

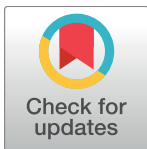
RESEARCH ARTICLE

A truncated PPAR gamma 2 localizes to mitochondria and regulates mitochondrial respiration in brown adipocytes

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Abstract

Peroxisome proliferator-activated receptor gamma (PPAR γ) is a key regulator of brown adipocyte differentiation and thermogenesis. The PPAR γ gene produces two isoforms, PPAR γ 1 and PPAR γ 2. PPAR γ 2 is identical to PPAR γ 1 except for additional 30 amino acids present in the N-terminus of PPAR γ 2. Here we report that the C-terminally truncated form of PPAR γ 2 is predominantly present in the mitochondrial matrix of brown adipocytes and that it binds to the D-loop region of mitochondrial DNA (mtDNA), which contains the promoter for mitochondrial electron transport chain (ETC) genes. Expression of mitochondrially targeted MLS-PPAR γ 2 in brown adipocytes increases mtDNA-encoded ETC gene expression concomitant with enhanced mitochondrial respiration. These results suggest that direct regulation of mitochondrially encoded ETC gene expression by mitochondrial PPAR γ 2, in part, underlies the isoform-specific role for PPAR γ 2 in brown adipocytes.

Introduction

A growing body of evidence from recent studies reveals that nuclear transcription factors translocate to mitochondria and play a role in a cell- or tissue-specific manner. Thyroid receptor (TR α isoform p43), MEF2D, STAT3, and CREB translocate to mitochondria and directly modulate mitochondrial DNA transcription in response to specific stimuli [1–5], indicating that nuclear transcription factors can control mitochondrial function via transcriptional regulation of mitochondrial genome. In addition, nuclear transcription factors have non-genomic function in mitochondria. Stress-induced p53 has been shown to trigger apoptosis in mitochondria by destabilizing the outer mitochondrial membrane through interaction with multi-domain Bcl-2 family members [6]. Estrogen receptors (ER α and ER β) can affect mitochondrial fatty acid β -oxidation by directly regulating mitochondrial HADHB enzyme activity [7–9]. Furthermore, a growing number of nuclear receptors such as glucocorticoid receptor (GR), vitamin D receptor (VDR), retinoid X receptor (RXR) and retinoic acid receptor (RAR) have been found in the mitochondria of various cells and tissues, although their function in mitochondria has not been explored [10].

Competing interests: The authors have declared that no competing interests exist.

Peroxisome proliferator-activated receptor (PPAR γ) is a key regulator of terminal fat cell differentiation [11, 12]. PPAR γ also regulates many genes involved in thermogenesis, lipid transport and metabolism, and insulin signaling in brown adipocytes [13, 14]. Alternative promoter usage and alternative splicing give rise to two different PPAR γ isoforms, PPAR γ 1 and PPAR γ 2. These two isoforms are identical except for additional 30 amino acids present in the N-terminus of PPAR γ 2 compared to PPAR γ 1 [15]. PPAR γ 1 and PPAR γ 2 are induced during differentiation of brown preadipocytes [12], but functional differences of these two isoforms have not been investigated. A previous study reported that a 45 kDa protein related to PPAR γ 2 is present in the mitochondria of several rat tissues including brown adipose tissue [16]. In the present study, we found that a C-terminally truncated form of PPAR γ 2 is predominantly present in the mitochondria of brown adipocytes and thus sought to investigate the possible isoform-specific role for PPAR γ 2 in brown adipocyte mitochondria.

Materials and methods

Mice

All animal experiments were performed according to the procedures reviewed and approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee (PBRC IACUC). C57BL/6J mice were housed on a 12-h light/12-h dark cycle. To collect brown adipose tissue from the interscapular region, mice were sacrificed with CO₂ inhalation, followed by cervical dislocation. The animal study was approved by the PBRC IACUC in the protocol 659 (03/23/2010) and protocol 740 (08/22/2011).

Cell culture

HeLa cells (ATCC) were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin and transfected using Fugene 6 (Roche Applied Science). Immortalized mouse brown preadipocytes [17] were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin and induced for differentiation as described previously [17, 18].

Plasmid construction

PPAR γ 2 was amplified from pCMX-PPAR γ 2 using primers containing Sall and NotI sites and subcloned into Sall/NotI sites of pCMV/mito that contains the mitochondrial localizing sequence (MLS). For a retroviral plasmid of pBABE-MLS-PPAR γ 2-HA, MLS-PPAR γ 2-HA was amplified from pCMV/mito-PPAR γ 2-HA using primers containing SnaBI and XhoI sites and subcloned into SnaBI/Sall sites of pBABE-neo. All plasmids generated were sequenced to rule out any mutations.

Subcellular fractionation

To obtain nuclear, cytosolic and mitochondrial fractions, tissues or cells were homogenized and subjected to subcellular fractionation by differential centrifugation as described previously [19].

Immunofluorescence

Brown adipocytes were seeded on glass coverslips, fixed, and subjected to indirect immunofluorescence as described previously [19]. The cells were analyzed with a Plan-Neofluar 40 \times /0.85 numerical aperture objective on a Zeiss LSM510 Meta confocal microscope.

Proteinase K digestion assay

Purified mitochondria were resuspended in SEM buffer (250mM sucrose, 1mM EDTA, 10mM MOPS, protease inhibitors) and incubated with increasing amounts of proteinase K for 15 min on ice in the absence or presence of 1% Triton X-100. After addition of 2mM PMSF, mitochondria were spun down, rinsed with SEM buffer containing PMSF, and resuspended in 2x Laemmli sample buffer.

Transmission electron microscopy and immunolabeling

Immuno-transmission electron microscopy (TEM) was carried out as described previously [19]. Briefly, brown adipocytes were fixed in 2% glutaraldehyde and 1% paraformaldehyde, followed by 1% osmium tetroxide. After dehydration, the cells were embedded in resin. Thin sections from the resin blocks were mounted on nickel grids. The grids were then subjected to immuno-labeling by incubating with PPAR γ (H-100) antibody or rabbit IgG. The grids were analyzed using a JEOL JEM 2011 transmission electron microscope at the LSU Socolofsky Microscopy Center.

Western blot

Cells were subjected to Western blot analysis as described previously [17]. Antibodies used were as follows: anti-PPAR γ 2 (PA1-824) from Thermo Fisher Scientific, anti-PPAR γ (H-100) (sc-7196), anti-PPAR γ (E-8) (sc-7273), anti-Tom20, anti-Lamin B1 from Santa Cruz, anti-UCP1 [20], and anti-HSP60 from Abcam.

