REVIEW

Modelling Mitochondrial Dysfunction in Mice

S. A. DOGAN[1], A. TRIFUNOVIC[1,2]

[1]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany, [2]Division of Metabolic Diseases, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden

Summary

Understanding mitochondrial role in normal physiology and pathological conditions has proven to be of high importance as mitochondrial dysfunction is connected with a number of disorders as well as some of the most common diseases (*e.g.* diabetes or Parkinson's disease). Modeling mitochondrial dysfunction has been difficult mainly due to unique features of mitochondrial genetics. Here we discuss some of the most important mouse models generated so far and lessons learned from them.

Corresponding author

A. Trifunovic, Cologne University Clinic, CECAD Cologne - Excellent in Aging Research at the Institute for Genetics, Zülpicher Str. 47, D-50674 Cologne, Germany. E-mail: aleksandra.trifunovic@uk-koeln.de

Introduction

Chinese philosophy explains the basis of nature through *yin* and *yang,* opposing forces, interdependent and able to exist only in relation to each other. The story of the cell and mitochondria is very similar. It is a story about two enemies that have become friends and allies. On this particular day, an ancient bacteria has invaded a single cell organism and instead of killing it, as many times before, it has found shelter and decided to stay inside it. Why not, there was a lot of food within and it was protected from the hostile environment of the ancient world. As for the host cell, beside the fact that it has survived the attack, benefits have also been great. Finally it had a way of fighting poisonous oxygen that was surrounding it, not to mention all this new energy produced within. This random encounter that happened around 1-2 billion years ago to give rise to a synergically superior eukaryote, is described by the endosymbiotic theory (Margulis 1975). The bacterium is believed to become integrated into the recipient cell and evolved into an organelle, the mitochondrion. Whether the random encounter was an invasion, infection or an unwilling indigestion event, the *yin* - the anaerobic cell - and the *yang* - the aerobic mitochondrion - left their differences behind and cooperated. The story, which started almost as a horror movie, turned into a romantic comedy in the end - or did it? Does the initial invasion of the bacteria still continue even if it lost its ability to live independently? A number of different diseases and mitochondrial involvement in aging and age-associated disease seem to prove this.

Mitochondrial involvement in disease

Mitochondria are small organelle found in almost every cell of the organism. They form a very dynamic network within eukaryotic cell and occupy roughly one fifth of its total volume (McBride *et al.* 2006). Mitochondria are considered to be the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP), through process of oxidative phosphorylation. Mitochondria are unique

because they are the only organelles in animal cells containing their own DNA, mitochondrial DNA (mtDNA).

Without mitochondria, higher animals would likely not exist because their cells would not be able to obtain enough energy. In fact, mitochondria enable cells to produce 15 times more ATP than they could otherwise. Mitochondrial energy production is a foundation for health and well-being. It is necessary for physical strength, stamina and consciousness (McBride *et al.* 2006). Even subtle insufficiency in mitochondrial function can cause weakness, fatigue and cognitive difficulties (Graff *et al.* 2002). Furthermore, chemicals that strongly interfere with mitochondrial function are known to be potent poisons.

Mitochondrial diseases are one of the most common inborn errors of metabolism with a frequency of about 1 in 5000 (Chinnery and Turnbull 2001). They are often called "mitochondrial encephalomyopathies" as it became apparent that brain and skeletal muscle are the most commonly affected tissues (Shapira *et al.* 1977). Today, the term "mitochondrial diseases" is almost exclusively used to describe diseases caused by defects in mitochondrial oxidative phosphorylation (OXPHOS) and not defects in numerous other cellular processes within mitochondria. Even within these boundaries, the classification of the mitochondrial diseases became quite complicated, as it was shown that mutations in either mtDNA or nDNA genes coding for mitochondrial proteins lead to major and catastrophic diseases in humans. The first patient suffering from a mitochondrial disorder was described by Luft *et al.* (1962). Since then thousands of patients have been diagnosed with different kinds of mitochondrial diseases. Due to the complexity of mitochondrial diseases, they are usually classified by their genetic defect rather then clinical manifestation.

