

Mitochondrial whims: metabolic rate, longevity and the rate of molecular evolution

Nicolas Galtier^{1,*}, Richard W. Jobson¹,
Benoît Nabholz¹, Sylvain Glémin¹
and Pierre U. Blier²

¹Institut des Sciences de l'Évolution, CNRS UMR 5554, Université
Montpellier 2, Place E. Bataillon, 34095 Montpellier, France

²Département de Biologie, Université du Québec à Rimouski,
300 allée des ursulines, Rimouski, Québec, Canada G5L 3A1
*Author for correspondence (galtier@univ-montp2.fr).

The evolutionary rate of mitochondrial DNA (mtDNA) is highly variable across lineages in animals, and particularly in mammals. This variation has been interpreted as reflecting variations in metabolic rate: mitochondrial respiratory activity would tend to generate mutagenic agents, thus increasing the mutation rate. Here we review recent evidence suggesting that a direct, mechanical effect of species metabolic rate on mtDNA evolutionary rate is unlikely. We suggest that natural selection could act to reduce the (somatic) mtDNA mutation rate in long-lived species, in agreement with the mitochondrial theory of ageing.

Keywords: ageing; mutation; substitution; soma; antagonistic pleiotropy; reactive oxygen species

1. INTRODUCTION

The rate at which DNA and proteins accumulate substitutions is an important aspect of molecular evolution. Substitution rates reflect the combined effects of mutation, natural selection and genetic drift, the respective roles of which have been sharply debated for decades. The so-called 'molecular clock' hypothesis, which states that the rate of molecular evolution is constant in time and across lineages, is only rarely met: some species evolve significantly faster than others as far as molecules are concerned (Bromham & Penny 2003). The causes of these variations are still largely mysterious. Mitochondrial DNA (mtDNA) has received particular attention as far as evolutionary rates are concerned. Mitochondrial evolution appears to be especially capricious. Sudden increases or decreases in the substitution rate have been reported in various lineages (Spradling *et al.* 2001; Nabholz *et al.* 2008). Given the popularity of mtDNA as a marker of population and species history, understanding the dynamics of this genome is of primary interest to the evolutionary community.

Several studies have reported a negative relationship between mtDNA substitution rate and species body mass: mtDNA tends to evolve faster in small

organisms (Martin & Palumbi 1993; Bromham *et al.* 1996). This is true of both non-synonymous (amino acid changing) and synonymous substitutions (Gillooly *et al.* 2007), which is indicative of a mutational mechanism—if only selection on proteins was at work, no effect would be expected at third-codon positions. The relationship could partly result from differences in generation time: assuming a constant per-generation mutation rate, species with a shorter generation time should undergo a higher per-year mutation rate. This hypothesis, however, cannot explain the whole pattern. The generation time effect must affect the mitochondrial and nuclear genomes to a similar extent; variations in substitution rate across species, however, are much stronger for mtDNA than for nuclear DNA, at least in mammals (Nabholz *et al.* 2008).

2. OXIDATIVE DAMAGES

An alternative explanatory model invokes variations in metabolic rate across species. Mitochondrial respiratory activity generates reactive oxygen species (ROS) as a by-product of membrane electron transport. These oxidative molecules are known to be mutagenic: they damage DNA by oxidizing nucleotides. Owing to its cytological location, mtDNA is the main target of these replication-independent mutations. Differences in mtDNA mutation rates across species could therefore reflect differences in metabolic rate, which is inversely related to body mass (Martin & Palumbi 1993). This hypothesis was recently championed by Gillooly *et al.* (2005) who modelled metabolic rate as a function of body mass and temperature, and reported a quasi-perfect match between mtDNA substitution rate and predicted metabolic rate.

The metabolic rate hypothesis, however, is not devoid of problems. First, it should be recalled that only germ-line mutations, which are transmitted to the next generation, are relevant from an evolutionary viewpoint. It is currently unclear whether the metabolic rate in female germ-line cells is correctly predicted by the metabolic rate in somatic tissues, for which we have measurements (Lanfear *et al.* 2007). Second, the metabolic rate hypothesis is difficult to reconcile with the existence of site-specific mutation hotspots in the mitochondrial genome (Galtier *et al.* 2006)—how could chemical mutagens hit specific nucleotide positions, but not their immediate neighbours? Third, the empirical evidence does not always conform to the predictions of the metabolic rate hypothesis (Lanfear *et al.* 2007). Finally, from a physiological point of view, the relationship between metabolic rate, ROS production and mtDNA mutation is far from clear, as we now discuss in more detail.

3. MITOCHONDRIAL MUTATION AND AGEING

Most of the literature about ROS production and mtDNA mutation has been achieved in the context of ageing studies. Harman (1957) introduced the free radical theory of ageing, which states that the ROS generated throughout the lifespan of an organism cause its senescence. In its modern form, the mitochondrial theory of ageing posits that somatic mutations occurring in mtDNA, by affecting the efficiency of respiratory proteins, result in an increase in the rate of ROS production, which in turn increases the mutation

One contribution of 11 to a Special Feature on 'Whole organism perspectives on understanding molecular evolution'.

