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Mouse models of oxidative phosphorylation dysfunction and disease

Uma D. Vempati^a, Alessandra Torraco^a, and Carlos T. Moraes^{a,b,*}

^a Department of Neurology, University of Miami, USA

^b Department of Cell Biology & Anatomy, University of Miami, USA

Abstract

Oxidative phosphorylation (OXPHOS) deficiency results in a number of human diseases, affecting at least one in 5000 of the general population. Altering the function of genes by mutations are central to our understanding their function. Prior to the development of gene targeting, this approach was limited to rare spontaneous mutations that resulted in a phenotype. Since its discovery, targeted mutagenesis of the mouse germline has proved to be a powerful approach to understand the *in vivo* function of genes. Gene targeting has yielded remarkable understanding of the role of several gene products in the OXPHOS system. We provide a “tool box” of mouse models with OXPHOS defects that could be used to answer diverse scientific questions.

Keywords

Mitochondria; Mitochondrial disease; Knockout mouse; Knockin mouse; Conditional knockout mouse

1. Introduction

The original knockout technology was developed by Mario R. Capecchi, Martin J. Evans and Oliver Smithies for which they were awarded the 2007 Nobel Prize in Physiology or Medicine. It allowed determination of the role of specific nuclear genes in development, physiology, and pathology. Several nuclear genes encoding mitochondrial proteins have now been studied by their modification in mice. The alteration of genes encoded by mtDNA continues to be a challenge. However, as described later in this article, cell biology techniques have allowed the transfer of existing mtDNA variants to fertilized eggs to generate mouse models of mtDNA-based disease.

Oxidative phosphorylation (OXPHOS) deficiency caused by mutations in either the nuclear or mitochondrial genome results in a number of human diseases [1–4]. In the mitochondria, electron transport across the five OXPHOS complexes (I–V) results in the generation of ATP. Each of the respiratory complexes are composed of several subunits, which are encoded either by the nuclear DNA or by the mitochondrial DNA (mtDNA), with the exception of complex II, which is encoded totally by the nucleus. The assembly of these complexes requires the coming together of all the constituent subunits in the mitochondria, which requires additional assembly factors and chaperones. Mutations in either mitochondrial or nuclear DNA can cause abnormalities in the OXPHOS. Since human samples are not available to conduct

*Corresponding author. Address: University of Miami Miller School of Medicine, 1095 NW 14th Terrace, Miami, FL 33136, USA. E-mail address: CMoraes@med.miami.edu (C.T. Moraes).

comprehensive mechanistic analysis, researchers have been using the mouse model to mimic these diseases. Gene targeting has yielded remarkable understanding of the role of several gene products in the OXPHOS system.

Several groups have chosen different nuclear encoded mitochondrial targeted proteins to create the respective knockout mice (summarized in Table 1) [5–7]. Here we summarize the various mouse models that exist for OXPHOS deficiency. Though it is not feasible to create knockouts of individual mtDNA encoded genes, a few groups have succeeded in propagating mutant mitochondrial DNA in mice zygotes and transferring these through the germline to generate mouse models [8–11].

As mentioned above, most of the available OXPHOS deficiency models have been created by standard homologous recombination techniques [12–14]. However, the availability of gene trap models, where a viral construct disrupts the gene of interest has also helped in the creation of new models [15]. Gene trap mutagenesis is cost-effective because of the commercial availability of mouse embryonic stem (ES) cell lines carrying insertional mutations. However, genes coding for OXPHOS components generally cause embryonic lethality when disrupted, so conditional gene disruption is usually required. Spatial and temporal regulation of gene expression can be achieved by placing the cre-recombinase under the control of a cell-specific or an inducible promoter, respectively [16].

2. Genes encoding OXPHOS structural subunits and assembly factors

2.1. Complex I

Complex I (NADH: ubiquinone oxidoreductase) is composed of 45-proteins, seven of which are encoded by the mitochondrial DNA (mtDNA) and the remaining encoded by the nuclear DNA. Complex I deficiency may cause early-onset progressive neurological disorder with lactic acidosis, Leigh syndrome, and cardiomyopathy.

2.1.1. *Ndufs4*—Mutations in *NDUFS4* subunit of complex I cause a Leigh-like phenotype in humans that result in death within 3–16 months after birth. In humans, *NDUFS4* appears to be essential for proper assembly of complex I. *Ndufs4* was inactivated in the germline by the deletion of floxed exon 2 using *Mox2-cre* transgenic mice. *Mox2* drives cre-expression throughout the epiblast following implantation. *Ndufs4* knockout (KO) mice are small, develop ataxia around 5 weeks, and die by 7 weeks. The phenotype included a retarded growth rate, lethargy, loss of motor skills, blindness, and elevated serum lactate [17]. It was surprising that the *Ndufs4* KO mice had no developmental abnormalities. The KO mice had some complex I activity in the muscle explaining why ATP levels were normal. This suggests that *NDUFS4* might play a role in the assembly, stability or activity of the complex.