Mitochondrial DNA diseases

About 200 mtDNA point mutations and innumerable single large-scale partial deletions have been associated with human diseases, most of which affect the central and peripheral nervous system (Anderson *et al.* 1981). Although genetically distinct, most mtDNA diseases share common features such as lactic acidosis, mosaic pattern of cells deficient in cytochrome c oxidase activity and massive mitochondrial proliferation in muscle resulting in ragged-red fibers (DiMauro *et al.* 1985). Mitochondrial DNA diseases commonly have a

delayed onset and progressive course. Mutations in mtDNA are divided into two groups: mtDNA point mutations and mtDNA rearrangements. A couple of unique features of mtDNA genetics and inheritance make it very difficult to predict the course of the disease, prenatal diagnosis and/or genetic counseling in everyday clinical practice:

(i) mtDNA does not follow the Mendelian rules of inheritance. In most animals, as in humans, mtDNA is maternally inherited. Therefore, a mother carrying an mtDNA mutation can transmit it to her children, but only her daughters can further transmit it to the next generation. As each cell contains ~10,000 copies of mtDNA, a pathogenic mutation could be present in all of them or just few of copies of the molecule. Existence of two or more different populations of mtDNA in a single cell is called heteroplasmy in contrast to homoplasmy where all mtDNA molecules are identical.

(ii) Threshold effect represents the minimal critical level of a pathogenic mutation in mtDNA that should be present in the cell or tissue to have a deleterious effect. A certain proportion of mutant mtDNA must be present before reduction of OXPHOS activity is observed, and the threshold is lower in tissues that are more dependent on oxidative metabolism. It has been shown that there are different thresholds for different types of mtDNA mutations, ranging from 90 % for some tRNA mutations (Chomyn *et al.* 1992, Hanna *et al.* 1995) to 60 % for mtDNA deletions (Bourgeron *et al.* 1993).

(iii) The last but not the least problem of mtDNA genetics is the mitotic segregation. Random distribution of mtDNA molecules during cell division can lead to increased amounts of mutant mtDNA molecules in one of the daughter cells. This can lead to a cell carrying low levels of mutated molecules giving rise to one of relatively high levels, which in turn will affect oxidative phosphorylation in that cell.

Nuclear DNA mutations

Mitochondrial diseases caused by a mutation in nuclear encoded genes are a very heterogeneous group. Not only are most of the ~80 structural proteins of the OXPHOS system encoded by nDNA, but all the proteins needed for their import from the cytoplasm and assembly in mitochondria are also encoded by nucleus. Defects in any of these proteins could lead to functionally impaired OXPHOS and therefore to mitochondrial disease. Furthermore, defects in any protein affecting stability and/or integrity of mtDNA could lead to the same

deleterious effect.

Certain tissues such as heart, brain, and skeletal muscle are very dependent on the OXPHOS for energy production; therefore it is not surprising that these tissues tend to be involved mitochondrial diseases. However, this cannot explain the diverse manifestations of mutations that would be predicted to have similar effects. For instance; central nervous system (CNS) involvement in mitochondrial diseases is clinically heterogeneous, manifesting as epilepsy, stroke-like episodes, migraine, ataxia, spasticity, extrapyramidal abnormalities, leukoencephalopathy, bulbar dysfunction, psychiatric abnormalities, neuropsychological deficits, or hypophysial abnormalities. The mechanisms that regulate the tissue-specific consequence of mitochondrial disease are still largely unknown. In fact, the different patterns of energy crisis are perhaps the most puzzling dilemma in the field. Therefore, many attempts were made in order to model the mitochondrial diseases in mice.

Introducing mutations into mtDNA: the mito-mouse and mito-PstI-mice

Generation of animal models carrying mtDNA mutations has proven to be extremely difficult mainly because DNA transformation of mitochondria inside living cells is basically unfeasible. The introduced DNA must cross not one, but three membranes (the plasma membrane, plus two mitochondrial membranes), while preserving cell and organelle integrity and viability. Furthermore, mtDNA exists in high copy number in each cell, meaning that numerous transformation events are required to ensure introduced mtDNA to reach meaningful levels. These technical difficulties have given rise to different approaches to manipulate the mtDNA in experimental animals.

Mito-mice

First attempt to generate a mouse model carrying mutated mtDNA was accomplished by introduction of exogenous mitochondria carrying a large deletion into mouse zygotes (Inoue *et al.* 2000). Mitochondria carrying somatic mtDNA mutations from aged mouse brain (synaptosomes) were fused to mouse ρ0 cells (cells without mtDNA) to create cybrid clones. A clone stably expressing a single large deletion (Δmt4696) was then enucleated and introduced into mouse zygotes using electrofusion. Following the implantation of those into pseudopregnant females, 5 heteroplasmic founder

