Ribosome Profiling Reveals a Dichotomy Between Ribosome Occupancy of Nuclear-Encoded and Mitochondrial-Encoded OXPHOS mRNA Transcripts in a Striatal Cell Model of Huntington Disease

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Abstract
Huntington disease (HD) is caused by an expanded polyglutamine mutation in huntingtin (mHTT), which promotes a prominent atrophy in the striatum and subsequent psychiatric, cognitive, and choreiform movements. Multiple lines of evidence point to an association between HD and aberrant striatal mitochondrial functions. However, present knowledge about whether (or how) mitochondrial mRNA translation is differentially regulated in HD remains unclear. We have recently applied ribosome profiling (Ribo-Seq), a technique based on the high-throughput sequencing of ribosome-protected mRNA fragments, to analyze detailed snapshots of ribosome occupancy of the mitochondrial mRNA transcripts in control and HD striatal cells. Ribo-seq data revealed almost unaltered ribosome occupancy on the nuclear-encoded mitochondrial transcripts involved in oxidative phosphorylation (OXPHOS) and only a mild reduction in ribosome occupancy on a few selected transcripts (SHDA, Ndufv1, Timm23, Tomm5, and Mrps22) in HD cells. By contrast, ribosome occupancy of mitochondrially encoded OXPHOS mRNAs (mtNd-1, mtNd-2, mtNd-4, mtNd-4l, mtNd-5, mtNd-6, mt-Co1, mtCyt b, and mt-ATP8) was dramatically increased, implying widespread dichotomous effects on ribosome occupancy and OXPHOS mRNA translation in HD. Thus, mHTT may command signals that specifically regulate translation of the mitochondrial OXPHOS transcripts and influence HD pathogenesis.

Key words
Dichotomy, energy metabolism, abnormality, brain disease, vulnerability, oxidative stress, mitoribosome, citoribosome.
Introduction

Expansion of the CAG repeat in the huntingtin (HTT) gene causes the motor disturbance, cognitive loss, and psychiatric manifestations of Huntington's Disease (HD), but the exact mechanism by which mutant HTT (mHTT) induces its pathological effect in the brain remains unclear. Previous studies found widespread mitochondrial abnormalities, including decreased complex activities [1-8], oxidative damage [9, 10], mitochondrial depolarization [11], calcium defects [12, 13], altered biogenesis [14-17], and mitophagy [18-20] in HD models and in patient samples. Despite several investigations, the precise cellular mode(s) of action of mHTT on mitochondria remains controversial. For example, mHTT had no effect on oxidative metabolism and mitochondrial Ca^{2+} handling in HD [21, 22], whereas mHTT directly interacted with mitochondria and altered mitochondrial proteostasis [23-27]. Consequently, the details of the mechanisms that relate mHTT to mitochondrial defects remain unclear.

Mitochondria are essential organelles that play a vital role in numerous cellular processes, and they contain their own semi-autonomous system of gene expression and mRNA translation machineries. The mitochondrial genome codes for components of the ribosomal-RNA genes and 22 transfer-RNA genes, as well as 13 proteins [28, 29] that participate in the oxidative phosphorylation (OXPHOS) reactions of complexes (I, III, IV). Additional genes required for mtDNA maintenance, replication, transcription, translation, post-translational modification, transport, assembly, and expression of OXPHOS complexes (II, V) are exclusively encoded by the nucleus [30]. Thus, mitochondrial functions, and particularly the translation and assembly of the OXPHOS complexes, are mechanistically daunting as they are encoded by different genomes. For these reasons, the molecular and biochemical control of mitochondrially encoded protein synthesis and the role of defects in this process in neurodegenerative diseases remain poorly understood.

The aim of the present study was to use our recently reported high-quality parallel RNA sequencing (RNA-seq) and ribosome profiling (Ribosome-seq) technique [31] to compare in vivo
genome-wide information on protein synthesis (GWIPS) in healthy and HD striatal cells. Here, we report our analysis of the relative expression levels of mitochondrially encoded mRNA transcripts, ribosome density and ribosome occupancy in the OXPHOS genes.

