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SHORT TAKE

Mitochondrial mutations and aging: random drift is insufficient to explain the accumulation of mitochondrial deletion mutants in short-lived animals

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Summary

Mitochondrial DNA deletions accumulate over the life course in post-mitotic cells of many species and may contribute to aging. Often a single mutant expands clonally and finally replaces the wild-type population of a whole cell. One proposal to explain the driving force behind this accumulation states that random drift alone, without any selection advantage, is sufficient to explain the clonal accumulation of a single mutant. Existing mathematical models show that such a process might indeed work for humans. However, to be a general explanation for the clonal accumulation of mtDNA mutants, it is important to know whether random drift could also explain the accumulation process in short-lived species like rodents. To clarify this issue, we modelled this process mathematically and performed extensive computer simulations to study how different mutation rates affect accumulation time and the resulting degree of heteroplasmy. We show that random drift works for lifespans of around 100 years, but for short-lived animals, the resulting degree of heteroplasmy is incompatible with experimental observations.

Key words: aging; mathematical model; mitochondrial mutation.

Introduction

The mitochondrial theory of aging has for many years been among the most popular, although recent questions have arisen concerning the magnitude of its contribution. Point mutations and deletions of mtDNA impair ATP production with negative consequences for all aspects of cellular homeostasis. Many studies have shown that mtDNA deletions accumulate with age in various mammalian species such as rats, monkeys and humans (Brierley *et al.*, 1998; Khrapko *et al.*, 1999; Cao *et al.*, 2001; Gokey *et al.*, 2004; Herbst *et al.*, 2007; McKiernan *et al.*, 2009). These single-cell studies have shown that the mitochondrial population of a cell is often overtaken by a single deletion mutant type via clonal expansion.

The mechanism behind the accumulation of defective mitochondria is currently unknown. The 'vicious cycle' hypothesis suggests that,

because defective mitochondria generate more radicals, this increases the production of mutants (Bandy & Davison, 1990; Arnheim & Cortopassi, 1992). However, this also implies that one should see many different mtDNA mutants in a single cell, quite the contrary of what has been observed. An alternative idea is called 'survival-of-the-slowest' (de Grey, 1997). This notes that the fate of a mutant depends on its rate of degradation as well as growth and proposes that defective mitochondria are degraded less frequently than wild-type. However, this hypothesis has problems with mitochondrial dynamics, as fission and fusion break the required link between genotype and phenotype (Kowald & Kirkwood, 2011), and evidence shows that dysfunctional mitochondria are preferentially degraded (Twig *et al.*, 2008; Kim & Lemasters, 2011), instead of being spared. It is also suggested that the reduced genome size of the deletion mutant confers a selection advantage (Wallace, 1992; Lee *et al.*, 1998). However, the time required for replication of mtDNA is only 1–2 h (Berk & Clayton, 1974; Clayton, 1982), while the half-life of mtDNA is in the order of 1–3 weeks (Gross *et al.*, 1969; Huemer *et al.*, 1971; Korr *et al.*, 1998). Therefore, it is difficult to see how mtDNA replication could be a rate limiting step for mitochondrial growth (de Grey, 1999; Elson *et al.*, 2001).

Finally, random drift might be sufficient to explain clonal expansion (Chinnery & Samuels, 1999; Elson *et al.*, 2001). Elson *et al.* (2001) simulated a population of 1000 mtDNAs with a 10 day half-life and a mutation probability between 10^{-6} and 10^{-4} per replication event. They defined COX-negative cells as those that contain more than 60% mutant mtDNA at the end of the simulation and showed that after 120 years, between 1 and 10% of COX-negative cells had a degree of heteroplasmy compatible with experimental observations. However, can such a process also work for short-lived animals like rodents, which show a similar pattern of accumulation as in humans but on a greatly accelerated timescale (Cao *et al.*, 2001; Herbst *et al.*, 2007)?