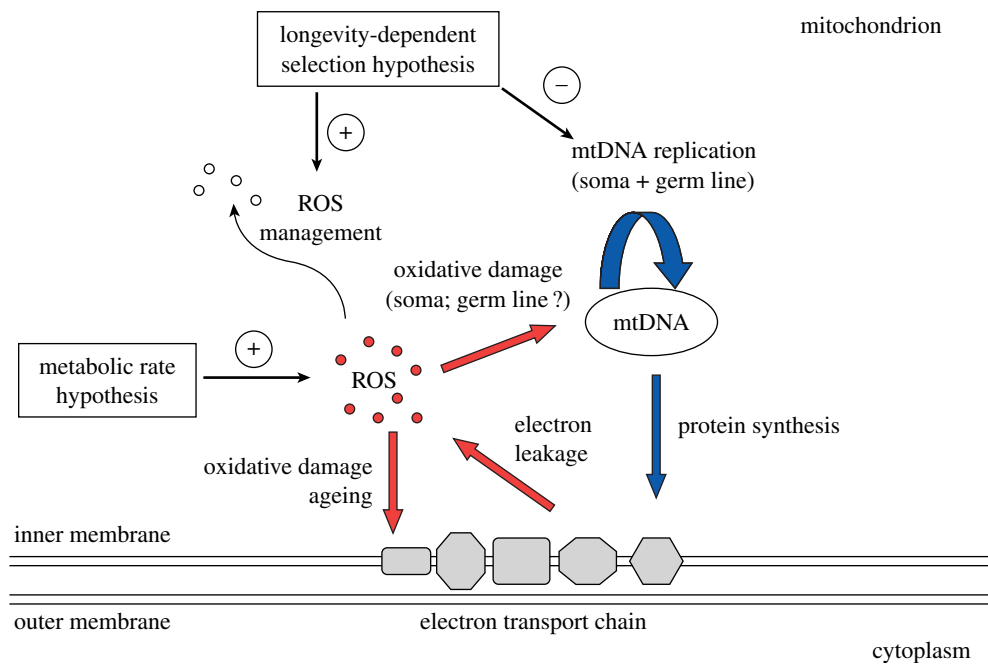


Figure 1. Major structures, processes and evolutionary forces involved in mitochondrial ageing.

rate—a vicious circle eventually causing ageing (figure 1). This model has been supported by various observations, including the inverse relationship between mitochondrial oxidative damage marker concentration and maximum lifespan in mammals (Barja & Herrero 2000), and the report of increased mtDNA mutation load in aged cells (Kujoth *et al.* 2007).

Closer examination of the evidence suggests that the situation might be more complex. Recently, two distinct groups have designed transgenic mice, in the genome of which the native mtDNA polymerase had been replaced by a modified, low-fidelity enzyme. These mtDNA-mutator mice showed strongly accelerated senescence, confirming the implications of mtDNA mutations in ageing (Trifunovic *et al.* 2004), but no increase in ROS production (Kujoth *et al.* 2005). In this case, ageing was apparently not caused by a positive feedback loop between mtDNA mutations and ROS production. ROS, furthermore, are not only a poison: they play a functional signalling role in healthy cells (Linnane *et al.* 2007), and could be under homeostatic control, irrespective of tissue preservation and ageing. Another (indirect) argument comes from the analysis of membrane lipid composition. In mammals and birds, a strong, negative correlation was reported between maximum lifespan and the proportion, among lipids, of *n*-3 polyunsaturated fatty acids (Hulbert *et al.* 2007). *n*-3 Polyunsaturated acyl chains are most sensitive to peroxidation, suggesting that lipid composition could have adapted to elongated lifespan. Lipid composition, however, is not significantly correlated to the mass-specific metabolic rate in mammals (Hulbert *et al.* 2007).

4. THE LONGEVITY HYPOTHESIS

This rapid review cast doubts on the simplistic, mechanical relationship between metabolic rate, ROS production, mtDNA mutation and ageing we outline above. One suggestion, however, is that long-lived

mammals and birds may have adapted to an increased lifespan by evolving macromolecular components more resistant to oxidative damages (Pamplona & Barja 2007; Min & Hickey 2008; Moosmann & Behl 2008). This conclusion points to a new hypothesis potentially explaining the variations of mtDNA substitution rate across species—longevity-dependent selection. According to this model, natural selection would act to reduce the mitochondrial mutation rate in long-lived species: a low somatic mutation rate would be required to achieve long lifespan. The low mtDNA mutation rate in long-lived species would not be a mechanical consequence of their reduced mass-specific metabolic rate, but rather a selected character (figure 1).

This hypothesis was first introduced by Samuels (2004) who reported a depletion of oligonucleotide direct repeats in the mitochondrial genomes of long-lived mammals. Direct repeats are known to be mutagenic. Their reduced number probably decreases the somatic mtDNA mutation rate in long-lived animals, which could be advantageous. Taking a phylogenetic approach, Nabholz *et al.* (2008) showed that the neutral mtDNA substitution rate is quite variable across the species of short-lived mammals, but constrained to low values in long-lived mammals, consistent with the longevity hypothesis. Correlation of mtDNA substitution rate and maximum lifespan was found to be stronger than that of either body mass or generation time (Nabholz *et al.* 2008; Welch *et al.* 2008).

