

Mitochondrial DNA mutations in disease and aging

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The small mammalian mitochondrial DNA (mtDNA) is very gene dense and encodes factors critical for oxidative phosphorylation. Mutations of mtDNA cause a variety of human mitochondrial diseases and are also heavily implicated in age-associated disease and aging. There has been considerable progress in our understanding of the role for mtDNA mutations in human pathology during the last two decades, but important mechanisms in mitochondrial genetics remain to be explained at the molecular level. In addition, mounting evidence suggests that most mtDNA mutations may be generated by replication errors and not by accumulated damage.

The origin of mtDNA

The mitochondrial oxidative phosphorylation system is remarkable in its dependence on both nuclear- and mitochondrial DNA (mtDNA)-encoded subunits (Falkenberg et al., 2007). The involvement of two distinct genomes creates a demand for elaborate regulatory processes to coordinate gene expression in response to cellular demands for ATP synthesis (Falkenberg et al., 2007). Mitochondria are related to α -protobacteria, and the eukaryotic cell arose approximately two billion years ago by some type of fusion event between ancient cells related to α -protobacteria and archaeabacteria (Yang et al., 1985; Lang et al., 1997; Andersson et al., 1998). Phylogenetic comparisons have shown that there is always cosegregation between the presence of mtDNA and a functional respiratory chain (Burger et al., 2003; Wallace, 2007). There is ongoing traffic of mtDNA fragments to the nucleus (Thorsness and Fox, 1990), and genes for many respiratory chain subunits have been transferred to the nucleus during evolution (Burger et al., 2003; Wallace, 2007). However, the genes for cytochrome *b* and cytochrome *c* oxidase subunit I are always maintained in mtDNA of the many organisms that have been studied to date (Wallace, 2007). The reason for the localization of these genes to mtDNA could be

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Abbreviations used in this paper: ES, embryonic stem; HSP, heavy strand promoter; LSP, light strand promoter; mtDNA, mitochondrial DNA; MTERF, mitochondrial transcription termination factor; ROS, reactive oxygen species; rRNA, ribosomal RNA.

the hydrophobicity of the gene products, which may prevent mitochondrial import if the gene is relocated to the nucleus. Approximately 25% of the yeast mitochondrial proteome of \sim 750–1,000 proteins is dedicated to maintenance and expression of mtDNA (Sickmann et al., 2003). This means \sim 200–250 nucleus-encoded proteins are needed to express a handful of mtDNA-encoded proteins, and it is unclear why this costly arrangement has been maintained throughout evolution if the only reason is the hydrophobicity of certain gene products. An interesting alternate hypothesis proposes that mtDNA has been kept for a regulatory purpose and that the biogenesis of the oxidative phosphorylation system requires direct interactions between the respiratory chain subunits and mtDNA (Allen, 2003).

Transcription and replication of mammalian mtDNA

Mammalian mtDNA encodes 13 proteins that all are subunits of the oxidative phosphorylation system and 22 tRNAs and 2 ribosomal RNAs (rRNAs; Fig. 1). The transcription of mtDNA is polycistronic and is initiated at one main promoter on each strand, the light strand promoter (LSP) and heavy strand promoter (HSP). The existence of a second HSP dedicated to the transcription of the rRNA genes has been reported (Montoya et al., 1983; Martin et al., 2005); however, its existence has been questioned, as transcription from this putative promoter cannot be reconstituted in vitro with known components of the basal transcription machinery (Litvin et al., 2010). The steady-state levels of rRNAs are much higher than the levels of the downstream mRNAs, but this is, in principle, compatible with polycistronic transcription from a single HSP as the rRNAs are incorporated into ribosomes and therefore may be much more stable than the downstream mRNAs.

The basal machinery needed for transcription initiation of mtDNA has been fully reconstituted in vitro and consists of a set of three proteins: mitochondrial RNA polymerase (POLRMT), mitochondrial transcription factor B2 (TFB2M), and mitochondrial transcription factor A (TFAM; Falkenberg et al., 2002). Interestingly, POLRMT is most closely related to bacteriophage RNA polymerases (Shutt and Gray, 2006a) and in addition contains a large N-terminal extension that may have a role in coupling transcription to translation (Rodeheffer and Shadel, 2003).

