Biochemical and Biophysical Research Communications 462 (2015) 201–207

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Identification and characterization of a nuclear localization signal of TRIM28 that overlaps with the HP1 box

Tetsuji Moriyama [a, 1], Percival Sangel [a, 1], Hiroki Yamaguchi [c], Chikashi Obuse [d], Yoichi Miyamoto [a], Masahiro Oka [a, e, *], Yoshihiro Yoneda [b, e, **]

[a] Laboratory of Nuclear Transport Dynamics, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka 567-0085, Japan
[b] National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka 567-0085, Japan
[c] School of Medicine, Osaka University, Osaka 565-0871, Japan
[d] Graduate School of Life Science, Hokkaido University, Sapporo 001-0021, Japan
[e] Laboratory of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 7 April 2015
Available online 7 May 2015

Keywords:
TRIM28
KAP1
TIF1β
NLS
Importin α
HP1

ABSTRACT

Tripartite motif-containing 28 (TRIM28) is a transcription regulator, which forms a repressor complex containing heterochromatin protein 1 (HP1). Here, we report identification of a nuclear localization signal (NLS) within the 462-494 amino acid region of TRIM28 that overlaps with its HP1 binding site, HP1 box. GST-pulldown experiments revealed the interaction of the arginine-rich TRIM28 NLS with various importin α subtypes (α1, α2 and α4). *In vitro* transport assay demonstrated that nuclear localization of GFP-TRIM28 NLS is mediated by importin αs, in conjunction with importin β1 and Ran. Further, we demonstrated that HP1 and importin αs compete for binding to TRIM28. Together, our findings suggest that importin α has an essential role in the nuclear delivery and preferential HP1 interaction of TRIM28.

1. Introduction

Tripartite motif-containing 28 (TRIM28), also known as Kruppel-box associated protein 1 (KAP1) and transcriptional intermediary factor-1β (TIF1β), was identified through its interaction with Kruppel-associated box (KRAB) domain found in many transcriptional factors [1–3]. All TIF1 family members, to which TRIM28 belongs, have a similar overall design characterized by an N-terminal tripartite motif (TRIM), which functions as a protein–protein and oligomerization interface. Specifically, the members of this family contain an RBCC (Ring finger, two B-box zinc fingers, and a coiled coil) domain, a TSS (central TIF1 signature sequence) domain consisting of tryptophan- and phenylalanine-rich sequence, and a C-terminal combination plant homeodomain (PHD) and bromodomain [4].

TRIM28 acts as a scaffold protein for recruiting chromatin-modifying enzymes (like SETDB1 [5] and Mi2α [6]) and a component of chromatin, heterochromatin protein 1 (HP1) [7–9]. HP1 interacts with various nuclear proteins through the pentapeptide PxVxL motif (HP1-box) [7–9], a structure observed in the diverse nuclear proteins including TRIM28 [10]. HP1 directly recognizes methylation of lysine 9 in histone H3 [11–13], and its interaction with TRIM28 is required for repression of reporter genes [8]. This methylation of histone H3 associated with TRIM28 repressor complex is lost when HP1 and TRIM28 interaction is disrupted, thus, suggesting the importance of both proteins and their interaction for gene silencing [14]. However, it is unknown how TRIM28 is imported into the nucleus and preferentially interacts with HP1 proteins after its nuclear translocation.

Importin α is a dynamic transport protein, which is known to transport cargo proteins from cytoplasm into the nucleus. Several subtypes of importin αs have been identified in mammals, and are classified into three subfamilies [15]. It forms a heterodimeric importin complex by binding with importin β1 and simultaneously recognizes the nuclear localization signal (NLS) of a cargo protein, thereby, forming a ternary complex. Translocation of this ternary

Abbreviations: NLS, nuclear localization signal; HP1, heterochromatin protein 1; TRIM28, tripartite motif-containing 28; GFP, green fluorescent protein; GSH, glutathione-Sepharose.

* Corresponding author. National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka 567-0085, Japan.
** Corresponding author. National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka 567-0085, Japan.
E-mail addresses: moka@nibiohn.go.jp (M. Oka), y-yoneda@nibiohn.go.jp (Y. Yoneda).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.04.108
0006-291X/

complex from the cytoplasm to the nucleus is mediated by the weak hydrophobic interaction of importin β1 with the nucleoporins of the nuclear pore complex (NPC). This importin α-importin β-cargo interaction is regulated by the small GTPase, Ran, through the creation of a RanGTP/GDP gradient across the nuclear envelope [16,17].

This paper exhibits the importin α's essential participatory role in TRIM28 nuclear transport and its potential function in assisting the interaction between TRIM28 and HP1, which promotes transcriptional repression.

2. Materials and methods

2.1. Cell culture

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, NY, USA) and maintained at 37 °C with 10% CO_2.

2.2. Plasmid constructs and transfection

TRIM28 and HP1β were generated by PCR amplification from HeLa cells cDNA, and subcloned into in the pENTR/D-TOPO cloning vector (Life Technologies, CA, USA). The Gateway® LR Clonase® II Enzyme mix (Life Technologies) was adapted for the generation of either HA or GFP tagged TRIM28 PCR-based mutagenesis. A series of deletion mutants of TRIM28 were made through site-specific recombination using an entry vector, pENTR plasmid containing the TRIM28 mutant oligonucleotide and prepared destination vector, pcDNA3.1-EGFP-DEST, pcDNA3.1-EGFPx2-DEST or p3xHA-CMV-DEST containing single or double GFP tags, or triple HA tags, respectively.