Results
Ribosome profiling of ribosome-protected fragments from 55S and 80S ribosomes.

We carried out systematic Ribo-Seq and RNA-seq analyses in well-established ST-Hdh-7/Q7 (WT control), ST-Hdh-Q7/Q111 (HD-het), ST-Hdh-Q111/Q111 (HD-homo) knock-in mouse striatal cell lines that express full-length wild-type (polyQ7) HTT and one (HTT-het) or two (HTT-homo) copies of mutant (polyQ111) HTT. Three replicates of WT control, HD-het, and HD-homo mutant striatal cells were subjected to ribosome profiling and the fractions contained 55S (mitoribosomes) and 80S (cytoribosomes) collected and prepared Ribo-Seq and matching RNA-Seq (Fig. 1). Through the use of multiple quality control measures, we have previously generated a high-quality global Ribo-Seq library of control, HD-het, and HD-homo cells [31].

Here, we explored ribosome occupancy by examining the ribosome profiles of mitochondrially encoded mRNAs in the Ribo-Seq. We combined the uploaded profiles, as a track hub in the University of California Santa Cruz (UCSC) Genome Browser 42, from the triplicate experiments and overlaid ribosome protected fragments (RPF, orange) and mRNA abundance (mRNA, blue). We then estimated the ribosome occupancy (R.O) as the ratio between CDS RPF abundance and mRNA abundance and for each gene (RPF/mRNA) from the raw read counts from the UCSC browser (Fig. 1).

Ribosome occupancy is increased on the mitochondrially encoded OXPHOS genes in HD

We evaluated the ribosome occupancy of all 13 mitochondrially encoded OXPHOS mRNA transcripts (mt-OXPHOS transcripts). We observed ribosome occupancy for 11 mt-OXPHOS mRNA transcripts. The complex I subunits mt-Nd1, mt-Nd2, mt-Nd3, mt-
Nd4, mt-Nd4l, mt-Nd5, and mt-Nd6 showed high RPF, low mRNA, and high R.O (RPF/mRNA) in HD cells compared to control; the R.O was also much higher in HD-het compared to HD-homo cells [Fig. 2(A-D), Fig. 3 (A-C)]. Mt-CyB, the only mitochondrially encoded complex III subunit, also showed high RPF and R.O (Fig. 4A). The complex IV subunit mt-Co1 and the complex V subunit mt-Atp8 showed high RFP and a significant trend of higher R.O in the HD-het and HD-homo cells (Fig. 4B, C). We were able to obtain R.O for the remaining three mt-OXPHOS transcripts (mt-Co3, mt-Co2, and mt-Atp6) and these showed a trend of higher R.O in HD cells (Supplementary Figure 1). However, we were unable to obtain statistically significant differences, as no RPF or mRNA reads were discernible in one or more replicates of the Ribo-Seq experiments (Supplementary Figure 1). Variation in the ribosome density across the transcripts was also observed; while Mt-Nd1 and mt-Nd3, mt-Nd4l showed ribosomes are preferentially located at the 5’ region mt-Nd3, mt-Nd6 and mt-CyB towards the 3’ region. These results indicate that almost all the mt-OXPHOS mRNA transcripts showed a trend of enhanced R.O with altered ribosome density in HD cells, and particularly in HD-het cells (See discussion).

**Ribosome occupancy is slightly decreased or unaltered on the mitochondrially encoded OXPHOS genes in HD**

We also investigated the RFP and R.O of selected nuclear-encoded complex I subunits. Ndufv1 showed ribosomes distributed throughout the 10 exons, whereas RPF is significantly diminished in HD-het cells and unaffected in HD-homo cells compared to controls (Fig. 5A). However, the R.O of the Ndufv1 transcript showed no differences (Fig. 5A). A selected example of nuclear encoded complex IV subunits (nu-Cox5a) showed no difference in the R.O between the control and HD cells (Fig. 5B). Similarly, the R.O of the complex III subunit Uqcrb was the same for control and HD cells (Fig. 5C), whereas the R.O of CytC1 was diminished in the HD-het cells compared to the controls (Fig. 6A). The Complex V subunits (ATP5o) showed no significant alterations in R.O among the cell types (Fig. 6B). For complex II, the Sdha showed a significant reduction in R.O in HD cells compared to controls (Fig. 6C), while the R.O of the Sdhc and Shhb subunits were similar between the control and HD cells (Data not shown).
**Ribosome occupancy is decreased or unaltered on the mitochondrial transcripts that code for the outer membrane, mitochondrial biogenesis, and mitochondrial ribosomes.**

