Five Friends of Methylated Chromatin Target of Protein-Arginine-Methyltransferase[Prmt]-1 (Chtop), a Complex Linking Arginine Methylation to Desumoylation

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Chromatin target of Prmt1 (Chtop) is a vertebrate-specific chromatin-bound protein that plays an important role in transcriptional regulation. As its mechanism of action remains unclear, we identified Chtop-interacting proteins using a biotinylation-proteomics approach. Here we describe the identification and initial characterization of Five Friends of Methylated Chtop (5FMC). 5FMC is a nuclear complex that can only be recruited by Chtop when the latter is arginine-methylated by Prmt1. It consists of the co-activator Pelp1, the Sumo-specific protease Senp3, Wdr18, Tex10, and Las1L. Pelp1 functions as the core of 5FMC, as the other components become unstable in the absence of Pelp1. We show that recruitment of 5FMC to Zbp-89, a zinc-finger transcription factor, affects its sumoylation status and transactivation potential. Collectively, our data provide a mechanistic link between arginine methylation and (de)sumoylation in the control of transcriptional activity. Molecular & Cellular Proteomics 11: 10.1074/mcp.M112.017194, 1263–1273, 2012.

Transcription factor activity is often controlled by post-translational modifications such as acetylation, phosphorylation, methylation, and sumoylation. Some modifications are associated with both gene activation and repression, whereas others appear to be more exclusive: asymmetrical dimethylation of arginine residues is restricted to transcriptional activation, whereas modification by sumoylation correlates with inhibition of transcription (1).

Arginine methylation occurs frequently within glycine-arginine-rich (GAR) regions and is catalyzed by members of the protein arginine methyltransferase (Prmt) family. These enzymes are subdivided in two major classes: type I enzymes catalyze the formation of asymmetrically dimethylated arginines (aDMA), whereas type II enzymes form symmetrically dimethylated arginines (sDMA) (2). Prmt1 and Prmt4/Carm1 (Coactivator-associated arginine methyltransferase 1) are the major type I enzymes and both are critical for mammalian development (3–4). Their substrates include RNA-binding proteins, nuclear matrix proteins, cytokines, and transcriptional regulators (2). Prmt1 methylates transcription factors such as Runx1 and STAT1 thereby promoting their transcriptional activity (5–6). Furthermore, Prmt1 and Prmt4 are recruited by nuclear hormone receptors and other transcription factors including YY1, p53, and NF-κB (7–10), resulting in the methylation of additional coactivators and histones. Prmt4 methylates histone H3 at arginine 17 and 26, whereas Prmt1 targets histone H4 at arginine 3 for methylation promoting subsequent acetylation of histone H3 at lysine 9 and histone H3 at lysine 14 (11) and further activating events (12).

Small ubiquitin-like modifier (SUMO) has an important regulatory function in several cellular processes, including DNA repair, cell cycle progression, signal transduction, chromatin structure and transcriptional regulation (13). Mammalian cells express four SUMO paralogs (SUMO-1 to SUMO-4). SUMO-1 differs in sequence by about 50% from SUMO-2 and 3, whereas SUMO-2 and SUMO-3 are 97% identical to each other. Conjugation of SUMO to target proteins occurs by a series of reactions conducted by the E1 activating enzyme, E2 conjugating enzyme, and an E3 SUMO ligase (14). The reverse desumoylation process is mediated by the isopeptidase activity of SUMO-specific proteases (Senps). In mammals, six members of Senps have been reported, known as Senp1–3 and Senp5–7. Sumoylation of multiple transcription factors, asymmetrically dimethylated arginines; sDMA, symmetrically dimethylated arginines; SUMO, Small ubiquitin-like modifier; SIMs, SUMO interacting motifs; Chtop, Chromatin target of Prmt1; HGNC, HUGO Gene Nomenclature Committee; MEL, mouse erythroleukemia.
5FMC: A Nuclear Desumoylation Complex

including Sp3, Sox6, Zeb1, and Zbp-89, has a negative effect on their transactivation potential, as it promotes the recruitment of repressive complexes (15–17). Many components of the repressor complexes CoREST1, NuRD, PRC1, S{	extit{et}}d3b1, and MEC themselves are also sumoylated, or have SUMO interacting motifs (SIMs). This suggests that sumoylation plays an important role in the formation and/or stabilization of these complexes (18).

