Acta Biochim Biophys Sin (2010): 173–176 | © The Author 2010. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmq001.
Advance Access Publication 2 February 2010

Involvement of CENP-F in histone methylation

Juan Du, Yan Li, and Xueliang Zhu*

Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
*Correspondence address. Tel: +86-21-54921406; Fax: +86-21-54921011; E-mail: xlzhu@sibs.ac.cn

CENP-F (also named mitosin) is a multifunctional protein of 350 kDa. In interphase, it is a nuclear protein, whereas in M phase it localizes to the kinetochore, the major microtubule-binding structure on chromosomes essential for chromosome segregation. CENP-F is also critical for myocyte differentiation through the interaction with Rb. It binds to ATF4 and negatively regulates the transcriptional activity of ATF4. It is also important for mitotic progression. Here we show that depletion of CENP-F by RNAi markedly downregulated the methylation of histone H3 at K4 and K9. Consistently, association of HP1α with mitotic chromosomes was largely decreased. These results uncover a novel role of CENP-F in regulation of epigenetic modification on histone H3.

Keywords CENP-F; mitosin; histone; methylation; HP1

Received: November 18, 2009 Accepted: January 3, 2010

Introduction

CENP-F (also named mitosin) is a 350-kDa multifunctional human nuclear/kinetochore protein [1,2]. Its chicken ortholog, CMF1, is involved in cardiac muscle differentiation [3,4]. Furthermore, its mouse ortholog, LEK1, plays an important role in cardiac muscle differentiation by stimulating expressions of key transcription factors including Nkx2.5 and Mef2C through interaction with Rb, though detailed mechanisms remain unclear [5]. CENP-F can also interact with and inhibit the activity of transcription factor ATF4 [6]. In M phase, CENP-F is located on the kinetochore [7,8]. Its importance in the M phase progression has also been documented [2,9].

The nucleosome of chromatin is composed of two superhelical turns of DNA wrapped around a histone octamer containing two each of H2A, H2B, H3, and H4. Histone modifications, including methylation and acetylation, have been shown to play a key role in epigenetic regulation of chromatin structures critical for gene transcription, DNA repair, and chromatin packing [10–12]. Histone methylation occurs at lysine (K) or arginine (R) residues by histone methyltransferases and is removed by histone demethylases [11,13].

Histone methylation is important for chromatin structure and function [10,14]. For instance, methylation of histone H3 at K9 is involved in heterochromatin formation and transcription silencing [10,11,14]. Dimethylated or trimethylated histone H3 (H3DM or H3TM) at K9 in fact serves as a docking site for heterochromatin protein 1 (HP1) [15]. The HP1 family in higher organisms contains three members, α, β, and γ, among which HP1α is the most widely studied one [16,17]. The *Drosophila* HP1α is involved in heterochromatin formation, gene expression, and telomere stability [17]. In mitosis, mammalian HP1α mainly remains associated with pericentromeric heterochromatin regions of chromosomes [18,19]. In contrast, methylation of histone H3 at K4 is usually associated with actively transcribed genes [11,14].

We previously noticed that depletion of CENP-F by RNAi appeared to trigger premature chromosome decondensation prior to anaphase onset [20]. Moreover, overexpression of its N-terminal deletion mutants altered nuclear chromatin organization [21,22]. In this study, we show that CENP-F plays an important role in histone methylation. Our results provided novel clues for CENP-F functions.

Materials and Methods

Plasmids

pBS/U6 was kindly provided by Dr. Y. Shi (Harvard Medical School, Boston, USA) and was used to construct pBS/U6/Mi-1 (herein referred to as RNAi-1) for RNA interference (RNAi) of CENP-F expression [20]. Another CENP-F RNAi plasmid (RNAi-2), which expresses a different hairpin RNA [23], was from Dr. D. Cleveland (University of California, San Diego, USA). FLAG-tagged full-length CENP-F was expressed with a construct described previously [6].

Cell culture

HEK293T and HeLa cells were maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10%

calf serum (Sijiqing Company, Hangzhou, China) at 37°C in an atmosphere containing 5% CO_2. Cells were transfected using the calcium phosphate method and assayed 48 h posttransfection. HEK293T cells were used for biochemical assays due to their high transfection efficiency. HeLa cells were used for microscopic studies due to their large cell size and superior adhesion to the substratum compared with HEK293T cells.

