OPINION

Is mitochondrial DNA turnover slower than commonly assumed?

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Abstract Mutations arise during DNA replication due to oxidative lesions and intrinsic polymerase errors. Mitochondrial DNA (mtDNA) mutation rate is therefore closely linked to the mitochondrial DNA turnover process, especially in post mitotic cells. This makes the mitochondrial DNA turnover rate critical for understanding the origin and dynamics of mtDNA mutagenesis in post mitotic cells. Experimental mitochondrial turnover quantification has been based on different mitochondrial macromolecules, such as mitochondrial proteins, lipids and DNA, and the experimental data suggested highly divergent turnover rates, ranging from over 2 days to about 1 year. In this article we argue that mtDNA turnover rate cannot be as fast as is often envisaged. Using a stochastic model based on the chemical master equation, we show that a turnover rate corresponding to mtDNA half-life in the order of months is the most consistent with published mtDNA mutation levels.

Keywords Mitochondrial DNA · Turnover rate · Ageing · Point mutations · POLG transgenic mice

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Mitochondrial and mitochondrial DNA turnover

Accumulation of mitochondrial DNA (mtDNA) mutations and mitochondrial dysfunction are often suggested to be important mechanisms of ageing (Harman 1972). Since mutations predominantly occur during

mtDNA replication, either as a consequence of oxidative lesions or of intrinsic polymerase errors (Kujoth et al. 2007), mtDNA turnover rate is a critical parameter determining de novo mtDNA mutation rate (Poovathingal et al. 2009). The mtDNA turnover rate may also impact clonal expansion dynamics of mtDNA mutations (Chinnery and Samuels 1999; Nicholas et al. 2009; Poovathingal et al. 2009). In post-mitotic tissues, mtDNA turnover is often considered to be mainly associated with the biogenesis of mitochondria that balances the mitochondrial autophagy (mitophagy) process, where the complete organelle and its constituents are catabolized by cellular double membrane structures known as autophagosomes (Nakatogawa et al. 2009). However, mitochondria do not generally exist as disconnected organelles, but undergo rapid fusion and fission, forming large, complex reticular structures (Chen and Chan 2005). Therefore, it is not trivial to define what exactly constitutes the "turnover of a mitochondrion". Despite of this, mitochondrial turnover rates have been proposed based on the turnover rates of various mitochondrial constituents such as mitochondrial proteins (Lipsky and Pedersen 1981; Menzies and Gold 1971; Miwa et al. 2008), mtDNA (Collins et al. 2003; Gross et al. 1969; Korr et al. 1998) or lipids (cardiolipin) (Fletcher and Sanadi 1961; Gross et al. 1969; Huemer et al. 1971).

Experimental data on the turnover of mitochondrial components are limited and divergent. Reported half-lives range from ∼2 to 350 days, depending on reference molecules and measurement techniques (Collins et al. 2003; Gross et al. 1969; Huemer et al. 1971; Korr et al. 1998; Miwa et al. 2008). Half-life values of about 2–17 days, derived from bulk protein turnover data, are often cited as the global mitochondrial turnover rate (Fletcher and Sanadi 1961; Menzies and Gold 1971; Miwa et al. 2008). However, as argued above, it is unclear if a global mitochondrial turnover can in fact be meaningfully defined. Importantly, if these short half-life values are assumed to be indicative of the mtDNA turnover rate, they would have dramatic impact on age-dependent mtDNA mutation accumulation dynamics (Chinnery and Samuels 1999; Elson et al. 2001; Kowald et al. 2005; Kowald and Kirkwood 2000; Poovathingal et al. 2009). It is the latter relationship that we will explore in more detail below.