2.1.2. Apoptosis inducing factor (AIF)—*AIF* is an inner mitochondrial membrane protein having a dual role in the cell. It was originally identified to be involved in apoptosis. Mutation analysis has established its primary physiological function to be maintenance of a functional OXPHOS complex I [18–20]. Conditional *AIF* KO mice were generated by inserting loxP sequences flanking exon 7. Deletion of *AIF* in skeletal muscle and the heart was achieved by crossing the *AIF* conditional KO mice with muscle-creatine kinase promoter driven-cre (*Mck-cre*) transgenic mice. Mutant animals develop severe dilated cardiomyopathy, heart failure, and skeletal muscle atrophy accompanied by lactic acidemia consistent with defects in the mitochondrial respiratory chain. Deletion of *AIF* in the liver and muscle was performed by crossing *AIF* conditional KO mice, which have been back-crossed several times with *albumin*- and *mck-cre* transgenic mice, respectively [21]. These mice exhibited increased insulin sensitivity and resistance to diabetes and obesity. Complex I activity was reduced to 60% in both knockouts, accompanied by a reduction in ATP levels.

2.2. Complex II

Complex II (succinate–cytochrome *c* reductase) is composed of four subunits (SDHA, SDHB, SDHC, and SDHD), all of which are encoded by the nuclear DNA. Mutations in *SDHA* cause Leigh syndrome, while mutations in *SDHB*, *SDHC*, and *SDHD* cause paragangliomas and pheochromocytomas.

SDHD gene encodes one of the two membrane-anchoring proteins of complex II and contains one heme and a ubiquinone binding site. *SDHD* is a tumor suppressor and mutations in which cause familial hereditary paraganglioma (PGL), a benign tumor of the carotid artery. *SDHD* knockout mice were created using a targeting vector with neomycin replacement cassette that eliminated exons 2–4 [22]. The *SDHD* null mice are embryonic lethal and the embryos die between E6.5 and E7.5 days. The heterozygous mice displayed SDHD deficiency, but not a deficiency in complex II.

2.3. Complex III

Complex III (ubiquinol–cytochrome *c* reductase) is composed of 11 subunits, of which all except cytochrome *b* are encoded by the nuclear DNA. Complex III deficiency in patients are associated with mutations in the mtDNA encoded cytochrome *b*, or the nuclear encoded *BCS1L*, a complex III assembly factor, and *UQCRB* and *UQCRCQ* subunits of complex III. Complex III deficiency can cause sporadic myopathy-rhabdomyolysis, neonatal renal tubulopathy, encephalopathy, liver failure, GRACILE and Bjornstad syndromes, and a neurological phenotype.

2.3.1. Rieske iron–sulfur protein (RISP)—RISP is an essential catalytic subunit of complex III. It contains an [2Fe–2S] cluster, elimination of which opens up an otherwise closed proton channel within the complex III. Possibly, in the normal complex, the [2Fe–2S] cluster may function as a proton-exiting gate [23]. A targeting vector consisting of the full length *RISP* gene with floxed exon 2 and floxed neomycin resistance cassette in the 3' UTR was inserted in mouse ES cells by homologous recombination. The knockin construct retained the neomycin resistance cassette in the 3' UTR that reduced RISP expression specifically in the skin [24]. Interestingly, although the knockin did not disrupt splicing or reading frame of *RISP*, it resulted in a pigment change in approximately 4–7 months old mice.

2.4. Complex IV

Complex IV (cytochrome *c* oxidase or COX) is composed of 13 subunits, three of which are encoded by mtDNA and constitute its catalytic core (COX I–III) and the remaining 10 are encoded in the nucleus. COX deficiency is associated with Leigh syndrome, severe cardiomyopathy, and complex encephalomyopathies.

2.4.1. Cytochrome c oxidase subunit VIaH (COX VIaH)—*COX VIaH* is implicated to modulate COX activity and in the dimerization of the two COX monomers. This muscle-specific subunit of COX was ablated in the mouse by inserting a neomycin cassette in the exon 2. The KO mice exhibited COX deficiency (reduced to 23%) and cardiac dysfunction as a consequence of impaired ventricular filling or diastolic dysfunction [25].

