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CBX8, a component of the Polycomb PRC1 complex, modulates DOT1L-mediated gene expression through AF9/MLLT3 ☆

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ABSTRACT

AF9 is known to interact with multiple proteins including activators and repressors of transcription. Our data indicate that other AF9 binding proteins compete with the histone methyltransferase DOT1L for AF9 binding thus diminishing its ability to methylate lysine 79 of histone 3. Specifically, we show that AF9 is part of a protein multimer containing members of Polycomb group (PcG) PRC1 complex, CBX8, RING1B, and BMI1. Interaction with CBX8 precludes AF9–DOT1L binding. Knock-down of *CBX8* with short-hairpin RNA (shRNA) leads to decreased expression of the AF9 target gene *ENaCα*. In contrast, *CBX8* overexpression results in increased *ENaCα* mRNA levels and this effect can be partially overcome by co-overexpression of AF9.

1. Introduction

AF9/MLLT3, located on chromosome band 9p22, was first identified as a fusion partner of the *Mixed Lineage Leukemia* (*MLL*) gene in acute lymphoid and myeloid leukemias [1–3]. The function of AF9 has not been completely characterized but a homozygous null mutation of *Af9* in mice is known to cause death in the perinatal period. These mice have axial skeleton defects suggesting a role for Af9 in normal embryogenesis through interplay with *HOX* gene function [4]. Apart from its role in development, recent studies indicate that AF9 is an important factor for the expression of genes that regulate normal hematopoiesis including *GATA1*. It has been shown that AF9 is highly expressed in hematopoietic stem cells and short-hairpin RNA (shRNA)-mediated knock down of AF9 in cord blood (CB) $CD34^+$ $CD38^-$ cells results in complete abrogation of erythrocyte colony formation [5]. Moreover, in hematopoietic precursor cells, expression of a chimeric protein comprising of the C-terminus of AF9 fused to the N-terminus of MLL leads to leukemic transformation [6,7]. Thus AF9 has role in both normal as well as neoplastic blood cell development.

Earlier studies have also shown that Af9 is important in renal salt homeostasis, brain development and HIV gene transcription by regulating the expression of *Enacα* and *Tbr1* genes and by indirect recruitment of HIV Tat protein, respectively [8–11]. Perhaps the best characterized target of Af9 is epithelial sodium channel gene α (*Enacα*). It has been shown in murine renal collecting ducts that Af9 together with the methyltransferase Dot1l result in histone H3 lysine 79 (H3K79) hypermethylation at the *Enacα* promoter region which then contributes to a repressed state of gene expression [8]. Similarly, Af9 in conjunction with Dot1l negatively regulates the expression of the *Tbr1* gene in the subventricular zone of mouse forebrain by governing the status of H3K79 methylation at its transcription start site [9]. This repressive activity of the H3K79 methylation mark by Dot1l is in contrast to canonical H3K79 methylation that is predominantly associated with actively transcribed genes [12].

In addition to DOT1L, AF9 has also been found to directly bind at least three other functionally and structurally unrelated proteins via its carboxy-terminal domain (CTD) [13]. AF9 has been shown to exist in a large multi-protein complex containing AF4, another commonly encountered MLL fusion partner, and positive transcription elongation factor b (P-TEFb) comprised of cyclin T1 and CDK9

Abbreviations: CTD, carboxy-terminal domain; ChIP, chromatin immunoprecipitation; H3K79, histone 3 lysine 79; PcG, polycomb group; PRC1, PcG repressive complex 1; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; shRNA, short-hairpin RNA

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[14,15]. This complex containing AF9–AF4–P-TEFb results in phosphorylation of Ser2 of the CTD of the large subunit of RNA Pol II which promotes productive transcriptional elongation of initiated mRNA transcripts [15,16]. It is through this process that AF9 is required for optimal transcription of HIV genes in conjunction with Tat. In this context, DOT1L is not a part of the AF9–AF4–P-TEFb complex [13]. AF9 is also known to directly interact with two repressors of transcription, the Polycomb protein CBX8, and the BCL6 co-repressor BCoR [17,19]. Here we investigate the AF9–CBX8 protein interaction. CBX8 is a component of the Polycomb-group (PcG) repressive complex 1 (PRC1). We find that an AF9–CBX8 complex also contains other PRC1 proteins. The AF9–DOT1L complex is distinct from the AF9–PRC1 complex. Experimentally manipulating levels of CBX8 has a direct effect on the abundance of AF9–DOT1L complexes and affects the expression of an AF9–DOT1L regulated gene. By way of these interactions overexpression of CBX8, typically a repressor of gene expression, leads to increased expression of the *ENaCα* gene.

2. Materials and methods

2.1. Cell culture and transfection

The MV4-11 human leukemia cell line (ATCC) expressing AF9 was maintained in RPMI 1640 medium with 10% fetal bovine serum, 1.1% Penicillin–Streptomycin (Pen/Strep) and 2.2% Glutamine. HEK293T cells (Clontech) were maintained in DMEM supplemented with 10% fetal bovine serum and 1.1% Pen/Strep. Cells were incubated at 37° C in room air plus 5% CO_2. For transient transfections of DNA expression vectors and shRNA, Lipofectamine2000™ reagent (Life Technologies) was used according to the manufacturer's protocol. Cells were harvested 48 h after transfection. GFP–RI3A–(AF4), GFP–DOT1L, pCMV3XFLAG–AF9, and HA–BCoR expression vectors were described previously [14,19]. Myc–DDK–CBX8, Myc–DDK–BMI1 expression vectors and CBX8 shRNA were purchased from OriGene.

