Asymmetric Segregation of Damaged Cellular Components in Spatially Structured Multicellular Organisms

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Abstract

The asymmetric distribution of damaged cellular components has been observed in species ranging from fission yeast to humans. To study the potential advantages of damage segregation, we have developed a mathematical model describing ageing mammalian tissue, that is, a multicellular system of somatic cells that do not rejuvenate at cell division. To illustrate the applicability of the model, we specifically consider damage incurred by mutations to mitochondrial DNA, which are thought to be implicated in the mammalian ageing process. We show analytically that the asymmetric distribution of damaged cellular components reduces the overall damage level and increases the longevity of the cell population. Motivated by the experimental reports of damage segregation in human embryonic stem cells, dividing symmetrically with respect to cell-fate, we extend the model to consider spatially structured systems of cells. Imposing spatial structure reduces, but does not eliminate, the advantage of asymmetric division over symmetric division. The results suggest that damage partitioning could be a common strategy for reducing the accumulation of damage in a wider range of cell types than previously thought.

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Introduction

Despite the numerous repair and degradation processes involved in cell maintenance, over time cells accumulate damaged and deteriorated material [1–3]. This accumulation of damage has been linked to the decline in replicative function and increased risk of apoptosis associated with cellular ageing [4–9].

In some unicellular organisms, damaged cellular components are segregated preferentially to one daughter cell during division and this asymmetry is thought to have evolved as a strategy for ensuring the longevity of the lineage [10,11]. In the budding yeast Saccharomyces cerevisiae, such differential inheritance has been documented for senescence factors including extra-chromosomal rDNA circles [9], oxidatively damaged proteins [6,10], and defective mitochondria [8,12]. When followed over successive generations, the division and growth rate of the mother cell receiving the damaged components is seen to decline [13,14], whereas the daughter cell displays full replicative potential.

Differential inheritance of damaged proteins has been documented for epithelial stem cells in the intestine, which divide asymmetrically to produce a stem cell and a differentiated cell [15]. With the exception of germ-line cells and differentiating stem cells, mitosis in mammalian cells is commonly thought to occur symmetrically with the equal distribution of cellular components between daughter cells [2]. However, human embryonic stem cells undergoing self-renewing divisions have been found to segregate irreversibly damaged proteins preferentially to one daughter cell [16]. In the study by Fuentesalba and colleagues (2008), cell-fate determinants were distributed equally between daughter cells, but proteins targeted for degradation were segregated asymmetrically. Likewise, several other species assumed to reproduce by morphologically symmetric division have been found to distribute damaged proteins asymmetrically [14,17].

The experimental evidence suggests that asymmetric partitioning of damage is a more common strategy than previously thought. Several mathematical models have been developed to study the role of damage segregation in unicellular organisms [11,18–21]. However, the effect of damage segregation on the ageing process of mammalian tissue - i.e. a multicellular system of somatic cells that do not rejuvenate at cell division - has not previously been explored. Furthermore, the existing computational models of damage segregation assume that the spatial organization of cells does not significantly impact the population dynamics. This may be a valid assumption for yeast, however, stem cells tend to inhabit microenvironments - known as stem cell niches - with a high degree of spatial structure that is instrumental in regulating stem cell fate [22].

We therefore developed a mathematical model to study damage segregation in cell populations with and without spatial structure. In the model, each daughter cell receives an amount of damage at least equal to the pre-divisional cell. This makes the model applicable to the description of cellular components where damage is duplicated during the cell cycle. In most cells, intracellular degradation processes are active and consequently the amount of...
damage accumulates more slowly than in our model. We consider the limiting case where damage is irreversible.

To illustrate the applicability of the model, we consider the specific case of damage incurred by mutations to mitochondrial DNA (mtDNA). The accumulation of defective mitochondria in ageing tissue is thought to be a major contributor to cellular ageing and has been linked to a variety of degenerative diseases [1]. Differential inheritance of defective mitochondria has been documented in budding yeast [3,5,8,12], but an active process of mitochondrial selection may also occur in mouse ovarian germ cells [23,24]. The model presented here demonstrates the potential benefits of such segregation.

Methods

To study the accumulation of damage and the associated decline in the longevity of a population of cells, we developed a mathematical model of \( N \) cells undergoing division and apoptosis, within a tissue that is homeostatic with respect to the number of cells. The damage level of a cell is denoted \( d \) and represents the accumulation of mitochondrial DNA mutations; \( d \) differs between cells and changes over time as each cell acquires additional damage. In the model, the probability of a cell going apoptotic is, in each time step, proportional to its damage level \( d \). The apoptotic cell is removed from the tissue and immediately afterwards another cell divides to replace it. This mechanism ensures tissue homeostasis. The damage level of the dividing cell is denoted \( d' \) to distinguish it from that of the apoptotic cell.