2.4.2. Cytochrome c oxidase subunit 10 (COX10)—This gene codes for a protoheme: heme-O-farnesyl transferase required for the synthesis of heme *a*, a prosthetic group in the catalytic core of COX [26,27]. Mutations in *COX10* have been found in patients with leukodystrophy and tubulopathy, anemia, Leigh syndrome, and fatal infantile hypertrophic cardiomyopathy. In order to create a conditional knockout mouse model, loxP sites were introduced into exon 6 of *COX10* gene by homologous recombination. The *COX10* gene was deleted in a tissue-specific manner, by crossing the conditional KO mouse with mice expressing

either *myosin light chain1f* (*Mlc-1f*), *albumin*-, or *calcium/calmodulin-dependent protein kinase II- α -cre* (*CamKII α -cre*) to delete *COX10* in the skeletal muscle, liver, and postnatal neurons in the central nervous system (CNS), respectively. In addition, the gene was deleted in cultured skin fibroblasts by stable transfection with a constitutive *cre*-recombinase plasmid. The KO fibroblasts were OXPHOS deficient and lacked both the complexes I and IV [28]. Deletion of *COX10* gene in the skeletal muscle resulted in a slowly progressing myopathy phenotype starting at 3 months of age associated with COX deficiency [29]. The liver KO mice exhibit mitochondrial hepatopathy between ages 23 and 56 days, with liver dysfunction, severe COX deficiency, mitochondrial proliferation and lipid accumulation [30]. Starting at around 4 months of age, mice with *COX10* KO in the CNS neurons exhibited behavioral abnormalities including biphasic hyper- and hypo-activities, compulsive devouring behavior, tail vibration, and excessive sensitivity to external stimuli. These changes in behavior were accompanied by shrinkage of the forebrain, with a reduced lifespan of 8–10 months of age. COX activity in the hippocampus and cortex was reduced to 30% of the controls [31].

The *CamKII α -Cre* driven *COX10* KO mouse was used to evaluate the contribution of COX activity to the formation and accumulation of amyloid plaques in a mouse model of Alzheimer's disease. Contrary to the initial expectations, brains deficient in COX activity showed reduced β -amyloid accumulation as well as reduced oxidative damage [31].

2.4.3. Cytochrome c (cyt c)—Cyt *c* shuttles electrons from OXPHOS complex III to complex IV. However, when it is released from mitochondria, it stimulates cell death by interacting with Apaf-1 and recruiting and activating pro-caspase-9. In mammals, two different isoforms of cyt *c* exist (somatic: cyt *c_s* and testis: cyt *c_t*) which unlike in yeast, are expressed in different compartments: the cyt *c_s* is expressed in all the tissues, while, cyt *c_t* is the sole cyt *c* expressed in postmeiotic male germ cells, including mature spermatozoa. Somatic cyt *c* KO was created by replacing the two coding exons with a neomycin resistance cassette. Deletion of the somatic cyt *c* is embryonic lethal and embryos die during mid-gestation [32], but derived embryonic fibroblasts were shown to be resistant to intrinsic apoptosis inducers. A mouse model with a disrupted testis cyt *c* gene created by insertional inactivation of exon 3 had also been reported [33]. Besides reduction in male fertility and early testicular atrophy, the cyt *c_t* KO mouse showed no other major abnormal phenotype. In an attempt to delineate the respiratory and cell death functions of cyt *c*, Hao et al. [34] developed a mouse with a mutated cyt *c* gene knocked in. The mutation (K72A) affected apoptosis but did not appear to have a major effect on respiration. The mutant mice were born at a lower frequency than expected due to partial embryonic lethality and they do not survive long after birth because of abnormal brain development (exencephaly). This study however did not assess the OXPHOS activity in the mice. They demonstrated that the apoptotic function of cyt *c* is required for normal brain development and lymphocyte homeostasis in mice.

We developed conditional cyt *c* KO mice that lacked both the somatic and testis isoforms of the gene to understand the role of this protein in postnatal animals [35]. In order to obtain the cyt *c* double KO mice, mice harboring a heterozygous somatic cyt *c* KO allele (cyt *c_s^{+/-}* [obtained from Jackson laboratories: strain name, B6; 129-Cycstm1Wlm/J]) were crossed with mice harboring a homozygous testis cyt *c* KO allele (Fig. 1A). The embryonic lethal phenotype of cyt *c_s^{-/-}* was rescued by introducing a ubiquitously expressed somatic cyt *c* transgene flanked by loxP sites (cyt *c^{fllox/o}*; Fig. 1B). Crosses of one of the transgenic animals with the cyt *c_t^{-/-}* cyt *c_s^{+/-}* animals gave rise to F1 mice that when backcrossed produced mice with the cyt *c_t^{-/-}* cyt *c_s^{-/-}* cyt *c^{fllox/o}* genotype, demonstrating that the transgene was able to rescue the embryonic lethal phenotype of the cyt *c_s* KO. A fibroblast line was derived from a 1 month-old homozygous cyt *c_s^{-/-}* cyt *c_t^{-/-}* mouse harboring the floxed cyt *c* transgene. The transgene was deleted in culture by infection with an adenovirus expressing cre-recombinase. Cyt *c* null cells thus generated lacked respiration and were resistant to both the extrinsic and intrinsic

apoptotic stimuli. Some OXPHOS complexes were reduced in the null cells (Vempati and Moraes, unpublished observations).

