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Negative transcriptional regulation of mitochondrial transcription factor A (TFAM) by nuclear TFAM

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ABSTRACT

The nuclear DNA-encoded mitochondrial transcription factor A (TFAM) is synthesized in cytoplasm and transported into mitochondria. TFAM enhances both transcription and replication of mitochondrial DNA. It is unclear, however, whether TFAM plays a role in regulating nuclear gene expression. Here, we demonstrated that TFAM was localized to the nucleus and mitochondria by immunostaining, subcellular fractionation, and TFAM-green fluorescent protein hybrid protein studies. In HT22 hippocampal neuronal cells, human TFAM (hTFAM) overexpression suppressed human *Tfam* promoter-mediated luciferase activity in a dose-dependent manner. The mitochondria targeting sequence-deficient hTFAM also repressed *Tfam* promoter activity to the same degree as hTFAM. It indicated that nuclear hTFAM suppressed *Tfam* expression without modulating mitochondrial activity. The repression required for nuclear respiratory factor-1 (NRF-1), but hTFAM did not bind to the NRF-1 binding site of its promoter. TFAM was co-immunoprecipitated with NRF-1. Taken together, we suggest that nuclear TFAM down-regulate its own gene expression as a NRF-1 repressor, showing that TFAM may play different roles depending on its subcellular localizations.

1. Introduction

Mitochondria possess several copies of mitochondrial DNA (mtDNA), which encodes 13 subunits of the oxidative phosphorylation system. Because mitochondria are not completely self-supporting organelles, the replication and transcription of mtDNA depend on trans-acting nucleus-encoded factors, including mitochondrial transcription factor A (TFAM), nuclear respiratory factors (NRFs), and peroxisome proliferator activated receptor γ-coactivator 1α (PGC-1α) [1]. Maintenance of mtDNA and normal ATP production are critical to mitochondrial function in eukaryotic cells.

TFAM is a 25-kDa DNA-binding protein consisting of two high mobility group box (HMG) domains and a basic carboxyl terminal tail that activates both mitochondrial RNA transcription [2] and mtDNA replication [3]. Approximately 1000 molecules of TFAM exist per mtDNA molecule, which is abundant enough to wrap mtDNA entirely to form a nucleoid structure similar to histones in the nucleosome [4]. TFAM binding to mtDNA is not sequence-specific yet their association may provide stability to both molecules. TFAM protects mtDNA from attack by reactive oxygen species (ROS) while mtDNA protects TFAM from degradation by Lon protease [5]. Studies demonstrate that TFAM knockout mice are embryonic lethal and lack mtDNA [6]. Loss of TFAM also leads to progressive degeneration of dopaminergic neurons [7]. Transgenic mice overexpressing human TFAM (hTFAM) exhibit an increased mtDNA copy number, which improves mitochondrial disease phenotypes and delays neuronal death [8]. Thus, TFAM is an attractive indicator of defects in mitochondrial function.

Recently, several contradictory functions have been reported for TFAM. Transgenic TFAM-overexpressing mice displayed an increased mtDNA copy number, enlarged nucleoid, and defective respiratory chain activity in the heart and muscles [9]. Moreover, the lifespan of TFAM-overexpressing fruit flies was shown to be shorter than that of controls under normal conditions, but longer under strong oxidative stress conditions [10]. These reports suggest that excess TFAM has both negative and positive effects on lifespan depending on the stress conditions. Nevertheless, increasing the amount of mtDNA by overexpression of TFAM, NRF-1, or PGC-1α has been suggested as therapeutic approaches to treat diseases with mitochondrial dysfunction [11].

TFAM has been found within the nuclei and mitochondria of various cancer cells, and regulates the expression of several nuclear genes involved in cancer cell growth [12]. Nuclear-localized TFAM

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has also been shown to enhance the transcription of *Serca2a* transcription in rat cardiac myocytes. However, except for the involvement of NRF-1, Sp1, and PGC-1α, details regarding the upstream regulators of TFAM-mediated transcription remain poorly understood [13]. NRFs activate several nuclear genes involved in multiple mitochondrial functions. NRF-1 binding sites were present in the promoters of cytochrome c, whereas the cytochrome c oxidase promoter possesses a NRF-2 binding site. The proximal promoter of *Tfam* contains both NRF-1 and NRF-2 recognition sites [14]. NRFs participate in regulating mtDNA-encoded gene expression through sequence-specific activation of *Tfam* transcription. NRF-mediated *Tfam* transcriptional activation may provide a link between nuclear and mitochondrial gene expression.