2.2. Immunoprecipitation

HEK293T cells transiently transfected with the designated gene expression vectors and/or shRNA were lysed in 1 ml lysis buffer (30 mM Tris pH 7.4, 150 mM NaCl, 0.5% Triton-X 100 (v/v), 1× protease inhibitor cocktail and 1 mM DTT). 48 h after transfection cleared cell lysates were incubated with anti-FLAG antibody (Sigma # F1804) or isotype control antibody. The immunoprecipitate was separated by SDS 4–12% PAGE followed by Western blotting to detect CBX8 (Bethyl # A300-882A), RING1A (Cell Signaling # 2820), RING1B (Santa Cruz # sc-101109), and BMI1 (Cell Signaling # 5856). To examine the interaction status between BMI1 and other AF9 interacting proteins, HEK293T cells co-expressing MYC–DDK–BMI1, GFP–AF4, GFP–DOT1L, or HA–BCoR were immunoprecipitated using either anti-Myc (Upstate # 06-549) or anti-DDK (Origene # TA50011). The immunoprecipitate was separated by SDS 4–12% PAGE followed by Western blotting to detect GFP (Life Technologies # A11122) or HA (Sigma # H3663) epitope tags that were fused to AF4, DOT1L and BCoR.

2.3. Size exclusion chromatography

1.0×10^9 MV4-11 cells were lysed by resuspension in 1 ml of lysis buffer (20 mM HEPES pH 7.2, 150 mM KCl, 2 mM EDTA, 1 mM DTT, 1× protease inhibitor cocktail, 1× PMSF) and passaged through a dounce homogenizer. 250 µl (approximately 1.5 mg total protein) of cleared cell lysate was then applied to a Superose 6 10/300 GL column (GE Healthcare) with a fractionation range of 5KDa–5MDa. Subsequently, 96250 µl fractions were collected using Fast Pressure Liquid Chromatography (FPLC) at a flow rate of 0.35 ml/min and maximum pressure of 1.5 MPa. The fractions were then analyzed by SDS 4–12%-PAGE followed by Western blotting for AF9 (Novus Biologicals # NB100-1566), RING1B (Santa Cruz # sc-101109) and CBX8 (Bethyl # A300-882A).

2.4. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

HEK293T cells were transiently transfected with the designated gene expression vectors and/or shRNA. Cells were harvested after 72 h and RNA was extracted using RNeasy kit (Qiagen). 2 µg RNA was then converted into cDNA using SuperScript III First Strand Synthesis Super Mix kit (Life Technologies). SYBR Green Supermix with ROX (BioRad) was used to perform quantitative PCR using 1:5 diluted cDNA and primers for the human *ENaCα* gene (F: ATGACTTCATTCCCCTGCTG, R: CATCACTGCCATTCTTGGTG) and *GAPDH* (primers provided in the EZ-Magna ChIPTM A/G protocol) [Milipore]).

2.5. Chromatin immunoprecipitation (ChIP)

HEK293T cells were transiently transfected with the indicated expression vectors. 48 h later, cells were fixed with 1% formaldehyde; ChIP was performed using the EZ-Magna ChIP™ A/G protocol (Milipore). The lysate was precipitated overnight at 4° C using anti-H3 (abcam # ab12079), anti-tri-methylated H3K79 (abcam # ab2621), anti-Myc and isotype control antibodies at concentrations of 3 µg/ml. The eluted DNA was then subjected to quantitative PCR using two different sets of primers for the *ENaCα* promoter regions (P1a F: ACCTCGAGCTGTGTCCTGAT, R: GCCCTGCTCACCTTTAATTG; P1b F: GTACTGGACCTGAGAAGGCG, R: CTTCTCCTTGTGTTGCCCTC).

3. Results

3.1. AF9 associates with PRC1 complex proteins

AF9 has been shown previously to interact directly with CBX8 and indirectly with RING1B through RING1B–CBX8 binding [17]. We examined whether AF9 interacts with other PRC1 components, including RING1A, BMI1, and HPH1. HEK293T cells expressing FLAG–AF9 efficiently precipitated endogenous CBX8, RING1A, RING1B and BMI1 using anti-FLAG antibody (Fig. 1A). In contrast, we did not detect HPH1 in the immunoprecipitate (data not shown). We next assessed whether the reciprocal is true, namely that immunoprecipitation of BMI1 can also capture AF9. We have previously demonstrated that (1) BMI1 binds RING1B through the RING finger domains of the two proteins. Furthermore, (2) CBX8 directly binds RINGB through its C-terminal C-box. Finally, (3) CBX8 binds the C-terminal domain of AF9 [17,18]. We predicted that these sequential protein interactions would link AF9 to BMI1. As shown in Fig. 1B, HEK293T cells expressing FLAG–AF9 contain AF9 in anti-BMI1 immunoprecipitates. These findings support a model in which a fraction of the total cellular AF9 exists within complexes containing at least four PcG proteins of the PRC1 complex.