To generate pcDNA3.1-EGFP-DEST and pcDNA3.1-EGFPx2-DEST expression vector, a EGFP or EGFP–EGFP DNA fragment cleaved by the *Nhe*I and *Hind*III was generated from pEGFP-C1 (Clontech, Cambridge, UK) or pEGFP-GFP expression vector previously described [13], and inserted into the *Nhe*I and *Hind*III sites of the pcDNA3.1/Zeo(+). The Reading Frame Cassette from the Gateway Conversion System (Life Technologies) was constructed into the *Eco*RV site of pcDNA3.1-EGFP and pcDNA3.1-EGFPx2.

To construct p3xHA-CMV-DEST expression vector, three tandem repeats of HA oligo (5′-ACCATGGCCTACCCATACGATGTTCCA-GATTACGCTTACCCATACGATGTTCCAGATTACGCTTACCCA-TACGATGTTCCAGATTACGC-3′) was inserted into the *Nhe*I and *Bam*HI sites of the pcDNA3.1/Zeo(+). The Reading Frame Cassette from the gateway Conversion System (Life Technologies) was inserted into the *Eco*RV site of p3xHA-CMV.

Sequences for oligonucleotides used in this study are listed in Supplementary Table S1. Purified plasmids were transfected to HeLa cells using Lipofectamine 2000 (Life Technologies, CA, USA), as according to the manufacturer's recommendations.

2.3. GST-pulldown assay and immunoblotting

HeLa cells were cultured on 100 mm dish and then at 60–80% confluence, and were transfected with the expression plasmid of TRIM28 or TRIM28 mutant subcloned in either pcDNA3.1-EGFP-DEST, pcDNA3.1-EGFPx2-DEST or p3xHA-CMV-DEST. At 48 h after transfection, the cells were washed with PBS and collected by scraping with 1 ml of lysis buffer (20 mM HEPES/NaOH [pH 7.3], 110 mM potassium acetate, 2 mM magnesium acetate, 5 mM sodium acetate, 1 mM EGTA/NaOH [pH 7.3], 2 mM dithiothreitol [DTT], 1 μg/ml aprotinin, pepstatin, leupeptin, 0.1% TritonX-100). The cell lysates were sonicated and centrifuged at 20,400 × g for 30 min at 4 °C.

Glutathione Sepharose beads (GSH beads, GE Healthcare Biosciences AB, Tokyo, JAPAN) were incubated with either recombinant protein GST, GST-mouse importin α1 (NPI1/KPNA1), GST-mouse importin α2 (Rch1/KPNA2), GST-mouse importin α4 (Qip1/KPNA4), GST-GFP, GST-SV40T NLS-GFP or GST-TRIM28 NLS-GFP for 1 h at 4 °C. The beads with GST proteins were washed several times with lysis buffer, and subsequently, GST-pulldown was carried out by incubating the prepared cell lysate or recombinant protein importin α1, importin α2, importin α4 or HP1β with the prepared beads for 4 h at 4 °C. After washing, the beads were resuspended in Laemmli sample buffer, and subjected to SDS-PAGE gel and each binding was detected by immunoblotting using antibodies specific for HA (3F10; Roche Diagnostics, Mannheim, Germany), GST (sc-138; Santa Cruz Biotechnology, CA, USA), HP1β (8676; Cell Signaling, Beverly, MA, USA), importin α1 (H00003836-M01; Abnova, Taipe, TAIWAN), importin α2 [18] or importin α4 (ab6039; Abcam, Tokyo, JAPAN) with peroxidase-conjugated secondary antibodies, followed by chemiluminescence imaging using AE 9300 EZ-capture MG (ATTO Corp.,Tokyo, JAPAN).

2.4. In Vitro Transport Assay

In Vitro Transport Assay was performed essentially as described [19]. HeLa cells grown on 8-well multitest slides (ICN Biomedicals, Inc., CA, USA) were washed twice with Transport Buffer (TB: 20 mM HEPES/NaOH [pH 7.3], 110 mM potassium acetate, 2 mM magnesium acetate, 5 mM sodium acetate, 1 mM EGTA/NaOH [pH 7.3], 2 mM DTT, 1 μg/ml each of aprotinin, pepstatin and leupeptin) and permeabilized with 40 μg/ml digitonin (Wako Pure Chemical Industries, Osaka, Japan) in TB for 5 min on ice. Import mixes were prepared in TB either with GST-TRIM28 NLS-GFP or GST-SV40T NLS-GFP, which was used as positive control. Each of these was further mixed with importin α, importin β1, RanGDP, p10/NTF2, ATP regeneration system (1 mM ATP, 5 mM phosphocreatine, and 20 U of creatine kinase), 0.5 mM GTP and 0.2% BSA in TB. The cells were incubated with the prepared import mixes for 30 min at 30 °C. The cell nuclei were stained with DAPI. GFP-fusion proteins were detected by Confocal Laser Scanning Microscope Leica TCS SP8 (Leica Microsystems, Mannheim, Germany).