In this study, we investigated whether TFAM possesses a novel function as a transcriptional repressor in the nuclei of HT22 mouse hippocampal neuronal cells. This is the first study demonstrating the function of TFAM on *Tfam* transcription within the nuclei of neuronal cells.

2. Materials and methods

2.1. Cell culture and transfection

HT22 mouse hippocampal neuronal cells,SK-Hep1 human hepatoma cells SH-SY5Y human neuroblastoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) or DMEM/F12 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin under 37 °C/5% CO_2 conditions.

For transient transfections, 70% confluent HT22 cells (3.5×10^4 cells/well) in 24-well plate were transfected with pTfam-luc (600 ng), pRL-TK (control renilla luciferase, 50 ng), and the indicated plasmid(s) (up to 250 ng) using SuperFect® Transfection Reagent (Qiagen, Valencia, CA). The total quantity of plasmid transfected was kept constant by adding pcDNA3.1, which was also used as a control plasmid for mock transfection. Transfected cells were maintained for 24 h in fresh complete media before harvest and determination of luciferase activity using a dual-luciferase assay kit (Promega, Madison, WI, USA) and luminometer (Berthold, Bad Wildbad, Germany). The transfection efficiencies were normalized against *Renilla* luciferase activity.

2.2. Preparation of plasmids

The pTfam-luc reporter vector containing human *Tfam* promoter (−2246 to +132 bp) [15] was sub-cloned in pGL3-Basic (Promega). The binding sites for NRF-1, Sp1, and NRF-2 (NSN) were deleted from the *Tfam* promoter to produce pΔNSN-hTfam-luc by oligonucleotide-directed mutagenesis PCR. Primer sequences are summarized in Supplementary Table 1). Plasmids for overexpressing hTFAM, NRF-1, and DN-NRF-1 were prepared in pcDNA3.1. The mitochondrial targeting sequence (MTS) was deleted by PCR to generate ΔMTS–hTFAM. The plasmids expressing hTFAM–EGFP or ΔMTS–hTFAM–EGFP fusion proteins were prepared by cloning the PCR-generated fragments of hTFAM or ΔMTS–hTFAM into pEGFP-N3 at HindIII/BamH1. All products from PCR-based cloning were sequenced to ensure the fidelity of the resulting constructs.

2.3. Subcellular fractionation

Subcellular organelle fractions from HT22, SH-SY5Y, or SK-Hep1 cells were prepared by differential centrifugation. The cells were harvested in PBS. Cell pellets were resuspended in each sub-organelle fractionation buffer and homogenized using a Dounce homogenizer. Cell lysates in nuclei preparation buffer (NB, 10 mM HEPES–KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride) were centrifuged at 2700*g* for 10 min at 4 °C. Cell pellets re-homogenized in NB containing 0.2% NP-40 were centrifuged at 200*g* for 5 min to prepare the nuclear fraction (Nu). Mitochondria (Mito) were isolated from cell lysates in mitochondria isolation buffer (MIB, 0.025 M Tris pH 7.4, 0.25 M sucrose, 1 mM EDTA) by centrifugation at 10,900*g* for 10 min. The cellular, nuclear, and mitochondrial pellets were resuspended in lysis buffer (PBS, pH 7.4, 2% SDS, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml pepstatin A and 2 μg/ml leupeptin) for Western blot analysis. Lysate protein concentration was determined by the BCA method (Pierce, Rockford, IL, USA).

2.4. Western blot analysis

Whole cell lysates (30 μg) or organelle extracts (10 μg) were prepared in (PBS, pH 7.4, 2% SDS, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml pepstatin A and 2 μg/ml leupeptin), separated using 12% or 15% SDS–PAGE, and transferred to PVDF membranes. Blots were probed with following primary antibodies: TFAM (homemade, 1:3000) [11], HDAC1 (Abcam) and Hsp60 (Santa Cruz Biotechnology) were used at 1:1000 dilution. Equivalent protein loading was verified by blotting with anti-β-actin antibody (Sigma–Aldrich, St. Louis, MO, USA). All blots were visualized using an enhanced chemiluminescence kit (Pierce).

