Decreased Mitochondrial Membrane Potential Activates the Mitochondrial Unfolded Protein Response

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Abstract

Mitochondria are ATP-producing organelles that also signal throughout the cell. Mitochondrial protein homeostasis is regulated through membrane potential-dependent protein import and quality control signaling. The mitochondrial unfolded protein response (UPR<sub>mt</sub>) is a specific program that responds to imbalances in nuclear and mitochondrial gene expression. Mounting evidence suggests that the electrochemical gradient that powers mitochondrial function, the mitochondrial membrane potential (Δψ<sub>m</sub>), is a core regulator of the UPR<sub>mt</sub>. Here we tested this notion directly by pharmacologically dissipating Δψ<sub>m</sub> and monitoring UPR<sub>mt</sub> activation. We found that chemical dissipation of Δψ<sub>m</sub> using FCCP indeed activated UPR<sub>mt</sub> dose-dependently in <i>C. elegans</i> assayed by the HSP-60::GFP reporter strain.

Figure 1. FCCP dose-dependently activates the mitochondrial unfolded protein response (UPR<sub>mt</sub>): A) Schematic depicting transcriptional control of the UPR<sub>mt</sub>. The UPR<sub>mt</sub> transcription factor (ATFS-1 in <i>C. elegans</i>) is imported to mitochondria according to the mitochondrial membrane potential (Δψ<sub>m</sub>) and degraded. Δψ<sub>m</sub> is denoted by “+” and “–” across the mitochondrial inner membrane. When the transcription factor enters the nucleus target genes such as hsp-60 are transcribed to respond to mitochondrial stress. FCCP is a protonophore that shuttles protons across the mitochondrial inner membrane resulting in decreased Δψ<sub>m</sub>. B) Adult <i>C. elegans</i> are shown after treatment with TMRE, a potentiometric Δψ<sub>m</sub> indicator. Quantification of fluorescence is shown below the representative images. From left to right, each bar represents the mean fluorescence from N = 17, 17, 8, 15, 16, 21, 11 animals. C) Adult <i>C. elegans</i> are shown expressing HSP-60::GFP. Control is compared to paraquat, a known UPR<sub>mt</sub> activator, and to FCCP treatment. Quantification is shown below the representative images. From left to right, each bar represents the mean fluorescence from N = 44, 42, 18, 42, 22, 28, 6, 33 animals. Raw fluorescence values are shown normalized to no treatment control in all cases. Scale bar is 250 μm in all images. Experimental N values are individual animals. Data were obtained across at least 3 different days. *p < 0.05 compared to control by one-way ANOVA with Bonferroni’s test in all cases. Data are means ± SEM.

Description
Mitochondria are organelles that make ATP by generating a proton gradient across the inner membrane through the action of the electron transport chain (ETC). The gradient is composed of a membrane potential ($\Delta \psi_m$) and a concentration gradient ($\Delta p$). In addition to energy production, mitochondria are recognized as signaling organelles (Chandel 2015), and $\Delta \psi_m$ powers diverse signaling outputs that mitochondria coordinate. One such signaling response is the mitochondrial unfolded protein response (UPR$^m$). Some ETC complex components are encoded by mitochondrial or nuclear genes and require coordination between the genomes for proper stoichiometry (Shpilka and Haynes 2018). The UPR$^m$ is a protein homeostasis response that is activated when nuclear and mitochondrial gene expression are not correctly coordinated (Melber and Haynes 2018). When the expression of mitochondrial and nuclear encoded proteins is mismatched, the UPR$^m$ is activated. In C. elegans, the UPR$^m$ is controlled by the transcription factor ATFS-1, which is normally trafficked to mitochondria and degraded. When nuclear:mitochondrial gene expression is perturbed, ATFS-1 is blocked from entering mitochondria and is instead trafficked to the nucleus where a host of chaperones and stress-response genes are activated and transcribed.

Recently, it was suggested that ATFS-1 entry to mitochondria for degradation depended on sufficient $\Delta \psi_m$ (Rolland et al. 2019). In vivo evidence supported this assertion, and implied that $\Delta \psi_m$ is a main regulator of mitochondrial protein homeostasis through its role in driving ATFS-1 import to mitochondria (Poveda-Huertes et al. 2021; Shpilka et al. 2021). Based on this premise, we sought to directly test if targeting $\Delta \psi_m$ could alter UPR$^m$ signaling. We predicted that decreased $\Delta \psi_m$ would result in activation of UPR$^m$ due to decreased driving force for ATFS-1 to enter mitochondria and be degraded. This would lead to ATFS-1 localization to the nucleus and activation of the UPR$^m$ target gene hsp-60 (Figure 1A). We used the protonophore FCCP at various doses to decrease $\Delta \psi_m$ in live C. elegans. $\Delta \psi_m$ was monitored in live, adult worms using the potentiometric fluorescent indicator, TMRE. At all doses tested $\Delta \psi_m$ was decreased compared to untreated control (Figure 1B). Using a similar dose range, we found that one intermediate dose was sufficient to activate UPR$^m$ signaling, assayed through the characterized transcriptional hsp-60 reporter strain (Melber and Haynes 2018; Rolland et al. 2019) (Figure 1C). Paraquat, a known UPR$^m$ positive control (Kim and Sieburth 2018), was used to confirm our results with FCCP. This study was repeated on at least 3 independent days.

