1   **A primer genetic toolkit for exploring mitochondrial biology and disease using**

2   **zebrafish**

3   Ankit Sabharwal[1], Jarryd M. Campbell[1,2], Zachary WareJoncas[1], Mark Wishman[1], Hirotaka

4   Ata[1,2], Wiebin Liu[1.3], Noriko Ichino[1], Jake D. Bergren[1], Mark D. Urban[1], Rhianna Urban[1], Tanya

5   L. Poshusta[1], Yonghe Ding[1,3], Xiaolei Xu[1,3], Karl J. Clark[1,2], Stephen C. Ekker[1,2*]

6   1. Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905,

7   USA

8   2. Center for Clinical and Translational Sciences, Mayo Clinic, Rochester, MN 55905, USA

9   3. Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN 55905, USA

10   *Corresponding author: Ekker.Stephen@mayo.edu

11   **Keywords:**

12   Mitochondria, Mitochondrial disorders, Zebrafish, gene editing,

13   TALEN, Gene Breaking Transposon

Abstract

Mitochondria are a dynamic eukaryotic innovation that play diverse roles in biology and disease. The mitochondrial genome is remarkably conserved in all vertebrates, encoding the same 37 gene set and overall genomic structure ranging from 16,596 base pairs (bp) in the teleost zebrafish (*Danio rerio*) to 16,569 bp in humans. Mitochondrial disorders are amongst the most prevalent inherited diseases affecting roughly 1 in every 5000 individuals. Currently, few effective treatments exist for those with mitochondrial ailments, representing a major unmet patient need. Mitochondrial dysfunction is also implicated to be a common component of a wide variety of other human illnesses ranging from neurodegenerative disorders like Huntington's disease and Parkinson's disease to autoimmune illnesses such as multiple sclerosis and rheumatoid arthritis. The electron transport chain (ETC) component of mitochondria is critical for mitochondrial biology and defects can lead to many mitochondrial disease symptoms. Here we present a publicly available collection of genetic mutants created in highly conserved, nuclear-encoded mitochondrial genes in *Danio rerio*. The zebrafish system represents a potentially powerful new opportunity for the study of mitochondrial biology and disease due to the large number of orthologous genes shared with humans and the many advanced features of this model system from genetics to imaging. This collection includes 24 mutant lines in 18 different genes created by locus-specific gene editing to induce frameshift or splice acceptor mutations leading to predicted protein truncation during translation. Also included are 7 lines created by the random insertion of the gene-breaking transposon (GBT) protein trap cassette. All of these targeted mutant alleles truncate conserved domains of genes critical to the proper function of the ETC or genes that have been implicated in human mitochondrial disease. This collection is designed to accelerate the use of zebrafish to study of many different aspects of

37   mitochondrial function with the goal of widening our understanding of their role in biology and

38   human disease.

Introduction

Mitochondria are semi-autonomous organelles critical for eukaryotic cell function. The mitochondrial endosymbiotic genesis origin hypothesis proposes its evolution from an alpha proteobacterial ancestor, *Rickettsia prowazekii* [1,2] that were harnessed by a eukaryotic cell as the host billions of years ago [3]. The proteobacterium became a symbiote of the host cell, bringing with it a system for more efficient generation of cellular energy in the form of ATP.

During the course of evolution, the genetic material of the mitochondria underwent reductive expansion and was transferred in a retrograde fashion to the nuclear genome. The retrograde movement of genes from the mitochondria to its eukaryotic host paved the way for the mitochondria to specialize as energy production organelles rather than consuming energy repetitively replicating its own multi-copy genome. The mitochondrial-encoded genetic material at present is a vestige of the original proteobacterial genome [4–6] meaning despite having DNA of their own, mitochondria rely heavily on the nuclear genes for most of their functions.

Mitochondria have critical functions in metabolism, organ homeostasis, apoptosis and aging. They also play important but still largely mysterious roles in human pathology, as demonstrated by the enormous biological variation and diverse disorders in patients with mitochondrial disease [7–10]. Nearly every organ system can be compromised, but with highly variable and complex physiological and biochemical outcomes. Imaging and basic science of mitochondria showcase how this highly dynamic organelle responds differentially to extrinsic and intrinsic biological signals. However, understanding how mitochondria function in normal biology, and how human mitochondrial DNA variations contribute to health and disease, has been hampered by a lack of effective approaches to manipulate the powerhouse of the cell.

61 The vertebrate mitochondrial chromosome is circular and includes 37 genes, 13 encoding for

62 protein subunits of the electron transport chain, 22 coding for transfer RNAs, and 2 encoding

63 ribosomal RNAs **(Figure 1)** [11,12]. The mitochondrial gene order, strand specific nucleotide

64 bias and codon usage is highly conserved [13]. However, mtDNA encoded genes lack introns

65 and utilize a divergent genetic code than their nuclear counterparts [14,15]. For instance, AUA

66 codon codes for methionine as per mitochondrial genetic code, whereas the same sequence codes

67 for isoleucine in the nuclear genetic code. Similarly, nuclear stop codon UGA is read as the

68 tryptophan amino acid by the mitochondrial codon cypher.

69 Mitochondria are unique cellular compartments with different DNA and RNA repair and editing

70 rules, hampering attempts at directly manipulating these nucleic acid components. For example,

71 DNA nucleases that introduce double stranded breaks and subsequent repair in nuclear DNA

72 induce the degradation of mtDNA [16–18]. Indeed, none of the canonical DNA repair pathways

73 found in the nucleus have been shown to be active in mitochondria [18–20]. Finally, no system

74 has demonstrated the ability to deliver exogenous DNA or RNA to mitochondria, restricting the

75 tools available for mtDNA editing [21,22]. All of these factors are distinct from the nuclear

76 genome, making mtDNA a far less accessible genome for traditional gene editing methods and

77 reagents.

78 The diverse functions that mitochondria are capable of, including oxidative phosphorylation,

79 would not be possible with the small subset of 13 proteins encoded in mtDNA. Discoveries led

80 by high throughput proteomic approaches have enhanced our knowledge of the mitochondrial

81 proteome [23–27]. There are approximately 1158 nuclear-encoded proteins that localize to the

82 mammalian mitochondria, exerting a dual genetic control via its nuclear counterpart. Nuclear-

83 encoded mitochondrial proteins on the basis of their mitochondrial function can be broadly

84   classified into different categories including oxidative phosphorylation, energy production,

85   membrane dynamics, genome maintenance, and ion/metabolite homeostasis.

86   Unlike the nuclear genome, where most cells have only two copies, each cell can harbor

87   thousands of copies of mtDNA, depending on environmental needs, making mitochondrial

88   genome engineering a population genetics challenge. When every mtDNA molecule is identical

89   with non-mutagenic variants, the cell is in a genetic state, called homoplasmy, and the

90   mitochondria functions normally. In people with mtDNA-based mitochondrial disease, mtDNA

91   genomes harboring pathogenic mutations typically co-exist with the healthy genomes in a state

92   called heteroplasmy. Homoplasmy may also arise when the cell harbors only mutant mtDNA

93   molecules, resulting in severe clinical manifestations. [28]. The ratio of pathogenic-harboring to

94   nonpathogenic-harboring mtDNA is critical to mitochondrial disease onset. A threshold exists

95   for most mitochondrial disease, at which, the healthy mtDNA genomes can no longer

96   compensate for the pathogenic genomes, resulting in disease onset leading to breakdown of

97   oxidative phosphorylation and other mitochondrial function(s) [29].

98   Mitochondrial disorders are a heterogeneous group of clinical manifestations resulting from

99   either inherited or spontaneous mutations in mtDNA or nDNA leading to altered structure or

100 function of the proteins or RNA that reside in mitochondria [6,10,30,31]. The pathophysiology

101 of mitochondrial disorders involves functions of both the nuclear and mitochondrial genomes,

102 conferring additional complexity to the manifestation of the syndromes. The prevalence of

103 mitochondrial disorders is about 1 in 5000 live births; however, the prevalence is often

104 influenced by the presence of founder mutations and consanguinity in populations [31–33].

105 These conditions range in severity dependent on the specific mutation and its prevalence and

106 localization within the patient. In addition to diseases specifically progenerated by mitochondrial

107 defects, mitochondrial dysfunction has also been implicated as a cofactor or by-product of many

108 other conditions; including cancer, immune diseases, developmental delays and

109 neurodegenerative condition such as Alzheimer's [34–38].

110 Mitochondrial dysfunction tends to affect primarily high energy systems and can therefore have

111 devastating effects on a wide range of body systems including brain function, liver function,

112 vision, hearing, immune function, and all muscle types [31,39]. The identification and

113 characterization of novel mitochondrial genes in human genetic disorders have also enhanced our

114 knowledge of mitochondrial function [40–44]. However, despite their known prevalence and

115 severity, mitochondrial diseases remain understudied as compared to other genetic conditions,

116 and the options for treatment are limited.

117 The conserved roles of mitochondria have been traditionally uncovered using accessible model

118 systems such as the yeast *S.cerevisiae* [45–48] and invertebrates including *C.elegans* [49–52]

119 and *D. melanogaster* [53–56]. Vertebrate mitochondria, however, are known to have additional

120 innovations and encode unique subunits not present in invertebrate models [5,45,57–67]. Most

121 exploration to date of vertebrate mitochondrial biology has been conducted either in cell culture

122 or in mouse models. However, cells in a dish are missing important environmental cellular and

123 organismal context, whereas mouse mitochondrial experimental work can be hindered by limited

124 population size and invasive imaging techniques.

125 We provide here an initial genetic toolkit for modeling mitochondrial biology and disease in

126 *Danio rerio* (zebrafish) in an effort to help catalyze the use of this invaluable model organism in

127 mitochondrial research. The zebrafish is a tropical freshwater teleost that has proven to be an

128 invaluable resource for the study of human disease and genetics [68–70]. Principal among the

7

129   many research amenable characteristics of zebrafish are their high genetic orthology to humans,

130   high fecundity, their optical transparency (in the embryonic stage) that lends itself to facile

131   imaging, and the ease of reagent delivery through microinjection into the single celled embryo,

132   which is a full millimeter across before the first mitotic division. Zebrafish also serve as a

133   potentially powerful vertebrate model organism to study human mitochondrial disorders because

134   of conserved mitochondrial genome and mitochondrial genetic machinery. Zebrafish and human

135   mitochondrial chromosomes display ~65% sequence identity at the nucleotide level, and share

136   the same codon usage, strand specific nucleotide bias and gene order [12] **(Figure 1)**.

137   Over the recent years, zebrafish research has helped shed light on mitochondrial biology and

138   further developed our understanding of the mechanisms of mitochondrial-associated pathology

139   [71–76]. Zebrafish have also been successfully used as a model system to study mitochondrial

140   targeting drugs with implications in development and cardiovascular function [71,77,78]. These

141   drugs can simply be dissolved in the water housing the larvae and offer an advantage of large

142   sample size with minimal volumes of drug administered. Drug-screening studies have aided in

143   understanding the pathogenesis of various diseases and helped to identify targets for treatment

144   [77].