2.5. Co-immunoprecipitation

Cell lysates (500 μg protein) in IP lysis buffer (50 mM Tris–Cl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, 2 μg/ml aprotinin, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin) were pre-cleared with 1 μg of normal rabbit IgG for 1 h at 4 °C and incubated with anti-TFAM (homemade, 1:400), anti-NRF-1 (Santa Cruz Biotechnology, 2 μg), or rabbit IgG (0.5 μg) antibody under constant rotation overnight at 4 °C. TrueBlot anti-Rabbit Ig IP Beads (20 μl, Rockland Immunochemicals) was added and the mixture was incubated at 4 °C for another 2 h. The slurry were washed three times in IP buffer, boiled in 40 μl IP buffer plus 10 μl SDS–PAGE loading buffer, and then subjected to 12% SDS–PAGE followed by Western blot analysis except using Rabbit TrueBlot anti-rabbit IgG HRP as a secondary antibody (1:1000, Rockland Immunochemicals).

2.6. Immunocytochemistry

Cells cultured on glass coverslips were stained with MitoTracker and immunostained with anti-mouse TFAM (1:500, Santa Cruz Biotechnology) antibodies overnight at 4 °C as described [16]. Nuclei were stained by incubation with Hoechst (2 μg/ml Molecular Probes, Eugene, OR) in PBS for 5 min at room temperature after secondary antibody treatment. Specimens were viewed using laser scanning confocal microscopy (Carl Zeiss, Germany) at 405 nm, 488 nm, and 555 nm for Hoechst, TFAM, and MitoTracker, respectively.

hTFAM–EGFP or ΔMTS–hTFAM–EGFP was transfected into SK-Hep1 cells using SuperFect® Transfection Reagent (Qiagen). To calculate the co-localization ratio, the transfected cells were immunostained with HDAC (nuclei) or Hsp60 (mitochondria) antibody, followed by an Alexa Fluor 555-conjugated secondary antibody (1:1000, Molecular Probes). Specimens were viewed and the percentage of co-localization was calculated using a laser scanning confocal microscope.

2.7. Chromatin immunoprecipitation (ChIP) assay

SH-SY5Y human neuroblastoma cells transfected with 10 μg of phTFAM or pΔMTS-hTFAM were subjected to formaldehyde

crosslinking for 15 min and soluble chromatin was prepared by sonication (Ultrasonic Processor VCX500 equipped with a 2 mm tip, six sets at 30% of maximum power, pulse ON – 15 s/OFF – 60 s). The cross-linked protein-DNA in the soluble chromatin was immunoprecipitated with anti-hTFAM antibody. Anti-mouse IgG (IgG) and anti-RNA polymerase II (Pol II) were used as negative and positive controls, respectively (Millipore, Billerica, MA). Ten percent of the soluble chromatin was used as the input control. The precipitated DNA fragments were analyzed by PCR using primers directed against the human *Tfam* promoter (5′-cca gag agc caa tgt ttt ca-3′ and 5′-tcc agc aat cac aac tgg aa-3′, 1205 bp).

2.8. Statistical analysis

Data shown are expressed as the mean ± standard error (SE). Statistical significance was evaluated by the Student's *t*-test and ANOVA for continuous variables. Significance was defined as $P < 0.05$.

3. Results

3.1. Localization of TFAM in nuclei and mitochondria

In order to regulate its own transcription, TFAM must be present in nuclei. Immunocytochemistry demonstrated that some endogenous TFAM was localized within the nuclei of HT22 (mouse hippocampal cell line) (Fig. 1A). The mitochondrial reticulum was visualized by MitoTracker staining. Western blots of subcellular fractions from HT22, SH-SY5Y, and SK-Hep1 cells confirmed that TFAM was present in mitochondria and nuclei (Fig. 1B). Each subcellular fraction was stringently monitored for contamination using the nuclear and mitochondrial markers HDAC1 and Hsp60, respectively. Mitochondrial TFAM was expected to exhibit a lower molecular weight than nuclear TFAM because, unlike the nuclear localization signal, the MTS is cleaved by metalloproteases in the mitochondrial matrix after translocation. The SDS–PAGE and Western blot analysis of a mixture of nuclear and mitochondrial lysates clearly shows that the apparent molecular weight of mitochondrial TFAM is smaller than that of nuclear TFAM (Fig. 1C).