Each cell contains multiple copies of mtDNA packaged into protein aggregates called nucleoids, with an average of 1.4 mtDNA molecules per nucleoid [25]. During the cell cycle, the number of nucleoids and mtDNA copies doubles. The mitochondrial genome is replicated and deletion-mutations and point-mutations are duplicated [26,27]. It has been shown that nucleoids, in general, do not exchange genomic material [25,28]. In the model, we consider the limiting case of irreversible damage from mutations to mitochondrial DNA. Consequently, each daughter cell, as a minimum, inherits the damage level \( d' \) of the parent cell.

Between cell divisions, the mitochondrial genome may acquire additional damage \( 2\Delta \), this occurs with a probability \( p \). The mechanisms for segregation of mitochondrial DNA at cell division are not fully understood [29]. Rather than attempting to model the segregation mechanism, we consider the limiting cases of completely symmetric or completely asymmetric segregation of additional damage. No cells are allowed a damage level that exceeds 1.

Thus, the three parameters of the model are (i) the initial damage level \( d_0 \) of all cells, (ii) the probability \( p \) of acquiring additional mutations between cell divisions, and (iii) the additional damage accumulated during the cell cycle \( 2\Delta \). We will also refer to \( \Delta \) as the fragility of the system, in accordance with previous work [30]. The dynamics of the model are shown schematically in figure 1.

In addition to the well-mixed system, we also consider the effect of spatial structure. This is done by considering a system of cells arranged on a one dimensional line, with periodic boundary conditions, and imposing the constraint that cells undergoing apoptosis can only be replaced by proliferating neighbouring cells.

![Figure 1. Schematic presentation of one time step of the computational model.](https://www.plosone.org/doi/10.1371/journal.pone.0087917.g001)
Mathematically, a system of cells arranged spatially in two or three dimensions falls in between the one-dimensional and the well-mixed (mean field) case. Consequently, results that hold for both the one-dimensional and the well-mixed system can be expected to generalize to the case of two and three dimensions.

The mathematical model was written in MATLAB and C++, and the code is available upon request. Simulations were carried out for different parameter values, system sizes, with symmetrical or asymmetrical replication, and with and without spatial structure.

Results

As the simulation progresses, cells gradually acquire mtDNA mutations and the average damage level of the system \( \langle d \rangle \) increases from the initial value of \( d_0 \). Since mutations are irreversible, the steady state where \( \langle d \rangle = 1 \) is absorbing. However, as seen in figure 2a, an additional transient steady state exists, characterized by a damage level \( d_{\text{sym}}^* \) for symmetric divisions and \( d_{\text{asym}}^* \) for asymmetric division. The steady state is sustained by cells with high levels of damage more frequently going apoptotic and being replaced by cells with fewer mtDNA mutations. As seen in figure 2a, the steady state damage level is higher when damage is distributed symmetrically upon cell divisions than when damage is segregated.

The steady state is only temporarily stable; eventually the fraction of cells, \( P_0 \), with the initial damage level, fluctuates down to zero and the system collapses to the state \( \langle d \rangle = 1 \). Biologically, this corresponds to the cell population having finite longevity. The characteristic time for this process is denoted by \( \tau_{\text{sym}} \) and \( \tau_{\text{asym}} \) for the symmetrically and asymmetrically dividing system, respectively. Comparing the dependence of \( \tau \) on the parameters \( p \) and \( \Delta \) in figure 2b in the symmetric and asymmetric case, it is evident that damage segregation dramatically increases the longevity of the cell population. This is in agreement with the results of Erjavec and co-workers [18]. As expected, a decrease in the mutation probability \( p \) or the initial damage level \( d_0 \) also increases the characteristic time for the collapse of the system, provided that \( \Delta < 0 \).

Somewhat counterintuitively, increasing the fragility \( \Delta \) leads to a more stable system, as may also be seen from figure 2b. As reported elsewhere [30], if the acquired damage is associated with a high likelihood of apoptosis, defective cells are more effectively replaced by less damaged cells, thereby reducing the overall damage level of the system.

