Biochemical and Biophysical Research Communications 441 (2013) 157–163

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of H3K18 deacetylation of Sirt7 by Myb-binding protein 1a (Mybbp1a)

Md. Fazlul Karim [a], Tatsuya Yoshizawa [a], Yoshifumi Sato [a], Tomohiro Sawa [b,c,1], Kazuhito Tomizawa [d], Takaaki Akaike [b,1], Kazuya Yamagata [a,*]

[a] Department of Medical Biochemistry, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
[b] Department of Microbiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
[c] PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan
[d] Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

ARTICLE INFO

Article history:
Received 3 October 2013
Available online 14 October 2013

Keywords:
Sirtuin
Sirt7
H3K18
Acetylation
Mybbp1a

ABSTRACT

Sirt7 localizes in the nucleus (enriched in the nucleolus) and is an NAD$^+$-dependent deacetylase with high selectivity for the acetylated lysine 18 of histone H3 (H3K18Ac). It has been reported that Sirt7 is necessary for maintaining the fundamental properties of the cancer cell phenotype and stabilizing the tumorigenicity of human cancer *via* deacetylation of H3K18Ac. However, the regulators of Sirt7 deacetylase activity are unknown. Myb-binding protein 1a (Mybbp1a) is reported to interact with and regulate the function of a number of transcription factors. In the present study, we demonstrated that Mybbp1a binds to Sirt7 *in vitro* and *in vivo*. Serial deletion studies indicated that N- and C-terminal regions of Sirt7 and C-terminal region of Mybbp1a are important for the binding. Furthermore, transfection experiments showed that Mybbp1a is capable of inhibiting the deacetylation activity of H3K18Ac by Sirt7. Our findings demonstrate that Mybbp1a is a novel negative regulator of Sirt7.

1. Introduction

Sirtuins are nicotinamide adenine dinucleotide oxidized form (NAD$^+$)-dependent deacetylases that target histones, transcription factors, and other proteins and regulate a wide range of biological pathways including energy metabolism, stress resistance, tumorigenesis, and aging. In mammals there are seven sirtuins, Sirt1 to Sirt7, which share a highly conserved NAD$^+$-binding and catalytic core domain, while having distinct flanking N- and C-terminal domains. The divergent N- and C-termini of sirtuins are responsible for their different functions and subcellular localization [1–4].

The physiological function of Sirt7 is the least well understood among the seven sirtuins. Ford et al. showed that Sirt7 is localized in the nucleus (enriched in the nucleolus) and its expression is abundant in highly proliferative tissues [5]. The authors showed that Sirt7 is associated with active ribosomal RNA (rRNA) genes, and interacts with RNA polymerase I (Pol I) machinery as well as with histones [5]. They also found that Sirt7 is a positive regulator of Pol I transcription and is required for cell viability [5]. Interaction of Sirt7 with the upstream binding factor (UBF), a component of the RNA Pol I machinery, has also been reported [6]. In addition to the role of Sirt7 on rRNA transcription, Barber et al. recently demonstrated that Sirt7 is an NAD$^+$-dependent deacetylase with high selectivity for the acetylated lysine 18 of histone H3 (H3K18Ac) and has a role in gene-specific transcriptional repression at a select subset of H3K18Ac-containing promoters [7]. They also demonstrated that deacetylation of H3K18Ac by Sirt7 is necessary for maintaining the fundamental properties of the cancer cell phenotype and stabilizing the tumorigenicity of human cancer [7].

These studies have shown the importance of the H3K18Ac deacetylase activity of Sirt7, but the regulation of Sirt7 activity is not well understood. To better understand the regulation of Sirt7, cellular proteins that interact with Sirt7 were investigated by biochemical purification. We found that Myb-binding protein 1a (Mybbp1a) bound to Sirt7 *in vitro* and *in vivo*, as reported in a recent proteomics study [8]. Serial deletion studies demonstrated that the N- and C-terminal regions of Sirt7 and the C-terminal region of Mybbp1a are important for the binding. Furthermore, transfection experiments indicated that Mybbp1a is capable of inhibiting the deacetylation activity of H3K18Ac by Sirt7. These results indicate that Mybbp1a is a novel negative regulator of Sirt7.

* Corresponding author. Address: Department of Medical Biochemistry, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, Kumamoto 860-8556, Japan. Fax: +81 96 364 6940.
E-mail address: k-yamaga@kumamoto-u.ac.jp (K. Yamagata).
[1] Present address: Department of Environmental Health Sciences and Molecular Toxicology, Tohoku University Graduate School of Medicine,Sendai, Japan.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.020

2. Methods

2.1. Plasmids

Detailed description of the plasmids used in this study is provided in the Supplementary data.

2.2. Antibodies and Western blotting

The following commercially available antibodies were used: anti-DYKDDDDK tag (anti-FLAG tag) (Wako), anti-HA (clone 3F10, Roche), anti-c-myc (Wako), anti-Histone H3 (Abcam), anti-H3K18Ac (Abcam), goat anti-rabbit IgG-HRP (Millipore), goat anti-rat IgG-HRP (Santa Cruz), donkey anti-mouse IgG-HRP (Millipore), Alexa Fluor 568 goat anti-rat IgG (Invitrogen), and Alexa Fluor 488 goat anti-mouse IgG (Invitrogen). For the detection of H3K18Ac, nuclear extracts were prepared from cells as described previously [9]. Western blotting was performed as described previously [10].