3.2. AF9 and CBX8 are found within a 300–400 kDa complex

Affinity purification of AF9 and its homolog ENL has shown that they associate, directly or indirectly, with a large number of proteins including activators and repressors of transcription [13,20,21]. However, it has not been fully determined to what extent AF9 forms mega-Dalton complexes collectively comprised of most of these proteins or whether it forms smaller distinct complexes each of which contain a specific subset of AF9 interacting

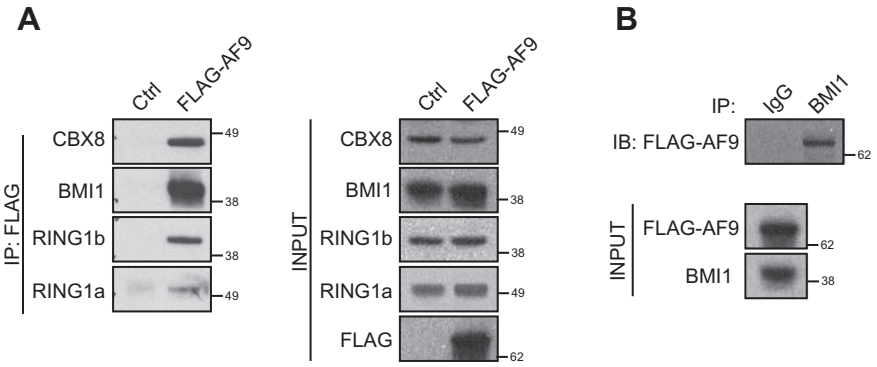

Fig. 1. AF9 interacts with components of the PRC1 complex. (A) HEK293T cells were transiently transfected with FLAG–AF9. Whole cell lysate was then immunoprecipitated with an anti-FLAG antibody. The immunoprecipitate was then analyzed by Western blot for PRC1 proteins. (B) HEK293T cells were transiently transfected with FLAG–AF9 and whole cell lysate was immunoprecipitated with an anti-BMI1 antibody. The immunoprecipitate was then analyzed by Western blot to detect AF9 with an anti-FLAG antibody. The figures are representative of three independent experiments.

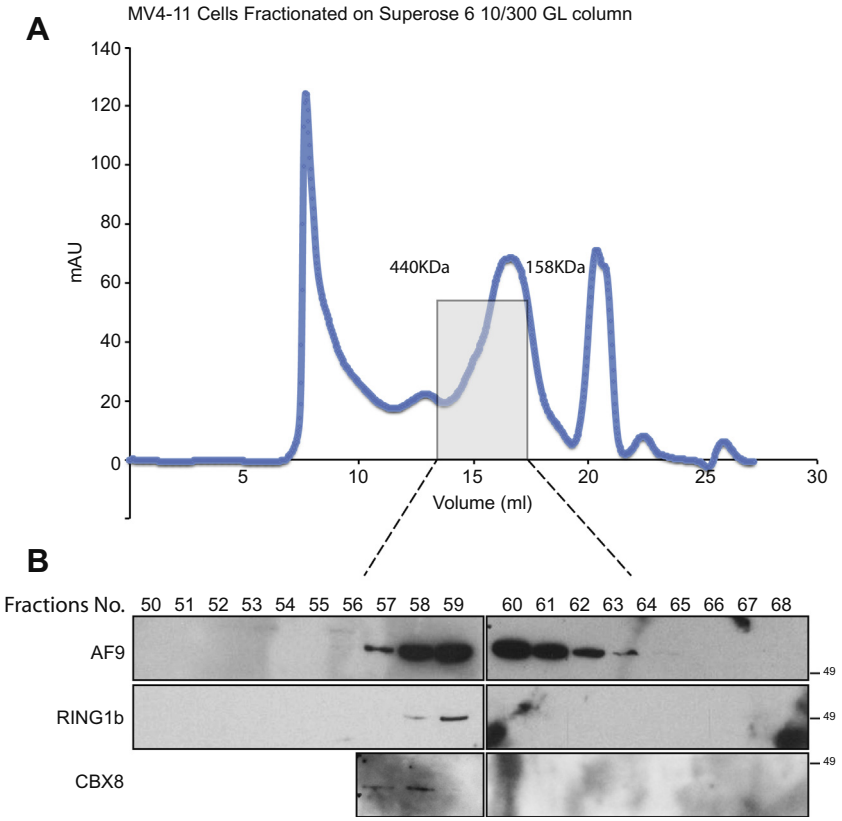

Fig. 2. AF9 complexes range in mass from 158 to 440 kDa. (A) MV4-11 whole cell extract was subjected to size exclusion chromatography using a Superose 6 10/300 GL column and fractions were collected using FPLC. Molecular weight corresponding to the volume of the fractions was detected using Calibration kit standards. (B) Individual fractions were subjected to SDS-4–12% PAGE and immunoblotted to detect the presence of AF9. Fractions containing AF9 were also immunoblotted with RING1B and CBX8 antibodies.

proteins. Here, we sought to determine the presence of discrete subsets of AF9–CBX8 interacting proteins by size exclusion chromatography. Whole cell lysate was prepared from the MV4-11 leukemia cell line that is known to express AF9. The lysate was then applied to a Superose 6 10/300GL column and fractions were collected using FPLC. The individual fractions were then subjected to SDS–PAGE and probed for the presence of AF9 by Western blot. As shown in Fig. 2, AF9 is present in higher molecular weight fractions ranging from 158–440 kDa. We then looked for the presence of other known AF9 interacting proteins focusing on members of the Polycomb group (PcG) PRC1 complex, CBX8 and RING1B. As shown, CBX8 is present in fractions 57 and 58, and RING1B is present in fractions 58 and 59. These results suggest that PRC1 proteins are found with AF9 as components of complexes restricted in mass to 300–400 kDa and that PRC1 proteins do not promiscuously associate with AF9.