2.4.4. *Surf1*—*Surf1* is a COX assembly factor that functions as an auxiliary chaperone-like factor, involved in the early assembly steps. Presence of a mutant *Surf1* protein causes accumulation of COX sub-complexes, reduction in fully assembled COX and the development of Leigh syndrome (LS). *Surf1* was deleted by replacing exons 5–7 with neomycin resistance gene. The KO mice displayed a high early embryonic lethality. The few surviving pups displayed early mortality due to COX deficiency and mitochondrial disease [36]. A conditional *Surf1* KO mouse was made by inserting floxed neomycin resistance cDNA into exon 7 of *Surf1* [37]. *Surf1* KO mice were generated by crossing the conditional KO mouse with a transgenic *cre* mouse that constitutively expresses the cre-recombinase. The KO mice exhibited COX deficiency which is milder than in humans harboring the *Surf1* mutation. Surprisingly, the KO mice displayed increased longevity and were also resistant to kainic acid-induced neurodegeneration.

3. Genes that regulate mtDNA

The mitochondrial DNA (mtDNA) is a multicopy genome of 16.5 kb, which is transmitted only through the maternal line. The mtDNA is a double-stranded circle which encodes 13 polypeptides, 22 transfer RNAs, and two ribosomal RNAs. In somatic cells, 6–10 mtDNA molecules are packed into nucleoids along with several different proteins. The nucleoids are attached to the mitochondrial inner membrane close to the OXPHOS system and the site of reactive oxygen species (ROS) production [38].

3.1. Mitochondrial transcription factor A (TFAM)

TFAM is a high mobility group (HMG)-box protein, which binds mtDNA and helps package it into nucleoids. Mutation analysis has established its primary physiological function to be the maintenance of mtDNA. It is also indispensable for mitochondrial transcription, where it functions as a basal transcription factor. The first *Tfam* KO was created by floxing exons 6 and 7 and deleting with a constitutively active β -actin-*cre*. The resulting KO mice were embryonic lethal and survive only until E8–E10.5. However, the heterozygous mice were born at normal frequency but exhibited reduced levels of *Tfam* expression, mtDNA and mitochondrial encoded proteins [39]. Subsequently, *Tfam* was specifically deleted in heart and skeletal muscle by crossing the conditional KO mouse (above) with *muscle creatinine kinase-cre* (*Ckmm-cre*) transgenic mouse that expresses cre-recombinase from E13. The KO mice developed a mosaic respiratory chain deficiency in the heart, dilated cardiomyopathy with atrioventricular conduction blocks. The KO mice had reduced levels of *Tfam* protein, mtDNA and mitochondrial encoded proteins, complexes I and IV activities in the skeletal muscle and more prominently in the heart [40].

Tfam was specifically deleted in heart by crossing the *Tfam*^{loxP} mouse (above) with the *myosin heavy chain* (*Myhca*)-*cre* transgenic mouse that expresses cre-recombinase from E8 [41]. The KO mice developed mitochondrial cardiomyopathy embryonically. 75% of the KO mice died in the neonatal period, but 25% continued to live up to 3 months, though they too develop dilated cardiomyopathy with atrioventricular conduction blocks. The reason for the longer survival of 25% of KO mice is attributed to the presence of certain modifying genes. The KO survivors showed little differences in the mtDNA copy number, mitochondrially encoded ATP8 protein and complex IV activity from littermate controls, which might explain their escape from neonatal mortality. *Tfam* was specifically deleted in skeletal muscle by crossing the *Tfam*^{loxP} mouse (above) with the *Mlc1f-cre* transgenic mouse that expresses cre-recombinase from E10 [42]. The KO mouse exhibits mitochondrial myopathy having ragged red fibers, abnormal mitochondria, progressive loss of OXPHOS function, and reduced muscle-

force. The onset of myopathy phenotype is between 3 and 4 months of age and they survive until 4–5 months. As in the other *Tfam* KO models, mtDNA levels are reduced in this KO starting at 1 month of age.

Tissue-specific disruption of *Tfam* in pancreatic- β cells was achieved by crossing *Tfam*^{loxP} mouse (above) with *insulin-2 cre* (*TgN ins2-cre*) transgenic mouse. The KO mice developed diabetes from around 5 weeks of age with decreased blood insulin concentration. They displayed mtDNA depletion, OXPHOS deficiency and loss of insulin-producing β -cells in the islets [43].

