Role of HSF1 in the mitochondrial unfolded protein response in mammals

（哺乳動物のミトコンドリア不良タンパク質応答における **HSF1** の役割）

A dissertation is submitted to Yamaguchi University Graduate School of Medicine, Department of Biochemistry and Molecular Biology in partial fulfillment for the degree of Doctor of Philosophy

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Abbreviations:

UPR: Unfolded Protein Response

HSF1: Heat Shock Transcription Factor 1

SSBP1: Single Strand DNA-Binding Protein 1

ATF5: Activating Transcription Factor 5

CHOP: C/EBP Homology Protein

MSR: Mitochondria Stress Response

GRP78: Glucose-Regulated Protein 78

HSP: Heat Shock Protein

OTC: Ornithine Transcarbamylase

MMP: Mitochondrial Membrane Potential

OCR: Oxygen Consumption Rate

ETC: Electron Transport Chain

GTPP: Gamitrinib-Triphenylphosphonium

CDDO: 2-Cyno-3, 12-dioxo-oleana-1, 9(11)-dien-28-oic acid

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1. Abstract

Protein homeostasis or proteostasis is highly related with organismal health and fitness. Mitochondria is a central hub of metabolism and signaling cascades, whose dysfunction is associated with age-related neurodegenerative diseases. The mitochondrial unfolded protein response (UPR^{mt}) is characterized by the transcriptional induction of mitochondrial chaperone and protease genes in response to impaired mitochondrial proteostasis, and is regulated by ATF5 and CHOP in mammalian cells. However, detailed mechanisms underlying the UPR^{mt} are currently unclear. In this study, we have shown that the heat shock transcription factor (HSF1) is required for activation of mitochondrial chaperone genes, including *HSP60*, *HSP10*, and *mtHSP70*, in mouse embryonic fibroblasts during the UPR^{mt}. Pharmacological inhibition of different functional sites of mitochondria, including matrix chaperone TRAP1, protease Lon, or electron transfer chain complex 1, or knockdown of TRAP1 induced the set of mitochondrial chaperone genes in a manner dependent on HSF1. Interestingly, mitochondrial single strand DNA-binding protein (SSBP1) is an important component of HSF1 transcription complex, and is differently required for the expression of different mitochondrial chaperone genes. HSF1 was significantly translocated into the nucleus, formed a DNA-binding trimer, and was phosphorylated at Ser326 during the UPR^{mt}. HSF1 occupied promoters of mitochondrial chaperone genes and its occupancy was enhanced during the UPR^{mt}. Of note, HSF1 occupancy was remarkably enhanced on *HSP60/HSP10* promoter. Furthermore, HSF1 supported maintenance of mitochondrial function in the same conditions. These results elucidated that HSF1 is required for induction of mitochondrial chaperones during the UPR^{mt}, and suggested that it is one of guardians for mitochondrial function under conditions of impaired mitochondrial proteostasis.

2. Introduction

Protein homeostasis or proteostasis in subcellular compartments is essential for cellular functions. Environmental and metabolic stresses constantly challenge the proteostasis capacity and create proteotoxic stress in cellular compartments including the mitochondria. Mitochondria is a central hub of cellular metabolism and signaling pathways, which includes ATP generation, nucleotide biosynthesis, lipid synthesis and amino acid metabolism (*Osman et al 2011; Wellen and Thompson, 2012*), and decline or loss in mitochondrial function is associated with age-related disorders and neurodegenerative diseases (*Vafai and Mootha, 2012*). An underlying mechanism by which cells respond to impaired mitochondrial proteostasis through activating transcription of mitochondrial genes, which thereby promotes matrix folding capacity, is known as the mitochondrial unfolded protein response (UPR^{mt}), and is not completed understood yet.

Genetic studies carried out in nematode *C. elegans* have revealed that a transcription factor ATFS-1, which is localized in the mitochondrial matrix under normal conditions, accumulates in the nucleus and activates the UPR^{mt} genes in response to mitochondrial proteotoxic stress. In addition, several factors including a mitochondrial transporter, transcription factors, and histone-modifying enzymes are also involved in the UPR^{mt}. In particular, histone demethylases, JMJD-3.1 and JMJD-1.2 are necessary, and their overexpression is sufficient for the UPR^{mt} (*Merkwerth et al, 2016; Melber et al, 2018*)(Fig. 1). In mammals, few studies have reported that the bZIP transcription factor ATF5 is regulated similarly to ATFS-1 and activates the UPR^{mt} genes during accumulation of truncated ornithine transcarbamylase (ΔOTC) in the mitochondria (*Fiorese et al, 2016*). Another bZIP transcription factor CHOP in complex with C/EBPβ also activates the UPR^{mt} genes, and its expression is induced via activation of JUN, which is mediated by c-Jun N-terminal kinase 2 during accumulation of ΔOTC (*Zhao et al, 2002; Horibe et al, 2007*). Considering that the UPR^{mt} is conserved in mammals, overexpression of mitochondria-localized ΔOTC induces mitochondrial chaperones, HSP60 (HSPD1) and HSP10 (HSPE1), in monkey cell lines

(*Zhao et al, 2002*). However, understanding the key factors that promotes matrix folding capacity by upregulating HSP60 and HSP10 is still rudimentary in mammals.

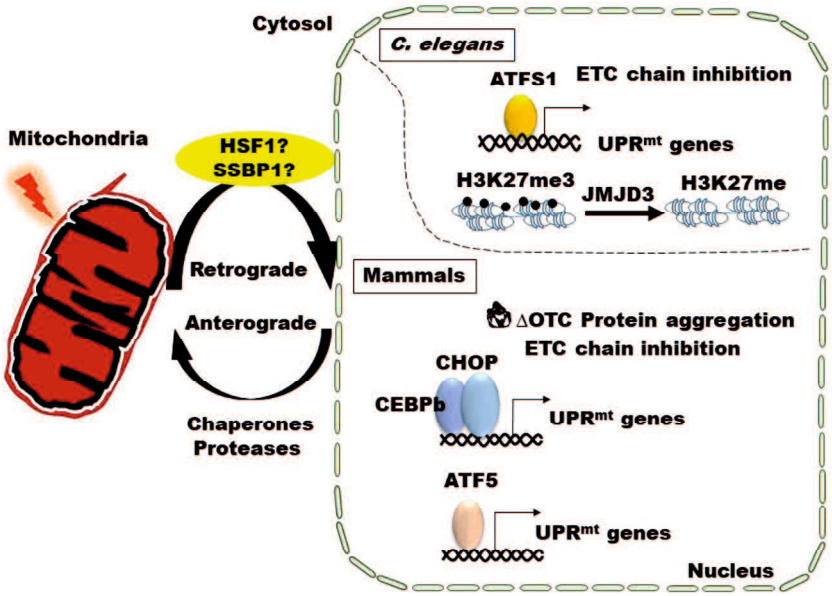

Fig1. Model of mitochondrial unfolded protein response pathways. A transcription factor ATFS1 regulates the UPR^{mt} genes in *C.elegans*. Chromatin modifying enzymes affects methylation pattern of UPR^{mt} genes. In mammals, ATF5 and CHOP (with C/EBPβ) is known to regulate the UPR^{mt} genes. HSF1 in co-operation with SSBP1 may play important roles during the UPR^{mt}.

At first, synthesis of a mammalian homolog of the bacterial GroEL protein was found to be elevated during heat shock and was referred to as HSP58 (thereafter HSP60), whereas that of a mitochondrial member of HSP70 family was increased in cells deprived of glucose and was referred to as glucose regulated protein GRP75 (also known as mtHSP70) (*Mizzen et al, 1989*). Mammalian *HSP60* and *HSP10* genes are linked head-to-head and share a bidirectional promoter, which is activated during heat shock (*Ryan et al, 1997*; *Hansen et al, 2003*). The heat shock response is regulated by heat shock transcription factor 1 (HSF1) in mammalian cells. However, HSF1 was not thought to be involved in the upregulation of *HSP60* and *HSP10* during the UPR^{mt}, because *HSP70* was not upregulated simultaneously (*Martinus et al, 1996*; *Zhao et al, 2002*; *Ryan et al, 1997*). We have been

analysing the HSF1 transcription complexes (*Fujimoto et al, 2012*; *Takii et al, 2015*), and one study suggested that HSF1 in complex with a coactivator, mitochondrial single stranded DNA-binding protein 1 (SSBP1), regulates the expression of mitochondrial chaperones, including HSP60, HSP10, and mtHSP70, during heat shock (*Tan et al, 2015*). Therefore, understanding the crucial role of HSF1 and its associated protein SSBP1 in mitochondria-nuclear communication during mitochondrial proteotoxic stress is enigmatic, hence we expanded our studies and focused on mitochondria specific stress response.

In this study, we have shown that treatment of mouse embryonic fibroblast (MEF) with reagents, which targets different functional sites within mitochondria, including GTPP (mtHSP90 inhibitor), CDDO (Lon protease inhibitor), and rotenone (ETC complex I inhibitor) (Fig. 2), induces HSF1-dependent expression of mitochondrial chaperones, HSP60, HSP10, and mtHSP70 as well as cytosolic HSP70. Collectively, we also demonstrated that HSF1 was activated, accumulated in the nucleus, converted to a trimer, modestly phosphorylated and remarkably recruited to *HSP60/HSP10* promoter. Furthermore, we show roles of HSF1 in maintenance of mitochondrial functions, including oxygen consumption and membrane potential. In summary, our results clearly indicate that HSF1 is required for activation of mitochondrial chaperone genes and is involved in the maintenance of mitochondrial function during the UPR^{mt}.