We calculated hTFAM co-localization ratio to HDAC or Hsp60 marker proteins using immunochemical images of hTFAM–EGFP and ΔMTS–hTFAM–EGFP -transfected cells (Fig. 1D). Approximately 70% of hTFAM–EGFP was overlapped with Hsp60 (mitochondria), while 86% of ΔMTS–hTFAM was overlapped with HDAC (nuclei).

3.2. Both hTFAM and ΔMTS-hTFAM repressed human Tfam promoter activity

Next, we investigated whether ectopic overexpression of hTFAM repressed transcription of human *Tfam* itself in HT22 cells. The human *Tfam* promoter contains binding sites for NRF-1, Sp1, and NRF-2 (NSN) (Fig. 2A). Transient co-transfection assay revealed that hTFAM decreased pTfam-luc activity in a dose-dependent manner (Fig. 2B). Enhanced TFAM expression may repress *Tfam* transcription via a negative feedback loop due to the increased mitochondrial activity or mtDNA copy number. To exclude this possibility, we assessed pTfam-luc activity after co-transfection of ΔMTS–hTFAM, a nuclear or non-mitochondrial form of hTFAM. ΔMTS–hTFAM repressed pTfam-luc activity in a dose-dependent manner (Fig. 2C) to a similar extent as hTFAM (Fig. 2D). These results suggest that TFAM exerts transcriptional repression primarily in the nucleus rather than through retrograde signaling from mitochondria to nucleus.

3.3. TFAM-mediated Tfam promoter repression is NRF-1-dependent

Because NRF-1 is the major *Tfam* transcription activator [14], we examined whether TFAM represses pTfam-luc activity by inhibiting NRF-1. NRF-1 overexpression restored both hTFAM-induced and ΔMTS–hTFAM-induced repression of pTfam-luc activity in a