Challenges with estimating global mitochondrial turnover rate—implications in estimating mtDNA turnover rate

Mitochondrial protein turnover rates are commonly used as a proxy for the turnover of the complete mitochondrial organelle (Fletcher and Sanadi 1961; Miwa et al. 2008) and thus for mtDNA turnover. Mitochondrial protein turnover rates are usually measured using pulse chase experiments (Fletcher and Sanadi 1961; Menzies and Gold 1971; Miwa et al. 2008). In these experiments, isotope-labeled protein precursor molecules are administered, followed by tracking the labeled mitochondrial protein. The first-order decay rate for the loss of isotope-label yields mitochondrial protein half-lives. However, such data must be interpreted with caution when extending this to estimate turnover rate of other mitochondrial components. For one, mitochondria contain ∼1,200 different polypeptides (Mootha et al. 2003), with reported half-lives ranging from a little over an hour to more than 10 days (Table 1). The presence of intra-mitochondrial proteolytic systems (Mijaljica et al. 2007) implies that mitochondrial proteins can be degraded independent of organelle turnover, resulting in protein-specific half-lives (Jaleel et al. 2008). In contrast to proteomics approaches, pulse chase experiments utilizing bulk protein will thus result in half-lives that reflect the average turnover of the population of proteins in mitochondria (Miwa et al. 2008). Therefore, protein turnover data from such experiments may be dominated by the turnover rate of few abundant proteins. This suggests that, even with the best experimental practices and even when artifacts, such as precursor reutilization (Burgess et al. 1978; Lipsky and Pedersen 1981; Miwa et al. 2008), are carefully controlled, bulk protein turnover might still overestimate the turnover of mitochondrial components, such as mtDNA.

Individual mitochondrial protein half-lives of more than 10 days have been reported (Table 1). If these experimental data are accepted, mitochondrial half-life, if meaningful at all, cannot be shorter than 10 days. However, more data from mitochondrial proteomics studies using modern methods would be desirable [e.g.:(Dai et al. 2009)]. Long half-lives have also been reported for other macromolecules in mitochondria. For instance, a half-life of 40 days has

Table 1 Turnover rates of some mammalian mitochondrial proteins

S. no.	Protein	Half life	Organism/tissue	Reference
1	Ornithine aminotransferase	1.9 days	Rat liver	Ip et al. (1974)
2	Cytochrome oxidase	5.7	Rat liver	Ip et al. (1974)
3	Carbamyl phosphate synthetase	7.7	Rat	Nicoletti et al. (1977)
4	Malate dehydrogenase	2.6	Rat	Nicoletti et al. (1977)
5	Aminolevulinate synthetase	70 min	Rat liver	Marver et al. (1966)
6	Cytochrome c	10.3 days	Rat liver	Fletcher and Sanadi (1961)
7	Ornithine transcarbamylase	7.7 days	Rat liver	Wallace et al. (1986)
8	Cytochrome c	11.8 days	Rabbit heart	Grozdova and Starostina (1973)
9	66 different mitochondrial proteins	2–8.5 days	Rat skeletal muscle	Jaleel et al. (2008)
10	Various	$\sim$18–95 days	Rat brain	Rodrijguez de Lores et al. (1971)

been reported for the mitochondrial lipid cardiolipin (Huemer et al. 1971). In this work, we are mainly concerned with the turnover of mtDNA. Importantly, attempts at direct measurement of mtDNA turnover have yielded half-life values ranging from 10 days up to as high as 300 days, again depending on tissue types and experimental techniques (Collins et al. 2003; Gross et al. 1969; Korr et al. 1998). However, even the fastest reported mtDNA turnover rate (10 days) (Gross et al. 1969) is significantly longer than the two-day mitochondrial turnover rate estimated based on the bulk protein measurements (Miwa et al. 2008).

Physiological levels of mtDNA turnover rate—mtDNA mutation data

The turnover rate of mtDNA critically determines how fast de novo mtDNA mutations can occur, as each replication event has a finite probability of introducing a new mutation (Poovathingal et al. 2009). For the purpose of this study, we considered age-related mtDNA point mutations in a *TaqI* recognition site (bp 634–637) in mouse heart tissues as reported by Vermulst et al. (2007). The majority of these mutations (86 %) are GC to AT transitions and for the *TaqI* site investigated by Vermulst et al. (2007), such mutations are silent, causing no change in amino acid sequence of the relevant gene product and therefore cannot be subjected to selective degradation. Furthermore, even for those mutations that are not silent, the reported burdens are several orders of magnitude lower than the levels that have been shown to affect the electron transport chain (ETC) function (Inoue

et al. 2000; Vermulst et al. 2007). If these low levels of mtDNA mutation burden are considered to be true, then these data provide a constraint on the magnitude of the mtDNA turnover rate, because ultimately mtDNA turnover directly affects the observed de novo mtDNA point mutation rate.