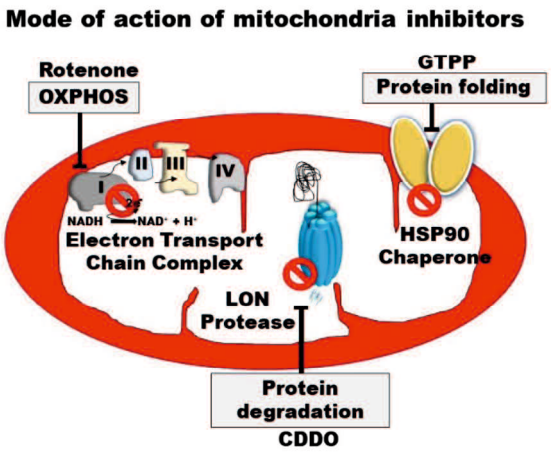

Figure 2. Reagents targeting different functional sites within mitochondria. Components of oxidative phosphorylation and mitochondrial proteins (matrix mitochondrial HSP90 chaperone, and LON protease) were selectively inhibited by rotenone, GTPP, or CDDO.

3. Materials and Methods

3.1 Cell cultures and treatments

Immortalized wild-type (clone #10) and HSF1-null (clone #4) mouse embryonic fibroblasts (MEF) (*Takii et al, 2015)*, HeLa (ATCC CCL-2) and HEK 293 (ATCC CRL-1573) cells were maintained at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Sigma-Aldrich). Cells were treated with mitochondria-specific stress reagents, 10 μM gamitrinibtriphenylphosphonium (GTPP) (a kind gift from Dr. D.C. Altieri), 5 μM synthetic triterpenoid 2-cyano-3, 12-dioxooleana-1, 9(11)-dien-28-oic acid (CDDO) (Cayman Chemicals, Ann Arbor, MI), and 20 μM rotenone (Sigma-Aldrich) for 6 h.

3.2 Assessment of mRNA

Total RNA was isolated from cells using TRIzol (Ambion). First-strand cDNA was synthesized using PrimeScript II Reverse Transcriptase and oligo dT primer in accordance with the manufacturer's instructions (TAKARA). Real-time quantitative PCR (qPCR) was performed using StepOnePlus (Applied Biosystems) with the Power SYBR Green PCR Master Mix (Applied Biosystems) using primers for mouse HSP60 (HSPD1), HSP10 (HSPE1), mtHSP70 (HSPA9), Lon, and HSP70 (HSPA1A and HSPA1B) (Table1). Relative quantities of mRNAs were normalized against GAPDH or RPLPO (large ribosomal protein) mRNA levels. All reactions were performed in triplicate with samples from three experiments.

Table 1. Primer sequences used for RT-qPCR

qPCR	Forward primer	Reverse primer
HSP70	5'-GGCTGGTGAGCCACTTCGT-3'	5'-GTTCTGGCTGATGTCCTTCTTGT-3'
mtHSP70	5'-AACGGCAAGCTGTCACCAA -3'	5'-ATCGTCGTCCAATAAGACGCTTA-3'
HSP60	5'-GGCACTGGCTCCTCATCTCA-3'	5'-GCGTCCGCACCAAATTTT-3'
HSP10	5'-TGCTGCCGAAACTGTAACCA-3'	5'TGCAACACTTTTCCTTGAGACTTT-3'
GAPDH	5'CGACTTCAACAGCAACTCCCACTC TTCC-3'	5'TGGGTGGTCCAGGGTTTCTTACTCCTT-3'
RNPL0	5'-GAGGACCTCACTGAGATTCGG-3'	5'-TTCTGAGCTGGCACAGTGAC-3'

3.3 RNA interference

To generate adenovirus vectors expressing short hairpin RNAs against mouse HSF1, SSBP1 and TRAP1, oligonucleotides containing each target sequence (Table 2) were annealed and inserted into pCR2.1-hU6 at the BamHI/HindIII sites, and then XhoI/HindIII fragments containing hU6-shRNA were inserted into a pShuttle-CMV vector (Stratagene)(*Fujimoto et al, 2018*). To knockdown HSF1, SSBP1, or TRAP1, MEF cells were infected with Ad-sh-mHSF1-KD, Ad-sh-mSSBP1-KD, or Ad-sh-mTRAP1-KD (1×10^8 pfu/ml) for 2 h and maintained in normal medium for 70 h. As a control, the cells were infected with an adenovirus vector expressing scrambled RNA (Ad-sh-SCR).

Table 2. Nucleotide sequences of shRNAs used for gene knockdown

shRNA	Sense strand	Antisense strand
SCR	5'GATCCATGTACTGCGCGTGGAGACTTCAAGAGAGTCTCCACGCGCAGTACATTCTTTTGGAAA3'	5'AGCTTTTCCAAAAGAATGTACTGCGCGTGGAGACTCTCTTGAAATCAGTCGTATTTCTCTTCG3'
TRAP1	5'GATCCGGTTCTGGAGTGTTTGAAATCAAGAGATTTCAAACACTCCAGAACCATTTTTTTGGAAA 3'	5'AGCTTTTCCAAAAAAATGGTTCTGGAGTGTTTGAAATCTCTTGAATTTCAAACACTCCAGAACCG3'
HSF1	5'GATCCGTGATCACCTGGATGCCATTTCAAGAGAATGGCATCCAGGTGATCACTTTTTTGGAAA 3'	5'AGCTTTTCCAAAAAAGTGATCACCTGGATGCCATTCTCTTGAAATGGCATCCAGGTGATCACG3'
SSBP1	5'GATCCAGTTTGGTTCTTGAACGATTTCAAGAGAATCGTTCAAGAACCAAACTGCTTTTGGAAA 3'	5'AGCTTTTCCAAAAGCAGTTTGGTTCTTGAACGATTCTCTTGAAATCGTCAAGACCAAACTG3'

3.4 Western blotting

Cells pellets were lysed with NP-40 buffer (150 mM NaCl, 1.0% Nonidet P-40, 50 mM Tris-HCl, pH 8.0) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) on ice for 10 min. After centrifugation at 16,000 × g for 10 min, supernatants were subjected to SDS-polyacrylamide gel electrophoresis. For HSP10 blot, a Real Gel Plate with 10-20% polyacrylamide gel (MDG-296, BIO CRAFT, Tokyo,

Japan) were used. After proteins were transferred onto nitrocellulose or PVDF (SSBP1 blot) membranes, the membranes were blocked in PBS/5% milk at a room temperature for 1 h, and then were immunoblotted using rabbit antibodies against HSF1 (anti-mHSF1j, Millipore ABE1044; dilution, 1:1,000)) (*Fujimoto et al, 2012)*, TRAP1 (anti-mTRAP1a; dilution, 1:1,000) (this study) HSP60 (anti-HSP60-1; 1:2,000)) [22], HSP10 (Santa Cruz sc-20958; 1:1,000), mtHSP70 (or GRP75) (Santa Cruz sc-13967; 1:1,000), and SSBP1 (anti-mSSBP1x; dilution, 1:1,000) (this study), and mouse antibody for HSP70 (Santa Cruz W27; 1:1,000) and β-actin (AC-15, Sigma) diluted in PBS/2% milk at a room temperature for 1 h or at 4°C overnight. The membranes was washed three times with PBS for 5 min, and incubated at room temperature for 1 h with secondary antibodies: peroxidase conjugated goat anti-rabbit or anti-mouse IgG. After wash with PBS/0.1% Tween 20 three times, chemiluminescent signals from ECL detection reagents (GE Healthcare) were captured on X-ray film (Super RX, Fujifilm). Intensity of the bands was quantified using NIH ImageJ. We generated rabbit antisera against mouse TRAP1 (anti-mTRAP1a) and SSBP1 (anti-mSSBP1x) by immunizing rabbits using TiterMax Gold adjuvant (CytRx, Los Angeles, CA) with bacterially expressed recombinant GST-mTRAP1 (full-length protein) and GST-mSSBP1 (full-length protein), respectively.

3.5 Cross-linking

MEF cells were treated with 10 μM GTPP, 5 μM CDDO, or 20 μM rotenone for 6 h, or heat shock at 42°C for 30 min. Whole cell extracts were prepared in buffer C (0.42 M NaCl, 20 mM HEPES-NaOH, pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 0.2 mM EDTA) containing protease inhibitors [21]. Aliquots containing 40 μg protein were mixed with a 0.05 volume of 100 mM disuccinimidyl glutarate (DSG) (final concentration of 5 mM) at room temperature for 30 min, and were subjected to western blotting using HSF1 antibody (anti-mHSF1j).

3.6 Immunofluorescence

HeLa cells were grown on coated glass coverslips in 35 mm culture dishes for 16 h at 37°C in 5% CO_2. Cells were fixed with 100% methanol at -20°C for 15 min, and then washed three times with PBS for 5 min each. Subsequently, they were permeabilized and blocked with PBS/0.1% Triton X-100/5% goat serum at room temperature for 1 h. After washing with PBS once, the coverslips were incubated with rat monoclonal IgG for HSF1 (10H8, ab61382, Abcam) (1:200 dilution) at 4°C overnight, and washed three times with PBS. They were then incubated with FITC-conjugated goat anti-rabbit IgG (Cappel) (1:200 dilution in PBS/2% milk) or Alexa Flour 546-conjugated goat anti rat IgG (Molecular Probe) (1:200 dilution) at room temperature for 1 h. Coverslips were washed three times with PBS for 5 min each, and then mounted in a VECTASHIELD with 4'-6-diamino-2-phenylindole (DAPI) mounting medium (Vector Laboratories). High resolution (×63 objective magnification) confocal images were taken using LSM510 META confocal microscope (Carl Zeiss), and were quantified by using a Zen lite software (Carl Zeiss). HSF1 fluorescence signals in a total cell and a nucleus were estimated by measuring the average intensities of pixels by manually tracing cellular periphery and the region stained with DAPI, respectively. Percentage of HSF1 fluorescence signal localized in the nucleus was calculated by normalizing the nuclear signal intensity to total fluorescence intensity from the cell.