We previously identified Friend of Prmt1 (Fop), also known as Small protein rich in arginine and glycine (SRAAG), encoded by the mouse 2500003M10Rik and human C1orf77 genes, respectively (19–20). Recently, the name Chromatin Target of Prmt1 (Chtop) has been assigned to this gene/protein by the HUGO Gene Nomenclature Committee (HGNC). For reasons of clarity, we will also use the name Chtop for the murine homolog. Chtop is a chromatin-associated protein that plays an important role in the ligand-dependent activation of estrogen target genes such as TFF1 (pS2) in breast cancer cells (19). In addition, it is a critical regulator of γ-globin gene expression (21). However, little is known about the molecular mechanism of transcriptional control mediated by Chtop.

Chtop contains a central GAR region that is recognized and methylated by Prmt1. Because arginine methyltransfer controls protein-protein interactions, we used a biotinylation-proteomics approach to identify proteins that bind Chtop in the presence and absence of Prmt1. In this study we identified and characterized a protein complex that binds specifically to methylated Chtop. As this nuclear complex consists of five proteins—SUMO1/sentrin/SMT3 specific peptide 3 (Senp3), proline-glutamate and leucine rich protein 1 (Pelp1), LAS1L-like protein (Las1L), Testis expressed 10 protein (Tex10), and WD repeat domain 18 protein (Wdr18)—we call it Five Friends of Methylated Chtop (5FMC). We show that Pelp1 is critical for the integrity of 5FMC and that Chtop and 5FMC are recruited by Zinc finger binding protein-89 (Zbp-89), thereby regulating both (de)sumoylation of, and transactivation by, Zbp-89.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Cells—The following plasmids have been described previously: GST-PELP1 deletions (22), T7-Pelp1 (23), pMT2_HA_Chtop, HA_mPRMT1 WT, and HA_mPRMT1 EQ (19). The cDNA of human SUMO-2 (hSUMO2) was kindly provided by Dr. Guntram Suske (Philipps-Universitaet Marburg, Germany). The cDNA of full-length LAS1L was obtained from Open Biosystems (Clone ID 1.5 cm × 100 μm, packed in-house) at a flow rate of 8 μl/min. Peptide separation was performed on a ReproSil C18 reversed phase column (Dr Maisch GmbH; column dimensions 1.5 cm × 100 μm, packed in-house) at a flow rate of 8 μl/min. Peptide separation was performed on ReproSil C18 reversed phase column (Dr Maisch GmbH; column dimensions 1.5 cm × 100 μm, packed in-house) using a linear gradient from 0 to 80% B (A = 0.1% formic acid; B = 80% (v/v) acetonitrile, 0.1% formic acid) in 70 min and at a constant flow rate of 200 nl/min using a splitter. The column eluent was directly sprayed into the electrospray ionization (ESI) source of the mass spectrometer. Mass spectra were acquired in positive mode and equipped with a nanospray source. Peptide mixtures were trapped on a ReproSil C18 reversed phase column (Dr Maisch GmbH; column dimensions 1.5 cm × 100 μm, packed in-house) at a flow rate of 8 μl/min. Peptide separation was performed on ReproSil C18 reversed phase column (Dr Maisch GmbH; column dimensions 1.5 cm × 50 μm, packed in-house) using a linear gradient from 0 to 80% B (0.1% formic acid; B = 80% (v/v) acetonitrile, 0.1% formic acid) in 70 min and at a constant flow rate of 200 nl/min using a splitter. The column eluent was directly sprayed into the electrospray ionization (ESI) source of the mass spectrometer. Mass spectra were acquired in positive mode; fragmentation of the peptides was performed in data-dependent mode. Peak lists were automatically created from raw data files using the Mascot Distiller software (version 2.3; MatrixScience). The Mascot search algorithm (version 2.2, MatrixScience) was used for searching against the IPI_mouse database (version 3.83, containing 60,010 sequences and 27,475,843 residues). The peptide tolerance was set to 10 ppm and the fragment ion tolerance was set to 0.8 Da. A maximum number of two missed cleavages by trypticin were allowed.
and carbamidomethylated cysteine and oxidized methionine were set as fixed and variable modifications, respectively. Search results were parsed into a home-built database system for further analysis. Entries were parsed if they had a minimum peptide Mascot score of 25, and a significance threshold of p < 0.05; the option “require red bold” was also selected. Using these parameters yielded an estimated peptide false discovery rate (FDR) of 3–5% against a target decoy database. The Mascot data have been uploaded to the PRIDE database (28) (www.ebi.ac.uk/pride) under accession numbers 21750–21767. The data was converted using PRIDE Converter (29) (http://pride-converter.googlecode.com).

