Chapter 10
Model Cells and Organisms in Mitochondrial Diseases

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Abstract The central role of mitochondria in cell metabolism and physiology is reflected in the diversity of functions that they perform within the cell. Alterations or imbalances in any of these cellular processes can cause mitochondrial dysfunction and disease. In this chapter we review the animal and cellular models used in the research of these diseases. We focus on genes that have been found to be mutated in patients with mitochondrial disease and comment on the potential of these models for use in identification and validation of novel therapies.

10.1 Introduction

The mitochondria present within our cells carry out important and diverse functions including oxidative phosphorylation and apoptotic cell death (Nunnari and Suomalainen 2012). Pathologies can arise from inhibition or imbalance in any of the cellular processes for which mitochondria are required. A healthy mitochondrion requires over a thousand proteins encoded by the nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) (Gorman et al. 2016). This means that there are over 1000 mutational targets from which mitochondrial dysfunction can result. Oxidative phosphorylation relies on the formation and stability of 5 multi subunit complexes

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of nuclearly and mitochondrially encoded components (Sanz and Stefanatos 2008). Accordingly, this process is particularly sensitive to mutation and as such, most mitochondrial disease patients carry mutations in genes encoding OXPHOS proteins (Lightowlers et al. 2015).

The current standard of care for mitochondrial disease patients is palliative, with interventions that mainly target specific symptoms (Viscomi et al. 2015; Lightowlers et al. 2015; Gorman et al. 2016). To facilitate the design, discovery and validation of curative treatments, a deeper understanding of disease pathogenesis is required. Predictive models, which recapitulate the clinical and molecular pleiotropy exhibited by mitochondrial disease patients will allow examination of the molecular effects of mitochondrial dysfunction. The mitochondrial research community has accumulated a collection of complementary models with just this aim.

In this chapter, we will attempt to summarise the wide range of models which have been created and used to study the effects of disrupting a variety of mitochondrial proteins, with a focus on those genes which have been found to be mutated in patients (Table 10.1). This will include *in vivo* models such as mice, zebrafish, flies and worms. Finally, we will comment on the potential of these models in investigating the pathophysiology of mitochondrial disease and the development and validation of novel therapies.

In vitro, cell culture models have been used to model the pathology of many patient derived mutations, using a variety of techniques. Most studies have used cytoplasmic hybrids (Cybrids). Cybrids allow the assessment of mtDNA alterations in a defined nuclear background. They have been used to study the bioenergetics of specific patient mutations and mtDNA heteroplasmy (Wilkins et al. 2014). Primary cells derived from patient biopsies have also been used to model mitochondrial diseases, however disease pathology is not always recapitulated in culture (Cameron et al. 2004). Finally, with the advent of induced pluripotency, the creation of stem cells from differentiated tissue also known as induced pluripotent stem cells (iPS), researchers can create patient specific iPS cells which can be differentiated into specific cell types, like neurons or muscle cells (Avior et al. 2016). This is particularly important for mitochondrial disease given the tissue specific effects of different mitochondrial syndromes. Despite the obvious limitations of an *in vitro* system, iPS cell models will be useful in validation of potential therapies. However, given the long standing and wide use of cell culture models we will focus this chapter on whole animal models of mitochondrial disease.

10.2 Mouse Models of Mitochondrial Disease

The mouse has been well established as the go to model in the study of several human diseases (Peters et al. 2007). Humans and mice share a large degree of genetic and physiological traits which make them an attractive model to study disease pathogenesis (Peters et al. 2007). In the last 20 years, technology for the

Table 10.1 Models of Mitochondrial disease

Mitochondrial disease	Gene	Model organism
CI deficiency, LS	*NDUFS4*	*M. musculus* (Kruse et al. 2008; Quintana et al. 2010, 2012)
	NDUFS6	*M. musculus* (Ke et al. 2012; Forbes et al. 2013)
	NDUFA5	*M. musculus* (Forbes et al. 2013)
	NDUFS3	*D. melanogaster* (Li 2013; Vos et al. 2012)
	NDUFAF1	*D. melanogaster* (Cho et al. 2012)
	NDUFAF6	*D. melanogaster* (Zhang et al. 2013)
	MT-ND2	*D. melanogaster* (Burman et al. 2014)
	NDUFS2	*C. elegans* (Kayser et al. 2001)
	NDUFV1	*C. elegans* (Grad and Lemire 2004)
CII deficiency	*SDHD*	*M. musculus* (Piruat et al. 2004)
	SDHB	*D. melanogaster* (Walker et al. 2006)
	SDHC	*C. elegans* (Ishii et al. 1998)
CoQ_{10} deficiency	*COQ2*	*D. melanogaster* (Fernandez-Ayala et al. 2014)
	COQ3	*D. melanogaster* (Fernandez-Ayala et al. 2014)
	COQ5	*D. melanogaster* (Fernandez-Ayala et al. 2014)
	COQ7	*M. musculus* (Wang et al. 2015), Drosophila (Fernandez-Ayala et al. 2014), C. elegans
	COQ8	*D. melanogaster* (Fernandez-Ayala et al. 2014)
	COQ9	*M. musculus* (Garcia-Corzo et al. 2013), Drosophila (Fernandez-Ayala et al. 2014)
	COQ10	*D. melanogaster* (Fernandez-Ayala et al. 2014)
	PDSS1	*D. melanogaster* (Grant et al. 2010)
	PDSS2	*M. musculus* (Hallman et al. 2006, Peng et al. 2008)
Cyt c deficiency	*CYCS*	*M. musculus* (Li et al. 2000b)
CIII deficiency	*BCS1L*	*M. musculus* (Leveen et al. 2011)
	UQCRB	*D. rerio* (Cho et al. 2013)
CIV deficiency, LS	*COX4I2*	*M. musculus* (Huttemann et al. 2012)
	COX10	*M. musculus* (Diaz et al. 2008, 2012b, 2005)
	COX15	*M. musculus* (Viscomi et al. 2011)
	COX5A	*D. rerio* (Baden et al. 2007)
	COX6A1/2	*D. melanogaster* (Kemppainen et al. 2014)
	COX6B1/2	*D. melanogaster* (Kemppainen et al. 2014)
	COX7A1/2	*D. melanogaster* (Kemppainen et al. 2014)
	CEP89	*D. melanogaster* (van Bon et al. 2013)
	SCO1	*D. melanogaster* (Porcelli et al. 2010)
	SCO2	*M. musculus* (Yang et al. 2010), Drosophila (Porcelli et al. 2010)
	SURF1	*M. musculus* (Agostino et al. 2003; Dell'agnello et al. 2007), *D. melanogaster* (Da-Re et al. 2014), *D. rerio* (Baden et al. 2007)

(continued)

Table 10.1 (continued)

Mitochondrial disease	Gene	Model organism
CV deficiency	*ATPIF1*	*M. musculus* (Formentini et al. 2014)
	MT-ATP6	*D. melanogaster* (Celotto et al. 2006; Palladino 2010)
French Canadian LS	*LRPPRC*	*M. musculus* (Xu et al. 2012; Ruzzenente et al. 2012)
Friedrich's Ataxia	*FXN*	*M. musculus* (Miranda et al. 2002; Martelli et al. 2012; Ristow et al. 2003; Puccio et al. 2001; Cossée et al. 2000)
Ethylmalonic encephalopathy (EE)	*ETHE1*	*M. musculus* (Di Meo et al. 2011)
	POLG	*M. musculus* (Hance et al. 2005; Lewis et al. 2007; Zhang et al. 2000; Bensch et al. 2009; Kasahara et al. 2006; Trifunovic et al. 2005, 2004), *D. melanogaster* (Bratic et al. 2015)
	POLG2	*M. musculus* (Humble et al. 2013), Drosophila (Baqri et al. 2009)
mtDNA depletion	*TWINKLE*	*M. musculus* (Tyynismaa et al. 2005; Milenkovic et al. 2013)
	TFAM	*M. musculus* (Ekstrand et al. 2007; Li et al. 2000a; Silva et al. 2000; Vernochet et al. 2012; Wang et al. 1999; Wredenberg et al. 2002; Ylikallio et al. 2010)
	ANT1	*M. musculus* (Graham et al. 1997), *D. melanogaster* (Vartiainen et al. 2014; Zhang et al. 1999)
	TK2	*M. musculus* (Zhou et al. 2008; Akman et al. 2008)
	TYMP	*M. musculus* (Haraguchi et al. 2002; Lopez et al. 2009)
	RRM2B	*M. musculus* (Kimura et al. 2003)
	MPV17	*M. musculus* (Clozel et al. 1999; Viscomi et al. 2008)
	MFN1	*M. musculus* (Chen et al. 2003, 2010, 2012)
	MFN2	*M. musculus* (Chen et al. 2007, 2010, 2012; Detmer et al. 2008; Gall et al. 2012; Sebastian et al. 2012; Papanicolaou et al. 2011; Cartoni et al. 2010), *D. melanogaster* (Dorn et al. 2011), *D. rerio* (Vettori et al. 2011)
	RNASEH1	*M. musculus* (Lima et al. 2016)
mtDNA dynamics	*OPA1*	*M. musculus* (Davies et al. 2007; Alavi et al. 2007, 2009; Moore et al. 2010; Sarzi et al. 2012), *D. melanogaster* (Dorn et al. 2011; Yarosh et al. 2008), *D. rerio* (Rahn et al. 2013)
	DRP1	*D. melanogaster* (Verstreken et al. 2005)

genetic modification of mice continues to improve, making the process quicker and cheaper (Van Der Weyden et al. 2011). In that time mouse models corresponding to over 40 nuclearly and mitochondrially encoded mitochondrial proteins have been created. Broadly these can be divided into two categories, those genes involved directly in oxidative phosphorylation (OXPHOS) i.e. subunits and assembly factors of the mitochondrial respiratory complexes, and those proteins required for normal mitochondrial function such as those regulating mitochondrial DNA maintenance.

10.2.1 Complex I Deficiency

10.2.1.1 NDUFS4

The first mouse model created which targeted complex I directly was the NADH: Ubiquinone Oxidoreductase Subunit S4 (*Ndufs4*) knockout (KO) mouse created in the Palmiter Lab (Kruse et al. 2008). *Ndufs4* whole body KO mice were created via Cre/loxP excision of exon 2 which resulted in undetectable levels of NDUFS4 protein (Kruse et al. 2008). Phenotypically *Ndufs4*$^{-/-}$ mice started to show signs of illness around 5 weeks of age, with symptoms of increasing lethargy, weight loss, progressive loss of motor ability and reduced survival at around 8 weeks, reminiscent of patients with isolated complex I (CI) deficiency or Leigh syndrome (Kruse et al. 2008; Dahl 1998). Leigh syndrome (LS) is a common infantile mitochondrial disorder which is characterized by progressive neurodegeneration (Dahl 1998). *Ndufs4*$^{-/-}$ mice had reduced levels of assembled CI and a corresponding decrease in CI activity as assayed by oxygen consumption (Kruse et al. 2008). Interestingly, when the same group conditionally removed *Ndufs4* from neurons and glia, they were able to phenocopy whole body KO mice (Quintana et al. 2010). This highlights the importance of the neuronal component in the *Ndufs4*$^{-/-}$ phenotype and suggests it could underlie that of LS associated with CI deficiency. Moreover, neuronal pathology in both models correlated with disease onset and progression in humans (Kruse et al. 2008; Quintana et al. 2010; Rahman et al. 1996). Removal of NDUFS4 globally or specifically from the central nervous system results in a LS-like phenotype which overlaps highly with that of patients suffering from LS, and as such could be useful in the validation of therapies for LS.

The Larsson and Tian groups went on to study the effects of conditional ablation of NDUFS4 in mouse heart, describing a mild phenotype of cardiomyopathy (Karamanlidis et al. 2013; Li et al. 2000a). Given that NDUFS4 levels were undetectable upon ablation, insights into the tissue specific phenotypes observed via conditional knockdown came from a study by Calvaruso and colleagues who measured the residual CI activity in various tissues of the whole-body KO (Calvaruso et al. 2012). The study found that residual CI activity was present in all tissues but that this varied greatly, and in fact that the heart possessed the highest level of residual CI activity (Calvaruso et al. 2012).

Using random transposon insertion Leong and colleagues created another model of NDUFS4 loss, *Ndufs4*$^{fky/fky}$, which in line with what had been observed in previous models displayed reduced CI activity in all tissues, progressive neuropathy and died prematurely at around 7 weeks of age (Leong et al. 2012).

Finally, the Pinkert lab created an NDUFS4 knock in (KI) mouse (*Ndufs4* $^{KI/KI}$) which carries a point mutation which results in the production of a truncated protein (Ingraham et al. 2009). This is a particularly interesting model, as most NDUFS4 mutations in humans lead to expression of truncated versions of the NDUFS4 protein (Budde et al. 2000; Iuso et al. 2006; Van Den Heuvel et al. 1998). Homozygous KI mice were embryonic lethal, while mice heterozygous for the KI allele were

viable and displayed a mild phenotype, characterized by a 30% decrease in CI linked respiration and CI activity, and increased levels of lactate, commonly seen in patients (Ingraham et al. 2009).

This group of studies using several independent models of *NDUFS4* dysfunction firmly establish the importance of this subunit in stability and regulation of respiratory complex I (Ingraham et al. 2009; Karamanlidis et al. 2013; Kruse et al. 2008; Leong et al. 2012; Quintana et al. 2010, 2012; Sterky et al. 2012). They also highlight how residual CI activity can vary greatly tissue to tissue, with implications not only on tissue specific pathology but also on organismal viability. As mutation of *NDUFS4* is associated with LS due to CI deficiency (Ruhoy and Saneto 2014), understanding the effects of its depletion can serve only to aid the development and assessment of targeted therapies.

10.2.1.2 NDUFS6

NADH: Ubiquinone Oxidoreductase Subunit S6 (*NDUFS6*) is part of the enzymatic core of complex I that has been found to be mutated in patients suffering from isolated CI deficiency (Kirby et al. 2004; Spiegel et al. 2009). Like many patients suffering from CI deficiency, patients carrying mutations in *NDUFS6* display a reduction in CI activity accompanied by acidosis, myopathy and encephalopathy. Several mutations of *NDUFS6* have been described to result in very severe phenotypes, and death within weeks of birth (Spiegel et al. 2009).

There is currently one mouse model of *NDUFS6* loss available, created via gene trap insertional mutagenesis (Forbes et al. 2013). The *Ndufs6*$^{gt/gt}$ mouse has decreased levels of assembled CI and CI activity across all tissues, however like in the case of *Ndufs4*, the degree of reduction varies from tissue to tissue, with the lowest residual activity found in cardiac tissue (Forbes et al. 2013). Interestingly, the authors describe a tissue specific splicing anomaly which may underlie this variability in *Ndufs6* knockdown. *Ndufs6*$^{gt/gt}$ animals develop cardiomyopathy, have reduced ATP synthesis from complex I linked substrates and a deregulation of fatty acid metabolism with sex bias, as males were more severely affected than females. Additionally, both homozygous *Ndufs6*$^{gt/gt}$ and heterozygous *Ndufs6*$^{gt/+}$ mice develop renal disease, with a greater severity in homozygous animals (Forbes et al. 2013).

Although this model displays classic phenotypes of isolated complex I deficiency many of the phenotypes associated with mutations in *NDUFS6* are missing. In contrast to the short survival of affected patients, *Ndufs6*$^{gt/gt}$ can live up to 4 months (Forbes et al. 2013; Spiegel et al. 2009; Kirby et al. 2004).

10.2.1.3 Mt-ND6

MT-ND6 is a mitochondrially encoded core subunit of CI (Wirth et al. 2016). Mutations and allelic variants of *MT-ND6* have been found in patients suffering from Leber hereditary optic atrophy (LHON), LS and mitochondrial encephalomyopathy lactic acidosis and stroke like episodes (MELAS) (Johns et al. 1992;

Chinnery et al. 2001; Ravn et al. 2001). A severe frameshift mutation of *MT-ND6* (13885insC) was shown to be actively removed from the oocyte in a mouse model of mitochondrial disease (Fan et al. 2008). Two further mouse models were created carrying the same missense mutation (G13997A) isolated from a lung carcinoma cell line, which corresponds to a mutation described in patients (G14600A) (Ishikawa et al. 2008). Homoplasmic mice created in the Hayashi lab had a very mild phenotype consisting of a 20–30% reduction in CI and CIII activity and a modest increase in serum lactate (Hashizume et al. 2012). Long term these mice failed to develop any neurological or ophthalmological pathologies (Hashizume et al. 2012).

The Wallace lab also introduced this mutation into a mouse model but in a different strain (Lin et al. 2012). They observed a clear recapitulation of LHON patient pathology (Lin et al. 2012). In addition to a ubiquitous reduction in CI activity, there were highly elevated levels of ROS in neuronal tissue of affected animals. Thus, the *MT-ND6*^{P25L} model provides an opportunity to investigate the molecular pathogenesis of LHON and test potential therapies (Lin et al. 2012).

