ARTICLE
Journal of Cellular Biochemistry 115:1967–1973 (2014)

Journal of Cellular Biochemistry

Two Distinct Nuclear Localization Signals in Mammalian MSL1 Regulate Its Function

Ruslan I. Dmitriev,[1,2]* Nikolay B. Pestov,[2] Mikhail I. Shakhparonov,[2] and Irina A. Okkelman[1,2]

[1]School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland

[2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 16/10, Miklukho-Maklaya 117997, Moscow, Russia

ABSTRACT

MSL1 protein regulates global histone H4 acetylation at residue K16 in stem and cancer cells, through interaction with KAT8. The functional significance of mammalian MSL1 isoforms, involved in various protein interactions, is poorly understood. We report the identification of a novel nuclear localization signal (NLS), common to all MSL1 isoforms, in addition to previously known bipartite NLS, located in domain PEHE. Isoforms having both NLS localize to sub-nuclear foci where they can target co-chaperone protein TTC4. However, all MSL1 isoforms also have ability to affect H4K16 acetylation. Thus, presence of two NLS in MSL1 protein can mediate activity of KAT8 in vivo. J. Cell. Biochem. 115: 1967–1973, 2014.

KEY WORDS: HISTONE ACETYLATION; KAT8; MSL1; NUCLEUS; SUB-NUCLEAR LOCALIZATION

The regulation of gene expression via histone tail modifications is of paramount importance for healthy and diseased conditions in mammalian cells, tissue and the whole organism [Barth and Imhof, 2010]. In particular, studies of histone acetylation recognized the role of histone-modifying enzymes such as acetyltransferases (HATs) and deacetylases (HDACs), often inter-regulated and linking gene expression with nutrient and energy production [Voss and Thomas, 2009; Bao and Sack, 2010]. Since been described as the prevalent posttranslational chromatin modification in eukaryotes, regulating its structure and protein interactions [Shogren-Knaak et al., 2006], the role of H4K16Ac was further revealed in such processes as response to DNA damage [Krishnan et al., 2011], autophagy [Füllgrabe et al., 2013], RNA polymerase II promoter-proximal pausing [Kapoor-Vazirani et al., 2011] and embryonic stem cell development [Taylor et al., 2013]. Reversible acetylation of residue K16 in histone H4 (H4K16Ac) is regulated through activity of NAD^+-dependent SIRT1 deacetylase [Mulligan et al., 2011] and a number of reported HATs, including GCN5 [Orpinell et al., 2010], TIP60 [Miyamoto et al., 2008] and MYST1 (KAT8) [Voss and Thomas, 2009]. While in different cell models different HATs are reportedly active towards H4K16Ac, expression analysis of tissues demonstrates KAT8 as the predominant HAT which is expressed ubiquitously [Thomas et al., 2007].

In mammalian cells, KAT8 is associated with two multi-protein complexes, based on MSL (from "male-specific lethal") and NSL ("non-specific lethal") proteins [Li et al., 2009]. The complex NSL-KAT8 is important for acetylation of p53 and regulation of pro-apoptotic events [Li et al., 2009] yet main function of MSL-KAT8 complex is the regulation H4K16Ac [Smith et al., 2005; Taipale et al., 2005; Mendjan et al., 2006]. This activity of KAT8 is fulfilled through highly conservative interaction with MSL1, MSL2, and MSL3 proteins, with MSL1 playing a central role in the assembly of the HAT complex [Smith et al., 2005; Kadlec et al., 2011].

Mammalian MSL1 (also known as "hampin") first identified as a homologue of Drosophila MSL1 protein [Marín, 2003], displays isoform diversity with tissue-specific expression [Dmitriev et al., 2005]. The primary structure of the longest isoforms (600–616 a.a.) includes 4 distinct domains, Pro-rich I, coiled coil IIcc, III and well-characterized IV PEHE, involved in interactions with MSL3 and KAT8 [Dmitriev et al., 2005; Kadlec et al., 2011]. In mouse, three of the ubiquitously expressed (A–C) and two testis-specific (D, E) isoforms of MSL1 were described [Dmitriev et al., 2005]. All isoforms

Abbreviations: a.a., amino acid; BP, bipartite; GFP, green fluorescent protein; MSL, male-specific lethal; NLS, nuclear localization signal; RFP, red fluorescent protein.

*Correspondence to: Ruslan I. Dmitriev, Cavanagh Pharmacy Building, College Road, Cork, Ireland.
E-mail: r.dmitriev@ucc.ie

Manuscript Received: 21 April 2014; Manuscript Accepted: 2 June 2014
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 10 June 2014
DOI 10.1002/jcb.24868 • © 2014 Wiley Periodicals, Inc.