In a well-mixed system, where an apoptotic cell can be replaced by any proliferating cell, the characteristic time for the transient steady state increases drastically with system size, as seen in figure 3. The figure also shows the results for a one-dimensional spatially structured system of cells with symmetric or asymmetric distribution of damage. In both cases, a transient steady state exists for some parameters \( d_0, p, \) and \( \Delta \). As in the well-mixed system, damage segregation increases the longevity of the cell population. However, imposing spatial structure diminishes the effect of system size and reduces the stability of the system since there is a tendency for defective cells to be replaced by other defective cells.

Figure 4a and 4b show how the damage level of the system develops over time for a spatially structured system where damage is distributed symmetrically and asymmetrically, respectively. Cells that go apoptotic can only be replaced by neighbouring cells, which locally leads to strong correlations in damage levels and to the propagation of mutant mtDNA in the population. In both plots, clusters of defective cells develop and expand in space, but it is more pronounced in the system without damage partitioning. The boundary between clusters with many mutations and clusters with few, will perform a random walk with a drift towards the defective group, since these are more likely to be removed by apoptosis.

As the simulations demonstrate, the system displays a transient steady state for both symmetric and asymmetric distribution of damage. For a well-mixed system in this regime, the mathematical model presented above is analytically tractable.

In a system proliferating by symmetric division, a cell with \( i \) mutations is assigned a damage level of \( d_i = d_0 + \Delta i \). The fraction of such cells in the system is denoted \( P_i^{\text{sym}} \). This fraction decreases if a cell goes apoptotic (which occurs at a rate of \( d_i P_i^{\text{sym}} \)) or if it acquires additional mutations before dividing to replace an apoptotic cell (rate \( d_i^{\text{sym}} (1 - p) P_i^{\text{sym}} \)). The fraction increases if the cell does not mutate prior to cell division (rate \( d_i^{\text{sym}} (1 - p) P_i^{\text{sym}} \)). If a cell with \( i - 1 \) mutations acquires an additional mutation before dividing, two new cells, each with \( i \) mutations, are generated. This
occurs at a rate of \( d \), and, consequently, \( P_{\text{sym}} \) increases at twice this rate. The equations governing the system are

\[
\dot{P}_{0,\text{sym}} = (-d_0 + d_{\text{sym}}(1-2p))P_{0,\text{sym}}
\]

(1)

\[
\dot{P}_{i,\text{sym}} = (-d_i + d_{\text{sym}}(1-2p))P_{i,\text{sym}} + 2d_{\text{sym}}P_{i-1,\text{sym}},
\]

(2)

Here the dot represents differentiation with respect to time.

In steady state the left hand side vanishes, turning (1) into an expression for the steady state damage level \( d_{\text{sym}} \), which may be inserted into (2) to yield a recurrence relation for the steady state.

\[
d_{\text{sym}} = \frac{d_0}{1-2p}
\]

(3)

The normalized solution to (4) is a Poisson distribution with mean \( \lambda_{\text{sym}} \).

\[
P_{i,\text{sym}} = \frac{\lambda_{\text{sym}}^i}{i!} \exp(-\lambda_{\text{sym}})
\]

(5)

\[
\lambda_{\text{sym}} = \frac{2p}{1-2p} \frac{d_0}{\Delta}
\]

(6)

From (3) it is evident that, for \( p < \frac{d_0}{1-2p} \), the steady state value \( d_{\text{sym}} \) only takes values between 0 and 1. This is in agreement with the observations in figure 2b.
Asymmetric Segregation of Cellular Components

\[ P_k^\text{asym} = (-d_k + d_k^\text{asym}(1 - p))P_k^\text{asym} + d_k^\text{asym}P_{k-1}^\text{asym}. \]  

As before, demanding that the time derivatives vanishes, yields a steady state damage level of \( d_{\text{asym}}^* \) and a Poisson distribution for the mean number of mutational increments per cell \( \lambda_{\text{asym}} \):

\[ d_{\text{asym}}^* = \frac{d_0}{1 - p} \]  

\[ \lambda_{\text{asym}} = \frac{p}{1 - p} \frac{d_0}{\Delta} \]  

\[ P_k^\text{asym} = \frac{\lambda_{\text{asym}}^k}{k!} \exp(-\lambda_{\text{asym}}). \]

From the analytical treatment of the model, the damage levels at steady state are \( d_{\text{sym}}^* = d_0 + \lambda_{\text{sym}} \Delta \) and \( d_{\text{asym}}^* = d_0 + 2\lambda_{\text{asym}} \Delta \). As seen in figure 2a, these values are in agreement with the results of the computational simulation.