**Lentivirus Mediated Knockdown—** The Chtop and Prmt1 shRNA lentiviral vectors were described previously (19, 21). For mouse Pelp1, Senp3, and human PELP1, SENP3, WDR18, LAS1L, clones from the TRC Mission shRNA library ((30); Sigma Aldrich, St. Louis, MO, USA) were used for knockdown experiments in MEL and 293T cells respectively, including a non-targeting shRNA control virus (SHC002). Lentivirus was produced by transient transfection of 293T cells as described before (31). The following clones were used from the TRC shRNA library: TRCN0000177043 (shPelp1 #1), TRCN0000187252 (shPelp1 #2), TRCN000031014 (shSenp3 #1), TRCN000031017 (shSenp3 #2), TRCN0000159617 (shPelp1 #2), TRCN0000159673 (shPelp1 #3), TRCN0000410406 (shSENP3 #1), TRCN00004104 (shSENP3 #2), TRCN0000780889 (shWDR18 #1), TRCN000078089 (shWDR18 #2), TRCN0000121835 (shLAS1L #2), TRCN0000142144 (shLAS1L #4), TRCN000035931 (shHPRMT1).

**Size-exclusion Chromatography and Subcellular Fractionation—** Nuclear extracts from MEL cells expressing BirA biotin ligase enzyme, were chromatographed over a Superose 6 column (Amersham Biosciences) using an AKTA fast-performance liquid chromatography apparatus. Fractions were collected and precipitated with trichloroacetic acid and analyzed by Western blotting. Subcellular fractionation was performed as described previously (19).

**RT, QPCR, ChiP Assay, and Statistical Analysis—** Reverse transcription (RT), RT-quantitative PCR (RT-QPCR), and ChiP were performed as described previously (32). Primers used for RT-QPCR and ChiP-QPCR are summarized in supplemental Table S2. ANOVA statistical analysis was performed by GraphPad Prism 5.02.