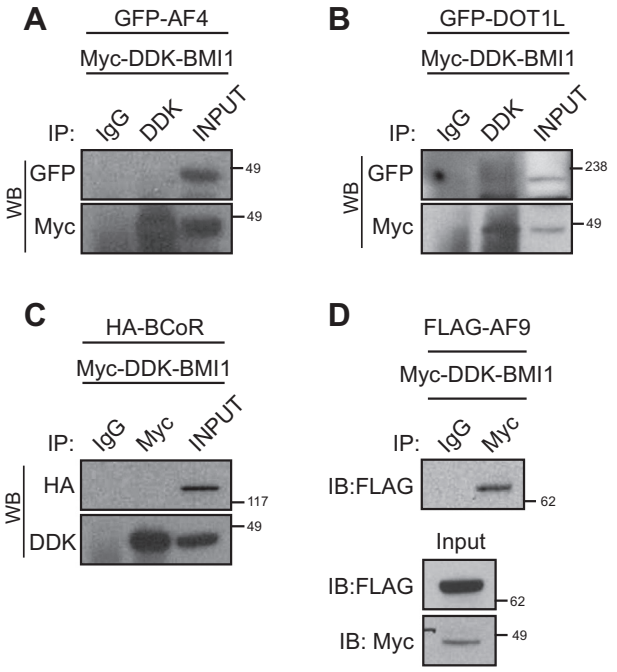

Fig. 3. Other AF9 binding partners are not detected in AF9–PRC1 complexes. (A) HEK293T cells co-expressing Myc–DDK–BMI1 and GFP–RI3A–(AF4: 647–871aa), GFP–DOT1L (B) or HA–BCoR (C) were immunoprecipitated with an anti-DDK antibody (A and B) or an anti-Myc antibody (C) to capture BMI1. Immunoprecipitates were then analyzed by Western blot using an anti-GFP antibody to detect the presence of AF4 or DOT1L (A and B), or an anti-HA antibody for the presence of BCoR (C). The figures are representative of three independent experiments.

3.3. AF9–PRC1 complexes do not contain other known AF9 binding partners

Our immunoprecipitation and gel filtration experiments suggest that AF9 is a component of several physically distinct complexes. In addition to CBX8, it has been shown that AF9 directly binds to AF4–P-TEFb, BCoR and DOT1L [8,15,19]. In order to determine whether AF9–PRC1 proteins exist as separate complexes that exclude the other known AF9-binding proteins, we performed co-immunoprecipitation experiments using HEK293T cells co-expressing Myc–DDK–BMI1 in conjunction with GFP–AF4 (Fig. 3A), GFP–DOT1L (Fig. 3B), HA–BCoR (Fig. 3C), or FLAG–AF9 (Fig. 3D). Since BMI1 efficiently precipitated FLAG–AF9 (Fig. 1B), we wished to analyze whether other AF9 interacting proteins could also be recovered with Myc–DDK–BMI1. As shown in Fig. 3A–C, Myc–DDK–BMI1 was not able to immunoprecipitate GFP–AF4, GFP–DOT1L or HA–BCoR. As a control to verify that proteins were effectively immunoprecipitated in the process, blots were probed with DDK (Fig. 3A and B) or Myc (Fig. 3C) antibodies to detect the presence of BMI1. As an additional positive control, immunoprecipitation of Myc–DDK–BMI1 revealed FLAG–AF9 (Fig. 3D). Therefore, these findings suggest that AF9–PRC1 complexes do not contain other proteins that directly bind AF9.

3.4. The association between AF9 and PRC1 proteins is mediated by CBX8

Using a yeast two-hybrid assay, we found that AF9 and RING1B can simultaneously bind CBX8 to form a ternary complex [17]. In order to determine whether CBX8 is also required for the association between AF9 and other PRC1 components, we knocked down CBX8 using shRNA in HEK293T cells and determined the effect on the interaction between AF9–PRC1 proteins. As shown in Fig. 4, knocking down CBX8 impaired the ability to co-precipitate FLAG–AF9 and RING1B as well as BMI1, without affecting the total levels of BMI1, RING1B or FLAG–AF9. We conclude that CBX8 functions, in part, as a scaffold to support the assembly of AF9–PRC1 complexes.

3.5. The association between AF9–CBX8 affects ENaCα gene expression

To examine the effect of AF9–CBX8 interactions on a known AF9 target gene, we performed qRT-PCR to measure the expression of *ENaCα*. We first knocked down CBX8 using shRNA in HEK293T cells with scrambled shRNA used as a control. Knocking down CBX8 decreased *ENaCα* gene expression approximately 25% compared to controls (Fig. 5A). Conversely, overexpression of CBX8 in HEK293T cells resulted in an increase in *ENaCα* gene expression of approximately 80% (Fig. 5B). Moreover, this increase was partially blocked by the co-expression of AF9. These findings suggest that although CBX8 is a part of the repressive PRC1 complex, it acts as an activator of *ENaCα* gene expression by decreasing the relative abundance of AF9–DOT1L complexes that repress *ENaCα* gene expression. To test this directly, we expressed epitope-tagged AF9 and DOT1L in