145   To help engender an expansion of zebrafish deployment in mitochondrial research, we present a

146   panel or "starter-pack" toolkit of genetic mutants made in nuclear-encoded mitochondrial genes

147   in zebrafish. This mutant panel, which we have named the Marriot Mitochondrial Collection

148   (MMC), consists of 30 zebrafish lines with mutations in 24 different mitochondrial genes. The

149   mutant collection focusses primarily on genes that encode for components of the energy

150   generating electron transport chain with at least one mutant in each complex of the ETC. Other

151   mutants consist of assembly factors, protein chaperones that manage mitochondrial membrane

152   traffic, and genes related to mitochondrial replication. These mutants were made either by the

153   use of targeted endonuclease [79], or curated from our research group's library of randomly

154   generated insertional mutants [80]. We hope that this collection, in addition to being intrinsically

155   useful, will also help serve as a primer to the modeling of mitochondrial biology and disease in

156   zebrafish.

Methods

Zebrafish Handling

159   All animal work was conducted under Mayo Clinic's institutional animal welfare approvals

160   (IACUC number: A34513-13-R16).

Identification of zebrafish orthologs having putative mitochondrial function

162   A previous study combining discovery and subtractive proteomics with computational,

163   microscopy identified 1098 mouse genes that could encode for proteins residing in mitochondria

164   [24]. They further identified 1013 human orthologs for these genes, providing an initial

165   inventory of the genes coding for proteins resident in mitochondria. Using literature assessment

166   and HUGO database curation approaches, we identified 97 proteins that are involved in the

167   biogenesis and assembly of electron transport chain in mitochondria. Using zebrafish orthologs

168   of human genes from ZFIN (Zebrafish Information Network), 93 zebrafish mitochondrial

169   orthologs were identified. These orthologs were systemically annotated with respect to clinical

170   phenotype by extensive mining from PubMed based published case reports and OMIM database

171   **(Supplementary Table 1)**.

172

173 **Mutant Generation**

174 Mutant lines were created by one of two methods, either through the targeted use of

175 Transcription Activator Like Effector Nucleases (TALENs) [79] or by screening Gene Breaking

176 Transposon (GBT) lines for integrations into mitochondrial genes [80]. Reagents were delivered

177 in both methods by the microinjection of either TALEN pairs or GBT transposon and Tol2

178 transposase into single cell zebrafish embryos.

179 **TALEN Design/Assembly/Delivery**

180 The TALEN mutants in this collection were originally generated as part of a previously

181 published study [79]. In brief, TALEN pairs were designed using the Mojohand software

182 platform [81] (www.talendesign.org) to target highly conserved, and therefore likely functionally

183 important, areas of nuclear-encoded mitochondrial genes. TALEN RVDs were then cloned into

184 pT3Ts-GoldyTALEN (TALEN vector with a T3 transcriptional promoter for in vitro

185 transcription) using the FusX rapid TALEN assembly system [79], which uses the RVD

186 definitions: HD=C, NN=G, NI=A, NG=T. Following assembly, mRNA was synthesized in vitro

187 using the mMessage Machine T3 kit (Ambion) and extracted by a phenol-chloroform extraction

188 as prescribed in the mMessage Machine manual. The extracted mRNA was then delivered into

189 single cell zebrafish embryos at 100pg doses (50pg per TALEN arm) by microinjection.

190 **TALEN Mutant Screening**

191 Following microinjection, genomic DNA was extracted from F0 larvae three days post

192 fertilization (dpf) by sodium hydroxide extraction. DNA for eight individual larvae was analyzed

193 for NHEJ activity at the TALEN target site by Restriction Fragment Length Polymorphism

194 (RFLP) analysis. Groups with high reported NHEJ activity by RFLP were raised to adulthood

195 and outcrossed to create an F1 generation. Suspected NHEJ mutants were further verified by

196 Sanger sequencing. F1 larvae demonstrating NHEJ mutations confirmed by both RFLP and

197 Sanger sequencing were raised to adulthood. Fin biopsies were performed on these adults and

198 DNA was extracted by sodium hydroxide extraction.

199 **Gene Breaking Transposon System**

200 The Gene Breaking Transposon System of protein trap system and a complete repository of

201 protocols for the creation and screening of GBT mutant lines has been described [80]. In short,

202 protein trap transposons were delivered in combination with mRNA for Tol2 transposase (25pg

203 each) into single cell zebrafish embryos by microinjection. Embryos were screened for GFP

204 fluorescence at 3-4dpf and classified broadly into three classes [80]. Class three embryos, those

205 with whole body GFP expression, were raised to adulthood and outcrossed to non-transgenic

206 lines to create an F1 generation. mRFP-expressing F1 embryos were sorted by expression

207 pattern, assigned a GBT number, and raised to adulthood. These adult fish were then outcrossed

208 to non-transgenic lines to create an F2 generation upon which all subsequent propagation, testing

209 and imaging was conducted.

210 To determine genes tagged by the protein trap system, rapid amplification of cDNA ends

211 (RACE) was performed as described [82] with minor updates to primer sequences. cDNA was

212 generated using a transposon-specific primer (5R-mRFP-P0) against 250ng of total mRNA in the

213 reverse transcription reaction. PCR was then performed using the following gene-specific

214 primers: 5R-mRFP-P1 and 5R-mRFP-P2. The resulting products were TA cloned for further

215 amplification and then sequence verified for in-frame mRFP fusions by Sanger sequencing.

216   In some cases, inverse PCR was also conducted to RACE PCR as described [80]. Whole

217   genomic DNA was extracted from individual F2 embryos using a sodium hydroxide extraction

218   and 800ng was digested in a combination reaction using AvrII, NheI, SpeI, and XbaI restriction

219   enzymes. Approximately 200ng of the product of this digestion was self-ligated and used as a

220   template for PCR using the following nested and primary primers. 5' side: 5R-mRFP-P1 and 5R-

221   mRFP-P2 paired with INV-OPT-P1 and INV-OPT-P2, respectively. 3' side: 5R-GFP-P1 and 5R-

222   GFP-P2 with Tol2-ITR(L)-O1 and Tol2-ITR(L)-O3, respectively. The products of the final

223   nested reaction were gel-extracted, cloned, and sequenced. Prospective in frame mRFP fusions

224   were further verified by comparing suspected protein trap allele to GBT expression pattern and

225   by PCR against DNA or cDNA from mRFP carrier siblings versus non-carrier siblings.

226   Protein trap lines as verified by these methods were catalogued for specific genomic fusions

227   using the National Center for Biotechnology Information's (NCBI) Homologene Database.

228   Human orthologs for tagged genes were further identified using blastX searches against the

229   human genome. GBT lines with protein trap fusions to nuclear encoded mitochondrial genes

230   with human orthologues have been included in this collection.

231   ***In silico* analysis to determine protein homology and alteration of DNA sequence by**

232   **TALENs and GBT in zebrafish mutants**

233   Human amino acid sequences were compared to both the zebrafish wild type sequence and each

234   specific zebrafish mutant sequence associated with each allele. Amino acid sequence information

235   for a particular gene was gathered for humans from https://www.uniprot.org/ along with the wild

236   type amino acid sequence for the zebrafish. The first/most common isoform for each entry was

237   used for this analysis. Any added information regarding functional domains or regions was also

238   gathered. A protein-protein BLAST was conducted using https://blast.ncbi.nlm.nih.gov/Blast.cgi

239   to compare the sequences of the human and wild type zebrafish amino acid sequences. BLAST

240   search settings used included the blastp algorithm. Regions of low and high homology were then

241   mapped out for the sequences.

242   For zebrafish mutant to human comparisons, the cDNA or DNA sequences were gathered via

243   sequencing and put through the translate tool at https://web.expasy.org/translate/. The standard

244   genetic code was used for conversion. Stop codons arising from the frameshifted DNA sequence

245   were found in all mutants. The BLAST analysis conducted on the mutants was taken from the

246   starting methionine to the first encountered stop codon. Regions of low and high homology were

247   then mapped for each sequence.

248   **Access to all reported reagents – zebrafish and sequences**

249   All listed tools are immediately available through the Mayo Clinic Zebrafish Facility, and all fish

250   lines will be available via ZIRC. Sequences needed for genotyping and related metadata are

251   currently on zfishbook [83].

252   **RESULTS**

253   **Generation of zebrafish mutant collection**

254   We generated zebrafish mutants for a wide selection of nuclear-encoded mitochondrial genes by

255   employing two genetic engineering approaches, gene breaking transposons and TALENs. The

256   collection of zebrafish mutants is referred as the Marriot Mitochondrial Collection (MMC) and

257   comprises of mutants for 24 nuclear-encoded mitochondrial proteins **(Figure 2)**. Out of these, 18

258   were created by TALEN indel mutagenesis and 6 were created by gene breaking trap

259   mutagenesis. The mutants include proteins from all known functional pathways involved in

260   mitochondrial homeostasis and ATP generation **(Figure 2)**.

13

261 Broadly, the pathways can be classified as subunits of oxidative phosphorylation complexes,

262 chaperones for assembly of oxidative phosphorylation proteins, maintenance proteins for the

263 mitochondrial genome (replication, mtRNA folding and translation), calcium homeostasis and

264 mitochondrial protein import. All of these genes have human orthologs, nearly all with known

265 mutations in which lead to severe clinical manifestations such as Leigh syndrome,

266 cardiomyopathy, progressive external ophthalmoplegia, and oxidative phosphorylation

267 deficiency (**Table 1**).

268 **Table 1: List of nuclear-encoded genes for which zebrafish mutants were generated as part**

269 **of MMC collection:** The current MMC resource list summarizes information on human gene,

270 zebrafish ortholog, mouse ortholog, relevant clinical phenotypes and diseases, protein function,

271 and OMIM ID. Green = Created by TALEN Indel Mutagenesis, Orange = Curated from GBT

272 protein trap lines (Keywords: Chr- Chromosome; OMIM ID: Online Mendelian Inheritance in

273 Man ID; NA-not available).

Approved symbol	Approved name	Human chr	Zebrafish orthologs	Zebrafish chr	Allele designator	Mouse orthologs	Mouse chr	Clinical phenotype observed in humans	Disease	Biological function	OMIM ID
NDUFAB1	NADH:ubiquinone oxidoreductase subunit AB1	16	*ndufab1a* *ndufab1b*	1 3	mn0135	Ndufab1b	7	-na-	-na-	Oxidative phosphorylation enzymes - Complex I	603836
NDUFAF6	NADH:ubiquinone oxidoreductase complex assembly factor 6	8	*ndufaf6*	16	mn0117	Ndufaf6	4	Focal right-hand seizures, ataxia, lactic acidosis, exercise intolerance, weakness and muscle tension	Leigh syndrome, mitochondrial complex I deficiency	Oxidative phosphorylation complex assembly - Complex I	612392
NDUFS4	NADH:ubiquinone oxidoreductase subunit S4	5	*ndufs4*	5	mn0118	Ndufs4	13	Mitochondrial complex I deficiency	Mitochondrial complex-I deficiency	Oxidative phosphorylation enzymes - Complex I	602694
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	5	*sdha*	19	mn0121	Sdha	13	Dyspnea, cardiomegaly, cardiomyopathy, nystagmus, hypotonia, gastrointestinal stromal tumors, paragangliomas, pheochromocytoma, psychomotor regression, severe hyperandrogenism	Mitochondrial complex-II deficiency; Cardiomyopathy, Leigh syndrome; Paraganglioma	Oxidative phosphorylation enzymes - Complex II	600857