**RESULTS**

**Methylation Dependent and Independent Interactions of Chtop—** To identify interaction partners of Chtop, an N-terminal double-tagged version of Chtop protein (Bio_HA_Chtop) was expressed in MEL cells. These cells also stably expressed BirA, a bacterial biotin ligase which efficiently biotinylates the Bio-tag (26). Note that tagged Chtop was not overexpressed, as endogenous levels were reduced in Bio_HA-Chtop transfected cells (Fig. 1C). Protein complexes from nuclear lysates were recovered by streptavidin pull down followed by nanoflow liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) and were compared with samples from cells expressing BirA alone (Fig. 1A, supplemental Table S2). This confirmed the association of Chtop with Prmt1 and Prmt5, factors that we have previously identified as Chtop binding proteins (19). MS analysis also revealed the binding of three members of the polycomb repressor complex 1 (PRC1; Pc2, Ring1B, and Phc2) (33–35), the SET nuclear oncoprotein (36), and the mRNA export protein 40-2-3 (37). Furthermore, high MASCOT scores were obtained for the proteins Pelp1, Las1L, Tex10, Senp3, and Wdr18, four of which were previously copurified with the MLL complex and were recently described as regulators of ribosome biogenesis (38–40). Pelp1 is a coactivator involved in nuclear hormone signaling (41), whereas Senp3 is a SUMO-specific protease (42). The WD-repeat protein Wdr18, Tex10, and Las1L had not been characterized previously. To investigate whether arginine methylation of Chtop played a role in these interactions, we performed lentiviral-mediated knockdown of Prmt1 in Bio_HA_Chtop expressing MEL_BirA cells and used a similar purification approach. When compared with cells transduced with a control lentivirus, no major differences were observed in the binding of Chtop to Prmt5, Pc2, Ring1B, Phc2, 40-2-3, and Set (Fig. 1B, supplemental Table S2). Interestingly, copurification of Las1L, Pelp1, Tex10, Senp3, and Wdr18 was strongly reduced or absent when Chtop was hypomethylated. Next, we confirmed the methylation (in)dependent interactions by streptavidin affinity purification, followed by Western blot analysis. Chtop is hypomethylated in the absence of Prmt1, as indicated by its faster migration pattern and by staining with an antibody that specifically recognizes aDMA (Asym24; Fig. 1C). Endogenous Chtop interactors were efficiently recovered in Bio_HA_Chtop pull downs, whereas no background staining was observed in MEL_BirA cells (Figs. 1D, 1E). In addition, no association was observed with Wdr18 in the absence of Prmt1, whereas the interactions with Pelp1, Las1L, Tex10 and Senp3 were strongly reduced. These associations do not depend on the presence if Prmt1, as only wildtype but not enzymatic inactive Prmt1 could rescue the knockdown (supplemental Fig. S1). Together, these results validate the interactions identified by MS and show that methylation of Chtop is required for the recruitment of Pelp1, Las1L, Tex10, Senp3, and Wdr18.