2.5. Immunofluorescence

HeLa cells grown on glass cover slides in a 12-well culture plates were fixed with 3.7% (v/v) formaldehyde in PBS, permeabilized with PBS containing 0.2% TritonX-100 and washed with PBS. After blocking in 3% skim milk in PBS, the cells were incubated with primary antibodies for overnight at 4 °C. After washing, the cells were subsequently incubated with secondary antibodies for 30 min at room temperature. Images were captured using a Confocal Laser Scanning Microscope Leica TCS SP8 (Leica Microsystems, Mannheim, Germany).

3. Results

3.1. Identification of TRIM28 NLS

TRIM28 has been known as a transcription regulator localized in the nucleus [9]. However, it remains unclear how TRIM28 is imported into the nucleus, and whether TRIM28 possesses a functional nuclear localization signal (NLS). To identify an NLS region of the TRIM28 protein, plasmids encoding various HA- or GFP-tagged full-length or truncated mutants were transfected into HeLa cells to assess their subcellular localization. At 24 h after transfection, the

HA-tagged full-length TRIM28 (1-836 amino acid [a.a.]) protein clearly appeared in the nucleus of transfected HeLa cells (Fig. 1A, full length). Among of the HA-tagged truncated mutants; 1–295 a.a., 296–836 a.a. and 561–836 a.a., the 296–836 a.a. were localized predominantly in the nucleus similar to the full-length protein (Fig. 1A). In contrast, the other two mutants exhibited diffusely in the cytoplasm as well as in the nucleus. This indicates the presence of NLS in the region between 296 a.a. and 560 a.a. of TRIM28.

We next tested the GFP-fused TRIM28 mutants which were truncated along 296–585 a.a. regions. As shown in Fig. 1B, the GFP-TRIM28 mutants 296–494 a.a., 395–494 a.a., and 395–585 a.a. clearly showed nuclear localization, as compared to GFP-TRIM28 296–394 a.a., 495–585 a.a., which were found equally both in the nucleus and cytoplasm. The nuclear localization was further observed in the truncated mutant containing tandem (2x) GFPs fused at the N-terminus, GFP-GFP-TRIM28 428–494 a.a. and GFP-GFP-TRIM28 462–494 a.a., indicating that NLS is specifically contained within the region 462–494 a.a.. However, further narrowing of this 33 a.a. residues abrogated clear nuclear translocation of the expressed GFP–GFP fusion protein (Fig. 1C). To further test whether this 33 a.a. (Fig. 1E) itself acts as an NLS, we examined the subcellular localization of a deletion mutant TRIM28 Δ462–494 a.a N-terminally tagged with HA. As shown in Fig 1D, the mutant diffusely appeared in the cells, in contrast to the clear nuclear localization of full-length TRIM28. Together, our results indicate that the region 462–494 a.a. of TRIM28 (Fig. 1E) functions as an NLS.

3.2. TRIM28 NLS interacts with various importin α subtypes

The TRIM28 NLS contains lysine and arginine residues which are potentially recognized by importin α as a classical NLS (cNLS). To assess whether TRIM28 is recognized by importin α through the NLS region, a GST-pulldown assay was conducted (Fig. 2A). Importin α family can be classified into three subfamilies [15], which show differential substrate specificity [20]. Thus, we tested if