Antibodies

Antibodies to FLAG (Sigma), H3TM(K4) (Millipore), or HP1α (Millipore) were mouse monoclonal. Rabbit antibodies to H3 or H3DM(K9) were from Millipore. Chicken anti-CENP-F IgY was described previously [24]. Secondary antibodies conjugated with Alexa 488 or 546 were purchased from Molecular Probes.

Fluorescence microscopy

Immunostaining for CENP-F and HP1α was performed as described previously [20]. Chromosomal DNA was labeled with 4′,6′-diamidino-2-phenylindole (DAPI). Fluorescence images were captured by using Leica TCS SP2 laser confocal microscope. Optical sections were scanned at 0.3-μm intervals. Z stack images were then formed by maximal projection. Statistic results were obtained in a blind fashion.

Flow cytometry

Cells were fixed in cold 70% ethanol for 1 h and then stained with propidium iodide (20 μg/ml in phosphate-buffered saline) in the presence of RNase A (200 μg/ml) for 30 min. Samples were analyzed using fluorescence-activated cell sorter (FACS) (Calibur, Becton Dickinson).

Immunoblotting

Cell lysates were subjected to 3–12% gradient SDS-PAGE. Immunoblots were developed in Western lightning chemiluminescence reagent plus (PerkinElmer Life and Analytical Sciences) and exposed to X-ray films (Kodak).

Results

CENP-F knockdown downregulates methylation of histone H3

For insights into possible roles of CENP-F in chromatin organization, we examined whether silencing CENP-F expression affected histone H3 modification. To avoid possible off-target effect, two different RNAi constructs capable of targeting different regions of the CENP-F mRNA [20,23], termed herein as RNAi-1 and RNAi-2, were used. CENP-F levels are cell cycle dependent [7,8]. To reduce potential influence of different cell cycle stages, we treated transfected HEK293T cells with nocodazole (5 μg/ml) for 12 h to block cells in M phase prior to preparation of cell lysates. FACS assays indicated successful synchronization of most cells in G2/M [**Fig. 1(A)**]. Immunoblotting indicated efficient silencing of CENP-F expression [**Fig. 1(B)**], consistent with previous reports [20,23]. Interestingly, the levels of H3DM at K9 and H3TM at K4 were markedly reduced in populations transfected with CENP-F RNAi constructs as well [**Fig. 1(B)**]. In contrast, total levels of histone H3 and HP1α were not affected [**Fig. 1(B)**].

To understand whether the changes in histone H3 modifications were specific for mitotic cells, we then enriched interphase cells by treating transfected HEK293T cells for 18 h with thymidine (2 mM). FACS assays confirmed accumulation of these cells in G1-S phases [**Fig. 1(C)**]. Immunoblotting showed that the levels of H3DM(K9) and H3TM(K4) also decreased in CENP-F-depleted cells [**Fig. 1(D)**]. Therefore, depletion of CENP-F by RNAi inhibited methylation of histone H3 independently of cell cycle progression.

CENP-F knockdown reduces the retention of HP1α on mitotic chromosomes

As H3DM at K9 can serve as a binding site for HP1α [15], we examined whether silencing CENP-F by RNAi diminished association of HP1α with chromatin in HeLa cells. Consistent with previous reports [18,25], HP1α exhibited punctate staining in the nucleus and chromosome staining in all stages of M phase [**Fig. 2(A)**], whereas CENP-F showed nuclear localization in interphase and kinetochore staining in M phase [**Fig. 2(A)**] [7,8]. Consistent with heterochromatin localization of HP1α [18,19], the immunostaining of HP1α in mitotic cells did not merge with the kinetochore staining of CENP-F [**Fig. 2(A)**, insets]. After transfection with RNAi-1, HP1α still showed punctate staining in interphase cells negative for CENP-F staining (data not shown). Nevertheless, in mitotic cells lacking kinetochore staining of CENP-F, chromosomal staining of HP1α was largely reduced [**Fig. 2(B)**]. Statistic results indicated that chromosomal HP1α was detected in 89% CENP-F-positive prometaphase cells but only in 35% CENP-F-depleted prometaphase cells ($n = 400$). In metaphase cells, the values were 92% and 40% ($n = 400$), respectively. Similar results were obtained after transfection of HeLa cells with RNAi-2 (data not shown).