**5FMC is a Nuclear Complex—** We have previously shown that the majority of Chtop is bound to chromatin (19). To elucidate where the newly identified methylation-specific partners of Chtop localize in the cell, we performed biochemical fractionation of MEL cells. This revealed that all five proteins were mainly found in the nucleoplasm, whereas low levels were also detected in the cytoplasmic and chromatin fractions (Fig. 2A). To examine whether the five proteins form a complex, we first performed size-exclusion chromatography of MEL nuclear extracts. The elution patterns of Pelp1, Las1L, Tex10, Senp3 and Wdr18, as well as Chtop, overlapped substantially (Fig. 2B). The molecular mass of the positive fractions was >1MDa, indicating that the factors were present in a high molecular weight protein complex or were bound to chromatin. Similar experiments in human 293T cells revealed comparable results, although larger proportions of Las1L, Tex10, and WDR18 were detected in fractions corresponding to lower molecular mass (supplemental Fig. S2). Next, double tagged (Bio_HA) Pelp1, Las1L, Senp3, and Wdr18 were stably expressed in MEL_BirA cells. Of note, we were not able to exogenously express the Tex10 protein, probably because of protein stability issues. Associated proteins were identified.
by streptavidin pull down in nuclear lysates followed by nanoLC-MS/MS and were compared with samples from MEL_BirA only cells. In all four experiments, the associated proteins with the highest MASCOT score were Pelp1, Las1L, Tex10, Senp3, and Wdr18 (Fig. 2C and supplemental Fig. S3 (Pelp1, Wdr18, and Las1L)). Moreover, in the MS analysis of Bio_HA_Senp3, Bio_HA_Wdr18 and Bio_HA_Pelp1 we observed an association with the Nol9 protein. The Nol9 ortholog in S. pombe (Grc3) was shown to associate with Las1 and the yeast IPI complex that consist of Rix1, Ipi1 and Crb3 (43). These proteins share homologous regions with Pelp1, Tex10 and Wdr18, respectively. Moreover, it was recently shown in human cells that NOL9 interacts with PELP1, TEX10, WDR18, LAS1L, and SENP3 (40). Chtop was only detected in the Bio_HA_Senp3 purification (Fig. 2C, left panel). The MS results were confirmed by immunoblot analysis of the streptavidin pull downs of tagged proteins (Fig. 2C, right panel and supplemental Fig. S3), and immunoprecipitations of endogenous proteins from both mouse and human cells (Fig. 2D and supplemental Fig. S4, respectively). Taken together, these results show that Pelp1, Las1L, Tex10, Senp3, and Wdr18 form a nuclear multi-protein complex. As this complex binds selectively to methylated Chtop, we named it Five Friend of Methylated Chtop, or 5FMC. Pelp1 is the Core Subunit and Critical for 5FMC Stability—To further study the composition of the 5FMC complex, we transiently cotransfected tagged 5FMC components in 293T cells, followed by coimmunoprecipitation (co-IP). We found that T7_Pelp1 is efficiently recovered in HA_Wdr18 IPs, whereas Myc_Senp3 interacts with HA_Wdr18 (Fig. 3A). Moreover, Myc_Senp3 co-purifies with HA_Wdr18 only when T7_Pelp1 is cotransfected (Fig. 3A, lanes 3 and 4). In addition, we observed that cotransfection of T7_Pelp1 resulted in higher protein levels of HA_Wdr18 and Myc_Senp3 (Fig. 3A, lanes 2...
and 4). Identical results were obtained when Myc_Senp3 was immunoprecipitated (data not shown). No conclusive results could be obtained with ectopically expressed Tex10 and Las1L, probably because of stability issues. These results show that Pelp1 is required for the interaction between Wdr18 and Senp3 and that Pelp1 might be required for the stability of these proteins. To further study the potential central role of Pelp1 within the 5FMC complex, we depleted endogenous mouse Pelp1 in MEL_BirA cells and endogenous human PELP1 in 293T cells by lentiviral-mediated knockdown using two different shRNAs (Fig. 3B and supplemental Fig. S5A).

Interestingly, the protein levels of Senp3 and Las1L were dramatically decreased, whereas Wdr18 and Tex10 could not be detected in the absence of endogenous Pelp1 (Fig. 3B, left panel). Quantitative RT-PCR showed that the reduced protein levels were caused by protein stability rather than reduced mRNA levels (Fig. 3B, right panel). The individual knockdown of LAS1L, SENP3, or WDR18 had no significant effect on the protein levels of the other 5FMC subunits. Furthermore, partial complexes exist upon depletion of LAS1L, SENP3, and WDR18 (supplemental Fig. S5). These results indicated that Pelp1 is the central component of the 5FMC complex and that the integrity of 5FMC is essential for the stability of its components. Analysis of the primary sequence of Pelp1 showed that Pelp1 contains a cysteine-rich region, two proline-rich regions and a C-terminal glutamine-rich region (41). To map the interactions between Pelp1 and other 5FMC components, we tested the ability of HA-tagged Las1L, Wdr18, and Senp3 proteins to bind to various domains of Pelp1 fused to GST. HA_Las1L and HA_Wdr18 interacted with the GST_Pelp1 fusion containing amino acids (aa) 401–600, whereas HA-Senp3 mainly interacted with domain 960–1130. Interactions were further studied using a series of deletion mutants of Las1L. These experiments showed that the C-terminal part of Las1L (aa 552–734) mediated the interaction with Pelp1, whereas the central domain (aa 370–552) mediated the interaction with Wdr18. The binding to Senp3 could not be mapped in detail: any deletion between aa 188 and 734 disrupted the interaction (supplemental Fig. S6A). Similar experiments with Wdr18 deletion constructs revealed that the region containing WD40 domains 4–6 were required for binding to Las1L, whereas deletion of any WD40 domain disrupted