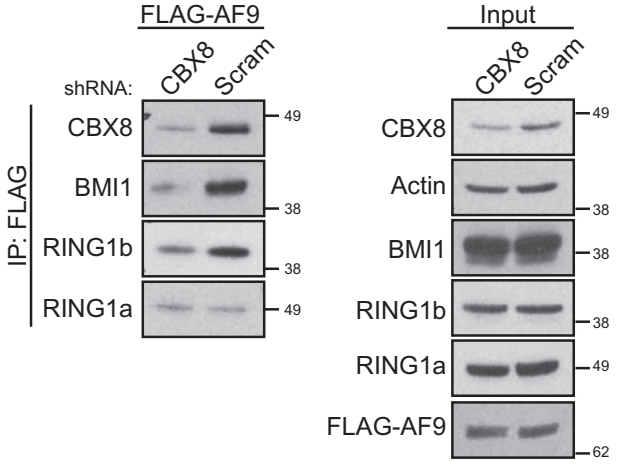

Fig. 4. The association of AF9 with PRC1 complexes is CBX8-dependent. HEK293T cells were co-transfected with FLAG–AF9 and CBX8 shRNA or scrambled (Scram) shRNA. Whole cell lysate was immunoprecipitated with an anti-FLAG antibody and then analyzed by Western blot to detect the presence different PRC1 proteins. The figure is representative of three independent experiments.

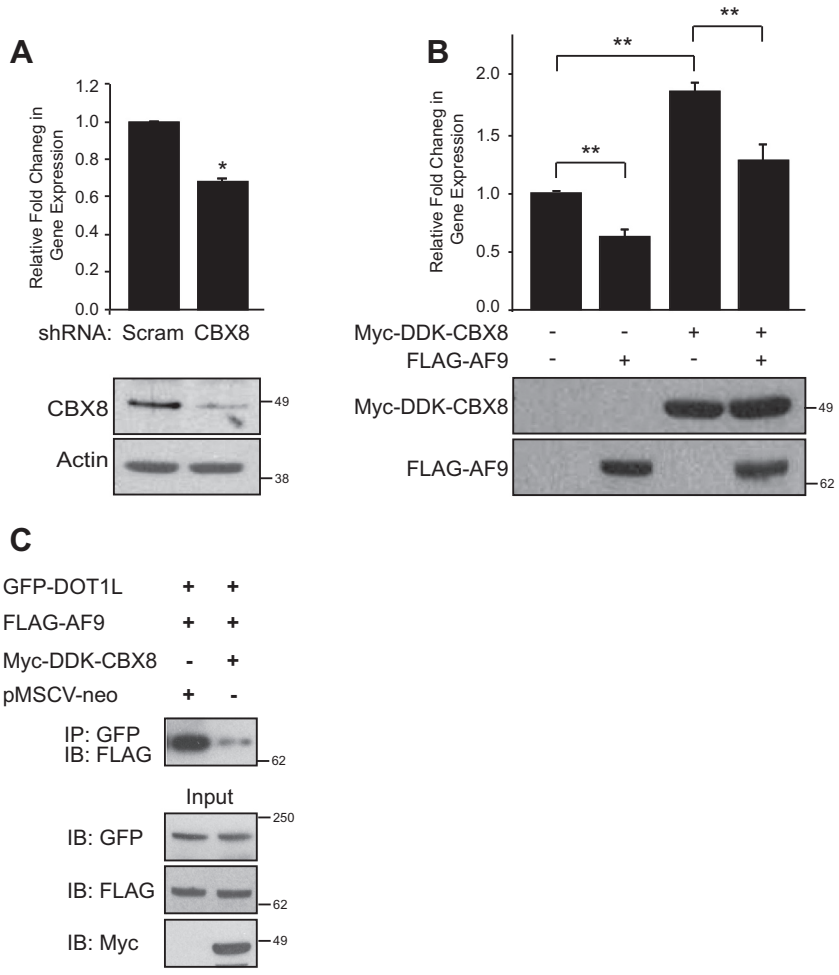

Fig. 5. Modulating AF9–CBX8 affects *ENaCα* gene expression. (A) CBX8 knock down with shRNA resulted in decreased *ENaCα* mRNA levels as shown by qRT-PCR. HEK293T cells were transiently transfected with CBX8 or scrambled/NT shRNA. Total RNA was analyzed for *ENaCα* mRNA levels after normalization to the *GAPDH* gene expression. Western blot analysis was done to determine the degree of CBX8 knock down. (B) CBX8 overexpression resulted in increased *ENaCα* mRNA levels, and co-overexpression of AF9 attenuates this effect. HEK293T cells were transiently transfected with FLAG–AF9, Myc–DDK–CBX8 or FLAG–AF9 and Myc–DDK–CBX8. Again total RNA was analyzed for *ENaCα* mRNA levels after normalization to the *GAPDH* gene. Western blot analysis of the same samples confirmed the presence of FLAG–AF9 and Myc–DDK–CBX8. The experiments were performed in triplicate and each experiment was independently repeated three times. Error bars are the standard deviations of the mean from the three independent experiments ($^{*}P = 0.0001$; $^{**}P < 0.003$). (C) CBX8 overexpression diminishes the interaction of AF9 and DOT1L. HEK293T cells were transiently transfected with FLAG–AF9 and GFP–DOT1L in the presence or absence of a CBX8 expression vector. Cell lysates were immunoprecipitated with an anti-GFP antibody to recover DOT1L. Immunoprecipitates were then analyzed for FLAG–AF9 by Western blot.

the presence and absence of a CBX8 expression vector. Immunoprecipitation of DOT1L yields substantially less AF9 when CBX8 is over-expressed confirming that CBX8 competes with DOT1L for AF9 binding (Fig. 5C).