display nuclear localization, although only A, B, and D contain domain IV PEHE and known bipartite (BP) nuclear localization signal (KR repeats at a.a. 507–521 of isoform A). The presence of N-terminal coiled-coil region suggests homo- or hetero-dimerization of protein isoforms in vivo. Apart from being an adaptor or scaffold protein for KAT8 association with chromatin [Kadlec et al., 2011] MSL1 is directly involved in multiple protein interactions with nuclear and cytoplasmic residents: Nupr1 [Aguado-Llera et al., 2013], NOP17/Pih1d1 [Dmitriev et al., 2007], TPR-containing molecular co-chaperone TTC4 [Dmitriev et al., 2007, 2009], Foxp3 [Rudra et al., 2012] and others. Through these interactions, physiological function of MSL1 should involve regulation of nucleo-cytoplasmic transport of molecular co-chaperones [Crevel et al., 2001, 2008], DNA repair [Aguado-Llera et al., 2013] or recruitment of tissue specific transcription factors [Dmitriev et al., 2007; Rudra et al., 2012]. The detailed analysis of the function of MSL1 protein and its isoform diversity must be beneficial for deeper understanding of regulation of histone acetylation (H4K16Ac) in developing and quiescent tissues. On the other hand, investigation of transport across nuclear envelope is important for understanding of numerous pathological and physiological conditions such as tumorigenesis or viral infection [Mor et al., 2014]. Here, we studied nuclear localization of MSL1 and identified a novel signal of nuclear localization, present in all protein isoforms. We found that the combination of both NLS allows for intra-nuclear focal accumulation of the protein and nuclear transport of TTC4 protein while all MSL1 isoforms affect H4K16Ac.

MATERIALS AND METHODS

CELL CULTURE

Mouse NIH-3T3 fibroblasts, human embryonic kidney HEK-293 cells, human colon cancer HCT116 and human HeLa cells were from ATCC (LGC Standards, Teddington, Middlesex, UK). Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (NIH-3T3, HEK-293, HeLa) or McCoy 5A medium supplemented with 10% fetal bovine serum (HCT116) and transfected with corresponding plasmid DNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) as described before [Dmitriev et al., 2009] or Mirus TransIT 2020 (Mirus Bio, MSC, Dublin, Ireland) as per manufacturer's instructions.

ANTIBODIES

Affinity purified rabbit polyclonal anti-mouse MSL1 antibodies were as described before [Dmitriev et al., 2007], rabbit polyclonal antibodies to mouse SKIP protein were prepared before [Pestov et al., 2007]. The anti-5′-bromo-2-deoxyuridine (BrdU) monoclonal antibodies BU33 were from Sigma-Aldrich (SIAL, Moscow, Russia). Rabbit anti-histone H4 (clone 62-141-13, No. 04-858) and H4K16Ac (No. 07-329) antibodies were from Millipore (Cork, Ireland).

CONSTRUCTS

Human PSP1 encoding sequence cloned into pEYFP-C1 vector was a gift from Prof. Angus Lamond (University of Dundee, UK). For experiments it was re-cloned into pEGFP-C1 using *Eco*RI and *Bam*HI sites. DsRed-Daxx plasmid DNA was a gift from Prof. Hiroshi Nishina (Tokyo Medical and Dental University, Japan). PRP3-RFP construct was obtained by cloning of full-length mouse PRP3 ORF (gene ID: 70767) into pDsRed-Express-N1 vector, TTC4-RFP plasmid DNA was described before [Dmitriev et al., 2009].

Full-length mouse MSL1 isoforms B, C, D, and E (accordingly to nomenclature proposed previously [Dmitriev et al., 2005]) were cloned into pEGFP-N3 vector as described before [Dmitriev et al., 2006]. N-terminal RFP-tagged chimera of isoform A was obtained by cloning into pDsRed-monomer-C1 vector. Deletion fragments of mouse MSL1 isoforms (Fig. 1) were obtained by cloning into pDsRed-monomer-C1 vector (a.a. 461–616 or 461–600, of RFP-tagged IV PEHE domains from isoforms A and B), pEGFP-N3 (a.a. 1–258, isoform A) or pEGFP-C1 (all other isoforms).

Immunofluorescence of transfected or BrdU-labeled cells using paraformaldehyde fixation protocol was performed as described before [Dmitriev et al., 2009].

PROTEIN EXTRACTION AND ANALYSIS

HCT116 cells were seeded on 25 cm^2 tissue culture flasks at 2.5×10^6 per flask, allowed to adhere (8 h) then transfected with plasmid DNAs encoding mouse MSL1 isoforms B, C, D, and E–GFP using Mirus TransIT 2020 reagent in Opti-MEM I medium (Invitrogen). Twenty three hours post-transfection, cells were washed with PBS supplemented with 5 mM sodium butyrate, lysed on ice in 500 μl of TEB buffer (PBS, 0.25% Triton X-100, 0.02% NaN_3, protease inhibitor cocktail Sigma P2714), centrifuged (600*g*, 10 min), crude nuclei were washed with 250 μl of TEB and then histones were acid-extracted in 125 μl of 0.2 M HCl for 3 h on rotary shaker. Extracts were cleared by centrifugation (600*g*, 10 min) and total protein was quantified by Bradford method. Normalized histone extracts were analyzed by Western blotting as described before [Zhdanov et al., 2013], using primary anti-histone H4 and H4K16Ac (Millipore) and infra-red dye labeled secondary antibodies (Odyssey, LI-COR). The densitometry was done using built-in software (Odyssey, LI-COR), with average signals shown and standard deviation presented as error bars (N = 2).