Using in silico simulations of random mtDNA turnover and de novo mutations, we argue that mtDNA turnover cannot be as rapid as is often envisaged based on the mitochondrial protein turnover data. A stochastic model based on chemical master equation (CME) (Poovathingal et al. 2009) was used to simulate the mitochondrial DNA turnover process and mtDNA mutagenesis process at different mtDNA turnover rates in cells. Using this stochastic model, we have simulated the accumulation of mtDNA point mutations burden in mouse heart tissue for several reported mitochondrial turnover rates and then compared the simulated burdens against the reported data.

In an earlier work (Poovathingal et al. 2009), we have demonstrated that the faster turnover promotes more rapid accumulation of mtDNA mutations. Here this model has been used to investigate the constraint on possible mtDNA turnover rate, given the observed mtDNA point mutation burden. We apply this model to the cases of wild-type and polymerase-γ (POLG) mutator mice, for which mtDNA point mutation data have been previously reported (Vermulst et al. 2007). The in silico mtDNA model tracks the accumulation of point mutations in cardiomyocytes (2.5×10^7 cells) during two stages of life: development and post-development (Fig. S1 in Online Resource 1). In the model, we assumed that the mtDNA population in each cell consists of only wild-type (W) and mutant

(*M*) molecules and that these mtDNA are well-mixed due to fast fusion-fission (Chinnery and Samuels 1999; Poovathingal et al. 2009). The stochastic model was formulated as a CME that describes the random mtDNA turnover process, comprising of relaxed replication and degradation of mtDNA (details in the Online Resource 1). Importantly, mutant and wild-type mtDNA are equally likely to replicated or degraded during mtDNA turnover. De novo point mutations are assumed to result from mtDNA replication errors due to two cumulative factors: oxidative lesions associated with ROS and the finite fidelity of the mtDNA polymerase-γ (POLG) enzyme (Kunkel 1992; Zhang et al. 2000) (Fig. S2 in Online Resource 1).

Oxidative lesions in mtDNA, including but not limited to 8OHdG, one of the oxidation products of guanine, facilitate the formation of point mutations during mtDNA replication because of alternative hydrogen bonding configuration of the oxidized bases leading to inappropriate base-paring. Therefore, the presence of oxidative lesions such as 8OHdG during mtDNA replication is an important determinant of mutation probability during replication. Essentially, mtDNA replication can turn oxidative lesions in the template strand (oxidative damage) into point mutations in the newly synthesized strand (Halliwell and Aruoma 1991; Halliwell and Gutteridge 1999). It is extremely challenging to accurately determine the rate of de novo oxidative damage events and of repair of such damage in vivo. The most widely available parameter related to oxidative DNA damage is therefore the steady state damage level of various oxidative DNA lesions, including 8OHdG. Steady state damage level results from the balance between the damage rate and repair rate. At any given time, there is a small fraction of the total damage events that remain unrepaired. Such steady state damage levels are typically very low, representing only a tiny fraction of the damage events that occur.

In vivo 8OHdG burden in nuclear DNA has been reported to be between 0.3 and 4.2 lesions per 10^6 bp based on different samples and different measurement techniques (ESCODD 2002a, b). However, such lesions make up only about 10–20 % of the complete damage spectra (Halliwell and Aruoma 1991; Halliwell and Gutteridge 1999) and thus, the overall oxidative lesions could range between 1.5 and 4.2 lesions per 10^6 bp. In our model simulations, we have made a conservative assumption that the oxidative damage to mtDNA is the same as that to nuclear DNA and have also used the lowest value in the range (1.5×10^{-6} bp^{-1}). This means that our model simulations likely underestimate the overall mutation rate during mtDNA replication. Importantly, oxidative lesions are converted into mutations only when mtDNA is replicated.