Tfam was also selectively deleted in the forebrain neurons by crossing the *Tfam*^{loxP} mouse (above) with *CamKII-cre* transgenic mouse [44]. The KO mouse was called mitochondrial late-onset neurodegeneration (MILON) mouse since they did not have overt phenotype until 5 months of age and die soon after. They displayed mtDNA and mitochondrial RNA depletion, OXPHOS deficiency, cell death, axonal degeneration, and gliosis in hippocampus and neocortex. Recently, the relationship between respiratory chain deficiency and the onset of mitochondrial disease symptoms was investigated by an embryo aggregation procedure [45]. Mouse chimeras were generated from *LacZ* and MILON embryos to obtain offspring having a mixture of normal and respiratory chain-deficient neurons in cerebral cortex. A low proportion (>20%) of respiratory chain-deficient neurons in the forebrain cause symptoms, whereas only a high proportion (>60–80%) cause premature death of the animal. *Tfam* was specifically deleted in mid-brain dopaminergic (DA) neurons by crossing the *Tfam*^{loxP} mouse (above) with *dopamine transporter-cre* (*DAT-cre*) transgenic mouse that expresses cre-recombinase from E10 [46]. The KO mice have reduced mtDNA levels, OXPHOS deficiency, which results in an adult onset of parkinsonism characterized by impaired motor function, intraneuronal inclusions, and dopamine neuron death.

3.2. DNA polymerase- γ (POLG)

Polg is the only DNA polymerase found in the mitochondria, and is responsible for both replication and repair. It is a heterodimeric enzyme containing a Pol I-like catalytic core (PolgA) and an accessory subunit. Defects in POLG result in mtDNA deletion and depletion syndromes such as progressive external ophthalmoplegia (PEO), Alper's syndrome, parkinsonism, and spinocerebellar ataxia. A knockin mutant of PolgA was created by substituting a critical aspartate residue (D257A) in the proofreading exonuclease domain two encoded by exon 3. The targeting construct containing the mutant and floxed exon 3 was inserted by homologous recombination and [47]. The knockin mice termed “mtDNA-mutator” mice lacked exonuclease activity, but had normal DNA polymerase activity. They developed 3- to 5-fold increase in point mutations and increased deletions in mtDNA with progressive respiratory chain deficiency. This resulted in the mutant mice developing a premature aging phenotype, (around 25 weeks) characterized by kyphosis (curvature of the spine), weight loss, alopecia, reduced fat content, anemia, osteoporosis, reduced fertility, and lifespan (die around 48 weeks).

Another group created a similar mutant of *PolgA* [48] and also found increased mtDNA mutations (3- to 8-fold increase) and premature aging phenotype beginning at 9 months. The ageing phenotype was characterized by thymic involution, testicular atrophy, loss of bone mass, loss of intestinal crypts, decrease in red blood cells and weight loss. Interestingly, the increased mtDNA mutations in the mutator mice do not result in an increase in reactive oxygen species (ROS) or oxidative stress. The premature aging was not caused by a decrease in cell proliferation, but rather due to an increase in the cell death. The lack of increased ROS in the mutator mice was later confirmed by a different study [49]. Although it is still unclear what drives the premature aging phenotype, it has been suggested that it is the level of large scale deletions.

PolgA knockout mice were generated by crossing floxed *PolgA* mouse (above) with the constitutively expressing β -actin *cre* transgenic mouse [50]. The KO mice were embryonic lethal and die around E7.5–E8.5 days due to severe mtDNA depletion. Even the heterozygous mice have reduced levels of *PolgA* and display mtDNA depletion.

A neuron-specific mutant *PolgA* transgenic mouse was created [51]. The aspartate at position 181 in the *PolgA* cDNA was replaced with alanine and cloned down stream of *CaMKII α* promoter. The transgenic mice accumulated mtDNA point mutations and deletions in the brain accompanied by mood disorders reminiscent of chronic progressive external ophthalmoplegia (CPEO) patients and bipolar disorder. Perturbation of calcium homeostasis was proposed to mediate the bipolar disorder-like behavioral in the mutant mice [52].

3.3. Adenine nucleotide transporter 1 (Ant1)

Ant1 is an inner mitochondrial membrane protein that transports ATP into the cell. In humans, mutations in *Ant1* result in CPEO. *Ant1* is expressed predominantly in skeletal muscle, heart and brain. *Ant1* KO mice were created by insertional inactivation by homologous recombination [53]. The targeting vector encompassed exon 4 of the *Ant1* gene and the neomycin resistance gene. The KO mice display mitochondrial myopathy with ragged red fibers, state III (ADP stimulated) respiration deficiency, and cardiomyopathy. The KO mice were further shown to have an increased expression of certain nuclear and mitochondrial genes encoding OXPHOS components, mitochondrial tRNA, and rRNA genes mimicking that seen in patients with mitochondrial diseases [54].

3.4. Twinkle

It is a mtDNA helicase, mutations in which cause multiple mtDNA deletions and an adult onset PEO. Two transgenic mice were created that harbored *twinkle* mutations reported in patients. One of the mutant mice carried a single amino acid substitution (A360T) in the cDNA, while another had an in-frame duplication of amino acids 353–365. The mutant mice accumulated mtDNA deletions in brain and heart, but not in the skeletal muscle. They developed minor COX deficiency in muscle fibers and brain [55].