3.7 Chromatin immunoprecipitation (ChIP) analysis

ChIP experiments were performed using a kit in accordance with the manufacturer's instructions (EMD Millipore). The antibody used for ChIP assays was anti-mHSF1j. Real-time qPCR of ChIP-enriched DNAs in *HSP60*, *mtHSP70*, *HSP70* (*HSPA1A*) and its intergenic region was performed using the primers listed in (Table 3). Percentage input was determined by comparing the cycle threshold value of each sample to a standard curve generated from a 5-point serial dilution of genomic input, and compensated by values obtained using normal IgG. IgG-negative control immunoprecipitations for all sites yielded

<0.05% input. All reactions were performed in triplicate with samples derived from three experiments.

Table 3. Primer sequences used for CHIP assay

CHIP-qPCR	Forward sequence	Reverse sequence
HSP60/ HSP10 promoter	5'-GCCGAGGTGAAAGAACGA-3'	5'-TCCCGTGGGTGAAAGGT-3'
mtHSP70 promoter	5'-ATGAGAGAACGGGAACTTTC-3'	5'GAGACTACGTGTCCGCTGCC-3'
HSP70.3 promoter (dHSE)	5'-ACCCTCCCCCTCAGGAATC 3'	5'TGTCCAGAACTCTCCAGAGGTTT-3'
HSP70.3 promoter (intergenic region)	5'-GTGGCGCATGCCTTTGAT-3'	5'CTTTGTAGAACAGGCTGACCTTGA 3'

3.8 Measurement of mitochondrial membrane potential and oxygen consumption

MEF cells, which were infected with Ad-sh-mHSF1-KD or Ad-sh-SCR, were seeded into plastic 96 well plates at a density of 5×10^4 cells/well and grown for 16 h. After treatment with each inhibitor for 3 h, the cells were stained with MitoTracker Red CMXRos (Molecular Probes) for 30 min. The wells were washed twice with PBS to remove excess fluorescent dye, and fluorescence signals were measured at 540 nm/615 nm (excitation /emission) using an ARVO X4 multilabel plate reader (PerkinElmer, Inc., MA). Alternatively, cells infected with Ad-sh-mHSF1-KD or Ad-sh-SCR were grown on glass coverslips in 35 mm culture dishes for 16 h, treated as described above, and were fixed with 100% methanol at -20°C for 15 min. Coverslips were washed three times with PBS for 5 min each, and then mounted in a VECTASHIELD with DAPI mounting medium (Vector Laboratories). High resolution (×63 objective magnification) confocal images were taken using LSM510 META confocal microscope (Carl Zeiss).

Oxygen consumption were examined by using MitoXpress Xtra Oxygen Consumption Assay (Agilent) in accordance with the manufacturer's instructions. MEF cells were treated as described above in plastic 96 well plates and maintained at 37°C on a thermoregulator. The cells were loaded with a reagent containing the oxygen sensitive MitoXpress Xtra fluorescent probe and treated with or without 500 nM FCCP (Cayman Chemical) or 5 μM antimycin A (Abcam), and was covered by mineral oil. Each sample well was then measured at 340 nm/ 642 nm (excitation /emission) repetitively every 5 min over 120 min using an ARVO X4 multilabel plate reader (PerkinElmer, Inc., MA), by taking TR-F intensity readings at delay time of 30 and 70 μs and gate time 100 μs. Measured TR-F intensity signals (counts/s) were converted into lifetime signals (μs). Relative oxygen consumption rate (OCR) was estimated as a value of MitoXpress Xtra fluorescence lifetime signal per hour per mg of protein (μs/h/mg).

3.9 Statistical analysis

Data were analyzed using Student's t-test for comparisons between two groups. Multiple-group differences were assessed by one-way ANOVA test followed by the Tukey's post hoc test (JMP Pro 14 software, SAS Institute Inc., Cary, NC, USA). Asterisks in figures indicate that differences were significant ($P < 0.01$ or 0.05). Error bars represent the standard deviations for more than three independent experiments.

4. Results

4.1 HSF1 is required for activation of mitochondrial chaperone genes

To examine the roles of HSF1 in the UPR^{mt}, we treated immortalized MEF cells with three reagents that target mitochondrial proteins and impair mitochondrial proteostasis. GTPP inhibits the matrix HSP90 chaperone TRAP1 (*Kang et al, 2009; Siegelin et al, 2011*), and CDDO inhibits the matrix protease Lon (*Bernstein et al, 2012*). Rotenone is an inhibitor of the electron transfer complex 1 (ETC1) and increases production of reactive oxygen species (ROS). Protein levels of HSP60 and HSP10 were increased by treatment with GTPP or CDDO at concentrations of 5 to 20 μM, but were not by treatment with rotenone (Fig. 3A). In contrast, mtHSP70 protein levels were increased by treatment with 10-50 μM rotenone, and was slightly increased by treatment with GTPP or CDDO. Thus, the treatment of MEF cells with these reagents induced at least some mitochondrial HSPs in a dose-dependent manner, as reported previously (*Runkel et al, 2013; Münch and Harper, 2016*). We then treated wild-type and HSF1-null MEF cells with 10μM GTPP, 5 μM CDDO, or 20 μM rotenone for 6 h, and found that HSP60, HSP10, and mtHSP70 mRNA levels were increased by 1.2- to 2.0-fold in wild-type cells treated with GTPP and CDDO, and only mtHSP70 mRNA levels were significantly increased in cells treated with rotenone (Fig. 3B). Remarkably, mRNA levels of these genes were not induced in HSF1-null cells at all. mRNA levels of HSP70 were simultaneously increased by 10- to 45-fold in wild-type cells in a manner dependent of HSF1, suggesting that cytoplasmic proteostasis was also impaired in these conditions (Fig. 3C) (*Kim et al, 2016*). In marked contrast, expression of mitochondrial protease Lon mRNA was induced in both wild-type and HSF1-null cells during the treatment (Fig. 3D).

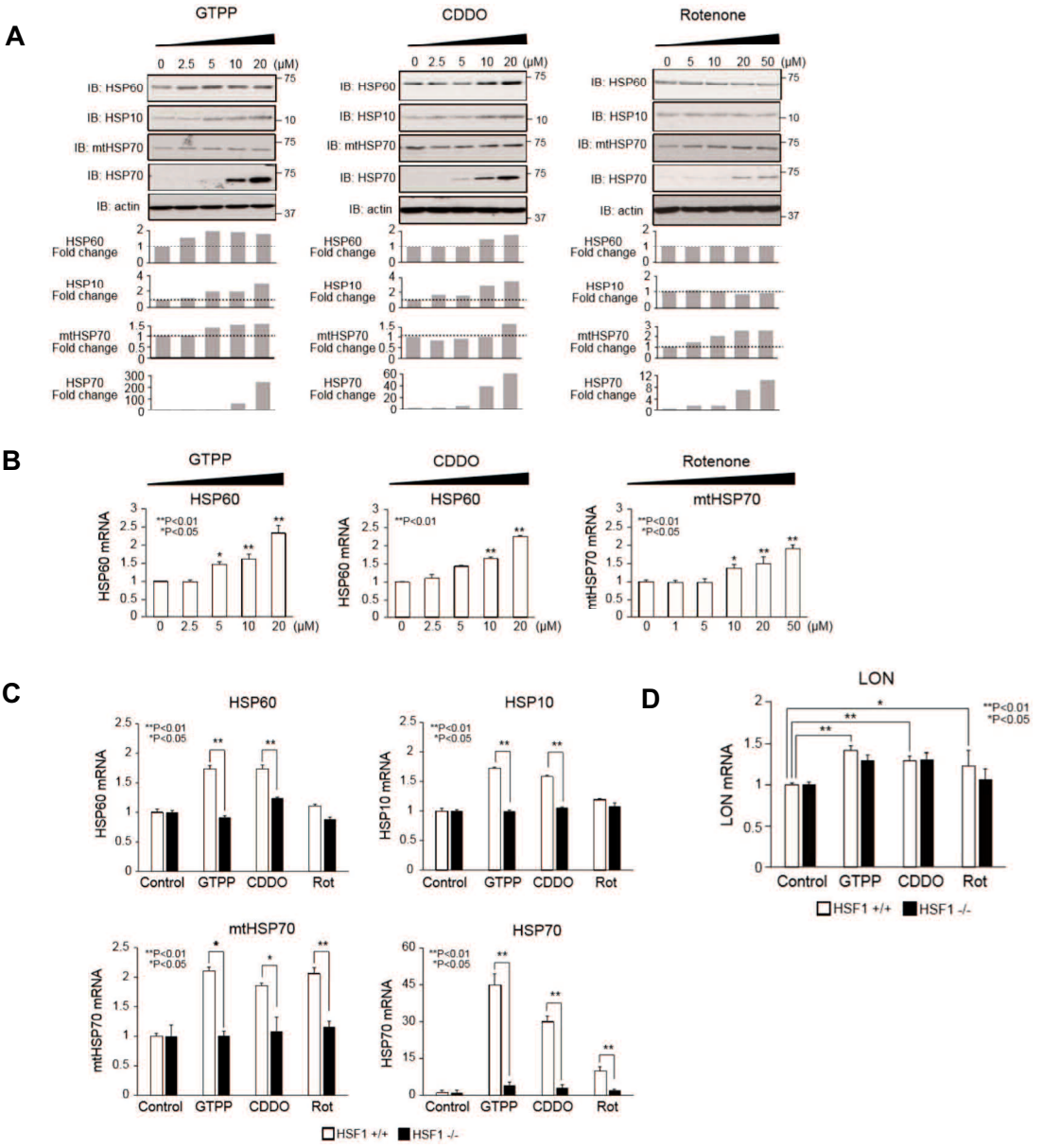

Figure 3. HSF1 is required for the activation of mitochondrial chaperone genes. (A) Induction of mitochondrial chaperones during treatment with reagents that target mitochondrial proteins. MEF cells were treated with GTPP, CDDO, or rotenone at the indicated concentrations for 6 h. Cell extracts were prepared using NP-40 lysis buffer, and were subjected to western blotting (upper). Intensity of HSP bands in representative blots was quantified using NIH ImageJ, and fold changes during treatments are shown (lower). **(B)** Induction of HSP60 and mtHSP70 mRNAs during treatment with reagents that target mitochondrial proteins. mRNA levels were quantified by RT-qPCR (n=3). Mean ± S.D. is shown. Asterisks indicate P < 0.01 or 0.05 by one-way ANOVA, compared with each mRNA level in untreated cells. **(C)** HSF1 is required for the induction of mitochondrial HSP mRNAs in cells treated with the indicated reagents. Wild-type (HSF1+/+) and HSF1-null (HSF1-/-)