Mitochondrial chromatin immunoprecipitation assay

Mitochondrial chromatin immunoprecipitation was carried out as described previously [19]. Briefly, mitochondria were isolated from brown adipocytes, crosslinked with 1% formaldehyde, and sheared to obtain chromatin fragments ranged from 400 bp to 1 kbp. After centrifugation at 10,000 \times g, the supernatant was pre-cleared with BSA-blocked Protein A agarose beads and incubated with a ChIP grade anti-PPAR γ (H-100) antibody or IgG at 4°C. The crosslinked DNA-protein complexes were released from BSA-blocked Protein A agarose beads and the DNA samples were purified. Quantitative real-time PCR was carried out using a pair of primers specific for the D-loop region and for ND1. D-loop fwd: 5'-gtggtgcatgcatttggtatct-3'; D-loop rev: 5'-catgaataattagccttagtgat-3'; ND1 fwd: 5'-cccattcgcgttattctt-3'; ND1 rev: 5'-aagtgatcgtaaggaagc-3'.

Retroviral infection

Retroviruses expressing an empty vector (pBABE-neo) or pBABE-MLS-PPAR γ 2-HA were produced as described previously [17]. Immortalized brown preadipocytes were infected in retrovirus-containing medium supplemented with 8 μ g/ml of polybrene for 8h as described previously [17]. After 48h, neomycin resistant clones were selected and pooled.

Quantitative real-time PCR

Total RNA was extracted from cells using the RNeasy mini kit with DNase I treatment (Qiagen). cDNA synthesis and quantitative real-time PCR were carried out as described previously [17, 18, 21]. Relative abundance of mRNA was determined after normalization to that of cyclophilin mRNA using the $\Delta\Delta$ Ct method.

Oxygen consumption assay

Cellular oxygen consumption assays were performed using the OROBOROS Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) as described previously [22]. Briefly, brown adipocytes (10^6 cells) were placed in a magnetically stirred respirometric chamber and measured for oxygen consumption rates (OCR) at baseline and after injection of antimycin A (a mitochondrial electron transport inhibitor). The value of mitochondrial respiration was determined by subtracting antimycin A-independent non-mitochondrial respiration as described in the Oroboros Operator's Manual.

Statistical analysis

All data are presented as mean \pm SEM. Student *t* tests were used to compare the difference between groups using Graphpad Prism 6 software. Values of *P* < 0.05 were considered statistically significant.

Results

A C-terminally truncated form of PPAR γ 2 is predominantly present in brown adipose tissue mitochondria

The PPAR γ gene produces two different isoforms, PPAR γ 1 and PPAR γ 2. PPAR γ 2 has additional 30 amino acids at the N-terminus compared to PPAR γ 1 (Fig 1A). Subcellular

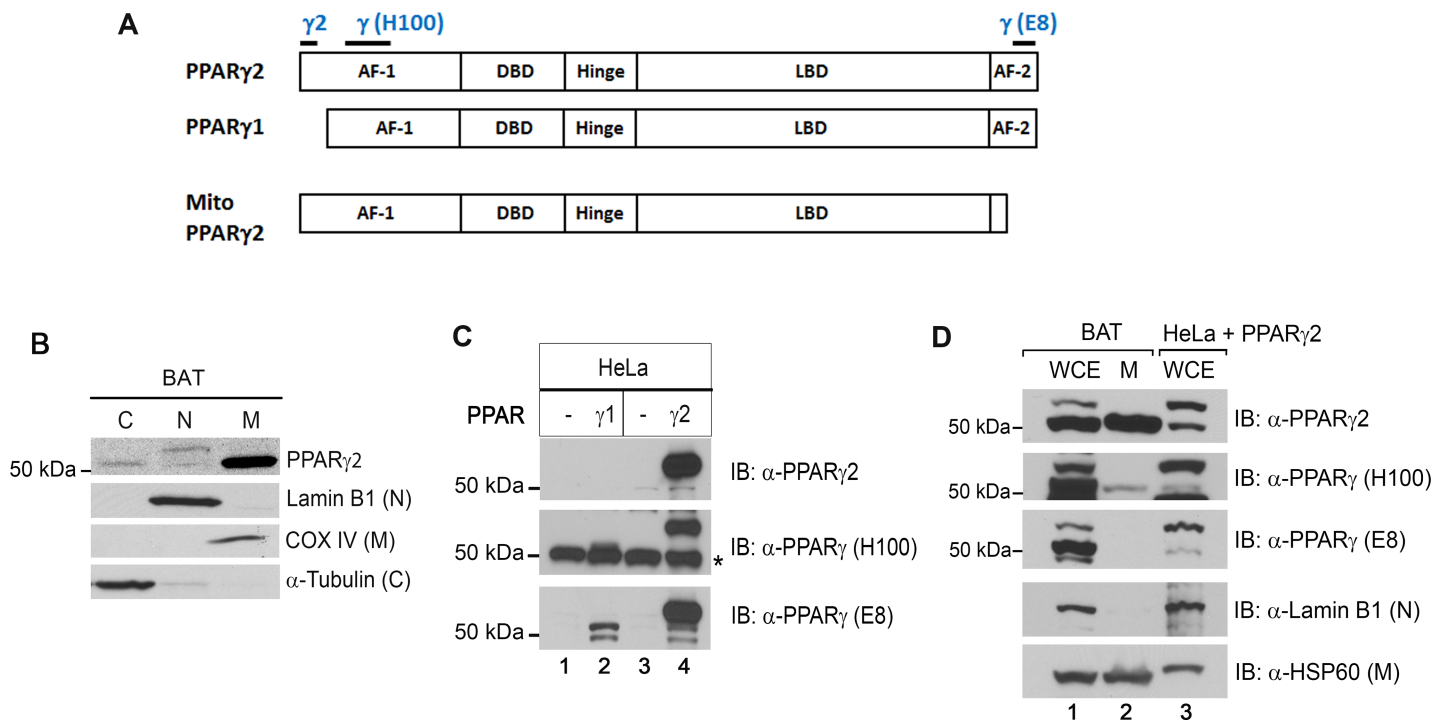


Fig 1. A C-terminally truncated form of PPAR γ 2 is enriched in brown adipose tissue mitochondria. (A) Schematic of PPAR γ 1 and PPAR γ 2 proteins. AF-1, activation function 1; DBD, DNA binding domain; LBD, ligand binding domain; AF-2, activation function 2. Blue letters represent specific regions recognized by three different PPAR γ antibodies. (B) Presence of a 52 kDa protein recognized by PPAR γ 2 antibody in the cytosolic and mitochondrial fractions. Brown adipose tissue was isolated from C57BL/6J mice and subjected to subcellular fractionation. Cytosolic (C), nuclear (N) and mitochondrial (M) markers were detected in their respective fractions. (C) Validation of three different PPAR γ antibodies. PPAR γ 1 and PPAR γ 2 were expressed in HeLa cells and analyzed with three different PPAR γ antibodies. *, a non-specific band at ~50kDa that is reacted with PPAR γ (H100) antibody in HeLa cells. (D) Western blot analysis of brown adipose tissue extracts (WCE) and mitochondrial lysates (M) with three different PPAR γ antibodies.