3.6. Increased expression of CBX8 impedes the recruitment of DOT1L to the ENaCα promoter

We hypothesized that overexpression of CBX8 increases *ENaCα* gene expression by reducing the recruitment of DOT1L to *ENaCα* promoter regions. To test this hypothesis, we performed ChIP using HEK293T cells transfected with Myc–DDK–CBX8. Antibodies recognizing tri-methylated H3K79 were used to identify the DOT1L-mediated chromatin mark. We used two primer sets for promoter regions as shown in Fig. 6A. Overexpression of CBX8 resulted in decreased H3K79 tri-methylation at both promoter regions. This was accompanied by increased recruitment of CBX8 to these regions (Fig. 6B). These findings suggest that a shift in the relative quantities of physically and functionally distinct AF9 complexes alters the regulation of AF9 target genes.

4. Discussion

Here, we show that AF9 associates with components of the PRC1, including CBX8, BMI1, RING1B and RING1A. More specifically, we show that CBX8 is required for the interaction between AF9 and other PRC1 components. Five different CBX proteins have been identified in mammals- CBX2, CBX4, CBX6, CBX7, and CBX8. The proteins share a homologous N-terminal chromodomain and a C-terminal C-box [22]. The chromodomain mediates interactions with methylated histone H3 and the different proteins recognize different methylation marks [23]. The C-box promotes binding to the Ring-finger proteins RING1A and RING1B which, in turn, mediate monoubiquitination of histone H2A at lysine 119 [24]. Of the five CBX proteins, only CBX8 is found in complexes containing AF9 or its homolog ENL [13,20]. We earlier reported that the AF9 binding domain of CBX8 is not conserved among the CBX proteins likely accounting for the specificity of the CBX8–AF9 interaction [17].

While CBX8 is the only CBX protein that binds AF9, AF9 in turn binds directly to at least three other proteins, AF4, DOT1L, and