RESULTS AND DISCUSSION

IDENTIFICATION OF ADDITIONAL NUCLEAR LOCALIZATION SIGNAL IN MOLECULE OF MSL1

Our preliminary experiments with full-length mouse MSL1 isoforms C, D, E [Dmitriev et al., 2006], and B [Dmitriev et al., 2009] pointed at nuclear localization for all of them, even during ectopic expression. Thus, the existence of unknown sequence motif in all of the isoforms responsible for nuclear transport was hypothesized. We therefore generated a series of deletion mutants fused with N- or C-terminal GFP (Fig. 1). Firstly, we confirmed that Δ1 fragment, comprising a.a. coordinates 259–460 (numbering for isoform A), localizes to nucleoplasm upon transfection of mouse embryonic fibroblast cells. Further analysis of deletion mutants showed that minimal sequence responsible for nuclear transport (NLS) represents fragment with coordinates a.a. 319–348 (3.3 kDa, pI 9.04), joining domains IIcc (coiled coil) and III (Fig. 1C). Shortening of this sequence, for example, in fragment a.a. 259–341 led to mixed nuclear and cytoplasmic location, while separately domains IIcc (8.9 kDa) and III (14.5 kDa) displayed whole cell distribution (Fig. 1C), due to the small

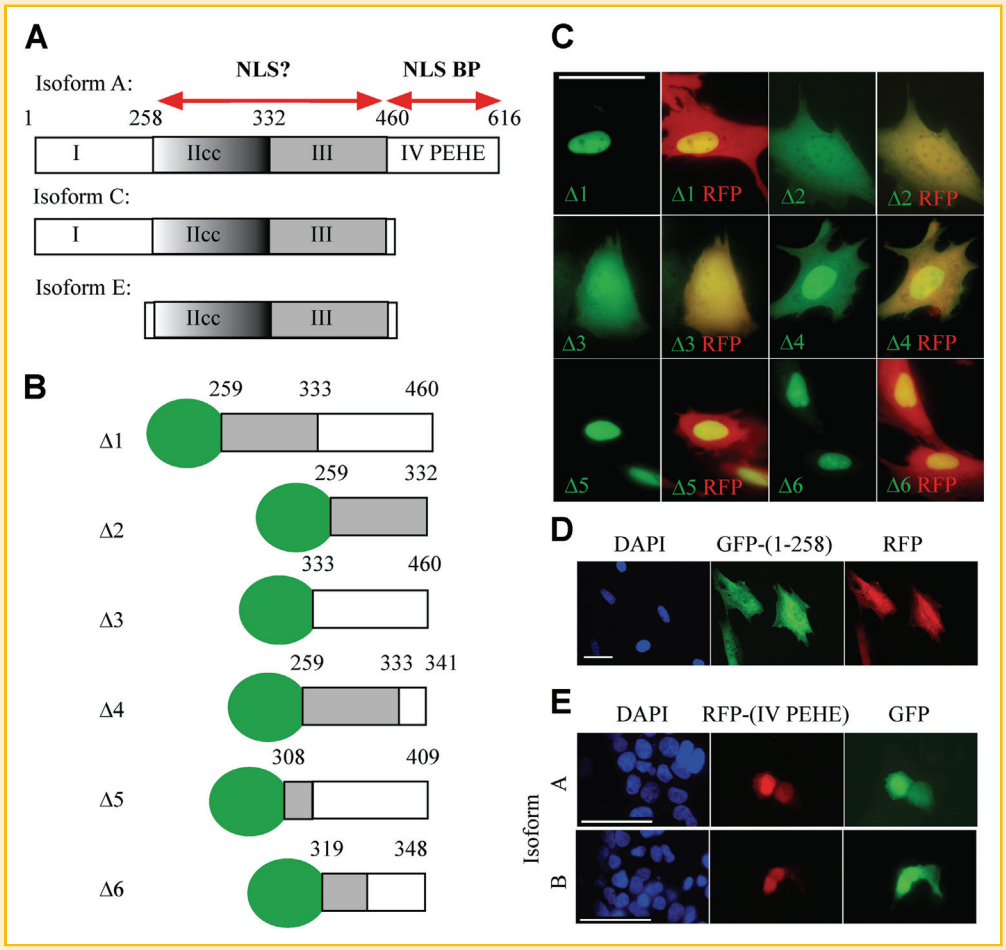

Fig. 1. Mapping of nuclear localization signals in molecule of mouse MSL1. A: Domain structure of mouse isoforms A, C and E with amino acid coordinates. B: Structure of deletion fragments Δ1–Δ6, based on domains IIcc and III with GFP fusion shown in green. C: localization of deletion mutants in cultured live 3T3 fibroblasts. Red fluorescent protein (RFP) used to visualize cytosol. D: localization of N-terminal fragment of isoform A (a.a. 1–258, green) in fixed 3T3 fibroblasts with nuclei stained with DAPI. E: localization of C-terminal domains IV PEHE of isoforms A and B, fused with red fluorescent protein, in fixed HeLa cells. Nuclei were stained with DAPI. GFP is shown to visualize cytosol. Amino acid coordinates correspond to isoform A (616 a.a.). Scale bar is 50 μm.

size of the resulting chimeric proteins, below 50 kDa threshold [Mor et al., 2014]. N-terminal portion present in isoforms A, B, and C (a.a. 1–258, domain I) also did not show any functional nuclear transport signals (Fig. 1D), while domains IV PEHE from mouse isoforms A and B showed nucleoplasmic localization even upon ectopic expression (human cells), through presence of known bipartite NLS BP (Fig. 1E).