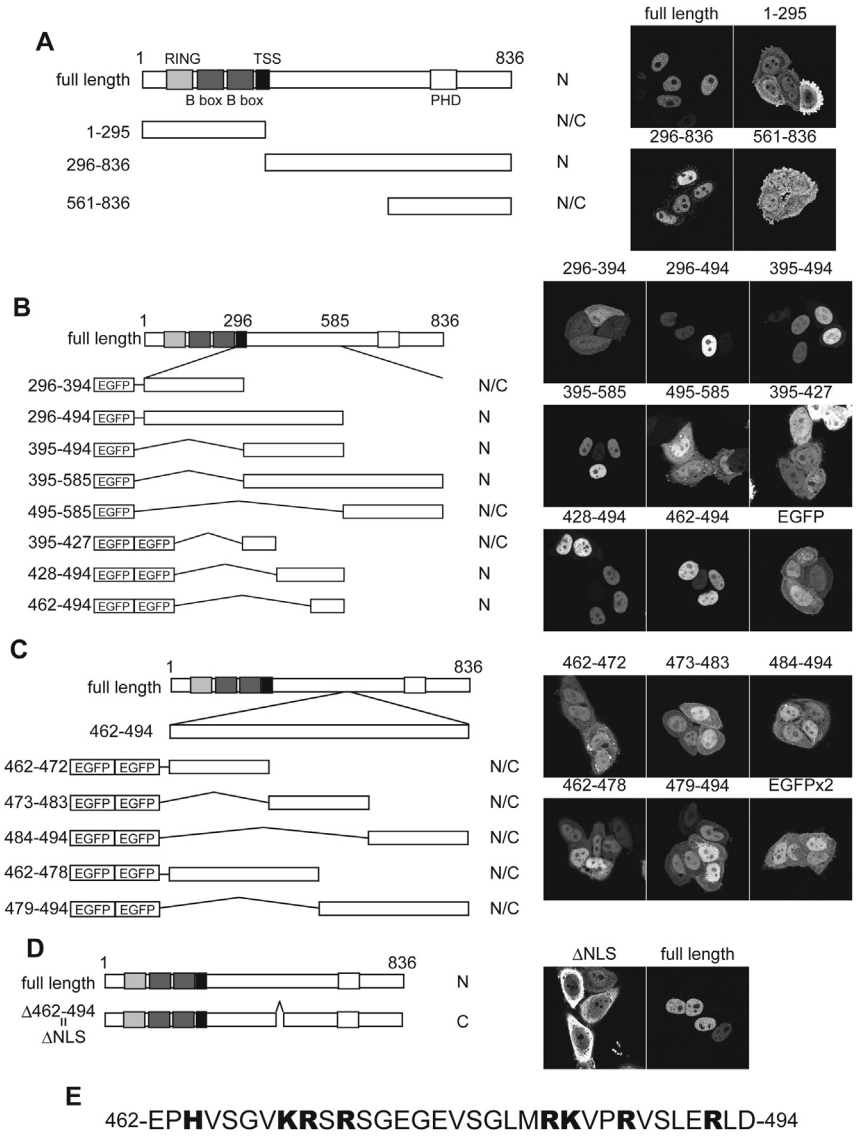

Fig. 1. Identification of an NLS in TRIM28. Immunofluorescence or fluorescence images of transfected HeLa cells expressing (A) 3xHA-tagged full-length or truncated TRIM28 mutants, (B) EGFP-fused truncated TRIM28 mutants within the 296-585 a.a. region, (C) EGFP-EGFP-fused truncated TRIM28 mutants within the 462–494 a.a. region (33 a.a. residue length), and (D) 3xHA-tagged full-length TRIM28 and 3xHA-tagged TRIM28 ΔNLS (462–494 a.a. deletion) mutant. (E) An amino acid sequence of the identified NLS region indicating the abundance of positively-charged amino acids (Bold).