10.2.2 Complex II Deficiency

10.2.2.1 SDHD

Succinate Dehydrogenase Complex Subunit D (*SDHD)* has been shown to be mutated in several cases of isolated CII deficiency (Jackson et al. 2014; Alston et al. 2012, 2015). Two separate attempts were made to create a mouse model for the study of this gene (Piruat et al. 2004; Bayley et al. 2009). *Sdhd*$^{-/-}$ mice created in the Barneo lab were embryonic lethal while mice heterozygous for the *Sdhd* KO allele had no observable phenotype, despite a decrease in CII activity (Piruat et al. 2004). As mutations in *Sdhd* have also been shown to be involved in a form of hereditary paraganglioma (Schiavi et al. 2006), the Taschner lab created *Sdhd*$^{-/-}$ mice to assess the role of *Sdhd* as tumour supressor. These mice were also embryonic lethal and aged heterozygous mice did not develop tumours (Bayley et al. 2009). As such these models are not useful for the study of *Sdhd* in isolated CII deficiency or as a tumour suppressor.

10.2.3 Complex III Deficiency

10.2.3.1 BCS1L

BCS1 like Ubiquinol-Cytochrome C Reductase Complex Chaperone (*BCS1L*) has been shown to be involved in the final steps of CIII assembly (Fernandez-Vizarra et al. 2007). Mutations in this gene are associated with cases of LS due to CIII deficiency, GRACILE syndrome (growth restriction, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death), Bjornstad syndrome and mitochondrial

CIII deficiency (Visapaa et al. 2002; Hinson et al. 2007; De Lonlay et al. 2001; Blazquez et al. 2009). The Fellman laboratory created a mouse model of GRACILE syndrome by using gene targeting to introduce the *Bcs1l* 232A > G point mutation, *Bcs1l*$^{g/g}$ (Leveen et al. 2011). The pathology they observed in *Bcs1l*$^{g/g}$ mice displayed remarkable similarities to that observed in patients with fatal GRACILE syndrome (Leveen et al. 2011). This included, growth restriction, progressive liver disease and premature death. Importantly, the model recapitulated the tissue specificity observed in patients with pathology arising only in the kidney and liver. Metabolic comparison of pre-symptomatic and symptomatic mice revealed a deterioration of carbohydrate and fatty acid metabolism leading to a starvation like state which may underlie disease onset (Kotarsky et al. 2012). Further analysis of the molecular pathogenesis underlying the *Bcs1l*$^{g/g}$ phenotype could provide key insights into GRACILE syndrome.

10.2.4 Complex IV Deficiency

10.2.4.1 COX4I2

Cytochrome C Oxidase Subunit 4 Isoform 2 (*COX4I2)* is a nuclearly encoded subunit of CIV which has been shown to be expressed in lung and pancreatic acinar cells, with patients carrying mutations in *Cox4I2* suffering from exocrine pancreatic insufficiency (Shteyer et al. 2009; Hüttemann et al. 2001). This subunit and that encoded by Cytochrome C Oxidase Subunit 4 Isoform 1 (*COX4I1)* is thought to play important roles the regulation and assembly of CIV. *Cox4i2* KO mice develop a progressive lung pathology with reduced levels of ATP and CIV activity (Huttemann et al. 2012). However, no pancreatic pathology was observed in these mice, this discrepancy could also result from an absence of *Cox4i2* expression in the pancreas of mice (Huttemann et al. 2012).

10.2.4.2 SURF1

SURF1 is an assembly factor involved in CIV biogenesis (Tiranti et al. 1998; Williams et al. 2001). Patients carrying mutations in *SURF1* suffer from LS due to CIV deficiency (Zhu et al. 1998; Tiranti et al. 1998). Two separate *Surf1* KO mice were created by the Zeviani lab which displayed an expected CIV deficiency but unexpectedly did not have any neuropathology, a hallmark of *SURF1* patients (Agostino et al. 2003; Dell'agnello et al. 2007). Although these models will be useful in understanding the biological role of *SURF1* they do not recapitulate the patient phenotype and therefore may not be suitable for the development and validation of novel therapies.

10.2.4.3 COX10

COX10 is a CIV assembly protein required for biosynthesis of the heme α prosthetic group required for functional CIV (Antonicka et al. 2003a). Mutations and allelic variants of *COX10* have been identified in patients suffering from isolated CIV deficiency and LS due to CIV deficiency (Antonicka et al. 2003a). A mouse model carrying a conditional allele ($Cox10^{cKO}$) was used to create model of mitochondrial myopathy, hepatopathy, neuropathy and encephalopathy (Diaz et al. 2008, 2005, 2012a). Conditional ablation of *Cox10* in the liver of mice resulted in phenotypes which recapitulated clinical features of mitochondrial hepatopathies including enlargement of the liver and steatosis (Diaz et al. 2008). Ablation of COX10 in the peripheral nervous system resulted in a severe neuropathy characterized by demyelination, also observed in several patients carrying *Cox10* mutations (Diaz et al. 2012a). Given the stark recapitulation of pathology seen in patients, these models will be important in understanding tissue specific pathogenesis and in the testing and validation of therapies which directly target the underlying mitochondrial dysfunction.

10.2.4.4 COX15

COX15 is a CIV assembly protein which plays a key role in the heme biosynthetic pathway (Petruzzella et al. 1998). Patients carrying mutations in this gene suffer from LS due to CIV deficiency and cardioencephalomyopathy (Antonicka et al. 2003b; Oquendo et al. 2004). COX15 is required for mouse embryonic development (Viscomi et al. 2011). Mice lacking COX15 specifically in muscle have acute CIV deficiency accompanied by a severe myopathy from 1 month of age (Viscomi et al. 2011).

10.2.4.5 SCO2

SCO2 is an assembly factor required for the assembly and function of CIV. Mutations in *SCO2* can result in fatal infant cardioencephalomyopathy due to CIV deficiency (Papadopoulou et al. 1999). The E140K amino acid change, corresponding to the n.1541G > A missense mutation most commonly found in patients, was introduced into a mouse model (Baxevanis et al. 2000). This model had a clear CIV defect but unfortunately did not recapitulate human disease pathology.

10.2.5 Complex V Deficiency

10.2.5.1 ATPIF1

Generation of patient relevant mouse models of CV deficiency will allow testing of therapeutic interventions. There are currently no mouse models of CV deficiency based on patient mutations available. To model CV deficiency, a mouse

overexpressing a modified form of human ATPase Inhibitory Factor 1 (*ATPIF1)* in neurons was created (Formentini et al. 2014). ATPIF1 is thought to play an inhibitory role in the hydrolysis of ATP by ATP synthase (CV) when mitochondrial membrane potential is decreased (Formentini et al. 2012). This mutant form of human ATPIF1 has increased affinity with the beta subunit of ATP synthase, inhibiting CV hydrolysis activity, which resulted in increased oxidative stress, reduced levels of respiration and CIV activation, as well as metabolic reprogramming that prevented cell death (Formentini et al. 2014). Generation of patient relevant mouse models of CV deficiency will allow a deeper investigation of molecular pathogenesis and testing of therapeutic interventions.

10.2.6 CoQ Deficiency

CoQ_{10} plays several roles in electron transport within mitochondria. It facilitates the flow of electrons from CI and CII to CIII, acts as an electron acceptor for several dehydrogenases, such as those involved in NADH shuttling from the cytosol to mitochondria (glycerol-3-phosphate dehydrogenase), in fatty acid beta-oxidation (ETF:QOR) and pyrimidine nucleotide synthesis (DHODH), and functions as a potent lipid soluble antioxidant in the plasma membrane and elsewhere (Lopez-Lluch et al. 2010).

Given the central role that CoQ plays in metabolism, it is unsurprising that mutations involving its biosynthesis lead to severe phenotypes and disorders. Human CoQ deficiency can result in several forms of mitochondrial disease including LS, myopathy, myoglobinuria, ataxia, cerebellar atrophy and severe infantile encephalomyopathy with renal failure (Fernandez-Ayala et al. 2014).

CoQ biosynthesis depends on a highly conserved multi-enzyme complex that involves at least eleven genes (named COQ1 to COQ10 plus PDSS2), although some of them have no catalytic activity (Bentinger et al. 2010). Below, we summarize three mouse models of CoQ deficiency.

COQ7 encodes a hydroxylase that takes part in the latter stages of CoQ biosynthesis. Tamoxifen-induced knockdown of *Coq7* led to weight loss a few days after drug administration, with reduced respiratory chain electron transfer, mitochondrial respiration and survival (Wang et al. 2015). However ROS production was unchanged (Wang et al. 2015).

COQ9 encodes a lipid-binding protein that is necessary for incorporation of COQ7 into the Q biosynthetic complex (Lohman et al. 2014) and has been shown to be mutated in a patient suffering with primary CoQ_{10} deficiency (Duncan et al. 2009). This patient mutation was used to create a KI mouse ($Coq9^{X/X}$) model of primary CoQ_{10} deficiency (Garcia-Corzo et al. 2013). Homozygous KI mice developed normally but developed neurological symptoms of paralysis and eventually death within 6 months (Garcia-Corzo et al. 2013). Accordingly, these mice had reduced levels of CoQ_{10} as well as decreased CI, mitochondrial respiration and ATP

synthesis (Garcia-Corzo et al. 2013). As such this KI mouse model is an excellent model of encephalopathy due to primary CoQ_{10} deficiency.

PDSS2 encodes part of the decaprenyl diphosphate synthase involved in CoQ biosynthesis, which has been found to be mutated in a case of infantile COQ deficiency type 3 (COQ10D3) (Lopez et al. 2006; Salviati et al. 2005). *Pdss2*$^{kd/kd}$ mice carry a missense mutation which results in kidney failure and decreased levels of CoQ_{10} (Peng et al. 2008). This can be rescued by CoQ supplementation (Saiki et al. 2008). Conditional ablation within the liver did not result in pathology despite depletion of CoQ_{10} (Peng et al. 2008). Suggesting that there may tissue specific thresholds for sensitivity to decreases in CoQ_{10}.

10.2.7 Mitochondrial DNA Maintenance

10.2.7.1 Twinkle

TWINKLE is the mtDNA helicase and plays a key role in mtDNA replication and maintenance (Spelbrink et al. 2001; Tyynismaa et al. 2004). Homozygous *Twinkle* KO mice were embryonic lethal but conditional ablation within skeletal and cardiac muscle resulted in depleted levels of mtDNA, OXPHOS defects and a reduced survival of only 19 weeks (Milenkovic et al. 2013). Mutation of *Twinkle* in humans can lead to several pathologies, including autosomal dominant progressive external ophthalmoplegia with mtDNA deletions (adPEOA) and mtDNA depletion syndromes (Spelbrink et al. 2001; Hakonen et al. 2008).

Mice carrying adPEOA associated alleles of *Twinkle* (*Twinkle*^{A360T}, *Twinkle*$^{dup353–365}$) recapitulate many aspects the human pathology (Tyynismaa et al. 2005). *Twinkle*^{A360T} and *Twinkle*$^{dup353–365}$ mice progressively accumulated mtDNA deletions, developing mitochondrial myopathy characterised by reduced CIV activity and increased mitochondrial proliferation (Tyynismaa et al. 2005). Interestingly, these "deletor" mice showed selective mitochondrial dysfunction in Purkinje cells, located within the cerebellum, and hippocampal CA2 pyramidal neurons which have been previously shown to be affected in adPEOA and other mtDNA depletion syndromes (Tyynismaa et al. 2005). Importantly, physiological analysis of muscle biopsies from these mice demonstrated that the accumulation of mtDNA deletions was associated with reduced OXPHOS function and induced a local and global starvation response (Tyynismaa et al. 2010). Using the *Twinkle* "deletor" mouse models, Nikkanen and colleagues outline a metabolic response to mitochondrial dysfunction which results in altered 1C metabolism and induction of serine directed glutathione synthesis as a possible mechanism to stabilise dNTP synthesis, which is also present in patients suffering from mitochondrial myopathy (Nikkanen et al. 2016). This validates the *Twinkle* "deletor" mouse as a disease model and provides several targets for therapy of mitochondrial myopathy.

10.2.7.2 *POLG* and *POLG2*

POLG encodes the catalytic subunit of the mitochondrial DNA polymerase γ, providing DNA polymerase and 3′-5′ exonuclease activity (Lamantea et al. 2002). Several hundred mutations of *POLG* have been described in patients which can result in several clinical presentations caused by mtDNA instability (Lamantea et al. 2002; Milone and Massie 2010). As such, accurately modelling disease pathogenesis of *POLG* mutation is challenging. Unsurprisingly, mtDNA stability is required during development, *Polg* KO mice are embryonic lethal and display severe mtDNA depletion (Hance et al. 2005).

Several mouse models have been created to address the pathology underlying *POLG* mutation (Lewis et al. 2007; Bensch et al. 2009; Kasahara et al. 2006; Zhang et al. 2000, 2005). The first *Polg* mouse model created by Zhang et al., used conditional expression of an exonuclease deficient *Polg*, $Polg^{D181A}$ within mouse cardiac muscle (Zhang et al. 2000). These mice developed cardiomyopathy associated with increased levels of mtDNA mutations (Zhang et al. 2000). Further analysis of this model revealed activation of a pro-survival response within the cardiac muscle, reasoned to be a response to activation of cell death pathways in the face of increased mtDNA instability (Zhang et al. 2005). This same mutation was also overexpressed in neurons and pancreatic β cells (Bensch et al. 2009). In neurons, expression of $Polg^{D181A}$ resulted in increased mtDNA instability in the forebrain leading to neurological dysfunction relating to behaviour and mood, in line with what has been observed in patients suffering from progressive external ophthalmoplegia (PEO) with mood disorder (Kasahara et al. 2006). In the pancreas, expression of $Polg^{D181A}$ within islets resulted in early onset diabetes due to β cell death and insulin insufficiency which associated with increased mtDNA mutations (Bensch et al. 2009).

To access the role of mtDNA mutations in aging two Knock IN mice for the $Polg^{D257A}$ mutation, which ablates proof reading of *Polg*, were created (Kujoth et al. 2005; Trifunovic et al. 2004). Although the role of mitochondrial dysfunction in aging is not the focus of this chapter, several relevant phenotypes where described in these mice (Kujoth et al. 2005, Trifunovic et al. 2004) . These mice, often referred to as "mutator" mice, accumulated mtDNA mutations and deletions which led to progressive OXPHOS deficiency (Kujoth et al. 2005; Trifunovic et al. 2005). Importantly, follow up analysis on this model revealed distinct tissue specific pathology due to this KI allele, $Polg^{D257A}$.

Finally, the Y995C mutation associated with PEO was transgenically expressed in mouse cardiac muscle and resulted in increased oxidative stress, reduced levels of mtDNA and cardiomyopathy, as seen with the expression of $Polg^{D181A}$, a proof reading deficient mutant, in mouse heart (Lewis et al. 2007).

POLG2 encodes the accessory subunit required for Polymerase γ function. Although rarer, mutations of *POLG2* have been described in patients (Longley et al. 2006). Like *Polg*, homozygous loss of *Polg2* results in embryonic lethality with severe mitochondrial defects, underlining the requirement of mtDNA stability for mammalian development (Humble et al. 2013). Importantly half the dose of either *Polg or Polg2* is sufficient for mitochondrial function, development and lifespan.

Given the number of described pathogenic mutations the mouse may not be the most suitable model for assessment of the molecular pathology of each disease causing lesion. This is an example where complementary models such as *Drosophila melanogaster* or *C. elegans* could play a role.

10.2.7.3 TFAM

Mitochondrial transcription factor A (*TFAM*) is required for mtDNA transcription and packaging and mitochondrial biogenesis (Stiles et al. 2016). In humans, the singular described mutation of *TFAM* resulted in mtDNA depletion syndrome (Stiles et al. 2016).

Due likely to its role in maintaining mtDNA stability, homozygous loss of *Tfam* is embryonic lethal (Larsson et al. 1998). *Tfam* has been conditionally ablated in skeletal muscle, cardiac muscle, pancreatic β cells, adipose tissue, forebrain and dopaminergic neurons (Wredenberg et al. 2002; Wang et al. 1999; Silva et al. 2000; Sörensen et al. 2001; Ekstrand et al. 2007; Vernochet et al. 2012). In all models, *Tfam* deletion resulted in mtDNA depletion and progressive OXHOS deficiency and except for the adipose tissue, this led to tissue specific pathologies. In the adipose tissue, this dysregulation of mitochondrial biology led to an increased insulin resistance and obesity prevention on a high fat diet.

Given the penetrance of the phenotypes seen in mouse models of *Tfam* deletion it is not surprising that only 2 cases of patients with *TFAM* mutations have been described so far.

10.2.7.4 LRPPRC

Leucine-rich pentatricopeptide repeat containing protein (*LRPPRC*) works in complex with other mitochondrial proteins to regulate post transcriptional gene expression of mitochondrial encoded transcripts (Ruzzenente et al. 2012; Sasarman et al. 2010). The French-Canadian variant form of LS (FCLS) is caused by the c.1119C > T mutation of *LRPPRC*, which is characterised by a severe defect in CIV with neuro and hepatopathy (Debray et al. 2011).