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fractionation of brown adipose tissue (BAT) and western blot analysis with anti-PPAR γ 2 antibody unexpectedly revealed that while a ~57 kDa protein representing PPAR γ 2 was enriched in the nuclear fraction, a ~52 kDa protein was predominantly present in the cytoplasmic and mitochondrial fractions (Fig 1B). The nuclear marker Lamin B1 was not detected in the cytoplasmic and mitochondrial fractions, indicating no contamination of these fractions by nuclei. To determine if a lower molecular weight band represents a variant of PPAR γ 2, we first tested the specificity of PPAR γ 2 antibody that recognizes an epitope corresponding to amino acids 1–16 of PPAR γ 2 (Fig 1A). PPAR γ 1 and PPAR γ 2 were separately expressed in HeLa cells and their protein expression was analyzed using PPAR γ 2 antibody. PPAR γ 1 was not detected by PPAR γ 2 antibody, but PPAR γ 2 was strongly detected at 57 kDa (Fig 1C, top panel), confirming that this antibody is highly specific to PPAR γ 2. Immunoblotting of BAT whole cell extracts with PPAR γ 2 antibody showed two bands at 57 and 52 kDa, and the lower molecular weight protein was enriched in the mitochondrial fraction (Fig 1D, lanes 1–2). Identity of the 52 kDa protein in the mitochondrial fraction was further analyzed using two different PPAR γ antibodies (H100 and E8). PPAR γ (H100) antibody recognizes amino acids 38–136 of PPAR γ 2, whereas PPAR γ (E8) antibody recognizes amino acids 486–505 mapping at the C-terminus of PPAR γ 2 (Fig 1A). PPAR γ 2 expressed in HeLa cells was detected by PPAR γ H100 and E8 antibodies at 57 kDa (Fig 1C, middle and bottom panels). In addition, PPAR γ 1 expressed in HeLa cells was detected by both PPAR γ antibodies (Fig 1C, middle and bottom panels). The 52 kDa band reacted with PPAR γ 2 antibody in the mitochondrial fraction of BAT was recognized by PPAR γ (H100) antibody but not by PPAR γ (E8) antibody (Fig 1D, lane 2). Taken together, these results demonstrate that the 52 kDa protein in the mitochondrial fraction is the C-terminally truncated form of PPAR γ 2 (Fig 1A).

To evaluate localization of the full-length and truncated PPAR γ 2 in brown adipocytes, fully differentiated brown adipocytes were subjected to indirect immunofluorescence using PPAR γ 2 antibody. PPAR γ 2 antibody-specific fluorescent signals were primarily found in the nucleus with high concentration at the nuclear envelope as well as in the cytoplasm in brown adipocytes (Fig 2A). To further determine subcellular localization of the full-length and truncated PPAR γ 2 during brown adipocyte differentiation, brown preadipocytes (day 0) were stimulated to undergo differentiation. The cells were collected at 2, 4 and 7 days of differentiation and subjected to nuclear and cytoplasmic fractionation. In agreement with increased PPAR γ 2 gene expression during brown adipocyte differentiation [12], PPAR γ 2 protein (57 kDa) began to become visible in the nuclear pellet at 2 days of differentiation and its levels were gradually increased during differentiation (Fig 2B). Similarly, the 52 kDa protein levels were elevated during brown adipocyte differentiation. While the full-length PPAR γ 2 was predominantly accumulated in the nuclear pellet (N), the truncated PPAR γ 2 (52 kDa) was present in both the nuclear pellet (N) and nuclei-free supernatant (S) of brown adipocytes undergoing differentiation (Fig 2B). Nuclear versus cytosolic localization is not likely regulated by cAMP-dependent signaling since treatment with cAMP did not affect localization of either the full-length or truncated PPAR γ 2 (Fig 2B, lanes 9–10). Next, the nuclei-free supernatant fractions were further centrifuged to isolate mitochondria. The truncated form of PPAR γ 2 was significantly enriched in the mitochondria of brown adipocytes undergoing differentiation (Fig 2C).

The C-terminally truncated form of PPAR γ 2 is localized in the mitochondrial matrix of brown adipocytes

To further confirm that the C-terminally truncated form of PPAR γ 2 localizes within the mitochondria, highly purified BAT mitochondria were treated with increasing concentrations of proteinase K, which cannot penetrate the mitochondrial membranes. The outer mitochondrial