Broad-scale comparisons can also help discriminate between the metabolic rate and the longevity models. Birds live longer, but have a higher mass-specific metabolic rate than mammals of similar body mass. Nabholz *et al.* (in press) showed that, on average, mtDNA substitution rate is lower in birds than mammals, favouring the longevity rather than the metabolic rate model. Cnidarians also provide interesting insights. The mtDNA substitution rate is very low in anthozoans (sea anemones, corals), which lack a mortal soma, and whose clonal colonies can

Table 1. Candidate genes potentially involved in the control of mtDNA mutation rate and ageing.

gene	protein product	function/process	involvement in mtDNA mutation and ageing
<i>POLG</i>	mtDNA polymerase	mtDNA replication and maintenance	protein defects leads to accumulation of mtDNA mutations
<i>POLG2</i>	Pol γ accessory subunit	mtDNA replication and maintenance	protein defects lead to accumulation of mtDNA mutations
<i>TFAM</i>	transcription factor A	mtDNA replication and maintenance	protein defects increase exposure of mtDNA to ROS
<i>TWINKLE</i> (<i>PEO1</i>)	mitochondrial helicase	mtDNA replication and maintenance	protein defects lead to multiple DNA deletions
<i>ANT1</i> (<i>SLC25A4</i>)	ADP/ATP translocase 1	ROS production	under-expression stalls electron transport chain and increases ROS production
<i>UCPs</i>	mitochondrial uncoupling proteins UCP1, UCP2, UCP3	ROS production	protein defects may increase ROS production
<i>SOD2</i>	superoxide dismutases [Mn]	protection against oxidative damage	protein defects increase oxidative stress
<i>MPV17I</i>	peroxisomal membrane protein family	protection against oxidative damage	downregulation leads to increased ROS damage
<i>HTRA2</i>	serine protease HTRA2	protection against oxidative damage	downregulation leads to increased ROS damage and apoptosis
<i>FOXO3A</i>	forkhead box protein O3	protection against oxidative damage	protein defects increase ROS and trigger apoptosis upon cellular stress
<i>PINK1</i>	serine/threonine-protein kinase	protection against oxidative damage	protein defects triggers mitochondrial dysfunction and apoptosis

live for hundreds of years (Hellberg 2006). Senescence is probably strongly counter-selected in these cells. Medusozoans (jellyfish, hydroids), by contrast, have a typical metazoan life cycle, with differentiated gonads in short-lived medusa; their mtDNA substitution rate is much higher than that of anthozoans (Huang *et al.* 2008), consistent with the longevity hypothesis. Similarly, plants, which lack a differentiated somatic line, generally harbour a low mitochondrial substitution rate (Wolfe *et al.* 1987), although plant mitochondria typically operate in the oxygen-rich environment resulting from photosynthesis (Noctor *et al.* 2007).

5. MITOCHONDRIAL DNA AND THE EVOLUTION OF SENESCENCE

Theoretical evolutionary biologists have been interested in the ultimate reasons for senescence evolution: why should the fitness of an individual decrease during its lifespan? The main models that were proposed to explain this apparent paradox invoke late-onset mutations (Hughes & Reynolds 2005). Deleterious mutations are less strongly counter-selected if they are expressed in late stages of life, because their impact on the life-average reproductive success is weaker. Such mutations have a higher probability of fixation than early-onset deleterious mutations. The accumulation of many late-onset mutations, each of weak effect, could explain senescence (mutation accumulation model). The effect is more pronounced for mutations that are advantageous in early stages of life, but deleterious in older individuals. Natural selection favours the fixation of such mutations if their life-average effect is beneficial (antagonistic pleiotropy model, Williams 1957). The relative contribution of these two kinds of mutation to senescence evolution has been debated, but few empirical data are available.

Being somatic, the mtDNA mutations invoked by the mitochondrial theory of ageing do not correspond to these two models. They do not affect heritable traits, and cannot be directly selected against. However, germline mutations that could modulate the mtDNA somatic mutation rate would potentially be under the influence of natural selection—our working hypothesis. According to this model, a mutation that increases the mtDNA mutation rate would be deleterious in late stages of life, when somatic mutations have had time to affect the mitochondrial proteome of a large number of cells, and induce substantial oxidative damage. If uncovered, such mutations would provide evidence for the ‘mutation accumulation’ model of senescence evolution.

Confirmation of the longevity-dependent selective model would require the identification of modifiers of the mtDNA mutation rate and the investigation of their influence on species longevity. The most obvious candidates are genes controlling the fidelity of mtDNA replication (e.g. DNA polymerase gamma), genes involved in the regulation of the mitochondrial metabolism and redox status (e.g. coupling/uncoupling proteins) and antioxidant genes regulating the amount of cellular ROS (e.g. superoxide dismutase; table 1). We suggest that molecular evolutionary analyses of these genes in long-lived versus short-lived mammals or birds would be worthwhile.

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