14

Approved symbol	Approved name	Human chr	Zebrafish orthologs	Zebrafish chr	Allele designator	Mouse orthologs	Mouse chr	Clinical phenotype observed in humans	Disease	Biological function	OMIM ID
BCS1L	BCS1 homolog, ubiquinol-cytochrome C reductase complex chaperone	2	bcs1l	9	mn0102	Bcs1l	1	Growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, early death, hypotonia, encephalopathy, microcephaly, psychomotor regression, hypoglycemia and hepatic failure	Björnstad syndrome: GRACILE syndrome; Leigh syndrome; Mitochondrial complex III deficiency, nuclear type 1	Oxidative phosphorylation complex assembly - Complex III	603647
UQCRQ	ubiquinol-cytochrome c reductase complex III subunit VII	5	uqcrq	14	mn0128 mn0129	Uqcrq	11	Severe psychomotor retardation and extrapyramidal signs, dystonia, athetosis and ataxia, mild axial hypotonia and marked global dementia	Mitochondrial complex III deficiency	Mitochondrial complex III: ubiquinol-cytochrome c reductase complex subunits	612080
COX10	Cytochrome c oxidase assembly factor heme A: farnesyltransferase COX10	17	cox10	12	mn0107 mn0108	Cox10	11	Muscle weakness, hypotonia, ataxia, ptosis, pyramidal syndrome, status epilepticus, lactic acidosis, hypertrophic cardiomyopathy, hypoglycemia and metabolic acidosis	Leigh syndrome due to mitochondrial COX IV deficiency; Mitochondrial complex IV deficiency	Oxidative phosphorylation complex assembly - Complex IV	602125
COX4I2	Cytochrome C Oxidase Subunit 4I2	20	cox4i2b	23	mn0104	Cox4i2	2	Exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis	Exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis	Oxidative phosphorylation complex enzymes - Complex IV	607976
SURF1	SURF1, Cytochrome C Oxidase Assembly Factor	9	surf1	5	mn0123	Surf1	2	Childhood onset neuropathy, lactic acidosis, mild intellectual disability ataxia, facial dysmorphism, encephalopathy, hypotonia, cerebellar ataxia, deafness, ophthalmoplegia, growth retardation and nystagmus	Charcot-Marie-Tooth disease, type 4K; Leigh syndrome, due to COX IV deficiency	Involved in biogenesis of cytochrome c oxidase complex	185620
ATP5F1E	ATP synthase F1 subunit epsilon	20	atp5f1e	6	mn0101	Atp5f1e	2	Neonatal-onset lactic acidosis, 3-methylglutaconic aciduria, mild mental retardation, and peripheral neuropathy	Mitochondrial complex (ATP synthase) deficiency	Oxidative phosphorylation complex assembly - ATP synthase	606153
TMEM70	Transmembrane protein 70	8	tmem70	2	mn0126 mn0127	Tmem70	1	Lactic acidosis, encephalopathy, histiocytoid cardiomyopathy, microcephaly, hypotonia, facial dysmorphism and 3-methylglutaconic aciduria psychomotor delay, hyperammonemia	Mitochondrial complex V (ATP synthase) deficiency	Biogenesis of mitochondrial ATP synthase	612418

15

Approved symbol	Approved name	Human chr	Zebrafish orthologs	Zebrafish chr	Allele designator	Mouse orthologs	Mouse chr	Clinical phenotype observed in humans	Disease	Biological function	OMIM ID
COQ2	Coenzyme Q2, Polyprenyltransfe--rase	4	coq2	5	mn0106	Coq2	5	Multiple system atrophy, unsteadiness of gait, nystagmus, gait ataxia, dysarthria, speech difficulty, dysmetria, lactic acidosis, urinary dysfunction and nystagmus	Coenzyme Q10 deficiency, primary; Multiple system atrophy	Biosynthesis of CoQ, coenzyme in mitochondrial respiratory chain	609825
PDSS2	Decaprenyl diphosphate synthase subunit 2	6	pdss2	13	mn0120	Pdss2	10	Hypotonia, seizures, cortical blindness, lactic acidosis, encephalopathy and nephrotic syndrome	Coenzyme Q10 deficiency	Involved in biosynthesis of coenzyme Q	610564
MICU1	Mitochondrial calcium uptake 1	10	micu1	13	mn0132	Micu1	10	Proximal muscle weakness and learning disabilities	Myopathy with extrapyramidal signs	Regulator of mitochondrial calcium uptake	605084
SMDT1	Single-pass membrane protein with aspartate rich tail	22	smdt1a smdt1b	3 1	mn0122	Smdt1	15	-na-	-na-	Core regulatory subunit of calcium channel in mitochondrial inner membrane	615588
MCU	Mitochondrial calcium uniporter	10	mcu	13	mn0111	Mcu	10	-na-	-na-	Calcium transporter and mediates calcium uptake in mitochondria	614197
TK2	Thymidine kinase 2, mitochondrial	16	tk2	7	mn0125	Tk2	8	Infantile onset fetal encephalomyopathy, failure to thrive, muscle weakness, mild facial weakness, myopathy, axonal neuropathy, brain atrophy, hypertrophic cardiomyopathy, regression of motor development, psychomotor arrest, dysphonia, dysphagia, ptosis, impaired gait, bilateral ptosis, hypotonia, lactic acidosis and epilepsy	Progressive external ophthalmoplegia; Mitochondrial DNA depletion syndrome 2 (myopathic type)	Required for mitochondrial DNA synthesis	188250
LRPPRC	Leucine rich pentatricopeptide repeat containing	2	lrpprc	13	mn0235gt	Lrpprc	17	Delayed psychomotor development, mental retardation, mild dysmorphic facial features, hypotonia, ataxia, and the development of lesions in the brainstem and basal ganglia, seizures, dysphagia and hypertrophic cardiomyopathy	Leigh syndrome, French-Canadian type	Involved in translation of mitochondrial encoded cox subunits and mediation of folding of mitochondrial transcriptome	607544
MTFMT	Mitochondrial methionyl-tRNA formyltransferase	15	mtfmt	7	mn0114 mn0115	Mtfmt	9	Psychomotor developmental delay, renal dysplasia, mild facial dysarthria and ataxia	Combined oxidative phosphorylation deficiency	Catalyzes the formylation of methionyl-tRNA	611766
MRPS18B	Mitochondrial ribosomal protein S18B	6	mrps18b	19	mn0425gt	Mrps18b	17	-na-	-na-	Part of small 28S subunit of mitoribosome	611982

16

Approved symbol	Approved name	Human chr	Zebrafish orthologs	Zebrafish chr	Allele designator	Mouse orthologs	Mouse chr	Clinical phenotype observed in humans	Disease	Biological function	OMIM ID
TIMM50	Translocase of inner mitochondrial membrane 50	19	*timm50*	15	mn0906gt	Timm50	7	Severe intellectual disability, seizure and 3-methylglutaconic aciduria	3-methylglutaconic aciduria, type IX; Mitochondrial complex V deficiency	Subunit of TIM23 inner mitochondrial membrane complex and recognizes mitochondrial targeting signal or pre-sequence	607381
OGDH	Oxoglutarate dehydrogenase	7	*ogdha* *ogdhb*	8 10	mn0281gt	Ogdh	11	Colorectal cancer	Alpha-ketoglutarate dehydrogenase deficiency	Catalyzes the conversion of 2-oxoglutarate to succinyl-CoA and CO_2	613022
IDH2	Isocitrate dehydrogenase (NADP (+)) 2, mitochondrial	15	*idh2*	18	mn0268gt	Idh2	7	Acute myeloid leukemia and abnormal production of D-2-hydroxyglutaric acid	D-2-hydroxyglutaric aciduria 2	Catalyzes the conversion of isocitrate to 2-oxoglutarate	147650

274

TALEN- and GBT-mediated targeting of nuclear-encoded mitochondrial genes

276 Amino acid analysis using protein-protein BLAST functions between the wild type human, wild

277 type zebrafish, and mutant zebrafish sequences showed consistent results between the three

278 conditions. The comparison of wild type human and wild type zebrafish sequences showed high

279 areas of homology following the mitochondrial targeting domain in almost every gene, with 80-

280 100% similarity in catalytic or active domains of the transcripts. Analysis of the mutant zebrafish

281 and human wild type comparison showed a range of difference with predicted frameshift

282 mutations leading to truncation of the protein very early in the transcript **(Figure 3A-X)**. Of the

283 alleles created by TALEN mutagenesis in the MMC collection, all but one (*micu1*) showed a

284 predicted frameshift mutation leading to a truncation event immediately following the NHEJ-

285 mediated insertion or deletion. The GBT mutants showed high levels of homology prior to the

286 transposon integration site followed by a nearly complete loss of normal transcript levels

287 following splicing into the GBT cassette.

288

Discussion

289

290   In a multicellular organism, each cell is able to carry out its functions due to well-orchestrated

291   cross-talk between the nuclear and mitochondrial genomes. Many mitochondrial functions like

292   energy production, genome maintenance, ion/metabolite homeostasis, membrane dynamics and

293   transport of biomolecules can be attributed to approximately 1158 known nuclear proteins

294   residing in the mitochondria [24,27]. Due to advancements in proteomic technologies in recent

295   years, there has been a surge in documentation of many characterized and uncharacterized

296   mitochondrial proteins. However, correlation between mitochondrial localization of these

297   proteins and their physiological significance in disease progression remains largely unexplored.

298   Out of the 1158 nuclear encoded proteins, only 245 have functional evidence in mitochondrial

299   clinical manifestations [39]. For the remaining 913 proteins, mitochondrial involvement in

300   disease progression has yet to be demonstrated. The obstacles in mitochondrial genetic research,

301   and thus delays in finding effective treatments, are primarily due to the limited tools available to

302   mitochondrial researchers, specifically the small number of available model systems and

303   animals.

304   Most mitochondrial research thus far has made use of model systems that each present their own

305   unique challenge to accurate study of human disease. Yeast, one of the most common laboratory

306   eukaryotes, have been incredibly useful in mitochondrial research, but unlike humans and other

307   animals they lack complex I [45]. Complex 1 deficiency is the most frequent among

308   mitochondrial disorders caused by mutations in 28 out of 48 genes contributing to its assembly

309   and biogenesis [84]. Also, complex 1 is responsible for the most ROS generation, which acts as a

310   cue in various signaling pathways [85].

18

311   Human cell culture has been valuable in exploring mitochondrial disease models, principally

312   through cytoplasmic hybrids that are made by replacing the mitochondria of an immortalized cell

313   line with mutated patient mitochondria [86–89]. However, while these cells are a more faithful

314   representation of human mitochondrial activity than yeast, they have different barriers to

315   accuracy. First, immortalized cells, and cultured cells in general, often have modified metabolic

316   pathways compared to cells *in vivo*. Second, as is common to all cell culture research, cells in a

317   dish cannot accurately represent the complex interactions and systems biology inherent to a

318   complete organism.

319   In this study, we aim to support the establishment of the zebrafish as a complementary animal

320   model for understanding the role of nuclear-encoded mitochondrial proteins in biology and the

321   pathophysiology of mitochondrial disorders. We propose that zebrafish can be an excellent

322   model organism to study mitochondrial biology primarily because of their conserved genome,

323   codon bias and synteny. Other advantages include their amenability to genetic manipulation and

324   optical clarity, which facilitates direct observation. New gene editing techniques such as

325   TALENs and CRISPRs have aided in the development of humanized disease models in

326   organisms such as zebrafish, mice, and even pigs [90–97].