Examination of nuclear-encoded mitochondrial outer membrane transcript Tomm5 showed significantly decreased RFA and RNA, but no significant alterations of R.O (one-way ANOVA, Tukey's multiple comparison), indicating that the decreased occupancy was due to diminished RNA levels (Fig. 7A). Timm23 showed lower RPF in HD-het cells than in HD-homo cells but no significant changes in RNA or R.O (Fig. 7B). Interestingly, the RPF of PCK2 was increased in HD-het due to increases in mRNA, but no change was observed in R.O (Fig. 7C).

We also compared the RFP, RNA, and R.O of various nuclear-encoded mitochondrial transcripts, as shown in Fig. 8. The Mrps22, a mitochondrial ribosome component, showed significantly decreased RFP and R.O (Fig. 8A), while Tim14, the inner membrane translocase, showed decreased RPF and RNA but no change in R.O (Fig. 8B). No other tested nuclear-coded mitochondrial transcripts (Mrpl3, Tufm, Rars2, Opa1, Fis1, and Vdac1) showed changes in ribosome occupancy (Fig. 8C-H). Additional examples of all the nuclear-coded mitochondrial mRNA transcripts in the control, HD-het, and HD-homo cells can be found in the UCSC browser at [https://genome.ucsc.edu/cgi-bin/hgTracks?hubUrl=Https://de.cyverse.org/anon-files/iplant/home/rmi2lab/Hub_Collaborations/Srini/hub.txt&genome=mm10](https://genome.ucsc.edu/cgi-bin/hgTracks?hubUrl=Https://de.cyverse.org/anon-files/iplant/home/rmi2lab/Hub_Collaborations/Srini/hub.txt&genome=mm10)

In summary, our ribosome profiling studies of mitochondrial transcripts in HD cells revealed an intriguing and previously unreported dichotomy: Substantial differences exist in the ribosome occupancy between nuclear-encoded vs mitochondrially encoded OXPHOS transcripts, and the ribosome occupancy levels of mitochondrially encoded OXPHOS genes is largely enhanced in HD cells. This dichotomy of ribosome occupancy implies the existence of a different mitochondrial translation mechanism in healthy versus HD cells. Understanding the possible mechanistic process behind this dichotomy
mediated by mHTT (Fig. 9) can help in understanding the complex disease process and in the identification of new therapeutic targets.

Discussion

Multiple aspects of mitochondrial dysfunction are linked to numerous brain diseases, and particularly neurodegenerative diseases [32-42]. Perturbed mitochondrial functions and disrupted protein synthesis have been reported in HD [3, 16, 43-51], but a detailed molecular and biochemical understanding is lacking. Here, we applied ribosome profiling and obtained new insight into mitochondrial protein synthesis in HD.

We found novel mechanistic defects of translational regulation that involve enhanced loading of ribosomes onto mitochondrially coded mRNAs that control the energy production, cell signaling, and cell death functions of the mitochondria [52, 53]. However, this regulation was absent from the nuclear-encoded mitochondrial mRNAs, revealing an intriguing mechanistic dichotomy in the translational regulation operating between the cytoplasm and the mitochondria in the HD context.