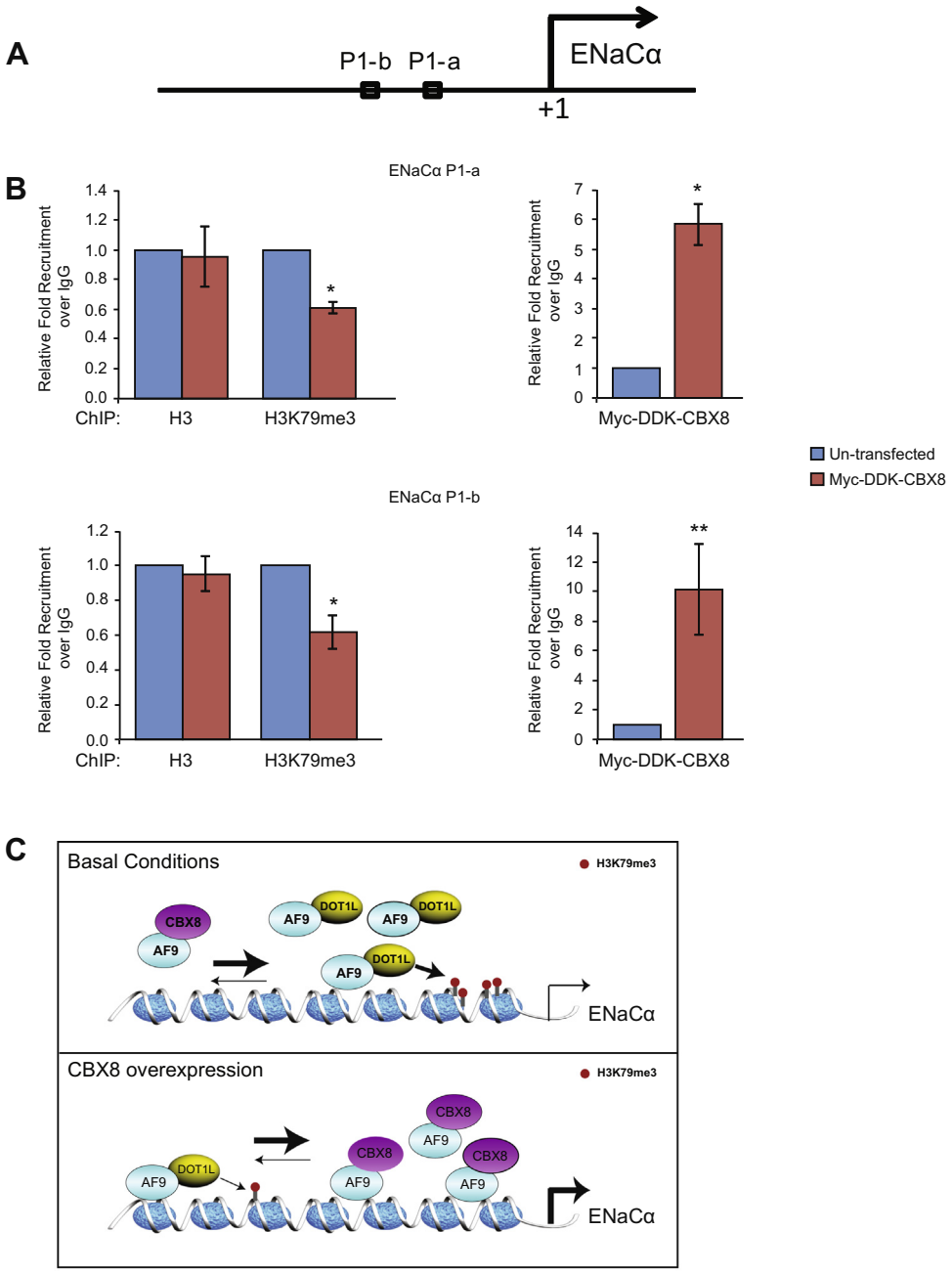

Fig. 6. Overexpression of CBX8 decreases histone H3 lysine K79 tri-methylation (H3K79me3) at *ENaCα* promoter regions. (A) Schematic diagram showing the location of primers in the *ENaCα* (*SCNN1A*) promoter region. (B) ChIP assay showing decreased H3K79me3 at the *ENaCα* promoter region with CBX8 overexpression. HEK293T cells were transiently transfected with Myc–DDK–CBX8 and ChIP assays were performed with the indicated antibodies. Immunoprecipitated DNA was amplified by q-PCR using the primer sets shown in panel (A). The experiments were performed in triplicate and independently repeated three times. Error bars are the standard deviations of the mean for three different experiments.(*$P = 0.008$; **$P < 0.03$). (C) Model demonstrating that the expression of the AF9 target gene *ENaCα* can be regulated by modulating the relative quantities of different AF9 complexes. Under basal conditions, the equilibrium favors AF9–DOT1L complexes causing H3K79 hypermethylation of the *ENaCα* promoter, thereby leading to its repressed state. Under conditions in which CBX8 is overexpressed, equilibrium is shifted to form AF9–CBX8 complexes at the expense of AF9–DOT1L. This results in lower levels of H3K79 methylation and increased recruitment of CBX8 to the *ENaCα*. Decreased H3K79 methylation of the *ENaCα* promoter is associated with its increased expression.

BCoR. Remarkably, these proteins all bind to a ~80 amino acid domain at the C-terminus of AF9 designated the AHD. The AHD itself is intrinsically disordered but adopts a unique structural conformation for each of the four proteins to which it binds [25]. In this report, we provide evidence that shifts in the abundance of different AF9 complexes directly affects AF9 target genes. Specifically, we show that changes in the expression of CBX8 alter the activity of another AF9-binding protein DOT1L whose function is dependent on AF9 binding [8]. In human embryonic kidney cells methylation of H3K79 by DOT1L represses *ENaCα* gene expression a known target of AF9–DOT1L. CBX8, by competing for AF9 binding, reduces H3K79 methylation at the *ENaCα* promoter and enhances expression of the gene. This leads to the seemingly paradoxical finding that overexpression of a repressive PcG protein actually up-regulates the expression of a gene. Our studies do not address whether other PRC1 proteins are required for CBX8 to modulate DOT1L activity, although overexpression of CBX8 alone (and not other PRC1 proteins) is sufficient for this effect. In the

pathological setting of MLL–AF9 induced leukemia in which CBX8 is required, it has been shown that CBX8 functions independently of other PRC1 proteins [26].

By way of dynamic changes with its four binding partners, AF9 serves to mediate the activities of protein complexes that function in essential cellular processes ranging from transcription elongation to epigenetic regulation. Drug-like molecules that are able to either promote or block the binding of specific AF9 interacting proteins are therefore predicted to have important effects on disease processes that subvert AF9.

Note added in proof

While this manuscript was under revision, Maethner et al. reported on the interaction of PRC1 with MLL-ENL [27].

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References

[1] Iida, S., Seto, M., Yamamoto, K., Komatsu, H., Tojo, A., Asano, S., Kamada, N., Ariyoshi, Y., Takahashi, T. and Ueda, R. (1993) MLLT3 gene on 9p22 involved in t(9;11) leukemia encodes a serine/proline rich protein homologous to MLLT1 on 19p13. Oncogene 8, 3085–3092.

[2] Schulze, J.M., Wang, A.Y. and Kobor, M.S. (2009) YEATS domain proteins: a diverse family with many links to chromatin modification and transcription. Biochem. Cell Biol. 87, 65–75.

[3] Nakamura, T., Alder, H., Gu, Y., Prasad, R., Canaani, O., Kamada, N., Gale, R.P., Lange, B., Crist, W.M. and Nowell, P.C. (1993) Genes on chromosomes 4, 9, and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. Proc. Natl. Acad. Sci. U. S. A. 90, 4631–4635.

[4] Collins, E.C., Appert, A., Ariza-McNaughton, L., Pannell, R., Yamada, Y. and Rabbitts, T.H. (2002) Mouse Af9 is a controller of embryo patterning, like Mll, whose human homologue fuses with Af9 after chromosomal translocation in leukemia. Mol. Cell Biol. 22, 7313–7324.

[5] Pina, C., May, G., Soneji, S., Hong, D. and Enver, T. (2008) MLLT3 regulates early human erythroid and megakaryocytic cell fate. Cell Stem Cell 2, 264–273.

[6] Cozzio, A., Passegue, E., Ayton, P.M., Karsunky, H., Cleary, M.L. and Weissman, I.L. (2003) Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes Dev. 17, 3029–3035.

[7] Chen, W., Kumar, A.R., Hudson, W.A., Li, Q., Wu, B., Staggs, R.A., Lund, E.A., Sam, T.N. and Kersey, J.H. (2008) Malignant transformation initiated by Mll–AF9: gene dosage and critical target cells. Cancer Cell 13, 432–440.