Homozygous loss of *Lrpprc* results in developmental delay, CIV deficiency and embryonic lethality (Ruzzenente et al. 2012; Xu et al. 2012). Conditional ablation of *Lrpprc* in the skeletal or cardiac muscle of mice led to reduced lifespan, development of progressive cardiomyopathy and CIV deficiency (Ruzzenente et al. 2012). The sensitivity of CIV function to mutation in *LRPPRC* could be a result of binding of LRPPRC to mRNA of CIV core subunit *COX1* (Xu et al. 2012). However, in a model of *Lrpprc* loss in the mouse heart, a defect in CV assembly was found to underlie the OXPHOS defect (Mourier et al. 2014). Our knowledge of *LRPPRC* function is modest and although those mouse models currently available recapitulate aspects of FCLS, we may need to build new (i.e. neuronal or liver specific

ablation) or utilise complementary models to have a more disease relevant model for analysis of molecular pathology.

10.2.7.5 ANT1

Adenine nucleotide translocator isoform 1 (*ANT1*) is a member of a family of adenine nucleotides translocators (ANTs) which are responsible for the exchange of ADP and ATP across the inner mitochondrial membrane (Li et al. 1989). Mutations in *ANT1* can be found in patients suffering from several types of mtDNA depletion syndromes with cardiomyopathy and myopathy, as well as adPEO (Kaukonen et al. 2000; Thompson et al. 2016). The Wallace laboratory created a *Ant1* KO mouse ($Ant1^{PGKneo}$) which faithfully recapitulated the features of a mitochondrial myopathy and cardiomyopathy (Graham et al. 1997). In line with patients, $Ant1^{PGKneo}$ mice had myopathy characterised by ragged red fibres (RRFs), COX negative fibres and dysfunctional mitochondria (Graham et al. 1997). These mice also displayed metabolic adaptations, including increased levels of lactate and Krebs cycle intermediates, as well as increased oxidative stress and accumulation of mtDNA deletions (Graham et al. 1997). Importantly and in contrast to patients, $Ant1^{PGKneo}$ mice do not display any neuronal or ophthalmological pathology which might be due to expression of another *Ant* family member (Lee et al. 2009; Phillips et al. 2010).

Despite the absence of a neuronal or ocular phenotype, the $Ant1^{PGKneo}$ mouse is a great model of mitochondrial myopathy and as such is an excellent model for molecular investigation of mitochondrial myopathy, as well as validation of targeted therapies.

10.2.7.6 TK2

The mitochondrial thymidine kinase is encoded by *TK2* (Johansson and Karlsson 1997). It catalyses the first step of the nucleotide salvage pathway and has been shown to be mutated in patients suffering from mitochondrial depletion syndrome type 2 (MTDS2) and autosomal recessive PEO (PEOB3) (Boustany et al. 1983; Moraes et al. 1991; Tyynismaa et al. 2012). Homozygous $Tk2^{-/-}$ mice are viable but develop encephalopathy and die within the first 4 weeks of life (Zhou et al. 2008). This was mirrored in a KI model where a point mutation corresponding to a human pathogenic mutation, H121N, was introduced (Akman et al. 2008). $Tk2^{KI}$ mice also displayed encephalopathy, early mortality and reduced levels of mtDNA (Akman et al. 2008). Importantly and in contrast to patients, neither mouse model developed myopathy which is a key characteristic of MTDS2. However, a detailed analysis of the $Tk2^{-/-}$ mouse described an altered transcriptional signature and pre-clinical phenotypes in heart and skeletal muscle (Paredes et al. 2013). Conditional or inducible models of *Tk2* loss specifically in the muscle may allow modelling of this myopathic mtDNA depletion syndrome.

10.2.7.7 TYMP

TYMP encodes thymidine phosphorylase which is essential for the nucleotide salvage pathway (Matsukawa et al. 1996). Mitochondrial neuro-gastrointestinal encephalopathy (MNGIE) is a mtDNA deletion syndrome characterised by gastrointestinal blockage, PEO and mitochondrial dysfunction (Hirano et al. 2012). Most MGNIE cases are a result of *TYMP* mutation (Hirano et al. 2012). The phenotype of a mouse model of *TYMP* deficiency (*Tymp* $^{-/-}$, *Up*$^{-/-}$) partially overlapped with that of MNGIE patients (Haraguchi et al. 2002). With increased levels of thymidine and deoxyuridine in several tissues and in the brain, progressive mtDNA depletion (Lopez et al. 2009).

10.2.7.8 RRM2B

RRM2B encodes the small subunit of the p53-controlled ribonucleotide reductase (Tanaka et al. 2000). Mutations in *RRM2B* have been associated with mtDNA depletion syndromes, PEO and MNGIE (Bourdon et al. 2007; Kollberg et al. 2009; Tyynismaa et al. 2009).

Rrm2b KO mice (*Rrm2b* $^{-/-}$) developed normally, had retarded growth after weaning and died at 14 weeks of severe kidney failure (Kimura et al. 2003). In a comparative analysis of *Rrm2b* $^{-/-}$ mice and patients carrying mutation in *RRM2B*, severe depletion of mtDNA in the absence of a OXPHOS defect was described (Bourdon et al. 2007). In both studies, *Rrm2b* mutation resulted in a decrease in the dNTP pool suggesting that *Rrm2b* is required for replenishing the dNTP pool (Bourdon et al. 2007; Kimura et al. 2003). *Rrm2b* $^{-/-}$ mice mirror the patient phenotype and given the association of *RRM2B* with several mitochondrial diseases this and future models of *Rrm2b* mutation could be used to validate therapies for mtDNA exhaustion resulting from dNTP deficiency.

10.2.7.9 MPV17

MPV17 encodes a protein of the mitochondrial inner membrane whose function has not yet been delineated. Work in yeast has shown that Sym1 (yeast orthologue of *MPV17*) is required for maintenance of mitochondrial morphology and mtDNA maintenance in stress conditions (Dallabona et al. 2010). In humans, mutation of *MPV17* leads to mtDNA depletion syndrome 6 (MTDPS6) which is an infantile syndrome characterised by liver failure. Unfortunately, *Mpv17* KO mice (*Mpv17*$^{-/-}$) did not develop liver failure despite a reduction in mtDNA levels leading to CI and CIV deficiency (Weiher et al. 1990). Liver failure was induced in *Mpv17*$^{-/-}$ mice fed a ketogenic diet (Bottani et al. 2014). Clues to the pathogenic mechanisms which underlie MTDPS6 may be found by focusing on understanding the function of *MPV17*.

10.2.8 Mitochondrial Dynamics

10.2.8.1 MFN1 and MFN2

The mitochondrial GTPases Mitofusin 1 and 2 (*Mfn1/2*) are essential for mouse development (Chen et al. 2003, 2007). However, they are partially redundant with overlapping and distinct roles. Loss of both MFN 1 and 2 in the skeletal muscle results in muscle atrophy, increased levels of lactate and premature death at 6–8 weeks of age (Chen et al. 2010). These mice have severely impaired muscle mitochondria, with a progressive reduction in mtDNA levels, despite a compensatory increase in mitochondrial biogenesis (Chen et al. 2010). Additionally, the remaining copies of mtDNA were found to have an increased number of point mutations and deletions. This work suggests that fusion is required to maintain mitochondrial integrity.

MFN2 has been conditionally deleted in several different tissues including the heart, kidney and liver (Papanicolaou et al. 2011; Gall et al. 2012; Sebastian et al. 2012). In all cases, loss of MFN2 resulted in dysfunction or tissue pathology, but did not mirror phenotypes observed in patients carrying pathological mutations of MFN2. To have a more patient relevant model, mice expressing mutant alleles of MFN2 associated with Type 2A Charcot Marie Tooth Disease (CMT2A), a peripheral polyneuropathy, were created (Detmer et al. 2008; Cartoni et al. 2010). As expected, in both cases animals displayed phenotypes consistent with those of CMT patients such as defective motor skills, axonopathy and incomplete disease penetrance (Detmer et al. 2008; Cartoni et al. 2010). However, the molecular pathogenesis may differ, as density and distribution of mitochondria in axons was not consistent between these models (Detmer et al. 2008, Cartoni et al. 2010). Nevertheless, these models have allowed examination of CMT2A pathogenesis and in future, could be utilised to further understand the interplay between mitochondrial distribution and neuronal function.

10.2.8.2 OPA1

Mutations in Optic atrophy 1 (*OPA1*) are associated with autosomal dominant optic atrophy (adOA) (Delettre et al. 2000). Mouse models homozygous for two mutant alleles which result in truncated forms of Opa1, die during early embryogenesis (Alavi et al. 2007; Davies et al. 2007). Heterozygous animals with around a 50% decrease in Opa1 displayed progressive vision loss as well as neurological symptoms, such as reduced locomotor activity. As seen in the case of MFN2, the phenotypes of these models did not completely overlap. However, further characterisation of both these models uncovered symptoms that were not restricted to retinal ganglion cells (RGCs), the cell type preferentially affected in adOA (Williams et al. 2012).

Mice homozygous for a common adOA deletion ($Opa1^{delTTAG}$) died at around E10.5, while heterozygous mice recapitulated phenotypes seen in patients with the syndromic form of adOA (adOA plus) (Sarzi et al. 2012). This included deafness,

encephalomyopathy, cardiomyopathy and defects of the peripheral nervous system (Sarzi et al. 2012). This was in addition to loss of RGCs and vision loss observed in previous mouse models and most adOA patients. At the molecular level these mice displayed reduced CIV activity and incorporation of CIV into stable supercomplexes (Sarzi et al. 2012).

Taken together these models clearly demonstrate the requirement of *OPA1* for normal mammalian development, where complete loss of *OPA1* leads to a fragmented mitochondrial network unable to support life. Analysis of dominant Opa1 alleles highlights the pleotropic effects Opa1 mutations. Mouse models which show RGC specific phenotypes will be useful in further understanding the sensitivity of this tissue to Opa1 loss. The more severe syndromic model of adOA ($Opa1^{delTTAG}$) not only mirrors adOA patients but also several clinical features seen across various forms of systemic mitochondrial disease. Validating this model for use in the identification of potential therapies.

10.2.9 Additional Mouse Models

10.2.9.1 AIFM1

Apoptosis Inducing Factor, Mitochondria Associated 1 (*AIFM1)* is a flavoprotein located in the mitochondrial intermembrane space, that acts both as a NADH:cytochrome c oxidoreductase in healthy cells and as an inducer of caspase independent apoptosis (Joza et al. 2009). Mutations in this gene can result in combined oxidative phosphorylation deficiency 6 (COXPD6) and Cowchock syndrome (Cowchock et al. 1985; Fischbeck et al. 1986; Ghezzi et al. 2010). A proviral insertion in the *Aifm1* gene resulted in an 80% reduction in the harlequin mouse model (*Hq*) (Klein et al. 2002). Further analysis of this model of neurodegeneration showed an isolated effect on CI function. Mitochondria from retinal and neuronal tissue of *Hq* mice had reduced CI activity, abundance and expression of CI subunits (Vahsen et al. 2004). The relationship between levels of *Aifm1* and CI was further underlined when conditional deletion within skeletal and cardiac muscle resulted in an 80% reduction in CI activity, atrophy of the skeletal muscles and cardiomyopathy (Joza et al. 2005). However, patients who carry mutations in *AIFM1* do not seem to carry complementary defects in CI but instead in respiratory CIII and CIV (Ghezzi et al. 2010). Further analysis of patients and available models will help elucidate the link between these two entities.

10.2.9.2 FXN

FRATAXIN (FXN) is a mitochondrial protein which has been shown to play roles in several processes, including heme and iron-sulphur cluster biosynthesis and antioxidant defence (Ristow et al. 2000; Shoichet et al. 2002; Martelli et al. 2007).

Friedreich's ataxia (FDRA) is a mitochondrial disease which is caused by expansion of a GAA triplet repeat in an intron of *FXN (*Campuzano et al. 1996*)*. Patients suffer from neurodegeneration, cardiomyopathy and diabetes, presenting with a distinct biochemical signature which includes a decrease in the levels of iron-sulphur cluster containing enzymes and an increase in markers of oxidative damage (Delatycki et al. 2000).

Homozygous *Fxn* null mice are early embryonic lethal, demonstrating the importance of *Fxn* in early embryogenesis (Cossée et al. 2000). Conditional KO of *Fxn* was performed in the heart, brain, liver and pancreatic β cells (Puccio et al. 2001; Ristow et al. 2003; Simon et al. 2004; Martelli et al. 2012). As in patients, tissues specific depletion of *Fxn* in the brain resulted in progressive neurodegeneration, in the heart in cardiomyopathy and in the pancreas, the onset of diabetes (Puccio et al. 2001, Ristow et al. 2003, Simon et al. 2004, Martelli et al. 2012). However, these phenotypes were accelerated and enhanced in mouse models when compared to patients but these mice failed to show any signs of oxidative damage (Puccio et al. 2001, Ristow et al. 2003, Simon et al. 2004, Martelli et al. 2012). Given what is known about other forms of repeat disease such as several forms of spinocerebral ataxia and myotonic dystrophy, the effects of expansion of the GAA triplet on the levels of FXN may be more nuanced than a complete KO of the gene. Accordingly a mouse model which overexpresses the human *FXN* gene with the GAA repeat mirrored the patient phenotype, developing a milder progressive form of ataxia with an accumulation of oxidative damage (Al-Mahdawi et al. 2006).

10.2.9.3 ETHE1

ETHE1 encodes a detoxification enzyme, present within mitochondria, preventing the accumulation of damaging hydrogen sulphide (H_2S) (Tiranti et al. 2004). Ethylmalonic encephalopathy (EE) is a severe childhood mitochondrial disorder characterised by encephalopathy, petechiae and gastrointestinal disturbances. Patients also excrete large quantities of ethylmalonic acid in their urine (Tiranti et al. 2004).

To gain a better understanding of EE pathology Tiranti *et al* created *Ethe1 KO* (*Ethe1*$^{-/-}$) mice and analysed them together with EE patients (Tiranti et al. 2009). They described a striking recapitulation of the patient phenotype, with *Ethe1*$^{-/-}$ mice showing symptoms of EE within the first two weeks of life and succumbing within the first 6 weeks (Tiranti et al. 2009). *Ethe1*$^{-/-}$ mice had arrested growth, pronounced CIV deficiency in muscle, brain and colon, increased levels of lactate, C4 and C5 acylcarnitines and increased levels of ethylmalonic acid and thiosulfate (Tiranti et al. 2009). Conditional ablation in muscle, brain and liver failed to phenocopy *Ethe1*$^{-/-}$ mice (Di Meo et al. 2011). While CIV deficiency was clear in targeted tissues, it was not sufficient to result in increased levels of ethylmalonic acid and thiosulfate in urine, observed in patients and *Ethe1*$^{-/-}$ mice (Di Meo et al. 2011).

The *Ethe1*$^{-/-}$ mouse model provides an excellent opportunity to develop targeted therapies for EE patients which can prevent or negate the accumulation of toxic H_2S.

10.2.9.4 RNASEH1

RNASEH1 encodes a ribonuclease which is required in mitochondria for mtDNA replication (Reyes et al. 2015). *RNASEH1* mutations have been associated with adult-onset encephalopathy with chronic PEO and mtDNA deletions (Reyes et al. 2015). *RNaseH1* null mice are embryonic lethal due to depletion of mtDNA resulting in cell death (Cerritelli et al. 2003). Conditional and inducible deletion of *RNaseH1* specifically in the liver also resulted in reduced levels of mtDNA, with clear defects in mitochondrial morphology. These mice also displayed progressive liver degeneration (Lima et al. 2016). These models have allowed the delineation and conformation of *RNASEH1* function. The liver specific models could in future allow modelling of liver dysfunction (hepatopathy) due to mtDNA depletion.

10.3 *Drosophila* Models of Mitochondrial Disease

The fruit fly, *Drosophila melanogaster,* has long been used in the study of several human pathologies (Vidal and Cagan 2006; Chan and Bonini 2000; Stefanatos and Vidal 2011). Despite its' relative simplicity, the fly genome shows remarkable conservation with that of humans, with the advantage of reduced genetic and functional redundancy. As a model organism to study mitochondrial disease, *Drosophila* present many advantages. There is a high degree of conservation of mitochondrial proteins and many patient phenotypes can be assessed in a whole animal model (Jacobs et al. 2004). The major advantage of the fly is the plasticity that the genetic tools available in *Drosophila* confer (Jennings 2011). While several mutations in mitochondrial proteins have been isolated in *Drosophila*, the true power of this model organism lies in the ability to utilise methods such as RNA interference (RNAi) in combination with the binary GAL4/UAS system for modulation of gene expression as well as the several methods of transgenesis available (Brand and Perrimon 1993; Lee and Luo 2001; St Johnston 2002; Dietzl et al. 2007; Perkins et al. 2015). Together these tools allow tissue specific or global, depletion or deletion of any gene of interest. Over the last 10 years the *Drosophila* research community has established several models of mitochondrial dysfunction. While we must keep in mind the differences between mammalian and insect physiology, the fly possesses similar if not the same cellular and physiological functions with a high degree of conservation. Importantly, flies with mutations in mitochondrial genes recapitulate several aspects of mitochondrial disease (Toivonen et al. 2001; Vartiainen et al. 2014).