Previous studies have indicated that both wtHTT and mHTT are localized to the mitochondria, but whether either HTT resides in the mitochondrial intermembrane space or the outer membrane remains controversial [18, 23, 26, 27, 54, 55]. Nevertheless, the mitochondrial localization raises an intriguing possibility that mitochondrial membrane-associated mHTT/wtHTT can induce signaling from the inner or outer membrane surface to regulate ribosome occupancy of translating OXPHOS mRNA and the insertion of OXPHOS peptides onto the inner mitochondrial membrane. By contrast, our recent model predicted that mHTT can directly bind to the polysomes and ribosomal proteins in the cytoplasm and thereby impede the speed of ribosome translocation and protein synthesis [31]. However, whether mHTT can bind to mitochondrial ribosomes (mitoribosomes) and proteins and use a similar mechanism to regulate mitochondrial mRNA translation is not clear. Nevertheless, as most (>95%) of the mitochondrial proteins are produced in the nucleus, one possibility is that mHTT may activate signals in the nucleus and cytoplasm
and these then communicate messages to the mitochondrion for its own mRNA translation (Fig. 9). Recent studies in yeast showed that cytosolic translational regulators control the mitochondrial OXPHOS genes [56, 57]. Moreover, a novel mitochondrial-associated quality control (mtRQC) pathway comprising of mtRF-R and MTES1 is shown to rescue mitochondrial ribosomes stalling[58]. Further identifying the molecular nature of cellular signals and ribosome stalling mechanisms in HD cells will provide new insights that could help to curtail mitochondrial translation defects and have a potential therapeutic impact in HD.

Enhanced ribosome occupancy is predicted to increase translation efficiency and protein levels. However, this assumption is not straightforward because ribosome occupancy can also increase due to slowly elongating ribosomes and the steady-state proteins levels are modulated by posttranslational mechanisms. A previous study indicated robust differences in the mitochondrial proteome between wtHTT and mHTT cells [27], with overall proteomics diminished in the HD, consistent with our studies [31]. However, they also found that the levels of selected proteins, such as mt-CO1, were increased while the levels of VDAC1 and TIM23 were diminished in mHTT-expressing cell lines. We found that the R.O of mt-CO1 is increased, the R.O of TIM23 is decreased; and the R.O of VDAC1 is unchanged. Thus, the enhanced ribosome occupancy of mRNA transcripts in HD is not directly correlated with enhanced protein levels by western blotting. This notion is further supported by the fact that the β–actin housekeeping gene shows diminished R.O and yet it shows enhanced β–actin protein levels by western blotting in HD-het cells (Supplementary Fig. 2A, B).

We also validated some of the mitochondrial proteins and found, for example, that mt-ND2 protein levels are increased (R.O is increased, Fig. 2B) in HD cells and mt-ND3 protein levels are decreased (R.O is increased, Fig. 2C), whereas mt-ND6 protein levels are unaltered (R.O is increased, Fig. 3C) (Supplementary Fig. 2B). The lack of a direct correlation between ribosome occupancy and protein levels indicates that the ribosome loading on mRNA transcripts and the steady-state-levels of fully translated proteins in HD are presumably controlled by independent mechanisms.
One important observation from this study is the differential influence of ribosome occupancy on mitochondrially coded mRNA by one copy of mHTT (HD-het) versus two copies of mHTT (HD-homo). The HD-het cells display a significantly higher ribosome occupancy on the mt-OXPHOS genes, with a minority of nu-coded OXPHOS transcripts exhibiting diminished occupancy compared to HD-homo cells (Figs. 2-4). This observation suggests that the combined presence of wtHTT and mHTT may lead to the emergence of an additive risk for translation regulatory phenotypes that readapt to the ongoing HD-related cellular demands. In addition, as wtHTT plays a key role as a negative regulator of ribosome translocation\cite{31}, this raises the possibility that enhanced ribosome occupancy in HD-het may be involved in the subtle loss of normal HTT function due to mHTT.

Alternatively, mHTT may exert a gain of function, so that the presence of two copies of the mHTT gene worsens the degree of ribosome occupancy at mt-coded RNAs and leads to stalling of ribosomes and possibly the eventual degradation of the stalled transcripts. This notion is supported by the data showing significantly reduced amounts of mitochondrial mRNA transcripts in the HD-homo versus the HD-het or control cells (Figs. 2-4). Future studies should investigate the mechanisms that may induce a decay of ribosome-stalled mitochondrial transcripts and associated factors in HD.