Fig. 2. 5FMC is a nuclear complex. A, Chtop methylation dependent interaction proteins are localized mainly in the nucleoplasm. MEL_BirA cells were biochemically fractionated as described under “Experimental Procedures”. Cytoplasmic, nucleoplasmic, chromatin and nuclear matrix were tested using Pelp1, Las1L, Tex10, Senp3, Wdr18 antibodies against endogenous proteins. Chtop, Prmt1, H4, and Lamin B served as controls for individual fractions. B, MEL_BirA cell nuclear extracts were analyzed by sized-exclusion chromatography on a Superose 6 column. Proteins eluted from the indicated fractions were blotted with the indicated antibodies. Molecular mass markers are indicated at the top. C, Senp3 interactions in MEL cells. Whole cell lysates (Input) and streptavidin pull downs (Strept. PD) from MEL_BirA (BirA) and MEL_BirA cells expressing biotinylated Senp3 (Bio_HA_Senp3) were analyzed by MS (table) and Western blotting. Immunoblot probed with the indicated antibodies. Arrows indicate endogenous (end.) and biotinylated (Bio_HA) Senp3. D, 5FMC is a nuclear complex. Endogenous association between the 5FMC components. MEL_BirA cell nuclear lysates were analyzed by immunoprecipitation (IP) and Western blotting with the antibodies indicated.
the binding to Pelp1 (supplemental Fig. S6B). These initial domain-mapping experiments suggest complex multi-intermolecular interactions and are in line with the proposed model shown in Fig. 3D.

**Chtop Recruits 5FMC to Zbp-89**—MS analysis of the Zbp-89 interactome revealed Chtop and several Chtop-associated factors as potential interaction partners of Zbp-89 in MEL cells (manuscript in preparation). To further explore the possibility that 5FMC interacts with Zbp-89, we performed bio-Zbp-89 pull downs in MEL_BirA cells followed by immunoblotting. We observed that Chtop, Prmt1 and 5FMC components associated with Zbp-89 (supplemental Fig. S7). These interactions were DNA independent, as degradation of DNA by Benzonase treatment did not affect the efficiency of co-purification. To investigate whether Chtop is required for the interaction between Zbp-89 and 5FMC, we examined the association of Zbp-89 and 5FMC complex components in Chtop knockdown cells. Bio_Zbp-89 was precipitated more efficiently when Chtop protein levels were reduced, suggesting that bio_Zbp-89 was more accessible in the absence of Chtop (Fig. 4A). Indeed, co-purification of 5FMC complex components was reduced to ~30% of control samples (Figs. 4A and 4B). These results indicate that Chtop is required for the association of Zbp-89 with the 5FMC complex.

**Senp3 Regulates the Sumoylation Status of Zbp-89**—It has been reported that Zbp-89 can be post-translationally modified by SUMO in transient overexpression experiments (44). To investigate whether endogenous Zbp-89 could be sumoylated, we first performed streptavidin pull downs from bio_HA_SUMO-2 expressing MEL_BirA nuclear extracts. Staining with an antibody recognizing Zbp-89 detects multiple sumoylated Zbp-89 species (Fig. 5A, lower panel), in line with the observation that Zbp-89 contains at least two domains that can be sumoylated (44). Knockdown of Senp3, using two different shRNAs, in these cells led to a significant increase of SUMO-2 detection in whole cell lysates, as well as of sumoylated Zbp-89. It should be noted that the reduction of Senp3 expression affected cell growth and survival, thereby limiting
the effect of the knockdown. Next, we performed similar experiments in MEL cells expressing bio_Zbp-89. Streptavidin pull downs probed with an anti-SUMO 2/3 antibody showed that Senp3 depletion resulted in an increase of the levels of SUMO modified bio_Zbp-89 and the appearance of a slower mobility form of bio_Zbp-89 when probed with anti-Zbp-89 antibody (Fig. 5B). The upper band is consistent with SUMO modification. Taken together, these results show that Zbp-89 is sumoylated in vivo, and that Senp3 plays a role in this process.

