Aging and stress

The role of the environment in cellular replication

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In single cell organisms, such as yeast and bacteria, cells age as they divide – a clock where time is counted by consecutive cell division events. Aging is characterized by a decrease in replicative fitness, which correlates with the increased probability of death. Although both in prokaryotes and eukaryotes asymmetries at cell division define the identity of the aging cell, setting the temporal and causative order of aging events, an important question has remained unsolved: how does the environment influence aging? It has been reported that under favourable conditions, cells avoid aging or age slowly, while under stress, aging is triggered or accelerated. Stress-induced changes in division morphology or damage segregation might modulate the rate of aging. This article explores the connection between aging and stress in unicellular organisms, highlighting evolutionarily conserved features.

Replicative aging and damage accumulation: segregation eliminates trash the cells cannot recycle

Unicellular organisms have a very simple mission: at each cell cycle they have to produce a copy of themselves, that itself has to retain the ability to replicate. However, this implies harvesting energy and nutrients from the environment, in order for complex enzymatic machinery to synthesize vital cell components (mitochondria, ribosomes, DNA). Then, a cell has to segregate those components to ensure each daughter cell has the minimum required set to grow and replicate. During this process errors accumulate and damaged mitochondria, ribosomes and proteins contribute to a decrease in the ability to replicate – the definition of cellular aging. So how do cells deal with damage they cannot repair? One solution is to split damage equally between cells at division, so each cell has approximately half of the damaged molecules present in the mother cell (Figure 1A). While splitting the damage equally seems to preclude aging if the rate of damage accumulation is low, under stressful environments, where damage accumulates faster, it can lead to aging and death (Figure 1B). Another solution is to segregate the damage to one of the cells at division, thus generating a cell clear of damage at every division (Figure 1C). Here, the cell that accumulates damage ages and dies, but at each division a clean cell which can withstand more damage is born. However, asymmetric segregation might also be insufficient in the presence of stress, either because stress interferes directly with the segregation mechanism, or because the damage accumulation is faster than the ability of the cell to segregate it efficiently (Figure 1D). In support of this, theoretical models predict that under high stress, asymmetric damage segregation is optimal, while under low stress, splitting damage equally between daughter cells is better[1].

A puzzling paradox is that while cells have evolved stress-response mechanisms (heat, osmotic, nutrient) to manage transiently high levels of damage, they seem unable to cope with a slow accumulation of damage during aging, which becomes lethal. Is it because this slow accumulating damage is undetected by the stress response pathways (Hsf1, Msn2/4, etc.) or due to its specific biochemical nature? A mild stress can in some cases delay aging (as is the case for nutrient depletion, or chemical stress) which indicates that the stress response machinery has the ability to clear age-related damage. In order to understand how aging is modulated by the growth environment and stress, in the following sections I will discuss specific examples from distinct unicellular organisms that shed light into these questions.

The effect of growth environment on replicative aging

The symmetry of division, which largely determines the mode of damage segregation is a key determinant of aging. Nonetheless, differences in growth conditions can also influence aging – sometimes very acutely. The following examples represent prokaryotic and eukaryotic organisms with different types of cell division.

Abbreviations: ERC, endonuclear-replicative circle; Hsf1, heat shock factor 1; Msn2/4, Stress response transcription factor; pma1, plasma membrane ATPase; TOR, target of rapamycin; sch9, S6 protein kinase; Sir2, sirtuin 2 transcription factor.