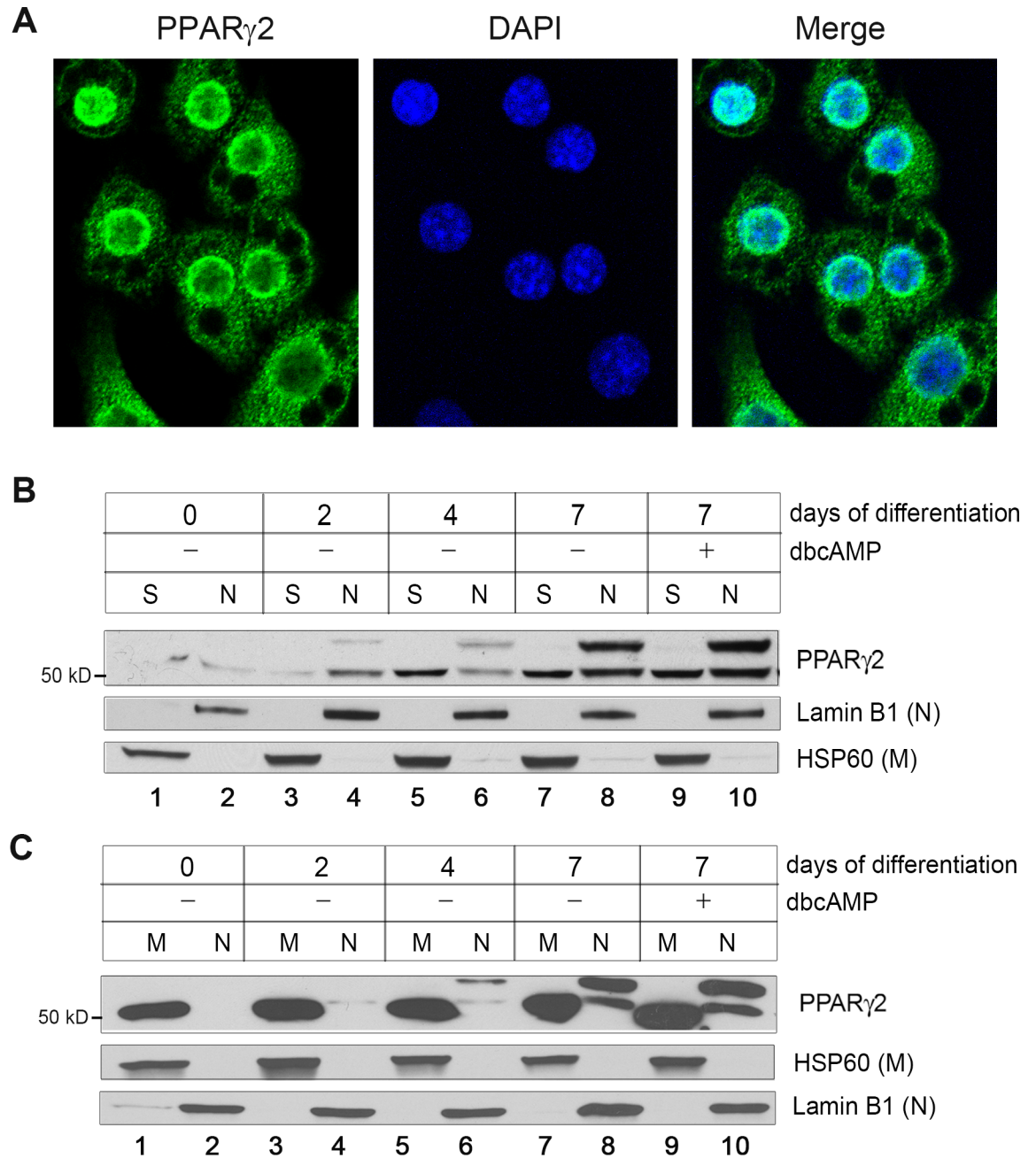


Fig 2. Localization of the full-length and truncated PPAR γ 2 during brown adipocyte differentiation. (A) Analysis of PPAR γ 2 localization in brown adipocytes. Brown preadipocytes were differentiated and subjected to indirect immunofluorescence using anti-PPAR γ 2 antibody. (B, C) Western blot analysis of the full-length and truncated PPAR γ 2 in subcellular fractions of brown adipocytes during differentiation. Brown preadipocytes (day 0) were differentiated for 2, 4, and 7 days, homogenized and subjected to centrifugation at $1,000 \times g$ (N, nuclear pellets; S, nuclei-free supernatant). The nuclei-free supernatant was further centrifuged at $10,000 \times g$ to isolate mitochondria (M).

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membrane protein Tom20 was completely digested by proteinase K, whereas inner mitochondrial membrane protein UCP1 and mitochondrial matrix protein HSP60 were resistant to proteinase K digestion (Fig 3A). Triton X-100-mediated solubilization of mitochondria resulted

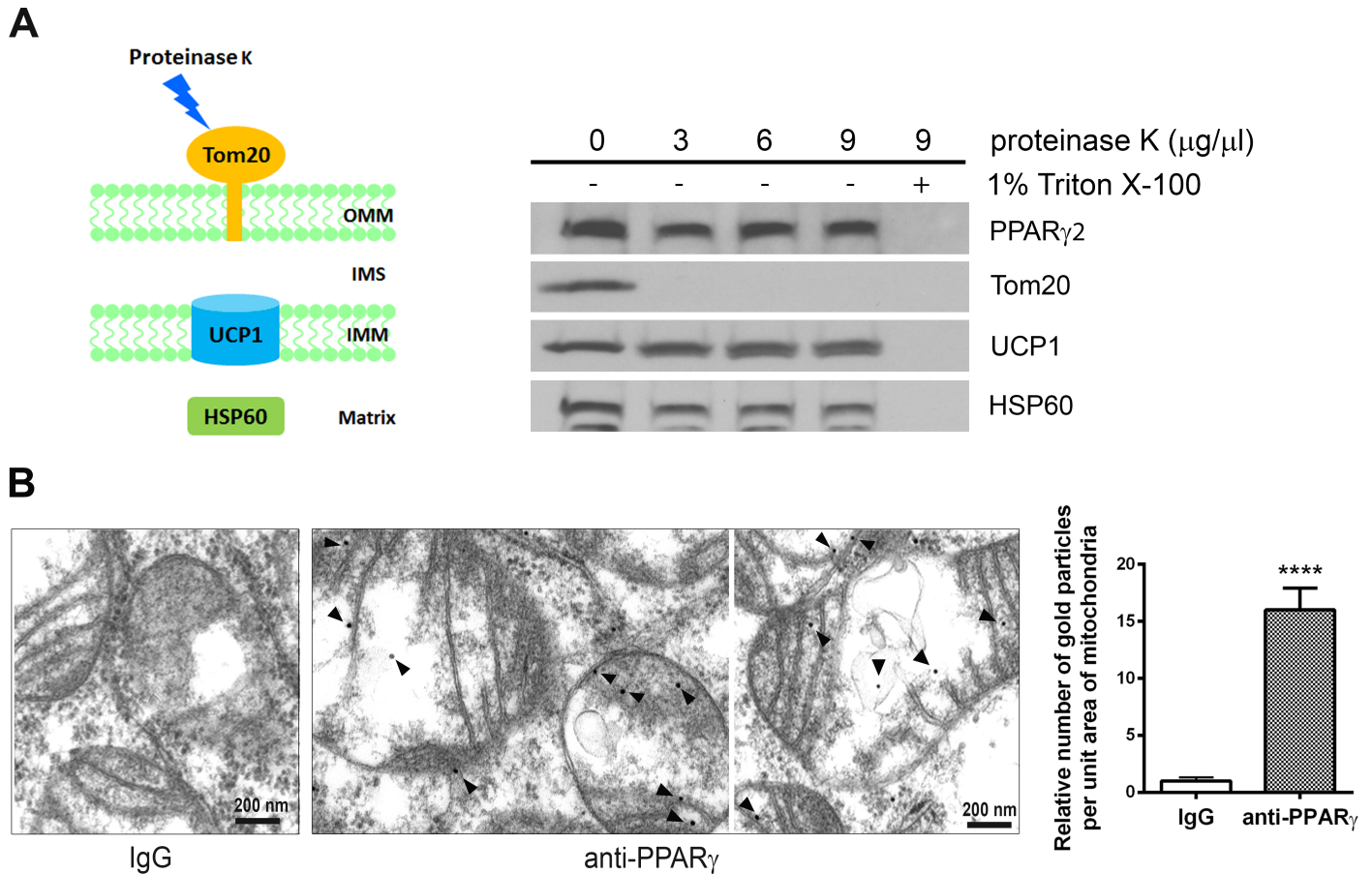


Fig 3. The truncated PPAR γ 2 localizes in the mitochondrial matrix. (A) The truncated form of PPAR γ 2 in mitochondria is protected from proteinase K digestion. Purified brown adipose tissue mitochondria (60 μg) were treated with increasing amounts of proteinase K in the absence or presence of 1% Triton X-100. (B) Immuno-transmission electron microscopic analysis of the truncated PPAR γ 2 in brown adipocytes. Black dots indicated by arrow heads represent immunogold particles reacted with PPAR γ (H100) antibody. Mitochondrial localization of immunogold particles was examined in 4–5 grids per group (20–30 mitochondria/grid), and the relative number of immunogold particles localized in the mitochondria was shown in the right panel. Data are presented as the mean \pm SEM. **** $P < 0.0001$.