2.3. Cell culture

HEK293 cells were purchased from RIKEN BRC CELL BANK, HEK293T from Clontech, and COS-7, and HeLa cells from the American Type Culture Collection. HEK293T, COS-7, and HeLa cells were cultured in Dulbecco's modified Eagle's medium containing 25 mM glucose, 10% fetal bovine serum, and 0.02% penicillin/streptomycin; Eagle's minimal essential medium containing 5.5 mM glucose, 10% fetal bovine serum, and 1× nonessential amino acids was used for culturing HEK293.

2.4. Expression and purification of Halo fusion Sirt7 proteins

Escherichia coli K12 (KRX) (Promega) was transformed with pFN18A-Halo or pFN18A-Halo-FLAG and pFN18A-Halo-Sirt7 or pFN18A-Halo-Sirt7 (FL/M1/M2/M3/M4)-FLAG plasmids. Colony selection was performed with 100 μg/ml ampicillin, and positive cells were inoculated in 5 ml LB medium (1% tryptone, 0.5% yeast extract, and 1% NaCl (pH 7.0)) supplemented with 100 μg/ml ampicillin and cultured overnight at 37 °C. This preculture was then diluted 1:1000 in 200 ml LB medium supplemented with 100 μg/ml ampicillin and cultured at 37 °C until the optical density at 600 nm (OD_{600}) was 0.5. Cells were further cultured at 20 °C until OD_{600} reached 0.7, and expression was induced overnight with 0.1% rhamnose at 20 °C. Bacteria were centrifuged at 4,000 rpm for 10 min at 4 °C and the pellet was resuspended in 10 ml Halo purification buffer containing 50 mM Hepes KOH (pH 7.4), 150 mM NaCl, 1% NP-40 and 1 mM PMSF. Cells were disrupted by sonication at 4 °C (pulse, 20 s; interval, 20 s; ×2). The soluble cell fractions were separated by centrifugation at 10,000 rpm for 10 min at 4 °C. Detection of Halo-Sirt7-FLAG proteins was performed by Western blotting using anti-FLAG antibody.

Five milliliters of the *E. coli* supernatant was mixed overnight with 100 μL HaloLink resin (Promega) at 4 °C. The resin was then precipitated by centrifugation at 2000 rpm for 30 s at 4 °C and the supernatant was removed. The resin was then washed five times with Halo purification buffer, resuspended in 100 μL Halo purification buffer, and used for the HaloTag pull-down assay.

Sirt7 proteins were removed from the HaloTag by adding 5 units of ProTev Plus protease (Promega) according to the manufacturer's protocol. After overnight incubation at 4 °C, the resin was precipitated by centrifugation at 2000 rpm for 1 min at 4 °C and the resulting supernatant containing both ProTev Plus and Sirt7 proteins was collected. The purity of the Sirt7 proteins was examined by SDS–PAGE and gel staining with Coomassie Brilliant Blue (CBB).

2.5. HaloTag pull-down assay

Halo-Sirt7 proteins were expressed in *E. coli* K12 (KRX) and purified with HaloLink resin. Nuclear extracts were prepared from C57BL/6 mouse liver as described previously [11]. COS-7 cells were transfected using JetPRIME transfection reagent (Polyplus, NY) with a pcDNA-$p160^{MBP}$-myc vector. After 24 h, cells were lysed in buffer containing 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.5% NP-40, 10 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM PMSF, and protease inhibitor cocktail (Nacalai Tesque). Halo or Halo-fusion Sirt7 proteins (30 μg) immobilized on the HaloLink resin were incubated with 500 μg mouse liver nuclear extract or 400 μg COS-7 lysate in the pull-down buffer containing 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% NP-40 (0.5% NP-40 for COS-7 cell lysate), 10 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM PMSF, and protease inhibitor cocktail. After binding overnight at 4 °C, the resins were washed five times with the pull-down buffer, and the bound proteins were separated by SDS–PAGE.

2.6. Proteomic analysis

Silver-stained gels were subjected to in-gel digestion with trypsin, followed by extraction of peptides and proteomic analysis by LC–MS/MS as described previously [12]. MS/MS data were processed in the form of Mascot MS/MS ion searches of the National Center for Biotechnology Information nonredundant database using the Matrix Science Web server Mascot version 2.2.

2.7. Immunoprecipitation

HEK293 cells were transfected using X-tremeGENE HP DNA transfection reagent (Roche) with pcDNA3-FLAG-Sirt7 and pcDNA-p160MBP-myc vectors. COS-7 cells were transfected using JetPRIME transfection reagent (Polyplus) with pcDNA3-FLAG-Sirt7 and pcDNA-p160C-myc vectors. After 24 h, cells were lysed in IP buffer (20 mM Tris–HCl (pH 7.4), 200 mM NaCl, 2.5 mM $MgCl_2$, 0.05% NP-40, 1 mM PMSF, and protease inhibitor cocktail). Next, lysed cells were passed through a 29Gx1/2″ (0.33 × 13 mm) needle (Terumo) 6 times and incubated on ice for 30 min. Cell lysate was collected after centrifugation at 15,000 rpm for 20 min at 4 °C. Then 700 μg cell lysate and FLAG tag antibody beads (Wako) were mixed and stirred at 4 °C for 18 h. After washing with IP buffer, proteins were eluted using 2× SDS sample buffer (100 mM Tris–HCl (pH 6.8), 4% SDS, 20% glycerol, and 0.2% Bromophenol Blue). Binding was examined by Western blotting.