3.5. Thymidine kinase 2 (TK2)

The nucleotides required for mtDNA replication are either imported from the cytosol or generated by phosphorylation of deoxyribonucleosides in the mitochondrial matrix. Phosphorylation of deoxyribonucleosides is catalyzed by the mitochondrial deoxyribonucleoside kinases thymidine kinase 2 (TK2) or deoxyguanosine kinase (DGUOK). TK2 is a pyrimidine deoxyribonucleoside kinase that phosphorylates deoxythymidine and deoxycytidine. Deficiency of mitochondrial DGUOK or TK2 causes mtDNA depletion syndromes in humans. *Tk2* was deleted in mice by replacing exon 4 and part of exon 5 with neomycin resistance gene [56]. The KO mice have progressive mtDNA depletion in several tissues by 2 weeks of age, without an increase in mutations; exhibited hypothermia and shivering. The *Tk2* KO mice died within a few weeks after birth. A knockin mouse with a pathogenic mutation was also characterized [57]. Homozygous *Tk2* mutant mice developed rapidly progressive weakness after age 10 days and died between ages 2 and 3 weeks. These mice had encephalomyelopathy with prominent vacuolar changes in the anterior horn of the spinal cord [57].

3.6. Cytosolic p53-inducible ribonucleotide reductase small subunit (RRM2B)

Bourdon and colleagues found that misense, nonsense, splice site and frameshift mutations in the RRM2B gene in four independent families with severe infantile disorders and mtDNA depletion [58]. Ribonucleotide reductase is a heterotetrameric enzyme responsible for de novo

modification of ribonucleoside 5'-diphosphates into deoxyribonucleoside 5'-diphosphates, a step essential for DNA synthesis. A mouse KO for the RRM2B gene showed a kidney dysfunction and a reduction in mtDNA levels [58].

3.7. Mitochondrial transcription termination factor 3 (MTERF3)

The basal mitochondrial transcription machinery is composed of three proteins, namely, mitochondrial RNA polymerase (POLRMT), TFAM, and mitochondrial transcription factor B1 or B2 (TFB1M or TFB2M) [59]. Members of the group of mitochondrial transcription termination factors are DNA-binding proteins including MTERF (also called MTERF1) that are implicated in the regulation of mitochondrial transcription. *Mterf3* knockout was created by crossing mice having floxed exon 2 of the *Mterf3* gene with the constitutively expressing β -actin-cre mice. The KO mice were embryonic lethal. Specific deletion of *Mterf3* in the heart was achieved by crossing the floxed mice (above) with mice expressing cre-recombinase in heart and skeletal muscle (*Ckmm-cre*). The KO mice had mitochondrial cardiomyopathy, and a shorter life span of 18 weeks. They displayed increased transcription initiation on both strands of mtDNA, decreased steady state levels of promoter-distal transcripts, and mitochondrial dysfunction in the heart [60]. This suggests that MTERF3 has a role in mitochondrial transcriptional repression.

4. Genes indirectly related to OXPHOS

4.1. Superoxide dismutase2 (SOD2)

SOD2 encodes the Mn (Manganese)-SOD, which is an intramitochondrial free radical scavenging enzyme that is a cell's defense against superoxide produced as a byproduct of oxidative phosphorylation. Defects in *SOD2* activity are found in ovarian cancer and type I diabetes. Several groups created the *SOD2* knockout mice. In the CD1 background, *SOD2* KO mice were created by replacing the exon 3, which encodes amino acids involved in homodimerization, tetramer formation and Mn-binding with a neomycin resistance cassette. The KO mice die within the first 10 days of life with a dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle, and metabolic acidosis. They display a severe reduction in succinate dehydrogenase (complex II) and aconitase (a TCA cycle enzyme) activities predominantly in the heart [61]. In the B6 background, *SOD2* KO mice were created by replacing the exons 1 and 2 with hypoxanthine phosphoribosyl-transferase (HPRT) minigene. The null mice survive up to 18 days and develop severe anemia, degeneration of neurons in the basal ganglia and brainstem, and progressive motor disturbances. The KO mice exhibit mitochondrial damage in CNS neurons and cardiac myocytes due to oxidative stress [62]. When the CD1 KO mice were backcrossed to the C57BL/6J background, more than 50% of the fetuses develop severe dilated cardiomyopathy by E15 and are embryonic lethal [63]. These differences between mice from different backgrounds suggest the existence of genetic modifiers for the manifestation of phenotype in *SOD2*-deficient mice. Interestingly, the heterozygous *SOD2* mice that had approximately half the normal levels of *SOD2* protein also exhibited oxidative stress [64,65].