327   The MMC collection described here encompasses mutations in 24 different nuclear-encoded

328   genes related to mitochondrial function enabling a diverse study of the important roles the

329   mitochondria play in cellular biology. The collection includes mutants in all five complexes of

330   the electron transport chain as well as many other crucial pathways related to protein transport,

331   metabolite synthesis, mtDNA replication and expression, and calcium homeostasis. The first set

332   of mutants were created using custom gene editing to exons coding for critical functional

333   domains of the protein product. These domains were targeted because they shared high levels of

334   homology to their human ortholog. The second group of mutants consisted of fish that had been

335   injected with a protein trap transposon system that truncates the expected protein products as

336   well as sorting and imaging through fluorescent expression patterns *in vivo*. Included here, we

337   have curated small group of mutants where the GBT integrated into a mitochondrial gene of

338   interest.

339   In recent years zebrafish have been used to understand the pathophysiology of human

340   mitochondrial disorders such as cardiovascular, multisystemic, neurological, and erythropoiesis,

341   to name a few. Studies for loss of function have been carried out to model left ventricular non-

342   compaction, a genetically heterogeneous cardiomyopathy caused by mutations in nicotinamide

343   nucleotide transhydrogenase (*NNT*) gene [98,99]. Transiently suppressing the levels of *nnt* gene

344   did mirror the symptoms, such as early ventricular malformation and contractility defects,

345   observed in human patients [99]. Transient suppression of *ndufb11* protein in zebrafish results in

346   cardiac anomalies, confirming the role of *NDUFB11* in histiocytoid cardiomyopathy

347   pathogenesis [41].

348   Parkinson disease (PD) is another example where *parkin* and *pink* knockdown larvae display

349   mitochondrial fragmentation accompanied by accumulation of oxidative species, mimicking the

350   phenotype observed in PD patients. Loss of dendritic arborization and peripheral neurons were

351   also observed in the knockdown animals [72,100–103]. The zebrafish model of Dravet syndrome

352   shows altered metabolism associated with the pathophysiology [104]. Downregulation of the

353   sodium channel, voltage-gated, type I like, alpha b (*scnl1ab*) in zebrafish leads to mitochondrial

354   structural abnormalities accompanied by accumulation of oxidative species [104–106].

355   Sideroblastic anemia, caused by mutations in mitoferrin (*mfrn1*) gene, has been successfully

356   modelled in zebrafish [107]. The elucidation of its role in zebrafish as iron importer does shed

20

357   some light on iron related defects in progression of anemia, accompanied by mitochondrial

358   dysfunction. Zebrafish erythrocytes are nucleated and possess organelles such as mitochondria

359   [108] in their cytoplasm, making them an ideal model organism to study ribosomopathies and

360   hematological malignancies [109–114]. Mitochondria in erythrocytes may offer valuable insight

361   into novel biological pathways resident in mitochondria.

362   Transient infantile liver failure and mitochondrial deafness, caused by mutations in tRNA 5-

363   methylaminomethyl-2-thiouridylate   methyltransferase   (*TRMU*)   gene   [115]   have   been

364   phenocopied in zebrafish [116]. Zebrafish *trmu* mutants exhibit abnormal mitochondrial

365   morphology, deficient oxidative phosphorylation, defects in hair cells and decreases in the steady

366   state levels of mt-tRNAs, resembling the observations made in patients suffering from this

367   syndrome [116].

368   Taking cues from these studies, many of the genes selected here were prioritized on the basis of

369   the mutations and clinical phenotype reported in patients. Detailed phenotypic analyses will be

370   described in subsequent studies. The zebrafish mutants encode for genes that have been involved

371   in myriad of clinical phenotypes such as encephalopathy, nephrotic syndrome, intellectual

372   disability, psychomotor developmental delay, respiratory chain deficiency, lactic acidosis, iron

373   overload, microcephaly, hypertrophic cardiomyopathy, etc. The superficial investigations of the

374   tissue-specific phenotypes associated with, for example, housekeeping nuclear encoded proteins,

375   have to be refined with organelle level investigations. The repository of mutants encoding for

376   proteins involved in respiratory chain biogenesis and assembly offers the potential to understand

377   the moonlighting role of these proteins. Mutations in these proteins are associated with organ-

378   specific phenotypes such as neurological, paraganglioma, cardiovascular etc. [41,117]. Extensive

379   energy demand by these tissues is one possible explanation for the progression of these

380   manifestations; however, the role of these proteins in organ-specific cellular niches or

381   homeostasis is also a possibility. These models can help to decipher the role of these proteins in

382   the mitochondrial interactome, when studied *in vivo*. This underpins the utility of this MMC

383   collection in deciphering the role of mitochondrial proteins in tissue-specific biological

384   pathways.

385   Zebrafish are also amenable to small molecule mediated pharmacological modelling of

386   mitochondrial phenotypes. To test hypoxia as a potential protective therapy in mitochondrial

387   disorders, Von Hippel-Lindau (*vhl*) null mutant zebrafish, when treated with antimycin mediated

388   mitochondrial insult, exhibited improved survival. In addition, FG-4592 was found to improve

389   survival in response to respiratory chain inhibition, possibly due to increase in hypoxia response

390   [118]. A series of ETC complex specific pharmacological inhibitors such as rotenone (complex

391   I), azide (complex IV), oligomycin (complex V) and chloramphenicol (mitochondrial protein

392   translation) have been used to model respiratory chain dysfunction in zebrafish [78,119,120].

393   Zebrafish larvae display a series of phenotypes, such as developmental arrest, when treated with

394   rotenone. Treatment with azide induced decreased heart rate, loss of motor function, inability to

395   respond to tactical stimulation, neurological damage and mortality. Organ specific manifestations

396   such as neurological and behavioral dysfunction have been reported in 6-7 days post fertilization

397   (dpf) zebrafish larvae upon administration of a titred drug concentration [120]. These

398   observations tilt the scales in favor of zebrafish for modeling mitochondrial clinical

399   manifestations. Gaining insights from these studies, our compendium of mutants provides an

400   excellent resource to test a large number of biological and chemical modulators as potential

401   therapies for mitochondrial disorders for which treatment remains elusive.

402   Zebrafish offer many unique advantages for *in vivo* imaging experiments, as compared to their

403   mammalian counterparts. A classical study corroborating this advantage is *in vivo* imaging of

404   mitochondrial transport in a single axon, as demonstrated by Yang and his colleagues [121].

405   Seok and his colleagues generated a transgenic zebrafish line, expressing green fluorescent

406   protein fused to mitochondrial localization sequence from cytochrome c oxidase [71].

407   Mitochondrial function is often measured by various parameters such as estimation of membrane

408   potential, mitochondrial superoxide species and energy production. Superoxide activity and

409   membrane potential in zebrafish has been measured by employing the use of cell permeable

410   chemical probes such as MitoSox [122] and Dihydrorhodamine 123 (DH123) [73], respectively.

411   Zebrafish transgenic lines expressing genetically encoded calcium and oxidation indicators, have

412   also served as an excellent model to measure calcium homeostasis and oxidative status *in vivo* in

413   models of mechanosensory hair cell damage and death [123]. Constructs such as Mitotimer allow

414   the measurement of mitochondrial turnover, transport, and changes in the redox history of

415   mitochondria during organogenesis events in zebrafish embryos [124]. This transgenic model

416   enables a sweeping picture of the mitochondrial network, helping in studying cellular processes

417   such as mitophagy and apoptosis.

418   Zebrafish have also provided novel mechanistic insights to interrogate the mitochondrial

419   dynamics, to be called as "*in-vivo* life cycle of mitochondria" in healthy and diseased conditions

420   [124]. One such example is that of Mitofish [74], that recapitulates mitochondrial network

421   biogenesis, unraveling the role of the organelle in cell-type specific niches of different organ

422   systems. Mitofish is a transgenic zebrafish line that fluorescently labels the mitochondria in the

423   neurons, enabling non-invasive *in vivo* observation. Advancement in adaptive optics and lattice

424   light sheet microscopy have empowered researchers to look at vibrant and colorful images of

425   organelles in zebrafish. These technologies have been applied in investigating the organellar

426   dynamics in brain during early development and in the eye of an adult zebrafish [125]. These

427   MMMC mutants provide an excellent platform to study mitochondrial remodeling and

428   homeostasis in the progression of various clinical disorders by mapping the mitochondrial wiring

429   of the cell.

430   These zebrafish mutants have been cryopreserved for the purpose of sharing the lines as a

431   resource with the field for expanding the study of mitochondrial genetics and biology. In

432   addition, our template for the creation of mitochondrial mutants in zebrafish should enable the

433   creation of higher animal models of specific variants of interest. For the purpose of accelerating

434   this research, all lines in this project have had their information deposited with ZIRC, and all of

435   the lines have been made available via zfishbook (www.zfishbook.com) [83].

436   The goal of this study is to disseminate the use of zebrafish as a *noblesse oblige* in the field of

437   mitochondrial biology and medicine, paving way for the development of novel insight for

438   diagnostic and therapeutic strategies. Ultimately, using this collection of mutants we hope to

439   unravel a small part of the mystery that shrouds one of the most crucial organelles in the

440   cell. We hope that this MMMC mutant collection and the primer we provide for adding to it,

441   will help to usher in a new mitochondrial research using the zebrafish.

Acknowledgements

447   by the Mayo Foundation, a gift by the Marriott Foundation and by NIH grants DK84567,

448   GM63904 and HG006431 to SCE.

Author Contributions

450   The paper was written by AS, MW, ZWJ and SCE. Experiments were executed by JMC, MW,

451   HA, NI, MDU, JDB, RMH, WL, YD and TLP with experimental guidance from XX, KJC and

452   SCE. Data analysis was completed by JMC, NI, JDB, MDU, RMH, MW, WL, YD and AS.

References

454   1.   Andersson SG, Zomorodipour A, Andersson JO, Sicheritz-Ponten T, Alsmark UC,

455   Podowski RM, et al. The genome sequence of Rickettsia prowazekii and the origin of

456   mitochondria. Nature. England; 1998;396: 133–140. doi:10.1038/24094

457   2.   Muller M, Martin W. The genome of Rickettsia prowazekii and some thoughts on the

458   origin of mitochondria and hydrogenosomes. Bioessays. United States; 1999;21: 377–381.

459   doi:10.1002/(SICI)1521-1878(199905)21:5<377::AID-BIES4>3.0.CO;2-W

460   3.   Gray MW, Burger G, Lang BF. Mitochondrial evolution. Science. United States;

461   1999;283: 1476–1481.

462   4.   Thorsness PE, Weber ER. Escape and migration of nucleic acids between chloroplasts,

463   mitochondria, and the nucleus. Int Rev Cytol. United States; 1996;165: 207–234.

464   5.   Lynch M, Koskella B, Schaack S. Mutation pressure and the evolution of organelle

465   genomic   architecture.   Science.   United   States;   2006;311:   1727–1730.

466   doi:10.1126/science.1118884

467   6.   Vafai SB, Mootha VK. Mitochondrial disorders as windows into an ancient organelle.