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in complete digestion of UCP1 and HSP60 by proteinase K. Similarly, the truncated form of PPAR γ 2 was protected from proteinase K digestion in the absence of Triton X-100, indicating that it does not adhere to the outer mitochondrial membrane in a nonspecific manner and is indeed localized within the mitochondria.

To determine the submitochondrial localization of the truncated form of PPAR γ 2, immuno-transmission electron microscopy (TEM) was carried out in fully differentiated brown adipocytes. PPAR γ (H100) antibody was used because no cellular signal was detected using PPAR γ 2 antibody for immuno-TEM analysis. PPAR γ (H100) antibody is able to detect the truncated form of PPAR γ 2 in mitochondria (Fig 1D). Immunogold particles corresponding to the truncated form of PPAR γ 2 were primarily localized in the mitochondrial matrix of brown adipocytes (Fig 3B). A small number of immunogold particles was also found closely associated with the inner mitochondrial membrane. The relative number of these immunogold particles in the brown adipocyte mitochondria was significantly higher compared with IgG control (Fig 3B).

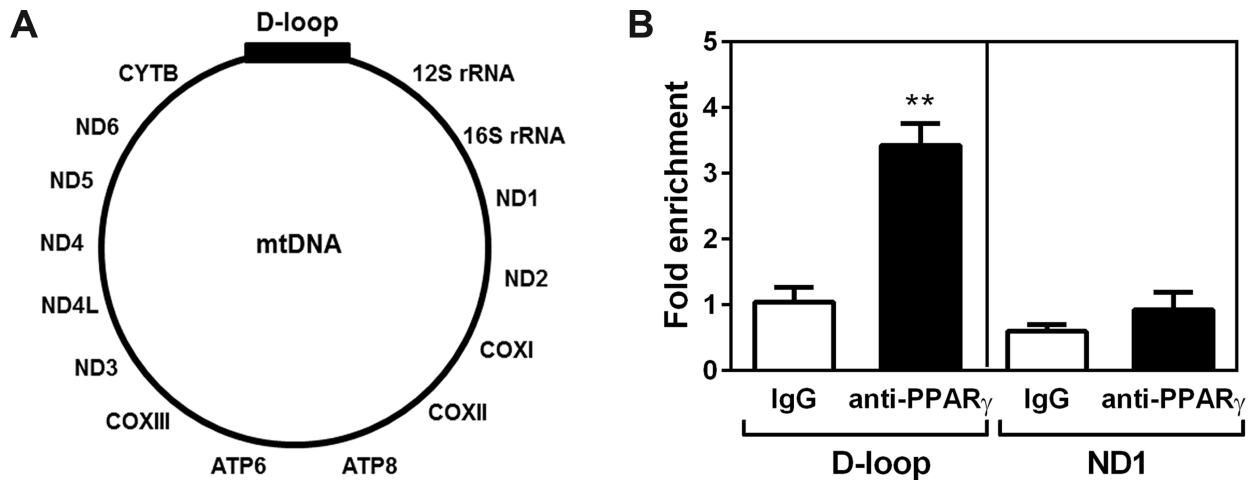


Fig 4. The truncated PPAR γ 2 binds to the D-loop region of mitochondrial DNA. (A) A schematic diagram of mitochondrial DNA (mtDNA). (B) Enrichment of the truncated PPAR γ 2 at the D-loop region of mtDNA in brown adipocyte mitochondria. Mitochondrial chromatin immunoprecipitation assay was carried out as described in Materials and Methods. The relative amounts of mtDNA immunoprecipitated with IgG or PPAR γ (H100) antibody were analyzed by quantitative real-time PCR analysis (n = 4). Data represent mean \pm SEM. **P<0.01.

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The C-terminally truncated form of PPAR γ 2 binds to the D-loop region of mitochondrial DNA

The finding that the truncated form of PPAR γ 2 was primarily localized in the mitochondrial matrix prompted us to ask whether it regulates mitochondrial DNA transcription. Mitochondrial DNA (mtDNA) is located in the mitochondrial matrix and encodes 11 key subunits of electron transport chain (ETC) complexes I, III and IV and 2 subunits of ATP synthase (Fig 4A). The D-loop region of mtDNA contains the promoter mediating bidirectional transcription [23, 24]. To test if the truncated form of PPAR γ 2 is recruited to the D-loop region of mtDNA, we isolated mitochondria from brown adipocytes and carried out mitochondrial chromatin immunoprecipitation (mtChIP) assays using a ChIP grade anti-PPAR γ (H100) antibody, which has been validated for its specificity and immunoprecipitation efficiency [25]. The truncated form of PPAR γ 2 bound to the D-loop region of mtDNA in mitochondria (Fig 4B). In contrast, no binding was detected at the coding region of ND1 gene, strengthening its specific binding to the D-loop region of mtDNA.

Mitochondrially targeted MLS-PPAR γ 2 enhances mtDNA-encoded ETC gene expression and mitochondrial respiration in brown adipocytes

Given that the truncated form of PPAR γ 2 bound to the D-loop region of mtDNA, we wanted to test if this protein regulates mtDNA-encoded ETC gene expression in mitochondria. We constructed MLS-PPAR γ 2 that contains a mitochondrial matrix localizing sequence (MLS) fused to the N-terminus of the protein. In frame insertion of the MLS directs the protein to the mitochondria and thus has been used to assess the function of nuclear transcription factors specifically in the mitochondria without its effect on gene expression in the nucleus [19, 26–28]. MLS-PPAR γ 2 was stably expressed in brown preadipocytes by retrovirus-mediated gene transfer. A previous study has shown that PPAR γ 2 expression in fibroblasts stimulates adipogenic differentiation [29]. We also observed that PPAR γ 2 expression in brown preadipocytes partially induced adipocyte differentiation without a differentiation cocktail, whereas no adipocyte differentiation was stimulated by MLS-PPAR γ 2 expression in brown preadipocytes (data not shown). This indicates that MLS-PPAR γ 2 has no effect on gene expression in the

