A viable hypomorphic mutation in the mitochondrial ribosome subunit, MRPS-31, exhibits mitochondrial dysfunction in *C. elegans*

Kylie M. Jozwik[1], James P. Held[1], Chloe A. Hecht[1], Maulik R. Patel[1,2,3,4§]

[1]Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, United States

[2]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee, United States

[3]Evolutionary Studies, Vanderbilt University, Nashville, Tennessee, United States

[4]Diabetes Research and Training Center, Vanderbilt University Medical Center, Nashville, Tennessee, United States

[§]To whom correspondence should be addressed: maulik.r.patel@vanderbilt.edu

Abstract

The mitochondrial ribosome (mitoribosome) translates mitochondrial genome encoded proteins essential for cellular energy production. Given this critical role, defects in the mitoribosome can cause mitochondrial stress and manifest as multisystemic diseases. In a screen for unique activators of the mitochondrial unfolded protein response (UPR^mt) in *Caenorhabditis elegans*, we recovered a strain harboring a missense mutation in the gene encoding mitochondrial ribosome protein S31 (MRPS-31)—a component of the mitoribosome small subunit. Herein, we confirm causality of the *mrps-31* allele and characterize its induction of UPR^mt and impact on organismal development, providing a valuable model for further study of the mitoribosome.
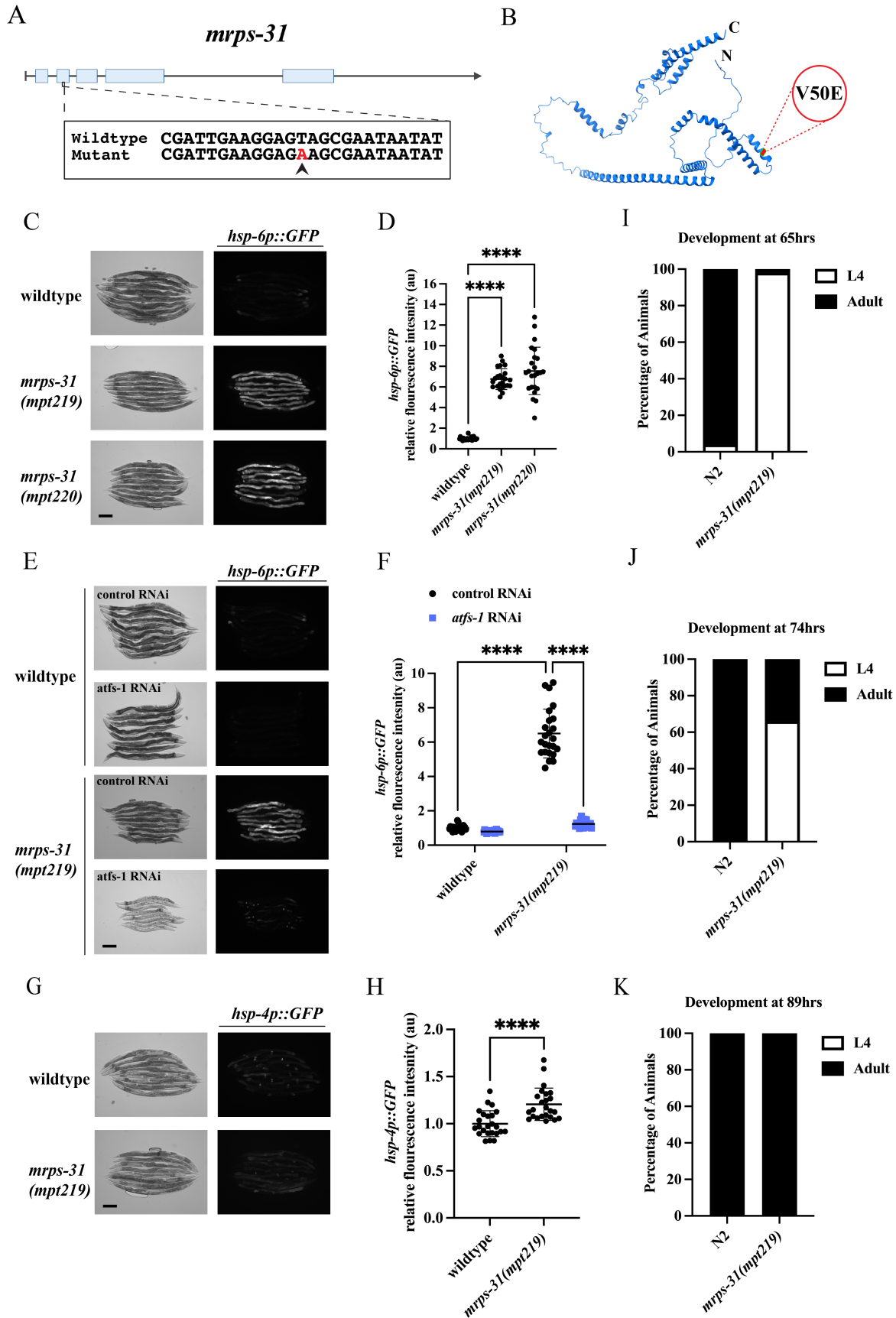
Figure 1.