From (3) and (9) it is evident that \( d_{\text{sym}}^* > d_{\text{asym}}^* \) for all parameter values. This implies that asymmetric segregation of damage is a more effective mechanism for reducing the amount of mtDNA damage accumulated in the population, than symmetric division.

The mean number of cells with the initial damage level is given by \( N_0^\text{sym} = N \cdot \exp(-\lambda_{\text{sym}}) \) and \( N_0^\text{asym} = N \cdot \exp(-\lambda_{\text{asym}}) \). Since \( \lambda_{\text{sym}} > \lambda_{\text{asym}} \), cell populations that partition damage have a larger proportion of unmutated cells at steady state and consequently \( \tau^\text{asym} > \tau^\text{sym} \) for the same set of parameters. Asymmetric segregation of damage thus increases the longevity of the population.

When the number of cells with the initial damage level fluctuates down to zero, a new steady state can be identified using the substitutions \( d_0 \rightarrow d_0 + \Delta \) and \( d_0 \rightarrow d_0 + 2\Delta \) for the symmetric and the asymmetric system, respectively. However, this increases the mean number of mutations, making these new steady states less stable. This explains the rapid collapse of the system observed in figure 2a.

Discussion

The mathematical model presented here demonstrates that the asymmetric distribution of damage reduces the overall damage level and increases the longevity of a population of cells in both spatially structured and unstructured systems. We have shown analytically that these results hold for all parameter values, implying that damage segregation, in this model, is always advantageous. This is in agreement with several computational studies published recently [11,18–21].

In their model, Erjavec and colleagues (2008) assume that damaged proteins have no intrinsic toxicity but that their accumulation prolongs the time it takes for the cell to acquire the critical number of intact proteins required for cytokinesis. Given that condition, damage partitioning enhances the popula-
tion fitness of a unicellular organism and allows the lineage to withstand higher levels of damage before system collapse for all damage rates analysed.

The work by Ackermann and colleagues (2007) demonstrates that the advantage of segregating damage depends on how the damage accumulated in a cell affects survival and reproduction. Their results indicate that asymmetry is selected for evolutionarily if asymmetric phenotypes have at least the same expected number of surviving progeny as symmetric phenotypes.

In the model presented here we have assumed that the probability of a cell going apoptotic is proportional to its damage level, which corresponds to the case of a linear survival function in [11] and our results are therefore in agreement. This form of the survival function has been validated experimentally in the bacterium Caulobacter crescentus [11]. Furthermore, even though Escherichia coli reproduces by morphologically symmetric division, studies have found that the cell inheriting old components exhibits a decreased reproductive output and an increased probability of apoptosis [14].

An important distinction between our work and that of [18] and [11] is that we consider cells that are not rejuvenated at cell division. Rather, each daughter cell receives an amount of damage at least equal to that of the predivisional cell. This makes the model applicable to defective mitochondrial DNA and other cellular components known to be duplicated during cell division. If damage is diluted at division - either by the repair of damage or the production of undamaged copies - a steady state similar to the one described here will occur when dilution balances the rate at which new damage is accumulated. In this case, the steady state will be permanent and the longevity of the cell population will no longer be finite.

The models presented in [11,18–20] describe a population of unicellular organisms and the focus is therefore on how damage segregation affects the growth rate. Our model, on the other hand, considers somatic cells within a tissue and hence the population size is kept constant.

Mechanisms for actively generating damage asymmetry are well established in several organisms [31] and these mechanisms appear to be able to accommodate changes in environmental conditions that affect the damage load cells are exposed to. In budding yeast, increasing the levels of oxidatively damaged proteins increases the damage asymmetry between the mother and daughter cell [10]. Furthermore, a mutant strain of budding yeast, characterized by the inability to segregate functioning mitochondria preferentially to the daughter cell, suffered an extensive loss of viability resulting in clonal senescence [8]. This is consistent with the expectations of the model presented here.

As well as damage asymmetry, the process of cell division may in itself be important for stabilizing the population. In mammalian cells, defective mitochondria have been shown to accumulate to a greater extent in post-mitotic tissues, such as brain and muscle, than in dividing tissue [32–34].

Our investigation of the effect of spatial structure was motivated by experimental reports of the asymmetric distribution of damage in dividing stem cells [13,16]. The result that segregation reduces the level of damage accumulated in spatially structured cell populations suggests that it could be a strategy for reducing stem cell ageing even for cells that divide symmetrically with respect to cell fate.

The model presented here extends the theoretical framework for investigating the potential benefits of damage partitioning to multicellular organisms and spatially structured systems. Our results indicate that the asymmetric distribution of damage - and in particular the segregation of defective mitochondria - is advantageous in both spatially structured and unstructured systems for all parameter values. Damage segregation might be a common strategy even for somatic cells that are not rejuvenated at cell division.