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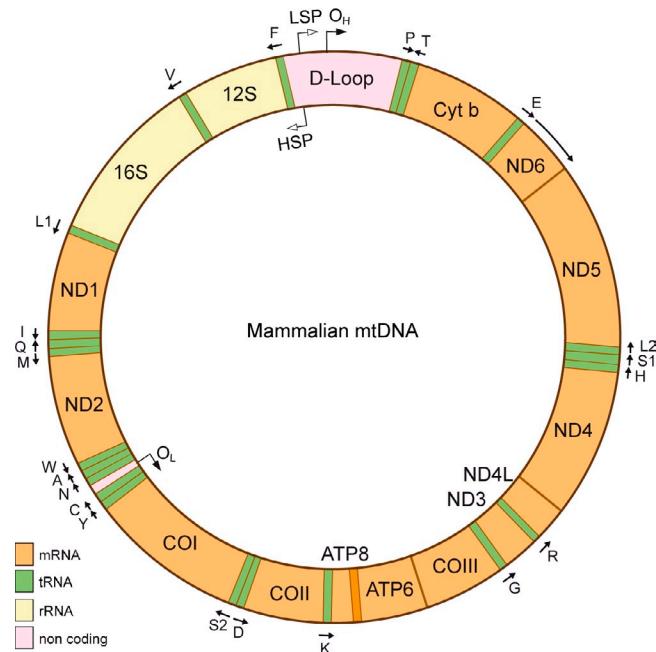


Figure 1. Schematic representation of mammalian mtDNA. The double-stranded circular mammalian mtDNA molecule of ~16.5 kb contains a single longer noncoding region, the displacement loop (D loop) region, harboring the promoters for transcription of both mtDNA strands (HSP and LSP) and the origin of leading strand replication (O_H). The origin of lagging strand replication (O_L) is embedded in a cluster of tRNA genes. The genes for the two rRNAs (12S and 16S rRNA), 13 mRNAs (ND1–6, ND4L, Cyt b, COI–III, ATP6, and ATP8), and 22 tRNAs (F, V, L1, I, M, W, D, K, G, R, H, S1, L2, T, P, E, S2, Y, C, N, A, and Q) are indicated by boxes. Illustration by Annika Röhl.

The paralogues TFB1M and TFB2M in mammalian cells are related to a highly conserved family of dimethyl adenosine methyltransferases (Falkenberg et al., 2002) that are present in bacteria, archaeabacteria, and eukaryotes (mitochondria and the nucleus), where they dimethylate two highly conserved adenines at a stem-loop structure at the 3' end of the small subunit rRNA (Shutt and Gray, 2006b). TFB1M was initially reported to have a role in mitochondrial transcription (Falkenberg et al., 2002; McCulloch and Shadel, 2003), but a conditional mouse knockout model has shown that it instead is an essential rRNA methyltransferase necessary for the integrity of the small subunit of the mitochondrial ribosome (Metodiev et al., 2009). TFB1M conditional knockout mice have severely impaired translation and are able to activate mitochondrial transcription, showing that there seems to be no functional redundancy between TFB1M and TFB2M (Metodiev et al., 2009). Biochemical studies further support the conclusion that TFB2M is essential for transcription, as it is a component of the catalytic site of POLRMT necessary for transcription initiation (Sologub et al., 2009; Litton et al., 2010).

TFAM is not only a transcription factor but also functions as an mtDNA packaging protein (Larsson et al., 1998; Ekstrand et al., 2004). It belongs to the high mobility group domain proteins (Parisi and Clayton, 1991) and can wrap, bend, and compact DNA (Fisher et al., 1992; Kaufman et al., 2007). There is approximately one TFAM molecule/10–20 bp mtDNA in mouse tissues (Ekstrand et al., 2004; Pellegrini et al., 2009), and

immunofluorescence experiments show a perfect colocalization between TFAM and mtDNA (Garrido et al., 2003), thus arguing that TFAM fully coats mtDNA *in vivo*. Genetic experiments in the mouse show that mtDNA levels *in vivo* are regulated according to levels of TFAM (Ekstrand et al., 2004). There are thousands of mtDNA copies in a mammalian somatic cell, and these genomes are packaged into mtDNA protein aggregates called nucleoids (Satoh and Kuroiwa, 1991; Kaufman et al., 2007; Bogenhagen et al., 2008). In budding yeast, a large number of proteins have been defined as components of the nucleoid based on a combination of biochemical experiments, showing that the studied protein can be cross-linked to and/or copurified with mtDNA, and genetic experiments, showing that the gene encoding the studied protein is essential for maintenance of mtDNA (Chen et al., 2005). This concept is not straightforward, as a protein may interact with mtDNA and be necessary for maintenance of mtDNA without having a role in packaging and organizing mtDNA. We propose that the definition of the nucleoid should focus on the role of the protein components in packaging and organizing mtDNA and that structural nucleoid proteins should comply with the following criteria: (a) the protein should interact with mtDNA (copurification or cross-linking); (b) the protein should have intrinsic biophysical properties consistent with packaging DNA; (c) the protein should be sufficiently abundant to coat mtDNA; (d) the protein should colocalize with mtDNA on high-resolution microscopy; and (e) the protein should be essential for maintenance of mtDNA *in vivo*. TFAM is currently the only mammalian protein that has been shown to fulfill all of these criteria. Definition of the structure of the nucleoid is a very fundamental question in mitochondrial genetics, and future studies are clearly warranted. Confocal microscopy experiments (Legros et al., 2004) have suggested that each nucleoid contains two to eight mtDNA molecules. This raises the interesting question of whether each nucleoid contains only one mtDNA genotype or whether there may be a mixture of mutant and wild-type mtDNA in a single nucleoid. Understanding this issue will provide novel insights into principles for mitotic segregation and maternal transmission of mtDNA, as discussed in the “Principles for maternal inheritance of mtDNA” section. The estimated size of the nucleoid in mammalian cells is ~250 nm, which is at the resolution limit of confocal microscopy. It is important to apply novel high-resolution optic microscopy techniques to determine whether 250 nm is the true size of the nucleoids and how uniform the size is. It is currently unknown whether the nucleoids in mammalian cells are free in the mitochondrial matrix or whether they are attached to the inner mitochondrial membrane. Another fundamental question is whether all nucleoids have an equal protein composition and how a particular nucleoid is selected for transcription and/or replication of mtDNA. Future studies of the nucleoid are therefore likely to give very fundamental insights into mammalian mitochondrial biology.