On the other hand, the reported POLG fidelity values range from 10^{-7} to 10^{-4} mutations bp^{-1} replication^{-1}, depending on cell types and quantification assays (Johnson and Johnson 2001; Kunkel 1985; Kunkel et al. 1987; Lee and Johnson 2006). In the simulations of the POLG transgenic mice, the replication error frequency due to POLG fidelity was set to 200 times higher than that of the wild-type mouse, based on the commonly reported literature data (Kunkel 1992; Zhang et al. 2000). Again, we have used the most conservative (lowest) values from the above range when computing the frequency of mtDNA replication error. Thus the mutation burden obtained by our model simulations should represent a lower limit (more details in Online Resource 1).

Many mitochondrial related processes were left out from the model, such as selective degradation of mitochondria with lower membrane potential (possibly due to mitochondria harboring high levels of detrimental mtDNA mutations) (Kim et al. 2007) and retrograde signaling (Liu and Butow 2006). While each of these processes may complicate or mask the inference of mtDNA turnover rate, we note that such processes are relevant only when mtDNA mutations become physically detectable, for instance through effects on the ETC activity, ATP or ROS levels or through impact on mitochondrial membrane potential (Kanki and Klionsky 2008; Narendra et al. 2008; Twig et al. 2008). Obviously, for highly deleterious mtDNA mutations, selective degradation of the affected mitochondria could serve as their clearance mechanism and by ignoring this process, our model simulation analysis may suggest an artificially slow mtDNA turnover rate. On the other hand, if retrograde signaling was prominent, the mtDNA turnover rate predicted by our basic model would be an exaggeration.

Mutant mtDNA carrying a single point mutation is indistinguishable from WT DNA unless the mutation causes some sort of phenotypes. Meanwhile, the mechanisms proposed for selective degradation and retrograde signaling assume functionally relevant

mutations (that is, mutations causing mitochondrial deficiency). This means that, while it is possible that such mechanisms operate to control the accumulation of some detrimental mutations such as mtDNA deletions or pathological point mutations, they cannot impact the accumulation of mutations that have no functional effect on mitochondria. In particular, selective degradation and retrograde signaling cannot impact the accumulation of *silent* point mutations. The stochastic drift of these mutations should arise only from mtDNA turnover, which was captured in our basic model as stated above. Such a simple model has other benefits, for example only a few model parameters need to be specified. In this case, the model involves only two free parameters, which are the mtDNA copy number at steady state and the de novo mutation frequency. The remaining model parameter is associated with the half-life of mtDNA. Therefore, model simulations reflected a direct causal relationship between mtDNA turnover and mutation burden.

Simulations of the model in this work were performed to predict the accumulation of mtDNA point mutations in post mitotic heart tissue of wild-type and POLG mutator mice. The steady state mtDNA copy number was set based on the reported value for mouse heart tissue (see supporting information in Online Resource 1), from which the mtDNA mutation burden were measured (Vermulst et al. 2007). Finally, model simulations were carried out using modified Gillespie's algorithm (see Online Resource 1, online for more details) and the complete model parameters used are included in the Online Resource 2, 3 and 4.

We have compared the mtDNA point mutation burden data from wild-type (WT) and POLG mutator mice (Vermulst et al. 2007) with those predicted by model simulations above with different mtDNA turnover rates, as shown in Fig. 1. The modeling results clearly show that, as long as neither mutant nor wild-type mtDNA are preferentially degraded or replicated, higher turnover rates always result in increased mtDNA mutation burden. This is not surprising, since de novo mtDNA mutations are tied to mtDNA replication. Note that we have explicitly made conservative assumptions regarding the occurrence of de novo mutations in this model, by setting the error rate arising due to limited POLG fidelity and lesions arising due to oxidative damage to the lowest plausible values that are consistent with the literature.