2.8. In vitro transcription and translation

After linearization with NotI, 1 μg of the pTD1-Mybbp1a-HA and pTD1-RLuc-HA constructs were transcribed at 37 °C for 4 h according to the manufacturer's protocol (ScriptMAX Thermo T7 Transcription Kit, Toyobo, Japan). mRNAs were purified using G50 Sephadex, and 10 μg of mRNA was translated at 25 °C for 5 h according to the manufacturer's protocol (Transdirect Insect Cell, Shimadzu Corporation). Fifty microliters of *in vitro* translated proteins (including translated machinery proteins) were incubated with 30 μg Halo or Halo-Sirt7 in the pull-down buffer containing 20 mM Hepes KOH (pH 7.4), 200 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM NaF, 1% NP-40, 1 mM PMSF, and protease inhibitor cocktail at 4 °C for 12 h. After washing with pull-down buffer, binding was examined by Western blotting with anti-HA antibody.

2.9. Immunocytochemistry

Both HA-tagged Sirt7 and myc-tagged Mybbp1a expression vectors (pcDNA-$p160^{MBP}$, pcDNA-$p67^{MBP}$, and pcDNA-p160C) were

transfected into HeLa cells with JetPRIME transfection reagent (Polyplus). After 24 h, cells were fixed in 10% neutralized formalin and permeabilized with 0.1% Triton X-100/3% BSA/PBS. Monoclonal rat anti-HA antibody (1:1000) and mouse anti-c-myc antibody (1:1000) were used as the primary antibodies, and Alexa Fluor 568 goat anti-rat IgG and Alexa Fluor 488 goat anti-mouse IgG were used as the secondary antibodies. Immunofluorescence was detected under a laser scanning confocal microscope (FV-1000, Olympus) [12].

2.10. RNA interference

For knockdown of Mybbp1a, HEK293T cells were transfected with control (Mission siRNA Universal Negative Control, SIC-001, Sigma) or Mybbp1a specific siRNA (target sequence; 5′-GCCGACUUGAAUAUAAUAC-3) (Sigma) [13] using HiPerfect transfection reagent (Qiagen) according to the manufacturer's protocol.

2.11. Quantitative reverse transcription-PCR

Total RNA was isolated from HEK293T cells using Sepasol RNA I super reagent (Nacalai Tesque). cDNA synthesis was performed with 1 μg total RNA using a PrimeScript RT reagent kit (TaKaRa). Quantitative reverse transcription-PCR was performed with SYBR Premix Ex TaqII (RR820A, TaKaRa) and an ABI 7300 thermal cycler (Applied Biosystems). The following primers were used. For mouse Mybbp1a: 5′-AGCACCTTCTGCTCCTCGT-3′ and 5′-ATGCAGGTCTGG ATGTCACC-3′; and for mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH): 5′-AGCCACATCGCTCAGACAC-3′ and 5′-GCCCA ATACGACCAAATCC-3′.

2.12. Statistical analysis

Values are expressed as the mean ± SD. Statistical significance was tested using two-tailed Student's *t*-test. A p value less than 0.05 was considered significant.