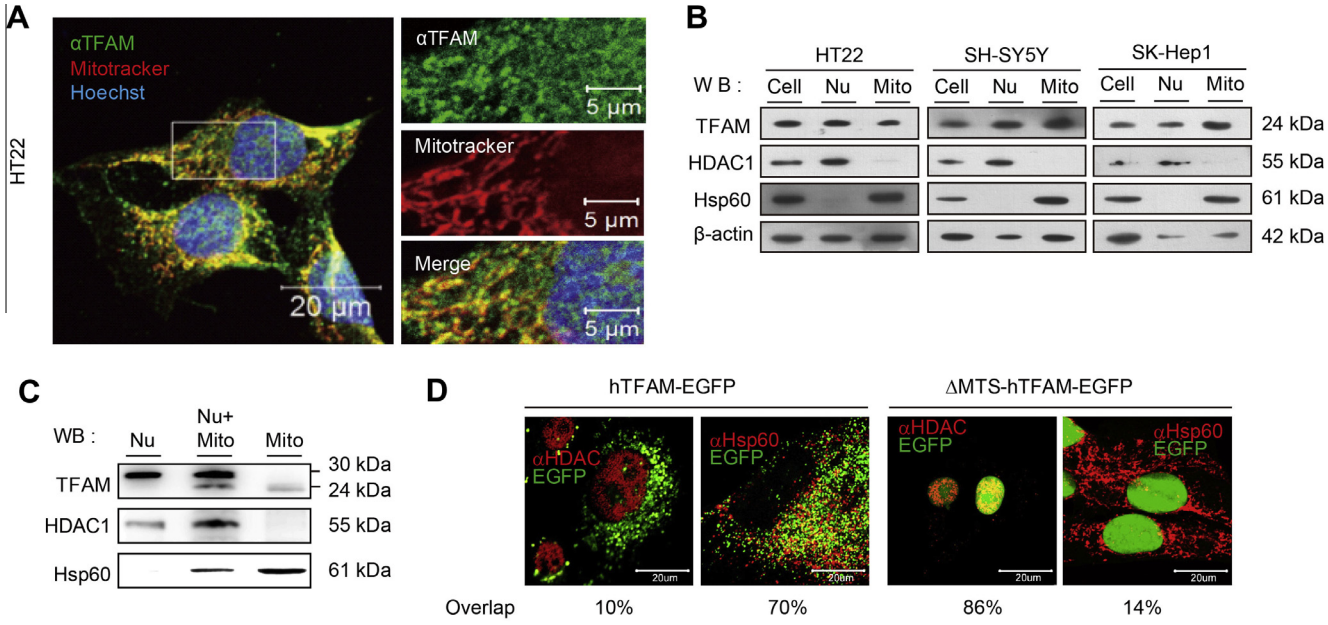

Fig. 1. TFAM localizes in the nucleus. (A) Confocal images of HT22 cells stained with MitoTracker (red), anti-mTFAM antibody (green) and Hoechst dye (blue). Scale bar = 5 or 20 μm. (B) Western blots of organelle extracts (10 μg) resolved by 12% SDS–PAGE. Cell, whole cell; Nu, nuclei; Mito, mitochondria. Markers: HDAC1, nuclei; Hsp60, mitochondria; β-actin, loading control. (C) Size comparison of nuclear versus mitochondrial TFAM. Extracts from nuclei (Nu, 10 μg) and/or mitochondria (Mito, 3 μg) were analyzed by 15% SDS–PAGE Western blot. (D) Stable cells overexpressing hTFAM–EGFP or ΔMTS–hTFAM–EGFP were immunostained with anti-HDAC or anti-Hsp60 antibody. The overlapping fluorescent intensities (yellow signal) were calculated as a percent (%) co-localization signals.

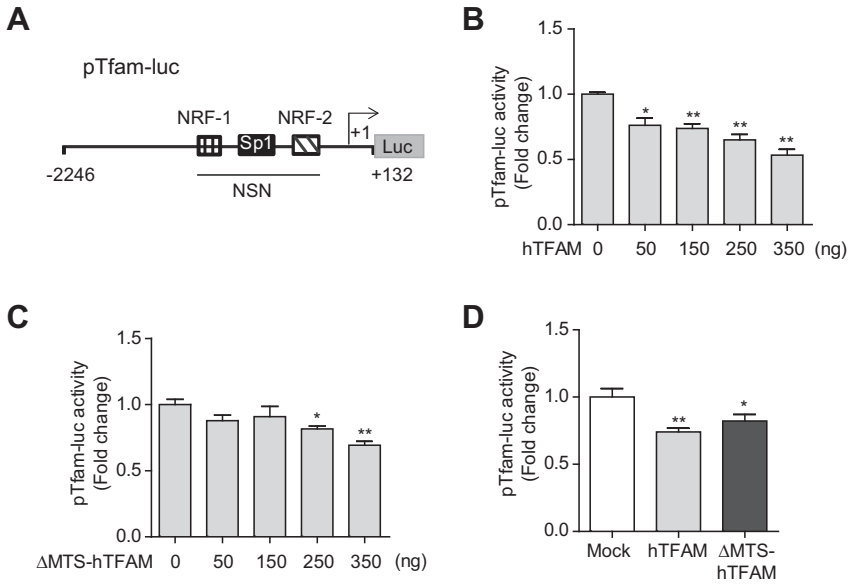

Fig. 2. Both hTFAM and ΔMTS–hTFAM repressed pTfam-luc activity. (A) The map of pTfam-luc (pGL3-hTfam(2378)-luc). (B, C) Co-transfection assay. HT22 cells were transiently co-transfected with pTfam-luc and pRL-TK along with various amounts of hTFAM (B) or ΔMTS–hTFAM (C). Luciferase activity of the harvested cells was measured at 24 h after transfection, and normalized against *Renilla* luciferase activity. (D) Comparison of pTfam-luc activity between HT22 cells co-transfected with 250 ng hTFAM or ΔMTS–hTFAM. Mean ± SE ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Mock.