10.3.1 Complex I

Li and colleagues have utilized P-element mediated excision of *CG12079/ND-30,* the fly orthologue of CI subunit *Ndufs3,* to create novel mutant alleles (Li 2013). They find that *CG12079/ND-30* mutants have severely reduced CI activity and aberrant assembly (Li 2013). Most developing larvae do not make it to adulthood but characterization of rare escapers revealed increased oxidative stress as well as activation of compensatory increases in mitochondrial biogenesis (Li 2013). These flies also display seizure sensitivity and defective climbing ability, phenotypes which can be extrapolated to mitochondrial disease patients (Jacobs et al. 2004). RNA interference (RNAi) mediated depletion of *Ndufs3* results in decreased ATP levels and membrane potential (Vos et al. 2012). Although this model was not further analysed it demonstrates the power of the availability of RNAi constructs against most of the genome to induce mitochondrial dysfunction (Dietzl et al. 2007, Perkins et al. 2015).

Mutants have also been isolated for two CI assembly factors *Ndufaf1* (*CG7598*) and *Ndufaf6* (*CG15738*) (Cho et al. 2012; Zhang et al. 2013). In the case of *Ndufaf1*, deletion resulted in developmental arrest with defects in mitochondrial structure and a loss of CI holoenzyme (Cho et al. 2012). RNAi mediated depletion of *Ndufaf1* (*CG7598*) also resulted in loss of CI holoenzyme and defects in mitochondrial structure (Cho et al. 2012). Two separate mutations of *Ndufaf6* isolated from an unbiased forward genetic screen resulted in decreased CI activity and increased ROS levels (Zhang et al. 2013).

Burman and colleagues took advantage of a *mt-ND2* mutant created using a mitochondrially targeted restriction enzyme (Burman et al. 2014). In humans, mutations in *MT-ND2* are associated with Leber Optic Atrophy (LHON) and CI deficiency (Johns and Berman 1991; Schwartz and Vissing 2002; Pulkes et al. 2005). $ND2^{del1}$ flies, which carry a small deletion in *ND2*, show an increase in heat, mechanical stress, hypoxic and hypercarbic induced paralysis as well as a reduced lifespan (Burman et al. 2014). Although there were no signs of muscle pathology, aged mutants displayed progressive neurodegeneration (Burman et al. 2014). Biochemically $ND2^{del1}$ flies have an isolated CI respiration defect, reduced levels of assembled CI, reduced membrane potential and ATP production. This model recapitulates many aspects of CI deficiency and provided insights into the role of *mt-ND2*. Importantly this model has also been recently used to parallel the therapeutic effects of rapamycin described in a mouse model of CI deficiency further validating its use in the investigation of mitochondrial disease pathogenesis (Johnson et al. 2013; Wang et al. 2016).

10.3.2 Complex II

SDHB is one of four subunits of CII. An insertional mutant for *Sdhb* (*CG3283)* in flies displayed a CII specific respiratory defect accompanied by a reduction in CII activity and an increase in mitochondrial ROS (Walker et al. 2006). Mutation

of *Sdhaf3* (*CG14898*) resulted in reduced CII activity, increased sensitivity to oxidative stress and motility defects (Na et al. 2014). Loss of *Sdhaf4* (*CG7224*) also resulted in a severe decrease in CII activity and increased sensitivity to oxidative stress as well as neurodegeneration and reduced survival (Van Vranken et al. 2014).

10.3.3 Coenzyme Q

In flies, the first model of CoQ deficiency was isolated in the Gould lab using a mosaic screen to identify modifiers of neuroblast size (Grant et al. 2010). This technique in fruit flies allows analysis of wild type and mutant cells within the same tissue, as well as allowing the study of mutations that are lethal, at any stage in development (Lee and Luo 2001). They identified *qless* (*CG31005*), the *Drosophila* orthologue of *PDSS1*, involved in the first step of COQ biosynthesis (Grant et al. 2010). Investigation of *qless* in the developing CNS revealed a growth defect in *qless* mutant clones which were smaller than wild type clones (Grant et al. 2010). *qless* mutant clones undergo caspase dependant apoptosis and express the marker of mitochondrial stress, Hsp60A. These phenotypes are rescued by dietary supplementation with CoQ10 (Grant et al. 2010). As well as being the first to study the effects of *PDSS1* loss in the fly, Grant and colleagues highlight how the power of mosaic analysis for the investigation of gene function.

COQ2 catalyses one of the final reactions in CoQ biosynthesis (Forsgren et al. 2004). Mutants of *Coq2* (*CG9613*) created using P-element mediated excision display developmental arrest and decreased levels of ATP (Liu et al. 2011). Animals heterozygous for mutant *CoQ2* had increased lifespan and inhibited insulin signalling which has been shown to have life extending effects (Liu et al. 2011). This model does not present a viable model of mitochondrial disease; however, mosaic analysis of this mutation may provide more insights into gene function.

Finally using RNAi technology, Fernandez-Ayala and colleagues have systematically depleted enzymes required for CoQ biosynthesis including, *Pdss1* (*CG31005*), *Coq2* (*CG9613*), *Coq3* (*CG9249*), *Coq5* (*CG2453*), *Coq6* (*CG2453*), *Coq7* (*CG14437*), *Coq8* (*CG32649*), *Coq9* (*CG30493*) and *Coq10* (*CG9410*) ubiquitously. Apart from *Coq6*, depletion of these enzymes resulted in development lethality (Fernandez-Ayala et al. 2014). *Coq6* knockdown flies had severe CoQ deficiency and the presence 5-dimethoxy ubiquinol (DMQ) a late stage CoQ biosynthesis intermediate was detected in COQ3, 6, and 9 knockdown animals (Fernandez-Ayala et al. 2014).

Mutation or RNAi mediated depletion of biosynthetic CoQ enzymes in flies results in primary CoQ deficiency. As such these models will allow study of the role of CoQ in development and mitochondrial biology in health and disease.

10.3.4 Complex III

The Zeviani Laboratory described mutations in tetratricopeptide repeat protein *TTC19* which resulted in severe CIII deficiency accompanied by progressive encephalopathy (Ghezzi et al. 2011). A fly KO line for *Ttc19* (*CG15173*) created by transposon insertion displayed a severe deficiency in CIII activity, reduced locomotor activity and an increased sensitivity to mechanical stress (Ghezzi et al. 2011). The parallels between the patient phenotype and that seen in the KO flies underlines the high degree of conservation of genes involved in mitochondrial respiration. Importantly, using RNAi technology to knockdown *Ttc19* down by 70% did not result in the same phenotypes observed in KO flies, highlighting that for some disease genes more than one model may be required (Ghezzi et al. 2011).

10.3.5 Complex IV

The first model of CIV deficiency described in *Drosophila* was the $levy^{1}$ mutation in the orthologue of *COX6A1*, *CG11015* (Liu et al. 2007). Initially identified in a screen of temperature sensitive paralytic mutants, $levy^{1}$ flies have a severe reduction in CIV activity, less ATP, increased sensitivity to mechanical stress and neurodegeneration as early as seven days old (Liu et al. 2007). Even though mutations in *Cox6a1* have not been described in patients we have included this model as the severity of the phenotype and clear neurodegeneration recapitulates the main features of CIV deficiency in humans. This is very important given that knockdown of *Surf1*, that is often found mutated in patients with LS due to CIV deficiency, does not result in neurodegeneration in flies or mice (Da-Re et al. 2014; Dell'agnello et al. 2007).

Ubiquitous depletion of several nuclearly encoded subunits of CIV via RNAi, including *Cox6b1,6c, and 7a,* results in developmental lethality (Kemppainen et al. 2014). Tissue specific depletion in the CNS resulted in a variety of phenotypes including no effect i.e. indistinguishable from wild type and severe neurodegeneration depending the targeted subunit (Kemppainen et al. 2014). Again, highlighting that for some disease genes RNAi knockdown models may not be appropriate.

The *SCO1* and *SCO2* genes, represented by one member in flies (*CG8885*), are required for the biogenesis of the core subunit of CIV, *MT-CO2*. Patients carrying mutations in *SCO1* suffer from CIV deficiency (Valnot et al. 2000). In flies, null mutations of *CG8885* result in developmental lethality while milder alleles result in female sterility (Porcelli et al. 2010). All mutants display a reduction in CIV activity with a reduction in locomotor activity in mutant adults (Porcelli et al. 2010). Given these robust phenotypes flies carrying patient relevant mutations in this gene may provide a more tractable system to study disease pathogenesis and therapeutic validation.

Finally, van Bon and colleagues studied the role of *Cep89* thought to modulate CIV activity *in vitro* in cell culture and *in vivo* in *Drosophila,* after isolating a mutation in a patient suffering from CIV deficiency (Van Bon et al. 2013). Ubiquitous RNAi depletion of the *Drosophila* orthologue of *Cep89* (*CG8214*), resulted in reduced CIV activity and developmental lethality (Van Bon et al. 2013). Tissue specific loss of *Cep89* in either the muscle or CNS also resulted in lethality with a few escapers who presented with severe motor defects. Closer inspection of viable knockdown flies revealed serious neuronal aberrations and learning defects. This study highlights how use of complementary models can allow molecular and pathological examination which can elucidate the role of novel mitochondrial disease genes identified in patients.

10.3.6 Complex V

Mutations of the mitochondrially encoded CV subunit *MT-ATP6* have been shown to cause maternally inherited LS (MILS), Neuropathy, ataxia, retinitis pigmentosa (NARP) and familial bilateral striatal necrosis (FBSN). An endogenous missense mutation of *mt-atp6* was isolated from *Drosophila ANT1* mutants, $sesb^{1}$ (Celotto et al. 2006; Palladino 2010). *mt-atp6* mutant flies ($ATP6^{1}$) had abnormal mitochondrial morphology, no detectable CV activity but respiration was not affected. $ATP6^{1}$ flies had a reduced survival, progressive neuromuscular pathology and locomotor deficiency, mirroring the main features observed in MILS patients (Celotto et al. 2006, Palladino 2010).

10.3.7 Associated Mitochondrial Proteins

Excluding those genes which are directly implicated in OXPHOS, knockout and knockdown models of several associated mitochondrial proteins have been created in *Drosophila*. In humans, mutation of *ANT1*, the ADP/ATP transporter, results in Progressive External Ophthalmoplegia with Mitochondrial DNA deletions (adPEO) and mitochondrial DNA depletion syndrome (Kaukonen et al. 2000; Thompson et al. 2016). Flies carrying a point mutation in *sesB* (*CG16944*), the *Drosophila ANT1* orthologue, are developmentally delayed, sensitive to mechanical stress and have compromised respiration (Zhang et al. 1999; Vartiainen et al. 2014). Analysis at the transcriptional and metabolic level revealed a glycolytic shift and increased mitochondrial stress (Vartiainen et al. 2014). Importantly, this phenotype overlapped with that of the fly model of mitochondrial disease, tko^{25t}, orthologous to human *MRPS12* (Royden 1987; Toivonen et al. 2001; Vartiainen et al. 2014). tko^{25t} flies are developmentally delayed, sensitive to mechanical stress, have a combined OXPHOS defect and display metabolic remodelling (Fernandez-Ayala et al. 2010;

Toivonen et al. 2001). This strongly suggests that some mitochondrial diseases could have common therapeutic targets.

Mutation of the DNA polymerase *POLG2* (*CG33650*) in developing larvae leads to depletion of mtDNA, increased mitochondrial biogenesis and altered mitochondrial neuronal transport (Baqri et al. 2009). This study provided key insights into how altered mitochondrial trafficking in neurons populated with mtDNA deficient mitochondria may underlie pathogenesis of mtDNA depletion syndromes. The Larsson lab have recently used genetic engineering to create flies with an exonuclease (DmPOLγ^{exo-}) deficient form of *POLG* (*CG8987*) (Bratic et al. 2015). They found that DmPOLγ^{exo-} flies accumulated mtDNA mutations, although several fold less than the equivalent "deletor" mouse model despite a clear developmental arrest at the larval stage (Bratic et al. 2015). Using this model, the authors could assess the transmission of these deletions and the existence of a mtDNA mutation threshold after which pathogenic effects were observed.

The fluid state of the mitochondrial network is regulated by the action of Dynamin related protein 1 (*DRP1*), which promotes mitochondrial fission, and Optic Atrophy 1 (*OPA1*) and Mitofusin 1 and 2 (*MFN1/2*) which promote mitochondrial fusion. Homozygous mutation of *Drp1* (CG3210), *Opa1*(*CG8479*) and *Marf* (*CG3869*) are developmentally lethal (Verstreken et al. 2005; Dorn et al. 2011; Debattisti and Scorrano 2013). Examination of *Drp1* mutant larvae uncovered a role for *DRP1* in maintaining cellular distribution of neuronal mitochondria and mitochondrial function as ATP levels were also depleted in *Drp1* mutants (Verstreken et al. 2005). UAS/GAL4 mediated depletion of *Opa1* or *Marf*, the single orthologue of MFN1 and 2, within cardiac muscle resulted in altered mitochondrial morphology and cardiomyopathy (Dorn et al. 2011). Confirming the functional redundancy between the human and fly genes, co-expression of MFN1 or MFN2 could rescue these phenotypes in *Marf* knockdown flies (Dorn et al. 2011). *Opa1* heterozygous flies have a reduced lifespan, increased oxidative stress, altered mitochondrial morphology and reduced activity of CII and III. Mosaic analysis of *Opa1* mutations demonstrated the requirement of Opa1 in the developing eye, where altered mitochondrial morphology and increased ROS levels resulted in a miss-patterned eye due to ectopic cell death (Yarosh et al. 2008).

10.4 Zebrafish Models of Mitochondrial Disease

Danio rerio, commonly referred to as the zebrafish, is a popular vertebrate model system used extensively in developmental studies (Eisen 1996; Fishman 1999). As tools for reverse genetic approaches in zebrafish have improved, their use to study the function of human disease genes has increased (Eisen 1996, Fishman 1999). There are several advantages to working with zebrafish including the availability of genetic tools for gene silencing and transgenesis, as well as high degree of genetic conservation (Wienholds et al. 2003; Soroldoni et al. 2009; Nasevicius and Ekker 2000).

To study mitochondrial disease using zebrafish, several tools and assays have been developed over the last ten years that allow real time visualization of mitochondrial networks and the assessment of physiological effects of mitochondrial dysfunction (Broughton et al. 2001; Kim et al. 2008; Plucinska et al. 2012). Below we highlight those cases where genes described to be mutated in patients have been modelled in zebrafish.

The first zebrafish model of mitochondrial dysfunction was described by Baden and colleagues (Baden et al. 2007). Using morpholinos against CIV subunit, COX5A (*cox5aa* and *cox5ab*) and CIV assembly factor, SURF1 (*surf1*), they could reduce CIV activity by around 50% (Baden et al. 2007). They also found a concomitant decrease in the levels of the mitochondrially encoded core subunit MT-CO1 (*mt-co1*). In all cases, morpholinos against *cox5aa*, *cox5ab*, or *surf1* resulted in severe developmental phenotypes and ectopic apoptosis (Baden et al. 2007). Mutant embryos displayed delayed and malformed development of the nervous system, gut and heart. As such these embryos die in embryogenesis. Although this model reveals details around the effects of CIV deficiency during development which may be informative for infantile syndromes associated with CIV deficiency there are many patient features that cannot be extrapolated here. However, given the strong developmental effects which have been so carefully characterised here, this model could be combined with the developmental screens for small molecules which alleviate the phenotypes described.

More recently zebrafish were used to study the role of CIII subunit Ubiquinol-Cytochrome C Reductase Binding Protein (UQCRB) *in vivo* (Cho et al. 2013). UQCRB has been shown to be mutated in a patient suffering from CIII deficiency (Haut et al. 2003). The focus of the study was the role of CIII in angiogenesis, which was impaired in embryos treated with a small molecule inhibitor of *uqcrb*, terpestacin or injected with morpholinos against *uqcrb* (Cho et al. 2013). Importantly, there were also clear developmental effects of *uqcrb* knockdown suggesting that this model could also be used for developmental screening of effective therapies.

Developmental defects were also observed when two regulators of mitochondrial dynamics, *MFN2* (*mfn2*) and *OPA1* (*opa1*), were depleted independently using specific morpholinos (Vettori et al. 2011; Rahn et al. 2013). *mfn2* knockdown resulted in decreased survival, with only 60% of injected embryos surviving (Vettori et al. 2011). Surviving animals had neuromuscular defects resulting in motility defects, reminiscent of patients carrying *MFN2* mutations which cause CMT2A (Vettori et al. 2011). As such this is a useful model for the investigation of CMT2A while the characterization of the developmental defects will allow easy screening of effective small molecules. Depletion of *opa1* also resulted in developmental defects with larvae dying at 7 days post fertilisation (dpf). *opa1* knockdown larvae had a fragmented mitochondrial network, reduced respiration and compensatory upregulation of mitochondrial biogenesis and transcription (Rahn et al. 2013). Although not directly extrapolatable to human patients with *OPA1* mutations, this like those zebrafish models described above could be used to identify active therapeutic small molecules.