Tissue specific deletion of *SOD2* in the liver was achieved by crossing *SOD2* conditional KO mice having floxed exon 3 with albumin-cre mice. The KO mice did not exhibit gross histological abnormalities or significant biochemical defects. In addition, lipid peroxidation was not higher in the KO liver [66]. This suggests that the liver dysfunction in the *SOD2*-deficient mice observed by other groups, including the heterozygous mice [67] were more due to the lack of *SOD2* in other tissues but not due to a liver-specific deficiency.

Deletion of *SOD2* in the motor neurons were achieved by crossing the *SOD2* conditional KO mice (above) with vesicular acetylcholine transporter-cre (VACHT-Cre) mice that express cre-

recombinase in cholinergic neurons in the somatomotor nuclei and medial habenular nucleus [68]. Surprisingly, null mice did not display oxidative damage in the motor neurons, but their axons were sensitized to disorganization following nerve injury.

5. Transmitochondrial mice

Mitochondrial DNA has a high mutation rate and the base substitutions cause several diseases, including myopathy, cardiomyopathy, neurological and endocrine disorders. Though it is not feasible to create knockouts of individual mitochondrially encoded genes, mutant mtDNA was introduced into mice using two techniques. In the first, enucleated cell cytoplasts were fused to embryonic stem cells, which were injected into blastocysts [11]. In the second technique, cytoplasts were directly fused to zygotes, which were transmitted through the germline [9, 10]. Hayashi's group created respiration-deficient cybrids carrying an mtDNA deletion and introduced into zygotes. Mutant mtDNA was transmitted maternally and its accumulation induced mitochondrial dysfunction in various tissues. The mutant mice showed COX deficiency and most of them died within 200 days due to renal failure. This was consistent with the presence of high amounts of mtDNA in the diseased i.e., dilated and anemic kidneys [9].

In another study, mtDNA from the NZB mice (resident to New Zealand) having a different haplotype was transmitted to the C57Bl/6 (B6) mice. The mtDNA from NZB mouse was transferred to cultured cells by fusing synaptosomes from the NZB mouse with a mtDNA deficient (p^0) cell line. After enucleation, the cybrids were electroporated into rhodamine-6 treated ES cells to create ES cell-cybrids, and transmitted through mouse B6 germ line [69]. Using this technique, mtDNA from a partially respiration-deficient cell line (mutation in *16s rRNA* gene) that was chloramphenicol resistant (CAP^R) were transmitted to the progeny. The respiratory deficient CAP^R chimeras developed ocular abnormalities, growth retardation, myopathy, and dilated cardiomyopathy [11]. Additionally, mice harboring a missense mutation in the mitochondrially encoded *COX1* gene (T6589C) were created [70] by cytoplasm fusion to ES cells. The progeny were respiration-deficient, and exhibited COX deficiency, lactic acidosis and growth retardation.

Recently, by cytoplasm fusion a mouse model was created that contains mtDNA point mutations of different severity: a pathogenic mutation in *ND6* (a complex I subunit) and a milder mutation in *COX1* [8]. The mutation in *ND6* (13885insC) inactivates complex I activity, while that in *COX1* (T6589C/V421A) reduces the activity of COX to 50%. Surprisingly, the *COX1* mutation could be faithfully transmitted to the progeny, but the *ND6* mutation, though present in the founder female was eliminated in successive generations by some filtering mechanism during oocyte maturation. The offspring harboring the *COX1* mutation developed a COX deficiency, mitochondrial myopathy, and cardiomyopathy.

6. Concluding remarks

Gene targeting has revolutionized life science and plays a key role in the development of medical therapy. Mouse models of OX-PHOS dysfunction have proved to be invaluable tools in enhancing our understanding of the mitochondrial disease. But some disease models are yet to be created. In some cases, we have to overcome the problem of embryonic and neonatal lethality of the mutant mice in order to examine if the mice faithfully reproduce the targeted disease that was. Overall, there is optimism that these models are leading us closer to a much awaited therapy.

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Fig. 1. Development of a null *cyt c* cell model. (A) illustrates the general approach used to develop a *cyt c* knockout cell line. We crossed mice with testis *cyt c* knockout with the somatic *cyt c* knockout. Backcrosses allowed the isolation of mice with appropriate alleles that were further crossed with a transgenic mouse harboring a loxP-.anked *cyt c* transgene. The structure of the transgene is depicted in (B).