[8] Zhang, W., Xia, X., Reisenauer, M.R., Hemenway, C.S. and Kone, B.C. (2006) Dot1a–AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaCalpha in an aldosterone-sensitive manner. J. Biol. Chem. 281, 18059–18068.

[9] Buttner, N., Johnsen, S.A., Kugler, S. and Vogel, T. (2010) Af9/Mllt3 interferes with Tbr1 expression through epigenetic modification of histone H3K79 during development of the cerebral cortex. Proc. Natl. Acad. Sci. U. S. A. 107, 7042–7047.

[10] He, N., Liu, M., Hsu, J., Xue, Y., Chou, S., Burlingame, A., Krogan, N.J., Alber, T. and Zhou, Q. (2010) HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. Mol. Cell 38, 428–438.

[11] Sobhian, B., Laguette, N., Yatim, A., Nakamura, M., Levy, Y., Kiernan, R. and Benkirane, M. (2010) HIV-1 Tat assembles a multifunctional transcription elongation complex and stably associates with the 7SK snRNP. Mol. Cell 38, 439–451.

[12] Steger, D.J., Lefterova, M.I., Ying, L., Stonestrom, A.J., Schupp, M., Zhuo, D., Vakoc, A.L., Kim, J.E., Chen, J., Lazar, M.A., Blobel, G.A. and Vakoc, C.R. (2008) DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. Mol. Cell Biol. 28, 2825–2839.

[13] Biswas, D., Milne, T.A., Basrur, V., Kim, J., Elenitoba-Johnson, K.S., Allis, C.D. and Roeder, R.G. (2011) Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes. Proc. Natl. Acad. Sci. U. S. A. 108, 15751–15756.

[14] Erfurth, F., Hemenway, C.S., de Erkenez, A.C. and Domer, P.H. (2004) MLL fusion partners AF4 and AF9 interact at subnuclear foci. Leukemia 18, 92–102.

[15] Bitoun, E., Oliver, P.L. and Davies, K.E. (2007) The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. Hum. Mol. Genet. 16, 92–106.

[16] Mueller, D., Garcia-Cuellar, M.P., Bach, C., Buhl, S., Maethner, E. and Slany, R.K. (2009) Misguided transcriptional elongation causes mixed lineage leukemia. PLoS Biol. 7, e1000249.

[17] Hemenway, C.S., de Erkenez, A.C. and Gould, G.C. (2001) The polycomb protein MPc3 interacts with AF9, an MLL fusion partner in t(9;11)(p22;q23) acute leukemias. Oncogene 20, 3798–3805.

[18] Hemenway, C.S., Halligan, B.W. and Levy, L.S. (1998) The Bmi-1 oncoprotein interacts with dinG and MPh2: the role of RING finger domains. Oncogene 16, 2541–2547.

[19] Srinivasan, R.S., de Erkenez, A.C. and Hemenway, C.S. (2003) The mixed lineage leukemia fusion partner AF9 binds specific isoforms of the BCL-6 corepressor. Oncogene 22, 3395–3406.

[20] Mueller, D., Bach, C., Zeisig, D., Garcia-Cuellar, M.P., Monroe, S., Sreekumar, A., Zhou, R., Nesvizhskii, A., Chinnaiyan, A., Hess, J.L. and Slany, R.K. (2007) A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. Blood 110, 4445–4454.

[21] Yokoyama, A., Lin, M., Naresh, A., Kitabayashi, I. and Cleary, M.L. (2010) A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription. Cancer Cell. 17, 198–212.

[22] Morey, L., Pascual, G., Cozzuto, L., Roma, G., Wutz, A., Benitah, S.A. and Di Croce, L. (2012) Nonoverlapping functions of the Polycomb group Cbx family of proteins in embryonic stem cells. Cell Stem Cell 10, 47–62.

[23] Bernstein, E., Duncan, E.M., Masui, O., Gil, J., Heard, E. and Allis, C.D. (2006) Mouse polycomb proteins bind differentially to methylated histone H3 and RNA and are enriched in facultative heterochromatin. Mol. Cell Biol. 26, 2560–2569.

[24] de Napoles, M., Mermoud, J.E., Wakao, R., Tang, Y.A., Endoh, M., Appanah, R., Nesterova, T.B., Silva, J., Otte, A.P., Vidal, M., Koseki, H. and Brockdorff, N. (2004) Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. Dev. Cell 7, 663–676.

[25] Leach, B.I., Kuntimaddi, A., Schmidt, C.R., Cierpicki, T., Johnson, S.A. and Bushweller, J.H. (2013) Leukemia fusion target AF9 is an intrinsically disordered transcriptional regulator that recruits multiple partners via coupled folding and binding. Structure 21, 176–183.

[26] Tan, J., Jones, M., Koseki, H., Nakayama, M., Muntean, A.G., Maillard, I. and Hess, J.L. (2011) CBX8, a polycomb group protein, is essential for MLL–AF9-induced leukemogenesis. Cancer Cell 20, 563–575..

[27] Maethner, E., Garcia-Cuellar, M., Breitinger, C., Takacova, S., Divoky, V., Hess, J.L. and Slany, R.K. (2013) MLL-ENL inhibits Polycomb repressive complex 1 to achieve efficient transformation of hematopoietic cells. Cell Reports 3, 1–14.