Our understanding of the regulation of the basal transcription initiation machinery is rather limited despite the fact that there is a long list of nuclear transcription regulators that are proposed to have intramitochondrial isoforms, e.g., nuclear hormone receptors, orphan receptors, and other transcription

factors (Shutt and Shadel, 2010). The mitochondrial transcription termination factors (MTERFs) are exclusively localized to mitochondria, and the first described member of this family is MTERF1, which has for >20 yr been implicated in transcription termination to enhance the production of rRNAs (Kruse et al., 1989; Yakubovskaya et al., 2010) and in transcription initiation (Martin et al., 2005). The paralogues MTERF2 and MTERF3 have roles in transcription initiation regulation (Park et al., 2007; Wenz et al., 2009a). Atomic structures of MTERF1 (Yakubovskaya et al., 2010) and MTERF3 (Spåhr et al., 2010) have unexpectedly shown that these proteins are very similar and contain repeated modules of a novel nucleic acid-binding fold, the MTERF motif.

Replication of mtDNA is dependent on POLRMT that forms the short RNA primers needed for initiation of mtDNA replication at the heavy and light strand replication origins (O_H and O_L ; Clayton, 1991; Fusté et al., 2010). The minimal replisome needed for replication of mtDNA consists of the mtDNA polymerase (Pol- γ ; Falkenberg and Larsson, 2009; Lee et al., 2009), the twinkle DNA helicase (Spelbrink et al., 2001), and the mitochondrial single-stranded DNA-binding protein (Falkenberg et al., 2007). The atomic structure for the Pol- γ was recently solved and showed that the complex is heterotrimeric and consists of one Pol- γ A subunit and two Pol- γ B subunits (Carrodeguas et al., 2001; Lee et al., 2009). The mode of mtDNA replication is controversial, as two competing models have been advocated: the strand-asymmetric (Clayton, 1982; Brown et al., 2005) and the strand-coupled replication modes (Holt et al., 2000; Yasukawa et al., 2006). According to the strand-asymmetric model, the leading strand replication is two thirds complete before lagging strand replication is initiated (Clayton, 1982; Brown et al., 2005). In contrast, the strand-coupled model argues that the replication of the leading and lagging strands is synchronous (Holt et al., 2000; Yasukawa et al., 2006). We have previously presented a more detailed discussion of these models (Falkenberg et al., 2007; Larsson, 2010), and only a few comments will be made here. There are many reported patients with high levels of mtDNA molecules with a single large deletion. The location of the deletion often varies between patients, but the deleted mtDNA molecule always retains O_H , O_L , and LSP, thus arguing that these sequences are essential for mtDNA replication (Moraes et al., 1991). Any valid model for mtDNA replication must therefore explain the role of these regulatory sequence elements in mtDNA replication. Recent data show that POLRMT specifically recognizes the stem-loop structure at O_L and provides a primer for initiation of lagging strand mtDNA synthesis at this site (Fusté et al., 2010). A combination of biochemical and genetic approaches will be necessary to further clarify the mode of mtDNA replication in mammalian mitochondria.

Pathogenic mutations of mtDNA are often heteroplasmic

Pathogenic mtDNA mutations were first reported in human patients >20 yr ago (Holt et al., 1988; Wallace et al., 1988; Zeviani et al., 1988). These discoveries were important breakthroughs that led to a genetic basis for the classification of mitochondrial disease and revealed novel insights into mitochondrial genetics.

A large number of pathogenic mtDNA mutations are known, and the reader is referred to specialized reviews on the associated clinical phenotypes (Larsson and Clayton, 1995; Munnich and Rustin, 2001; Zeviani and Di Donato, 2004).