10.5 *Caenorhabditis elegans* Models of Mitochondrial Disease

The transparent nematode *C. elegans* has been used extensively over the last 50 years to study diverse and complex biological processes (Kenyon 1988). Its short life cycle, defined cellular lineage and array of tools for genetic manipulation have made it a powerful force in the study of gene function (Kenyon 1988). The simplicity of its nervous system coupled with conservation of neuronal subtypes and communication make it an attractive model to study neurological disorders. Given the severe effects that mitochondrial dysfunction has on the nervous system of patients with mitochondrial disease, using *C .elegans* to study mutations identified in patients will allow investigation of the neuropathology of several mitochondrial disorders.

Several classical genetic mutants which correspond to mitochondrial genes found mutated in patients have been isolated in *C. elegans* (Kayser et al. 2001; Kayser et al. 2004; Ishii et al. 1998; Grad and Lemire 2004; Tsang et al. 2001). Mutants of the *NDUFS2* orthologue, *gas-1* have a reduce lifespan, retarded growth, increased sensitivity to temperature, oxidative stress and hyperoxia, as well as a reduced CI dependant respiration (Kayser et al. 2001). Mutation of *mev-1*, the orthologue of *SDHC* result in increased oxygen sensitivity and accelerated ageing. Analysis of the CII function demonstrated the requirement of this subunit for the participation of CII in electron transport (Ishii et al. 1998). Grad and Lemire created 3 models expressing distinct patient mutations of *nuo-1*, the orthologue of *NDUFV1* (Grad and Lemire 2004). In all three cases, mutations of *nuo-1* resulted in decreased lifespan, premature ageing, compromised respiration and lactic acidosis (Grad and Lemire 2004). *clk-1*, *orthologue* of *COQ7*, mutants are unable to produce CoQ_9, accumulate the intermediate DMQ_9 and display defects in CoQ dependant activities of CI and CIII, as has been described in other animal models of *COQ7* loss (Kayser et al. 2004; Fernandez-Ayala et al. 2014).

These studies clearly demonstrate that mutation of genes found in patients with mitochondrial disease can induce many of same phenotypes in worms, validating their use as a model for the study of molecular pathogenesis and the effectiveness of available therapies.

10.6 Conclusion

As can be appreciated from the studies summarised, the extent of mitochondrial disease models currently available is impressive. Each with its advantages and limitations. The last 25 years of mitochondrial research has elucidated many important aspects of these organelles functions in health and disease. Despite this progress patients remain without treatments. As we move forward the focus remains on understanding the molecular pathology underlying mitochondrial disease but increasingly it will be on the development and validation of therapies. These aims both rely on a foundation of disease relevant and genetically defined models. Models

of mitochondrial disease in flies, worms and fish display complex and pleiotropic phenotypes of mitochondrial syndromes which can be extrapolated to human patients. Although the mouse is an excellent model for human disease, the use of complementary models suitable for high throughput screening, where patient specific mutations can be introduced using state of the art genome editing will accelerate progress in both these areas.

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References

Agostino A, Invernizzi F, Tiveron C, Fagiolari G, Prelle A, Lamantea E, Giavazzi A, Battaglia G, Tatangelo L, Tiranti V, Zeviani M (2003) Constitutive knockout of Surf1 is associated with high embryonic lethality, mitochondrial disease and cytochrome c oxidase deficiency in mice. Hum Mol Genet 12:399–413

Akman HO, Dorado B, López LC, García-Cazorla A, Vilà MR, Tanabe LM, Dauer WT, Bonilla E, Tanji K, Hirano M (2008) Thymidine kinase 2 (H126N) knockin mice show the essential role of balanced deoxynucleotide pools for mitochondrial DNA maintenance. Hum Mol Genet 17:2433–2440

Alavi MV, Bette S, Schimpf S, Schuettauf F, Schraermeyer U, Wehrl HF, Ruttiger L, Beck SC, Tonagel F, Pichler BJ, Knipper M, Peters T, Laufs J, Wissinger B (2007) A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. Brain J Neurol 130:1029–1042

Alavi MV, Fuhrmann N, Nguyen HP, Yu-Wai-Man P, Heiduschka P, Chinnery PF, Wissinger B (2009) Subtle neurological and metabolic abnormalities in an Opa1 mouse model of autosomal dominant optic atrophy. Exp Neurol 220:404–409

Al-Mahdawi S, Pinto RM, Varshney D, Lawrence L, Lowrie MB, Hughes S, Webster Z, Blake J, Cooper JM, King R, Pook MA (2006) GAA repeat expansion mutation mouse models of Friedreich ataxia exhibit oxidative stress leading to progressive neuronal and cardiac pathology. Genomics 88:580–590

Alston CL, Davison JE, Meloni F, Van Der Westhuizen FH, He L, Hornig-Do HT, Peet AC, Gissen P, Goffrini P, Ferrero I, Wassmer E, Mcfarland R, Taylor RW (2012) Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. J Med Genet 49:569–577

Alston CL, Ceccatelli Berti C, Blakely EL, Olahova M, He L, Mcmahon CJ, Olpin SE, Hargreaves IP, Nolli C, Mcfarland R, Goffrini P, O'sullivan MJ, Taylor RW (2015) A recessive homozygous p.Asp92Gly SDHD mutation causes prenatal cardiomyopathy and a severe mitochondrial complex II deficiency. Hum Genet 134:869–879

Antonicka H, Leary SC, Guercin GH, Agar JN, Horvath R, Kennaway NG, Harding CO, Jaksch M, Shoubridge EA (2003a) Mutations in COX10 result in a defect in mitochondrial heme A biosynthesis and account for multiple, early-onset clinical phenotypes associated with isolated COX deficiency. Hum Mol Genet 12:2693–2702

Antonicka H, Mattman A, Carlson CG, Glerum DM, Hoffbuhr KC, Leary SC, Kennaway NG, Shoubridge EA (2003b) Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. Am J Hum Genet 72:101–114

Avior Y, Sagi I, Benvenisty N (2016) Pluripotent stem cells in disease modelling and drug discovery. Nat Rev Mol Cell Biol 17:170–182

Baden KN, Murray J, Capaldi RA, Guillemin K (2007) Early developmental pathology due to cytochrome c oxidase deficiency is revealed by a new zebrafish model. J Biol Chem 282:34839–34849

Baqri RM, Turner BA, Rheuben MB, Hammond BD, Kaguni LS, Miller KE (2009) Disruption of mitochondrial DNA replication in Drosophila increases mitochondrial fast axonal transport in vivo. PLoS One 4:e7874

Baxevanis CN, Voutsas IF, Tsitsilonis OE, Tsiatas ML, Gritzapis AD, Papamichail M (2000) Compromised anti-tumor responses in tumor necrosis factor-alpha knockout mice. Eur J Immunol 30:1957–1966

Bayley J-P, Van Minderhout I, Hogendoorn PCW, Cornelisse CJ, Van Der Wal A, Prins FA, Teppema L, Dahan A, Devilee P, Taschner PEM (2009) Sdhd and SDHD/H19 knockout mice do not develop paraganglioma or pheochromocytoma. PLoS One 4:e7987

Bensch KG, Mott JL, Chang S-W, Hansen PA, Moxley MA, Chambers KT, De Graaf W, Zassenhaus HP, Corbett JA (2009) Selective mtDNA mutation accumulation results in beta-cell apoptosis and diabetes development. Am J Physiol Endocrinol Metab 296:E672–E680

Bentinger M, Tekle M, Dallner G (2010) Coenzyme Q–biosynthesis and functions. Biochem Biophys Res Commun 396:74–79

Blazquez A, Gil-Borlado MC, Moran M, Verdu A, Cazorla-Calleja MR, Martin MA, Arenas J, Ugalde C (2009) Infantile mitochondrial encephalomyopathy with unusual phenotype caused by a novel BCS1L mutation in an isolated complex III-deficient patient. Neuromuscul Disord 19:143–146

Bottani E, Giordano C, Civiletto G, di Meo I, Auricchio A, Ciusani E, Marchet S, Lamperti C, D'amati G, Viscomi C, Zeviani M (2014) AAV-mediated liver-specific MPV17 expression restores mtDNA levels and prevents diet-induced liver failure. Mol Ther 22:10–17

Bourdon A, Minai L, Serre V, Jais J-P, Sarzi E, Aubert S, Chretien D, De Lonlay P, Paquis-Flucklinger V, Arakawa H, Nakamura Y, Munnich A, Rötig A (2007) Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. Nat Genet 39:776–780

Boustany RN, Aprille JR, Halperin J, Levy H, Delong GR (1983) Mitochondrial cytochrome deficiency presenting as a myopathy with hypotonia, external ophthalmoplegia, and lactic acidosis in an infant and as fatal hepatopathy in a second cousin. Ann Neurol 14:462–470

Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415

Bratic A, Kauppila TE, Macao B, Gronke S, Siibak T, Stewart JB, Baggio F, Dols J, Partridge L, Falkenberg M, Wredenberg A, Larsson NG (2015) Complementation between polymerase- and exonuclease-deficient mitochondrial DNA polymerase mutants in genomically engineered flies. Nat Commun 6:8808

Broughton RE, Milam JE, Roe BA (2001) The complete sequence of the zebrafish (Danio rerio) mitochondrial genome and evolutionary patterns in vertebrate mitochondrial DNA. Genome Res 11:1958–1967

Budde SM, Van Den Heuvel LP, Janssen AJ, Smeets RJ, Buskens CA, Demeirleir L, Van Coster R, Baethmann M, Voit T, Trijbels JM, Smeitink JA (2000) Combined enzymatic complex I and III deficiency associated with mutations in the nuclear encoded NDUFS4 gene. Biochem Biophys Res Commun 275:63–68

Burman JL, Itsara LS, Kayser EB, Suthammarak W, Wang AM, Kaeberlein M, Sedensky MM, Morgan PG, Pallanck LJ (2014) A Drosophila model of mitochondrial disease caused by a complex I mutation that uncouples proton pumping from electron transfer. Dis Model Mech 7:1165–1174

Calvaruso MA, Willems P, Van Den Brand M, Valsecchi F, Kruse S, Palmiter R, Smeitink J, Nijtmans L (2012) Mitochondrial complex III stabilizes complex I in the absence of NDUFS4 to provide partial activity. Hum Mol Genet 21:115–120

Cameron JM, Levandovskiy V, Mackay N, Robinson BH (2004) Respiratory chain analysis of skin fibroblasts in mitochondrial disease. Mitochondrion 4:387–394

Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Canizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, di Donato S, Mandel JL, Cocozza S, Koenig M, Pandolfo M (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271:1423–1427

Cartoni R, Arnaud E, Medard JJ, Poirot O, Courvoisier DS, Chrast R, Martinou JC (2010) Expression of mitofusin 2(R94Q) in a transgenic mouse leads to Charcot-Marie-tooth neuropathy type 2A. Brain 133:1460–1469

Celotto AM, Frank AC, Mcgrath SW, Fergestad T, Van Voorhies WA, Buttle KF, Mannella CA, Palladino MJ (2006) Mitochondrial encephalomyopathy in Drosophila. J Neurosci 26:810–820

Cerritelli SM, Frolova EG, Feng C, Grinberg A, Love PE, Crouch RJ (2003) Failure to produce mitochondrial DNA results in embryonic lethality in Rnaseh1 null mice. Mol Cell 11:807–815

Chan HY, Bonini NM (2000) Drosophila models of human neurodegenerative disease. Cell Death Differ 7:1075–1080

Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 160:189–200

Chen H, Mccaffery JM, Chan DC (2007) Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell 130:548–562

Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, Mccaffery JM, Chan DC (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. Cell 141:280–289

Chen Y, Csordas G, Jowdy C, Schneider TG, Csordas N, Wang W, Liu Y, Kohlhaas M, Meiser M, Bergem S, Nerbonne JM, Dorn GW, Maack C (2012) Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca(2+) crosstalk. Circ Res 111:863–875

Chinnery PF, Brown DT, Andrews RM, Singh-Kler R, Riordan-Eva P, Lindley J, Applegarth DA, Turnbull DM, Howell N (2001) The mitochondrial ND6 gene is a hot spot for mutations that cause Leber's hereditary optic neuropathy. Brain 124:209–218

Cho J, Hur JH, Graniel J, Benzer S, Walker DW (2012) Expression of yeast NDI1 rescues a Drosophila complex I assembly defect. PLoS One 7:e50644

Cho YS, Jung HJ, Seok SH, Payumo AY, Chen JK, Kwon HJ (2013) Functional inhibition of UQCRB suppresses angiogenesis in zebrafish. Biochem Biophys Res Commun 433:396–400

Clozel M, Hess P, Fischli W, Löffler BM, Zwacka RM, Reuter A, Weiher H (1999) Age-dependent hypertension in Mpv17-deficient mice, a transgenic model of glomerulosclerosis and inner ear disease. EXG 34:1007–1015

Cossée M, Puccio H, Gansmuller A, Koutnikova H, Dierich A, Lemeur M, Fischbeck K, Dollé P, Koenig M (2000) Inactivation of the Friedreich ataxia mouse gene leads to early embryonic lethality without iron accumulation. Hum Mol Genet 9:1219–1226

Cowchock FS, Duckett SW, Streletz LJ, Graziani LJ, Jackson LG (1985) X-linked motor-sensory neuropathy type-II with deafness and mental retardation: a new disorder. Am J Med Genet 20:307–315

Dahl HH (1998) Getting to the nucleus of mitochondrial disorders: identification of respiratory chain-enzyme genes causing Leigh syndrome. Am J Hum Genet 63:1594–1597

Dallabona C, Marsano RM, Arzuffi P, Ghezzi D, Mancini P, Zeviani M, Ferrero I, Donnini C (2010) Sym1, the yeast ortholog of the MPV17 human disease protein, is a stress-induced bioenergetic and morphogenetic mitochondrial modulator. Hum Mol Genet 19:1098–1107

Da-Re C, Von Stockum S, Biscontin A, Millino C, Cisotto P, Zordan MA, Zeviani M, Bernardi P, De Pitta C, Costa R (2014) Leigh syndrome in Drosophila melanogaster: morphological and biochemical characterization of Surf1 post-transcriptional silencing. J Biol Chem 289:29235–29246

Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, Votruba M (2007) Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. Hum Mol Genet 16:1307–1318

De Lonlay P, Valnot I, Barrientos A, Gorbatyuk M, Tzagoloff A, Taanman J-W, Benayoun E, Chretien D, Kadhom N, Lombes A, De Baulny HO, Niaudet P, Munnich A, Rustin P, Rotig A (2001) A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. Nat Genet 29:57–60

Debattisti V, Scorrano L (2013) D. melanogaster, mitochondria and neurodegeneration: small model organism, big discoveries. Mol Cell Neurosci 55:77–86

Debray FG, Morin C, Janvier A, Villeneuve J, Maranda B, Laframboise R, Lacroix J, Decarie JC, Robitaille Y, Lambert M, Robinson BH, Mitchell GA (2011) LRPPRC mutations cause a phenotypically distinct form of Leigh syndrome with cytochrome c oxidase deficiency. J Med Genet 48:183–189

Delatycki MB, Williamson R, Forrest SM (2000) Friedreich ataxia: an overview. J Med Genet 37:1–8

Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J, Hamel CP (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet 26:207–210

Dell'agnello C, Leo S, Agostino A, Szabadkai G, Tiveron C, Zulian A, Prelle A, Roubertoux P, Rizzuto R, Zeviani M (2007) Increased longevity and refractoriness to ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Hum Mol Genet 16:431–444

Detmer SA, Velde CV, Cleveland DW, Chan DC (2008) Hindlimb gait defects due to motor axon loss and reduced distal muscles in a transgenic mouse model of Charcot-Marie-tooth type 2A. Hum Mol Genet 17:367–375

Di Meo I, Fagiolari G, Prelle A, Viscomi C, Zeviani M, Tiranti V (2011) Chronic exposure to sulfide causes accelerated degradation of cytochrome c oxidase in ethylmalonic encephalopathy. Antioxid Redox Signal 15:353–362

Diaz F, Thomas CK, Garcia S, Hernandez D, Moraes CT (2005) Mice lacking COX10 in skeletal muscle recapitulate the phenotype of progressive mitochondrial myopathies associated with cytochrome c oxidase deficiency. Hum Mol Genet 14:2737–2748

Diaz F, Garcia S, Hernandez D, Regev A, Rebelo A, Oca-Cossio J, Moraes CT (2008) Pathophysiology and fate of hepatocytes in a mouse model of mitochondrial hepatopathies. Gut 57:232–242

Diaz F, Garcia S, Padgett KR, Moraes CT (2012a) A defect in the mitochondrial complex III, but not complex IV, triggers early ROS-dependent damage in defined brain regions. Hum Mol Genet 21:5066–5077

Diaz F, Garcia S, Padgett KR, Moraes CT (2012b) A defect in the mitochondrial complex III, but not complex IV, triggers early ROS-dependent damage in defined brain regions. Hum Mol Genet 21:5066–5077