468   Nature. England; 2012;491: 374–383. doi:10.1038/nature11707

469   7.   Elson JL, Samuels DC, Turnbull DM, Chinnery PF. Random intracellular drift explains

470   the clonal expansion of mitochondrial DNA mutations with age. Am J Hum Genet. United

471   States; 2001;68: 802–806. doi:10.1086/318801

472   8.   DiMauro S, Hirano M. Mitochondrial DNA Deletion Syndromes. In: Adam MP, Ardinger

473   HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. Seattle (WA); 1993.

474   9.   Wallace DC. Mitochondrial DNA mutations in disease and aging. Environ Mol Mutagen.

475   United States; 2010;51: 440–450. doi:10.1002/em.20586

476   10. Elson JL, Smith PM, Greaves LC, Lightowlers RN, Chrzanowska-Lightowlers ZMA,

477   Taylor RW, et al. The presence of highly disruptive 16S rRNA mutations in clinical

478   samples indicates a wider role for mutations of the mitochondrial ribosome in human

479   disease. Mitochondrion. Netherlands; 2015;25: 17–27. doi:10.1016/j.mito.2015.08.004

480   11. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N.

481   Reanalysis and revision of the Cambridge reference sequence for human mitochondrial

482   DNA. Nature genetics. United States; 1999. p. 147. doi:10.1038/13779

483   12. Broughton RE, Milam JE, Roe BA. The complete sequence of the zebrafish (Danio rerio)

484   mitochondrial genome and evolutionary patterns in vertebrate mitochondrial DNA.

485   Genome Res. United States; 2001;11: 1958–1967. doi:10.1101/gr.156801

486   13. Wolstenholme DR. Animal mitochondrial DNA: structure and evolution. Int Rev Cytol.

487   United States; 1992;141: 173–216.

488   14. Osawa S, Jukes TH, Watanabe K, Muto A. Recent evidence for evolution of the genetic

489   code. Microbiol Rev. United States; 1992;56: 229–264.

490   15.   Richter R, Pajak A, Dennerlein S, Rozanska A, Lightowlers RN, Chrzanowska-

491   Lightowlers ZMA. Translation termination in human mitochondrial ribosomes. Biochem

492   Soc Trans. England; 2010;38: 1523–1526. doi:10.1042/BST0381523

493   16.   Bayona-Bafaluy MP, Blits B, Battersby BJ, Shoubridge EA, Moraes CT. Rapid directional

494   shift of mitochondrial DNA heteroplasmy in animal tissues by a mitochondrially targeted

495   restriction endonuclease. Proc Natl Acad Sci U S A. United States; 2005;102: 14392–

496   14397. doi:10.1073/pnas.0502896102

497   17.   Srivastava S, Moraes CT. Double-strand breaks of mouse muscle mtDNA promote large

498   deletions similar to multiple mtDNA deletions in humans. Hum Mol Genet. England;

499   2005;14: 893–902. doi:10.1093/hmg/ddi082

500   18.   Nissanka N, Minczuk M, Moraes CT. Mechanisms of Mitochondrial DNA Deletion

501   Formation. Trends Genet. England; 2019; doi:10.1016/j.tig.2019.01.001

502   19.   Alexeyev M, Shokolenko I, Wilson G, LeDoux S. The maintenance of mitochondrial

503   DNA integrity--critical analysis and update. Cold Spring Harb Perspect Biol. United

504   States; 2013;5: a012641. doi:10.1101/cshperspect.a012641

505   20.   Zinovkina LA. Mechanisms of Mitochondrial DNA Repair in Mammals. Biochemistry

506   (Mosc). United States; 2018;83: 233–249. doi:10.1134/S0006297918030045

507   21.   Patananan AN, Wu T-H, Chiou P-Y, Teitell MA. Modifying the Mitochondrial Genome.

508   Cell Metab. United States; 2016;23: 785–796. doi:10.1016/j.cmet.2016.04.004

509   22.   Gammage PA, Moraes CT, Minczuk M. Mitochondrial Genome Engineering: The

510   Revolution May Not Be CRISPR-Ized. Trends Genet. England; 2018;34: 101–110.

511   doi:10.1016/j.tig.2017.11.001

512 23. Lopez MF, Kristal BS, Chernokalskaya E, Lazarev A, Shestopalov AI, Bogdanova A, et

513   al. High-throughput profiling of the mitochondrial proteome using affinity fractionation

514   and automation. Electrophoresis. Germany; 2000;21: 3427–3440. doi:10.1002/1522-

515   2683(20001001)21:16<3427::AID-ELPS3427>3.0.CO;2-L

516 24. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong S-E, et al. A mitochondrial

517   protein compendium elucidates complex I disease biology. Cell. United States; 2008;134:

518   112–123. doi:10.1016/j.cell.2008.06.016

519 25. Elstner M, Andreoli C, Klopstock T, Meitinger T, Prokisch H. The mitochondrial

520   proteome database: MitoP2. Methods Enzymol. United States; 2009;457: 3–20.

521   doi:10.1016/S0076-6879(09)05001-0

522 26. Smith AC, Robinson AJ. MitoMiner, an integrated database for the storage and analysis of

523   mitochondrial proteomics data. Mol Cell Proteomics. United States; 2009;8: 1324–1337.

524   doi:10.1074/mcp.M800373-MCP200

525 27. Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian

526   mitochondrial proteins. Nucleic Acids Res. England; 2016;44: D1251-7.

527   doi:10.1093/nar/gkv1003

528 28. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev

529   Genet. England; 2005;6: 389–402. doi:10.1038/nrg1606

530 29. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat J-P, Letellier T. Mitochondrial

531 threshold effects. Biochem J. England; 2003;370: 751–762. doi:10.1042/BJ20021594

532 30. Chinnery PF. Mitochondrial Disorders Overview. In: Adam MP, Ardinger HH, Pagon RA,

533   Wallace SE, Bean LJH, Stephens K, et al., editors. Seattle (WA); 1993.

534 31. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, et al.

535   Mitochondrial diseases. Nature reviews. Disease primers. England; 2016. p. 16080.

536   doi:10.1038/nrdp.2016.80

537 32. Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial

538   respiratory chain disorders in children. Brain. England; 2003;126: 1905–1912.

539   doi:10.1093/brain/awg170

540 33. Schaefer AM, McFarland R, Blakely EL, He L, Whittaker RG, Taylor RW, et al.

541   Prevalence of mitochondrial DNA disease in adults. Ann Neurol. United States; 2008;63:

542   35–39. doi:10.1002/ana.21217

543 34. Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, et al. Mitochondrial

544   DNA mutation associated with Leber's hereditary optic neuropathy. Science. United

545   States; 1988;242: 1427–1430.

546 35. Swerdlow RH, Wooten GF. A novel deafness/dystonia peptide gene mutation that causes

547   dystonia in female carriers of Mohr-Tranebjaerg syndrome. Ann Neurol. United States;

548   2001;50: 537–540.

549 36. Garone C, Tadesse S, Hirano M. Clinical and genetic spectrum of mitochondrial

550   neurogastrointestinal encephalomyopathy. Brain. England; 2011;134: 3326–3332.

551   doi:10.1093/brain/awr245

552   37.   Urrutia PJ, Mena NP, Nunez MT. The interplay between iron accumulation, mitochondrial

553   dysfunction, and inflammation during the execution step of neurodegenerative disorders.

554   Front Pharmacol. Switzerland; 2014;5: 38. doi:10.3389/fphar.2014.00038

555   38.   Singh RK, Saini S, Verma D, Kalaiarasan P, Bamezai RNK. Mitochondrial ND5 mutation

556   mediated elevated ROS regulates apoptotic pathway epigenetically in a P53 dependent

557   manner for generating pro-cancerous phenotypes. Mitochondrion. Netherlands; 2017;35:

558   35–43. doi:10.1016/j.mito.2017.05.001

559   39.   Craven L, Alston CL, Taylor RW, Turnbull DM. Recent Advances in Mitochondrial

560   Disease. Annu Rev Genomics Hum Genet. United States; 2017;18: 257–275.

561   doi:10.1146/annurev-genom-091416-035426

562   40.   Ghezzi D, Saada A, D'Adamo P, Fernandez-Vizarra E, Gasparini P, Tiranti V, et al.

563   FASTKD2 nonsense mutation in an infantile mitochondrial encephalomyopathy

564   associated with cytochrome c oxidase deficiency. Am J Hum Genet. United States;

565   2008;83: 415–423. doi:10.1016/j.ajhg.2008.08.009

566   41.   Shehata BM, Cundiff CA, Lee K, Sabharwal A, Lalwani MK, Davis AK, et al. Exome

567   sequencing of patients with histiocytoid cardiomyopathy reveals a de novo NDUFB11

568   mutation that plays a role in the pathogenesis of histiocytoid cardiomyopathy. Am J Med

569   Genet A. United States; 2015;167A: 2114–2121. doi:10.1002/ajmg.a.37138

570   42.   Wesolowska M, Gorman GS, Alston CL, Pajak A, Pyle A, He L, et al. Adult Onset Leigh

571   Syndrome in the Intensive Care Setting: A Novel Presentation of a C12orf65 Related

572   Mitochondrial Disease. J Neuromuscul Dis. Netherlands; 2015;2: 409–419.

573   doi:10.3233/JND-150121

574   43.   Floyd BJ, Wilkerson EM, Veling MT, Minogue CE, Xia C, Beebe ET, et al.

575   Mitochondrial Protein Interaction Mapping Identifies Regulators of Respiratory Chain

576   Function. Mol Cell. United States; 2016;63: 621–632. doi:10.1016/j.molcel.2016.06.033

577   44.   Kohda M, Tokuzawa Y, Kishita Y, Nyuzuki H, Moriyama Y, Mizuno Y, et al. A

578   Comprehensive Genomic Analysis Reveals the Genetic Landscape of Mitochondrial

579   Respiratory Chain Complex Deficiencies. PLoS Genet. United States; 2016;12: e1005679.

580   doi:10.1371/journal.pgen.1005679

581   45.   Boumans H, Grivell LA, Berden JA. The respiratory chain in yeast behaves as a single

582   functional unit. J Biol Chem. United States; 1998;273: 4872–4877.

583   46.   Schwimmer C, Rak M, Lefebvre-Legendre L, Duvezin-Caubet S, Plane G, di Rago J-P.

584   Yeast models of human mitochondrial diseases: from molecular mechanisms to drug

585   screening. Biotechnol J. Germany; 2006;1: 270–281. doi:10.1002/biot.200500053

586   47.   Altmann K, Durr M, Westermann B. Saccharomyces cerevisiae as a model organism to

587   study mitochondrial biology: general considerations and basic procedures. Methods Mol

588   Biol. United States; 2007;372: 81–90. doi:10.1007/978-1-59745-365-3_6

589   48.   Denoth Lippuner A, Julou T, Barral Y. Budding yeast as a model organism to study the

590   effects of age. FEMS Microbiol Rev. England; 2014;38: 300–325. doi:10.1111/1574-

591   6976.12060

592   49.   Addo MG, Cossard R, Pichard D, Obiri-Danso K, Rotig A, Delahodde A. Caenorhabditis

593   elegans, a pluricellular model organism to screen new genes involved in mitochondrial

594   genome maintenance. Biochim Biophys Acta. Netherlands; 2010;1802: 765–773.