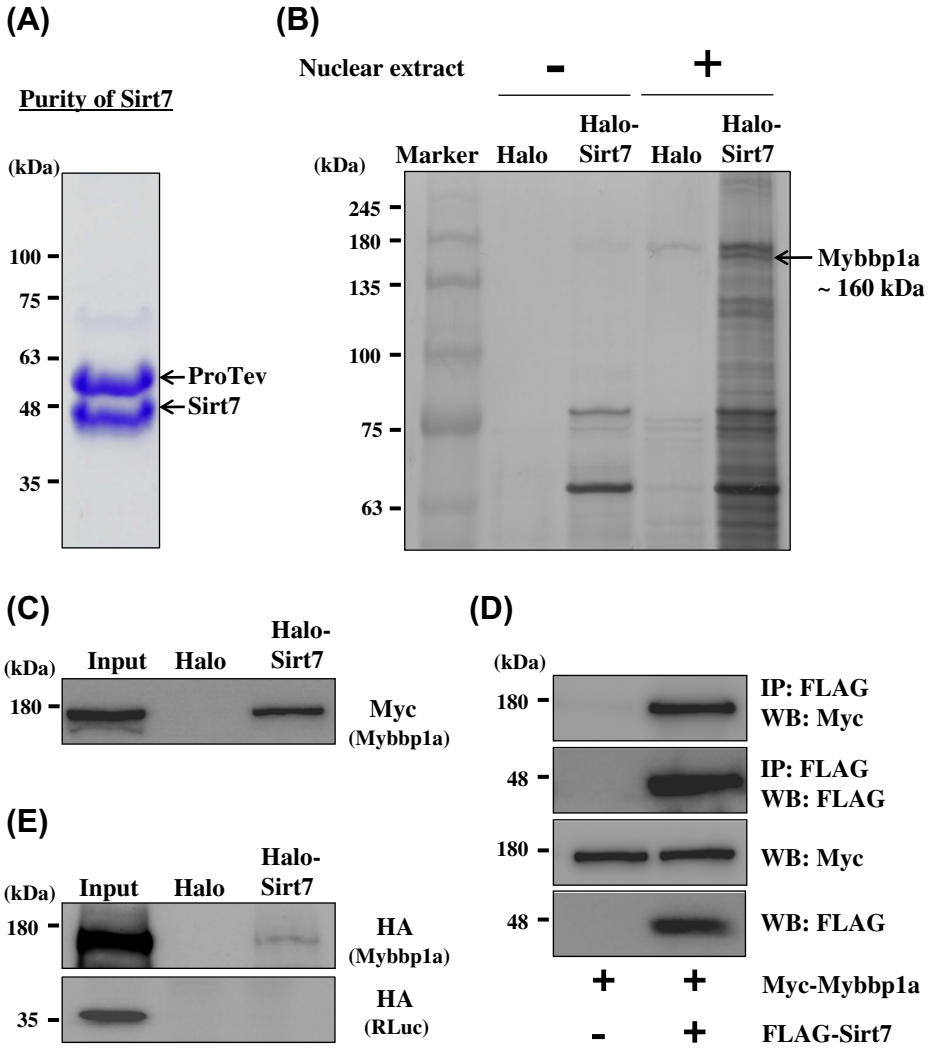

Fig. 1. Interaction between Sirt7 and Mybbp1a *in vitro* and *in vivo*. (A) Purification of Sirt7. Halo-Sirt7 immobilized on HaloLink resin was digested with ProTev Plus protease (51 kDa) to cleave Sirt7 (46 kDa) from Halo which is covalently bound to resin. Proteins were resolved by SDS–PAGE and the gel was stained with Coomassie Brilliant Blue. (B) Mouse liver nuclear lysates were subjected to the Halo pull-down assay using Halo-Sirt7, and the eluted proteins were resolved by SDS–PAGE and silver stained. Mass spectrometry identified a protein of approximately 160 kDa as Mybbp1a. (C) COS-7 cells were transfected with a pcDNA-p160^{MBP}-myc expression plasmid, and cell lysates were incubated with Halo fused with Sirt7. HaloLink resin-bound proteins were separated by SDS–PAGE and Western blotting was performed with anti-myc antibody. (D) HEK293 cells were transfected with FLAG-tagged Sirt7 and myc-tagged Mybbp1a expression plasmids. The interaction was evaluated by immunoprecipitation with FLAG resin, followed by immunoblotting. (E) HA-tagged Mybbp1a (top) and HA-tagged Renilla luciferase (bottom) were translated *in vitro* and incubated with Halo-Sirt7 immobilized on HaloLink resin. Eluted proteins were separated by SDS–PAGE and Western blotting was performed with anti-HA antibody. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Mybbp1a interacts with Sirt7 both in vitro and in vivo

To identify Sirt7-interacting proteins, we used Halo-fused FL mouse Sirt7 for the pull-down assay with mouse liver nuclear lysate. Expression of FL-Sirt7 was confirmed by CBB staining (Fig. 1A). Protein of approximately 160 kDa that specifically precipitated with Halo-Sirt7 was repeatedly found and identified as Mybbp1a by mass spectrometry analysis (Fig. 1B and Supplementary Fig. 1). Mybbp1a was originally found to interact with the c-Myb proto-oncogene product (c-Myb) [14]. Mybbp1a has also been shown to interact with a number of transcription factors, including PGC-1α, aryl hydrocarbon receptor, RelA/p65, Prep1, and p53 [15–19].

Mybbp1a is found predominantly in the nucleolus [14] where Sirt7 is also localized. In addition, since a recent proteomics study revealed that Mybbp1a is an interactive partner of Sirt7 [8], we focused on Mybbp1a for further investigation. COS-7 cells were transfected with the myc-tagged Mybbp1a expression plasmid, and Mybbp1a-transfected cell lysates and Halo-Sirt7 fusion proteins were used to assess the interaction between Mybbp1a and the fusion proteins. As shown in Fig. 1C, Halo-Sirt7, but not Halo, interacted strongly with Mybbp1a.