MEF cells were treated with 10 μM GTPP, 5 μM CDDO, and 20 μM rotenone for 6 h. mRNA levels of HSP60, HSP10, mtHSP70, and HSP70 were quantified by RT-qPCR (n=3). Mean ± S.D. is shown. Asterisks indicate P < 0.01 or 0.05 by Student's t-test. **(D)** HSF1 is not required for the induction of Lon mRNAs in cells treated with the indicated reagents. Cells were treated as was shown in B. mRNA levels of Lon were quantified by RTqPCR (n=3). Mean ± S.D. is shown. Asterisks indicate P < 0.01 or 0.05 by Student's t-test.

4.2 TRAP 1 Inhibition induces mitochondrial chaperones via HSF1-dependent pathway

To exclude non-specific effects of GTPP, we knocked down TRAP1 and confirmed that both HSP60 and HSP70 protein levels were increased in TRAP1-knockdown cells (Fig. 4A) (*Baqri et al, 2014; Münch and Harper, 2016*). HSP60, HSP10, and HSP70 mRNA levels were also increased by about 1.5-fold. However, they were not increased at all in TRAP1-knockdown cells deficient in HSF1 (Fig. 4B, C). These results demonstrated that HSF1 is required for activation of mitochondrial chaperone genes, but not for that of *Lon* protease gene, during the UPR^{mt} in mouse cells, when mitochondrial proteostasis is impaired by targeting a mitochondrial chaperone.

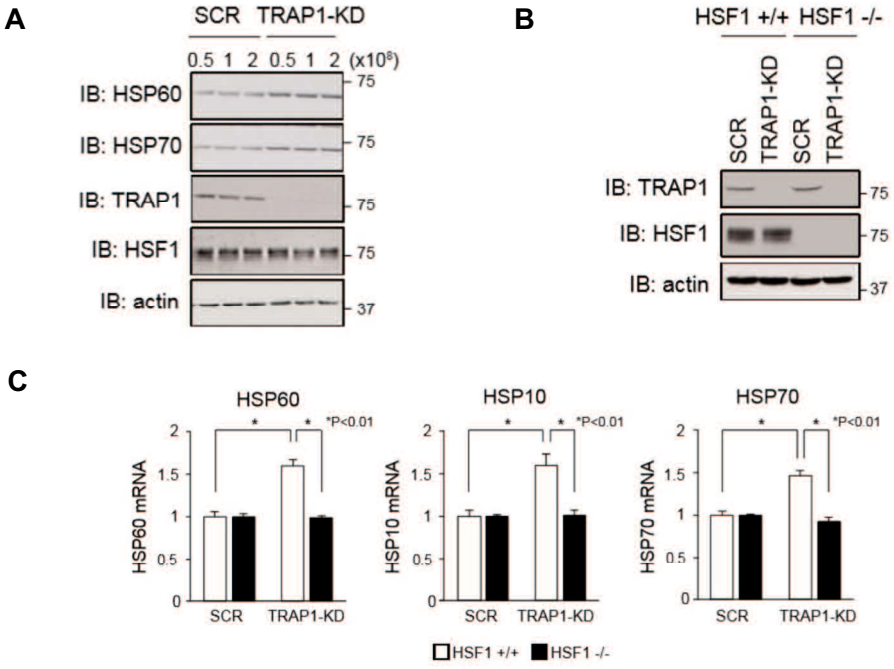

Figure 4. HSP60 is induced by TRAP1 knockdown. (A) MEF cells were infected with an adenovirus expressing scrambled RNA (SCR) or shRNA for TRAP1 (TRAP-KD) at the indicated concentration (0.5 to 2 × 10^8 PFU/ml) for 2 h, maintained with normal medium for 70 h. Cell extracts were prepared and subjected to western blotting. (B, C) HSF1 is required for the induction of HSP60 mRNAs in TRAP1 knockdown cells. Wild-type (HSF1+/+) and HSF1-null (HSF1-/-) MEF cells were treated as described in (A), and cell extracts were subjected to western blotting (B). mRNA levels of HSP60 and HSP70 were quantified by RT-qPCR (n=3) (C). Mean ± S.D. is shown (left). Asterisks indicate $P < 0.05$ by Student's t-test. Cell extracts were prepared and subjected to western blotting (right).

4.3 Different requirement of SSBP1 for activation of the mitochondrial chaperone genes

We then investigated the effects of SSBP1 on the activation of UPR^{mt} genes in response to impaired mitochondrial proteostasis. MEF cells were infected for 72 h with an adenovirus expressing short hairpin RNA for SSBP1 or HSF1, or scrambled RNA (SCR) as a control, and protein level of SSBP1 or HSF1 was transiently reduced (Fig. 5A). We confirmed that the expression of HSP60, HSP10, and mtHSP70 mRNAs as well as HSP70 mRNA were not increased at all in HSF1 knockdown cells during treatment with GTPP, CDDO, or rotenone (Fig. 5B-D, black bars). In SSBP1 knockdown cells, the expression of HSP70 mRNA was partially increased during the same treatment (Fig. 5B-D, gray bars. In marked contrast, HSP60 mRNA expression was not increased at all in SSBP1 knockdown cells during treatment with GTPP or CDDO. On the other hand, HSP10 mRNA expression levels were partially increased in SSBP1 knockdown cells during GTTP treatment, whereas they were not increased at all in the same cells treated with CDDO (Fig. 5B and C, gray bars). mtHSP70 mRNA expression was fully increased in SSBP1 knockdown cells during CDDO treatment, whereas it was not increased at all in the same cells treated with GTPP or rotenone (Fig. 5B-D, gray bars). These results suggested different requirements of SSBP1 on the activation of mitochondrial chaperone genes during the UPR^{mt}.