Figure 1d shows that the mtDNA mutation burden obtained using a short mitochondrial half-life [∼2 days (Miwa et al. 2008)] does not fit well with the point mutation burden experimental data (Vermulst et al. 2007). Simulations using other reported half-lives, ranging from 10 to 300 days (Collins et al. 2003; Gross et al. 1969; Korr et al. 1998; Menzies and Gold 1971) as shown in Fig. 1a–c, indicate that the observed mtDNA mutation burden data for both the WT and POLG mice are most consistent with a mtDNA turnover rate corresponding to a half-life between 30 and 300 days. However, as we have taken a conservative assumption regarding the frequency of de novo mutations in this model, the actual mtDNA turnover rate could even be slower.

A slow mtDNA turnover rate during aging implies that de novo somatic mtDNA mutations are rare events. As we have discussed elsewhere (Poovathingal et al. 2009), for 300 day mtDNA half-life, the number of de novo mutations during the developmental stage of mice (where mtDNA replication frequency is tied to cell divisions) is roughly equal to that during the entire adult life of the organisms. In simulations, each somatic cell experiences on average between 2 and 3 de novo mutations during 3 years of mouse lifespan. Therefore, most mtDNA mutations may already exist in early life, consistent with findings from a deep sequencing study (Ameur et al. 2011). Also, these single mutational events would become the starting point for the (random) accumulation of mtDNA mutations during aging, a process known clonal expansion (Larsson 2011). An increase in either mtDNA turnover rate or replication error rate (e.g. due to increased oxidative lesions) would mean a higher de novo mtDNA mutation frequency. A more detailed exposition on the relationship between mtDNA turnover and age-related mtDNA mutation dynamics has also been given in an earlier work (Poovathingal et al. 2009).

Concluding remarks

Attributing a global mitochondrial half-life parameter based on the turnover of any individual mitochondrial components requires strong justification and given the dynamic characteristics of mitochondria, the very concept of "whole organelle" turnover may be questionable. We believe that there are strong arguments for mtDNA half-lives to be longer than a month.
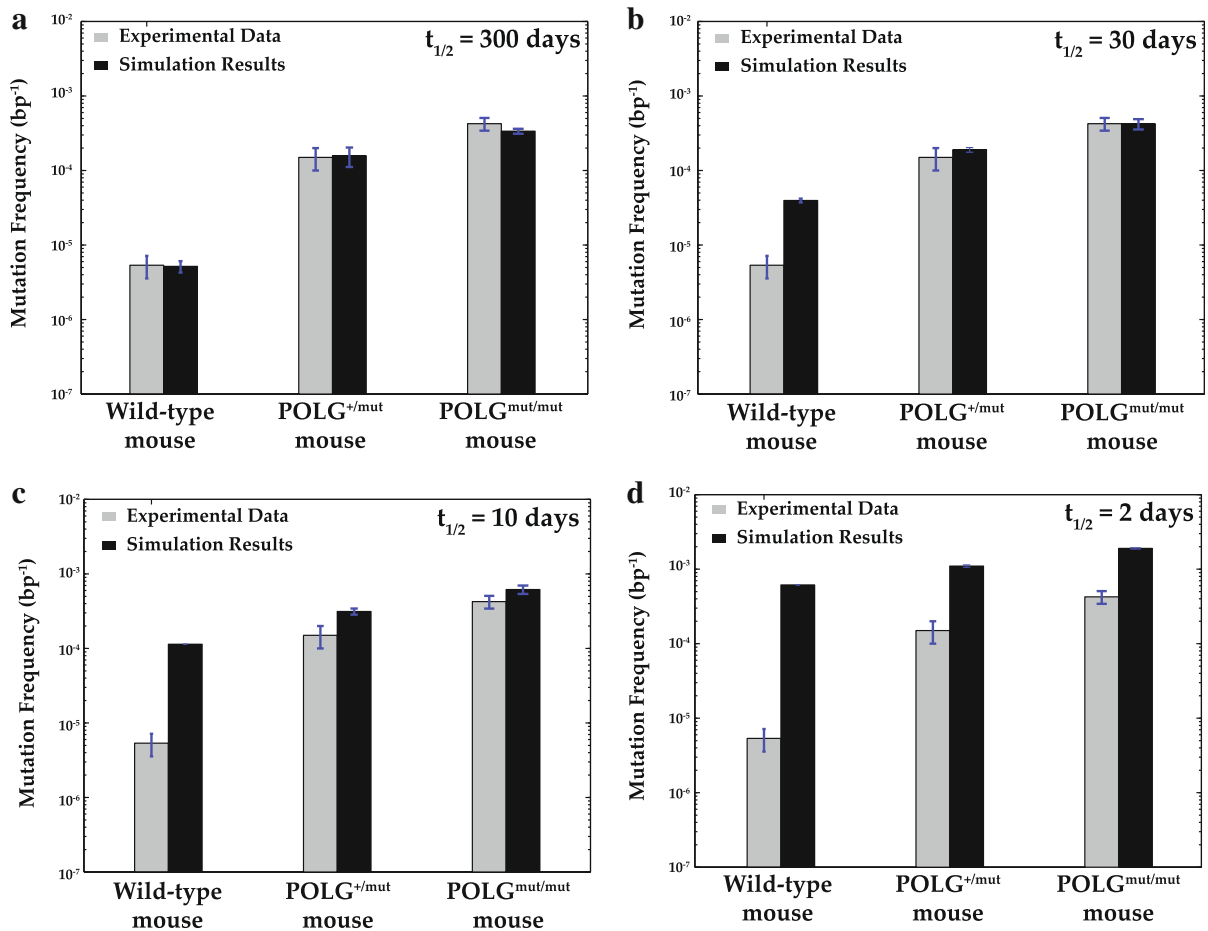
Fig. 1 Comparison of experimental data and simulations of point mutation frequency in wild-type and POLG mice ($n = 10$) (Vermulst et al. 2007) for different values of mtDNA turnover rate. The frequencies of the accumulated point mutations in mouse heart at the end of 29 months for wild-type mouse and at the end of 6 months of age for POLG$^{+/mut}$ and POLG$^{mut/mut}$ mice. Please note that the mutation frequency coordinate is in log-scale. Results of point mutation frequencies are obtained using mtDNA turnover rates of **a**. $t_{1/2} = 2$ days, (Miwa et al. 2008) **b**. $t_{1/2} = 10$ days, (Gross et al. 1969) **c**. $t_{1/2} = 30$ days, (Gross et al. 1969) and **d**. $t_{1/2} = 300$ days (Collins et al. 2003)