Discussion

In this study, we found that CENP-F is important for methylation of histone H3. CENP-F depletion by RNAi markedly reduced methylation levels of histone H3 at K4

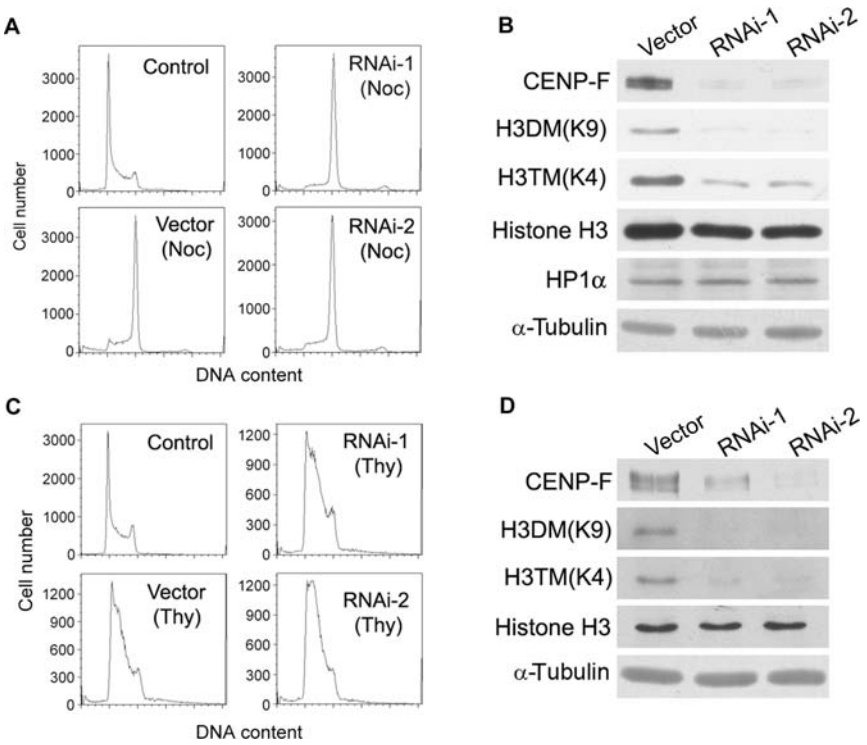

Figure 1 Effect of CENP-F RNAi on histone H3 modification HEK293T cells were transfected with vector, RNAi-1, or RNAi-2 for 36 h, followed by nocodazole (Noc) treatment for an additional 12 h to block cells in prometaphase (A, B) or by thymidine (Thy) treatment for an additional 18 h to arrest cells at the G1/S boundary or in S phase (C, D). (A, C) Flow cytometry results of each cell population. (B, D) Immunoblotting results. Cells were lysed and subjected to 3–12% gradient SDS-PAGE followed by immunoblotting to visualize the indicated proteins. Antibodies specific to dimethylated histone H3 (H3DM) at K9 or trimethylated histone H3 (H3TM) at K4 were used to assess the methylation status, respectively.