Dietzl G, Chen D, Schnorrer F, Su K-C, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, Couto A, Marra V, Keleman K, Dickson BJ (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 448:151–156

Dorn GW, Clark CF, Eschenbacher WH, Kang MY, Engelhard JT, Warner SJ, Matkovich SJ, Jowdy CC (2011) MARF and Opa1 control mitochondrial and cardiac function in Drosophila. Circ Res 108:12–17

Duncan AJ, Bitner-Glindzicz M, Meunier B, Costello H, Hargreaves IP, Lopez LC, Hirano M, Quinzii CM, Sadowski MI, Hardy J, Singleton A, Clayton PT, Rahman S (2009) A nonsense mutation in COQ9 causes autosomal-recessive neonatal-onset primary coenzyme Q10 deficiency: a potentially treatable form of mitochondrial disease. Am J Hum Genet 84:558–566

Eisen JS (1996) Zebrafish make a big splash. Cell 87:969–977

Ekstrand MI, Terzioglu M, Galter D, Zhu S, Hofstetter C, Lindqvist E, Thams S, Bergstrand A, Hansson FS, Trifunovic A, Hoffer B, Cullheim S, Mohammed AH, Olson L, Larsson N-G (2007) Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proc Natl Acad Sci 104:1325–1330

Fan W, Waymire KG, Narula N, Li P, Rocher C, Coskun PE, Vannan MA, Narula J, Macgregor GR, Wallace DC (2008) A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. Science 319:958–962

Fernandez-Ayala DJ, Chen S, Kemppainen E, O'dell KM, Jacobs HT (2010) Gene expression in a Drosophila model of mitochondrial disease. PLoS One 5:e8549

Fernandez-Ayala DJ, Jimenez-Gancedo S, Guerra I, Navas P (2014) Invertebrate models for coenzyme q10 deficiency. Mol Syndromol 5:170–179

Fernandez-Vizarra E, Bugiani M, Goffrini P, Carrara F, Farina L, Procopio E, Donati A, Uziel G, Ferrero I, Zeviani M (2007) Impaired complex III assembly associated with BCS1L gene mutations in isolated mitochondrial encephalopathy. Hum Mol Genet 16:1241–1252

Fischbeck KH, Ar-Rushdi N, Pericak-Vance M, Rozear M, Roses AD, Fryns JP (1986) X-linked neuropathy: gene localization with DNA probes. Ann Neurol 20:527–532

Fishman MC (1999) Zebrafish genetics: the enigma of arrival. Proc Natl Acad Sci 96:10554–10556

Forbes JM, Ke BX, Nguyen TV, Henstridge DC, Penfold SA, Laskowski A, Sourris KC, Groschner LN, Cooper ME, Thorburn DR, Coughlan MT (2013) Deficiency in mitochondrial complex I activity due to Ndufs6 gene trap insertion induces renal disease. Antioxid Redox Signal 19:331–343

Formentini L, Sanchez-Arago M, Sanchez-Cenizo L, Cuezva JM (2012) The mitochondrial ATPase inhibitory factor 1 triggers a ROS-mediated retrograde prosurvival and proliferative response. Mol Cell 45:731–742

Formentini L, Pereira MP, Sanchez-Cenizo L, Santacatterina F, Lucas JJ, Navarro C, Martinez-Serrano A, Cuezva JM (2014) In vivo inhibition of the mitochondrial H+-ATP synthase in neurons promotes metabolic preconditioning. EMBO J 33:762–778

Forsgren M, Attersand A, Lake S, Grunler J, Swiezewska E, Dallner G, Climent I (2004) Isolation and functional expression of human COQ2, a gene encoding a polyprenyl transferase involved in the synthesis of CoQ. Biochem J 382:519–526

Gall JM, Wang Z, Liesa M, Molina A, Havasi A, Schwartz JH, Shirihai O, Borkan SC, Bonegio RGB (2012) Role of mitofusin 2 in the renal stress response. PLoS One 7:e31074

Garcia-Corzo L, Luna-Sanchez M, Doerrier C, Garcia JA, Guaras A, Acin-Perez R, Bullejos-Peregrin J, Lopez A, Escames G, Enriquez JA, Acuna-Castroviejo D, Lopez LC (2013) Dysfunctional Coq9 protein causes predominant encephalomyopathy associated with CoQ deficiency. Hum Mol Genet 22:1233–1248

Ghezzi D, Sevrioukova I, Invernizzi F, Lamperti C, Mora M, D'adamo P, Novara F, Zuffardi O, Uziel G, Zeviani M (2010) Severe X-linked mitochondrial encephalomyopathy associated with a mutation in apoptosis-inducing factor. Am J Hum Genet 86:639–649

Ghezzi D, Arzuffi P, Zordan M, Da Re C, Lamperti C, Benna C, D'adamo P, Diodato D, Costa R, Mariotti C, Uziel G, Smiderle C, Zeviani M (2011) Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies. Nat Genet 43:259–263

Gorman GS, Chinnery PF, Dimauro S, Hirano M, Koga Y, Mcfarland R, Suomalainen A, Thorburn DR, Zeviani M, Turnbull DM (2016) Mitochondrial diseases. Nat Rev Dis Primers 2:16080

Grad LI, Lemire BD (2004) Mitochondrial complex I mutations in Caenorhabditis elegans produce cytochrome c oxidase deficiency, oxidative stress and vitamin-responsive lactic acidosis. Hum Mol Genet 13:303–314

Graham BH, Waymire KG, Cottrell B, Trounce IA, Macgregor GR, Wallace DC (1997) A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator. Nat Genet 16:226–234

Grant J, Saldanha JW, Gould AP (2010) A Drosophila model for primary coenzyme Q deficiency and dietary rescue in the developing nervous system. Dis Model Mech 3:799–806

Hakonen AH, Goffart S, Marjavaara S, Paetau A, Cooper H, Mattila K, Lampinen M, Sajantila A, Lonnqvist T, Spelbrink JN, Suomalainen A (2008) Infantile-onset spinocerebellar ataxia and mitochondrial recessive ataxia syndrome are associated with neuronal complex I defect and mtDNA depletion. Hum Mol Genet 17:3822–3835

Hallman TM, Peng M, Meade R, Hancock WW, Madaio MP, Gasser DL (2006) The mitochondrial and kidney disease phenotypes of kd/kd mice under germfree conditions. J Autoimmun 26:1–6

Hance N, Ekstrand MI, Trifunovic A (2005) Mitochondrial DNA polymerase gamma is essential for mammalian embryogenesis. Hum Mol Genet 14:1775–1783

Haraguchi M, Tsujimoto H, Fukushima M, Higuchi I, Kuribayashi H, Utsumi H, Nakayama A, Hashizume Y, Hirato J, Yoshida H, Hara H, Hamano S, Kawaguchi H, Furukawa T, Miyazono K, Ishikawa F, Toyoshima H, Kaname T, Komatsu M, Chen Z-S, Gotanda T, Tachiwada T, Sumizawa T, Miyadera K, Osame M, Yoshida H, Noda T, Yamada Y, Akiyama S-I (2002) Targeted deletion of both thymidine phosphorylase and uridine phosphorylase and consequent disorders in mice. Mol Cell Biol 22:5212–5221

Hashizume O, Shimizu A, Yokota M, Sugiyama A, Nakada K, Miyoshi H, Itami M, Ohira M, Nagase H, Takenaga K, Hayashi J-I (2012) Specific mitochondrial DNA mutation in mice regulates diabetes and lymphoma development. Proc Natl Acad Sci U S A 109:10528–10533

Haut S, Brivet M, Touati G, Rustin P, Lebon S, Garcia-Cazorla A, Saudubray JM, Boutron A, Legrand A, Slama A (2003) A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis. Hum Genet 113:118–122

Hinson JT, Fantin VR, Schonberger J, Breivik N, Siem G, Mcdonough B, Sharma P, Keogh I, Godinho R, Santos F, Esparza A, Nicolau Y, Selvaag E, Cohen BH, Hoppel CL, Tranebjaerg L, Eavey RD, Seidman JG, Seidman CE (2007) Missense mutations in the BCS1L gene as a cause of the Bjornstad syndrome. N Engl J Med 356:809–819

Hirano M, Garone C, Quinzii CM (2012) CoQ(10) deficiencies and MNGIE: two treatable mitochondrial disorders. Biochim Biophys Acta 1820:625–631

Humble MM, Young MJ, Foley JF, Pandiri AR, Travlos GS, Copeland WC (2013) Polg2 is essential for mammalian embryogenesis and is required for mtDNA maintenance. Hum Mol Genet 22:1017–1025

Hüttemann M, Kadenbach B, Grossman LI (2001) Mammalian subunit IV isoforms of cytochrome c oxidase. Gene 267:111–123

Huttemann M, Lee I, Gao X, Pecina P, Pecinova A, Liu J, Aras S, Sommer N, Sanderson TH, Tost M, Neff F, Aguilar-Pimentel JA, Becker L, Naton B, Rathkolb B, Rozman J, Favor J, Hans W, Prehn C, Puk O, Schrewe A, Sun M, Hofler H, Adamski J, Bekeredjian R, Graw J, Adler T, Busch DH, Klingenspor M, Klopstock T, Ollert M, Wolf E, Fuchs H, Gailus-Durner V, Hrabe De Angelis M, Weissmann N, Doan JW, Bassett DJ, Grossman LI (2012) Cytochrome c oxidase subunit 4 isoform 2-knockout mice show reduced enzyme activity, airway hyporeactivity, and lung pathology. FASEB J 26:3916–3930

Ingraham CA, Burwell LS, Skalska J, Brookes PS, Howell RL, Sheu SS, Pinkert CA (2009) NDUFS4: creation of a mouse model mimicking a complex I disorder. Mitochondrion 9:204–210

Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394:694–697

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J-I (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664

Iuso A, Scacco S, Piccoli C, Bellomo F, Petruzzella V, Trentadue R, Minuto M, Ripoli M, Capitanio N, Zeviani M, Papa S (2006) Dysfunctions of cellular oxidative metabolism in patients with mutations in the NDUFS1 and NDUFS4 genes of complex I. J Biol Chem 281:10374–10380

Jackson CB, Nuoffer JM, Hahn D, Prokisch H, Haberberger B, Gautschi M, Haberli A, Gallati S, Schaller A (2014) Mutations in SDHD lead to autosomal recessive encephalomyopathy and isolated mitochondrial complex II deficiency. J Med Genet 51:170–175

Jacobs HT, Fernandez-Ayala DJ, Manjiry S, Kemppainen E, Toivonen JM, O'dell KM (2004) Mitochondrial disease in flies. Biochim Biophys Acta 1659:190–196

Jennings BH (2011) Drosophila – a versatile model in biology & medicine. Mater Today 14:190–195

Johansson M, Karlsson A (1997) Cloning of the cDNA and chromosome localization of the gene for human thymidine kinase 2. J Biol Chem 272:8454–8458

Johns DR, Berman J (1991) Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 174:1324–1330

Johns DR, Neufeld MJ, Park RD (1992) An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 187:1551–1557

Johnson SC, Yanos ME, Kayser EB, Quintana A, Sangesland M, Castanza A, Uhde L, Hui J, Wall VZ, Gagnidze A, Oh K, Wasko BM, Ramos FJ, Palmiter RD, Rabinovitch PS, Morgan PG, Sedensky MM, Kaeberlein M (2013) mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science 342:1524–1528

Joza N, Oudit GY, Brown D, Benit P, Kassiri Z, Vahsen N, Benoit L, Patel MM, Nowikovsky K, Vassault A, Backx PH, Wada T, Kroemer G, Rustin P, Penninger JM (2005) Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. Mol Cell Biol 25:10261–10272

Joza N, Pospisilik JA, Hangen E, Hanada T, Modjtahedi N, Penninger JM, Kroemer G (2009) AIF: not just an apoptosis-inducing factor. Ann N Y Acad Sci 1171:2–11

Karamanlidis G, Lee CF, Garcia-Menendez L, Kolwicz SC Jr, Suthammarak W, Gong G, Sedensky MM, Morgan PG, Wang W, Tian R (2013) Mitochondrial complex I deficiency increases protein acetylation and accelerates heart failure. Cell Metab 18:239–250

Kasahara T, Kubota M, Miyauchi T, Noda Y, Mouri A, Nabeshima T, Kato T (2006) Mice with neuron-specific accumulation of mitochondrial DNA mutations show mood disorder-like phenotypes. Mol Psychiatry 11:577–593

Kaukonen J, Juselius JK, Tiranti V, Kyttala A, Zeviani M, Comi GP, Keranen S, Peltonen L, Suomalainen A (2000) Role of adenine nucleotide translocator 1 in mtDNA maintenance. Science 289:782–785

Kayser EB, Morgan PG, Hoppel CL, Sedensky MM (2001) Mitochondrial expression and function of GAS-1 in Caenorhabditis elegans. J Biol Chem 276:20551–20558

Kayser E-B, Sedensky MM, Morgan PG, Hoppel CL (2004) Mitochondrial oxidative phosphorylation is defective in the long-lived mutant clk-1. J Biol Chem 279:54479–54486

Ke B-X, Pepe S, Grubb DR, Komen JC, Laskowski A, Rodda FA, Hardman BM, Pitt JJ, Ryan MT, Lazarou M, Koleff J, Cheung MMH, Smolich JJ, Thorburn DR (2012) Tissue-specific splicing of an Ndufs6 gene-trap insertion generates a mitochondrial complex I deficiency-specific cardiomyopathy. Proc Natl Acad Sci U S A 109:6165–6170

Kemppainen KK, Rinne J, Sriram A, Lakanmaa M, Zeb A, Tuomela T, Popplestone A, Singh S, Sanz A, Rustin P, Jacobs HT (2014) Expression of alternative oxidase in Drosophila ameliorates diverse phenotypes due to cytochrome oxidase deficiency. Hum Mol Genet 23:2078–2093

Kenyon C (1988) The nematode Caenorhabditis elegans. Science 240:1448

Kim MJ, Kang KH, Kim CH, Choi SY (2008) Real-time imaging of mitochondria in transgenic zebrafish expressing mitochondrially targeted GFP. BioTechniques 45:331–334

Kimura T, Takeda S, Sagiya Y, Gotoh M, Nakamura Y, Arakawa H (2003) Impaired function of p53R2 in Rrm2b-null mice causes severe renal failure through attenuation of dNTP pools. Nat Genet 34:440–445

Kirby DM, Salemi R, Sugiana C, Ohtake A, Parry L, Bell KM, Kirk EP, Boneh A, Taylor RW, Dahl HH, Ryan MT, Thorburn DR (2004) NDUFS6 mutations are a novel cause of lethal neonatal mitochondrial complex I deficiency. J Clin Invest 114:837–845

Klein JA, Longo-Guess CM, Rossmann MP, Seburn KL, Hurd RE, Frankel WN, Bronson RT, Ackerman SL (2002) The harlequin mouse mutation downregulates apoptosis-inducing factor. Nature 419:367–374

Kollberg G, Darin N, Benan K, Moslemi AR, Lindal S, Tulinius M, Oldfors A, Holme E (2009) A novel homozygous RRM2B missense mutation in association with severe mtDNA depletion. Neuromuscul Disord 19:147–150

Kotarsky H, Keller M, Davoudi M, Leveen P, Karikoski R, Enot DP, Fellman V (2012) Metabolite profiles reveal energy failure and impaired beta-oxidation in liver of mice with complex III deficiency due to a BCS1L mutation. PLoS One 7:e41156

Kruse SE, Watt WC, Marcinek DJ, Kapur RP, Schenkman KA, Palmiter RD (2008) Mice with mitochondrial complex I deficiency develop a fatal encephalomyopathy. Cell Metab 7:312–320

Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla TA (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 309:481–484

Lamantea E, Tiranti V, Bordoni A, Toscano A, Bono F, Servidei S, Papadimitriou A, Spelbrink H, Silvestri L, Casari G, Comi GP, Zeviani M (2002) Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. Ann Neurol 52:211–219

Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS, Clayton DA (1998) Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat Genet 18:231–236

Lee T, Luo L (2001) Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci 24:251–254

Lee J, Schriner SE, Wallace DC (2009) Adenine nucleotide translocator 1 deficiency increases resistance of mouse brain and neurons to excitotoxic insults. Biochim Biophys Acta 1787:364–370

Leong DW, Komen JC, Hewitt CA, Arnaud E, Mckenzie M, Phipson B, Bahlo M, Laskowski A, Kinkel SA, Davey GM, Heath WR, Voss AK, Zahedi RP, Pitt JJ, Chrast R, Sickmann A, Ryan MT, Smyth GK, Thorburn DR, Scott HS (2012) Proteomic and metabolomic analyses of mitochondrial complex I-deficient mouse model generated by spontaneous B2 short interspersed nuclear element (SINE) insertion into NADH dehydrogenase (ubiquinone) Fe-S protein 4 (Ndufs4) gene. J Biol Chem 287:20652–20663

Leveen P, Kotarsky H, Morgelin M, Karikoski R, Elmer E, Fellman V (2011) The GRACILE mutation introduced into Bcs1l causes postnatal complex III deficiency: a viable mouse model for mitochondrial hepatopathy. Hepatology 53:437–447