(A) Schematic of the *C. elegans mrps-31* gene structure, highlighting the T>A mutation responsible for UPR^mt reporter activation in strains harboring the *mpt219* and *mpt220* alleles. (B) AlphaFold predicted structure of the *C. elegans* MRPS-31 from UniProt (UniProt Consortium, 2023), with the V50E mutation site marked in red. (C) Corresponding brightfield and fluorescence images of UPR^mt reporter (*hsp-6p::GFP*) activation in day 2 adult (D2A) wildtype, *mrps-31*(*mpt219*), and *mrps-31*(*mpt220*) animals. Scale bar 200 μm. (D) Fluorescence intensity quantification of *hsp-6p::GFP* reporter activation in individual D2A wildtype, *mrps-31*(*mpt219*), and *mrps-31*(*mpt220*) animals normalized to *hsp-6p::GFP* in a wildtype background (n=24 for each condition, au=arbitrary units, mean and SD shown, ordinary one-way ANOVA with Tukey's multiple comparison test). (E) Corresponding brightfield and fluorescence images of UPR^mt reporter (*hsp-6p::GFP*) activation in D2A wildtype and *mrps-31*(*mpt219*) animals on control and *atfs-1* RNAi. Scale bar 200 μm. (F) Fluorescence intensity quantification of *hsp-6p::GFP* in individual D2A wildtype and *mrps-31*(*mpt219*) animals on control and *atfs-1* RNAi normalized to *hsp-6p::GFP* in a wildtype background on control RNAi (n=24 for each condition, au=arbitrary units, mean and SD shown, two-way ANOVA with Tukey's multiple comparisons test). (G) Corresponding brightfield and fluorescence images of UPR^ER reporter (*hsp-4p::GFP*) activation in day 2 adult (D2A) wildtype and *mrps-31*(*mpt219*) animals. Scale bar 200 μm. (H) Fluorescence intensity quantification of *hsp-4p::GFP* reporter activation in individual D2A wildtype and *mrps-31*(*mpt219*) animals normalized to *hsp-4p::GFP* in a wildtype background (n=24 for each condition, au=arbitrary units, mean and SD shown, unpaired t-test). (I-K) Percentage of N2 and *mrps-31(mpt219)* animals that have reached L4/adulthood at 65hrs, 74hrs, and 89hrs respectively (n=50).

Description

Over 1,100 proteins are necessary to make a functional mitochondrion (Rath et al., 2021). Of these, 99% are encoded by the nucleus and thereafter targeted to the mitochondria while the remaining few are encoded by the mitochondrial genome (mtDNA) (Weinhouse, 2017). The 13 proteins encoded by mammalian mtDNA are critical components of the electron transport chain, necessary for creating the electrochemical gradient that results in the production of ATP—the major source of cellular energy. Unlike nuclear-encoded mitochondrial proteins, the translation of mtDNA encoded proteins depends on the mitochondrial ribosome (mitoribosome), a distinct and specialized ribosome unique from its cytosolic counterpart (Greber & Ban, 2016).