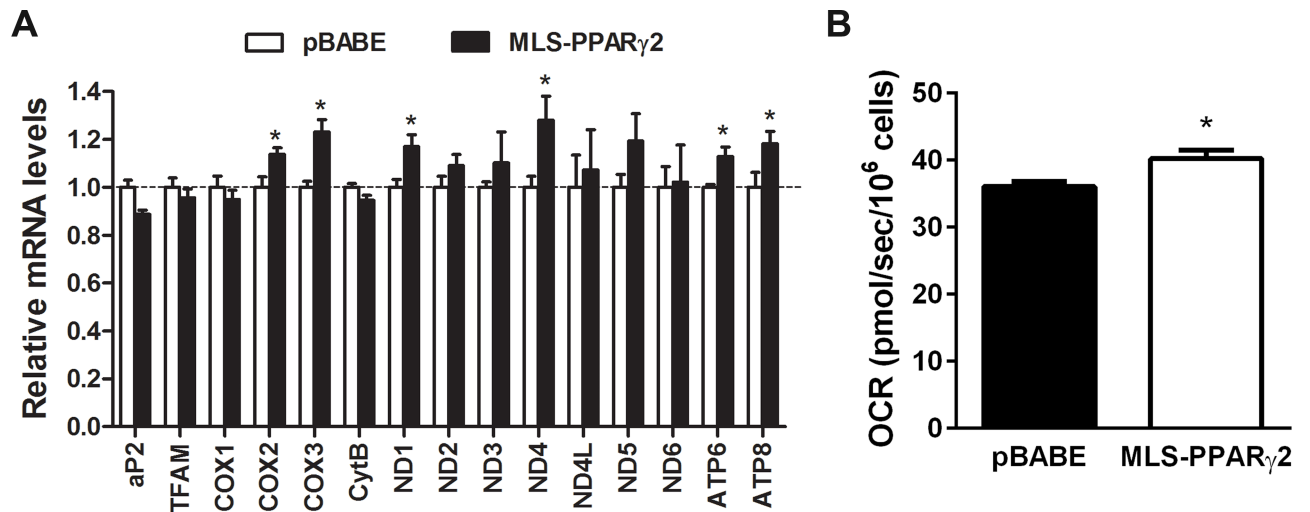


Fig 5. MLS-PPAR γ 2 increases mitochondrial respiration by modulating mtDNA-encoded ETC gene expression. (A) Expression of MLS-PPAR γ 2 in brown adipocytes increases mtDNA-encoded ETC gene expression. Quantitative real-time PCR was carried out in brown adipocytes expressing pBABA or MLS-PPAR γ 2 (n = 5). Data represent mean \pm SEM. *P<0.05. (B) MLS-PPAR γ 2 enhances mitochondrial respiration in brown adipocytes. Cellular oxygen consumption rates (OCR) were measured at baseline and after injection of antimycin A (n = 6). The value of mitochondrial respiration was determined by subtracting antimycin A-independent non-mitochondrial respiration. Data represent mean \pm SEM. *P<0.05.

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nucleus. Further supporting this, expression levels of aP2, which is a PPAR γ target gene and an adipogenic marker, were not different between MLS-PPAR γ 2- and empty vector-expressing brown adipocytes (Fig 5A). Mitochondrial transcription factor A (TFAM) is a key regulator of mtDNA transcription [24]. TFAM is encoded by nuclear genome and imported to mitochondria. Gene expression analysis showed that TFAM was comparably expressed in MLS-PPAR γ 2- and empty vector-expressing brown adipocytes. Despite the same levels of TFAM, expression of several mtDNA-encoded ETC genes was elevated in MLS-PPAR γ 2-expressing brown adipocytes compared to control brown adipocytes (Fig 5A), suggesting a direct effect of MLS-PPAR γ 2 on mtDNA-encoded ETC gene expression. Next, to determine the effect of increased ETC gene expression on mitochondrial respiration, cellular oxygen consumption rates (OCR) were measured at baseline and after injection of a mitochondrial electron transport inhibitor, antimycin A. Mitochondria-dependent respiration was determined by subtracting antimycin A-independent non-mitochondrial respiration. In agreement with an increase in mtDNA-encoded ETC gene expression by MLS-PPAR γ 2, mitochondrial respiration was slightly enhanced in MLS-PPAR γ 2-expressing brown adipocytes compared to control brown adipocytes (Fig 5B). Taken together, these results indicate that mitochondrial PPAR γ 2 plays a role in mitochondrial respiration via regulating mtDNA-encoded ETC gene expression in brown adipocytes.

Discussion

The present study identifies the truncated form of PPAR γ 2 (52 kDa) that is highly enriched in brown adipocyte mitochondria and regulates mtDNA-encoded ETC gene expression. While the full-length PPAR γ 2 was predominantly localized in the nucleus, the truncated form of PPAR γ 2 was located in the nucleus, cytoplasm, and mitochondria of brown adipocytes. Cytoplasmic localization of this truncated protein is not surprising because nuclear-cytoplasmic shuttling of PPAR γ 1 and PPAR γ 2 has been reported. PPAR γ 1 and PPAR γ 2 contain nuclear import and export signals at the DNA-binding domain (DBD) and the ligand-binding domain

(LBD), respectively [30]. Accordingly, their nuclear-cytoplasmic shuttling is mediated by a nuclear importer importin α/β and a nuclear exporter CRM1 [30]. The truncated PPAR γ 2 lacks the C-terminal end of PPAR γ 2 (at least amino acids 486–505) that in part consists of the activation function 2 (AF2) domain (amino acids 436–505). The AF2 domain is required for ligand-dependent activation of PPAR γ 2 via interaction with coactivators and subsequent degradation via the ubiquitin proteasome system [31, 32]. It is not likely that lack of the C-terminal end of PPAR γ 2 increases cytoplasmic translocation since the AF2 domain does not regulate nuclear-cytoplasmic shuttling of PPAR γ 1 [33]. Rather, it is more probable that the C-terminal end of PPAR γ 2 retains the full-length PPAR γ 2 better in the nucleus via interaction with nuclear coactivators. How does the truncated form of PPAR γ 2 translocate to mitochondria? There are growing number of evidence that nuclear transcription factors translocate to mitochondria despite the absence of a mitochondrial localization sequence. Several studies have shown that mitochondrial heat shock protein 70 (mtHSP70) and voltage-dependent anion channel (VDAC) serve as a transport route for proteins that lack a mitochondrial localization sequence [2, 3, 34, 35]. Thus it would be interesting to examine whether mtHSP70 and/or VDAC assist mitochondrial import of the truncated form of PPAR γ 2.