5FMC is Critical for Zbp-89 Dependent Gene Regulation

The observations that 5FMC is a desumoylating complex that is recruited by Zbp-89 suggest that it is involved in transcriptional regulation. To examine whether 5FMC is recruited to Zbp-89 target genes we performed chromatin immunoprecipitation (ChIP) experiments for Pelp1, the core component of the complex. We used MEL_BirA cells that ectopically expressed bio_Pelp1, as this increased sensitivity (not shown). After chromatin precipitation, the promoter regions of three (Dusp6, Zbp-89, Atf5) and the coding region of one
5FMC is involved in the regulation of Zbp-89 target genes. A, Pelp1 is recruited at the promoter regions of Dusp6, Zbp-89, Atf5 and the coding region of Tubb1. MEL_BirA cells that ectopically expressed bio_Pelp1 analyzed by ChIP using Pelp1 antibody for the indicated gene promoter or coding regions. The promoter region of the Osm gene and the region upstream of Gata-1 promoter (Gata-1 upstream) were used as negative controls. *** indicates \( p < 0.001 \), ** indicates \( p < 0.01 \). Error bars: S.D. of triplicate experiment. B, Knockdown of Pelp1, Senp3 and Chtop in MEL_BirA cells. MEL_BirA cells were treated with the indicated shRNAs. Cell lysates were analyzed by Western blotting with the indicated antibodies. Actin staining serves as a loading control. C, Pelp1, Senp3 and Chtop knockdowns reduced RNA polymerase II (Pol II) occupancy at the promoter regions of Dusp6, Zbp-89, Atf5 and the coding region of Tubb1. MEL_BirA cells were treated as in (B), ChIP analysis at the indicated regions was performed using Pol II antibody. Error bars: S.D. of triplicate experiment. D, Regulation of Zbp-89 target genes by Senp3 and Pelp1. Total RNA was extracted from MEL_BirA cells treated as in (B) and analyzed by RT-QPCR for Dusp6, Tubb1, Zbp-89, Atf5, Senp3 and Pelp1. *** indicates \( p < 0.001 \), ** indicates \( p < 0.01 \), * indicates \( p < 0.05 \). Error bars: S.D. of triplicate experiment.
Chtop was because of reduced protein levels of Zbp-89 (Fig. 6B, lower panel). The same regions were also tested for changes in the histone modifications H3K4 and H3K27, but no changes were detected (not shown). Collectively, our data indicate that 5FMC is recruited to Zbp-89 target genes and that it is involved in their transcriptional activation by Zbp-89. This most likely involves desumoylation of Zbp-89 and possibly of other factors.

**DISCUSSION**

In the present study, we have identified 5FMC, a desumoylating protein complex that exclusively binds to arginine-methylated Chtop. It acts as a key regulator of Zbp-89 sumoylation and is required for full transcriptional activation of Zbp-89 dependent genes. To our knowledge, this is the first description of a mechanism that adds specificity to desumoylation processes.

The 5FMC complex is composed of five proteins: Pelp1, Las1L, Tex10, Senp3, and Wdr18. These factors are among the most abundant proteins present in Chtop purifications, but only when Chtop is methylated by Prmt1. Little is known about most of the components of the 5FMC complex. Pelp1 is a coactivator involved in nuclear hormone signaling (41), Senp3 is a SUMO-specific protease (42), whereas Wdr18, Las1L, and Tex10 have not been characterized yet. In yeast, the proteins Rix1, Ipi1, and Ipi3 (S. cerevisiae)/Crb3 (S. pombe) share conserved regions with Pelp1, Tex10, and Wdr18, respectively. They form the IPI complex and have been shown to function in ribosomal RNA processing (45). It was recently shown that Las1, the yeast ortholog of Las1L, is also associated with the IPI complex (43). Furthermore, PELP1, TEX10, LAS1L, SENP3, and WDR18 were recently linked to ribosome biogenesis in human cells, indicating an evolutionary conserved complex with a role in ribosomal RNA processing. In human cells, the complex was localized mainly in the nucleoplasm with a subfraction present in the nucleolus (39–40). In this study, we observed a similar distribution in mouse cells.