If a global organelle turnover parameter were to be at all meaningful, it would have to be much slower than commonly quoted mitochondrial turnover time of 2–10 days (Gross et al. 1969; Miwa et al. 2008). Interestingly, there are several studies reporting longer half-lives (>30 days) for cardiolipin, mtDNA and some individual proteins. As shown here, support for slow turnover of mtDNA comes from the quantification of age-related mtDNA mutations. Using a stochastic computational model based on minimal and conservative assumptions regarding the mtDNA turnover process and the associated de novo mutagenesis, we found that simulations using a short mtDNA half-life of 2 days grossly overestimate

mtDNA point mutations when compared with published data for mutation burden (Vermulst et al. 2007). The available experimental data were most consistent with a mtDNA half-life of months. In parallel to our conclusion, a previous computational study suggested that mitochondrial deoxyribonucleoside salvage pathway alone could not support a rapid mtDNA replication (∼2 h mtDNA) and that the kinetics of this pathway could only provide for a much slower mtDNA replication of longer than 10 h (Gandhi and Samuels 2011).

The rate of mtDNA turnover is a crucial parameter, determining the rates of de novo mutations and the consequent age-dependent loss of mtDNA integrity

and mitochondrial function (Gruber et al. 2008; Poovathingal et al. 2009; Wiesner et al. 2006). Given the fundamental role of mtDNA turnover rate for the understanding of the basic mechanism(s) of ageing, we feel that the accurate quantification of this rate is an important issue worthy of further careful experimental studies, if possible, by a direct and sensitive experimental estimation of mtDNA turnover. While some components of mitochondria are turned over rapidly, the global mitochondrial turnover rate, if meaningful at all, could not be faster than that of the most persistent macromolecules, e.g. mtDNA. Certainly, bulk protein turnover rate should not be used as a surrogate for mtDNA turnover.

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