Taken together, our study findings demonstrate an unexpected dichotomy of ribosome occupancy among nuclear and mitochondrially coded OXPHOS transcripts due to the presence of mHTT. Defining the underlying mechanisms that create this dichotomy of OXPHOS mRNA translation and their influence on mitochondrial structure and function will reveal new understanding of HD pathogenesis and identify new therapeutic targets.
Figure legends

**Figure. 1.** A schematic diagram showing the experimental design for performing the ribosome profiling (Ribo-Seq) and mRNA sequencing (RNA-Seq) in indicated mouse striatal cells. RNAase digested polysomes collected as 80S peaks most likely comprised of mitoribosomes with lower sedimentation coefficients (~55S, brown arrow).

**Figure 2.** (A-D) Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for mt-ND1 (A), mt-ND2 (B), mt-ND3 (C) and mt-ND4 (D), extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), ****P< 0.0001, ***P< 0.001, **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 3.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for mt-Nd4l (A), mt-Nd5 (B), and mt-Nd6 (C) extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 4.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for mt-Co1 (A), mt-CyB (B), and mt-ATP8 (C) extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 5.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for
RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for Ndufv1 (A), Cox5a (B), and Uqcrb (C) extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 6.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for Cytc1 (A), ATP5o (B), and Sdha (C) extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), ****P< 0.0001, ***P< 0.001, **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 7.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for Tomm5 (A), Timm23 (B), and Pck2 (C) extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), ****P< 0.0001, **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 8.** Representative individual raw reads for RPF and mRNA from the triple experiments for the indicated mRNA transcripts extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P< 0.01, *P< 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 9.** Model for mHTT-mediated ribosome stalling of mitochondrially coded OXPHOS genes. We reported widespread ribosome stalling in HD cells and our model predicted that HTT inhibits ribosome movement and mHTT gains this function and further impedes the ribosome movements and inhibits protein synthesis. Here we report the observation that an intriguing dichotomy in OXPHOS transcript ribosome loading in HD.
Almost all mitochondrially encoded OXPHOS transcripts, but not the nuclear-encoded OXPHOS transcripts, show a higher ribosome occupancy in HD cells. We predict that the unique dichotomy and the associated signaling mechanisms that HTT or mHTT has on inducing mt OXPHOS transcript ribosome loading may emanate from the cytoplasm or from the inner or outer mitochondrial membrane. The possible mechanistic process for the dichotomy of ribosome occupancy in HD remains unknown.

**Supplementary Figure 1.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for mt-Co2 (A), mt-Co3 (B), and mt-Atp6 (C) extracted from UCSC Genome Browser.

**Supplementary Figure 2.** A. β-actin raw reads for RPF and mRNA from the triple experiments extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P < 0.01, one-way ANOVA followed by Tukey's multiple comparison test. B. Representative Western blot for the indicated proteins from control, HD-het and HD-homo cells.

**Material and methods**

**Cell culture:**
Mouse striatal cells (STHdh) expressing knock-in wild-type Htt\textsuperscript{exon1} with 7 Glu repeats (control; STHdh\textsuperscript{Q7/Q7}) or expressing knock-in mutant human Htt\textsuperscript{exon1} with 111 Glu repeats (HD-het; STHdh\textsuperscript{Q7/Q111}, and HD-homo; STHdh\textsuperscript{Q111/Q111}) \cite{59} were purchased from Coriell Institute for Medical Research (Camden, New Jersey, USA) and cultured in 10% FBS, DMEM, high glucose, 5% CO\textsubscript{2}, at 33°C, as described before \cite{60}.

