter plasmid pMr1930-BH and TTF-TIP5/PB or TTF-GFP. Crosslinked chromatin was precipitated with anti-tetra-acH4 antibodies and coprecipitated rDNA (promoter and endogenous 28S RNA) were amplified by PCR.

(C) Binding of the PHD finger/bromodomain to the promoter-proximal terminator T_0 is required for histone H4 deacetylation. Cells were cotransfected with pMr1930 or pMr1930ΔT_0 and with TTF-TIP5/PB or TTF-GFP as indicated. The association of tetra-acetylated histone H4 with the reporter plasmids was examined by ChIP and quantified by real-time PCR. The bars represent the relative level of histone acetylation at the reporter plasmids after overexpression of TTF-TIP5/PB and TTF-GFP, respectively. A scheme of the reporter plasmids and primers used is shown above.

(D) Targeting the C terminus of TIP5 to rDNA mediates methylation of the rDNA promoter. NIH3T3 cells overexpressing TTF-TIP5/PB and TTF-GFP were analyzed for de novo rDNA methylation by resistance to HpaII digestion.

(E) The PHD finger/bromodomain of TIP5 interacts with HDAC1, DNMT1, and DNMT3b. Ten micrograms of bead-bound GST, GST-TIP5/PB, GST-TIP5/PHD, and GST-TIP5/BD was incubated with 500 μg nuclear extract from cells overexpressing HDAC1, DNMT1, or DNMT3b, and associated proteins were visualized on Western blots. Ten percent of input is shown in lane 1.

(F) The PHD finger of TIP5 mediates the interaction with SNF2h. Lysates from HEK293T cells overexpressing Flag-tagged TIP5 were incubated with anti-Flag antibodies and co-precipitated SNF2h was visualized on Western blots. Ten percent of input is shown in lanes 1–4.

that all important enzymatic activities that mediate rDNA silencing (e.g., histone hypoacetylation, DNA methylation, and ATP-dependent nucleosome remodeling) are recruited by the bipartite PHD finger/bromodomain of TIP5.

Experimental Procedures

Plasmids

pcDNA-TIP5 and pcDNA-TIP5ΔC1509 have been described [2, 4]. C-terminal fragments of TIP5 (aa 1579–1850, 1579–1682, or 1728–1850) were inserted into pGEX to yield pGST-TIP5/PB, pGST-TIP5/PHD, and pGST-TIP5/BD. Point mutations in the bromodomain of TIP5 (Y1775F) and ATPase domain of SNF2h (K211R) were generated with Quick Change PCR mutagenesis strategies (Stratagene). TIP5 mutants lacking the bromodomain (TIP5ΔBD) or the PHD finger (TIP5ΔPHD) were constructed by polymerase chain reaction (PCR). pGST-SNF2-SANT contains SNF2h aa 837–989 inserted into pGEX. A fragment encoding the C-terminal part of TIP5 (aa 1579–1850) was inserted into pcDNA-TTFΔN306 [17] to generate pcDNA-TTF-TIP5/PB. The reporter plasmid pMr1930-BH contains 5′-terminal mouse rDNA sequences from −1930 to +292 fused to a 3′-terminal rDNA fragment [2]. In pMr1930ΔT_0, the upstream terminator T_0 has been deleted. pMr131 contains mouse rDNA sequences from −167 to +131.

Transient Transfections and RNA Analysis

5×10^5 NIH3T3 cells were cotransfected with 2.5 μg pMr1930-BH and different amounts of expression vectors encoding Flag-tagged wild-type or mutant TIP5. After 48 hr, transcripts were isolated and analyzed on Northern blots by hybridization to a ^{32}P-labeled riboprobe that is complementary to pUC9 sequences that have been inserted between the 5′- and 3′-terminal rDNA fragments in pMr1930-BH.

Chromatin Immunoprecipitations

Antibodies were incubated with crosslinked chromatin overnight at 4°C and collected with protein A/G agarose beads for 2 hr. After reversal of the crosslink and digestion with proteinase K, DNA was extracted with phenol-chloroform and amplified by PCR [18]. PCR products were visualized on agarose gels or subjected to real-time PCR. The enrichment of immunoprecipitated DNA was quantified by comparison to input DNA.

Protein-Protein Interaction Assays

Glutathione-Sepharose containing GST-fusion proteins was incubated with fractionated nuclear extract (DEAE-280 fraction), and associated proteins were detected as described [2]. For coimmunoprecipitation assays, lysates from HEK293T cells overexpressing the respective proteins were incubated for 4 hr at 4°C with anti-Flag antibodies (M2, Sigma). After washing with binding buffer (20 mM Tris-HCl [pH 7.8]; 200 mM NaCl; 1 mM EDTA; 1 mM EGTA; 1%

Triton X-100), precipitated proteins were analyzed on Western blots.

Histone and Peptide Binding Assays

One hundred micrograms of histones from HEK293T cells that were cultured for 24 hr in the presence of 10 mM sodium butyrate were incubated with 10 μg of immobilized GST-TIP5/BD or GST-TIP5/BD^{Y1775F} for 4 hr at 4°C in 500 μl binding buffer. After washing with 200 and 800 mM NaCl, captured proteins were analyzed on triton-acetic acid-urea (TAU) gels [6, 7]. For peptide binding assays, 10 μg of immobilized histone H4 peptides (aa 1–24) were incubated for 90 min with in vitro-translated TIP5 and washed with binding buffer containing 500 mM NaCl, and bound proteins were analyzed by 6% SDS-PAGE and autoradiography [19].