We performed detailed analysis of a novel NLS (termed NLS1) primary structure (Fig. S1). Compared to NLS BP, this 29 a.a. fragment displays slightly less cationic nature: pI 9 contrasting to pI 11. Phylogenetic analysis showed 100% of identity between NLS1 fragments in mouse and rat proteins, 93% to human and chimpanzee, 86% to opossum and 72% to chicken proteins (Fig. S1). Analysis of homologues from amphibian and fish did not show significant similarities (only 30% identity). Multiple alignments allowed identifying "canonic" sequence for this novel NLS:

^{319}S(P)-KPFSCGRSG***KGH******KR***K-T(S)-PFG-N(S)-*TERKTPV*KK^{348};

The database search demonstrated that similar sequence regions are present in other nuclear proteins, such as *Bos taurus* TDG thymine-DNA glycosylase (MGC140185) (identical residues are shown in italic) or mouse zinc-finger protein Zfp239 (Mok-2) (identical residues are shown in bold) [Arranz et al., 2001].

Taken together, these results confirm the presence of two functional NLS in MSL1 protein isoforms leading to nuclear protein targeting in cells from different species (mouse, human). Newly identified NLS1 is present in all isoforms and is highly conservative in mammals, while NLS BP resides in highly conservative PEHE domain, is specific only to isoforms A, B, and D.

SUB-NUCLEAR LOCALIZATION OF MSL1 IN CELLS AND IN VIVO

In contrast to nucleoplasmic location of nuclear localization sequences NLS1 and NLS BP (Fig. 1), the additional, sub-nuclear localization was noticed for full-length isoforms A and B upon overexpression or immunostaining of endogenous MSL1 in cultured cells (Fig. 2). This speckle-like localization was evident in some of transfected cells, while in others MSL1 was located exclusively to the nucleoplasm (not shown). Since the histone acetylation changes during cell cycle [Barth and Imhof, 2010], this sub-nuclear localization can be attributed to cell cycle dependent events such