Several studies have reported mitochondrial translocation of truncated nuclear receptors such as thyroid receptor (TR α 1 p43), progesterone receptor (PR-M), and retinoid X receptor (RXR α p44) in a tissue- or cell-specific manner [36–38]. These truncated proteins are produced by multiple pathways including translation at the internal initiation site, alternative splicing, and enzymatic cleavage. Casas et al. previously reported that a 45 kDa protein related to PPAR γ 2 is present in the mitochondria of brown adipose tissue [16]. It's not clear whether this protein is identical to the truncated PPAR γ 2 we found in the present study. However, both findings demonstrate that the truncated forms of PPAR γ 2 are predominantly located in brown adipose tissue mitochondria. Cytoplasmic PPAR γ 2 has been shown in 3T3-L1 adipocytes, but its truncated form has not been reported [39]. We did not detect the truncated form of PPAR γ 2 at 52 kDa in subcutaneous and visceral adipose tissue (data not shown). This may suggest that the truncated form of PPAR γ 2 is specifically produced in brown adipocytes. The mechanism by which the truncated form of PPAR γ 2 originates in brown adipocytes remains to be determined.

Collectively, our data suggest that mitochondrial PPAR γ 2 affects mitochondrial respiration through influencing the expression of mtDNA-encoded ETC genes in brown adipocytes. Many ETC subunits encoded by nuclear DNA assemble as subcomplexes in the mitochondrial matrix and their redistribution to the inner mitochondrial membrane are accelerated by mtDNA-encoded ETC subunits [40, 41]. Thus the mechanism by which mitochondrial PPAR γ 2 regulates mtDNA-encoded ETC gene expression may provide an additional level of control for efficient ETC complex formation in the inner mitochondrial membrane of brown adipocytes. Enhanced mitochondrial respiration through the ETC complexes is critical for UCP1-mediated heat production in brown adipocytes.

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Author Contributions

Conceptualization: Ji Suk Chang.

Data curation: Ji Suk Chang.

Formal analysis: Ji Suk Chang.

Funding acquisition: Ji Suk Chang.

Investigation: Ji Suk Chang.

Methodology: Ji Suk Chang, Kyoungsoo Ha.

Project administration: Ji Suk Chang.

Supervision: Ji Suk Chang.

Writing – original draft: Ji Suk Chang.

Writing – review & editing: Ji Suk Chang.

References

1. Casas F, Rochard P, Rodier A, Cassar-Malek I, Marchal-Victorion S, Wiesner RJ, et al. A variant form of the nuclear triiodothyronine receptor c-ErbAalpha 1 plays a direct role in regulation of mitochondrial RNA synthesis. *Mol Cell Biol*. 1999; 19(12):7913–24. PMID: [10567517](#)
2. She H, Yang Q, Shepherd K, Smith Y, Miller G, Testa C, et al. Direct regulation of complex I by mitochondrial MEF2D is disrupted in a mouse model of Parkinson disease and in human patients. *J Clin Invest*. 2011; 121(3):930–40. <https://doi.org/10.1172/JCI43871> PMID: [21393861](#)
3. De Rasmio D, Signorile A, Roca E, Papa S. cAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. *FEBS J*. 2009; 276(16):4325–33. <https://doi.org/10.1111/j.1742-4658.2009.07133.x> PMID: [19614745](#)
4. Ryu H, Lee J, Impey S, Ratan RR, Ferrante RJ. Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. *Proc Natl Acad Sci U S A*. 2005; 102(39):13915–20. <https://doi.org/10.1073/pnas.0502878102> PMID: [16169904](#)
5. Macias E, Rao D, Carbajal S, Kiguchi K, Digiovanni J. Stat3 Binds to mtDNA and Regulates Mitochondrial Gene Expression in Keratinocytes. *J Invest Dermatol*. 2014.
6. Vaseva AV, Moll UM. The mitochondrial p53 pathway. *Biochim Biophys Acta*. 2009; 1787(5):414–20. <https://doi.org/10.1016/j.bbabi.2008.10.005> PMID: [19007744](#)
7. Yang SH, Liu R, Perez EJ, Wen Y, Stevens SM Jr., Valencia T, et al. Mitochondrial localization of estrogen receptor beta. *Proc Natl Acad Sci U S A*. 2004; 101(12):4130–5. <https://doi.org/10.1073/pnas.0306948101> PMID: [15024130](#)
8. Zhou Z, Zhou J, Du Y. Estrogen receptor alpha interacts with mitochondrial protein HADHB and affects beta-oxidation activity. *Mol Cell Proteomics*. 2012; 11(7):M111 011056.
9. Zhou Z, Zhou J, Du Y. Estrogen receptor beta interacts and colocalizes with HADHB in mitochondria. *Biochem Biophys Res Commun*. 2012; 427(2):305–8. <https://doi.org/10.1016/j.bbrc.2012.09.047> PMID: [23000159](#)
10. Psarra AM, Sekeris CE. Steroid and thyroid hormone receptors in mitochondria. *IUBMB Life*. 2008; 60(4):210–23. <https://doi.org/10.1002/iub.37> PMID: [18344181](#)
11. Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, et al. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell*. 1999; 4(4):611–7. PMID: [10549292](#)
12. Nedergaard J, Petrovic N, Lindgren EM, Jacobsson A, Cannon B. PPARgamma in the control of brown adipocyte differentiation. *Biochim Biophys Acta*. 2005; 1740(2):293–304. <https://doi.org/10.1016/j.bbadis.2005.02.003> PMID: [15949696](#)
13. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. 1998; 92(6):829–39. PMID: [9529258](#)
14. Lehrke M, Lazar MA. The many faces of PPARgamma. *Cell*. 2005; 123(6):993–9. <https://doi.org/10.1016/j.cell.2005.11.026> PMID: [16360030](#)
15. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, et al. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc Natl Acad Sci U S A*. 1995; 92(17):7921–5. PMID: [7644514](#)
16. Casas F, Domenjoud L, Rochard P, Hatier R, Rodier A, Daury L, et al. A 45 kDa protein related to PPARgamma2, induced by peroxisome proliferators, is located in the mitochondrial matrix. *FEBS Lett*. 2000; 478(1–2):4–8. PMID: [10922459](#)