Lewis W, Day BJ, Kohler JJ, Hosseini SH, Chan SSL, Green EC, Haase CP, Keebaugh ES, Long R, Ludaway T, Russ R, Steltzer J, Tioleco N, Santoianni R, Copeland WC (2007) Decreased mtDNA, oxidative stress, cardiomyopathy, and death from transgenic cardiac targeted human mutant polymerase gamma. Lab Investig 87:326–335

Li Z (2013) Pathogenic study of mitochondrial complex I deficiency and Leigh syndrome in Drosophila model. Am Soc Human Genet 2013:2396F

Li K, Warner CK, Hodge JA, Minoshima S, Kudoh J, Fukuyama R, Maekawa M, Shimizu Y, Shimizu N, Wallace DC (1989) A human muscle adenine nucleotide translocator gene has four exons, is located on chromosome 4, and is differentially expressed. J Biol Chem 264:13998–14004

Li H, Wang J, Wilhelmsson H, Hansson A, Thoren P, Duffy J, Rustin P, Larsson NG (2000a) Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. Proc Natl Acad Sci U S A 97:3467–3472

Li K, Li Y, Shelton JM, Richardson JA, Spencer E, Chen ZJ, Wang X, Williams RS (2000b) Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. Cell 101:389–399

Lightowlers RN, Taylor RW, Turnbull DM (2015) Mutations causing mitochondrial disease: what is new and what challenges remain? Science 349:1494–1499

Lima WF, Murray HM, Damle SS, Hart CE, Hung G, De Hoyos CL, Liang X-H, Crooke ST (2016) Viable RNaseH1 knockout mice show RNaseH1 is essential for R loop processing, mitochondrial and liver function. Nucleic Acids Res 44:5299–5312

Lin CS, Sharpley MS, Fan W, Waymire KG, Sadun AA, Carelli V, Ross-Cisneros FN, Baciu P, Sung E, Mcmanus MJ, Pan BX, Gil DW, Macgregor GR, Wallace DC (2012) Mouse mtDNA mutant model of Leber hereditary optic neuropathy. Proc Natl Acad Sci U S A 109:20065–20070

Liu W, Gnanasambandam R, Benjamin J, Kaur G, Getman PB, Siegel AJ, Shortridge RD, Singh S (2007) Mutations in cytochrome c oxidase subunit VIa cause neurodegeneration and motor dysfunction in Drosophila. Genetics 176:937–946

Liu J, Wu Q, He D, Ma T, Du L, Dui W, Guo X, Jiao R (2011) Drosophila sbo regulates lifespan through its function in the synthesis of coenzyme Q in vivo. J Genet Genomics 38:225–234

Lohman DC, Forouhar F, Beebe ET, Stefely MS, Minogue CE, Ulbrich A, Stefely JA, Sukumar S, Luna-Sanchez M, Jochem A, Lew S, Seetharaman J, Xiao R, Wang H, Westphall MS, Wrobel RL, Everett JK, Mitchell JC, Lopez LC, Coon JJ, Tong L, Pagliarini DJ (2014) Mitochondrial COQ9 is a lipid-binding protein that associates with COQ7 to enable coenzyme Q biosynthesis. Proc Natl Acad Sci U S A 111:E4697–E4705

Longley MJ, Clark S, Yu Wai Man C, Hudson G, Durham SE, Taylor RW, Nightingale S, Turnbull DM, Copeland WC, Chinnery PF (2006) Mutant POLG2 disrupts DNA polymerase gamma subunits and causes progressive external ophthalmoplegia. Am J Hum Genet 78:1026–1034

Lopez LC, Schuelke M, Quinzii CM, Kanki T, Rodenburg RJ, Naini A, Dimauro S, Hirano M (2006) Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. Am J Hum Genet 79:1125–1129

Lopez LC, Akman HO, Garcia-Cazorla A, Dorado B, Marti R, Nishino I, Tadesse S, Pizzorno G, Shungu D, Bonilla E, Tanji K, Hirano M (2009) Unbalanced deoxynucleotide pools cause mitochondrial DNA instability in thymidine phosphorylase-deficient mice. Hum Mol Genet 18:714–722

Lopez-Lluch G, Rodriguez-Aguilera JC, Santos-Ocana C, Navas P (2010) Is coenzyme Q a key factor in aging? Mech Ageing Dev 131:225–235

Martelli A, Wattenhofer-Donze M, Schmucker S, Bouvet S, Reutenauer L, Puccio H (2007) Frataxin is essential for extramitochondrial Fe-S cluster proteins in mammalian tissues. Hum Mol Genet 16:2651–2658

Martelli A, Friedman LS, Reutenauer L, Messaddeq N, Perlman SL, Lynch DR, Fedosov K, Schulz JB, Pandolfo M, Puccio H (2012) Clinical data and characterization of the liver conditional mouse model exclude neoplasia as a non-neurological manifestation associated with Friedreich's ataxia. Dis Model Mech 5:860–869

Matsukawa K, Moriyama A, Kawai Y, Asai K, Kato T (1996) Tissue distribution of human gliostatin/platelet-derived endothelial cell growth factor (PD-ECGF) and its drug-induced expression. Biochim Biophys Acta 1314:71–82

Milenkovic D, Matic S, Khl I, Ruzzenente B, Freyer C, Jemt E, Park CB, Falkenberg M, Larsson N-G (2013) TWINKLE is an essential mitochondrial helicase required for synthesis of nascent D-loop strands and complete mtDNA replication. Hum Mol Genet 22:1983–1993

Milone M, Massie R (2010) Polymerase gamma 1 mutations. Neurologist 16:84–91

Miranda CJ, Santos MM, Ohshima K, Smith J, Li L, Bunting M, Cossée M, Koenig M, Sequeiros J, Kaplan J, Pandolfo M (2002) Frataxin knockin mouse. FEBS Lett 512:291–297

Moore BA, Gonzalez Aviles GD, Larkins CE, Hillman MJ, Caspary T (2010) Mitochondrial retention of Opa1 is required for mouse embryogenesis. Mamm Genome 21:350–360

Moraes CT, Shanske S, Tritschler HJ, Aprille JR, Andreetta F, Bonilla E, Schon EA, Dimauro S (1991) mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. Am J Hum Genet 48:492–501

Mourier A, Ruzzenente B, Brandt T, Kuhlbrandt W, Larsson NG (2014) Loss of LRPPRC causes ATP synthase deficiency. Hum Mol Genet 23:2580–2592

Na U, Yu W, Cox J, Bricker DK, Brockmann K, Rutter J, Thummel CS, Winge DR (2014) The LYR factors SDHAF1 and SDHAF3 mediate maturation of the iron-sulfur subunit of succinate dehydrogenase. Cell Metab 20:253–266

Nasevicius A, Ekker SC (2000) Effective targeted gene/'knockdown/' in zebrafish. Nat Genet 26:216–220

Nikkanen J, Forsstrom S, Euro L, Paetau I, Kohnz RA, Wang L, Chilov D, Viinamaki J, Roivainen A, Marjamaki P, Liljenback H, Ahola S, Buzkova J, Terzioglu M, Khan NA, Pirnes-Karhu S, Paetau A, Lonnqvist T, Sajantila A, Isohanni P, Tyynismaa H, Nomura DK, Battersby BJ,

Velagapudi V, Carroll CJ, Suomalainen A (2016) Mitochondrial DNA replication defects disturb cellular dNTP pools and remodel one-carbon metabolism. Cell Metab 23:635–648

Nunnari J, Suomalainen A (2012) Mitochondria: in sickness and in health. Cell 148:1145–1159

Oquendo CE, Antonicka H, Shoubridge EA, Reardon W, Brown GK (2004) Functional and genetic studies demonstrate that mutation in the COX15 gene can cause Leigh syndrome. J Med Genet 41:540–544

Palladino MJ (2010) Modeling mitochondrial encephalomyopathy in Drosophila. Neurobiol Dis 40:40–45

Papadopoulou LC, Sue CM, Davidson MM, Tanji K, Nishino I, Sadlock JE, Krishna S, Walker W, Selby J, Glerum DM, Coster RV, Lyon G, Scalais E, Lebel R, Kaplan P, Shanske S, de Vivo DC, Bonilla E, Hirano M, Dimauro S, Schon EA (1999) Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. Nat Genet 23:333–337

Papanicolaou KN, Khairallah RJ, Ngoh GA, Chikando A, Luptak I, O'shea KM, Riley DD, Lugus JJ, Colucci WS, Lederer WJ, Stanley WC, Walsh K (2011) Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. Mol Cell Biol 31:1309–1328

Paredes JA, Zhou X, Höglund S, Karlsson A (2013) Gene expression deregulation in postnatal skeletal muscle of TK2 deficient mice reveals a lower pool of proliferating myogenic progenitor cells. PLoS One 8:e53698

Peng M, Falk MJ, Haase VH, King R, Polyak E, Selak M, Yudkoff M, Hancock WW, Meade R, Saiki R, Lunceford AL, Clarke CF, L Gasser, D. (2008) Primary coenzyme Q deficiency in Pdss2 mutant mice causes isolated renal disease. PLoS Genet 4:e1000061–e1000014

Perkins LA, Holderbaum L, Tao R, Hu Y, Sopko R, Mccall K, Yang-Zhou D, Flockhart I, Binari R, Shim H-S, Miller A, Housden A, Foos M, Randkelv S, Kelley C, Namgyal P, Villalta C, Liu L-P, Jiang X, Huan-Huan Q, Wang X, Fujiyama A, Toyoda A, Ayers K, Blum A, Czech B, Neumuller R, Yan D, Cavallaro A, Hibbard K, Hall D, Cooley L, Hannon GJ, Lehmann R, Parks A, Mohr SE, Ueda R, Kondo S, Ni J-Q, Perrimon N (2015) The transgenic RNAi project at Harvard Medical School: resources and validation. Genetics 201:843–852

Peters LL, Robledo RF, Bult CJ, Churchill GA, Paigen BJ, Svenson KL (2007) The mouse as a model for human biology: a resource guide for complex trait analysis. Nat Rev Genet 8:58–69

Petruzzella V, Tiranti V, Fernandez P, Ianna P, Carrozzo R, Zeviani M (1998) Identification and characterization of human cDNAs specific to BCS1, PET112, SCO1, COX15, and COX11, five genes involved in the formation and function of the mitochondrial respiratory chain. Genomics 54:494–504

Phillips MJ, Webb-Wood S, Faulkner AE, Jabbar SB, Biousse V, Newman NJ, Do VT, Boatright JH, Wallace DC, Pardue MT (2010) Retinal function and structure in Ant1-deficient mice. Invest Opthalmol Vis Sci 51:6744–6749

Piruat JI, Pintado CO, Ortega-Saenz P, Roche M, Lopez-Barneo J (2004) The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. Mol Cell Biol 24:10933–10940

Plucinska G, Paquet D, Hruscha A, Godinho L, Haass C, Schmid B, Misgeld T (2012) In vivo imaging of disease-related mitochondrial dynamics in a vertebrate model system. J Neurosci 32:16203–16212

Porcelli D, Oliva M, Duchi S, Latorre D, Cavaliere V, Barsanti P, Villani G, Gargiulo G, Caggese C (2010) Genetic, functional and evolutionary characterization of scox, the Drosophila melanogaster ortholog of the human SCO1 gene. Mitochondrion 10:433–448

Puccio H, Simon D, Cossée M, Criqui-Filipe P, Tiziano F, Melki J, Hindelang C, Matyas R, Rustin P, Koenig M (2001) Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. Nat Genet 27:181–186

Pulkes T, Liolitsa D, Wills AJ, Hargreaves I, Heales S, Hanna MG (2005) Nonsense mutations in mitochondrial DNA associated with myalgia and exercise intolerance. Neurology 64:1091–1092

Quintana A, Kruse SE, Kapur RP, Sanz E, Palmiter RD (2010) Complex I deficiency due to loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh syndrome. Proc Natl Acad Sci U S A 107:10996–11001

Quintana A, Zanella S, Koch H, Kruse SE, Lee D, Ramirez JM, Palmiter RD (2012) Fatal breathing dysfunction in a mouse model of Leigh syndrome. J Clin Invest 122:2359–2368

Rahman S, Blok RB, Dahl HH, Danks DM, Kirby DM, Chow CW, Christodoulou J, Thorburn DR (1996) Leigh syndrome: clinical features and biochemical and DNA abnormalities. Ann Neurol 39:343–351

Rahn JJ, Stackley KD, Chan SS (2013) Opa1 is required for proper mitochondrial metabolism in early development. PLoS One 8:e59218

Ravn K, Wibrand F, Hansen FJ, Horn N, Rosenberg T, Schwartz M (2001) An mtDNA mutation, 14453G-->A, in the NADH dehydrogenase subunit 6 associated with severe MELAS syndrome. Eur J Hum Genet 9:805–809

Reyes A, Melchionda L, Nasca A, Carrara F, Lamantea E, Zanolini A, Lamperti C, Fang M, Zhang J, Ronchi D, Bonato S, Fagiolari G, Moggio M, Ghezzi D, Zeviani M (2015) REPOR T RNASEH1 mutations impair mtDNA replication and cause adult-onset mitochondrial Encephalomyopathy. Am J Hum Genet 97:186–193

Ristow M, Pfister MF, Yee AJ, Schubert M, Michael L, Zhang C-Y, Ueki K, Michael MD, Lowell BB, Kahn CR (2000) Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. Proc Natl Acad Sci 97:12239–12243

Ristow M, Mulder H, Pomplun D, Schulz TJ, Müller-Schmehl K, Krause A, Fex M, Puccio H, Müller J, Isken F, Spranger J, Müller-Wieland D, Magnuson MA, Möhlig M, Koenig M, Pfeiffer AFH (2003) Frataxin deficiency in pancreatic islets causes diabetes due to loss of beta cell mass. J Clin Invest 112:527–534

Royden C (1987) The tko locus, site of a behavioral mutation in D. melanogaster, codes for a protein homologous to prokaryotic ribosomal protein S12. Cell 51:165–173

Ruhoy IS, Saneto RP (2014) The genetics of Leigh syndrome and its implications for clinical practice and risk management. Appl Clin Genet 7:221–234

Ruzzenente B, Metodiev MD, Wredenberg A, Bratic A, Park CB, Cámara Y, Milenkovic D, Zickermann V, Wibom R, Hultenby K, Erdjument-Bromage H, Tempst P, Brandt U, Stewart JB, Gustafsson CM, Larsson N-G (2012) LRPPRC is necessary for polyadenylation and coordination of translation of mitochondrial mRNAs. EMBO J 31:443–456

Saiki R, Lunceford AL, Shi Y, Marbois B, King R, Pachuski J, Kawamukai M, Gasser DL, Clarke CF (2008) Coenzyme Q10 supplementation rescues renal disease in Pdss2kd/kd mice with mutations in prenyl diphosphate synthase subunit 2. Am J Physiol Ren Physiol 295:F1535–F1544

Salviati L, Sacconi S, Murer L, Zacchello G, Franceschini L, Laverda AM, Basso G, Quinzii C, Angelini C, Hirano M, Naini AB, Navas P, Dimauro S, Montini G (2005) Infantile encephalomyopathy and nephropathy with CoQ10 deficiency: a CoQ10-responsive condition. Neurology 65:606–608

Sanz A, Stefanatos RK (2008) The mitochondrial free radical theory of aging: a critical view. Curr Aging Sci 1:10–21

Sarzi E, Angebault C, Seveno M, Gueguen N, Chaix B, Bielicki G, Boddaert N, Mausset-Bonnefont AL, Cazevieille C, Rigau V, Renou JP, Wang J, Delettre C, Brabet P, Puel JL, Hamel CP, Reynier P, Lenaers G (2012) The human OPA1delTTAG mutation induces premature age-related systemic neurodegeneration in mouse. Brain 135:3599–3613

Sasarman F, Brunel-Guitton C, Antonicka H, Wai T, Shoubridge EA (2010) LRPPRC and SLIRP interact in a ribonucleoprotein complex that regulates posttranscriptional gene expression in mitochondria. Mol Biol Cell 21:1315–1323

Schiavi F, Savvoukidis T, Trabalzini F, Grego F, Piazza M, Amista P, Dematte S, del Piano A, Cecchini ME, Erlic Z, de Lazzari P, Mantero F, Opocher G (2006) Paraganglioma syndrome: SDHB, SDHC, and SDHD mutations in head and neck paragangliomas. Ann N Y Acad Sci 1073:190–197

Schwartz M, Vissing J (2002) Paternal inheritance of mitochondrial DNA. N Engl J Med 347:576–580

Sebastian D, Hernandez-Alvarez MI, Segales J, Sorianello E, Munoz JP, Sala D, Waget A, Liesa M, Paz JC, Gopalacharyulu P, Oresic M, Pich S, Burcelin R, Palacin M, Zorzano A (2012) Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. Proc Natl Acad Sci U S A 109:5523–5528