**Isolation of ribosomes (mitoribosomes and cytoribosomes) for profiling:**
Global RNase footprintings were performed during three independent rounds of cell cultures (n=3). For each round of global footprinting, mouse immortalized striatal cells
(i.e. control, HD-het, and HD-homo cells) were plated in 15 cm dishes at a confluency of 70%. The following day the mediums were changed and after 2 hours the cells were incubated with cycloheximide (CHX, 100 µg/ml) for 10 min as in previous studies[31, 61, 62]. Cells were then scraped and washed with cold PBS (containing 100 µg/ml CHX) twice. During the second wash 5% of cells were transferred to new tubes and were lysed by adding 700 µl of QiAozol lysis reagent. Total RNAs of these samples were isolated using miRNeasy Mini Kit (Qiagen) for total mRNA sequencing. After the second wash, the rest of the cells were lysed in a lysis buffer containing 20 mM HEPES pH 7.3, 150 mM KCl, 10 mM MgCl$_2$, 2 mM DTT, 100 µg/ml CHX, 0.5% v/v Triton X-100, 20 U/ml RNasin and EDTA free protease inhibitor cocktail (Roche). The cell lysates were passed 20 times through a 26G needle and incubated on ice for 15 minutes, then centrifuged at 21000 rpm for 15 minutes. Supernatants were transferred to new tubes. Equal total RNA amount of each sample was used for global RNase foot printing as follow; for each A260 absorbance unit of the lysates 60 units of RNaseT1 (ThermoFisher Scientific) and 0.6 µl of RNaseA (Ambion) were added and the samples were incubated at 25°C for 30 min. RNase treated samples were immediately loaded on 10-50% sucrose gradients and centrifuged at 40000 RPM (SW41Ti rotor) at 4°C for 2 hours. Gradients were fractionated using a gradient fractionator and UA-6 detector, 254 nm filter (ISCO/BRANDEL). Fractions containing both 55S (mitoribosomes) and 80S (cytoribosomes) peaks of each sample were collected and their RNAs were isolated using a miRNeasy Mini Kit (Qiagen).

**Generation of cDNA libraries from ribosome protected mRNAs:**
The following procedure were performed for all the RNA samples simultaneously. 20 µg of each sample was run on a 15% TBE-Urea gel (Novex) along with 26 and 32 nt RNA markers. The gel containing each sample was excised between two markers. RNAs were extracted from gel pieces by incubating gel slurries with nuclease-free water overnight at 4°C and precipitated using RNase-free isopropanol and then eluted in nuclease-free water. T4 Polynucleotide Kinase (NEB) was used to catalyze the addition of 5' monophosphate and removal of the 3' phosphate in the RNA fragments to leave a 3' hydroxyl terminal needed for adapter ligation. RNA was purified using the Zymo clean and conc-5 kit (Zymo Research, Cat. # R1013). Ribosomal RNA was depleted from the
samples using TruSeq total RNA rRNA-depletion protocol (Illumina, Cat. #RS-122-2201) and then RNA samples were purified using Agencourt RNAClean XP beads (Beckman Coulter).

**Generation of cDNA libraries and sequencing:**
NEXTflex small RNA-seq Kit v3 (Perkin Elmer) was used to ligate 5’ and 3’ adapters to purified RPF fragments, which then were reverse transcribed and amplified (14 cycles) to generate cDNA libraries. Libraries were cleaned up using NEXTflex Cleanup beads, pooled and sequenced in the NextSeq 500 (V2) using single-end 50bp chemistry at the Scripps Genomic Core, at Florida, USA.

**Generation of mRNA-seq libraries:**
NEBNext Ultra II Directional kit (NEB, Cat. # E776) with the NEBNext poly(A) mRNA Magnetic isolation module (NEB, Cat. # E7490) was used generate mRNA-seq libraries. Briefly, 400ng of high-quality total RNA was used to purify poly(A) mRNA, fragmented, reverse-transcribed with random primers, adapter ligated, and amplified according to manufacturer recommendations. The final libraries were validated on the bioanalyzer, pooled, and sequenced on the NextSeq 500 using paired-end 40bp chemistry.