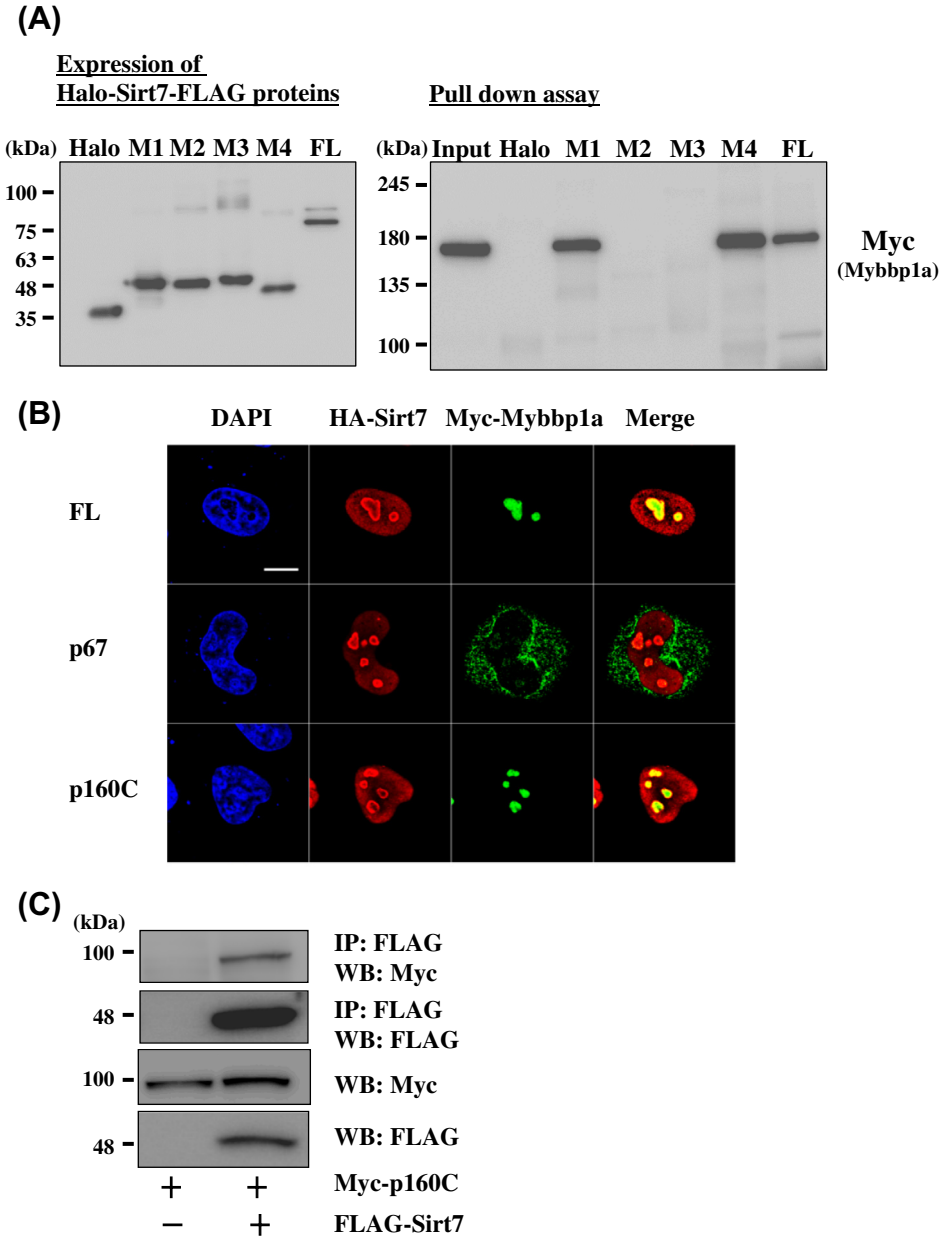

Fig. 2. Mapping the interaction regions between Sirt7 and Mybbp1a. (A) Expression of Halo-FLAG and Halo-Sirt7-FLAG proteins (full-length and M1–4). Protein expression was determined by Western blotting with anti-FLAG antibody (left panel). COS-7 cells were transfected with a pcDNA-p160^{MBP}-myc expression plasmid. Cell lysates were incubated with Halo fused with Sirt7. Halo-Sirt7-FLAG proteins immobilized on HaloLink resin were incubated with COS-7 lysates. Eluted proteins were separated by SDS–PAGE and Western blotting was performed with anti-myc antibody (right panel). (B) Intracellular localization of Sirt7 and Mybbp1a. HeLa cells were cotransfected with the pcDNA3-HA-Sirt7 and myc-tagged Mybbp1a expression vectors (FL, p67, and p160C). Cells were double stained with anti-HA antibody (Alexa Fluor 563; red) and anti-Myc antibody (Alexa Fluor 488; green). DAPI (blue) was used for nuclear staining. Scale bar = 10 μm. (C) COS-7 cells were transfected with pcDNA3-FLAG-Sirt7 and pcDNA-p160C-Myc expression vectors. Sirt7 was immunoprecipitated using FLAG tag antibody beads and separated by SDS–PAGE, and p160C or Sirt7 was detected by Western blotting.

Next, we evaluated the interaction between Sirt7 and Mybbp1a in cultured cells. HEK293 cells were transfected with the FLAG-Sirt7 expression plasmid alone or with FLAG-Sirt7 plus myc-Mybbp1a expression plasmids, and cell lysates were immunoprecipitated with FLAG resin. FLAG-Sirt7 was able to co-immunoprecipitate Mybbp1a (Fig. 1D), indicating that Sirt7 binds to Mybbp1a in cells. Halo-Sirt7 bound to *in vitro*-translated HA-tagged Mybbp1a, but not to unrelated HA-tagged RLuc, suggesting direct binding of Mybbp1a to Sirt7 (Fig. 1E).

3.2. Mapping the interaction regions between Sirt7 and Mybbp1a

Sirt7 contains a conserved NAD^+-binding and catalytic core domain (amino acids 91–332), flanking N-terminal region (amino acids 1–90), and C-terminal (amino acids 333–402) region. To more precisely map the interaction domain between Sirt7 and Mybbp1a, we generated deletion mutants of Sirt7 (Supplementary Fig. 2). Halo-FL-Sirt7-FLAG and Halo-Sirt7-FLAG deletion mutants (M1–4) were incubated with COS-7 lysate expressing myc-tagged Mybbp1a, and their interactions were investigated by pull-down assay. Both the N-terminal (M1) and C-terminal (M4) regions of Sirt7 bound to Mybbp1a as well as to FL-Sirt7, but no interaction was observed between the core domain (M2 and M3) of Sirt7 and Mybbp1a (Fig. 2A), indicating that the N- and C-terminal flanking regions of Sirt7 are involved in the interaction.