Given the role of the mitoribosme in translating mtDNA-encoded proteins, dysregulation of any of the 82 nuclear-encoded mitoribosomal proteins (MRPs) can lead to significant mitochondrial stress (De Silva et al., 2015). In fact, mutations in MRPs can manifest as multisystemic human diseases including sensorineural hearing loss, hypertrophic cardiomyopathy, and neurological deterioration, due to disrupted mitochondrial bioenergetics (Rötig, 2011; De Silva et al., 2015). Therefore, continued study and characterization of MRPs remain important for understanding basic mitochondrial biology and associated human health.

In response to mitochondrial dysregulation, cells activate retrograde signaling pathways to mitigate stress (Ng et al., 2021). One such mechanism is the conserved mitochondrial unfolded protein response (UPR^mt). UPR^mt promotes survival and recovery of the mitochondrial network by activating signaling cascades that result in the upregulation of nuclear-transcribed chaperones and proteases (Shpilka & Haynes, 2018). In *Caenorhabditis elegans*, where UPR^mt is well characterized, UPR^mt activation is dependent on the dual-targeted transcription factor, ATFS-1. In healthy cells, ATFS-1 is predominantly imported into the mitochondria (Nargund et al., 2012). However, under conditions of mitochondrial stress, ATFS-1 traffics to the nucleus where it drives the expression of mitochondrial-protective genes (Nargund et al., 2015). This mechanism has been leveraged in *C. elegans* to develop a transgenic reporter strain in which one of the targets of ATFS-1, the *hsp-6* promoter, drives the expression of GFP (Yoneda et al., 2004). Therefore, GFP expression in this strain is a proxy for UPR^mt activation.

Previously, we utilized the *hsp-6p::GFP* reporter to conduct a forward genetic screen for mutations that activate the mitochondrial unfolded protein response (UPR^mt) specifically in the intestine of *C. elegans* (Held et al., 2024). Six independent mutants were recovered, one of which (MRP650), harbored a putative causal missense mutation in mitochondrial ribosome protein S31 (*mrps-31*), homolog of the human *MRPS31*, which encodes a component of the small subunit of the mitoribosome. However, as is typical with random mutagenesis, there are other mutations linked to the *mrps-31* variant. To determine if the MRPS-31 V50E mutation found in MRP650 was causal in activating UPR^mt (Figure 1A, 1B), we introduced it into a N2 wildtype genetic background using CRISPR/Cas9 and assessed *hsp-6p::GFP* reporter activation in live animals. We recovered and sequence verified two independent, MRPS-31 V50E CRISPR hits (alleles *mpt219* and *mpt220*, denoted *mrps-31*(*mpt219*) and *mrps-31*(*mpt220*)), and fluorescence microscopy revealed similarly robust UPR^mt reporter activation in adult animals of both alleles (Figure 1C, 1D). Thus, we further characterized one of these alleles: *mrps-31*(*mpt219*). Knockdown of ATFS-1, the central transcription factor of UPR^mt, completely abrogates UPR^mt activation in *mrps-31*(*mpt219*)

animals (Figure 1E, 1F). In addition, mrps-31(mpt219) animals on atfs-1 RNAi are smaller and display developmental defects. These data suggest that MRPS-31 V50E is the causal mutation in strain MRP650 recovered from the screen and that it activates canonical ATFS-1-dependent UPR^mt.

To determine whether the stress induced by mrps-31(mpt219) is specific to the mitochondria, we also assessed activation of the endoplasmic reticulum unfolded protein response (UPR^ER) reporter, *hsp-4p::GFP*—a proxy for ER stress and compromised cellular proteostasis (Calfon et al., 2002). We find that *hsp-4p::GFP* reporter is only very slightly induced in mrps-31(mpt219) animals, much less than what is observed by known activators of UPR^ER (Figure 1G, 1H) (Calfon et al., 2002; Hou et al., 2014). These data suggest that stress induced by mrps-31(mpt219) is predominantly specific to the mitochondria.