Additionally, several studies suggested a role for 5FMC components in transcriptional regulation and (de)sumoylation events, although this had not been explored further. Doseff and Arndt proposed in their initial identification of Las1 in S. cerevisiae that it functions as a transcription factor (46), whereas it was recently shown to localize to heterochromatic regions (43). In human cells, components of the 5FMC complex were first detected in the MS analysis of the MLL1-WDR5 complex, a complex that regulates transcription activation by H3K4 methylation (38). The 5FMC complex was also found in a recent study on human coregulator protein complex networks obtained from integrative mass spectrometry-based analysis of 3290 antibody-based affinity purifications (47). Thus, the data obtained with this approach independently support the composition of the 5FMC complex reported here. Furthermore, PELP1, LAS1L, TEX10, and SENP3 were also detected together with components of the CoREST1/HDAC1 corepressor complex in a MS study for proteins that interact with SUMO-2 (48). Sumoylation is important for stability and recruitment of repressive complexes such as CoREST, NuRD, and SetDB1 (18), indicating that desumoylation of transcription factors and corepressors is required for derepression.

Our results indicate that Pelp1 is the core component of 5FMC. Pelp1 has the ability to interact with nuclear receptors (NRs) and enhances transcription of their target genes (23). Pelp1 has been shown to interact with the acetyltransferases CBP and p300 (22), KDM1, a member of the CoREST1 repressor complex (49), and deacetylases, including components of the NuRD repressor complex (50). Interestingly, Pelp1 was also identified in a blind screen for SUMO-2 interacting proteins (51). This opens the possibility that Pelp1 acts, in addition to its scaffold function within 5FMC, as a Sumo-2 sensor to detect Senp3 substrates.

As Chtop is strongly associated with chromatin (19), whereas 5FMC mainly resides in the nucleoplasm (this paper), the interactions between Chtop and 5FMC are most likely transient and highly dynamic. Possibly, Chtop recruits 5FMC
complex, to desumoylate its substrates, in a “hit-and-run” manner. In addition, 5FMC may very well act as a desumoylation complex outside the chromatin environment and independent of Chtop, e.g. in ribosome biogenesis (39).

In line with our previous observation that Chtop colocalizes to H3K27me3 (19), we found that Chtop interacts with the Prc1 complex. In contrast to 5FMC, this association does not depend on the methylation status of Chtop. Intriguingly, the Pc2 component of Prc1 (also known as Cbx4) has been identified as a SUMO-ligase for several transcriptional regulators (52–53). Although these studies mainly focused on SUMO-1 modification, Chtop may recruit 5FMC as an antagonist of Pc2/Cbx4.

We have shown that Chtop recruits 5FMC to Zbp-89 and that Zbp-89 is subsequently desumoylated by Senp3, resulting in higher Pol II levels on Zbp-89 target genes. We anticipated that depletion for Senp3 and Pelp1 would also affect H3K4 and/or H3K27 methylation of these regions, as 5FMC might be connected with the MLL1-WDR5 (38) and Prc1 complexes (this paper). However, no changes were observed, which is in line with the observation that overexpression of catalytically inactive Senp3 does not affect dIleH3K4 of specific promoters (48).

Collectively, these observations suggest a model where methylated Chtop recruits the 5FMC complex to factors like Zbp-89. Subsequently, the Senp3 protease desumoylates Zbp-89 and possibly additional components of repressor complexes, resulting in the stimulation of transcription of target genes (Fig. 7).

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** This article contains supplemental Tables S1 and S2 and Figs. S1 to S8.

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