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Author Contributions

Analyzed the data: CS JJ KB. Contributed reagents/materials/analysis tools: CS JJ KB. Wrote the paper: CS JJ KB.

References

1. Wallace D (1999) Mitochondrial diseases in man and mouse. Science 283: 1482–1488.

2. Neumüller R, Knoblich J (2009) Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. Genes & development 23: 2675–2699.

3. Klinger H, Rinnerthaler M, Lam Y, Laun P, Heeren G, et al. (2010) Quantification of (a) symmetric inheritance of functional and of oxidatively damaged mitochondrial aconitase in the cell division of old yeast mother cells. Experimental gerontology 45: 533–542.

4. Passos J, Saretzki G, Alhoed S, Nelson G, Richter T, et al. (2007) Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. PLoS biology 5: e110.

5. McElhinny-Figueroa JR, Veeva J, Swaye TC, Zhou C, Liu C, et al. (2011) Mitochondrial quality control during inheritance is associated with lifespan and mother–daughter age asymmetry in budding yeast. Aging cell 10: 883–893.

6. Erjavec N, Larsson I, Grantham J, Nystrom T (2007) Accelerated aging and failure to segregate damaged proteins in mtΔ cells can be suppressed by overproducing the protein aggregation-remodeling factor hsp104p. Genes & development 21: 2410–2421.

7. Roux A, Charrad P, Ferbeyre G, Rokach L (2010) Fusion yeast and other yeasts as emergent models to unravel cellular aging in eukaryotes. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 65: 1–13.

8. Lai C, Jaruga E, Borghouts C, Jazwinski S (2002) A mutation in the atp2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae. Cell motility and the cytoskeleton 37: 199–210.

9. Sinclair D, Guarente L (1997) Extrachromosomal rDNA circles—a cause of aging. Cell 91: 1033–1042.

10. Aguilaniu H, Gustafsson I, Rigoulet M, Nystrom T (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science 299: 1751–1753.
22. Li L, Xie T (2005) Stem cell niche: structure and function. Annu Rev Cell Dev Biol 21: 605–631.
23. Pepling M, Spradling A (2001) Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. Developmental biology 234: 339–351.
24. Mantel C, Messina-Graham S, Broxmeyer H (2010) Upregulation of nascent mitochondrial biogenesis in mouse hematopoietic stem cells parallels upregulation of cd34 and loss of pluripotency: A potential strategy for reducing oxidative risk in stem cells. Cell cycle (Georgetown, Tex) 9: 2008.
25. Kukat C, Wurm CA, Spähr H, Falkenberg M, Larsson NG, et al. (2011) Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. Proceedings of the National Academy of Sciences 108: 13534–13539.
26. Kowald A, Kirkwood T (2000) Accumulation of defective mitochondria through delayed degradation of damaged organelles and its possible role in the ageing of post-mitotic and dividing cells. Journal of theoretical biology 202: 145–160.
27. Twig G, Elezza A, Molina A, Mohamed H, Wikstrom J, et al. (2000) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. The EMBO journal 27: 433–446.
28. Gilkerson RW, Schon EA, Hernandez E, Davidson MM (2008) Mitochondrial nucleoids maintain genetic autonomy but allow for functional complementation. The Journal of cell biology 181: 1117–1128.
29. Spellman JN (2010) Functional organization of mammalian mitochondrial dna in nucleoids: history, recent developments, and future challenges. IUBMB life 62: 19–32.
30. Bendtsen K, Juhl J, Trusina A (2012) Fragile dna repair mechanism reduces ageing in multicellular model. PloS one 7: e36018.
31. Ouellet J, Barral Y (2012) Organelle segregation during mitosis: Lessons from asymmetrically dividing cells. The Journal of Cell Biology 196: 305–313.
32. Cortopassi G, Shibata D, Soong N, Aruheim N (1992) A pattern of accumulation of a somatic deletion of mitochondrial dna in aging human tissues. Proceedings of the National Academy of Sciences 89: 7370.
33. Lee H, Pang C, Hsu H, Wei Y, et al. (1994) Differential accumulations of 4,977 bp deletion in mitochondrial dna of various tissues in human ageing. Biochimica et biophysica acta 1226: 37.
34. Liu V, Zhang C, Nagley P (1998) Mutations in mitochondrial dna accumulate differentially in three different human tissues during ageing. Nucleic acids research 26: 1268–1275.