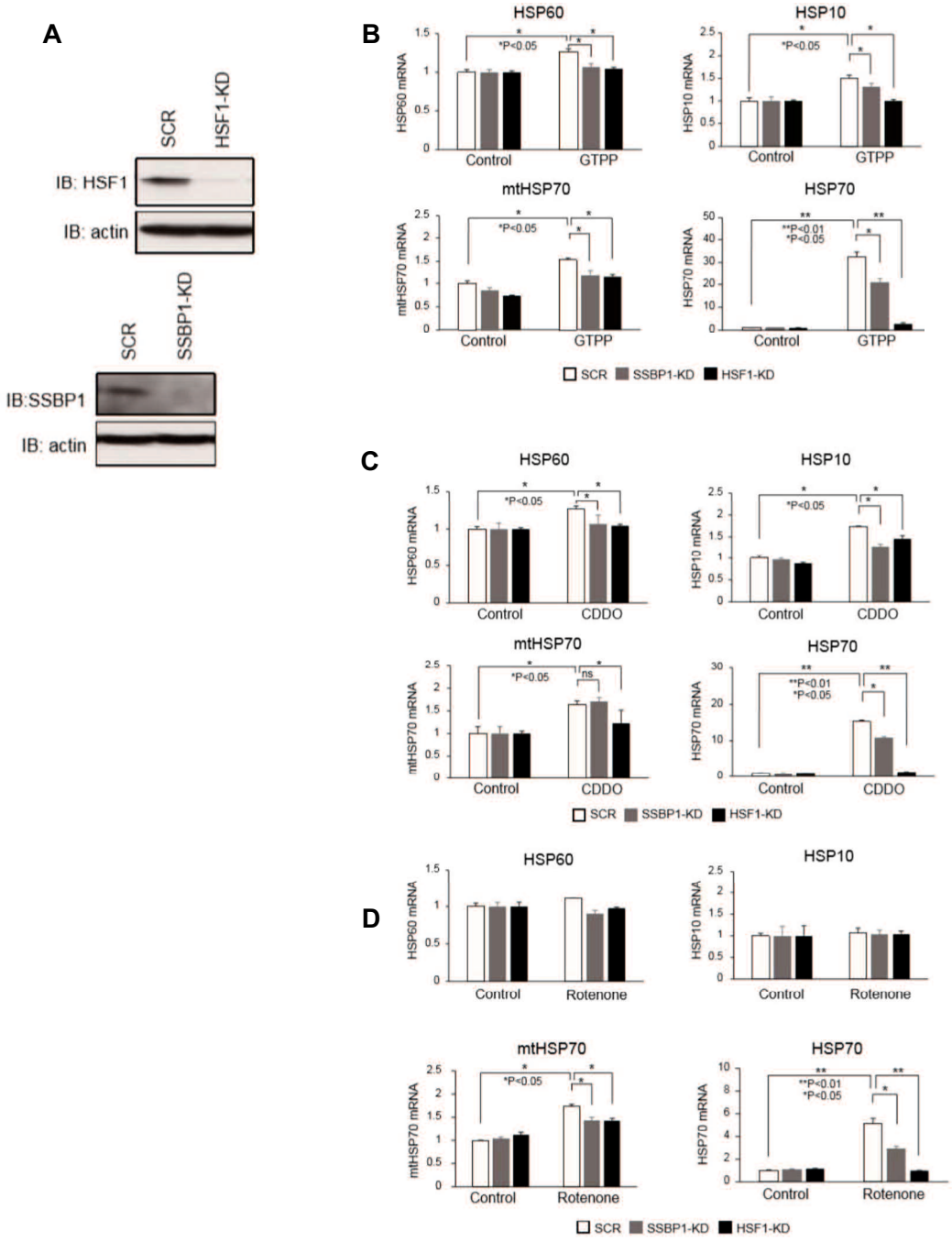

Figure 5. Different requirements of SSBP1 for activation of mitochondrial chaperone genes. (A) Knockdown of HSF1 or SSBP1. MEF cells were infected with an adenovirus expressing scrambled RNA (SCR) or shRNA for SSBP1 (SSBP1-KD) or HSF1 (HSF1-KD) for 2 h, maintained with normal medium for 70 h. Cell extracts were prepared and subjected to western blotting. (B-D) Activation of the mitochondrial chaperone genes in SSBP1 or HSF1 knockdown cells. SSBP1 or HSF1 were knocked down as described in (A). The cells were treated with 10 μM GTPP (B), 5 μM CDDO (C), and 20 μM rotenone (D) for 6 h. mRNA levels of HSP60, HSP10, mtHSP70, and HSP70

were quantified by RT-qPCR (n=3). Mean ± S.D. is shown. Asterisks indicate P < 0.01 or 0.05 by Student's t-test.

4.4 Nuclear translocation, trimer formation, and phosphorylation of HSF1

We investigated whether HSF1 is activated directly or indirectly during treatment with GTPP, CDDO, or rotenone. HSF1 activation involves its nuclear translocation, trimer formation, and phosphorylation of a specific residue (*Sarge et al, 1993; Baler et al, 1993*). We found that HSF1 localizes to both the cytoplasm and nucleus in unstressed cells, and slightly accumulates in the nucleus during treatment with GTPP, CDDO, or rotenone (Fig. 6A and B). Nuclear foci termed HSF1 granules were detected in cells treated with heat shock but not in cells treated with these reagents. Second, we examined the oligomeric form of HSF1 using DSG cross-linking experiments. Monomeric HSF1 shifted to a trimeric form during treatment of MEF cells with heat shock, and was partly shifted to a trimeric form during treatment with GTPP, CDDO, or rotenone (Fig. 6C). Third, we studied HSF1-Ser326 phosphorylation, which is an active mark of HSF1 transcriptional activity (*Guettouche et al, 2005*). Because a specific antibody for human HSF1Ser326, but not for mouse HSF1-Ser326, is available, we replaced endogenous HSF1 with human HSF1 in MEF cells. It was revealed that hHSF1-Ser326 was phosphorylated at lower levels in cells treated with GTPP, CDDO, or rotenone than in cells treated with heat shock at 42°C 90 min (Fig. 6D). Hyper phosphorylation of HSF1, which is detected as retarded bands on a gel, is often correlated with the activation of HSF1, but was not evident in the same cells. These results suggested that HSF1 is modestly activated in response to impaired mitochondrial proteostasis.

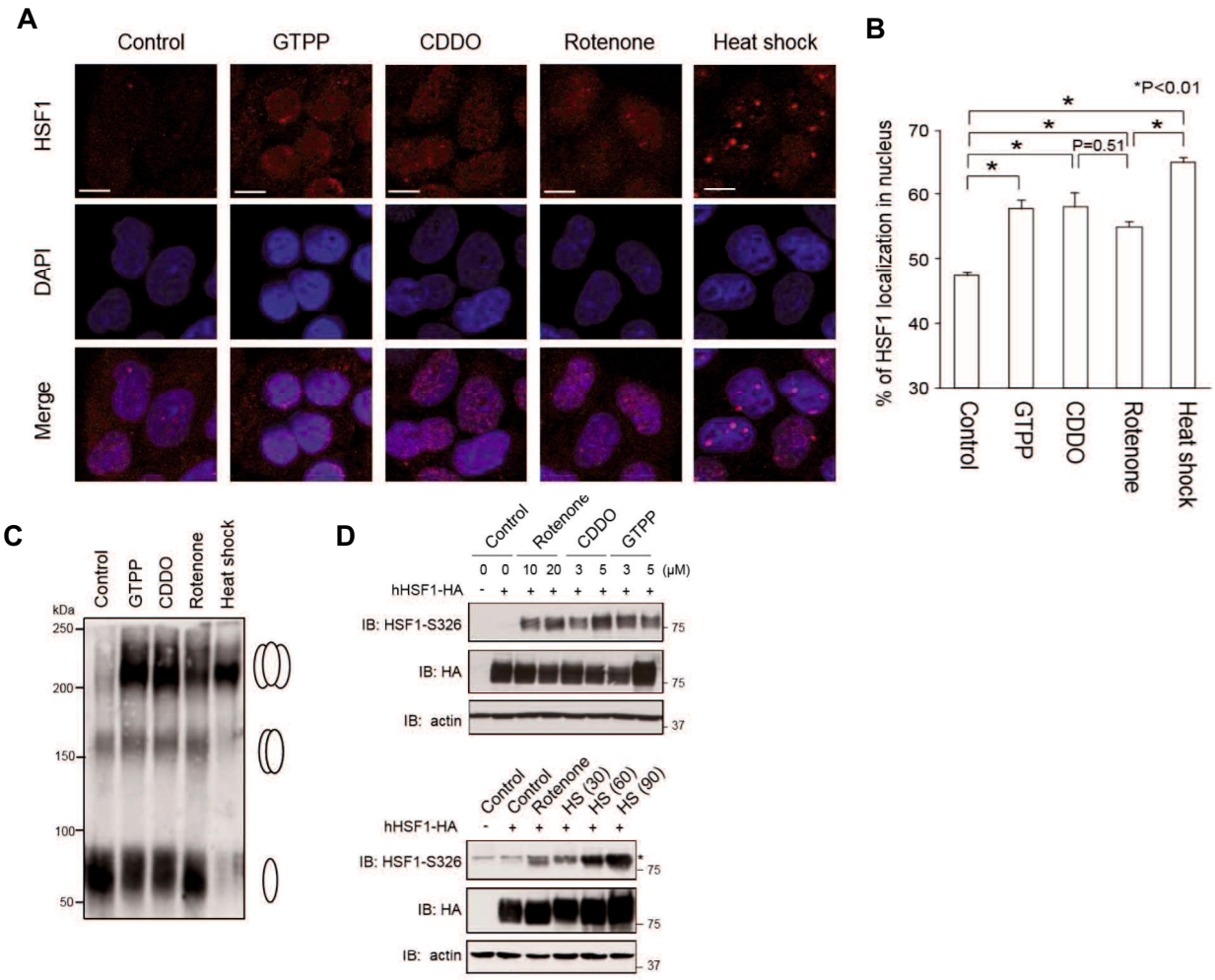

Figure 6. Nuclear translocation of HSF1 in response to impaired mitochondrial proteostasis. (A) HeLa cells were treated with 10 μM GTPP, 5 μM CDDO, or 20 μM rotenone for 6 h. The cells were co-stained with an antibody for HSF1 and the nuclear marker DAPI, and fluorescence images were merged (Merge). Bars, 20 μm. (B) Quantitative estimation of HSF1 signals in the nucleus. The fluorescent signals in the nucleus (arbitrary unit) were estimated by using Zen 2.5 software (n=20). Mean ± S.D. is shown. Asterisks indicate $P < 0.05$ by Student's t-test. (C) Trimer formation of HSF1. MEF cells were treated with 10 μM GTPP, 5 μM CDDO, or 20 μM rotenone for 6 h, or heat shock at 42°C for 30 min. Whole cell extracts were prepared, and aliquots containing 40 μg protein were mixed with a cross-linking reagent DSG at a final concentration of 5 mM at room temperature for 30 min, and were subjected to western blotting using HSF1 antibody. Positions of HSF1 monomer, dimer, and trimer are indicated on the right. (D-E)) Phosphorylation of HSF1-Ser326. HSF1-null (HSF1-/-) cells were infected with adenovirus expressing HA-tagged human HSF1 (1x108 pfu/ml) for 2 h, and maintained with normal medium for 46 h. The cells were treated with GTPP, CDDO, or rotenone at the indicated concentrations for 6 h (left), or treated with 20 μM rotenone for 6 h or heat shock (HS) at 42°C for 30, 60, or 90 min (right). Cell extracts were prepared and subjected to western blotting using HSF1-phosphoS326 (S326P), HA, or β-actin antibody. An asterisk indicates non-specific bands.