**Ribo-Seq, RNA-seq quality control and mapping the reads to UCSC browser:**
RNAseq reads were trimmed using Cutadapt[^63] with the following parameters: -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA -A AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT --minimum-length=15 --pair-filter=any. For Riboseq reads, 3’ adapters were trimmed using Cutadapt with the following parameters: -a TGGAATTCTCGGGTGCCAAGG --minimum-length 23. The reads were further trimmed using Cutadapt to remove 4 bases from either side of each read accordingly to the NEXTflex™ Small RNA Trimming Instructions (cutadapt -u 4 -u -4). Fastq files were checked for quality control with FastQC. Both RNAseq and Riboseq reads were next mapped to a library of mouse rRNA and tRNA sequences using Bowtie v1.1.2. Any reads mapping to these abundant contaminants were filtered out. Remaining reads were then aligned to the mouse transcriptome with RSEM v1.3.0[^64] using the GRCm38.p5 genome annotation and the comprehensive gene annotation from Gencode.
(M16 release) as transcriptome reference. Reads with a mapping quality <5 were
discarded. Cleaned bam files were converted to bigWig files with Bedtools\textsuperscript{[65]} for
visualisation using the UCSC Genome Browser. For the euclidian distance analyses,
gene expression was quantified with RSEM and comparison plots were generated in R
using DESeq\textsuperscript{2}[66] and ggplot2 packages.

**Western blot analysis:**
The cells were lyzed in the lysis buffer containing 20 mM HEPES pH 7.3, 150 mM KCl,
10 mM MgCl\textsubscript{2}, 2 mM DTT, 100 µg/ml CHX, 0.5% v/v Triton X-100, 20 U/ml RNasin and
EDTA free protease inhibitor cocktail (Roche) and an RNA concentration A\textsubscript{260} reading
of 10 OD, loaded on a 30-50% sucrose gradient. Individual fractions (250 µl) were
collected, the protein was precipitated using methanol/chloroform method, and loaded for
Western blots analysis using antibodies to detect indicated endogenous protein. The
antibodies against Actin (sc47778, 1:20000) was from Santa Cruz and against mitochondrial
proteins, Mt-CO\textsubscript{2} (A11154, 1:1000), Mt-ND\textsubscript{2} (A6180, 1:1000), Mt-ND\textsubscript{3} (A9940, 1:1000),
Mt-ND\textsubscript{6} (A17991, 1:1000) were from ABclonal.

**Statistical analysis:**
Data were expressed as mean ± SEM as indicated. Experiments were performed in
biological triplicates. Statistical analysis was performed with a Student’s \( t \)-test or one-way
ANOVA followed by Tukey’s multiple comparison test.

**Author contributions:**
S.S made the initial observations and conceptualized the project. N.S contributed to the
Western blotting. S.S analyzed the data and wrote the paper with input from N.S.

**Competing interests:** Authors declare no competing interests.

**Data and materials availability:** All the data is available in the main text or the
supplementary materials.
**Figure Legends**

**Figure. 1.** A schematic diagram showing the experimental design for performing the ribosome profiling (Ribo-Seq) and mRNA sequencing (RNA-Seq) in indicated mouse striatal cells.

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**Figure 7.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for Tomm5 (A), Timm23 (B), and Pck2 (C) extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), ****P < 0.0001, **P < 0.01, *P < 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 8.** Representative individual raw reads for RPF and mRNA from the triple experiments for the indicated mRNA transcripts extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P < 0.01, *P < 0.05, one-way ANOVA followed by Tukey’s multiple comparison test.

**Figure 9.** Model for mHTT-mediated ribosome stalling of mitochondrially coded OXPHOS genes. We reported widespread ribosome stalling in HD cells, and our model predicts that HTT inhibits ribosome movement. The mutant mHTT gains this function and further impedes the ribosome movements, ultimately inhibiting protein synthesis. Here, we report an intriguing dichotomy in OXPHOS transcript ribosome loading in HD. Almost all the mitochondrially encoded OXPHOS transcripts, but not the nuclear-encoded OXPHOS transcripts, show a higher ribosome occupancy in HD cells than in control cells.
We predict that this unique dichotomy and the associated signaling mechanisms triggered by HTT and/or mHTT to induce mt OXPHOS transcript ribosome loading may emanate from the cytoplasm or from the inner or outer mitochondrial membrane. The underlying mechanistic process that results in this dichotomy of ribosome occupancy in HD remains to be determined in the future work.

**Supplementary Figure 1.** Representative graphs showing overlay of mitochondrially-encoded transcripts Ribo-Seq (RPF)/mRNA-Seq and their corresponding average individual raw reads for RPF and mRNA and the ratio of RPF/mRNA (Ribosome occupancy) from the triple experiments for mt-Co2 (A), mt-Co3 (B), and mt-Atp6 (C) extracted from UCSC Genome Browser.