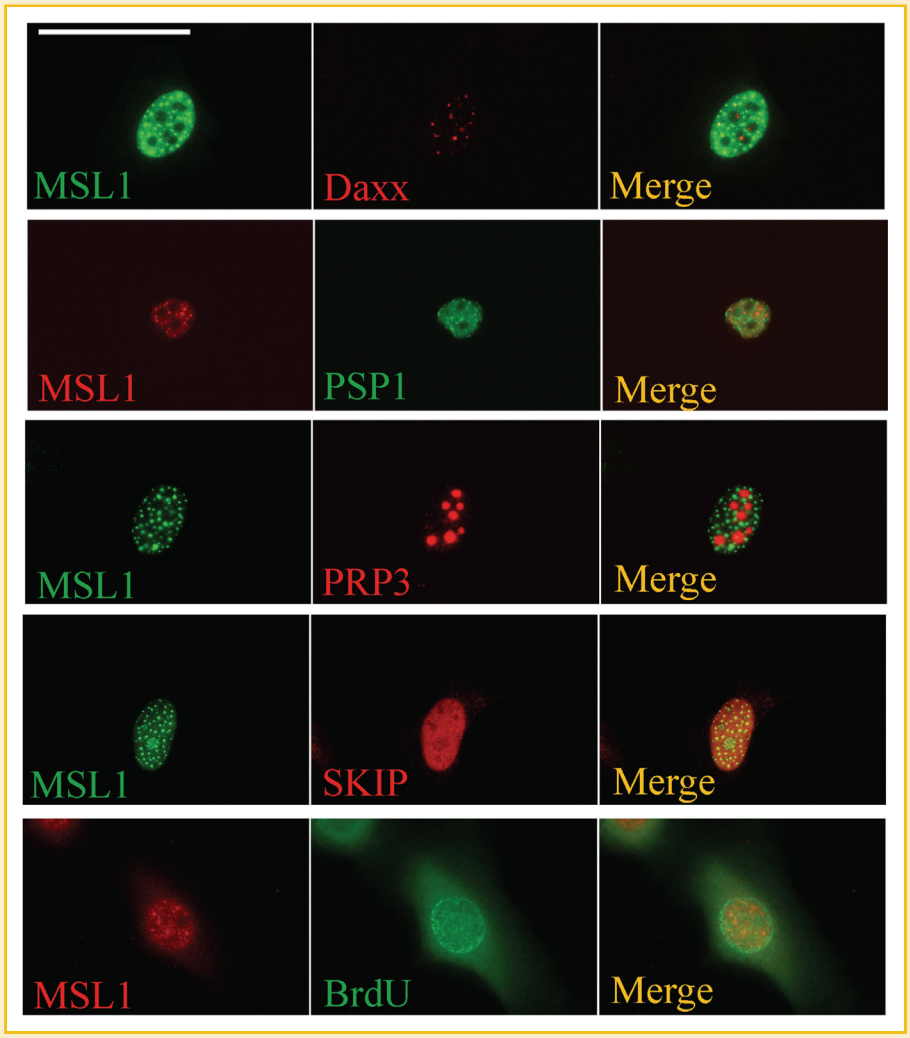

Fig. 2. MSL1 localizes to nucleoplasm and distinct sub-nuclear foci. Fluorescence microscopy of overproduced MSL1 isoforms B (GFP, green), A (RFP, red), or endogenous (lower panel, red) in 3T3 fibroblasts co-localized with markers of nuclear bodies: PML (DsRed-Daxx, red), paraspeckles (GFP-PSP1, green), nucleoli and spliceosomes (PRP3-RFP, red), spliceosomes (SKIP, red) and DNA replication foci (BrdU, green). Scale bar is 50 μm.

as DNA synthesis. This localization was not an artifact as it was observed also with intact (untransfected) cells (immunofluorescence, Fig. 2, lower panel), while protein overproduction can definitely lead to local protein aggregation, especially in case of GFP-tagged MSL1 (Fig. 2, top panel).

We attempted to co-localize the MSL1-specific speckles with some known markers of nuclear bodies [Dundr, 2012], including nucleoli (Surf-6 [Polzikov et al., 2005], PRP3 [Comitato et al., 2007]), PML bodies (Daxx [Kitagawa et al., 2006]), paraspeckles (PSP1 [Fox et al., 2002]), DNA replication foci (BrdU labeling), heat shock granules [Cotto et al., 1997], spliceosomes (SKIP [Leong et al., 2004], PRP3) and others (not shown), by using co-transfection with fluorescent protein-tagged markers or immunostaining (Fig. 2). This speckle-like localization is also different from that reported for H4K16Ac in the cells (stains whole nucleoplasm) and histone γH2A.X (DNA damage foci) [Li et al., 2010]. Although, we were not able to co-localize sub-nuclear MSL1 with any of tested nuclear body markers, this localization can be attributed to local chromatin unwrapping (active chromatin regions), adjacent to sites with acetylated H4K16 [Smith et al., 2005; Shogren-Knaak et al., 2006].

The sub-nuclear speckle localization of MSL1 was observed only with the longest MSL1 isoforms (A and B), consisting of all four domains I–IV (Fig. 1A), and containing both NLS. We speculate that simultaneous presence of two NLS mediates such intranuclear trafficking of MSL1 in vivo. This explains why isoforms C and E, containing only NLS1, localized to nucleoplasm in cultured 3T3 fibroblasts and HEK-293 cells, as well as isoform D (not shown). However, testis-specific isoform D may need additional factors in other cell types, to be transported in such sub-nuclear bodies.

To analyze the localization of MSL1 in vivo, we performed immunofluorescence of mouse testis samples (Fig. S1). Staining with polyclonal antiserum demonstrated heterogeneous nuclear distribution of MSL1 in the cells from testis, confirming data obtained with cultured cells. Such heterogeneity can also reflect diversity of MSL1 isoforms, present in testis [Dmitriev et al., 2005].

FUNCTIONAL ROLE OF MSL1 SUB-NUCLEAR LOCALIZATION

TTC4 protein represents molecular co-chaperone involved in regulation of DNA replication through interaction with CDC6 and heat shock proteins [Crevel et al., 2008]. Previously we showed that interaction with MSL1 can lead to nuclear transport of TTC4 in mouse cells [Dmitriev et al., 2009]. Thus, we looked if different MSL1 isoforms and fragments lacking sub-nuclear accumulation, such as isoform C or fragment Δ1 (a.a. 259–460), can affect the TTC4 localization. To minimize the possibility of effects of endogenous isoforms, we used ectopic expression in human HEK293 cells (Fig. 3). We found that only sub-nuclear MSL1 (isoforms A and B) could transport TTC4-RFP into the nucleus (to the same sub-nuclear foci) while nucleoplasm-localized isoforms did not show any nuclear transport of TTC4. Our previous data demonstrated that the region responsible for interaction with TTC4 resides in fragment Δ1 [Dmitriev et al., 2007]. Thus, interaction with TTC4 might potentially interfere with the functional activity of NLS1 in isoform C, explaining the observed lack of MSL1-mediated TTC4 nuclear retention. In contrast, two functional NLS present in isoforms A and B simultaneously can anchor TTC4 in the nucleus.