Mitochondrial stress often correlates with delayed organismal development (Feng et al., 2001; Yang & Hekimi, 2010). Thus, we assessed development of mrps-31(mpt219) animals. The N2 wildtype *C. elegans* strain develop from a fertilized egg into a reproductively mature, egg laying hermaphrodite adults in approximately 65hrs at 20°C (Byerly et al., 1976). Therefore, developmental stage was assessed at 65hrs at which time 96% of N2 animals were reproductive adults while only 2% of mrps-31(mpt219) animals had reached adulthood (Figure 1I). Developmental stage was also assessed at 74hrs where 34% of mrps-31(mpt219) animals reached reproductive maturity and at 89hrs, where all animals had reached reproductive adulthood (Figure 1J, 1K).

It is known that compromising mitoribosome function leads to mitochondrial stress and dysfunction (Rolland et al., 2019; Lopez Sanchez et al., 2021). However, mitoribosome defects can be challenging to study because these genes are essential thus rendering homozygous mutations non-viable. For example, the National BioResource Project isolated a *C. elegans* strain with a 450bp deletion in exon 1 of mrps-31 (allele: tm1314) but these animals have a sterile/lethal phenotype (Mitani, 2009). Herein, we have identified and characterized a hypomorphic mrps-31 allele in *C. elegans*, which induces mitochondrial stress yet is reproductively viable and able to be maintained as a homozygous population. UPR^mt reporter activation and slowed development phenotype of mrps-31(mpt219) animals is consistent with a loss-of-function mutation. Interestingly, mrps-31(mpt219) exhibits robust UPR^mt reporter activation predominantly in the intestine of *C. elegans*, which is unique compared to other systemic mutations that impair electron transport chain function which activate UPR^mt in all somatic tissues (Baker et al., 2012; Held et al., 2024). This tissue specific activation suggests that MRPS-31 may not be required equally across all tissues or that mrps-31(mpt219) selectively impairs function in the intestine. Our recovery of a mutation in a mitoribosome subunit that exhibits features of mitochondrial stress yet is reproductively viable provides a valuable system for studying mitoribosome biology and has potential to serve as a model for disorders associated with mitoribosome dysfunction.

Methods

Worm Maintenance- Animals were grown on nematode growth media (NGM) plates seeded with OP50 *E.coli* obtained from the Caenorhabditis Genetics Center. All strains were maintained at 20°C.

CRISPR/Cas9- CRISPR was conducted using Alt-R S.p. Cas9 Nuclease V3 (IDT#1081058) and tracrRNA (IDT#1072532) as previously described (Dokshin et al., 2018). Instead of using rol-6 plasmid, we used dpy-10 endogenous editing as a co-injection marker as previously described (Paix et al., 2015). Once the desired edit was recovered, the dpy-10 injection marker was outcrossed using a wildtype background (N2).

Genetic Crosses- Strains resulting from genetic crosses were generated by crossing 15-20 heterozygous males of a given strain to 5-8 larval stage 4 (L4) hermaphrodites of another strain (heterozygous males were first generated by crossing wildtype N2 males to L4 hermaphrodites of a strain). F1 generation L4s were cloned out from the cross plates. Once F2 progeny were laid, the F1 adult was genotyped and screened for the alleles of interest. F2 progeny were cloned out from F1 plates harboring the allele(s) of interest and once F3 progeny were laid, the F2 animals were genotyped and screened again for the alleles of interest.

RNAi- RNAi by feeding was conducted as previously described (Held et al., 2022). Briefly, atfs-1 and empty vector RNAi clones were grown up overnight at 37°C shaking from a single colony in 2mL liquid culture of LB supplemented with 50μg/ml ampicillin. To make 16 RNAi plates, 50mL of LB supplemented with 50μg/ml ampicillin was inoculated with 500μL of overnight culture and then incubated shaking overnight at 37°C. Following overnight incubation, cultures were induced by adding an additional 50mL of LB supplemented with 50μg/ml ampicillin in addition to 8mM IPTG and then incubated shaking at 37°C for 3.5-4hrs (to an $OD_{550-600}$ of approximately .8). After incubation, the $OD_{550-600}$ was taken and the cultures were pelleted by centrifugation at 3900 rpm for 6min. Supernatant was removed and a mixture of 4mL of M9 supplemented with 8mM IPTG was made. Pellets were resuspended in the M9+IPTG mixture such that the final OD was standardized to 0.8.