17. Chang JS, Huypens P, Zhang Y, Black C, Kralli A, Gettys TW. Regulation of NT-PGC-1alpha subcellular localization and function by protein kinase A-dependent modulation of nuclear export by CRM1. *J Biol Chem.* 2010; 285(23):18039–50. <https://doi.org/10.1074/jbc.M109.083121> PMID: 20351112
18. Zhang Y, Huypens P, Adamson AW, Chang JS, Henagan TM, Lenard NR, et al. Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1{alpha}. *J Biol Chem.* 2009; 284(47):32813–26. <https://doi.org/10.1074/jbc.M109.037556> PMID: 19773550
19. Chang JS, Ha K. An unexpected role for the transcriptional coactivator isoform NT-PGC-1alpha in the regulation of mitochondrial respiration in brown adipocytes. *J Biol Chem.* 2017; 292(24):9958–66. <https://doi.org/10.1074/jbc.M117.778373> PMID: 28473468
20. Commins SP, Watson PM, Padgett MA, Dudley A, Argyropoulos G, Gettys TW. Induction of uncoupling protein expression in brown and white adipose tissue by leptin. *Endocrinology.* 1999; 140(1):292–300. <https://doi.org/10.1210/endo.140.1.6399> PMID: 9886838
21. Kim J, Fernand VE, Henagan TM, Shin J, Huypens P, Newman S, et al. Regulation of Brown and White Adipocyte Transcriptome by the Transcriptional Coactivator NT-PGC-1alpha. *PLoS One.* 2016; 11(7): e0159990. <https://doi.org/10.1371/journal.pone.0159990> PMID: 27454177
22. Jun HJ, Joshi Y, Patil Y, Noland RC, Chang JS. NT-PGC-1alpha activation attenuates high-fat diet-induced obesity by enhancing brown fat thermogenesis and adipose tissue oxidative metabolism. *Diabetes.* 2014; 63(11):3615–25. <https://doi.org/10.2337/db13-1837> PMID: 24848065
23. Taanman JW. The mitochondrial genome: structure, transcription, translation and replication. *Biochim Biophys Acta.* 1999; 1410(2):103–23. PMID: 10076021
24. Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev.* 2008; 88(2):611–38. <https://doi.org/10.1152/physrev.00025.2007> PMID: 18391175
25. Siersbaek MS, Loft A, Aagaard MM, Nielsen R, Schmidt SF, Petrovic N, et al. Genome-wide profiling of peroxisome proliferator-activated receptor gamma in primary epididymal, inguinal, and brown adipocytes reveals depot-selective binding correlated with gene expression. *Mol Cell Biol.* 2012; 32(17):3452–63. <https://doi.org/10.1128/MCB.00526-12> PMID: 22733994
26. Lee J, Kim CH, Simon DK, Aminova LR, Andreyev AY, Kushnareva YE, et al. Mitochondrial cyclic AMP response element-binding protein (CREB) mediates mitochondrial gene expression and neuronal survival. *J Biol Chem.* 2005; 280(49):40398–401. <https://doi.org/10.1074/jbc.C500140200> PMID: 16207717
27. Gough DJ, Corlett A, Schlessinger K, Wegrzyn J, Larner AC, Levy DE. Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. *Science.* 2009; 324(5935):1713–6. <https://doi.org/10.1126/science.1171721> PMID: 19556508
28. Szczepanek K, Chen Q, Derecka M, Salloum FN, Zhang Q, Szelag M, et al. Mitochondrial-targeted Signal transducer and activator of transcription 3 (STAT3) protects against ischemia-induced changes in the electron transport chain and the generation of reactive oxygen species. *J Biol Chem.* 2011; 286(34):29610–20. <https://doi.org/10.1074/jbc.M111.226209> PMID: 21715323
29. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell.* 1994; 79(7):1147–56. PMID: 8001151
30. Umemoto T, Fujiki Y. Ligand-dependent nucleo-cytoplasmic shuttling of peroxisome proliferator-activated receptors, PPARalpha and PPARgamma. *Genes Cells.* 2012; 17(7):576–96. <https://doi.org/10.1111/j.1365-2443.2012.01607.x> PMID: 22646292
31. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem.* 2008; 77:289–312. <https://doi.org/10.1146/annurev.biochem.77.061307.091829> PMID: 18518822
32. Hauser S, Adelmant G, Sarraf P, Wright HM, Mueller E, Spiegelman BM. Degradation of the peroxisome proliferator-activated receptor gamma is linked to ligand-dependent activation. *J Biol Chem.* 2000; 275(24):18527–33. <https://doi.org/10.1074/jbc.M001297200> PMID: 10748014
33. von Knethen A, Tzieply N, Jennewein C, Brune B. Casein-kinase-II-dependent phosphorylation of PPARgamma provokes CRM1-mediated shuttling of PPARgamma from the nucleus to the cytosol. *J Cell Sci.* 2010; 123(Pt 2):192–201. <https://doi.org/10.1242/jcs.055475> PMID: 20026644
34. Neupert W, Herrmann JM. Translocation of proteins into mitochondria. *Annu Rev Biochem.* 2007; 76:723–49. <https://doi.org/10.1146/annurev.biochem.76.052705.163409> PMID: 17263664
35. Zalk R, Israelson A, Garty ES, Azoulay-Zohar H, Shoshan-Barmatz V. Oligomeric states of the voltage-dependent anion channel and cytochrome c release from mitochondria. *Biochem J.* 2005; 386(Pt 1):73–83. <https://doi.org/10.1042/BJ20041356> PMID: 15456403
36. Bigler J, Eisenman RN. c-erbA encodes multiple proteins in chicken erythroid cells. *Mol Cell Biol.* 1988; 8(10):4155–61. PMID: 3054510

37. Dai Q, Shah AA, Garde RV, Yonish BA, Zhang L, Medvitz NA, et al. A truncated progesterone receptor (PR-M) localizes to the mitochondrion and controls cellular respiration. *Mol Endocrinol*. 2013; 27(5):741–53. <https://doi.org/10.1210/me.2012-1292> PMID: 23518922
38. Casas F, Daury L, Grandemange S, Busson M, Seyer P, Hatier R, et al. Endocrine regulation of mitochondrial activity: involvement of truncated RXRalpha and c-Erb Aalpha1 proteins. *FASEB J*. 2003; 17(3):426–36. <https://doi.org/10.1096/fj.02-0732com> PMID: 12631582
39. Thuillier P, Baillie R, Sha X, Clarke SD. Cytosolic and nuclear distribution of PPARgamma2 in differentiating 3T3-L1 preadipocytes. *J Lipid Res*. 1998; 39(12):2329–38. PMID: 9831621
40. Bourges I, Ramus C, Mousson de Camaret B, Beugnot R, Remacle C, Cardol P, et al. Structural organization of mitochondrial human complex I: role of the ND4 and ND5 mitochondria-encoded subunits and interaction with prohibitin. *Biochem J*. 2004; 383(Pt. 3):491–9. <https://doi.org/10.1042/BJ20040256> PMID: 15250827
41. Antonicka H, Ogilvie I, Taivassalo T, Anitori RP, Haller RG, Vissing J, et al. Identification and characterization of a common set of complex I assembly intermediates in mitochondria from patients with complex I deficiency. *J Biol Chem*. 2003; 278(44):43081–8. <https://doi.org/10.1074/jbc.M304998200> PMID: 12941961