Mybbp1a contains several short basic amino acid repeat sequences in the C-terminal, which are responsible for nucleolar localization (Supplementary Fig. 3) [14,15,20]. HeLa cells were transfected with HA-tagged Sirt7 and myc-tagged Mybbp1a expression vectors (Mybbp1a (FL), N-terminal half of Mybbp1a (p67), and C-terminal half of Mybbp1a (p160C)) [15], and the intracellular localization of Mybbp1a was investigated by immunofluorescence microscopy. Sirt7 was localized predominantly in the nucleolus [5,6], and Mybbp1a (FL) was also located within the nucleolus as described previously [14]. Double staining for Sirt7 and Mybbp1a (FL and p160C) revealed colocalization in the nucleolus, while p67-Mybbp1a was localized in the cytoplasm and did not colocalize with Sirt7 (Fig. 2B), indicating that the C-terminal of Mybbp1a is required for proper targeting to the nucleolus to bind with Sirt7.

Next, FLAG-tagged Sirt7 and myc-tagged p160C were cotransfected into COS-7 cells to investigate whether Sirt7 binds to the C-terminal of Mybbp1a in cells. Sirt7 was then immunoprecipitated and the complex was run on SDS–PAGE and analyzed by Western blotting. As shown in Fig. 2C, p160C was immunoprecipitated with Sirt7. These results indicate that the C-terminal of Mybbp1a binds to Sirt7 within the nucleolus.

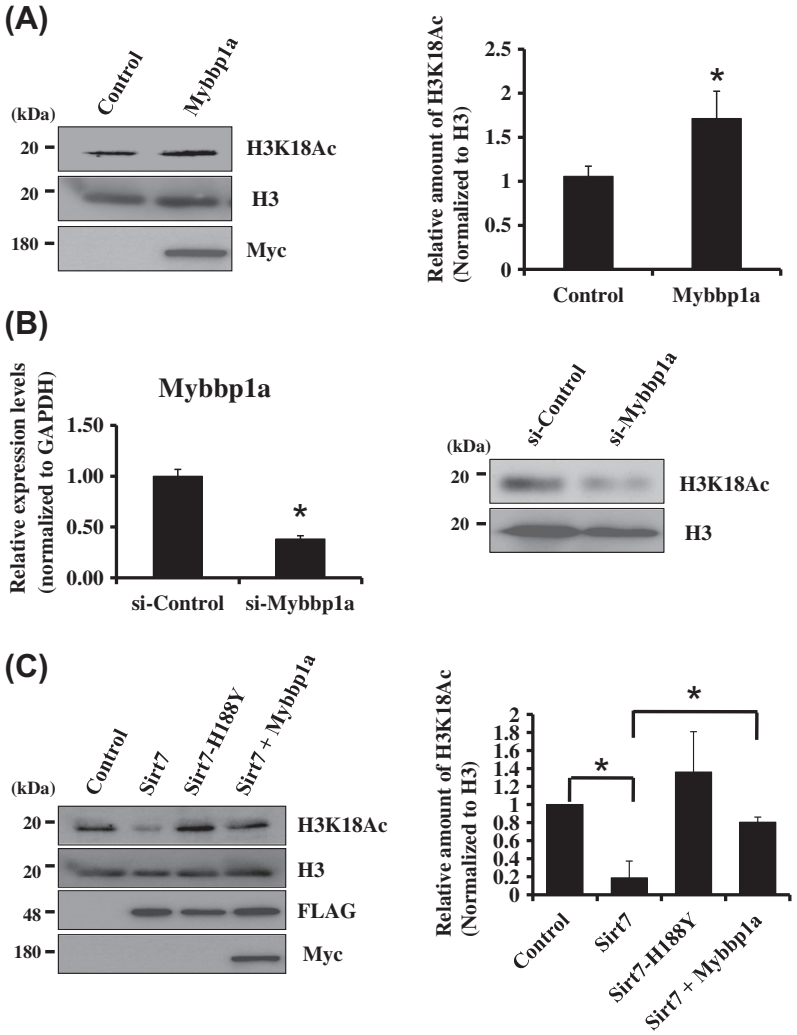

Fig. 3. Mybbp1a inhibits the deacetylation of H3K18Ac by Sirt7. (A) Western blot analysis showing H3K18Ac levels in HEK293 cells transfected with myc-tagged Mybbp1a or control empty vector. Data are the mean ± SD of 3 experiments. $p < 0.05$. (B) HEK293T cells were transfected with Mybbp1a siRNA or control siRNA. Data are expressed as the mean ± SD of 6 experiments, $p < 0.05$ (left). Western blotting showing H3k18Ac levels in HEK293T cells transfected with Mybbp1a siRNA (right). (C) Western blot analysis showing H3K18Ac levels in HEK293T cells transfected with FLAG-tagged Sirt7 (WT or H188Y) and myc-tagged Mybbp1a expression vectors. Data are the mean ± SD of 3 experiments. $p < 0.05$.

3.3. Mybbp1a inhibits the deacetylation of H3K18Ac by Sirt7

To investigate the functional relevance of the interaction between Sirt7 and Mybbp1a, HEK293 cells were transfected with Mybbp1a expression vector and H3K18Ac levels were evaluated by Western blotting. Mybbp1a overexpression significantly increased H3K18Ac levels in cells (Fig. 3A). We also investigated the effect of knockdown of Mybbp1a in HEK293T cells. Suppression of endogenous Mybbp1a mRNA by siRNA (reduced to 38.2% of the control level) decreased H3K18Ac levels (Fig. 3B). These results suggest that Mybbp1a has a role in regulating H3K18Ac levels. Next, HEK293T cells were transfected with FLAG-tagged Sirt7 (wild-type (WT) and H188Y mutant) and/or myc-tagged Mybbp1a expression vectors. H188Y is a loss-of-function mutation of Sirt7, located in the sirtuin core domain [5]. Overexpression of WT-Sirt7 significantly decreased the H3K18Ac level in cells, whereas H188Y-Sirt7 lacked deacetylation activity (Fig. 3C) as described previously [7]. Co-expression of Mybbp1a and Sirt7 did not affect the expression of FLAG-Sirt7 (Fig. 3C (left), lane 4). However, the reduction in Sirt7-dependent levels of H3K18Ac was significantly increased by the co-expression of Mybbp1a (Fig. 3C (right), lane 4), indicating that Mybbp1a inhibits the deacetylation activity of Sirt7.