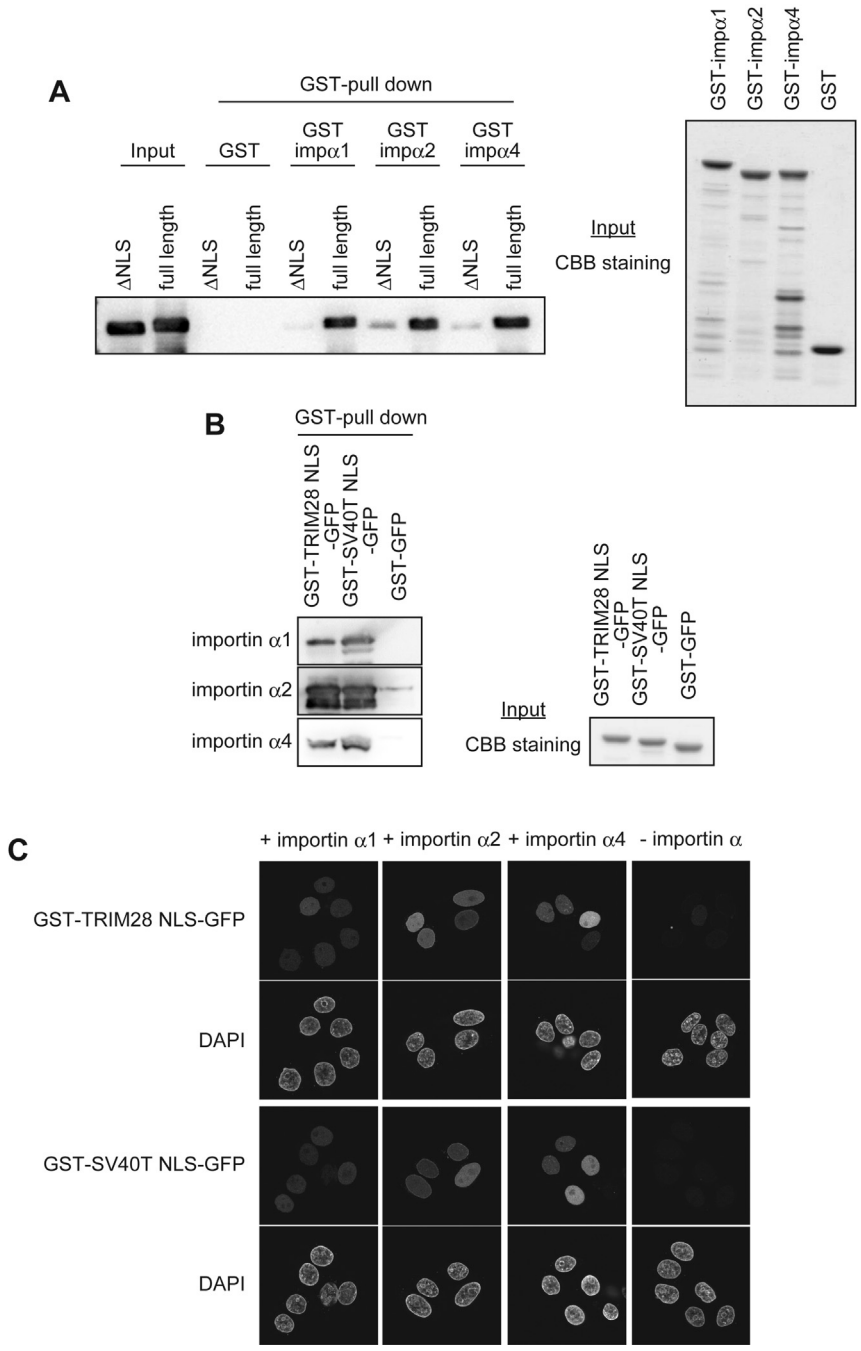

Fig. 2. Importin α/β1 mediates nuclear import of TRIM28. (A) A full-length TRIM28 but not ΔNLS mutant interacts with importin αs. GST-importin αs were immobilized on the GSH beads, and tested for interaction with 3xHA-tagged full-length TRIM28 or 3xHA-tagged NLS TRIM28 from transfected HeLa cell lysate. The input and the bound fractions were analyzed by SDS-PAGE followed by CBB staining or immunoblotting with HA antibody. (B) Direct interaction of the TRIM28 NLS with importin αs. GST-pulldown using immobilized GST-TRIM28 NLS-GFP, GST-SV40T NLS-GFP (as positive control) or GST-GFP (as negative control) with recombinant importin αs. The input and the bound fractions were analyzed by SDS-PAGE followed by CBB staining or immunoblotting with importin α1, importin α2 or importin α4 antibody. (C) TRIM28 nuclear transport by importin α/β1. An *in vitro* transport assay showing the nuclear localization of GST-TRIM28 NLS-GFP is dependent on importin α1, importin α2 or importin α4. GST-SV40T NLS-GFP was used as a positive control.

TRIM28 interacts with a representative member of each subfamily, namely, α1, α2, or α4. Either the full-length or the NLS deletion mutant of TRIM28 fused 3xHA was transiently overexpressed in HeLa cells and then the cell lysate was incubated either with GST-importin α1, α2, or α4 immobilized on glutathione-Sepharose (GSH) beads. As expected, the 3xHA-full-length protein interacted with all tested importin αs, while the NLS deletion mutant (3xHA ΔNLS) showed only weak signals (Fig. 2A).

Next, to further assess whether the binding between the NLS of TRIM28 and importin αs occurs directly, GST and GFP fused TRIM28 NLS sequences (GST-TRIM28 NLS-GFP) was immobilized on GSH beads and then mixed either with recombinant importin α1, α2, or α4. GST-SV40T NLS-GFP or GST-GFP proteins were used as positive or negative controls, respectively. As shown in Fig. 2B, the TRIM28 NLS protein was recognized with all importin αs examined like GST-SV40T NLS-GFP.

3.3. Importin α/β mediates the nuclear import of TRIM28 NLS

To demonstrate the functional ability of the TRIM28 NLS, we performed an *in vitro* nuclear transport assay using digitonin-permeabilized semi-intact HeLa cells. GST-TRIM28 NLS-GFP clearly accumulated in the nucleus of HeLa cells in the presence of importin α1, α2 or α4, together with importin β1 and Ran (Fig. 2C). These results were comparable to the nuclear localization of GST-SV40T NLS-GFP (positive control) with the respective importin α subtypes used in assay. No nuclear localization was conferred by the TRIM28 NLS in the absence of importin α (Fig. 2C control). Taken together, we conclude that the TRIM28 NLS has an ability to be transported by importin α/β1, where all the subtypes of importin αs tested (α1, α2, or α4) are potentially functional.

3.4. HP1 box of TRIM28 overlaps with the identified NLS

TRIM28 contains a protein motif called HP1 box [7,9], which serves as a binding site for the HP1, a highly conserved protein found in chromatin [21]. Indeed, the binding of HP1 to HP1 box of TRIM28 causes the transcriptional repression [9]. After careful scrutiny, the reported HP1 box contained in 476–513a.a. region of TRIM28 was seen to overlap with the NLS region (462–494 a.a.) identified in this study (Fig. 3A). Therefore, we examined the intracellular distribution of HA-tagged TRIM28 HP1 box mutant (R487E, V488E), which is defective for HP1 binding [9], in HeLa cells (Fig. 3B). The results showed the failure in the nuclear delivery of the HA-tagged TRIM28 HP1 box mutant. In a separated GST-pulldown assay, no interaction was observed between the HA-tagged TRIM28 HP1 box mutant and GST-importin αs (Fig. 3C). Together, these findings clearly indicated that the identified NLS could functionally pertain to a part of HP1 box within TRIM28. Finally, we examined if HP1 and importin α could compete with binding to TRIM28 through the HP1 box/NLS region. For this purpose, GST-TRIM28 NLS-GFP bound to GSH beads were first incubated with importin α2, and then recombinant HP1β were added to the reactions. As shown in Fig. 3D, importin α2 was dissociated from the TRIM28 NLS by the addition of HP1β. Collectively, our findings revealed the unexpected overlapping between the NLS and HP1 box in TRIM28.

4. Discussion

TRIM28 does not bind directly to DNA but exerts transcriptional regulation as part of a protein complex [4]. This TRIM28 complex has been implicated in many cellular events and processes such as self-renewal and maintenance of pluripotency of stem cells [22,23], cell differentiation [24], DNA repair [25–27], and gene silencing [8,9,28]. Before TRIM28 forms the functional complex, it has to be delivered to the nucleus. This study is the first report to characterize the nucleocytoplasmic transport mechanism of TRIM28. First of all, we identified an NLS of TRIM28 that consists of a unique 33 a.a. arginine-rich residue located between 462 and 494 a.a.. We also demonstrated that TRIM28 nuclear import is mainly dependent on importin α/β1 through our identified NLS. Interestingly, we also noticed that this NLS overlaps with the reported HP1 box domain in TRIM28, and the mutation in the HP1 box domain drastically disrupts the nuclear transport of TRIM28. Furthermore, we demonstrated that HP1 and importin α competitively binds to TRIM28.