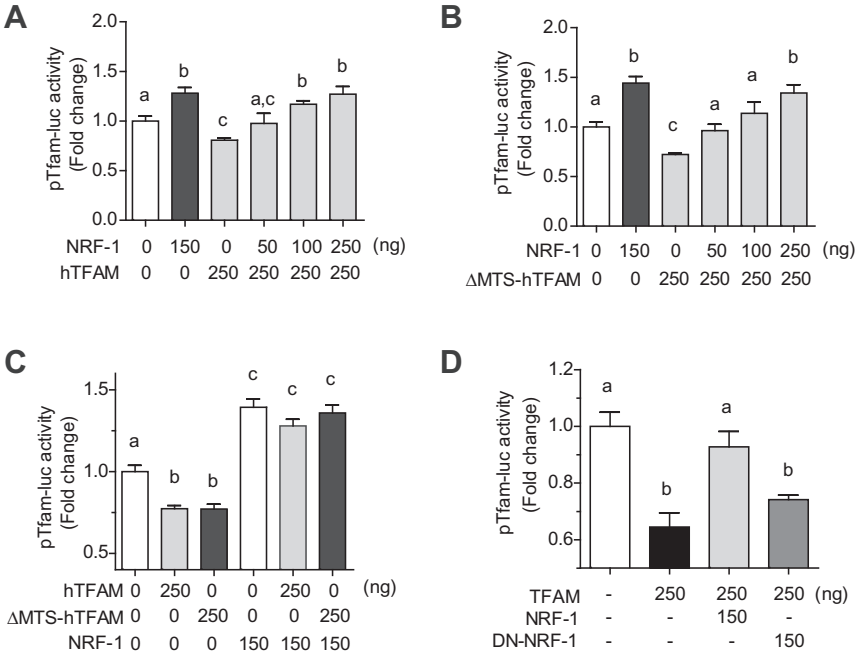

Fig. 3. NRF-1 restored hTFAM-mediated repression of *Tfam* promoter activity. Co-transfection luciferase assay of HT22 cells with various amounts of NRF-1 in the presence of hTFAM (A) or ΔMTS–hTFAM (B). (C) Comparison of NRF-1 effects between hTFAM- and ΔMTS–hTFAM-transfected cells ($n = 12$). (D) DN-NRF-1 did not further decrease the hTFAM-induced repression. Mean ± SE ($n = 6$). Different letters on the graphs indicate that statistical differences were observed ($P < 0.01$, ANOVA).

dose-dependent manner (Fig. 3A and B). The extent of recovery was comparable with both constructs (Fig. 3C). However, a dominant-negative NRF-1 (DN-NRF-1) consisting of the DNA binding domain without the transactivation domain failed to further decrease this transcriptional repression (Fig. 3D). It suggests that DN-NRF-1 interact with excess hTFAM to release endogenous NRF-1 trans-activating Tfam transcription. Therefore, NRF-1 is required to exert nuclear TFAM-mediated inhibition of transcription.

3.4. Requirement of NSN sites for the TFAM-mediated suppression

To determine if hTFAM displaces NRF-1 on NSN binding sites of the promoter, we measured the luciferase activity of pΔNSN-Tfam-luc-transfected cells. We found that pΔNSN-Tfam-luc exhibited a greater than 70% loss of *Tfam* promoter activity and addition of hTFAM did not further reduce the activity (Fig. 4A). This result demonstrates that the NSN sites on the *Tfam* promoter were required for repression by nuclear TFAM.