250μL of suspension was seeded onto standard 60mm NGM plates containing 1mM of IPTG. Plates were left to dry overnight and then used within 1 week. Bacterial RNAi feeder strain was from the Ahringer RNAi Feeding Library, grown from a single colony and identity confirmed by Sanger sequencing.

Fluorescence Microscopy- All animal imaging was performed using a Zeiss Axio Zoom V16 stereo zoom microscope. Worms were immobilized on 2% agarose pads on microscope slides in ~1μL of 100mM levamisole (ThermoFisher #AC187870100) and then a coverslip was applied.

Image Quantification- All fluorescent microscopy images were analyzed with FIJI. First, a region of interest was drawn around the body wall of each worm using the brightfield image. Subsequently, the corresponding fluorescent image was opened in the program and average fluorescent intensity of each individual biological replicate was obtained using the measure function (which calculates the average pixel intensity by dividing the sum total fluorescent intensity by the total number of pixels within the bounds of the trace) for each region of interest.

Development Assay- 25 gravid adult animals were plated on OP50 seeded 60mm NGM plates and allowed to lay embryos for 90min at room temperature. Following egg laying, 50 embryos per genotype were moved to a clean OP50 seeded NGM plate and incubated at 20°C for 65 hrs. After incubation, development of wildtype and mutant animals were assessed. At this time, all animals were L4 stage (characterized by the developing vulva) or later. Adulthood was determined by the presence of a line of embryos flanking the vulva or the presence of vulval eversion (the final stage of vulva morphogenesis).

Reagents

Strain	Genotype	Available from
N2	*wildtype*	CGC
MRP650	*mpt140*; *zcIs13* V	Held et al., 2024
GL347	*zcIs13*[*hsp-6p*::GFP] V	CGC
SJ4005	*zcIs4*[*hsp-4p*::GFP] V	CGC
MRP1054	*mrps-31*(*mpt219*[V50E]) III	This study
MRP1055	*mrps-31*(*mpt219*[V50E]) III; *zcIs13* V	This study
MRP1057	*mrps-31*(*mpt220*[V50E]) III; *zcIs13* V	This study
MRP1056	*mrps-31*(*mpt219*[V50E]) III; *zcIs4* V	This study

References

Baker BM, Nargund AM, Sun T, Haynes CM. 2012. Protective coupling of mitochondrial function and protein synthesis via the eIF2α kinase GCN-2. PLoS Genet 8(6): e1002760. PubMed ID: 22719267

9/30/2024 - *Open Access*

Byerly L, Cassada RC, Russell RL. 1976. The life cycle of the nematode Caenorhabditis elegans. I. Wild-type growth and reproduction. Dev Biol 51(1): 23-33. PubMed ID: 988845

Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. 2002. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature 415(6867): 92-6. PubMed ID: 11780124

Davis P, Zarowiecki M, Arnaboldi V, Becerra A, Cain S, Chan J, et al., Sternberg PW. 2022. WormBase in 2022-data, processes, and tools for analyzing Caenorhabditis elegans. Genetics 220(4). PubMed ID: 35134929

De Silva D, Tu YT, Amunts A, Fontanesi F, Barrientos A. 2015. Mitochondrial ribosome assembly in health and disease. Cell Cycle 14(14): 2226-50. PubMed ID: 26030272

Dokshin GA, Ghanta KS, Piscopo KM, Mello CC. 2018. Robust Genome Editing with Short Single-Stranded and Long, Partially Single-Stranded DNA Donors in Caenorhabditis elegans. Genetics 210(3): 781-787. PubMed ID: 30213854

Feng J, Bussière F, Hekimi S. 2001. Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. Dev Cell 1(5): 633-44. PubMed ID: 11709184

Greber BJ, Ban N. 2016. Structure and Function of the Mitochondrial Ribosome. Annu Rev Biochem 85: 103-32. PubMed ID: 27023846

Held JP, Dbouk NH, Strozak AM, Grub LK, Ryou H, Schaffner SH, Patel MR. 2024. Germline status and micronutrient availability regulate a somatic mitochondrial quality control pathway via short-chain fatty acid metabolism. bioRxiv. PubMed ID: 38826313