595   doi:10.1016/j.bbadis.2010.05.007

31

596 50. Harrington AJ, Hamamichi S, Caldwell GA, Caldwell KA. C. elegans as a model

597 organism to investigate molecular pathways involved with Parkinson's disease. Dev Dyn.

598 United States; 2010;239: 1282–1295. doi:10.1002/dvdy.22231

599 51. Moreno-Arriola E, Cardenas-Rodriguez N, Coballase-Urrutia E, Pedraza-Chaverri J,

600 Carmona-Aparicio L, Ortega-Cuellar D. Caenorhabditis elegans: A useful model for

601 studying metabolic disorders in which oxidative stress is a contributing factor. Oxid Med

602 Cell Longev. United States; 2014;2014: 705253. doi:10.1155/2014/705253

603 52. Tissenbaum HA. Using C. elegans for aging research. Invertebr Reprod Dev. England;

604 2015;59: 59–63. doi:10.1080/07924259.2014.940470

605 53. Sanchez-Martinez A, Luo N, Clemente P, Adan C, Hernandez-Sierra R, Ochoa P, et al.

606 Modeling human mitochondrial diseases in flies. Biochim Biophys Acta. Netherlands;

607 2006;1757: 1190–1198. doi:10.1016/j.bbabio.2006.05.008

608 54. Fernandez-Moreno MA, Farr CL, Kaguni LS, Garesse R. Drosophila melanogaster as a

609 model system to study mitochondrial biology. Methods Mol Biol. United States;

610 2007;372: 33–49. doi:10.1007/978-1-59745-365-3_3

611 55. Guo M. Drosophila as a model to study mitochondrial dysfunction in Parkinson's disease.

612 Cold Spring Harb Perspect Med. United States; 2012;2. doi:10.1101/cshperspect.a009944

613 56. Sen A, Cox RT. Fly Models of Human Diseases: Drosophila as a Model for

614 Understanding Human Mitochondrial Mutations and Disease. Curr Top Dev Biol. United

615 States; 2017;121: 1–27. doi:10.1016/bs.ctdb.2016.07.001

616 57. Lightowlers RN, Rozanska A, Chrzanowska-Lightowlers ZM. Mitochondrial protein

617   synthesis: figuring the fundamentals, complexities and complications, of mammalian

618   mitochondrial translation. FEBS Lett. England; 2014;588: 2496–2503.

619   doi:10.1016/j.febslet.2014.05.054

620   58.   Pett W, Lavrov D V. Cytonuclear Interactions in the Evolution of Animal Mitochondrial

621   tRNA Metabolism. Genome Biol Evol. England; 2015;7: 2089–2101.

622   doi:10.1093/gbe/evv124

623   59.   Lavrov D V, Pett W. Animal Mitochondrial DNA as We Do Not Know It: mt-Genome

624   Organization and Evolution in Nonbilaterian Lineages. Genome Biol Evol. England;

625   2016;8: 2896–2913. doi:10.1093/gbe/evw195

626   60.   Brown WM, George MJ, Wilson AC. Rapid evolution of animal mitochondrial DNA.

627   Proc Natl Acad Sci U S A. United States; 1979;76: 1967–1971.

628   61.   Moyes CD, Suarez RK, Hochachka PW, Ballantyne JS. A comparison of fuel preferences

629   of mitochondria from vertebrates and invertebrates. Can J Zool. NRC Research Press;

630   1990;68: 1337–1349. doi:10.1139/z90-201

631   62.   Grossman LI, Wildman DE, Schmidt TR, Goodman M. Accelerated evolution of the

632   electron transport chain in anthropoid primates. Trends Genet. England; 2004;20: 578–

633   585. doi:10.1016/j.tig.2004.09.002

634   63.   Gershoni M, Fuchs A, Shani N, Fridman Y, Corral-Debrinski M, Aharoni A, et al.

635   Coevolution predicts direct interactions between mtDNA-encoded and nDNA-encoded

636   subunits of oxidative phosphorylation complex i. J Mol Biol. England; 2010;404: 158–

637   171. doi:10.1016/j.jmb.2010.09.029

33

638   64.   Haen KM, Pett W, Lavrov D V. Parallel loss of nuclear-encoded mitochondrial
639   aminoacyl-tRNA synthetases and mtDNA-encoded tRNAs in Cnidaria. Mol Biol Evol.
640   United States; 2010;27: 2216–2219. doi:10.1093/molbev/msq112

641   65.   Rea SL, Graham BH, Nakamaru-Ogiso E, Kar A, Falk MJ. Bacteria, yeast, worms, and
642   flies: exploiting simple model organisms to investigate human mitochondrial diseases.
643   Dev Disabil Res Rev. United States; 2010;16: 200–218. doi:10.1002/ddrr.114

644   66.   Kuzmenko A V, Levitskii SA, Vinogradova EN, Atkinson GC, Hauryliuk V, Zenkin N, et
645   al. Protein biosynthesis in mitochondria. Biochemistry (Mosc). United States; 2013;78:
646   855–866. doi:10.1134/S0006297913080014

647   67.   Zhao J, Lendahl U, Nister M. Regulation of mitochondrial dynamics: convergences and
648   divergences between yeast and vertebrates. Cell Mol Life Sci. Switzerland; 2013;70:
649   951–976. doi:10.1007/s00018-012-1066-6

650   68.   Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat
651   Rev Genet. England; 2007;8: 353–367. doi:10.1038/nrg2091

652   69.   Lin C-Y, Chiang C-Y, Tsai H-J. Zebrafish and Medaka: new model organisms for modern
653   biomedical research. J Biomed Sci. England; 2016;23: 19. doi:10.1186/s12929-016-0236-
654   5

655   70.   Blanco-Sanchez B, Clement A, Phillips JB, Westerfield M. Zebrafish models of human
656   eye and inner ear diseases. Methods Cell Biol. United States; 2017;138: 415–467.
657   doi:10.1016/bs.mcb.2016.10.006

658   71.   Kim MJ, Kang KH, Kim C-H, Choi S-Y. Real-time imaging of mitochondria in transgenic

659   zebrafish expressing mitochondrially targeted GFP. Biotechniques. England; 2008;45:

660   331–334. doi:10.2144/000112909

661   72.   Flinn L, Mortiboys H, Volkmann K, Koster RW, Ingham PW, Bandmann O. Complex I

662   deficiency and dopaminergic neuronal cell loss in parkin-deficient zebrafish (Danio rerio).

663   Brain. England; 2009;132: 1613–1623. doi:10.1093/brain/awp108

664   73.   Song Y, Selak MA, Watson CT, Coutts C, Scherer PC, Panzer JA, et al. Mechanisms

665   underlying metabolic and neural defects in zebrafish and human multiple acyl-CoA

666   dehydrogenase deficiency (MADD). PLoS One. United States; 2009;4: e8329.

667   doi:10.1371/journal.pone.0008329

668   74.   Plucinska G, Paquet D, Hruscha A, Godinho L, Haass C, Schmid B, et al. In vivo imaging

669   of disease-related mitochondrial dynamics in a vertebrate model system. J Neurosci.

670   United States; 2012;32: 16203–16212. doi:10.1523/JNEUROSCI.1327-12.2012

671   75.   Steele SL, Prykhozhij S V, Berman JN. Zebrafish as a model system for mitochondrial

672   biology   and   diseases.   Transl   Res.   United   States;   2014;163:   79–98.

673   doi:10.1016/j.trsl.2013.08.008

674   76.   Byrnes J, Ganetzky R, Lightfoot R, Tzeng M, Nakamaru-Ogiso E, Seiler C, et al.

675   Pharmacologic modeling of primary mitochondrial respiratory chain dysfunction in

676   zebrafish. Neurochem Int. England; 2017; doi:10.1016/j.neuint.2017.07.008

677   77.   Pinho BR, Santos MM, Fonseca-Silva A, Valentao P, Andrade PB, Oliveira JMA. How

678   mitochondrial dysfunction affects zebrafish development and cardiovascular function: an

679   in vivo model for testing mitochondria-targeted drugs. Br J Pharmacol. England;

680   2013;169: 1072–1090. doi:10.1111/bph.12186

681   78.   Polyak E, Ostrovsky J, Peng M, Dingley SD, Tsukikawa M, Kwon YJ, et al. N-

682   acetylcysteine and vitamin E rescue animal longevity and cellular oxidative stress in pre-

683   clinical models of mitochondrial complex I disease. Mol Genet Metab. United States;

684   2018;123: 449–462. doi:10.1016/j.ymgme.2018.02.013

685   79.   Ma AC, McNulty MS, Poshusta TL, Campbell JM, Martinez-Galvez G, Argue DP, et al.

686   FusX: A Rapid One-Step Transcription Activator-Like Effector Assembly System for

687   Genome   Science.   Hum   Gene   Ther.   United   States;   2016;27:   451–463.

688   doi:10.1089/hum.2015.172

689   80.   Clark KJ, Balciunas D, Pogoda H-M, Ding Y, Westcot SE, Bedell VM, et al. In vivo

690   protein trapping produces a functional expression codex of the vertebrate proteome. Nat

691   Methods. United States; 2011;8: 506–515. doi:10.1038/nmeth.1606

692   81.   Neff KL, Argue DP, Ma AC, Lee HB, Clark KJ, Ekker SC. Mojo Hand, a TALEN design

693   tool for genome editing applications. BMC Bioinformatics. England; 2013;14: 1.

694   doi:10.1186/1471-2105-14-1

695   82.   Clark KJ, Geurts AM, Bell JB, Hackett PB. Transposon vectors for gene-trap insertional

696   mutagenesis   in   vertebrates.   Genesis.   United   States;   2004;39:   225–233.

697   doi:10.1002/gene.20049

698   83.   Clark KJ, Argue DP, Petzold AM, Ekker SC. zfishbook: connecting you to a world of

699   zebrafish   revertible   mutants.   Nucleic   Acids   Res.   England;   2012;40:   D907-11.

700   doi:10.1093/nar/gkr957

701   84.   Sharma LK, Lu J, Bai Y. Mitochondrial respiratory complex I: structure, function and

702   implication in human diseases. Curr Med Chem. United Arab Emirates; 2009;16: 1266–

703   1277.

704   85.   Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial

705   electron transport chain. J Neurochem. England; 2002;80: 780–787.

706   86.   King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous

707   mitochondria by complementation. Science. United States; 1989;246: 500–503.

708   87.   Koga Y, Davidson M, Schon EA, King MP. Analysis of cybrids harboring MELAS

709   mutations in the mitochondrial tRNA(Leu(UUR)) gene. Muscle Nerve Suppl. United

710   States; 1995;3: S119-23.

711   88.   Vithayathil SA, Ma Y, Kaipparettu BA. Transmitochondrial cybrids: tools for functional

712   studies of mutant mitochondria. Methods Mol Biol. United States; 2012;837: 219–230.

713   doi:10.1007/978-1-61779-504-6_15

714   89.   Wilkins HM, Carl SM, Swerdlow RH. Cytoplasmic hybrid (cybrid) cell lines as a practical

715   model   for   mitochondriopathies.   Redox   Biol.   Netherlands;   2014;2:   619–631.