4. Discussion

Sirt7 localizes to the nucleus (enriched in the nucleolus), where it binds to rRNA genes and activates RNA Pol I transcription by interacting with Pol I and UBF [5,6]. H3K18Ac is a general marker of active transcription, and H3K18 hypoacetylation has been reported as a marker of malignancy in various human cancers [21–23]. It was recently shown that Sirt7 is a highly selective H3K18 deacetylase, and that ELK4, a MAPK signaling-dependent ETS transcription factor, targets Sirt7 to specific promoters for H3K18 deacetylation [7]. Sirt7 is also necessary for maintaining features of the human cancer cell phenotype by suppressing the expression of tumor suppressor genes via locus-specific deacetylation of H3K18Ac [7]. These findings confirm the importance of the H3K18Ac deacetylase activity of Sirt7, but how Sirt7 H3K18Ac deacetylation activity is regulated remains unknown.

Mybbp1a is a component of chromatin remodeling complex B-WICH [23] and has been reported to suppress rRNA transcription and maintain rDNA repeats in a silenced state [13,24]. Moreover, increasing evidence indicates that Mybbp1a interacts with a number of nuclear factors and regulates target gene transcription by RNA polymerase II. In the present study, we demonstrated that Mybbp1a interacts with Sirt7 both *in vitro* and *in vivo*, which is consistent with the results of a recent proteomics study that showed an interaction between SIRT7 and the B-WICH complex including Mybbp1a [8]. Our study extends this previous research by showing that Mybbp1a binds to Sirt7 with an affinity for the N- and C-terminal domains of Sirt7 (M1 and M4; Fig. 2A). Because the flanking regions are divergent within the sirtuin family, the binding of Mybbp1a to Sirt7 is likely specific. The interaction was not dependent on the conserved enzymatic core domain (Fig. 2A, M2 and M3), suggesting that the deacetylation activity of Sirt7 might not be necessary for interacting with Mybbp1a.

We also demonstrated inhibition of H3K18Ac deacetylase activity of Sirt7 by Mybbp1a. The mechanism by which Mybbp1a inhibits Sirt7 activity is presently unknown; however, the following mechanisms might be involved: (1) Mybbp1a might directly inhibit the interaction between Sirt7 and H3K18Ac; (2) Mybbp1a might inhibit the binding of ELK4 to its binding site; and (3) Mybbp1a might recruit proteins with H3K18 acetylation activity. Further experiments are necessary to examine the precise mechanism of inhibition of Sirt7 by Mybbp1a.

It has been reported that Mybbp1a-downregulated NIH3T3 cells are more susceptible to Ras-induced transformation and cause more potent Ras-driven tumors [25]. Mybbp1a may act as a tumor suppressor, at least in part, by inhibiting Sirt7. Our findings that Mybbp1a suppresses H3K18Ac deacetylation activity by Sirt7 may have significant implications for the treatment of cancer.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas, a Grant-in-Aid for Scientific Research (C), a Grant from Banyu Life Science Foundation International, and a Grant from Japan Diabetes Foundation. Md. Fazlul Karim is supported by the scholarship for the International Priority Graduate Programs; Advanced Graduate Courses for International Students, MEXT.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.020.