Shoichet SA, Bäumer AT, Stamenkovic D, Sauer H, Pfeiffer AFH, Kahn CR, Müller-Wieland D, Richter C, Ristow M (2002) Frataxin promotes antioxidant defense in a thiol-dependent manner resulting in diminished malignant transformation in vitro. Hum Mol Genet 11:815–821

Shteyer E, Saada A, Shaag A, Al-Hijawi FA, Kidess R, Revel-Vilk S, Elpeleg O (2009) Exocrine pancreatic insufficiency, Dyserythropoeitic Anemia, and Calvarial hyperostosis are caused by a mutation in the COX4I2 gene. Am J Hum Genet 84:412–417

Silva JP, Köhler M, Graff C, Oldfors A, Magnuson MA, Berggren PO, Larsson NG (2000) Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. Nat Genet 26:336–340

Simon D, Seznec H, Gansmuller A, Carelle N, Weber P, Metzger D, Rustin P, Koenig M, Puccio H (2004) Friedreich ataxia mouse models with progressive cerebellar and sensory ataxia reveal autophagic neurodegeneration in dorsal root ganglia. J Neurosci 24:1987–1995

Sörensen L, Ekstrand M, Silva JP, Lindqvist E, Xu B, Rustin P, Olson L, Larsson NG (2001) Late-onset corticohippocampal neurodepletion attributable to catastrophic failure of oxidative phosphorylation in MILON mice. J Neurosci 21:8082–8090

Soroldoni D, Hogan BM, Oates AC (2009) Simple and efficient Transgenesis with Meganuclease constructs in Zebrafish. In: Lieschke GJ, Oates AC, Kawakami K (eds) Zebrafish: methods and protocols. Humana Press, Totowa

Spelbrink JN, Li FY, Tiranti V, Nikali K, Yuan QP, Tariq M, Wanrooij S, Garrido N, Comi G, Morandi L, Santoro L, Toscano A, Fabrizi GM, Somer H, Croxen R, Beeson D, Poulton J, Suomalainen A, Jacobs HT, Zeviani M, Larsson C (2001) Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. Nat Genet 28:223–231

Spiegel R, Shaag A, Mandel H, Reich D, Penyakov M, Hujeirat Y, Saada A, Elpeleg O, Shalev SA (2009) Mutated NDUFS6 is the cause of fatal neonatal lactic acidemia in Caucasus Jews. Eur J Hum Genet 17:1200–1203

St Johnston D (2002) The art and design of genetic screens: Drosophila melanogaster. Nat Rev Genet 3:176–188

Stefanatos RKA, Vidal M (2011) Tumor invasion and metastasis in Drosophila: a bold past, a bright future. J Genet Genomics 38:431–438

Sterky FH, Hoffman AF, Milenkovic D, Bao B, Paganelli A, Edgar D, Wibom R, Lupica CR, Olson L, Larsson NG (2012) Altered dopamine metabolism and increased vulnerability to MPTP in mice with partial deficiency of mitochondrial complex I in dopamine neurons. Hum Mol Genet 21:1078–1089

Stiles AR, Simon MT, Stover A, Eftekharian S, Khanlou N, Wang HL, Magaki S, Lee H, Partynski K, Dorrani N, Chang R, Martinez-Agosto JA, Abdenur JE (2016) Mutations in TFAM, encoding mitochondrial transcription factor A, cause neonatal liver failure associated with mtDNA depletion. Mol Genet Metab 119:91–99

Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y, Nakamura Y (2000) A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404:42–49

Thompson K, Majd H, Dallabona C, Reinson K, King MS, Alston CL, He L, Lodi T, Jones SA, Fattal-Valevski A, Fraenkel ND, Saada A, Haham A, Isohanni P, Vara R, Barbosa IA, Simpson MA, Deshpande C, Puusepp S, Bonnen PE, Rodenburg RJ, Suomalainen A, Ounap K, Elpeleg O, Ferrero I, Mcfarland R, Kunji ER, Taylor RW (2016) Recurrent De novo dominant mutations in SLC25A4 cause severe early-onset mitochondrial disease and loss of mitochondrial DNA copy number. Am J Hum Genet 99:860–876

Tiranti V, Hoertnagel K, Carrozzo R, Galimberti C, Munaro M, Granatiero M, Zelante L, Gasparini P, Marzella R, Rocchi M, Bayona-Bafaluy MP, Enriquez JA, Uziel G, Bertini E, Dionisi-Vici C, Franco B, Meitinger T, Zeviani M (1998) Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. Am J Hum Genet 63:1609–1621

Tiranti V, D'adamo P, Briem E, Ferrari G, Mineri R, Lamantea E, Mandel H, Balestri P, Garcia-Silva MT, Vollmer B, Rinaldo P, Hahn SH, Leonard J, Rahman S, Dionisi-Vici C, Garavaglia B, Gasparini P, Zeviani M (2004) Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. Am J Hum Genet 74:239–252

Tiranti V, Viscomi C, Hildebrandt T, di Meo I, Mineri R, Tiveron C, Levitt MD, Prelle A, Fagiolari G, Rimoldi M, Zeviani M (2009) Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. Nat Med 15:200–205

Toivonen JM, O'dell KM, Petit N, Irvine SC, Knight GK, Lehtonen M, Longmuir M, Luoto K, Touraille S, Wang Z, Alziari S, Shah ZH, Jacobs HT (2001) Technical knockout, a Drosophila model of mitochondrial deafness. Genetics 159:241–254

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlöf S, Oldfors A, Wibom R, Törnell J, Jacobs HT, Larsson N-G (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429:417–423

Trifunovic A, Hansson A, Wredenberg A, Rovio AT, Dufour E, Khvorostov I, Spelbrink JN, Wibom R, Jacobs HT, Larsson NG (2005) Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. Proc Natl Acad Sci 102:17993–17998

Tsang WY, Sayles LC, Grad LI, Pilgrim DB, Lemire BD (2001) Mitochondrial respiratory chain deficiency in Caenorhabditis elegans results in developmental arrest and increased life span. J Biol Chem 276:32240–32246

Tyynismaa H, Sembongi H, Bokori-Brown M, Granycome C, Ashley N, Poulton J, Jalanko A, Spelbrink JN, Holt IJ, Suomalainen A (2004) Twinkle helicase is essential for mtDNA maintenance and regulates mtDNA copy number. Hum Mol Genet 13:3219–3227

Tyynismaa H, Mjosund KP, Wanrooij S, Lappalainen I, Ylikallio E, Jalanko A, Spelbrink JN, Paetau A, Suomalainen A (2005) Mutant mitochondrial helicase Twinkle causes multiple mtDNA deletions and a late-onset mitochondrial disease in mice. Proc Natl Acad Sci U S A 102:17687–17692

Tyynismaa H, Ylikallio E, Patel M, Molnar MJ, Haller RG, Suomalainen A (2009) A heterozygous truncating mutation in RRM2B causes autosomal-dominant progressive external ophthalmoplegia with multiple mtDNA deletions. Am J Hum Genet 85:290–295

Tyynismaa H, Carroll CJ, Raimundo N, Ahola-Erkkila S, Wenz T, Ruhanen H, Guse K, Hemminki A, Peltola-Mjosund KE, Tulkki V, Oresic M, Moraes CT, Pietilainen K, Hovatta I, Suomalainen A (2010) Mitochondrial myopathy induces a starvation-like response. Hum Mol Genet 19:3948–3958

Tyynismaa H, Sun R, Ahola-Erkkila S, Almusa H, Poyhonen R, Korpela M, Honkaniemi J, Isohanni P, Paetau A, Wang L, Suomalainen A (2012) Thymidine kinase 2 mutations in autosomal recessive progressive external ophthalmoplegia with multiple mitochondrial DNA deletions. Hum Mol Genet 21:66–75

Vahsen N, Cande C, Briere JJ, Benit P, Joza N, Larochette N, Mastroberardino PG, Pequignot MO, Casares N, Lazar V, Feraud O, Debili N, Wissing S, Engelhardt S, Madeo F, Piacentini M, Penninger JM, Schagger H, Rustin P, Kroemer G (2004) AIF deficiency compromises oxidative phosphorylation. EMBO J 23:4679–4689

Valnot I, Osmond S, Gigarel N, Mehaye B, Amiel J, Cormier-Daire V, Munnich A, Bonnefont JP, Rustin P, Rotig A (2000) Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. Am J Hum Genet 67:1104–1109

Van Bon BW, Oortveld MA, Nijtmans LG, Fenckova M, Nijhof B, Besseling J, Vos M, Kramer JM, De Leeuw N, Castells-Nobau A, Asztalos L, Viragh E, Ruiter M, Hofmann F, Eshuis L, Collavin L, Huynen MA, Asztalos Z, Verstreken P, Rodenburg RJ, Smeitink JA, De Vries BB,

Schenck A (2013) CEP89 is required for mitochondrial metabolism and neuronal function in man and fly. Hum Mol Genet 22:3138–3151

Van Den Heuvel L, Ruitenbeek W, Smeets R, Gelman-Kohan Z, Elpeleg O, Loeffen J, Trijbels F, Mariman E, de Bruijn D, Smeitink J (1998) Demonstration of a new pathogenic mutation in human complex I deficiency: a 5-bp duplication in the nuclear gene encoding the 18-kD (AQDQ) subunit. Am J Hum Genet 62:262–268

Van Der Weyden L, White JK, Adams DJ, Logan DW (2011) The mouse genetics toolkit: revealing function and mechanism. Genome Biol 12:224

Van Vranken JG, Bricker DK, Dephoure N, Gygi SP, Cox JE, Thummel CS, Rutter J (2014) SDHAF4 promotes mitochondrial succinate dehydrogenase activity and prevents neurodegeneration. Cell Metab 20:241–252

Vartiainen S, Chen S, George J, Tuomela T, Luoto KR, O'dell KM, Jacobs HT (2014) Phenotypic rescue of a Drosophila model of mitochondrial ANT1 disease. Dis Model Mech 7:635–648

Vernochet C, Mourier A, Bezy O, Macotela Y, Boucher J, Rardin MJ, An D, Lee KY, Ilkayeva OR, Zingaretti CM, Emanuelli B, Smyth G, Cinti S, Newgard CB, Gibson BW, Larsson N-G, Kahn CR (2012) Adipose-specific deletion of TFAM increases mitochondrial oxidation and protects mice against obesity and insulin resistance. Cell Metab 16:765–776

Verstreken P, Ly CV, Venken KJT, Koh T-W, Zhou Y, Bellen HJ (2005) Synaptic mitochondria are critical for mobilization of reserve Pool vesicles at Drosophila neuromuscular junctions. Neuron 47:365–378

Vettori A, Bergamin G, Moro E, Vazza G, Polo G, Tiso N, Argenton F, Mostacciuolo ML (2011) Developmental defects and neuromuscular alterations due to mitofusin 2 gene (MFN2) silencing in zebrafish: a new model for Charcot-Marie-tooth type 2A neuropathy. Neuromuscul Disord 21:58–67

Vidal M, Cagan RL (2006) Drosophila models for cancer research. Curr Opin Genet Dev 16:10–16

Visapaa I, Fellman V, Vesa J, Dasvarma A, Hutton JL, Kumar V, Payne GS, Makarow M, Van Coster R, Taylor RW, Turnbull DM, Suomalainen A, Peltonen L (2002) GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. Am J Hum Genet 71:863–876

Viscomi C, Spinazzola A, Maggioni M, Fernandez-Vizarra E, Massa V, Pagano C, Vettor R, Mora M, Zeviani M (2008) Early-onset liver mtDNA depletion and late-onset proteinuric nephropathy in Mpv17 knockout mice. Hum Mol Genet 18:12–26

Viscomi C, Bottani E, Civiletto G, Cerutti R, Moggio M, Fagiolari G, Schon EA, Lamperti C, Zeviani M (2011) In vivo correction of COX deficiency by activation of the AMPK/PGC-1alpha axis. Cell Metab 14:80–90

Viscomi C, Bottani E, Zeviani M (2015) Emerging concepts in the therapy of mitochondrial disease. Biochim Biophys Acta 1847:544–557

Vos M, Esposito G, Edirisinghe JN, Vilain S, Haddad DM, Slabbaert JR, Van Meensel S, Schaap O, De Strooper B, Meganathan R, Morais VA, Verstreken P (2012) Vitamin K2 is a mitochondrial electron carrier that rescues pink1 deficiency. Science 336:1306–1310

Walker DW, Hajek P, Muffat J, Knoepfle D, Cornelison S, Attardi G, Benzer S (2006) Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. Proc Natl Acad Sci U S A 103:16382–16387

Wang J, Wilhelmsson H, Graff C, Li H, Oldfors A, Rustin P, Brüning JC, Kahn CR, Clayton DA, Barsh GS, Thorén P, Larsson NG (1999) Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. Nat Genet 21:133–137

Wang Y, Oxer D, Hekimi S (2015) Mitochondrial function and lifespan of mice with controlled ubiquinone biosynthesis. Nat Commun 6:6393

Wang A, Mouser J, Pitt J, Promislow D, Kaeberlein M (2016) Rapamycin enhances survival in a Drosophila model of mitochondrial disease. Oncotarget 7:80131–80139

Weiher H, Noda T, Gray DA, Sharpe AH, Jaenisch R (1990) Transgenic mouse model of kidney disease: insertional inactivation of ubiquitously expressed gene leads to nephrotic syndrome. Cell 62:425–434

Wienholds E, Van Eeden F, Kosters M, Mudde J, Plasterk RH, Cuppen E (2003) Efficient target-selected mutagenesis in zebrafish. Genome Res 13:2700–2707

Wilkins HM, Carl SM, Swerdlow RH (2014) Cytoplasmic hybrid (cybrid) cell lines as a practical model for mitochondriopathies. Redox Biol 2:619–631

Williams SL, Taanman JW, Hansikova H, Houst'kova H, Chowdhury S, Zeman J, Houstek J (2001) A novel mutation in SURF1 causes skipping of exon 8 in a patient with cytochrome c oxidase-deficient leigh syndrome and hypertrichosis. Mol Genet Metab 73:340–343

Williams PA, Piechota M, VON Ruhland C, Taylor E, Morgan JE, Votruba M (2012) Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. Brain 135:493–505

Wirth C, Brandt U, Hunte C, Zickermann V (2016) Structure and function of mitochondrial complex I. Biochim Biophys Acta 1857:902–914

Wredenberg A, Wibom R, Wilhelmsson H, Graff C, Wiener HH, Burden SJ, Oldfors A, Westerblad H, Larsson N-G (2002) Increased mitochondrial mass in mitochondrial myopathy mice. Proc Natl Acad Sci 99:15066–15071

Xu F, Addis JBL, Cameron JM, Robinson BH (2012) LRPPRC mutation suppresses cytochrome oxidase activity by altering mitochondrial RNA transcript stability in a mouse model. Biochem J 441:275–283

Yang H, Brosel S, Acin-Perez R, Slavkovich V, Nishino I, Khan R, Goldberg IJ, Graziano J, Manfredi G, Schon EA (2010) Analysis of mouse models of cytochrome c oxidase deficiency owing to mutations in Sco2. Hum Mol Genet 19:170–180

Yarosh W, Monserrate J, Tong JJ, Tse S, Le PK, Nguyen K, Brachmann CB, Wallace DC, Huang T (2008) The molecular mechanisms of OPA1-mediated optic atrophy in Drosophila model and prospects for antioxidant treatment. PLoS Genet 4:e6

Ylikallio E, Tyynismaa H, Tsutsui H, Ide T, Suomalainen A (2010) High mitochondrial DNA copy number has detrimental effects in mice. Hum Mol Genet 19:2695–2705

Zhang YQ, Roote J, Brogna S, Davis AW, Barbash DA, Nash D, Ashburner M (1999) Stress sensitive B encodes an adenine nucleotide translocase in Drosophila melanogaster. Genetics 153:891–903

Zhang D, Mott JL, Chang SW, Denniger G, Feng Z, Zassenhaus HP (2000) Construction of transgenic mice with tissue-specific acceleration of mitochondrial DNA mutagenesis. Genomics 69:151–161

Zhang D, Mott JL, Chang S-W, Stevens M, Mikolajczak P, Zassenhaus HP (2005) Mitochondrial DNA mutations activate programmed cell survival in the mouse heart. Am J Physiol Heart Circ Physiol 288:H2476–H2483

Zhang K, Li Z, Jaiswal M, Bayat V, Xiong B, Sandoval H, Charng WL, David G, Haueter C, Yamamoto S, Graham BH, Bellen HJ (2013) The C8ORF38 homologue Sicily is a cytosolic chaperone for a mitochondrial complex I subunit. J Cell Biol 200:807–820

Zhou X, Solaroli N, Bjerke M, Stewart JB, Rozell B, Johansson M, Karlsson A (2008) Progressive loss of mitochondrial DNA in thymidine kinase 2-deficient mice. Hum Mol Genet 17:2329–2335

Zhu Z, Yao J, Johns T, Fu K, De Bie I, Macmillan C, Cuthbert AP, Newbold RF, Wang J, Chevrette M, Brown GK, Brown RM, Shoubridge EA (1998) SURF1, encoding a factor involved in the biogenesis of cytochrome c oxidase, is mutated in Leigh syndrome. Nat Genet 20:337–343