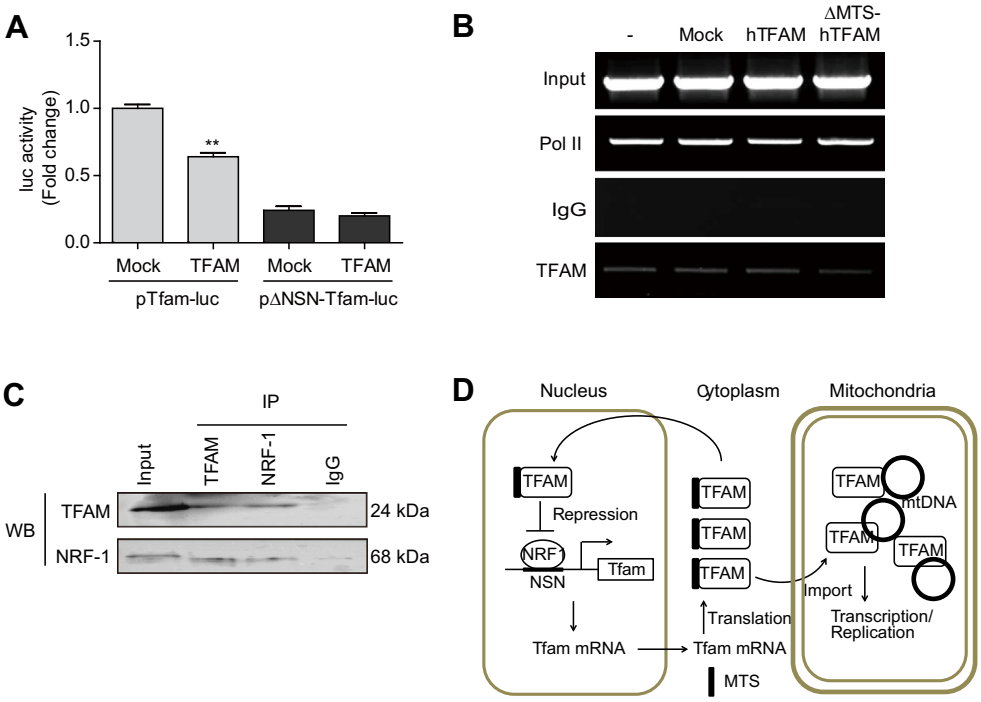

Fig. 4. TFAM repressed NRF-1 activity without binding directly to the *Tfam* promoter. (A) Requirement of NSN sites for repression. Luciferase activities were determined after co-transfection of pTfam-luc or pΔNSN-hTfam-luc along with hTFAM. Mean ± SE ($n = 6$) $^{**}P < 0.01$ vs. Mock. (B) Chromatin immunoprecipitation (ChIP) assay using anti-hTFAM antibodies (TFAM) and specific PCR primers for the hTFAM promoter. SH-SY5Y cells were transiently transfected with pcDNA3.1 (mock), phTFAM, or pΔMTS-hTFAM. Anti-RNA polymerase II (Pol II), positive control; rabbit IgG, negative controls. Input was 1/10 of the soluble chromatin. (C) Co-immunoprecipitation of TFAM with NRF-1. The hTFAM-transfected cell lysates were immunoprecipitated (IP) with anti-hTFAM, anti-hNRF-1, or rabbit IgG antibody and then incubated with TrueBlot Rabbit Ig IP beads. The washed precipitates were analyzed by 12% SDS–PAGE and Western blot (WB). (D) Schematic illustration of two different roles for TFAM in the nucleus and mitochondria. Nuclear TFAM contains MTS which is removed in mitochondrial matrix. MTS, mitochondrial targeting sequence.

3.5. Direct binding of TFAM to the Tfam promoter is not required for repression

To verify whether TFAM binds to NSN sites of the *Tfam* promoter, we performed the ChIP assay using SH-SY5Y human cells. Because hTFAM-mediated repression was demonstrated to require NRF-1 activity and the NSN site, we amplified the *Tfam* promoter proximal region (−1137 to +69, 1205 bp) that encompasses the NSN sites from anti-hTFAM-precipitated chromatin. Basic properties of the TFAM HMG box produced sequence-independent weak binding of hTFAM to the promoter DNA much like wrapping mtDNA [4,17]. But overexpression of hTFAM or ΔMTS–hTFAM did not alter the binding efficiency onto the proximal promoter region (Fig. 4B). Therefore, we conclude that direct binding of hTFAM onto the promoter is not necessary for suppressing *Tfam* transcription. In fact, co-immunoprecipitation analysis revealed that TFAM interacted directly with NRF-1 (Fig. 4C). Taken together, we suggest that nuclear TFAM possessing MTS may block NRF-1 transactivation activity via a protein–protein interaction, but mitochondrial TFAM binds mtDNA to control the transcription and replication (Fig. 4D).

4. Discussion

The typical mitochondrial matrix protein TFAM is encoded by nuclear DNA. The TFAM precursor protein possessing MTS is synthesized in the cytosol. Upon recognition of the MTS by transport machinery on the mitochondrial outer membrane, the precursor molecule is imported into mitochondria, where it is processed to generate mature TFAM. This mature TFAM plays a role as a regulator of transcription and replication of mtDNA.