To address this question, we performed a stochastic simulation of degradation and replication of 1000 mtDNA molecules inside a post-mitotic cell. Each mtDNA has a half-life of 10 days, and after a molecule has been degraded, one of the remaining molecules is randomly chosen to replicate to maintain the population level. Mitochondrial deletions are assumed to happen via slippage replication at perfect and imperfect direct repeats (Shoffner *et al.*, 1989; Guo *et al.*, 2010b), and thus, each replication leads, with a certain probability, P_{mut} , to a deletion. A mutant has the same probabilities of degradation and replication as the wild-type, so its numbers in the population follow a process of random drift. The simulation is performed for a certain number of years during which degradation and replication are calculated on an hourly basis. To get statistical insight into the results, we performed 3000 repetitions of each single simulation and calculated average values. The simulation program was written in Java and the corresponding Eclipse project is available from the authors.

To test the effect of different mutation rates, we performed simulations over a time span of 120 years with mutation rates ranging

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from 10^{-6} to 10^{-4} (Fig. 1A). Like Elson *et al.* (2001), we define COX-negative cells as those that contain more than 60% of mutant mtDNAs. Our results are in excellent agreement with Fig. 1C of those authors, except that we used more repetitions to get a better estimate of the mean. This was especially necessary for a mutation rate of 10^{-6} , which resulted in a very low number of COX-negative cells. A mutation rate of $P_{\text{mut}} = 10^{-5}$ resulted in 2.7% of COX_{neg} cells and a mutation rate of $P_{\text{mut}} = 10^{-4}$ gave 22.3% of COX_{neg} cells at 120 years, a range compatible with experimental observations in older people (Brierley *et al.*, 1998).

To investigate whether random drift can also account for the accumulation of mtDNA mutants in shorter-lived animals, we calculated the mutation rate needed to produce $5 \pm 0.5\%$, $10 \pm 0.5\%$ and $15 \pm 0.5\%$ COX-negative cells after a certain number of years. As clonally expanded mtDNA mutants are found in rats (Cao *et al.*, 2001; Herbst *et al.*, 2007), we started calculations for a lifespan as low as 3 years. As can be seen in Fig. 1B, it is possible to reach 5–15% of COX-negative cells after just 3 years, but only with a mutation rate 200-fold higher than what is required for a lifespan of 120 years (7.6×10^{-3} vs. 4×10^{-5}).

An interesting further question concerns the degree of heteroplasmy in the COX-negative cells. We therefore calculate the number of different types of mtDNA molecules present at the end of the simulation. Figure 1C shows the degree of heteroplasmy to increase dramatically with decreasing lifespan. On average, more than 30 types of mtDNA molecules are present in each COX-negative cell after 3 years of simulation.

Could this heteroplasmy be observed experimentally? If there is one dominant mutant, while all other types only exist in very small numbers, it might be that standard PCR techniques only detect the dominant form. To test this, we also calculated how frequent is the most dominant mutant in COX-negative cells (Fig. 1D). For a 120 year lifespan, the most frequent mutant is represented at well above 90%, but this drops rapidly with decreasing lifespan and is below 20% for a 3 year lifespan.

A hallmark of mtDNA mutation accumulation in humans is the low degree of heteroplasmy, that is, in the cells overtaken by a mutant mtDNA, hardly any other mutant can be found. This is clearly also the case in rats (Cao *et al.*, 2001; Herbst *et al.*, 2007). In mice, mitochondrial deletions have also been found (Tanhauser & Laipis, 1995; Eimon *et al.*, 1996; Guo *et al.*, 2010a), but no single-cell studies have yet been reported, so that the degree of heteroplasmy is unknown. However, even for a timeframe of 10–40 years, the number of mutants per cell is too high to agree with observations. In rhesus monkeys, Gokey *et al.* (2004) examined single muscle fibres in 29- and 39-year-old animals and detected only a single, clonally expanded, deletion mutant per COX-negative fibre. Yet, the simulations show that even for a 40-year lifespan the most frequent mutant accounts for less than 80% of all mtDNAs in a COX-negative cell. Thus, the heteroplasmy is readily detectable. For a more complete overview, Table 1 summarizes the values displayed in Fig. 1. As the measured values for mtDNA half-lives range from 1–3 weeks and were quantified only for rodents but never for humans, we also performed simulations with half-lives ranging from 5 to 20 days. In general, an increasing half-life leads to a reduction in the number of