Our attempts to co-express MSL1 isoforms with KAT8 histone acetyltransferase were not successful (not shown), possibly due to perturbing acetylation of H4K16, which is essential for cell survival [Taipale et al., 2005; Kapoor-Vazirani et al., 2011; Taylor et al., 2013]. We therefore attempted to see the effect of MSL1 overproduction on total acetylation levels of H4K16. Some MSL1 isoforms lack highly conservative PEHE domain IV, involved in interaction with KAT8, and second NLS (NLS BP) and we tested if they still are involved in regulation of H4K16 acetylation. We transfected human colon cancer HCT116 cells with full-length MSL1 isoforms B–E and looked at H4K16Ac, normalized to total levels of detected histone H4 (Fig. 4). Despite the normalization for total protein, transfected cells exhibited decreased levels of H4 (possibly

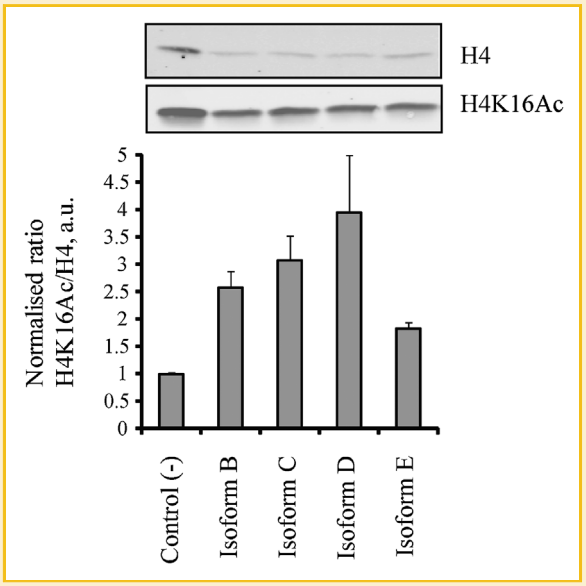

Fig. 4. Effect of over-production of MSL1 isoforms on endogenous levels of H4K16Ac. HCT116 cells were transfected with GFP chimeras corresponding to isoforms B–E, then subjected to histone extraction and analyzed by Western blotting using anti-histone H4 and H4K16Ac antibodies (top). Normalized ratios of H4K16Ac/ H4 are shown on bottom graph.

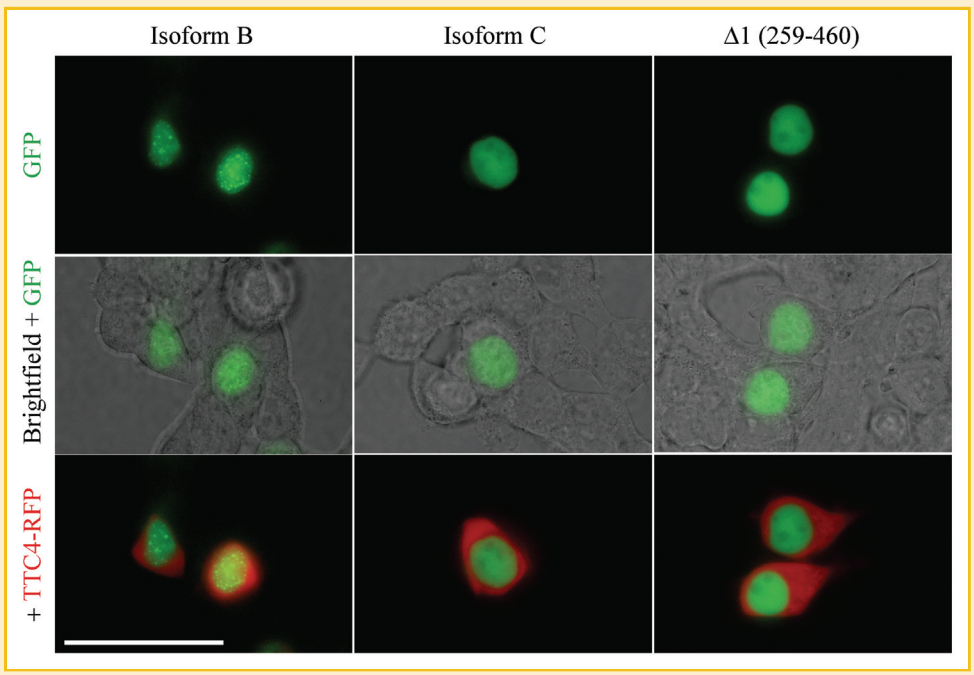

Fig. 3. Different effects of sub-nuclear MSL1 localization on TTC4. Fluorescence microscopy of live HEK-293 cells co-transfected with GFP chimeras of MSL1 isoforms B, C, and fragment 259–460 (green) and TT4-RFP (red). Scale bar is 50 μm.

due to effect of transfection procedure), although ratios of H4K16Ac/H4 were significantly increased (1.5- to 4-fold). Although we did not normalize increase of H4K16Ac to overall amount of produced MSL1 per cell (cells exhibited 25–30% transfection efficiency), this data confirm that all MSL1 isoforms can affect histone acetylation.