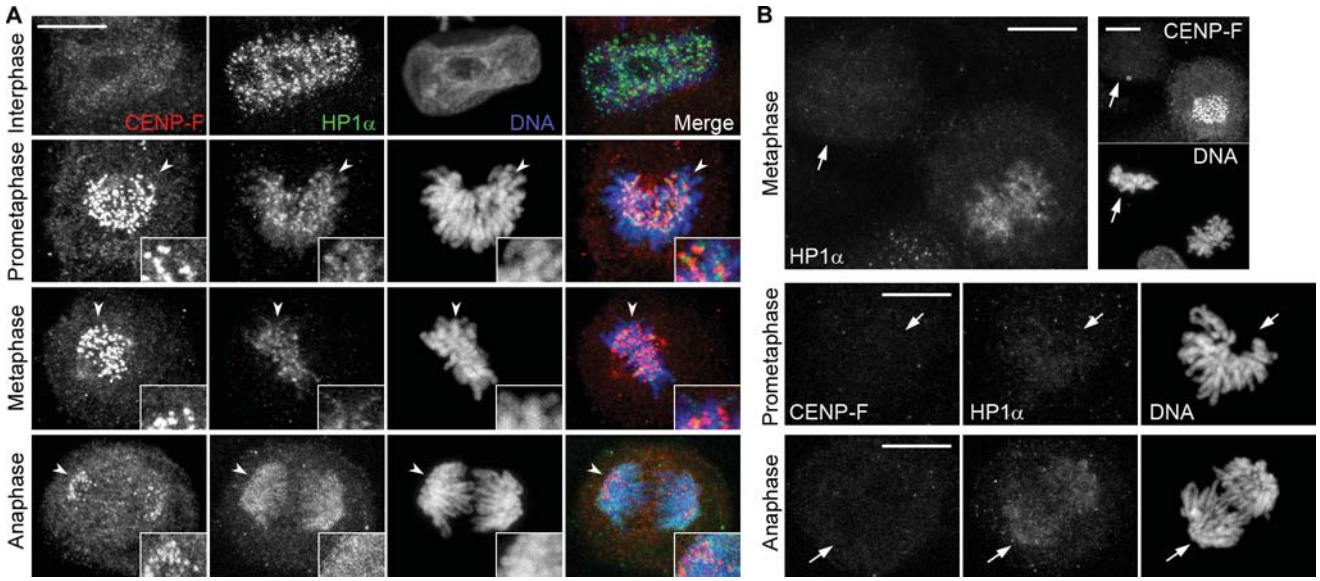

Figure 2 CENP-F depletion reduces chromosome association of HP1α in mitotic cells HeLa cells transfected with vector (A) or RNAi-1 (B) for 48 h were subjected to immunostaining to visualize CENP-F and HP1α. Representative regions in (A) (arrowheads) are magnified 2× to show details (insets). Arrows in (B) indicate CENP-F-depleted cells. Scale bar, 10 μm.

and K9 (**Figs. 1** and **2**). It is possible that CENP-F depletion also affect methylation at other sites in histone H3 and/or even sites in other histones. For instance, the reduced association of HP1 with chromosome (**Fig. 2**) suggests a decrease in trimethylation of histone H3 at K9 as well [15].

How CENP-F functions in histone methylation is currently not clear. Given that the static levels of histone

methylation are governed by both histone methyltransferases and demethylases [11,26], loss of CENP-F might either attenuate the activity of certain methyltransferase(s) or enhance the activity of certain demethylase(s). Alternatively, as a nuclear matrix protein in interphase [7], CENP-F might contribute to micro-environment that facilitates histone methylation.

Alterations in histone methylation may affect chromatin structure and gene transcription [14]. CENP-F is a nuclear matrix protein [7] and may potentially be involved in chromatin remodeling by affecting the global epigenetic modification patterns. Indeed, attenuated retention of HP1α on heterochromatin in CENP-F-depleted mitotic cells (**Fig. 2**) suggests alterations in chromosome packing or conformation [14]. The tendency for chromosome decondensation in CENP-F-depleted cells and chromosome localization of certain CENP-F mutants provides further supports [20,21]. On the other hand, the interaction of CENP-F with Rb has been shown to be critical for mouse ES cell differentiation into cardiac myocytes [5]. As Rb is also a regulator of chromatin remodeling [27], CENP-F might cooperate with Rb in chromatin remodeling to modulate transcription of certain differentiation-related genes. Certainly, further investigations are still needed to verify these speculations.

Acknowledgements

We thank Qiongping Huang, Lele Xie, and Wei Bian for technical assistance and Drs Yang Shi (Harvard Medical School, Boston, USA), Don Cleveland (University of California, San Diego, USA), and Stuart Schreiber (Broad Institute of Harvard and MIT, Cambridge, USA) for kindly providing plasmids used in this study. We also thank Dr Gang Pei for providing antibodies to histone H3.

Funding

This work was supported by the grants of the Ministry of Science and Technology of China (2007CB947100 and 2006CB943900) and the Shanghai Municipal Council for Science and Technology (08XD14048 and 09JC1416200).