Figure 1 (A) Time course of the abundance of COX-negative cells over a simulation period of 120 years for various mutation rates. The time points are in monthly intervals and are the mean of 3000 simulations for $P_{\text{mut}} = 10^{-4}$ and 10^{-5} , and 15000 simulations for $P_{\text{mut}} = 10^{-6}$. COX-negative cells are defined as those with more than 60% mutant mtDNA. (B) Mutation rates that are required to result in 5, 10 and 15% of COX-negative cells after a certain number of years. The data points represent the mean of 3000 simulations for a lifespan of 3, 10, 40, 80 and 120 years. (C) Degree of heteroplasmy given by the average number of different mtDNAs in COX-negative cells after a certain number of years. (D) Percentage of most frequent mutant in COX-negative cells after 3, 10, 40, 80 and 120 years of simulation. Mutation rates were adjusted so that 5, 10 and 15% of COX-negative cells resulted after the indicated number of years of simulation (see B).

Table 1 The first three columns give the mutation rate necessary to achieve a certain percentage of COX-negative cells after the indicated number of years. The middle three columns show the mean number of different types of mutants per COX-negative cell, and the right most three columns display the percentage of the most frequent mutant type in COX-negative cells after the indicated number of years

Age years	MutRate 5% OX ⁻	MutRate 10% COX ⁻	MutRate 15% COX ⁻	MutTypes 5% COX ⁻	MutTypes 10% COX ⁻	MutTypes 15% COX ⁻	MostFreq 5% COX ⁻	MostFreq 10% COX ⁻	MostFreq 15% COX ⁻
3	6.5×10^{-3}	7.6×10^{-3}	8.5×10^{-3}	31.5	35.7	38.7	17	16	15
10	9.5×10^{-4}	1.45×10^{-3}	1.8×10^{-3}	7.8	10.4	12.2	44	40	37
40	9×10^{-5}	1.7×10^{-4}	2.7×10^{-4}	2.3	2.8	3.5	81	76	72
80	3×10^{-5}	7×10^{-5}	1.1×10^{-4}	1.44	1.75	2.08	92	90	86
120	2×10^{-5}	4×10^{-5}	8×10^{-5}	1.35	1.46	1.61	95	93	92

COX-negative cells, as mutation-prone replications occur less frequently (results not shown). The degree of heteroplasmy changes very little. Therefore, the specific value of the mtDNA half-life does not affect the main message of the simulations.

What do our results mean for understanding the accumulation of mitochondrial deletion mutants? In principle, random drift is a viable explanation for the accumulation pattern in humans and other long-lived species, but it is not a general explanation for the vast majority of mammalian species (nor has its contribution been investigated in very short-lived invertebrates). As mentioned in the introduction, neither the vicious cycle nor survival-of-the-slowest-hypotheses are compatible with experimental findings. This leaves the idea that deletion mutants might have a selection advantage because they have a shorter replication time. But this idea has also been criticized and a simulation study that we recently performed (Kowald, A., unpublished data) confirms that the large discrepancy between the half-life and replication time of mtDNA molecules results in unrealistic predictions regarding the time required for the accumulation process.

Thus, intriguingly, there currently exists no idea that satisfactorily explains the observed clonal expansion of mtDNA deletion mutants in short-lived as well as long-lived animals, confirming the importance of careful quantitative study of the mitochondrial aging hypothesis. But what properties are required for this unknown mechanism? To explain the wide inter-species range of accumulation times displayed by deletion mutants at the tissue level, two scenarios are possible. One is that the hypothetical mechanism controls the speed of the accumulation process itself at the single-cell level. If the rate of accumulation of a mutant inside a cell is species specific, being faster for short-lived than long-lived species, then the accumulation rate in a tissue will show the same time dependence. An alternative is that the accumulation process in all mammalian cells is similar and very fast (i.e. shorter than a rodent lifespan) but that the initiation of the accumulation is triggered in individual cells at different time points, and that it is the likelihood of such initiation that differs between species. In the second scenario, although individual cells experience rapid accumulation, the statistical variability among the cells in time point when the accumulation begins will result in the same continuous pattern of tissue accumulation as in the first. The challenge is to identify which aspects of mitochondrial biochemistry can provide the mechanistic basis for these scenarios.

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