females out of 111 newborn mice were bred and germline transmission was obtained in F_0 generation. Mutant mtDNA accumulated in F_1 - F_3 progenies and caused mitochondrial dysfunction. The mitochondrial-transgenic mice obtained in this way inherited various levels (5-90 %) of deleted mtDNA and were named delta-mtDNA (ΔmtDNA) mice or mito-mice. There was a strong correlation between pathological phenotypes and the level of deleted mtDNA. Mice with high levels of Δmt4696 presented mitochondrial disease phenotypes such as low body weight, lactic acidosis, systemic ischemia, auriculoventricular block with Wenckebach periodicity, hearing loss, renal failures, and male infertility (Inoue *et al.* 2000, Nakada *et al.* 2001, 2006). Early symptoms of the lactic acidosis, low body weight, and sperm abnormalities could be detected in mito-mice carrying approximately more than 75 % mtDNA. Surprisingly, the deleted mtDNA was transmitted through the maternal line to the offspring, although single mtDNA deletions are not usually inherited. Mito-mice with predominant levels of deleted mtDNA molecules died around seven months of age of renal failure. This came as a bit of surprise as renal failure is not a common lesion in mitochondrial diseases. Nevertheless, phenotype of Mito-mice mimic early-onset Pearson syndrome in humans that is caused by a single heteroplasmic mtDNA deletion and leads to anaemia, mitochondrial myopathy and pancreatic and renal insufficiency.

Mitochondrial PstI mice

An elegant way of inducing mtDNA depletion into mouse model was accomplished by expression of mitochondrially-targeted Restriction Endonuclease *(Pst*I), specifically in skeletal muscle. Mouse mtDNA harbors two *Pst*I sites, and transgenic founders had high levels of double-strand brakes leading to mtDNA depletion associated with development of mitochondrial myopathy (Srivastava and Moraes 2001). The founders showed a chimeric pattern of transgene expression and their residual level of wild-type mtDNA in muscle was ~ 40 % of controls. Beside high levels of mtDNA depletion, these mice carried multiple, large mtDNA deletions in muscle. Most of these deletions involved one of the *Pst*I sites and the 3′-end of the D-loop region and were characterized by no or small direct repeats at the breakpoint region. This implies that double-strand breaks may be involved in the generation of multiple-deletion syndromes in humans, as these deletions share basically identical features.

Many lessons learned from Tfam-deficient mice

Facing with challenges in direct mitochondrial DNA manipulations, scientists chose to take another approach and they have generated mutations in components that are needed for the mtDNA maintenance and/or expression. Maintenance and expression of mtDNA are completely dependent on nuclear genes and it is therefore possible to produce a global reduction of mtDNA expression, similar to the reduction observed in patients with mtDNA mutations, by disruption of nuclear genes.

One of the very first attempts of mimicking mitochondrial dysfunction in mice was developed by disrupting the nuclear *Tfam* gene, which encodes a transcriptional activator that is imported to mitochondria (Larsson *et al.* 1996, 1998). The TFAM protein specifically binds mtDNA promoters and activates transcription (Larsson *et al.* 1996). TFAM has the ability to bend and unwind DNA and may activate transcription by facilitating binding of mitochondrial RNA polymerase and other factors to the mtDNA promoters. Mitochondrial transcription is not only necessary for gene expression but also for mtDNA replication by providing the RNA primers necessary for initiation of mtDNA replication by mitochondrial DNA polymerase. Transcription is thus a prerequisite for mtDNA replication. Furthermore, TFAM is a very abundant protein, fully coating mtDNA, therefore it plays important role in mtDNA maintenance in mammals (Larsson *et al.* 1996). *Tfam* is absolutely required for mtDNA maintenance *in vivo,* and homozygous germ line *Tfam* knockouts lack mtDNA and die during embryogenesis (Larsson *et al.* 1998). Characterization of tissue-specific *Tfam* knockouts has demonstrated that TFAM deficiency leads to mtDNA depletion, reduction in levels of mitochondrial transcripts, and severe respiratory chain deficiency (Wang *et al.* 1999, Silva *et al.* 2000, Sorensen *et al.* 2001, Ekstrand *et al.* 2007). Furthermore, selective disruption of *Tfam* in different tissues has shown that diverse cell types vary considerably in their tolerance to reduced mtDNA levels. Interestingly, the phenotype of tissue-specific *Tfam* knockouts faithfully reproduces pathology found in humans with mtDNA disorders. It is thus likely that impaired mtDNA expression is a key pathogenesis feature of mtDNA disorders and that the distribution of mtDNA and, as a consequence, the distribution of the respiratory chain deficiency is the main determinant of the phenotype.