Patients harboring pathogenic mtDNA mutations often have a mixture of wild-type and mutated molecules, a condition called heteroplasmy. The mutation load can vary widely between members of the same family and in different cells of particular tissues. This uneven distribution of mutated mtDNA leads to a mosaic respiratory chain deficiency in affected tissues. This mosaicism is likely an important determinant in disease pathophysiology, but few relevant animal models are available (Table I), and the mechanisms are therefore poorly understood. In an experimental study, mouse chimeras with mosaic respiratory chain deficiency in pyramidal neurons of the cerebral cortex were generated (Dufour et al., 2008). These mice developed clinical symptoms if they had a proportion of >20% respiratory chain-deficient neurons, and the defect caused mortality if there were >60–80% defective neurons (Dufour et al., 2008). Interestingly, a transneuronal degeneration phenomenon, whereby respiratory chain-deficient neurons induce death of neighboring neurons with normal respiratory chain function, was also observed (Dufour et al., 2008).

Segregation of mutated mtDNA in somatic tissues

The mitotic segregation of heteroplasmic mtDNA mutations in somatic tissues and the principles for maternal transmission of mtDNA (the bottleneck phenomenon and the purifying selection) are cornerstones in mammalian mitochondrial genetics. Unfortunately, these phenomena are poorly understood at the cell biological level despite their fundamental importance. Increased molecular understanding of these processes would lead to new understanding of disease pathophysiology and would also improve genetic counseling in families with mitochondrial disease.

Replication of mtDNA is not linked to the cell cycle, and a particular mtDNA molecule may be replicated many times or not at all as a cell divides (Bogenhagen and Clayton, 1977). This mode of replication makes it possible for a single mutational event to expand clonally or be lost as the cell divides (Fig. 2). The turnover of mtDNA in differentiated tissues has not been extensively studied, and half-lives of ~14 d have been reported in the rat brain and other tissues (Menzies and Gold, 1971). Thus, there is likely continuous replication of mtDNA in all tissues, which makes segregation of mtDNA mutations possible also in postmitotic cells. Heteroplasmic mtDNA mutations will only cause respiratory chain dysfunction if present above a certain threshold (Fig. 2), which varies depending on the type of mutation and the type of affected tissue. It is generally assumed that mitotic segregation of pathogenic mtDNA mutations is a largely random process and that high mutation levels may be selected against in rapidly dividing cells. However, there are also reports that seemingly neutral polymorphisms in mtDNA may undergo directional selection in certain tissues (Jenuth et al., 1997; Battersby and Shoubridge, 2001; Jokinen et al., 2010).

Table I. Mouse models for mtDNA mutation

Model	Experimental manipulation	Phenotype	References
mtDNA point mutation			
Mutator mouse (POLGA D257A)	Knockin modification of DNA polymerase γ exonuclease domain	Impaired respiratory chain function, premature aging phenotypes	Trifunovic et al., 2004; Kujoth et al., 2005
Trans-mitochondrial mice (CAP ^R)	Cytoplasmic transfer of chloramphenicol-resistant mtDNAs to ES cells	Growth retardation, myopathy, cardiomyopathy	Marchington et al., 1999; Sligh et al., 2000
Trans-mitochondrial mouse (T6589C)	Cytoplasmic transfer of mtDNA with T6589C missense mutation to ES cells	Impaired respiratory chain function, growth retardation	Kasahara et al., 2006
Trans-mitochondrial mouse (T6589C + 13885insCdelT)	Cytoplasmic transfer of mtDNA with T6589C, 13885insC, and 13885insCdelT mutation to ES cells	Decreased complex IV activity, myopathy, cardiomyopathy	Fan et al., 2008
mtDNA deletion			
Trans-mitochondrial mouse (Δ mtDNA4696)	Cytoplasmic transfer of deleted mtDNA [A7,759–12,454] to pronuclear stage embryo	Impaired respiratory chain function, renal failure, early mortality	Inoue et al., 2000
Deletor mouse (Twinkle dup353–365)	Transgenic expression of mutant mtDNA helicase twinkle	Progressive decrease of respiratory chain function	Tyynismaa et al., 2005
Mito-PstI mouse	Skeletal muscle–specific expression of mitochondrially targeted PstI restriction endonuclease	Impaired respiratory chain function in skeletal muscle, myopathy	Srivastava and Moraes, 2005
mtDNA depletion			
TFAM ^{−/−}	Knockout of TFAM in whole body	Respiratory chain deficiency, embryonic lethality	Larsson et al., 1998
TFAM ^{−/−} heart	Knockout of TFAM in cardiomyocyte	Dilated cardiomyopathy	Wang et al., 1999
TFAM ^{−/−} forebrain neuron	Knockout of TFAM in forebrain	Neurodegenerative symptoms	Sørensen et al., 2001
TFAM ^{−/−} dopaminergic neuron	Knockout of TFAM in dopaminergic neuron	Parkinson’s disease symptoms	Ekstrand et al., 2007
TFAM ^{−/−} pancreatic β cell	Knockout of TFAM in pancreatic β cell	Mitochondrial diabetes	Silva et al., 2000
POLGA ^{−/−}	Knockout of POLGA	Respiratory chain deficiency, embryonic lethality	Hance et al., 2005
MFN1 ^{−/−} + MFN2 ^{−/−} skeletal muscle	Skeletal muscle–specific knockout of MFN1 and MFN2, GTPases essential for mitochondrial fusion	Respiratory chain deficiency in skeletal muscle, muscle atrophy, early mortality	Chen and Chan, 2010
TK2 ^{−/−}	Knockout of thymidine kinase 2 (TK2)	Growth retardation, early mortality	Zhou et al., 2008
TK2 H126N	Knockin modification of TK2 found in human mtDNA depletion syndrome patients (TK2 H126N)	Impaired respiratory chain function in brain, growth retardation, early mortality	Akman et al., 2008
RRM2B ^{−/−}	Knockout of RRM2B gene, encoding p53-controlled ribonucleotide reductase (p53R2)	Growth retardation, renal failure, muscle atrophy, early mortality	Bourdon et al., 2007; Kimura et al., 2003
RNASEH1 ^{−/−}	Knockout of mitochondrial ribonuclease H1 (RNaseH1)	Embryonic lethality, respiratory chain deficiency	Cerritelli et al., 2003
mtDNA increase			
PAC-hTFAM	Transgenic expression of P1 artificial chromosome containing human TFAM	No apparent pathophysiology	Ekstrand et al., 2004
TFAM-EGFP	Transgenic expression of TFAM-EGFP fusion protein	Improve mitochondrial disease phenotypes	Nishiyama et al., 2010
CAG-hTFAM	Transgenic expression of mouse TFAM cDNA under CAG promoter	Improve cardiac failure after myocardial infarction, delay neuronal cell death	Ikeuchi et al., 2005; Hokari et al., 2010
TWINKLE	Transgenic expression of mouse TWINKLE cDNA	No apparent pathophysiology	Tyynismaa et al., 2004
Twinkle + hTFAM	Transgenic expression of mouse TWINKLE cDNA and human TFAM cDNA	Progressive respiratory chain dysfunction	Ylikallio et al., 2010