In conclusion, we investigated the mechanism of nuclear retention of MSL1 and found the presence of two functional NLS. The second and previously unknown NLS motif is specific to mammalian MSL1 orthologs and, when present together with bipartite NLS (in PEHE domain, conservative from mammals to flies [Marín, 2003; Dmitriev et al., 2005]), leads to speckle-like sub-nuclear localization of protein. This sub-nuclear localization is important for nuclear targeting of molecular co-chaperone TTC4 and is possibly related to chromosomal sites with acetylated H4K16. We also found that independent of the number of NLS in molecule, any isoform can positively affect acetylation of H4K16. Thus, complex structure, presence of two active NLS and different domain composition of MSL1 isoforms points at the multitude of its activities in nucleus and complexity of the regulation of KAT8.

ACKNOWLEDGMENTS

We are grateful to A. Kazakova, R. Abdulin, A. Vasilevskaja and K. Klinova (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry) for technical assistance, Prof. A. Lamond (University of Dundee, UK) for the gift of PSP1-YFP and Prof. H. Nishina (Tokyo Medical and Dental University, Japan) for the gift of DsRed-Daxx expression constructs. We thank Alicia H. Waters for critical reading of the manuscript. This work was supported through Science Foundation Ireland (SFI), MCB RAS and Russian Foundation for Basic Research (RFBR) grants.

REFERENCES

Aguado-Llera D, Hamidi T, Doménech R, Pantoja-Uceda D, Gironella M, Santoro J, Velázquez-Campoy A, Neira JL, Iovanna JL. 2013. Deciphering the binding between Nupr1 and MSL1 and their DNA-repairing activity. PloS ONE 8:e78101.

Arranz V, Dreuillet C, Crisanti P, Tillit J, Kress M, Ernoult-Lange M. 2001. The zinc finger transcription factor, MOK2, negatively modulates expression of the interphotoreceptor retinoid-binding protein gene, IRBP. J Biol Chem 276:11963–11969.

Bao J, Sack MN. 2010. Protein deacetylation by sirtuins: Delineating a post-translational regulatory program responsive to nutrient and redox stressors. Cell Mol Life Sci 67:3073–3087.

Barth TK, Imhof A. 2010. Fast signals and slow marks: The dynamics of histone modifications. Trends Biochem Sci 35:618–626.

Comitato A, Spampanato C, Chakarova C, Sanges D, Bhattacharya SS, Marigo V. 2007. Mutations in splicing factor PRPF3, causing retinal degeneration, form detrimental aggregates in photoreceptor cells. Hum Mol Genet 16: 1699–1707.

Cotto J, Fox S, Morimoto R. 1997. HSF1 granules: A novel stress-induced nuclear compartment of human cells. J Cell Sci 110:2925–2934.

Crevel G, Bates H, Huikeshoven H, Cotterill S. 2001. The Drosophila Dpit47 protein is a nuclear Hsp90 co-chaperone that interacts with DNA polymerase α. J Cell Sci 114:2015–2025.

Crevel G, Bennett D, Cotterill S. 2008. The human TPR protein TTC4 is a putative Hsp90 co-chaperone which interacts with CDC6 and shows alterations in transformed cells. PloS ONE 3:e0001737.

Dmitriev R, Pestov N, Korneenko T, Gerasimova A, Zhao H, Modyanov N, Kostina M, Shakhparonov M. 2005. Tissue specificity of alternative splicing of transcripts encoding hampin, a new mouse protein homologous to the Drosophila MSL-1 protein. Russ J Bioorg Chem 31:325–331.

Dmitriev RI, Pestov NB, Korneenko TV, Shakhparonov MI. 2006. Intracellular location of hampin isoforms. Dokl Biochem Biophys 408:130–132.

Dmitriev RI, Korneenko TV, Bessonov AA, Shakhparonov MI, Modyanov NN, Pestov NB. 2007. Characterization of hampin/MSL1 as a node in the nuclear interactome. Biochem Biophys Res Commun 355:1051–1057.

Dmitriev RI, Okkelman IA, Abdulin RA, Shakhparonov MI, Pestov NB. 2009. Nuclear transport of protein TTC4 depends on the cell cycle. Cell Tissue Res 336:521–527.

Dundr M. 2012. Nuclear bodies: Multifunctional companions of the genome. Curr Opin Cell Biol 24:415–422.

Fox AH, Lam YW, Leung AKL, Lyon CE, Andersen J, Mann M, Lamond AI. 2002. Paraspeckles: A Novel Nuclear Domain. Current Biology 12:13–25.

Füllgrabe J, Lynch-Day MA, Heldring N, Li W, Struijk RB, Ma Q, Hermanson O, Rosenfeld MG, Klionsky DJ, Joseph B. 2013. The histone H4 lysine 16 acetyltransferase hMOF regulates the outcome of autophagy. Nature.

Kadlec J, Hallacli E, Lipp M, Holz H, Sanchez-Weatherby J, Cusack S, Akhtar A. 2011. Structural basis for MOF and MSL3 recruitment into the dosage compensation complex by MSL1. Nat Struct Mol Biol 18:142–149.

Kapoor-Vazirani P, Kagey JD, Vertino PM. 2011. SUV420H2-mediated H4K20 trimethylation enforces RNA polymerase II promoter-proximal pausing by blocking hMOF-dependent H4K16 acetylation. Mol Cell Biol 31:1594–1609.