Selective *Tfam* disruption in heart and skeletal muscle leads to development of a mosaic cardiac-specific progressive respiratory chain deficiency, dilated cardiomyopathy, atrioventricular heart conduction blocks. These animals die very early, at around 2-4 weeks of age, providing genetic evidence that the respiratory chain is critical for normal heart function. Furthermore, these animals reproduce biochemical, morphological and physiological features of the dilated cardiomyopathy of Kearns-Sayre syndrome (Schon *et al.* 1995, Wang *et al.* 1999).

It has been estimated that around 0.5-1 % of all cases of diabetes mellitus are caused by mtDNA mutations. A mouse model engineered to have *Tfam* deficiency specifically in pancreatic beta-cells faithfully mimics features of mitochondrial diabetes in humans (Silva *et al.* 2000). The mice developed diabetes from the age of 5 weeks and displayed severe mtDNA depletion, deficient oxidative phosphorylation and abnormal appearing mitochondria in islets at the ages of 7-9 weeks. Physiological studies revealed reduced hyperpolarization of the mitochondrial membrane potential, impaired Ca^{2+}-signalling and lowered insulin release in response to glucose stimulation. This animal model provides genetic evidence for a critical role of the respiratory chain in insulin secretion.

Mitochondrial late-onset neurodegeneration (MILON) mice were generated after postnatal deletion of *Tfam* gene in forebrain neurons (Sorensen *et al.* 2001). MILON mice appeared perfectly normal and showed no abnormalities until the age of 5-6 months. Following this time period, their physical condition deteriorates pretty rapidly and death occurs within 1-2 weeks. MILON mice displayed a progressive, marked neuronal cell loss, degeneration, severe disruption of cortical organization and axonal degeneration in neocortex and hippocampus. This was accompanied by a significant reduction in mtDNA copy number and mtRNA levels in neocortex of MILON mice. The importance of MILON mice comes from the fact that it successfully mimics the late-onset and progressive nature of human neurodegenerative diseases.

Genetic evidence for the role of mitochondrial dysfunction in development of parkinsonian phenotype came from the MitoPark mouse. This mouse model have a midbrain, dopamine (DA) neuron specific *Tfam* deficiency and recapitulate several features of Parkinson's Disease (PD) in humans such as adult-onset degeneration

of nigrostriatal dopamine circuitry; motor deficits that are ameliorated by L-DOPA administration; progressive course of phenotypic manifestations and neurodegeneration; and altered response to L-DOPA treatment dependent on disease stage. The mice appear normal until adolescence, followed by development of PD-like motor problems and clinical features similar to PD patients who respond well to L-DOPA treatment (Ekstrand *et al.* 2007). Despite showing signs of motor deficits at 14 to 20 weeks of age, the mice survive until 45 weeks at which point they need to be sacrificed due to their poor general condition. Maybe the most important feature of MitoPark mice was that they fully mimic delayed onset and progressive course of symptoms of PD, something that was very difficult to reproduce with other animal models.

Mouse models mimicking neurodegenerative diseases

The lack of mouse models presents a major obstacle to study the effect of mitochondrial dysfunction in postmitotic tissues, such as the brain. Over the years attempts were made to generate models that will replicate neurodegenerative diseases such as Leigh syndrome, ethylmalonic encephalopathy, mitochondrial neurogastrointestinal encephalomyopathy and encephalomyelopathy. We will discuss just a few of them.

Surf1 deficient mice

Leigh syndrome (LS) or subacute necrotizing encephalomyelopathy, is an early-onset progressive neurodegenerative disorder characterized by predominant involvement of the central nervous system (CNS) (Leigh 1951). Usually affects infants under the age of two and has a very poor prognosis, as most of the patients die within few years. Mutations in both nuclear and mitochondrial genes have been identified in LS patients. Mutations in the nuclear *Surf1* gene, coding for a putative complex IV (cytochrome c oxidase (COX)) assembly factor are one of the main cause of LS (Tiranti *et al.* 1998, 1999). In these patients, SURF1p mutations or depletions cause a reduction in the fully assembled COX (Tiranti *et al.* 1999). Surprisingly, Surf1 deficient mice displayed mild reduction of COX activity in all tissues, with no Leigh's syndrome lesions in the CNS. The Surf1 deficient mice have a slightly smaller size at birth that is corrected later in life and show only mild differences in