ES, embryonic stem.

Mitochondria form a dynamic network undergoing continuous fusion and fission (Detmer and Chan, 2007; Hoppins et al., 2007). These processes ensure mixing of the gene product content of the mitochondrial network in a cell and are necessary for maintaining function, as exemplified by the finding that blocked fusion leads to loss of mtDNA (Chen and Chan, 2010). Respiratory chain deficiency leads to a fragmentation of the mitochondrial network in cell lines and mouse tissues (Duvezin-Caubet et al., 2006). Autophagocytosis has an important role in vivo to remove mitochondria during normal erythrocyte differentiation

(Sandoval et al., 2008). Studies of mammalian cell lines have shown that Parkin, an E3-ubiquitin ligase mutated in juvenile-onset Parkinson’s disease, translocates to dysfunctional mitochondria to promote degradation by autophagocytosis (Narendra et al., 2008, 2009, 2010; Suen et al., 2010). The evidence for the existence of this mitochondrial quality control mechanism in mammals is at present based on forced expression of Parkin in transformed cell lines in combination with the use of rather drastic mitochondrial toxins (Narendra et al., 2008, 2009, 2010; Suen et al., 2010). Human patients with mitochondrial disease

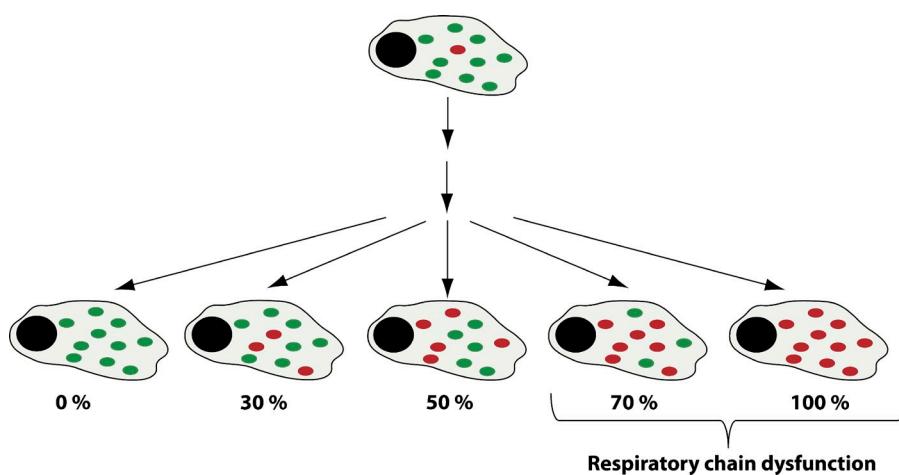


Figure 2. Mitotic segregation of mtDNA. A single mutational event creates heteroplasmy in a cell, but the level of mutated mtDNA (red dots) is very low in comparison with normal mtDNA (green dots). There is no synchronization between cell division and mtDNA replication, and a particular mtDNA molecule may be replicated many times or not at all during a single cell cycle. Repeated cell division will lead to mitotic segregation of normal and mutated mtDNA, and accumulation of mutated mtDNA above a certain threshold level will lead to impaired respiratory chain function.

often have a deteriorating respiratory chain function and may accumulate mutated mtDNA with time (Larsson et al., 1990), thus questioning the efficiency of this proposed mitochondrial quality control mechanism. There is a clear need to verify that the mitochondrial quality control mechanism observed in cell lines is relevant for mammals *in vivo*.