Held JP, Feng G, Saunders BR, Pereira CV, Burkewitz K, Patel MR. 2022. A tRNA processing enzyme is a key regulator of the mitochondrial unfolded protein response. Elife 11. PubMed ID: 35451962

Hou NS, Gutschmidt A, Choi DY, Pather K, Shi X, Watts JL, Hoppe T, Taubert S. 2014. Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo. Proc Natl Acad Sci U S A 111(22): E2271-80. PubMed ID: 24843123

Lopez Sanchez MIG, Krüger A, Shiriaev DI, Liu Y, Rorbach J. 2021. Human Mitoribosome Biogenesis and Its Emerging Links to Disease. Int J Mol Sci 22(8). PubMed ID: 33917098

Meng EC, Goddard TD, Pettersen EF, Couch GS, Pearson ZJ, Morris JH, Ferrin TE. 2023. UCSF ChimeraX: Tools for structure building and analysis. Protein Sci 32(11): e4792. PubMed ID: 37774136

Mitani S. 2009. Nematode, an experimental animal in the national BioResource project. Exp Anim 58(4): 351-6. PubMed ID: 19654432

Nargund AM, Fiorese CJ, Pellegrino MW, Deng P, Haynes CM. 2015. Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPR(mt). Mol Cell 58(1): 123-33. PubMed ID: 25773600

Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. 2012. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science 337(6094): 587-90. PubMed ID: 22700657

Ng MYW, Wai T, Simonsen A. 2021. Quality control of the mitochondrion. Dev Cell 56(7): 881-905. PubMed ID: 33662258

Paix A, Folkmann A, Rasoloson D, Seydoux G. 2015. High Efficiency, Homology-Directed Genome Editing in Caenorhabditis elegans Using CRISPR-Cas9 Ribonucleoprotein Complexes. Genetics 201(1): 47-54. PubMed ID: 26187122

Rath S, Sharma R, Gupta R, Ast T, Chan C, Durham TJ, et al., Mootha VK. 2021. MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. Nucleic Acids Res 49(D1): D1541-D1547. PubMed ID: 33174596

Rolland SG, Schneid S, Schwarz M, Rackles E, Fischer C, Haeussler S, et al., Conradt B. 2019. Compromised Mitochondrial Protein Import Acts as a Signal for UPR(mt). Cell Rep 28(7): 1659-1669.e5. PubMed ID: 31412237

Rötig A. 2011. Human diseases with impaired mitochondrial protein synthesis. Biochim Biophys Acta 1807(9): 1198-205. PubMed ID: 21708121

Shpilka T, Haynes CM. 2018. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. Nat Rev Mol Cell Biol 19(2): 109-120. PubMed ID: 29165426

UniProt Consortium. 2023. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Res 51(D1): D523-D531. PubMed ID: 36408920

Weinhouse C. 2017. Mitochondrial-epigenetic crosstalk in environmental toxicology. Toxicology 391: 5-17. PubMed ID: 28855114

Yang W, Hekimi S. 2010. Two modes of mitochondrial dysfunction lead independently to lifespan extension in Caenorhabditis elegans. Aging Cell 9(3): 433-47. PubMed ID: 20346072

Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D. 2004. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J Cell Sci 117(Pt 18): 4055-66. PubMed ID: 15280428

Author Contributions: Kylie M. Jozwik: writing - original draft, investigation, methodology, validation, formal analysis, visualization, conceptualization. James P. Held: conceptualization, investigation, methodology, writing - review editing. Chloe A. Hecht: investigation, validation, writing - review editing. Maulik R. Patel: supervision, writing - review editing, funding acquisition, project administration, conceptualization.

Reviewed By: Anonymous

Nomenclature Validated By: Anonymous

WormBase Paper ID: WBPaper00067288

Citation: Jozwik, KM; Held, JP; Hecht, CA; Patel, MR (2024). A viable hypomorphic mutation in the mitochondrial ribosome subunit, MRPS-31, exhibits mitochondrial dysfunction in *C. elegans*. microPublication Biology. 10.17912/micropub.biology.001344