4.5 HSF1 occupancy in *HSP60/HSP10* promoter is remarkably high

It was assumed that HSF1 mildly occupies mitochondrial chaperone gene promoters in vivo in impaired mitochondrial proteostasis conditions, because it is activated only modestly. As shown previously, HSF1 heavily bound to HSP60/HSP10 promoter as well as HSP70 (HSPA1A) promoter, and a little to mtHSP70 promoter during heat shock at 42°C for 30 min In contrast, HSF1 moderately bound to HSP70 promoter in cells treated with GTPP and CDDO, and bound to it at a lower level in cells treated with rotenone. HSF1 constitutively bound to mtHSP70 promoter to some extent, and its binding was induced moderately in cells treated with rotenone, and was little induced in cells treated with GTPP and CDDO (Fig. 7A and B).HSF1 also constitutively bound to HSP60/HSP10 promoter to some extent, and levels of HSF1 binding were little induced in cells treated with rotenone. Contrary to our expectation, the levels of HSF1 binding were heavily induced in cells treated with GTPP and CDDO, like in cells treated with heat shock. Furthermore, we confirmed that levels of HSF1 binding to HSP60/HSP10 promoter were markedly induced in TRAP1 knockdown cells (Fig. 7C). These results indicated that HSF1 occupancy on the mitochondrial chaperone gene promoters is induced at different levels. HSF1 occupancy in HSP60/HSP10 promoter was remarkably high during the treatment with GTPP and CDDO, whereas that in mtHSP70 or HSP70 promoter was moderate. It was assumed that HSF1 mildly occupies mitochondrial chaperone gene promoters in vivo in impaired mitochondrial proteostasis conditions, because it is activated only modestly. As shown previously, HSF1 heavily bound to *HSP60/HSP10* promoter as well as *HSP70* (*HSPA1A*) promoter, and moderately to *mtHSP70* promoter in response to heat shock at 42°C for 30 min (Fig. 7A and B). In contrast, HSF1 moderately bound to HSP70 promoter in cells treated with GTPP and CDDO, and bound to it at a lower level in cells treated with rotenone. HSF1 constitutively bound to *mtHSP70* promoter to some extent, and its binding was induced moderately in cells treated with rotenone, and induced at lower levels in cells treated with GTPP and CDDO. HSF1 also constitutively bound to *HSP60/HSP10* promoter to some extent, and levels of HSF1 binding were moderately induced in cells treated with rotenone. Contrary to our expectation, the

levels of HSF1 binding were heavily induced in cells treated with GTTP and CDDO, like in cells treated with heat shock (Fig. 7A and B). Furthermore, we confirmed that levels of HSF1 binding to *HSP60/HSP10* promoter were markedly induced in TRAP1 knockdown cells (Fig. 7C). These results indicated that HSF1 occupancy on the mitochondrial chaperone gene promoters is induced at different levels. HSF1 occupancy in *HSP60/HSP10* promoter was remarkably high during the UPR^{mt}, whereas that in *mtHSP70* or *HSP70* promoter was moderate.

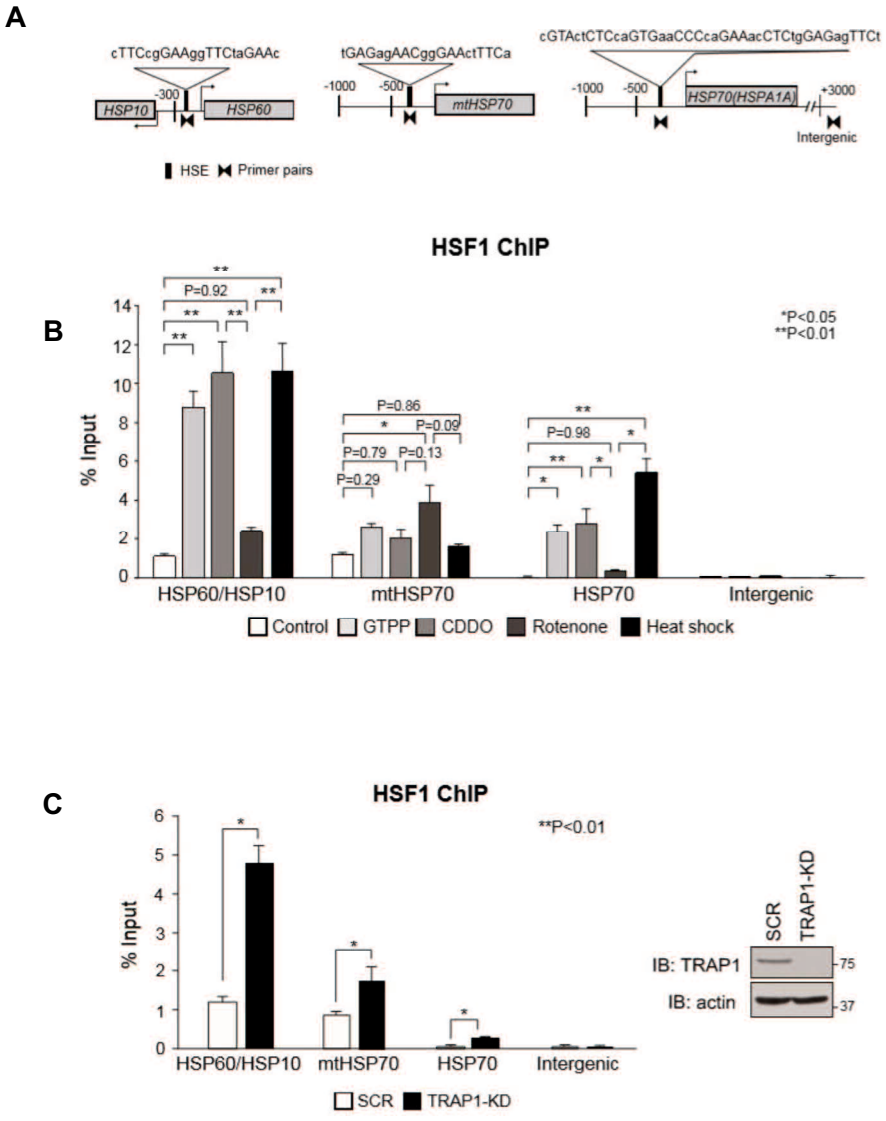

Figure 7. HSF1 occupancy on the mitochondrial chaperone gene promoters is induced at different levels. (A) Schematic representation of mouse HSP60/HSP10, mtHSP70, and HSP70 (HSPA1A) loci. Nucleotide sequences of each HSE and consensus nGAAn sequences

are shown. Amplified promoter regions by qPCR using each specific primer pairs are indicated. **(B)** HSF1 occupancy is induced in response to impaired mitochondrial proteostasis. MEF cells were treated with 10 μM GTPP, 5 μM CDDO, 20 μM rotenone for 6 h, or heat shock at 42°C for 30 min. ChIP-qPCR performed on each promoter region using HSF1 antibody (n = 3). Mean ± S.D. is shown. Asterisks indicate $P < 0.01$ or 0.05 by one-way ANOVA, compared between two groups. **(C)** TRAP1 knockdown enhances HSF1 occupancy on HSP60/HSP10 promoter. MEF cells were infected for 72 h with Ad-sh-mTRAP1-KD, and ChIP-qPCR analyses were performed using HSF1 antibody. Mean ± S.D. is shown (n=3) (left). Asterisks indicate $P < 0.01$ by Student's t-test. Cell extracts were prepared and subjected to western blotting using TRAP1, β-actin antibody (right).

4.6 HSF1 promotes maintenance of mitochondrial function

To test whether HSF1-mediated expression of UPRmt genes is related with mitochondrial function, we first examined mitochondrial membrane potential using a fluorescent probe MitoTracker Red. The intensity of MitoTracker fluorescence was not affected when MEF cells were treated with 10 μM GTPP, 5 μM CDDO, or 20 μM rotenone for 3 h (Fig. 8A). However, it was significantly reduced in HSF1 knockdown cells treated with GTPP or rotenone, but not in those cells treated with CDDO. We next examined the basal (-FCCP) and maximal (+FCCP) oxygen consumption in the same cells (Fig. 8B). The relative oxygen consumption rate (OCR) was not significantly reduced by HSF1 knockdown, but was reduced in cells treated with GTPP, CDDO, or rotenone for 3 h. Remarkably, levels of the relative OCR were more reduced in HSF1 knockdown cells than those in scrambled RNA-treated cells in the presence or absence of FCCP. These results suggested that HSF1 promotes maintenance of mitochondrial function in response to impaired mitochondrial proteostasis.