The UPR$^m$ is linked to many stress-resistance signaling responses, including hypoxia resistance (Peña et al. 2016). We previously showed that FCCP protected against hypoxia (Berry et al. 2020b), and this work suggests that UPR$^m$ may have been involved. Our other work, however, implicated energy-sensing signaling downstream of decreased $\Delta \psi_m$ and hypoxia resistance (Berry et al. 2020a). The requirement of UPR$^m$ for hypoxia resistance downstream of decreased $\Delta \psi_m$ remains untested. Further, the UPR$^m$ has been implicated in many disease models that are not fully characterized (Jovaisaite et al. 2014). It is likely that many changes occur with decreased $\Delta \psi_m$, and it will be necessary to characterize changes in $\Delta \psi_m$ and other mitochondrial functions in a wide range of contexts to uncover molecular mechanisms of mitochondrial signaling. For example, it is unclear why only a certain dose (10 nM) of FCCP activated the UPR$^m$ here when all doses resulted in mitochondrial depolarization. This result suggests that other cellular mechanisms likely control UPR$^m$ activation in addition to $\Delta \psi_m$. In addition, the level of mitochondrial depolarization may differentially result in UPR$^m$ or mitochondrial autophagy, a process that is also responsive to decreased $\Delta \psi_m$. The mechanism that controls which cellular response is elicited likely depends on the degree of mitochondrial stress. This work is complementary to the established role of mitochondrial ETC stress and UPR$^m$. Inhibition of the ETC results in activation of UPR$^m$, but only if initiated before the L3/L4 stage in C. elegans (Durieux et al. 2011). Our results suggest that the mechanism of ETC mediated UPR$^m$ activation may be somewhat distinct from the $\Delta \psi_m$ control over UPR$^m$, because decreased $\Delta \psi_m$ was able to induce UPR$^m$ after the L3/L4 stage.

Methods

Request a detailed protocol

Fluorescence imaging. C. elegans were fed OP50 bacteria on nematode growth media (NGM) plates. L4 animals were synchronized by egg lay and were stained by exposure to 100 nM TMRE for 24 hours that was applied to plates (Cho et al. 2017; Berry et al. 2020B). Fluorescence was photographed in day 1 adult animals on 2% agarose pads with tetramisole (0.1% w/v) for anesthetic. Red TMRE fluorescence was captured using TexasRed filter sets, and green GFP was captured using GFP filter sets on an epifluorescence microscope (Olympus MVX) equipped with a Lumenera camera and acquisition software (Infinity Analyze; Lumenera). Fluorescence of individual animals was quantified using ImageJ. ROIs were whole animals, and background was manually subtracted from final measurements in ImageJ. Mean intensity was measured, and after background subtraction, was normalized to control intensity. Paraquat was dissolved in water and applied to plates at a final concentration of 4 mM. FCCP was dissolved in ethanol (< 0.1% final concentration, consistent across all concentrations) and applied to plates at various concentrations (see figure). Animals were exposed to paraquat or FCCP for 24 hours.
Reagents

| Strain | Genotype | Source |
|--------|----------|--------|
| N2     | wildtype | CGC    |
| SJ4058 | zcIs9 [hsp-60::GFP + lin-15(+)] V | CGC |

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References

Berry BJ, Baldzizhar A, Nieves TO, Wojtovich AP. 2020. Neuronal AMPK coordinates mitochondrial energy sensing and hypoxia resistance in C. elegans. FASEB J 34: 16333-16347. PMID: 33058299.

Berry BJ, Trewin AJ, Milliken AS, Baldzizhar A, Amirano AM, Lim Y, Kim M, Wojtovich AP. 2020. Optogenetic control of mitochondrial proton motive force to impact cellular stress resistance. EMBO Rep 21: e49113. PMID: 32043300.

Chandel NS. 2015. Evolution of Mitochondria as Signaling Organelles. Cell Metab 22: 204-6. PMID: 26073494.

Cho I, Song HO, Cho JH. 2017. Mitochondrial Uncoupling Attenuates Age-Dependent Neurodegeneration in C. elegans. Mol Cells 40: 864-870. PMID: 29081084.

Durieux J, Wolff S, Dillin A. 2011. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 144: 79-91. PMID: 21215371.

Jovaisaite V, Mouchiroud L, Auwerx J. 2014. The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. J Exp Biol 217: 137-43. PMID: 24353213.

Kim S, Sieburch D. 2018. Sphingosine Kinase Activates the Mitochondrial Unfolded Protein Response and Is Targeted to Mitochondria by Stress. Cell Rep 24: 2932-2945.e4. PMID: 30208318.

Melber A, Haynes CM. 2018. UPRmt regulation and output: a stress response mediated by mitochondrial-nuclear communication. Cell Res 28: 281-295. PMID: 29424373.

Peña S, Sherman T, Brookes PS, Nehrke K. 2016. The Mitochondrial Unfolded Protein Response Protects against Anoxia in Caenorhabditis elegans. PLoS One 11: e0159989. PMID: 27459203.

Poveda-Huertes D, Taskin AA, Dhausadi I, Myketin L, Marada A, Habernig L, Büttner S, Vögtle FN. 2021. Increased mitochondrial protein import and cardiolipin remodelling upon early mtUPR. PLoS Genet 17: e1009664. PMID: 34214073.

Rolland SG, Schneid S, Schwarz M, Rackles E, Fischer C, Haeussler S, Regmi SG, Yeroslaviz A, Habermann B, Mokranjac D, Lambie E, Conradt B. 2019. Compromised Mitochondrial Protein Import Acts as a Signal for UPRmt. Cell Rep 28: 1659-1669.e5. PMID: 31412237.

Shpilka T, Haynes CM. 2018. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. Nat Rev Mol Cell Biol 19: 109-120. PMID: 29145624.

Shpilka T, Du Y, Yang Q, Melber A, Uma Naresh N, Lavelle J, Kim S, Liu P, Weidberg H, Li R, Yu J, Zhu LJ, Strittmatter L, Haynes CM. 2021. UPRmt scales mitochondrial network expansion with protein synthesis via mitochondrial import in Caenorhabditis elegans. Nat Commun 12: 479. PMID: 33473112.

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