Our data suggest that importin α binding to TRIM28 may affect TRIM28-HP1 interaction, which is important for the targeting of TRIM28 to its sites of action, including heterochromatin region [29]. We speculate that importin α, aside from its well-known role as a nuclear transport factor, masks the HP1 box for TRIM28 to interact with the HP1 protein preferentially, possibly by preventing the access of other low-affinity HP1 box-binding proteins [10], during its nuclear transport and/or chromatin targeting within the nucleus. Thus, we propose a scenario as follows (Fig. 4); in the cytoplasm, importin αs recognize the TRIM28 NLS and simultaneously binds with importin β1 to form a ternary complex that migrates into the nucleus through the NPC. Although the binding of RanGTP to importin β1 triggers the dissociation of the complex importin α may not readily release TRIM28 NLS due to high affinity binding between importin α and TRIM28 NLS. Within the nucleus, importin α may associate with TRIM28 to mask the HP1-box of TRIM28 until they find the target binding site, where is (or will become) enriched

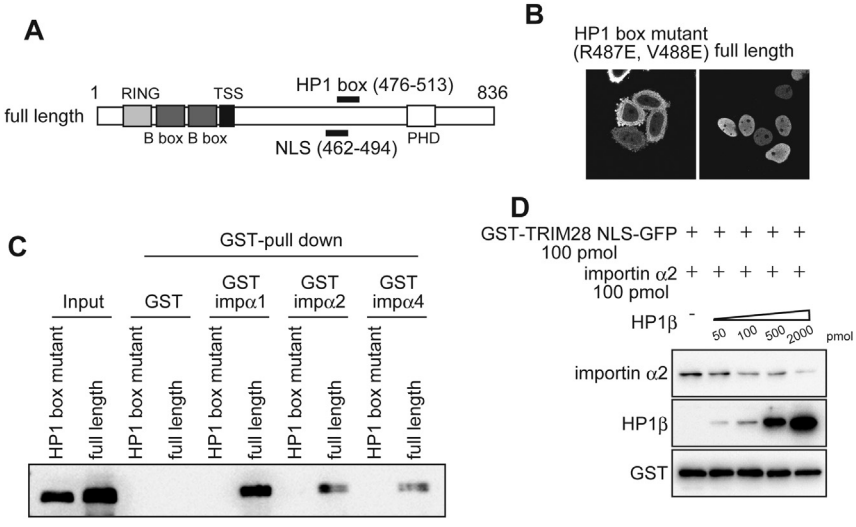

Fig. 3. Functional overlap between HP1 box and NLS in TRIM28. (A) Schematic representation of HP1 box and the newly identified NLS of TRIM28. Note that both domains overlap. (B) Immunofluorescence images showing the subcellular localization of 3xHA-tagged TRIM28 (full-length) or 3xHA-tagged TRIM28 HP1 box mutant (R487E, V488E) expressed in HeLa cells. (C) The TRIM28 HP1 box mutant fails to interact with importin αs. Either GST-importin α1, GST-importin α2 or GST-importin α4 immobilized on the GSH beads was incubated with HA-tagged TRIM28 and 3xHA-tagged TRIM28 HP1 box mutants from lysates of transfected HeLa cells, and analyzed by immunoblotting with HA antibody. (D) Competitive binding of importin α2 and HP1β to the TRIM28 NLS. GST-TRIM28 NLS-GFP immobilized on the GSH beads was incubated with importin α2. After washing, the beads were further incubated with various amounts of HP1β for 4 h at 4 °C. The bound fraction was analyzed by immunoblotting with anti-GST, HP1β and importin α2 antibodies.

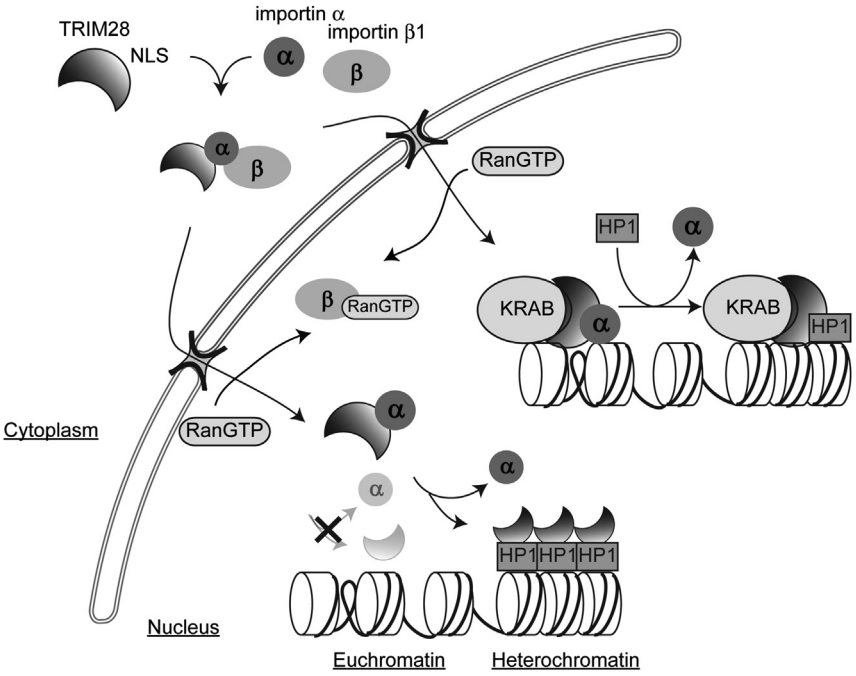

Fig. 4. A model for TRIM28 nuclear transport and chromatin targeting within the nucleus.