Kitagawa D, Kajiho H, Negishi T, Ura S, Watanabe T, Wada T, Ichijo H, Katada T, Nishina H. 2006. Release of RASSF1C from the nucleus by Daxx degradation links DNA damage and SAPK/JNK activation. EMBO J 25:3286–3297.

Krishnan V, Chow MZY, Wang Z, Zhang L, Liu B, Liu X, Zhou Z. 2011. Histone H4 lysine 16 hypoacetylation is associated with defective DNA repair and premature senescence in Zmpste24-deficient mice. Proc Natl Acad Sci 108:12325–12330.

Leong GM, Subramaniam N, Issa LL, Barry JB, Kino T, Driggers PH, Hayman MJ, Eisman JA, Gardiner EM. 2004. Ski-interacting protein, a bifunctional nuclear receptor coregulator that interacts with N-CoR/SMRT and p300. Biochem Biophys Res Commun 315:1070–1076.

Li X, Corsa CAS, Pan PW, Wu L, Ferguson D, Yu X, Min J, Dou Y. 2010. MOF and H4 K16 acetylation play important roles in DNA damage repair by modulating recruitment of DNA damage repair protein Mdc1. Mol Cell Biol 30:5335–5347.

Li X, Wu L, Corsa CAS, Kunkel S, Dou Y. 2009. Two mammalian MOF complexes regulate transcription activation by distinct mechanisms. Mol Cell 36:290–301.

Marín I. 2003. Evolution of chromatin-remodeling complexes: Comparative genomics reveals the ancient origin of "novel" compensasome genes. J Mol Evol 56:527–539.

Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K. 2006. Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. Mol Cell 21:811–823.

Miyamoto N, Izumi H, Noguchi T, Nakajima Y, Ohmiya Y, Shiota M, Kidani A, Tawara A, Kohno K. 2008. Tip60 is regulated by circadian transcription factor clock and is involved in cisplatin resistance. J Biol Chem 283:18218–18226.

Mor A, White MA, Fontoura BMA. 2014. Nuclear trafficking in health and disease. Curr Opin Cell Biol 28:28–35.

Mulligan P, Yang F, Di Stefano L, Ji J-Y, Ouyang J, Nishikawa JL, Toiber D, Kulkarni M, Wang Q, Najafi-Shoushtari SH. 2011. A SIRT1-LSD1 corepressor complex regulates Notch target gene expression and development. Mol cell 42:689–699.

Orpinell M, Fournier M, Riss A, Nagy Z, Krebs AR, Frontini M, Tora L. 2010. The ATAC acetyl transferase complex controls mitotic progression by targeting non-histone substrates. EMBO J 29:2381–2394.

Pestov NB, Ahmad N, Korneenko TV, Zhao H, Radkov R, Schaer D, Roy S, Bibert S, Geering K, Modyanov NN. 2007. Evolution of Na,K-ATPase βm-subunit into a coregulator of transcription in placental mammals. Proc Natl Acad Sci 104:11215–11220.

Polzikov M, Zatsepina O, Magoulas C. 2005. Identification of an evolutionary conserved SURF-6 domain in a family of nucleolar proteins extending from human to yeast. Biochem Biophys Res Commun 327:143–149.

Rudra D, deRoos P, Chaudhry A, Niec RE, Arvey A, Samstein RM, Leslie C, Shaffer SA, Goodlett DR, Rudensky AY. 2012. Transcription factor Foxp3 and its protein partners form a complex regulatory network. Nat Immunol 13(10):1010–1019.

Shogren-Knaak M, Ishii H, Sun J-M, Pazin MJ, Davie JR, Peterson CL. 2006. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 311:844–847.

Smith ER, Cayrou C, Huang R, Lane WS, Côté J, Lucchesi JC. 2005. A human protein complex homologous to the Drosophila MSL complex is responsible for the majority of histone H4 acetylation at lysine 16. Mol Cell Biol 25:9175–9188.

Taipale M, Rea S, Richter K, Vilar A, Lichter P, Imhof A, Akhtar A. 2005. HMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. Mol Cell Biol 25:6798–6810.

Taylor GCA, Eskeland R, Hekimoglu-Balkan B, Pradeepa MM, Bickmore WA. 2013. H4K16 acetylation marks active genes and enhancers of embryonic stem cells, but does not alter chromatin compaction. Genome Res 23:2053–2065.

Thomas T, Loveland KL, Voss AK. 2007. The genes coding for the MYST family histone acetyltransferases, Tip60 and Mof, are expressed at high levels during sperm development. Gene Expr Patterns 7:657–665.

Voss AK, Thomas T. 2009. MYST family histone acetyltransferases take center stage in stem cells and development. Bioessays 31:1050–1061.

Zhdanov AV, Dmitriev RI, Golubeva AV, Gavrilova SA, Papkovsky DB. 2013. Chronic hypoxia leads to a glycolytic phenotype and suppressed HIF-2 signaling in PC12 cells. Biochim Biophys Acta 1830:3553–3569.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.