motor skills and blood lactate levels, comparing to littermates (Dell'agnello *et al.* 2007). The mild phenotype could be explained by the fact that SURF1 is not a fundamental COX assembly factor in mice as it is in humans. Unexpectedly, these animals displayed broad protection from *in vivo* neurodegeneration induced by exposure to high doses of kainic acid. It was suggested that these findings are associated with reduced mitochondrial uptake of calcium ions and cellular calcium homeostasis. Maybe the most striking result of this unusual model system was the prolonged lifespan that is observed in those animals. This is in great contrast with the severe and fatal encephalomyelopathy in humans with Surf1 mutations. However, it goes very well with the findings in both roundworm, *Caenorhabditis elegans* and fruit fly, *Drosophila melanogaster*, that mild mitochondrial dysfunction prolongs lifespan (Dillin *et al.* 2002, Rea *et al.* 2007, Copeland *et al.* 2009).

TK2 deficient mice

Thymidine kinase 2 (TK2) is an enzyme that phosphorylates pyrimidine nucleosides inside mitochondria. Mutations in TK2 cause the impairment in mitochondrial nucleotide salvage pathway and leads to mtDNA depletion syndrome (MDS), an autosomal recessive disorder of infancy or childhood characterized by decreased mitochondrial DNA copy number in affected tissues (Mancuso *et al.* 2002). To further elucidate the tissue-specific effects of TK2 deficiency, two different TK2 mouse models were generated: the knock-out (TK2-deficient mice) (Zhou *et al.* 2008) and the knock-in mice (Akman *et al.* 2008). A week after birth TK2-deficient mice exhibited failure to thrive, severe growth retardation, lipodystrophy, and hypothermia resulting in a very early death (Zhou *et al.* 2008). These changes were mirrored by progressive mtDNA depletion in multiple organs including skeletal muscle, heart, brain, liver, and spleen. Although TK2 deficiency caused severe mtDNA depletion, the remaining copies were intact and showed no increase in number of mtDNA mutations or deletions. Approximately at the same time a TK2 mouse model carrying the H126N mutation (homolog to human H121N pathogenic mutation) was generated (Akman *et al.* 2008). These mice showed severe and rapidly progressive encephalomyelopathy after 10 days and died between ages 2 and 3 weeks. Further investigation revealed that TK2 deficiency in these animals leads to unbalanced dNTP pools and general mtDNA depletion. However, in

contrast to the widespread defects of mtDNA, only brain showed decreased activities of respiratory chain enzymes. It seems that in most tissues, but brain, levels of mtDNA-encoded polypeptides were maintained at levels similar to controls indicating transcriptional or translational compensation for the mtDNA defect. Transcriptional compensation for partial mtDNA depletion has been observed in skeletal muscle of patients with TK2 mutations (Vila *et al.* 2003).

Deletor mice

Twinkle is a nuclear-encoded mitochondrial helicase, which, together with mitochondrial DNA polymerase (POLG) and mitochondrial single-stranded DNA-binding protein forms minimal mitochondrial replisome (Korhonen *et al.* 2004). In humans, dominant mutations in Twinkle are cause of adult-onset progressive external ophthalmoplegia (PEO), which is associated with multiple mtDNA deletions (Spelbrink *et al.* 2001). Transgenic mice expressing a mutant murine Twinkle carrying a duplication-mutation (dup353–365) homologous to the one found in PEO patients, very accurately replicates human disease. These 'Deletor' mice (due to multiple mtDNA deletion accumulation in their tissues) show progressive respiratory deficiency and chronic late-onset mitochondrial disease in skeletal muscle starting at around one year of age. Respiratory dysfunction is also apparent in distinct neuronal populations such as cerebellar Purkinje cells and hippocampal neurons. Although Deletor-mice accumulate mtDNA deletions and displays clear signs of adult-onset mitochondrial myopathy and neurodegeneration, the lifespan was not affected.

Mitochondrial involvement in aging: mtDNA mutator mice

The involvement of mitochondrial DNA (mtDNA) in the aging process mainly stems from their location, in close proximity to the mitochondrial respiratory chain, which is the main source of reactive oxygen species (ROS) generation and therefore helps explain their susceptibility to oxidative damage. This vulnerability of mtDNA has led to the suggestion that the accumulation of somatic mtDNA mutations might play an important role in the aging process by producing cells with a decreased oxidative capacity (Harman 1972). We developed a model that provided evidence in direct support for the involvement of mitochondria in the