with HP1 that competitively interacts with TRIM28 NLS (HP1 box) to release importin α from TRIM28, resulting in the efficient TRIM28-HP1 complex formation on the target sites. That is, the dynamic interaction between the TRIM28 NLS and importin α is required not only for efficient nuclear transport of TRIM28 but also for appropriate interaction between HP1 and TRIM28 and/or targeting of TRIM28 onto HP1-rich heterochromatin region, causing the efficient and proper transcriptional regulation and/or chromatin remodeling in the nucleus.

Previously, it has been suggested that Kap114p, the yeast homologue of mammalian importin 9, may play roles in the intranuclear targeting of TATA-binding protein (TBP) after its nuclear translocation, based on the observation that the TATA-containing DNA stimulates the RanGTP-dependent dissociation of TBP from Kap114p [30]. In addition, it was also shown that GAL4-NLS binds to both DNA and importin β1 in a mutually exclusive manner [31]. Indeed, NLSs often overlap with a DNA-binding site [32,33]. Furthermore, several importin β family members were shown to function not only as import factors but also as chaperones to prevent the aggregation of highly basic proteins, such as ribosomal proteins [34]. These observations, together with our findings in this study, suggest the significance of an NLS-importin binding beyond nuclear transport processes.

Conflict of interest

None.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.108.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.108.

References

[1] J.R. Friedman, W.J. Fredericks, D.E. Jensen, D.W. Speicher, X.P. Huang, E.G. Neilson, F.J. Rauscher 3rd, KAP-1, a novel corepressor for the highly conserved KRAB repression domain, Genes. Dev. 10 (1996) 2067–2078.
[2] S.S. Kim, Y.M. Chen, E. O'Leary, R. Witzgall, M. Vidal, J.V. Bonventre, A novel member of the RING finger family, KRIP-1, associates with the KRAB-A transcriptional repressor domain of zinc finger proteins, Proc. Natl. Acad. Sci. U S A 93 (1996) 15299–15304.
[3] P. Moosmann, O. Georgiev, B. Le Douarin, J.P. Bourquin, W. Schaffner, Transcriptional repression by RING finger protein TIF1 beta that interacts with the KRAB repressor domain of KOX1, Nucleic Acids Res. 24 (1996) 4859–4867.
[4] S. Iyengar, P.J. Farnham, KAP1 protein: an enigmatic master regulator of the genome, J. Biol. Chem. 286 (2011) 26267–26276.
[5] D.C. Schultz, K. Ayyanathan, D. Negorev, G.G. Maul, F.J. Rauscher 3rd, SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins, Genes. Dev. 16 (2002) 919–932.
[6] D.C. Schultz, J.R. Friedman, F.J. Rauscher 3rd, Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD, Genes. Dev. 15 (2001) 428–443.
[7] B. Le Douarin, A.L. Nielsen, J.M. Garnier, H. Ichinose, F. Jeanmougin, R. Losson, P. Chambon, A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors, EMBO J. 15 (1996) 6701–6715.
[8] A.L. Nielsen, J.A. Ortiz, J. You, M. Oulad-Abdelghani, R. Khechumian, A. Gansmuller, P. Chambon, R. Losson, Interaction with members of the heterochromatin protein 1 (HP1) family and histone deacetylation are differentially involved in transcriptional silencing by members of the TIF1 family, EMBO J. 18 (1999) 6385–6395.
[9] R.F. Ryan, D.C. Schultz, K. Ayyanathan, P.B. Singh, J.R. Friedman, W.J. Fredericks, F.J. Rauscher 3rd, KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Kruppel-associated box-zinc finger proteins in heterochromatin-mediated gene silencing, Mol. Cell Biol. 19 (1999) 4366–4378.
[10] M.S. Lechner, D.C. Schultz, D. Negorev, G.G. Maul, F.J. Rauscher 3rd, The mammalian heterochromatin protein 1 binds diverse nuclear proteins through a common motif that targets the chromoshadow domain, Biochem. Biophys. Res. Commun. 331 (2005) 929–937.