Principles for maternal inheritance of mtDNA

Maternal transmission of mammalian mtDNA has been recognized for >35 yr (Hutchison et al., 1974; Giles et al., 1980). It came as a true surprise when it was observed that the mtDNA genotype could shift completely in a few generations in cows (Olivo et al., 1983). A similar rapid segregation was soon observed in many human pedigrees transmitting heteroplasmic pathogenic mtDNA mutations (Larsson et al., 1992). Heteroplasmic mice containing mtDNA from two different mouse strains, created by experimental manipulation of embryos, exhibited a similar rapid, apparently random, germline segregation of mtDNA (Jenuth et al., 1996). This segregation phenomenon is attributed to a bottleneck mechanism in the female germline, whereby only a small fraction of all mtDNA copies in the primordial germ cell is transmitted to the oocyte. Several studies have produced conflicting results proposing that the bottleneck for mtDNA transmission occurs at different stages in the maternal germline, and results are also conflicting about whether there is a concomitant mtDNA copy number reduction in primordial germ cells (Cao et al., 2007, 2009; Cree et al., 2008; Wai et al., 2008; Samuels et al., 2010).

The transmission of mtDNA mutations through the mouse maternal germline is not neutral, but there is rather a strong purifying selection against deleterious mtDNA mutations (Fan et al., 2008; Stewart et al., 2008b). The mtDNA mutator mouse expresses a catalytic subunit of Pol- γ with deficient proofreading capacity (Table I), which leads to accumulation of high levels of acquired point mutations in mtDNA (Trifunovic et al., 2004). Maternal transmission of this apparently random set of point mutations generated in the mtDNA mutator mouse (Trifunovic et al., 2004) shows a strong selection against amino acid replacement mutations, whereas mutations affecting tRNA or rRNA coding genes are better tolerated (Stewart et al., 2008a,b).

Surprisingly, the spectrum of mutations present in offspring to the mtDNA mutator mouse has clear similarities to the naturally occurring spectrum of mutations observed in human populations (Stewart et al., 2008a,b). These results suggest that the naturally occurring spectrum of mtDNA mutations in humans can be explained by mtDNA replication errors that have been subjected to purifying selection in the maternal germline. The molecular mechanism of purifying selection is not understood. Formally, such a selection could occur at different levels (Fig. 3). A mechanism for selection at the level of the mtDNA is not easy to envision, as it involves blocking replication or destroying mutant mtDNA genomes without the need for testing the function of the gene products encoded by the mutant genome. A more plausible mechanism is functional testing of mtDNA at the level of a single organelle (Shoubridge and Wai, 2008). Such a mechanism could involve fragmentation of the mitochondrial network during some stage of oocyte development to permit expression of individual mtDNA molecules and subsequent functional readout of respiratory chain function in single mitochondria (Stewart et al., 2008a). It is also possible that there is a competition between cells during germ cell development and that cells with high levels of mutated mtDNA will be disfavored or undergo apoptosis. Mutations of tRNA or rRNA genes are more likely than amino acid substitutions to escape the purifying selection in the mouse maternal germline, which is consistent with the observation that the majority of pathogenic mutations of human mtDNA affects tRNA genes, although they only occupy ~9% of the genome (Stewart et al., 2008a). The bottleneck mechanism may be linked to the purifying selection, but it could also represent an independent protective mechanism. It is, of course, possible that a down-regulation of mtDNA copy number and fragmentation of the mitochondrial network may facilitate purifying selection in the maternal germline. However, the bottleneck mechanism could also prevent the spread of low levels of deleterious mtDNA mutations in maternal lineages (Stewart et al., 2008a). Available data on the nature of the bottleneck are mainly correlative, and it will be important to experimentally investigate possible mechanisms, e.g., by introducing pathogenic mtDNA mutations into mice concomitant with experimental manipulation of mtDNA copy number and mitochondrial dynamics in the female germline.