716   doi:10.1016/j.redox.2014.03.006

717   90.   Bedell VM, Wang Y, Campbell JM, Poshusta TL, Starker CG, Krug RG 2nd, et al. In vivo

718   genome editing using a high-efficiency TALEN system. Nature. England; 2012;491: 114–

719   118. doi:10.1038/nature11537

720   91.   Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, et al. Efficient genome

721   editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol. United States; 2013;31:

722   227–229. doi:10.1038/nbt.2501

723   92.   Jiang   W,   Zhou   H,   Bi   H,   Fromm   M,   Yang   B,   Weeks   DP.   Demonstration   of

724   CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco,

725   sorghum and rice. Nucleic Acids Res. England; 2013;41: e188. doi:10.1093/nar/gkt780

726 93. Fujihara Y, Ikawa M. CRISPR/Cas9-based genome editing in mice by single plasmid

727   injection. Methods Enzymol. United States; 2014;546: 319–336. doi:10.1016/B978-0-12-

728   801185-0.00015-5

729 94. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for

730   genome   engineering.   Cell.   United   States;   2014;157:   1262–1278.

731   doi:10.1016/j.cell.2014.05.010

732 95. Zhang F, Wen Y, Guo X. CRISPR/Cas9 for genome editing: progress, implications and

733   challenges. Hum Mol Genet. England; 2014;23: R40-6. doi:10.1093/hmg/ddu125

734 96. Hashimoto M, Bacman SR, Peralta S, Falk MJ, Chomyn A, Chan DC, et al. MitoTALEN:

735   A General Approach to Reduce Mutant mtDNA Loads and Restore Oxidative

736   Phosphorylation Function in Mitochondrial Diseases. Mol Ther. United States; 2015;23:

737   1592–1599. doi:10.1038/mt.2015.126

738 97. Niu D, Wei H-J, Lin L, George H, Wang T, Lee I-H, et al. Inactivation of porcine

739   endogenous retrovirus in pigs using CRISPR-Cas9. Science. United States; 2017;357:

740   1303–1307. doi:10.1126/science.aan4187

741 98. Meimaridou E, Kowalczyk J, Guasti L, Hughes CR, Wagner F, Frommolt P, et al.

742   Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial

743   glucocorticoid   deficiency.   Nat   Genet.   United   States;   2012;44:   740–742.

744   doi:10.1038/ng.2299

745 99. Bainbridge MN, Davis EE, Choi W-Y, Dickson A, Martinez HR, Wang M, et al. Loss of

746 Function Mutations in NNT Are Associated With Left Ventricular Noncompaction. Circ

747 Cardiovasc Genet. United States; 2015;8: 544–552.

748 doi:10.1161/CIRCGENETICS.115.001026

749 100. Anichtchik O, Diekmann H, Fleming A, Roach A, Goldsmith P, Rubinsztein DC. Loss of

750 PINK1 function affects development and results in neurodegeneration in zebrafish. J

751 Neurosci. United States; 2008;28: 8199–8207. doi:10.1523/JNEUROSCI.0979-08.2008

752 101. Fett ME, Pilsl A, Paquet D, van Bebber F, Haass C, Tatzelt J, et al. Parkin is protective

753 against proteotoxic stress in a transgenic zebrafish model. PLoS One. United States;

754 2010;5: e11783. doi:10.1371/journal.pone.0011783

755 102. Sallinen V, Kolehmainen J, Priyadarshini M, Toleikyte G, Chen Y-C, Panula P.

756 Dopaminergic cell damage and vulnerability to MPTP in Pink1 knockdown zebrafish.

757 Neurobiol Dis. United States; 2010;40: 93–101. doi:10.1016/j.nbd.2010.06.001

758 103. Xi Y, Ryan J, Noble S, Yu M, Yilbas AE, Ekker M. Impaired dopaminergic neuron

759 development and locomotor function in zebrafish with loss of pink1 function. Eur J

760 Neurosci. France; 2010;31: 623–633. doi:10.1111/j.1460-9568.2010.07091.x

761 104. Kumar MG, Rowley S, Fulton R, Dinday MT, Baraban SC, Patel M. Altered Glycolysis

762 and Mitochondrial Respiration in a Zebrafish Model of Dravet Syndrome. eNeuro. United

763 States; 2016;3. doi:10.1523/ENEURO.0008-16.2016

764 105. Baraban SC, Dinday MT, Hortopan GA. Drug screening in Scn1a zebrafish mutant

765 identifies clemizole as a potential Dravet syndrome treatment. Nat Commun. England;

766 2013;4: 2410. doi:10.1038/ncomms3410

767   106.   Grone BP, Qu T, Baraban SC. Behavioral Comorbidities and Drug Treatments in a
768   Zebrafish scn1lab Model of Dravet Syndrome. eNeuro. United States; 2017;4.
769   doi:10.1523/ENEURO.0066-17.2017

770   107.   Guernsey DL, Jiang H, Campagna DR, Evans SC, Ferguson M, Kellogg MD, et al.
771   Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic
772   autosomal recessive congenital sideroblastic anemia. Nat Genet. United States; 2009;41:
773   651–653. doi:10.1038/ng.359

774   108.   Bronnimann D, Annese T, Gorr TA, Djonov V. Splitting of circulating red blood cells as
775   an in vivo mechanism of erythrocyte maturation in developing zebrafish, chick and mouse
776   embryos. J Exp Biol. England; 2018;221. doi:10.1242/jeb.184564

777   109.   Berman J, Hsu K, Look AT. Zebrafish as a model organism for blood diseases. Br J
778   Haematol. England; 2003;123: 568–576.

779   110.   Danilova N, Sakamoto KM, Lin S. Ribosomal protein S19 deficiency in zebrafish leads to
780   developmental abnormalities and defective erythropoiesis through activation of p53
781   protein family. Blood. United States; 2008;112: 5228–5237. doi:10.1182/blood-2008-01-
782   132290

783   111.   Butler MG, Gore A V, Weinstein BM. Zebrafish as a model for hemorrhagic stroke.
784   Methods Cell Biol. United States; 2011;105: 137–161. doi:10.1016/B978-0-12-381320-
785   6.00006-0

786   112.   Kulkeaw K, Sugiyama D. Zebrafish erythropoiesis and the utility of fish as models of
787   anemia. Stem Cell Res Ther. England; 2012;3: 55. doi:10.1186/scrt146

788   113. Kretz CA, Weyand AC, Shavit JA. Modeling Disorders of Blood Coagulation in the

789   Zebrafish. Curr Pathobiol Rep. United States; 2015;3: 155–161. doi:10.1007/s40139-015-

790   0081-3

791   114. Avagyan S, Zon LI. Fish to Learn: Insights into Blood Development and Blood Disorders

792   from Zebrafish Hematopoiesis. Hum Gene Ther. United States; 2016;27: 287–294.

793   doi:10.1089/hum.2016.024

794   115. Prezant TR, Agapian J V, Bohlman MC, Bu X, Oztas S, Qiu WQ, et al. Mitochondrial

795   ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic

796   deafness. Nat Genet. United States; 1993;4: 289–294. doi:10.1038/ng0793-289

797   116. Zhang Q, Zhang L, Chen D, He X, Yao S, Zhang Z, et al. Deletion of Mtu1 (Trmu) in

798   zebrafish revealed the essential role of tRNA modification in mitochondrial biogenesis

799   and hearing function. Nucleic Acids Res. England; 2018;46: 10930–10945.

800   doi:10.1093/nar/gky758

801   117. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med. United

802   States; 2003;348: 2656–2668. doi:10.1056/NEJMra022567

803   118. Jain IH, Zazzeron L, Goli R, Alexa K, Schatzman-Bone S, Dhillon H, et al. Hypoxia as a

804   therapy for mitochondrial disease. Science. United States; 2016;352: 54–61.

805   doi:10.1126/science.aad9642

806   119. Bretaud S, Lee S, Guo S. Sensitivity of zebrafish to environmental toxins implicated in

807   Parkinson's disease. Neurotoxicol Teratol. United States; 2004;26: 857–864.

808   doi:10.1016/j.ntt.2004.06.014

41

809   120.   Byrnes J, Ganetzky R, Lightfoot R, Tzeng M, Nakamaru-Ogiso E, Seiler C, et al.

810   Pharmacologic modeling of primary mitochondrial respiratory chain dysfunction in

811   zebrafish. Neurochem Int. England; 2018;117: 23–34. doi:10.1016/j.neuint.2017.07.008

812   121.   Xu Y, Chen M, Hu B, Huang R, Hu B. In vivo Imaging of Mitochondrial Transport in

813   Single-Axon Regeneration of Zebrafish Mauthner Cells. Front Cell Neurosci. Switzerland;

814   2017;11: 4. doi:10.3389/fncel.2017.00004

815   122.   Dowling JJ, Arbogast S, Hur J, Nelson DD, McEvoy A, Waugh T, et al. Oxidative stress

816   and successful antioxidant treatment in models of RYR1-related myopathy. Brain.

817   England; 2012;135: 1115–1127. doi:10.1093/brain/aws036

818   123.   Pickett SB, Thomas ED, Sebe JY, Linbo T, Esterberg R, Hailey DW, et al. Cumulative

819   mitochondrial activity correlates with ototoxin susceptibility in zebrafish mechanosensory

820   hair cells. Elife. England; 2018;7. doi:10.7554/eLife.38062

821   124.   Mandal A, Pinter K, Drerup CM. Analyzing Neuronal Mitochondria in vivo Using

822   Fluorescent Reporters in Zebrafish. Front cell Dev Biol. Switzerland; 2018;6: 144.

823   doi:10.3389/fcell.2018.00144

824   125.   Liu T-L, Upadhyayula S, Milkie DE, Singh V, Wang K, Swinburne IA, et al. Observing

825   the cell in its native state: Imaging subcellular dynamics in multicellular organisms.

826   Science. United States; 2018;360. doi:10.1126/science.aaq1392

827

828 **Figures Legends:**

829 **Figure 1: Circular representation of the zebrafish (NC_002333.2) and human**

830 **mitochondrial genomes (NC_012920.1):** Both genomes share the same synteny, number of

831 genes and are nearly identical in size.

832 **Figure 2: Zebrafish Marriot mitochondrial mutant collection:** Schematic representation of

833 various mitochondrial resident pathways for which zebrafish mutants were generated. The

834 nuclear-encoded mitochondrial proteins have been illustrated according to the function they are

835 involved in mitochondrial maintenance and homeostasis. Mutants generated by gene editing are

836 depicted as grey, whereas those generated by the GBT system are depicted in pink.