Antibodies

Antibodies against TIP5 (α-mTIP5N1-18) have been described [4]. Anti-Flag antibodies (M2) were from Sigma and antibodies to modified histones from either Upstate Biotechnology (α-tetra-acH4, α-acH4K12, α-acH4K16, α-acH3K9, α-diMeH3K9) or Abcam (α-acH4K5, α-acH4K8).

Supplemental Data

Supplemental Data include one figure and are available with this article online at: http://www.current-biology.com/cgi/content/full/15/15/1434/DC1/.

Acknowledgments

We thank Asifa Akhtar for histone H4 peptides. This work was supported by the Deutsche Forschungsgemeinschaft (SFB/Transregio 5, SP "Epigenetics"), the EU-Network "Epigenome," and the Fonds der Chemischen Industrie.

Received: March 31, 2005
Revised: June 9, 2005
Accepted: June 27, 2005
Published: August 9, 2005

References

1. Santoro, R., Li, J., and Grummt, I. (2002). The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat. Genet. *32*, 393–396.
2. Zhou, Y., Santoro, R., and Grummt, I. (2002). The chromatin remodeling complex NoRC targets HDAC1 to the ribosomal gene promoter and represses RNA polymerase I transcription. EMBO J. *21*, 4632–4640.
3. Li, J., Santoro, R., Koberna, K., and Grummt, I. (2005). The chromatin remodeling complex NoRC controls replication timing of rRNA genes. EMBO J. *24*, 120–127.
4. Strohner, R., Nemeth, A., Jansa, P., Hofmann-Rohrer, U., Santoro, R., Längst, G., and Grummt, I. (2001). NoRC—a novel member of mammalian ISWI-containing chromatin remodeling machines. EMBO J. *20*, 4892–4900.
5. Ornaghi, P., Ballario, P., Lena, A.M., Gonzalez, A., and Filetici, P. (1999). The bromodomain of Gcn5p interacts in vitro with specific residues in the N terminus of histone H4. J. Mol. Biol. *287*, 1–7.
6. Ladurner, A.G., Inouye, C., Jain, R., and Tjian, R. (2003). Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. Mol. Cell *11*, 365–376.
7. Matangkasombut, O., and Buratowski, S. (2003). Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. Mol. Cell *11*, 353–363.
8. Kanno, T., Kanno, Y., Siegel, R.M., Jang, M.K., Lenardo, M.J., and Ozato, K. (2004). Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. Mol. Cell *13*, 33–43.
9. Kasten, M., Szerlong, H., Erdjument-Bromage, H., Tempst, P., Werner, M., and Cairns, B.R. (2004). Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. EMBO J. *23*, 1348–1359.
10. Strohner, R., Nemeth, A., Nightingale, K.P., Grummt, I., Becker, P.B., and Längst, G. (2004). Recruitment of the nucleolar remodeling complex NoRC establishes ribosomal DNA silencing in chromatin. Mol. Cell. Biol. *24*, 1791–1798.
11. Zhang, K., Williams, K.E., Huang, L., Yau, P., Siino, J.S., Bradbury, E.M., Jones, P.R., Minch, M.J., and Burlingame, A.L. (2002). Histone acetylation and deacetylation: Identification of acetylation and methylation sites of HeLa histone H4 by mass spectrometry. Mol. Cell. Proteomics *1*, 500–508.
12. Schultz, D.C., Friedman, J.R., and Rauscher, F.J. (2001). Targeting histone deacetylase complexes via KRAB-zinc finger proteins: The PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. Genes Dev. *15*, 428–443.
13. Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G., and Rauscher, F.J. (2002). SETDB1: A novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. *16*, 919–932.
14. Ragvin, A., Valvatne, H., Erdal, S., Arskog, V., Tufteland, K.R., Breen, K., Oyan, A.M., Eberharter, A., Gibson, T.J., Becker, P.B., et al. (2004). Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300. J. Mol. Biol. *337*, 773–788.
15. Nemeth, A., Strohner, R., Grummt, I., and Längst, G. (2004). The chromatin remodeling complex NoRC and TTF-I cooperate in the regulation of the mammalian rRNA genes in vivo. Nucleic Acids Res. *32*, 4091–4099.
16. Santoro, R., and Grummt, I. (2005). Epigenetic mechanism of rDNA silencing: Temporal order of NoRC-mediated histone modification, chromatin remodeling and DNA methylation. Mol. Cell. Biol. *25*, 2539–2546.
17. Evers, R., and Grummt, I. (1995). Molecular coevolution of mammalian ribosomal gene terminator sequences and the transcription termination factor TTF-I. Proc. Natl. Acad. Sci. USA *92*, 5827–5831.
18. Santoro, R., and Grummt, I. (2001). Molecular mechanisms mediating methylation-dependent silencing of ribosomal gene transcription. Mol. Cell *8*, 719–725.
19. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creats a binding site for HP1 proteins. Nature *410*, 116–120.