process of aging (Trifunovic *et al.* 2004). We created homozygous knock-in mice expressing a error prone form of the nuclear-encoded mitochondrial DNA polymerase (POLG). As the proofreading in the knock-in mice is efficiently prevented, these mice develop the mtDNA mutator phenotype (mtDNA mutator mice) with a three to fivefold increase in the levels of point mutations. The mtDNA mutator mice display a completely normal phenotype at birth and in early adolescence until the age of ~25 weeks when first signs of kyphosis and hair loss were noticeable. As the animals got older the kyphosis became marked, varying degrees of alopecia appeared and the animals became progressively leaner and less active. The median life span of the mtDNA mutator mice was approximately 48 weeks and all of them died or became moribund before the age of 61 weeks. Measurements of whole body bone mineral density (BMD) showed a clear reduction with increasing age, consistent with the clinical features of osteoporosis, commonly detected in aging humans (Kalu 1995). MtDNA mutator hearts shoved signs of dilated cardiomyopathy with marked cardiac hypertrophy and mosaic distribution of COX-deficient cardiomyocytes (Trifunovic *et al.* 2004). Furthermore, a profound reduction in fertility of mtDNA-mutator mice of both sexes was detected. MtDNA mutator females usually produced 1 or 2 liters of normal size, but they never became pregnant again after the age of 25 weeks despite being continuously exposed to males for several months. Studies on the auditory system pathology of mutator mice and humans with presbyacusis (age-related hearing loss) showed striking similarities (Niu *et al.* 2007). Apoptotic cell loss in the spiral ganglion and progressive degeneration of neurons in the cochlear nucleus were the most probable cause of the presbyacusis-like phenotype observed in aging mtDNA mutator mice.

Confirmation of our results came from another, independently developed mtDNA mutator mice (Kujoth *et al.* 2005). Surprisingly, both models showed that increased levels of mtDNA mutations were not associated with increased ROS production or increased oxidative stress in mtDNA mutator mice (Kujoth *et al.* 2005, Trifunovic *et al.* 2005). In agreement with this, we observed normal sensitivity to oxidative stress-induced cell death in mouse embryonic fibroblasts from mtDNA mutator mice, despite the presence of a severe respiratory-chain dysfunction (Trifunovic *et al.* 2005).

The main consequence the mitochondrial proof reading deficiency in mtDNA mutator mice is the

massive, progressive, random accumulation of mtDNA mutations during the course of mitochondrial biogenesis (Trifunovic *et al.* 2004). MtDNA mutator mice accumulate mutations in a linear fashion, suggesting no involvement of a vicious circle predicted by the mitochondrial theory of aging (Trifunovic *et al.* 2005). We believe that the premature aging phenotypes in mtDNA mutator mice are not generated by massively increased oxidative stress accompanied by exponential accumulation of mtDNA mutations, but that respiratory-chain dysfunction *per se* is the primary inducer of premature aging (Trifunovic *et al.* 2005). However, it is still possible that mutation load might be an underestimate since cells with the highest levels of deleterious mutations may be lost due to cell death and/or replicative disadvantage.

Due to constant abortive replication, demonstrated by the presence of linear deleted mtDNA molecules, mtDNA mutator mice display ~25 % decrease in the levels of full-length mtDNA (Trifunovic *et al.* 2004). Although reduced copy number of full-length mtDNA does not affect overall mtDNA expression we cannot completely exclude a possibility that described reduction in the levels of full-length mtDNA could contribute to the phenotypes (Edgar *et al.* 2009).

Our latest results strongly argue that the observed phenotypes are a direct consequence of the accumulation of mtDNA point mutations (Edgar *et al.* 2009). We believe that even though mtDNA mutator mice randomly accumulate point mutations, these mutations have deleterious impact primarily on the protein-coding genes. This is in agreement with our recent finding of strong purifying selection against mutations in the protein-coding genes during germline transmission of mutated mtDNA in the mouse (Stewart *et al.* 2008).

Conclusion

Animal models are invaluable tools for in depth study of human pathologies *in vivo*. Development of models for mitochondrial diseases has proved to be very challenging, but a great deal of progress has been made in the last decade. The existing mouse models will still be very useful for studies of pathogenesis and, in some cases, for testing potential treatments for mitochondrial disorders. To further elucidate the role of mtDNA maintenance and expression mechanisms and their effect on human pathologies and disease, more models carrying patient mutations would need to be generated. Furthermore, mitochondrial dysfunction is more and more associated with common diseases such as neurodegeneration and metabolic syndrome, as well as aging, which emphasizes the role mouse models in understanding the role of mitochondrial dysfunction in common pathologies.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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