References

[1] L. Guarenete, Sir2 links chromatin silencing, metabolism, and aging, Genes Dev. 14 (2000) 1201–1206.
[2] X. Li, N. Kazgan, Mammalians sirtuins and energy metabolism, Int. J. Biol. Sci. 7 (2011) 575–587.
[3] R.H. Houtkooper, E. Pirinen, J. Auwerx, Sirtuins as regulators of metabolism and healthspan, Nat. Rev. Mol. Cell Biol. 13 (2012) 225–238.
[4] M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance, Annu. Rev. Pathol. 5 (2010) 253–295.
[5] E. Ford, R. Voit, G. Liszt, C. Magin, I. Grummt, L. Guarente, Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription, Genes Dev. 20 (2006) 1075–1080.
[6] A. Grob, P. Roussel, J.E. Wright, B. Mcstay, D. Hernandez-Verdun, V. Sirri, Involvement of SIRT7 in resumption of rDNA transcription at the exit from mitosis, J. Cell Sci. 15 (122) (2009) 489–498.
[7] M.F. Barber, E. Michishita-Kioi, Y. Xi, L. Tasselli, M. Kioi, Z. Moqtadiri, R.I. Tennen, S. Paredes, N.L. Young, K. Chen, K. Struhl, B.A. Garcia, O. Gozani, W. Li, K.F. Chua, SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation, Nature 487 (2012) 114–118.
[8] Y.C. Tsai, T.M. Greco, A. Boonmee, Y. Miteva, I.M. Cristea, Functional proteomics establishes the interaction of SIRT7 with chromatin remodeling complexes and expands its role in regulation of RNA polymerase I transcription, Mol. Cell. Proteomics 11 (2012) 60–76.
[9] E. Schreiber, P. Matthias, M.M. Müller, W. Schaffner, Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells, Nucleic Acids Res. 17 (1989) 6419.
[10] K. Yamagata, Q. Yang, K. Yamamoto, H. Iwahashi, J. Miyagawa, K. Okita, I. Yoshiuchi, J. Miyazaki, T. Noguchi, H. Nakajima, M. Namba, T. Hanafusa, Y. Matsuzawa, Mutation P291fsinsC in the transcription factor hepatocyte nuclear factor-1alpha is dominant negative, Diabetes 47 (1998) 1231–1235.
[11] H.T. Tran, A. Ulke, N. Morrice, C.J. Johannes, G.B. Moorhead, Proteomic characterization of protein phosphatase complexes of the mammalian nucleus, Mol. Cell. Proteomics 3 (2004) 257–265.
[12] Y. Sato, M. Hatta, M.F. Karim, T. Sawa, F.Y. Wei, S. Sato, M.A. Magnuson, F.J. Gonzalez, K. Tomizawa, T. Akaike, T. Yoshizawa, K. Yamagata, Anks4b, a novel target of HNF4α protein interacts with GRP78 protein and regulates endoplasmic reticulum stress-induced apoptosis in pancreatic β-cells, J. Biol. Chem. 287 (2012) 23236–23245.
[13] J. Hochstatter, M. Hölzel, M. Rohrmoser, L. Schermelleh, H. Leonhardt, R. Keough, T.J. Gonda, A. Imhof, D. Eick, G. Längst, A. Németh, Myb-binding protein 1a (Mybbp1a) regulates levels and processing of pre-ribosomal RNA, J. Biol. Chem. 287 (2012) 24365–24377.
[14] F.J. Tavner, R. Simpson, S. Tashiro, D. Favier, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, E.M. Macmillan, J. Lutwyche, R.A. Keough, S. Ishi, T.J. Gonda, Molecular cloning reveals that the p160 Myb-binding protein is a novel, predominantly nucleolar protein which may play a role in transactivation by Myb, Mol. Cell. Biol. 18 (1998) 989–1002.
[15] M. Fan, J. Rhee, J. St-Pierre, C. Handschin, P. Puigserver, J. Lin, S. Jaeger, H. Erdjument-Bromage, P. Tempest, B.M. Spiegelman, Suppression of mitochondrial respiration through recruitment of p160 Myb binding protein to PGC-1α: modulation by p38 MAPK, Genes Dev. 18 (2004) 278–289.
[16] L.C. Jones, S.T. Okino, T.J. Gonda, J.P. Whitlock Jr., Myb-binding protein 1a augments AhR-dependent gene expression, J. Biol. Chem. 21 (2002) 22515–22519.

[17] H.R. Owen, M. Elser, E. Cheung, M. Gersbach, W.L. Kraus, M.O. Hottiger, MYBBP1a is a novel repressor of NF-kappaB, J. Mol. Biol. 366 (2007) 725–736.
[18] V.M. Diaz, S. Mori, E. Longobardi, G. Menendez, C. Ferrai, R.A. Keough, A. Bachi, F. Blasi, P160 Myb-binding protein interacts with Prep1 and inhibits its transcriptional activity, Mol. Cell. Biol. 27 (2007) 7981–7990.
[19] T. Kuroda, A. Murayama, N. Katagiri, Y.M. Ohta, E. Fujita, H. Masumoto, M. Ema, S. Takahashi, K. Kimura, J. Yanagisawa, RNA content in the nucleolus alters p53 acetylation via MYBBP1A, EMBO J. 30 (2011) 1054–1066.
[20] R.A. Keough, E.M. Macmillan, J.K. Lutwyche, J.M. Gardner, F.J. Tavner, D.A. Jans, B.R. Henderson, T.J. Gonda, Myb-binding protein 1a is a nucleocytoplasmic shuttling protein that utilizes CRM1-dependent and independent nuclear export pathways, Exp. Cell Res. 289 (2003) 108–123.
[21] S.K. Kurdistani, S. Tavazoie, M. Grunstein, Mapping global histone acetylation patterns to gene expression, Cell 117 (2004) 721–733.
[22] D.B. Seligson, S. Horvath, M.A. McBrian, V. Mah, H. Yu, S. Tze, Q. Wang, D. Chia, L. Goodglick, S.K. Kurdistani, Global levels of histone modifications predict prognosis in different cancers, Am. J. Pathol. 174 (2009) 1619–1628.
[23] P. Martínez-Redondo, I. Santos-Barriopedro, A. Vaquero, A big step for SIRT7, one giant leap for sirtuin in cancer, Cancer Cell 21 (2012) 719–721.
[24] B.C. Tan, C.C. Yang, C.L. Hsieh, Y.H. Chou, C.Z. Zhong, B.Y. Yung, H. Liu, Epigenetic silencing of ribosomal RNA genes by Mybbp1a, J. Biomed. Sci. 19 (2012) 57.
[25] S. Mori, R. Bernardi, A. Laurent, M. Resnati, A. Crippa, A. Gabrieli, R. Keough, T.J. Gonda, F. Blasi, Myb-binding protein 1A (MYBBP1A) is essential for early embryonic development, controls cell cycle and mitosis, and acts as a tumor suppressor, PLoS One 7 (2012) e39723.