Table 1

Mouse models of OXPHOS deficiencies

Complex subunit	Inactivation/transgene	cre Promoter	Phenotype	References
CI/Ndufs4	Insertion exon 2	Mox2	Ataxia, retarded growth rate, blindness	[1]
CI/AIF	Floxed exon 7	Mck	Dilated cardiomyopathy, skeletal muscle atrophy	[19]
CI/AIF	Floxed exon 7 mice back-crossed	Albumin	Insulin sensitivity, resistance to diabetes and obesity	[21]
CII/SDHD	Exons 2–4 replaced with neo gene		Embryonic lethality	[22]
CIII/RISP	Floxed exon 2		Skin pigment change	[24]
CIV/COX VIa	Insertion exon 2		Cardiac dysfunction	[25]
<i>Assembly factor</i>				
CIV/Surf1	Replacement of exons 5–7 with neo gene	Constitutive	High embryonic lethality; COX deficiency	[36]
CIV/Surf1	Neo inserted in exon 7	Constitutive	Increased longevity	[37]
CIV/COX 10	Floxed exon 6	Mlc-1f	Mitochondrial myopathy	[29]
CIV/COX 10	Floxed exon 6	Constitutive cre in fibroblasts	OXPHOS deficiency	[28]
CIV/COX 10	Floxed exon 6	Albumin	Hepatopathy	[30]
CIV/COX 10	Floxed exon 6	CamKII α	Cortical atrophy; Decreased β -amyloid plaques in COX10 deficient Alzheimer's mouse model	[31]
CIV/Cyt c_s	Exons 2 and 3 replaced by neo-cassette		Embryonic lethal	(32)
CIV/Cyt c_i	Insertional inactivation of exon 3		Testicular atrophy	[33]
CIV/Cyt c knockin	Mutant cyt c gene knockin (K72A)		Partial embryonic lethality, exencephaly	[34]
CIV/Cyt c_s , Cyt c_i , Cyt c_{Tg}	Cyt c_s : exons 2 and 3 replaced by neo-cassette; Cyt c_i : insertional inactivation of exon 3	Constitutive cre in fibroblasts	Apoptosis resistance; OXPHOS deficiency	[35]
<i>MtDNA regulator</i>				
TFAM	Floxed exons 6 and 7	β -actin cre	Embryonic lethal	[39]
TFAM	Floxed exons 6 and 7	Ckmm-cre	Dilated cardiomyopathy with atrioventricular conduction blocks	[40]
TFAM	Floxed exons 6 and 7	Myhca-cre	Dilated cardiomyopathy with atrioventricular conduction blocks	[41]
TFAM	Floxed exons 6 and 7	Insulin-2 cre	Diabetes; decreased blood insulin	[43]
TFAM	Floxed exons 6 and 7	CamKII-cre	Axonal degeneration, gliosis in forebrain	[44]
TFAM	Floxed exons 6 and 7	Mlc1f-cre	Mitochondrial myopathy	[42]
TFAM	Floxed exons 6 and 7	DAT-cre	Parkinsonism	[46]
PolgA	Point mutation in exon 3		Increased mtDNA mutations, premature aging	[47]
PolgA	Point mutation in exon 3		Increased mtDNA mutations, premature aging	[48]
PolgA	Floxed exon 3	β -actin cre	Embryonic lethal	[50]
PolgA	CaMKII α -Point mutant transgenic		Increased mtDNA mutations, mood disorders	[51]
Ant1	Insertional inactivation		Mitochondrial myopathy, cardiomyopathy	[53]
MTERF3	Floxed exon 2	β -actin-cre Ckmm-cre	Embryonic lethal Mitochondrial cardiomyopathy	[60]

Complex subunit	Inactivation/transgene	cre Promoter	Phenotype	References
Twinkle	Two mutants: A360T and duplication of aa353–365		mtDNA deletions in brain and heart	[55]
TK2	KO and KI		mtDNA depletion; Encephalomyelopathy (KI); early death (KO)	[57,71]
RRM2B	KO		mtDNA depletion; Kidney Dysfunction	[58]
SOD2	Replacement of exon 3 with neo-cassette		Dilated cardiomyopathy, accumulation of lipid	[61]
SOD2	Replacement of exons 1 and 2 with HPRT gene		Anemia, degeneration of neurons, and motor disturbances	[62]
SOD2	Floxed exon 3	Albumin-cre	No phenotype	[66]
SOD2	Floxed exon 3	VChT mice	No oxidative damage in neurons; axons sensitized to disorganization	[68]
<i>Transmitochondrial mice</i>				
Deleted mtDNA (4,696 bp deletion)	Cytoplasm fused to zygote		COX negative fibers; systemic ischemia and enlarged kidneys	[9]
CAP ^R (16S rRNA mutant)	Cybrid fused to ES cell		Respiration deficiency	[11]
COX1 gene mutant (T6589C)	Cytoplasm fused to ES cell		COX deficiency; growth retardation	[70]
COX1 gene mutant (T6589C/V421A)	Cybrid fused to ES cell		COX deficiency, mitochondrial myopathy, and cardiomyopathy.	[8]

Abbreviations: CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; neo, neomycin; KO, knockout; KI, knockin.