[11] A.J. Bannister, P. Zegerman, J.F. Partridge, E.A. Miska, J.O. Thomas, R.C. Allshire, T. Kouzarides, Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain, Nature 410 (2001) 120–124.
[12] M. Lachner, D. O'Carroll, S. Rea, K. Mechtler, T. Jenuwein, Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins, Nature 410 (2001) 116–120.
[13] J. Nakayama, J.C. Rice, B.D. Strahl, C.D. Allis, S.I. Grewal, Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly, Science 292 (2001) 110–113.
[14] R. Riclet, M. Chendeb, J.L. Vonesch, D. Koczan, H.J. Thiesen, R. Losson, F. Cammas, Disruption of the interaction between transcriptional intermediary factor 1{beta} and heterochromatin protein 1 leads to a switch from DNA hyper- to hypomethylation and H3K9 to H3K27 trimethylation on the MEST promoter correlating with gene reactivation, Mol. Biol. Cell 20 (2009) 296–305.
[15] D.S. Goldfarb, A.H. Corbett, D.A. Mason, M.T. Harreman, S.A. Adam, Importin alpha: a multipurpose nuclear-transport receptor, Trends Cell Biol. 14 (2004) 505–514.
[16] M. Stewart, Molecular mechanism of the nuclear protein import cycle, Nat. Rev. Mol. Cell Biol. 8 (2007) 195–208.
[17] H. Fried, U. Kutay, Nucleocytoplasmic transport: taking an inventory, Cell Mol. Life Sci. 60 (2003) 1659–1688.
[18] N. Yasuhara, R. Yamagishi, Y. Arai, R. Mehmood, C. Kimoto, T. Fujita, K. Touma, A. Kaneko, Y. Kamikawa, T. Moriyama, T. Yanagida, H. Kaneko, Y. Yoneda, Importin alpha subtypes determine differential transcription factor localization in embryonic stem cells maintenance, Dev. Cell 26 (2013) 123–135.
[19] Y. Miyamoto, N. Imamoto, T. Sekimoto, T. Tachibana, T. Seki, S. Tada, T. Enomoto, Y. Yoneda, Differential modes of nuclear localization signal (NLS) recognition by three distinct classes of NLS receptors, J. Biol. Chem. 272 (1997) 26375–26381.
[20] Y. Yoneda, Nucleocytoplasmic protein traffic and its significance to cell function, Genes. Cells 5 (2000) 777–787.
[21] J.C. Eissenberg, S.C. Elgin, The HP1 protein family: getting a grip on chromatin, Curr. Opin. Genet. Dev. 10 (2000) 204–210.
[22] Y. Seki, A. Kurisaki, K. Watanabe-Susaki, Y. Nakajima, M. Nakanishi, Y. Arai, K. Shiota, H. Sugino, M. Asashima, TIF1beta regulates the pluripotency of embryonic stem cells in a phosphorylation-dependent manner, Proc. Natl. Acad. Sci. U S A 107 (2010) 10926–10931.
[23] G. Hu, J. Kim, Q. Xu, Y. Leng, S.H. Orkin, S.J. Elledge, A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal, Genes. Dev. 23 (2009) 837–848.
[24] F. Cammas, M. Herzog, T. Lerouge, P. Chambon, R. Losson, Association of the transcriptional corepressor TIF1beta with heterochromatin protein 1 (HP1): an essential role for progression through differentiation, Genes. Dev. 18 (2004) 2147–2160.
[25] D.E. White, D. Negorev, H. Peng, A.V. Ivanov, G.G. Maul, F.J. Rauscher 3rd, KAP1, a novel substrate for PIKK family members, colocalizes with numerous damage response factors at DNA lesions, Cancer Res. 66 (2006) 11594–11599.
[26] A.A. Goodarzi, A.T. Noon, D. Deckbar, Y. Ziv, Y. Shiloh, M. Lobrich, P.A. Jeggo, ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin, Mol. Cell 31 (2008) 167–177.
[27] Y. Ziv, D. Bielopolski, Y. Galanty, C. Lukas, Y. Taya, D.C. Schultz, J. Lukas, S. Bekker-Jensen, J. Bartek, Y. Shiloh, Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway, Nat. Cell Biol. 8 (2006) 870–876.
[28] M.S. Lechner, G.E. Begg, D.W. Speicher, F.J. Rauscher 3rd, Molecular determinants for targeting heterochromatin protein 1-mediated gene silencing: direct chromoshadow domain-KAP-1 corepressor interaction is essential, Mol. Cell Biol. 20 (2000) 6449–6465.
[29] F. Cammas, M. Oulad-Abdelghani, J.L. Vonesch, Y. Huss-Garcia, P. Chambon, R. Losson, Cell differentiation induces TIF1beta association with centromeric heterochromatin via an HP1 interaction, J. Cell Sci. 115 (2002) 3439–3448.
[30] L.F. Pemberton, J.S. Rosenblum, G. Blobel, Nuclear import of the TATA-binding protein: mediation by the karyopherin Kap114p and a possible mechanism for intranuclear targeting, J. Cell Biol. 145 (1999) 1407–1417.
[31] C.K. Chan, S. Hubner, W. Hu, D.A. Jans, Mutual exclusivity of DNA binding and nuclear localization signal recognition by the yeast transcription factor GAL4: implications for nonviral DNA delivery, Gene Ther. 5 (1998) 1204–1212.
[32] M. Cokol, R. Nair, B. Rost, Finding nuclear localization signals, EMBO Rep. 1 (2000) 411–415.
[33] E.C. LaCasse, Y.A. Lefebvre, Nuclear localization signals overlap DNA- or RNA-binding domains in nucleic acid-binding proteins, Nucleic Acids Res. 23 (1995) 1647–1656.
[34] S. Jakel, J.M. Mingot, P. Schwarzmaier, E. Hartmann, D. Gorlich, Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains, EMBO J. 21 (2002) 377–386.