References

1 Ma L, Zhao X and Zhu X. Mitosin/CENP-F in mitosis, transcriptional control, and differentiation. J Biomed Sci 2006, 13: 205–213.
2 Varis A, Salmela AL and Kallio MJ. CENP-F (mitosin) is more than a mitotic marker. Chromosoma 2006, 115: 288–295.
3 Pabon-Pena LM, Goodwin RL, Cise LJ and Bader D. Analysis of CMF1 reveals a bone morphogenetic protein-independent component of the cardiomyogenic pathway. J Biol Chem 2000, 275: 21453–21459.
4 Wei Y, Bader D and Litvin J. Identification of a novel cardiac-specific transcript critical for cardiac myocyte differentiation. Development 1996, 122: 2779–2789.
5 Papadimou E, Menard C, Grey C and Puceat M. Interplay between the retinoblastoma protein and LEK1 specifies stem cells toward the cardiac lineage. EMBO J 2005, 24: 1750–1761.
6 Zhou X, Wang R, Fan L, Li Y, Ma L, Yang Z and Yu W, *et al*. Mitosin/CENP-F as a negative regulator of activating transcription factor-4. J Biol Chem 2005, 280: 13973–13977.
7 Liao H, Winkfein RJ, Mack G, Rattner JB and Yen TJ. CENP-F is a protein of the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. J Cell Biol 1995, 130: 507–518.
8 Zhu X, Mancini MA, Chang KH, Liu CY, Chen CF, Shan B and Jones D, *et al*. Characterization of a novel 350-kilodalton nuclear phosphoprotein that is specifically involved in mitotic-phase progression. Mol Cell Biol 1995, 15: 5017–5029.
9 Liang Y, Yu W, Li Y, Yu L, Zhang Q, Wang F and Yang Z, *et al*. Nudel modulates kinetochore association and function of cytoplasmic dynein in M phase. Mol Biol Cell 2007, 18: 2656–2666.
10 Margueron R, Trojer P and Reinberg D. The key to development: interpreting the histone code? Curr Opin Genet Dev 2005, 15: 163–176.
11 Shilatifard A. Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Curr Opin Cell Biol 2008, 20: 341–348.
12 Sims RJ, 3rd, Nishioka K and Reinberg D. Histone lysine methylation: a signature for chromatin function. Trends Genet 2003, 19: 629–639.
13 Klose RJ and Zhang Y. Regulation of histone methylation by demethylimination and demethylation. Nat Rev Mol Cell Biol 2007, 8: 307–318.
14 Gelato KA and Fischle W. Role of histone modifications in defining chromatin structure and function. Biol Chem 2008, 389: 353–363.
15 Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J and Hunt DF, *et al*. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature 2005, 438: 1116–1122.
16 Lomberk G, Wallrath L and Urrutia R. The heterochromatin protein 1 family. Genome Biol 2006, 7: 228.
17 Fanti L and Pimpinelli S. HP1: a functionally multifaceted protein. Curr Opin Genet Dev 2008, 18: 169–174.
18 Minc E, Allory Y, Worman HJ, Courvalin JC and Buendia B. Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. Chromosoma 1999, 108: 220–234.
19 Hayakawa T, Haraguchi T, Masumoto H and Hiraoka Y. Cell cycle behavior of human HP1 subtypes: distinct molecular domains of HP1 are required for their centromeric localization during interphase and metaphase. J Cell Sci 2003, 116: 3327–3338.
20 Yang Z, Guo J, Chen Q, Ding C, Du J and Zhu XL. Silencing mitosin induces misaligned chromosomes, premature chromosome decondensation before anaphase onset, and mitotic cell death. Mol Cell Biol 2005, 25: 4062–4074.
21 Zhu X. Structural requirements and dynamics of mitosin-kinetochore interaction in M phase. Mol Cell Biol 1999, 19: 1016–1024.
22 Zhu XL, Ding L and Pei G. Carboxyl terminus of mitosin is sufficient to confer spindle pole localization. J Cell Biochem 1997, 66: 441–449.
23 Bomont P, Maddox P, Shah JV, Desai AB and Cleveland DW. Unstable microtubule capture at kinetochores depleted of the centromere-associated protein CENP-F. EMBO J 2005, 24: 3927–3939.
24 Yang ZY, Guo J, Li N, Qian M, Wang SN and Zhu XL. Mitosin/CENP-F is a conserved kinetochore protein subjected to cytoplasmic dynein-mediated poleward transport. Cell Res 2003, 13: 275–283.
25 Terada Y. Aurora-B/AIM-1 regulates the dynamic behavior of HP1alpha at the G2-M transition. Mol Biol Cell 2006, 17: 3232–3241.
26 Shi Y and Whetstine JR. Dynamic regulation of histone lysine methylation by demethylases. Mol Cell 2007, 25: 1–14.
27 Macaluso M, Montanari M and Giordano A. Rb family proteins as modulators of gene expression and new aspects regarding the interaction with chromatin remodeling enzymes. Oncogene 2006, 25: 5263–5267.