837 **Figure 3: A depiction of homology of the mutants created for proteins involved in different**

838 **mitochondrial resident pathways: A-C: Complex 1 of mitochondrial respiratory chain;** A:

839 NADH: ubiquinone oxidoreductase subunit AB1 (Ndufab1b); B: NADH dehydrogenase

840 association factor 6 (Ndufaf6); C: NADH dehydrogenase iron sulfur protein 4 (Ndufs4); **D:**

841 **Complex 2 of mitochondrial respiratory chain,** Succinate dehydrogenase subunit A (Sdha),

842 subunit; **E-F: Complex 3 of mitochondrial respiratory chain;** E: Mitochondrial chaperone

843 protein BCS1 (Bcs1l); F: Ubiquinol-cytochrome c reductase subunit VIII (Uqcrq); **G-I:**

844 **Complex 4 of mitochondrial respiratory chain;** G: Protoheme IX farnesyltransferase (Cox10);

845 H: Cytochrome c oxidase IV subunit 1 (Cox4i2b); I: Surfeit locus protein 1 (Surf1); **J-K:**

846 **Complex 5 of mitochondrial respiratory chain;** J: ATP synthase subunit epsilon (Atp5e); K:

847 Transmembrane protein 70 (Tmem70); **L-M: Biosynthesis of coenzyme Q;** L: 4-

848 hydroxybenzoate polyprenyltransferase (Coq2) M: Decaprenyl-disphosphate synthase subunit 2

849 (Pdss2); **N-Q: Mitochondrial calcium homeostasis;** N: Mitochondrial calcium uptake protein 1

43

850   (Micu1); O: Calcium uniporter protein (Mcu) generated by TALEN and P; GBT; Q: Essential

851   MCU regulator (Smdt1); **R-U: Mitochondrial genome maintenance;** R: Thymidine kinase 2

852   (Tk2); S: Leucine-rich pentatricopeptide repeat-containing (Lrpprc); T: Mitochondrial

853   methionyl-tRNA formyltransferase (Mtfmt); U: 28s Ribosomal protein s18b, mitochondrial

854   (Mrps18b); **V: Mitochondrial protein import;** Mitochondrial import inner membrane

855   translocase subunit TIM50 (Timm50); **W-X: Mitochondrial metabolite synthesis;** W: 2-

856   Oxoglutarate dehydrogenase (Ogdh); X: Isocitrate dehydrogenase (Idh2).
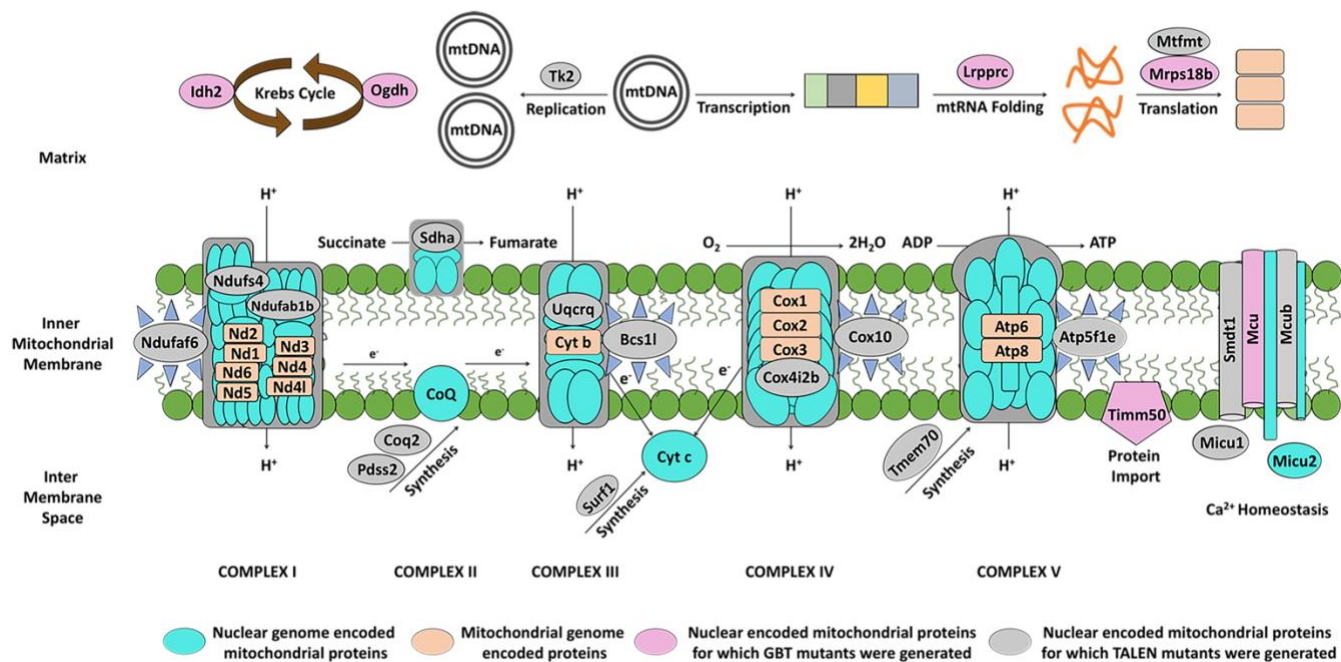
857 **Figures:**

858 **Figure 1:**
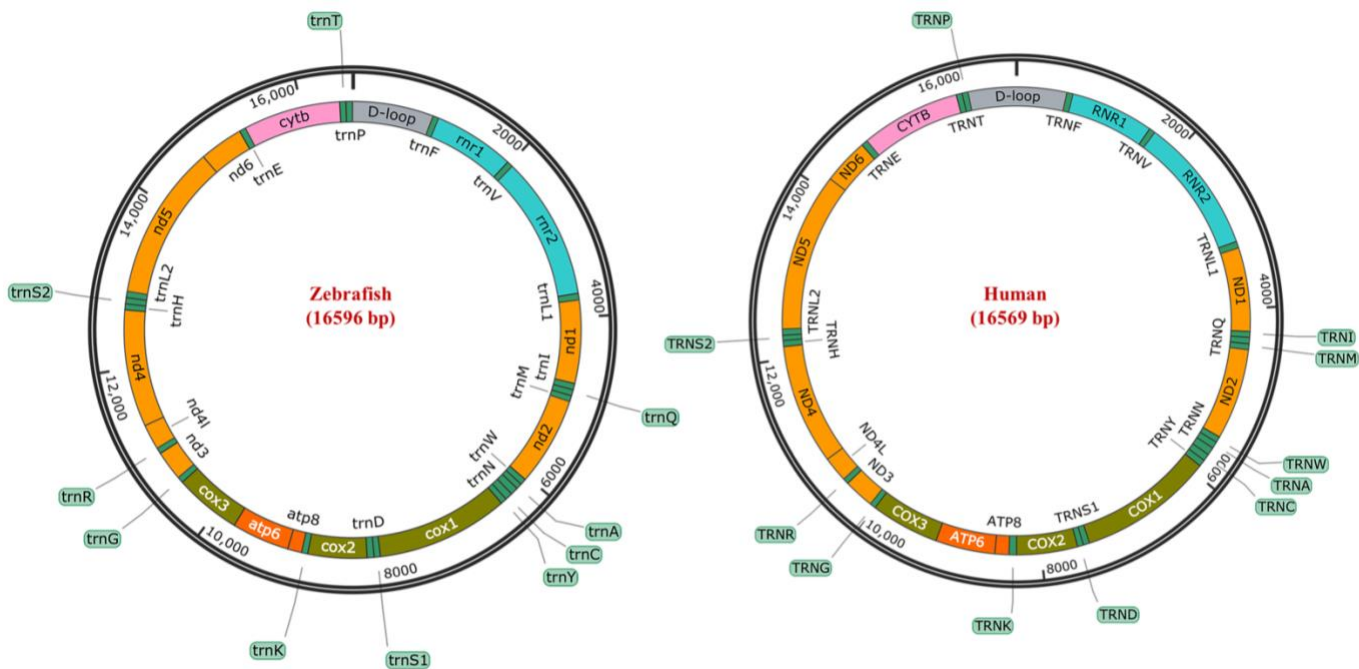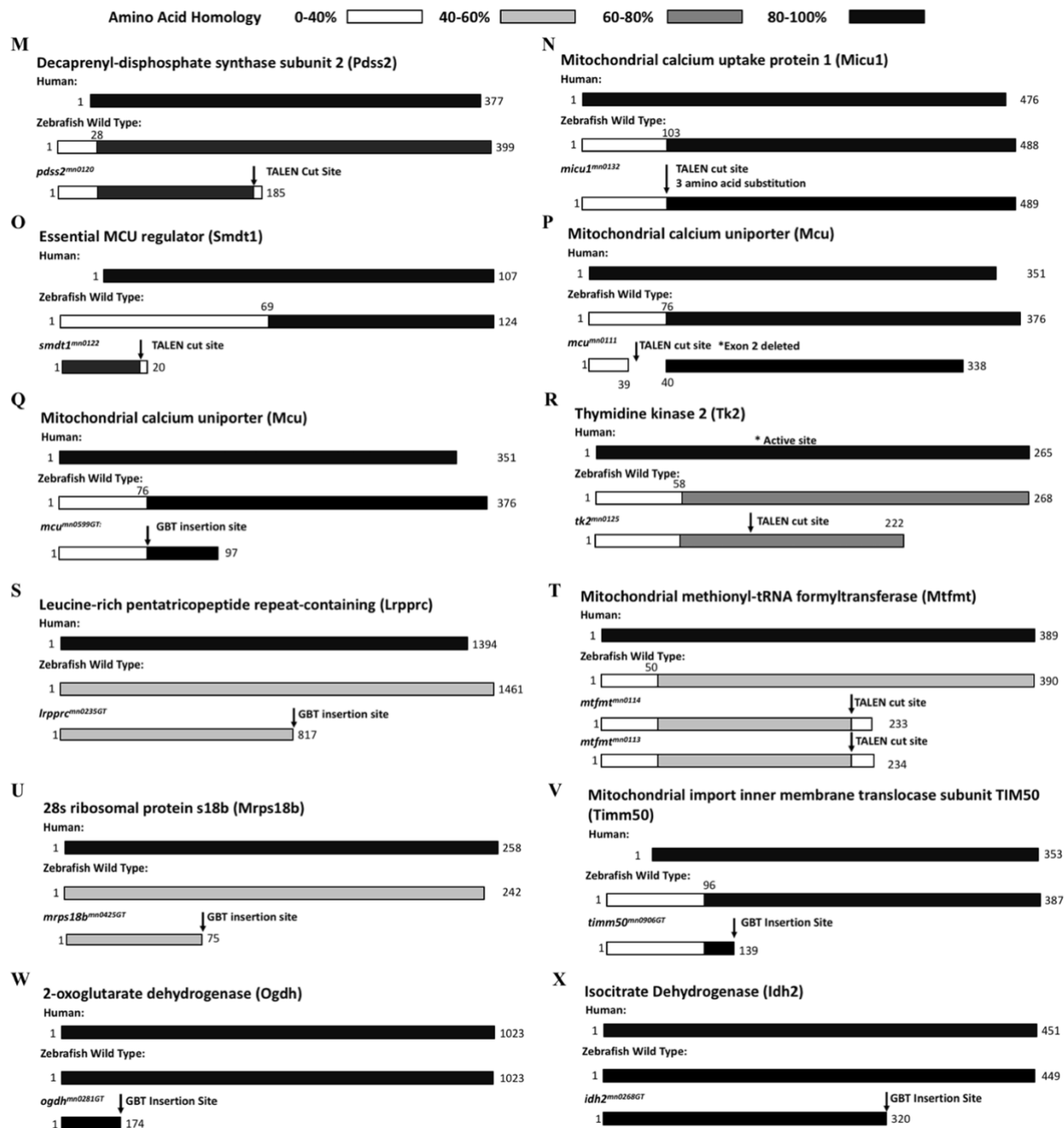

859

860

861 **Figure 2:**

862

863

864 **Figure 3:**