Level of Selection

mtDNA

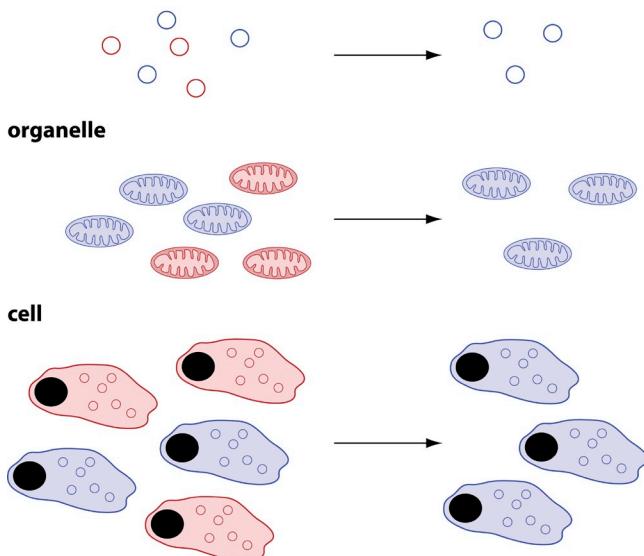


Figure 3. Different levels at which purifying selection can occur in the maternal germline. (top) Genomes with mutations could be blocked from replication or selectively destroyed without the need for gene expression. (middle) A fragmented mitochondrial network would allow functional testing of individual mtDNA molecules. The presence of a mutated mtDNA molecule would result in a mitochondrion with deficient respiratory chain function, which, in turn, would lead to selection against and/or destruction of this mitochondrion. (bottom) Cells with high levels of mutated mtDNA may fail to compete with respiratory chain-competent cells and may be selected against or undergo apoptosis. The colors indicate mutant (red) and wild-type (blue) mtDNA (top); respiratory chain-deficient (red) and normal (blue) mitochondria (middle); and respiratory chain-deficient (red) and normal (blue) cells (bottom).

Aging and somatic mtDNA mutations

Patients with mitochondrial syndromes have a monoclonal expansion of mutated mtDNA leading to high levels of a single type of mutation, whereas aging is associated with polyclonal expansions leading to low levels of many different types of mtDNA mutations. It is important to emphasize that one should not expect fundamental differences in the pathophysiology of mtDNA mutations regardless of whether they occur in genetic syndromes or in aging. Important principles in mitochondrial genetics, e.g., mitotic segregation of heteroplasmic mtDNA mutations, are also applicable to aging. Furthermore, the consequence of an mtDNA mutation at the cellular level will not depend on whether the mtDNA mutation is spontaneous or acquired but will rather depend on the type of mtDNA mutation and how it impairs respiratory chain function and the consequences this impairment, in turn, has on specialized functions of the affected cell. The role of somatic mtDNA mutations in mammalian aging has recently been extensively reviewed (Krishnan et al., 2007; Larsson, 2010), and only some issues will be discussed here. It is well established that somatic mtDNA mutations in human aging undergo clonal expansion and cause a mosaic respiratory chain dysfunction in different tissues (Krishnan et al., 2007; Larsson, 2010). It has generally been assumed that age-associated somatic mtDNA mutations are caused by accumulated damage during the aging process (Wallace, 2001). However, an alternative

hypothesis is that most of the mutations are created as replication errors during embryogenesis and then undergo clonal expansion in adult life (Larsson, 2010). Mosaic respiratory chain deficiency caused by clonal expansion of mtDNA mutations is ubiquitously observed in human aging, but the extent to which this aberration impairs organ function is unknown. The mtDNA mutator mice (Table I) develop a premature aging syndrome, which is caused by the accumulation of point mutations and the presence of linear deleted mtDNA molecules, which likely represent a continuously formed replication intermediate (Trifunovic et al., 2004; Bailey et al., 2009; Edgar et al., 2009). There has been one study arguing that a third type of mutation, circular mtDNA molecules with deletions, drives the phenotype of mtDNA mutator mice (Vermulst et al., 2008). However, this study has been refuted in several independent studies, in which sensitive PCR assays (Edgar et al., 2009; Kraytsberg et al., 2009) or deep sequencing (Williams et al., 2010; Ameur et al., 2011) could not detect any significant levels of circular mtDNA molecules with deletions in various tissues of mtDNA mutator mice. In addition, the biochemical phenotype in mtDNA mutator mice is fully consistent with the idea that point mutations of mtDNA create amino acid substitutions of respiratory chain subunits, which explains the observed decline in the stability of the respiratory chain complexes (Edgar et al., 2009).

Interestingly, the point mutation load is high already during embryogenesis in mtDNA mutator mice, but aging symptoms are not observed until early adult life, and there is no evidence for increased oxidative stress (Kujoth et al., 2005; Trifunovic et al., 2005). The results from the mtDNA mutator mouse thus lend some experimental support to the hypothesis that somatic mtDNA mutations created during embryogenesis may contribute to the creation of aging phenotypes in adult life.

Pathophysiological effects of mtDNA mutations

The studies of pathophysiological effects of altered mtDNA expression have to a large extent been dependent on the use of mouse models (Table I). It is important to emphasize that there is as yet no transfection system for mammalian mitochondria, and mouse models have therefore been developed by introducing naturally occurring mtDNA mutations into mouse embryos or by manipulating nuclear genes that control the maintenance and expression of mtDNA.