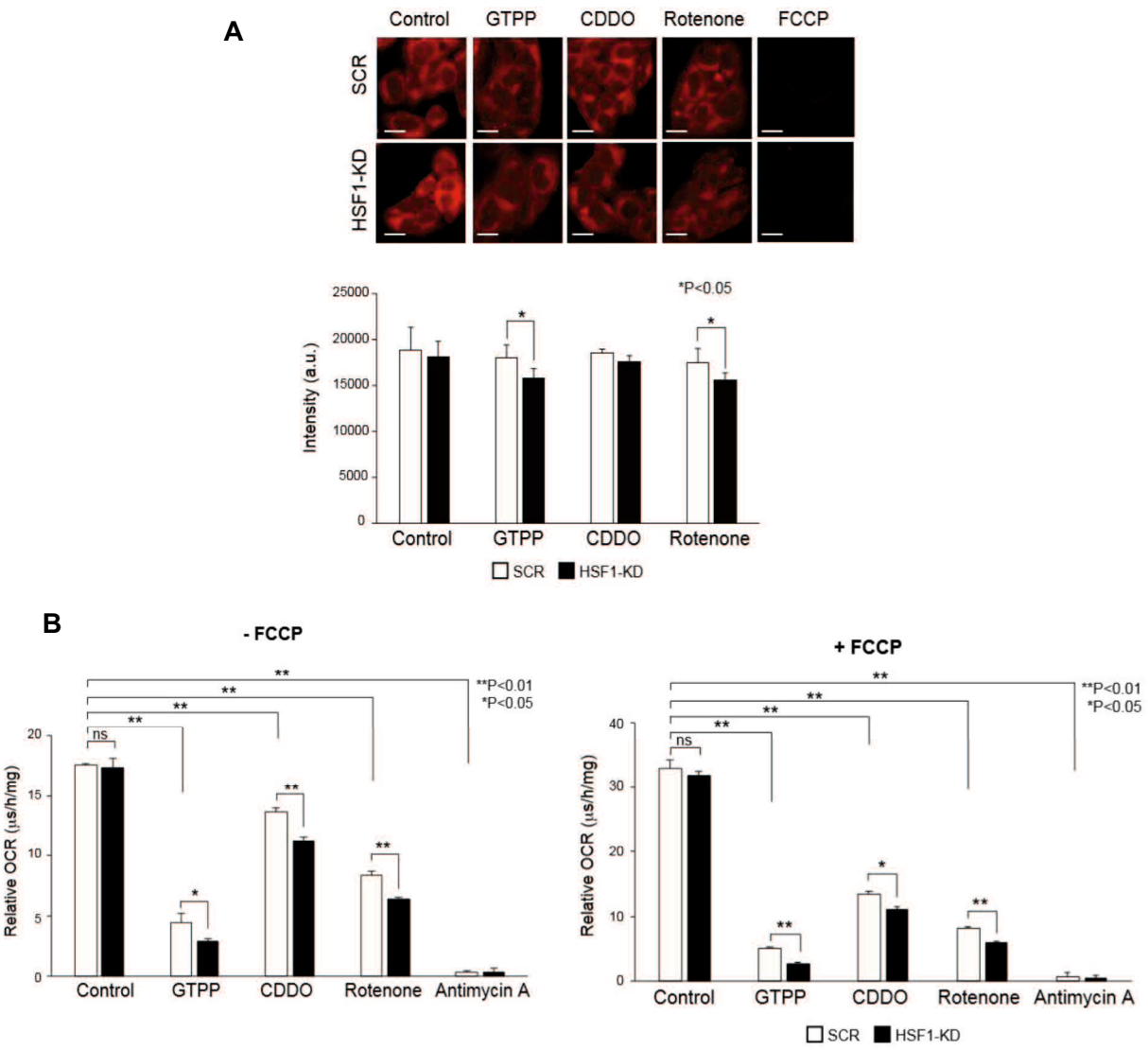

Figure 8. HSF1 is involved in the maintenance of mitochondrial function. **(A)** HSF1 supports the maintenance of mitochondrial membrane potential. Control (SCR) and HSF1 knockdown (HSF1-KD) MEF cells were treated with 10 μM GTPP, 5 μM CDDO, 20 μM rotenone for 3 h. The cells were stained with MitoTracker Red CMXRos, and MitoTracker fluorescent signals were measured (arbitrary fluorescence unit). Mean ± S.D. is shown (n=3). Asterisks indicate $p<0.05$ by Student's t-test. **(B)** HSF1 supports the maintenance of oxygen consumption. MEF cells were treated as described in (A) and mixed with MitoXpress Xtra reagent containing an oxygen sensitive fluorescence probe. Fluorescent signals were measured and oxygen consumption (arbitrary fluorescence unit per an hour) is shown. Mean ± S.D. is shown (n=6). Asterisks indicate $p<0.05$ by Student's t-test.

5. Discussion

Mitochondria are the central hub of metabolic and signaling processes including ATP production and apoptotic cell death (*Pagliarini et al, 2013; Friedman et al, 2014*) , and declines in mitochondrial function are associated with aging and disorders, such as neurodegenerative diseases and cancer (*Vafai and Mootha, 2012; Campisi et al, 2019*). Cells must adapt to a large variety of mitochondrial dysfunctions by changing nuclear-encoded mitochondrial gene expression. Among these homeostatic mechanisms, the UPR^{mt} is an adaptive response to accumulation of misfolded proteins in mitochondria. ATF5 and CHOP have been shown to be required for the activation of UPR^{mt} genes during accumulation of ΔOTC in human HEK293 and monkey COS-7 cells, respectively (*Zhao et al, 2002; Fiorese et al, 2016*). In this study, we used immortalized MEF cells for analysis of the UPR^{mt}, and mechanisms of the UPR^{mt} were analyzed during treatment with GTPP, CDDO, or rotenone (*Runkel et al, 2013; Münch et al, 2016; Quirós et al, 2017*), which induces the expression of HSP60, HSP10, mtHSP70, or Lon as well as cytoplasmic HSP70. We showed that both disruption of *HSF1* gene and transient HSF1 knockdown abolished the upregulation of mitochondrial chaperone genes, but not for that of protease *Lon*, during the UPR^{mt} (Figs. 3C and 3D). In contrast, SSBP1 is required for the upregulation of only *HSP60*. Even in unstressed conditions, HSF1 constitutively occupied *HSP60/HSP10* and *mtHSP70* promoters (Fig. 6). Furthermore, a very small part of HSF1 accumulated in the nucleus, shifted to a trimeric form, and was phosphorylated at Ser326, suggesting that HSF1 was activated directly or indirectly in response to impaired mitochondrial proteostasis (Fig. 5). Although treatment with the inhibitors may also cause proteostasis impairment in the cytoplasm, our observation indicated that HSF1 is required for activation of mitochondrial chaperone genes during the UPR^{mt} (Fig. 9).

HSF1 has been shown to plays roles in the maintenance of mitochondrial function through different pathways. HSF1 deficiency causes reduced constitutive expression of cytoplasmic HSPs including HSP25, which is associated with a decrease in cellular GSH/GSSG ratio and an increase in mitochondrial oxidative stress in the heart, kidney, and

oocytes (*Yan et al, 2002; Metchat et al, 2009; Bierkamp et al, 2010*). Induction of HSPs, including HSP60 and HSP10, by HSF1 and SSBP1 promotes the maintenance of mitochondrial membrane potential in proteotoxic stress conditions, which are caused by heat shock or proteasome inhibition (*Tan et al, 2015*). Furthermore, activation of HSF1 is associated with increased mitochondrial function by enhancing the expression of PGC1α, which is a central regulator of mitochondrial biogenesis and function (*Ma et al, 2015*). Consistently, mitochondrial function such as mitochondrial membrane potential is suggested to be more reduced by the expression of an aggregation-prone polyglutamine protein in HSF1 knockdown cells (*Intihar et al, 2019*). Here, we showed that mitochondrial membrane potential and oxygen consumption were more reduced in HSF1 knockdown cells than those in scrambled RNA-treated cells during treatment with GTTP, CDDO, or rotenone (Fig. 8). Our observations suggested that mitochondrial function in conditions of impaired mitochondrial proteostasis is maintained in part by the HSF1-dependent upregulation of mitochondrial chaperone genes.

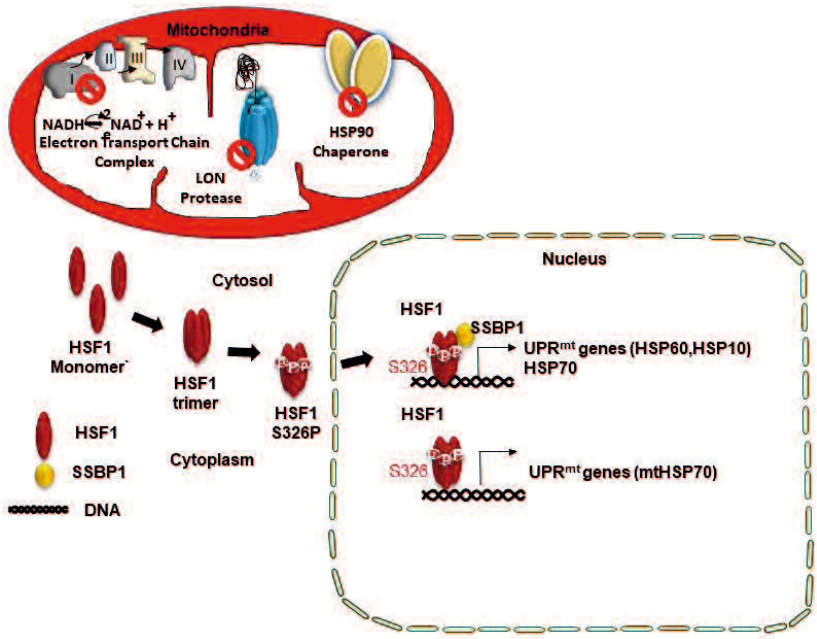

Figure 9. HSF1 is an essential UPR^{mt} regulator in mammals.

It is worth noting that *HSP60* and *HSP10* uniquely share a bidirectional promoter containing an HSE, which consisted of at least four inverted repeats of an exceptionally conserved consensus nGAAn unit (*Ryan et al, 1997; Hansen et al, 2003*). ChIP-seq and ChIP-qPCR data analysis showed that HSF1 constitutively binds to the bidirectional promoter at a much higher level than to the promoters of other *HSP* genes including *HSP70* in MEF cells, and the level of HSF1 binding to this promoter was dramatically elevated during heat shock (*Tan et al, 2015; Takii et al, 2015*). HSF1 was mostly converted to a DNA-binding trimer during heat shock, whereas a small part of HSF1 shifted to a trimer during the UPR^{mt} (Fig. 6C). Unexpectedly, in vivo HSF1 binding to the bidirectional promoter was induced in cells treated with GTPP and CDDO at the same levels as that in cells treated with heat shock (Fig.7B). Level of HSF1 binding to this promoter was also moderately elevated in cells treated with rotenone. Thus, analysis of in vivo HSF1 binding to the unique bidirectional promoter of *HSP60/HSP10* could be a sensitive marker of the UPR^{mt}.

6. Summary

In summary, we here show that HSF1 is required for activation of mitochondrial chaperone genes, including *HSP60*, *HSP10*, and *mtHSP70* during inhibition of matrix chaperone TRAP1, protease Lon, or electron transfer complex 1 activity. SSBP1 partially tunes the expression of *HSP60* and *HSP10* in cooperation with HSF1 under the same conditions. HSF1 is translocated into nucleus, oligomerized and phosphorylated at Ser326. HSF1 occupancy is enhanced at UPR^{mt} gene promoters during this response. Furthermore, HSF1 supports maintenance of mitochondrial function probably by enhancing matrix folding capacity. Therefore, HSF1 may be one of guardians for mitochondrial function under conditions of impaired mitochondrial proteostasis.