In this study, we demonstrated a novel function of TFAM as a transcriptional repressor of its own expression in nuclei. In this case, TFAM must be transported into the nucleus as well as the mitochondria. We confirmed that a portion of total TFAM localized to the nucleus (Fig. 1). Recently, a study identified two putative nuclear localization signals within the HMG-boxes of TFAM and that the proportion of nuclear-localized TFAM varied among different cancer cells [12]. Both nuclear and mitochondrial TFAM has been shown to promote cancer cell growth via p21-dependent G1 cell cycle progression. Other groups have also demonstrated that nuclear localization of TFAM and mitochondrial transcription factor B2 (mtTFB2), which binds two proximal regions of the rat sarcoplasmic reticulum Ca^{2+}-ATPase 2a (*Serca2*) promoter to enhance transcription in rat cardiac myocytes [18]. The putative binding site for TFAM on the *Serca2* promoter was suggested as GGGGGCGGG. Here, we also observed nuclear localization of both endogenous and overexpressed TFAM in HT22 cells, which are not cancer cells but immortalized hippocampal neurons. Therefore, the nuclear presence of TFAM may not be due to carcinogenic changes or disease pathogenesis, but rather possibly observed in most normal eukaryotic cells. Estimation of the proportion of nuclear TFAM revealed that approximately 10–20% was found in nuclear fractions of control and TFAM–EGFP fusion protein-expressing HT22 cells (Fig. 1).

Elucidating the molecular mechanism of TFAM transcription has important implications for understanding mitochondrial dysfunction-associated disorders, including neurodegeneration, aging, and diabetes. However, why and how increasing TFAM down-regulates its own expression is poorly understood. Our results suggest that TFAM may suppress its own expression without modulating mitochondrial activity because the non-mitochondrial form of ΔMTS–hTFAM repressed *Tfam* promoter activity to the same degree as hTFAM (Fig. 2).

Early studies of *Tfam* transcriptional regulation indicated the involvement of NRF-1 and NRF-2 [19]. Pro-oxidant condition has been shown to augment NRF-1 phosphorylation and *Tfam* transcription [20]. PGC-1α is a co-activator of NRF-1 transcriptional activity on the *Tfam* promoter [21]. Negative feedback inhibition of its own transcription implies that TFAM plays a role as a repressor in the nucleus, distinct from its role as an activator in the mitochondria (Fig. 4D). In mitochondria, TFAM interacts with mtTFB to activate mitochondrial RNA polymerase [22]. Alternatively, TFAM may interact with NRF-1 to suppress its activity in the nucleus. It is not likely that the inhibitory function of TFAM is exerted on the *Tfam* promoter, which is different from its activity on mitochondrial D-loop DNA. Co-transfection of pΔNSN-Tfam-luc and ChIP assays demonstrate that direct DNA binding was not required for TFAM self-repression (Fig. 4). Additional investigation is necessary to identify the molecular interaction between TFAM and NRF-1 on the *Tfam* promoter.

TFAM regulates the amount of mtDNA present in cells and tissues [23]. Abnormalities of mtDNA, such as deletion, depletion, and mutation, have been shown to cause various diseases such as progressive neurodegeneration and aging [24–26]. Nevertheless, the expression level of TFAM has not been directly reported in these diseases yet. We observed that TFAM expression did not change notably in response to external stimulants in normal cells (unpublished data). Our results support that the negative feedback loop controlling TFAM expression may function to maintain its level precisely in normal cells. For example, augmentation of nuclear-localized TFAM may repress its transcription back to a basal level even though mitochondrial TFAM enhances the transcription/replication of mtDNA. Therefore, we suggest that autoregulation of *Tfam* in the nucleus would be another important regulatory mechanism for maintaining homeostatic levels of TFAM, as well as mutually protect TFAM and mtDNA by forming TFAM:mtDNA complexes in mitochondria [5].

In conclusion, TFAM may play different roles depending on its subcellular localization. In mitochondria, it activates the transcription and replication of mtDNA, whereas TFAM suppresses its own transcription to maintain its expression level in the nucleus (Fig. 4D). This novel function of TFAM in the nucleus may only be one of its roles [27]. Understanding TFAM-mediated communication between the nucleus and mitochondria may enable us to identify the cause of mitochondrial diseases, including neurodegenerative diseases.

Acknowledgments

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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.2014.05.082.

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