Expression of mtDNA is essential for oxidative phosphorylation (Larsson et al., 1998), and the most straightforward prediction is therefore that mtDNA mutations should have no other primary effects besides impairing cellular energy production. There are indeed a large number of different types of mtDNA mutations that have effects on respiratory chain function and ATP synthesis (Larsson and Clayton, 1995; Munnich and Rustin, 2001; Zeviani and Di Donato, 2004). Mutations of mtDNA have several downstream effects that are a consequence of deficient oxidative phosphorylation, and those will be further discussed in the next paragraphs.

It is important to remember that there are possibilities that mtDNA mutations could have effects unrelated to oxidative phosphorylation. Amino acid replacements of mtDNA-encoded

proteins can create novel histocompatibility antigens in the mouse (Loveland et al., 1990; Morse et al., 1996). These antigens are derived by degradation of mtDNA-encoded respiratory chain subunits, and the corresponding peptides are released from mitochondria and reach the cell surface (Loveland et al., 1990; Morse et al., 1996). There is also evidence that mtDNA sequence variants may elicit an innate immune response against tumor cells (Ishikawa et al., 2010).

It was initially reported that mtDNA is not required for execution of apoptosis in cell lines (Jacobson et al., 1993). However, a subsequent study showed that depletion of mtDNA caused by knockout of TFAM in the mouse leads to increased in vivo apoptosis (Wang et al., 2001). Increased apoptosis and/or cell death has now been reported in a variety of mtDNA-depleted tissues in the mouse: the heart (Wang et al., 2001), pyramidal neurons (Sørensen et al., 2001), dopamine neurons (Ekstrand et al., 2007), and pancreatic β cells (Silva et al., 2000). In addition, there is extensive apoptosis in different tissues of the mtDNA mutator mouse (Kujoth et al., 2005; Niu et al., 2007). Human patients with genetic mitochondrial diseases often have extensive cell death in the nervous system (Alpers, 1931; Leigh, 1951; Oldfors et al., 1990). Cell loss is also a common problem in aging, and, thus, it is possible that the observed clonal expansion of somatic mtDNA mutations may contribute to cell loss in human aging (Krishnan et al., 2007; Larsson, 2010).

Patients with pathogenic mtDNA mutations often accumulate increased levels of mitochondria, e.g., manifested as ragged-red muscle fibers in skeletal muscle. Also, tissue-specific knockout mice with impaired mtDNA maintenance (Wang et al., 1999; Wredenberg et al., 2002), defective mitochondrial transcription (Park et al., 2007), or impaired mitochondrial translation (Metodiev et al., 2009) have increased mitochondrial mass in affected tissues. It is generally assumed that this accumulation of mitochondria is caused by activation of mitochondrial biogenesis in response to deficient energy production. Experiments in mice with mtDNA depletion in skeletal muscle have shown that activation of mitochondrial biogenesis at least partly can compensate for the deficient mitochondrial function and maintain close to normal overall ATP production in the affected tissue (Wredenberg et al., 2002). Similarly, forced expression of PGC1- α in mouse skeletal muscle induces mitochondrial biogenesis, which, in turn, protects against mitochondrial myopathy (Wenz et al., 2008) and age-related loss of muscle mass (Wenz et al., 2009b).

Increased production of reactive oxygen species (ROS) and oxidative damage are heavily implicated in human pathology and aging, but the role this type of damage plays is much debated (Bokov et al., 2004). This controversy is to some extent explained by the complex chemistry of ROS formation (Murphy, 2009) and uncertainties about the performance of methods used to measure ROS (Bokov et al., 2004; Murphy, 2009; Murphy et al., 2011). There are also some intriguing studies showing that a substantial increase of oxidative damage does not shorten lifespan in mice (Zhang et al., 2009). It is often argued that mitochondrial dysfunction increases ROS production (Wallace, 2005), but this concept has poor experimental support. On the contrary, it has been reported that ROS production and oxidative

damage are not increased in different tissues of mice with depletion (Wang et al., 2001) or point mutations of mtDNA (Kujoth et al., 2005; Trifunovic et al., 2005). An impairment of respiratory chain function leads to a variety of metabolic consequences, e.g., deficient ATP production, increased NADH/NAD $^{+}$ ratios, and alterations in Ca $^{2+}$ homeostasis (Tavi et al., 2005; Aydin et al., 2009), which all may be more relevant for disease pathophysiology than ROS.

Future perspectives

During the last 25 yr, we have seen a remarkable increase in our understanding of mitochondrial genetics in mammals. We know today that maternal transmission of mtDNA is associated with a bottleneck phenomenon, which allows rapid shifts of genotypes between generations, and a purifying selection, which weeds out the most deleterious mutations. However, we still have no molecular understanding of these two processes despite their fundamental importance for mtDNA transmission. The different types of pathologies that are caused by mtDNA mutations are remarkable, and in many cases there is a reasonably good correlation between genotype and phenotype. However, the underlying pathophysiology is not understood in any depth. Important challenges for the future involve understanding the downstream effects of mitochondrial dysfunction on cell physiology in disease and aging.

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