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7. References

Baler R, Dahl G, Voellmy R (1993) Activation of human heat shock genes is accompanied by oligomerization, modification, and rapid translocation of heat shock transcription factor HSF1. Mol Cell Biol **13**, 2486-2496.

Baqri RM, Pietron AV, Gokhale RH, Turner BA, Kaguni LS, Shingleton AW, Kunes S, Miller KE (2014) Mitochondrial chaperone TRAP1 activates the mitochondrial UPR and extends health span in Drosophila. Mech Ageing Dev **141-142**, 35-45.

Bernstein SH, Venkatesh S, Li M, Lee J, Lu B, Hilchey SP, Morse KM, Metcalfe HM, Skalska J, Andreeff M, Brookes PS, Suzuki CK (2012) The mitochondrial ATP-dependent Lon protease: a novel target in lymphoma death mediated by the synthetic triterpenoid CDDO and its derivatives. Blood **119**, 3321-3329.

Bierkamp C, Luxey M, Metchat A, Audouard C, Dumollard R, Christians E (2010) Lack of maternal Heat Shock Factor 1 results in multiple cellular and developmental defects, including mitochondrial damage and altered redox homeostasis, and leads to reduced survival of mammalian oocytes and embryos. Dev Biol **339**, 338-353.

Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E (2019) From discoveries in ageing research to therapeutics for healthy ageing. Nature **571**, 183-192.

Fiorese CJ, Schulz AM, Lin YF, Rosin N, Pellegrino MW, Haynes CM (2016) The transcription factor ATF5 mediates a mammalian mitochondrial UPR. Curr Bio **26**, 2037-2043.

Friedman JR, Nunnari J (2014). Mitochondrial form and function. Nature **505**, 335-343.

Fujimoto M, Izu H, Seki K, Fukuda K, Nishida T, Yamada S, Kato K, Yonemura S, Inouye S, Nakai A (2004) HSF4 is required for normal cell growth and differentiation during mouse lens development. EMBO J **23**, 4297-4306.

Fujimoto M, Takaki E, Takii R, Tan K, Prakasam R, Hayashida N, Iemura S, Natsume T, Nakai A (2012) RPA assists HSF1 access to nucleosomal DNA by recruiting histone chaperone FACT. Mol Cell **48**, 182-194.

Fujimoto M, Takii R, Katiyar A, Srivastava P, Nakai A (2018) Poly(ADP-Ribose) Polymerase 1 Promotes the Human Heat Shock Response by Facilitating Heat Shock Transcription Factor 1 Binding to DNA. Mol Cell Biol **38**, e00051-18.

Fujimoto M, Takii R, Takaki E, Katiyar A, Nakato R, Shirahige K, Nakai A. (2017) The HSF1-PARP13-PARP1 complex facilitates DNA repair and promotes mammary tumorigenesis. Nat Commun **8**,1638.

Gomez-Pastor R, Burchfiel ET, Thiele DJ (2018) Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol **19**, 4-19.

Guettouche T, Boellmann F, Lane WS, Voellmy R (2005) Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. BMC Biochem **6**, 4.

Hansen JJ, Bross P, Westergaard M, Nielsen M, Eiberg H, Børglum AD, Mogensen J, Kristiansen K, Bolund L, Gregersen N (2003) Genomic structure of the human mitochondrial chaperonin genes: HSP60 and HSP10 are localised head to head on chromosome 2 separated by a bidirectional promoter. Hum Genet **112**, 436.

Haynes CM, Ron D (2010) The mitochondrial UPR - protecting organelle protein homeostasis. J Cell Sci **123**, 3849-3855.

Horibe T, Hoogenraad NJ (2007) The chop gene contains an element for the positive regulation of the mitochondrial unfolded protein response. PLoS One **2**, e835.

Intihar TA, Martinez EA, Gomez-Pastor R (2019) Mitochondrial Dysfunction in Huntington's Disease; Interplay Between HSF1, p53 and PGC-1α Transcription Factors. Front Cell Neurosci **13**, 103.

Kang BH, Plescia J, Song HY, Meli M, Colombo G, Beebe K, Scroggins B, Neckers L, Altieri DC (2009) Combinatorial drug design targeting multiple cancer signaling networks controlled by mitochondrial Hsp90. J Clin Invest **119**, 454-464.

Kim HE, Grant AR, Simic MS, Kohnz RA, Nomura DK, Durieux J, Riera CE, Sanchez M, Kapernick E, Wolff S, Dillin A (2016) Lipid Biosynthesis Coordinates a Mitochondrial-toCytosolic Stress Response. Cell **166**, 1539-1552.

Ma X, Xu L, Alberobello AT, Gavrilova O, Bagattin A, Skarulis M, Liu J, Finkel T, Mueller E (2015) Celastrol Protects against Obesity and Metabolic Dysfunction through Activation of a HSF1-PGC1α Transcriptional Axis. Cell Metab **22**, 695-708.

Martinus RD, Garth GP, Webster TL, Cartwright P, Naylor DJ, Høj PB, Hoogenraad NJ (1996) Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. Eur J Biochem **240**, 98-103.

Melber A, Haynes CM (2018) UPRmt regulation and output: a stress response mediated by mitochondrial-nuclear communication. Cell Res **28**, 281-295.

Merkwirth C, Jovaisaite V, Durieux J, Matilainen O, Jordan SD, Quiros PM, Steffen KK, Williams EG, Mouchiroud L, Tronnes SU, Murillo V, Wolff SC, Shaw RJ, Auwerx J, Dillin A (2016) Two Conserved Histone Demethylases Regulate Mitochondrial Stress-Induced Longevity. Cell **165**, 1209-1223.

Metchat A, Akerfelt M, Bierkamp C, Delsinne V, Sistonen L, Alexandre H, Christians ES (2009) Mammalian heat shock factor 1 is essential for oocyte meiosis and directly regulates Hsp90alpha expression. J Biol Chem **284**, 9521-9528.

Mizzen LA, Chang C, Garrels JI, Welch WJ (1989) Identification, characterization, and purification of two mammalian stress proteins present in mitochondria, grp 75, a member of the hsp 70 family and hsp 58, a homolog of the bacterial groEL protein. J Biol Chem **264**, 20664-20675.

Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev **12**, 3788-3796.

Münch C, Harper JW (2016) Mitochondrial unfolded protein response controls matrix preRNA processing and translation. Nature **534**, 710-713.

Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM (2012) Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science **6094**:587-590.

Osman C, Voelker DR, Langer T (2011) Making heads or tails of phospholipids in mitochondria. J Cell Bio **192**(1):7-16.

Pagliarini DJ1, Rutter J (2013) Hallmarks of a new era in mitochondrial biochemistry. Genes Dev **27**, 2615-2627.

Pellegrino, M.W.,and Haynes, C.M. (2015) Mitophagy and the mitochondrial unfolded protein response in neurodegeneration and bacterial infection. BMC Biol. **13**, 22

Quirós PM, Mottis A, Auwerx J (2016) Mitonuclear communication in homeostasis and stress. Nat Rev Mol Cell Biol **17**, 213-226.

Quirós PM, Prado MA, Zamboni N, D'Amico D, Williams RW, Finley D, Gygi SP, Auwerx J (2017) Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. J Cell Biol **216**, 2027-2045.

Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. **8**, 519-529.

Runkel ED, Liu S, Baumeister R, Schulze E (2013) Surveillance-activated defenses block the ROS-induced mitochondrial unfolded protein response. PLoS Genet **9**, e1003346.

Ryan MT, Herd SM, Sberna G, Samuel MM, Hoogenraad NJ, Høj PB (1997) The genes encoding mammalian chaperonin 60 and chaperonin 10 are linked head-to-head and share a bidirectional promoter. Gene **196**, 9-17.

Sarge KD, Murphy SP, Morimoto RI (1993) Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Mol Cell Biol **13**, 1392-1407.

Siegelin MD, Dohi T, Raskett CM, Orlowski GM, Powers CM, Gilbert CA, Ross AH, Plescia J, Altieri DC (2011) Exploiting the mitochondrial unfolded protein response for cancer therapy in mice and human cells. J Clin Invest **121**, 1349-1360.

Takii R, Fujimoto M, Tan K, Takaki E, Hayashida N, Nakato R, Shirahige K, Nakai A. 2015. ATF1 modulates the heat shock response by regulating the stress-inducible HSF1-transcription complex. Mol Cell Biol **35**:11–25.

Tan K, Fujimoto M, Takii R, Takaki E, Hayashida N, Nakai A (2015) Mitochondrial SSBP1 protects cells from proteotoxic stresses by potentiating stress-induced HSF1 transcriptional activity. Nat Commun **6**, 6580.

Vafai SB, Mootha VK (2012) Mitochondrial disorders as windows into an ancient organelle. Nature **491**, 374-383.

Wellen KE, Thompson CN (2012) A two-way street: reciprocal regulation of metabolism and signalling. Nat Rev Mol Cell Biol **4**, 270-276.

Wolff S, Weissman JS, Dillin A (2014) Differential scales of protein quality control. Cell **157**, 52-64.

Yan LJ, Christians ES, Liu L, Xiao X, Sohal RS, Benjamin IJ (2002) Mouse heat shock transcription factor 1 deficiency alters cardiac redox homeostasis and increases mitochondrial oxidative damage. EMBO J **21**, 5164-5172.

Yan LJ, Rajasekaran NS, Sathyanarayanan S, Benjamin IJ (2005) Mouse HSF1 disruption perturbs redox state and increases mitochondrial oxidative stress in kidney. Antioxid Redox Signal **7**, 465-471.

Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D (2004) Compartmentspecific perturbation of protein handling activates genes encoding mitochondrial chaperones. J Cell Sci **117**, 4055-4066.

Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ(2002) A mitochondrial specific stress response in mammalian cells. EMBO J. **17**, 4411-4419.