**Supplementary Figure 2.** A. β-actin raw reads for RPF and mRNA from the triple experiments extracted from UCSC Genome Browser. Error bar represents mean ± SEM (n = 3 independent experiments), **P< 0.01, one-way ANOVA followed by Tukey's multiple comparison test. B. Representative Western blot for the indicated proteins from control, HD-het and HD-homo cells.

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striatal cells (control) → striatal cells (HD-het) → striatal cells (HD-homo) → Cycloheximide → Total cytoplasmic lysate → mitochondrial coding RNAs (RNA-seq)

5' 3' 5' 3' 5' 3'
→ RNAase treatment → Sucrose gradient → Collection of monosome

Gel extraction of ribosome protected mRNA fragments (RPFs) (20-40nt) → Deep sequencing → Mitochondrial ribosome footprinting (Ribo-Seq)

Fig. S8
### Figure 2

A. Mitochondrially Encoded NADH-Ubiquinone Oxidoreductase Core Subunit 1 (Mt-Nd1)

B. Mitochondrially Encoded NADH-Ubiquinone Oxidoreductase Core Subunit 2 (Mt-Nd2)

C. Mitochondrially Encoded NADH-Ubiquinone Oxidoreductase Core Subunit 3 (mt-Nd3)

D. Mitochondrially Encoded NADH-Ubiquinone Oxidoreductase Core Subunit 4 (mt-Nd4)
Figure 3

A Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 4L (mt-Nd4L)

B Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 5 (mt-Nd5)

C Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 6 (mt-Nd6)
Figure 4

A Mitochondrially Encoded Cytochrome B (mt-CyB)

B Mitochondrially Encoded Cytochrome C Oxidase I (mt-Co1)

C Mitochondrially Encoded ATP Synthase 8 (mt-ATP8)
Figure 5

A. NADH:Ubiquinone Oxidoreductase Core Subunit V1 (Ndufv1)

B. Cytochrome c oxidase subunit 5A (Cox5a)

C. Ubiquinol-Cytochrome C Reductase Binding Protein (Uqcrb)
Figure 6

A. Mitochondrial cytochrome c1 protein (CytC1)

B. ATP Synthase Subunit O, Mitochondrial (ATP5o)

C. Succinate Dehydrogenase Complex Flavoprotein Subunit A (Sdha)
Figure 8

A. Mitochondrial ribosomal protein L22 (Mrps22)

B. Mitochondrial Import Inner Membrane Translocase Subunit (Tim14)

C. Mitochondrial ribosomal protein L3 (MrpL3)

D. Tu Translation Elongation Factor, Mitochondrial (Tufm)

E. Arginyl-tRNA Synthetase 2, Mitochondrial (Rars2)

F. OPA1 Mitochondrial Dynamin Like GTPase (Opa1)

G. Mitochondrial fission 1 protein (Fis1)

H. Voltage-dependent anion channel 1 (Vdac1)

** Figure 8: Expression levels of various mitochondrial proteins under different conditions. The graphs show raw read counts and RPF/RNA ratios for each condition. **
Widespread ribosome stalling in the cytoplasm of HD cells

Selected examples of unaltered ribosome occupancy of nuclear-encoded OXPHOS transcripts in HD cells

Signals for ribosome stalling of mt-OXPHOS transcripts?

Selected examples of unaltered ribosome occupancy of nuclear-encoded OXPHOS transcripts in HD cells

Ndufs1
Ndufaf1
Ndufv1
Uqcrb
Cyt1
ATP5o
ATP5f1
ATP5k
Sdhb
Sdhc
Supplementary Figure 1

A  Mitochondrially Encoded Cytochrome C Oxidase II (mt-Co2)

B  Mitochondrially Encoded Cytochrome C Oxidase III (mt-Co3)

C  Mitochondrially Encoded ATP Synthase Membrane Subunit 6 (mt-Atp6)
**Supplementary Fig 2**

**A**

![Graph](image)

**B**

![Blot](image)