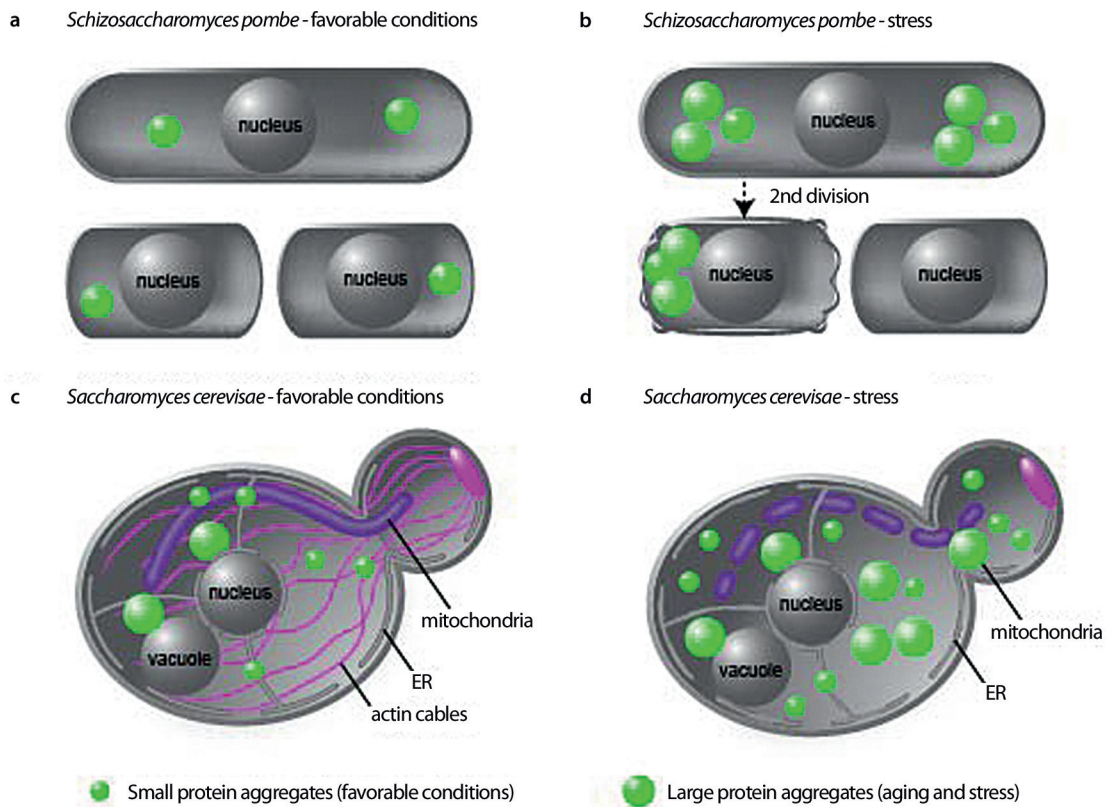

Figure 1. Stress influences damage segregation and aging in different unicellular organisms. (A) Scheme representing a symmetrically dividing cell under favourable conditions, where the damage is split between both daughter cells. Aging is not present. (B) During stress, damage fuses together and the symmetrically dividing cell that retains the majority of the damage after two divisions ages and dies, while its sister is born clean. (C) Scheme representing an asymmetrically dividing cell, where the damage is retained in the aging mother cell, by tethering to cell components. The daughter cell is born free of damage. (D) During stress, the asymmetrically dividing cell loses its ability to retain damage, contributing to the premature aging of the daughter cell.

In asymmetrically dividing prokaryotes, such as *Caulobacter crescentus*, the stalked mother cell divides to give rise to a daughter swarmer cell[2]. As the stalked cell divides, its replicative time increases, and the cell eventually stops dividing. Although the total lifespan of stalked cells is hard to quantify (at 130 generations imaging was stopped), experiments have reported different rates of decline in reproductive output, which might depend on temperature or imaging conditions. It is likely that the stalked mother cell accumulates damaged molecules, such as aggregated proteins which contribute to the decline in replicative fitness.

In symmetrically dividing prokaryotes, such as *Escherichia coli*, it was reported that when grown to form a microcolony in a solid substrate, the cell that inherits the old cell pole or an inclusion body composed of aggregated proteins exhibits a consecutive decrease in growth rate and death over 10 generations[3]. Later it was shown that when *E. coli* grow in liquid media in microfluidic channels, the cell that inherits the old pole does not exhibit a decrease in growth rate for 200 generations, although there is a periodic formation of filamentous growth[4]. It is possible that a solid, non-renewable media where cells are growing together in a microcolony is more stressful and contributes to generate more damage and aging[5], while a renewable liquid media delays damage accumulation and prevents aging. Along these lines, it would be interesting to quantify the accumulation of protein aggregates in cells grown inside microfluidic channels, to determine if the difference in aging is a direct consequence of slower damage accumulation.

For symmetrically dividing eukaryotes, such as the fission yeast *Schizosaccharomyces pombe*, previous reports have indicated that upon micromanipulation in solid media, *S. pombe* cells that inherited the old pole would lose its rod-shape morphology and stop dividing after 12–15 divisions[14]. Recently, it was shown that when *S. pombe* cells are grown in microcolonies (seven generations) or the cell that retains the old pole is followed for up to 30 divisions using micromanipulation, there is no cumulative increase

in division time or increased probability of death[6]. These contradictory reports might result from differences in growth conditions, since *S. pombe* cells exposed to oxidative or heat stress exhibit aging: cells that inherit a large amount of damage exhibit a cumulative increase in division time and a higher probability of death (over 4 divisions)[6] .

After a pioneering study that demonstrated that asymmetrically dividing eukaryotic budding yeast cells have a finite lifespan (25 divisions)[7], several groups studied replicative aging of *Saccharomyces cerevisiae* and identified a hierarchy of mechanisms: first, the asymmetric retention of a proton pump (pma1) allows the vacuole to re-acidify in the daughter – resetting the aging clock[8]. Then, as pma1 builds up in the cell membrane, the progressive increase in cytosolic pH results in loss of vacuolar and mitochondrial function, which correlates with the accumulation of ERCs, old vacuoles, damaged mitochondria and later aggregated proteins in the mother. In general, mutants that rescue or slow down the decline of these cell components, increase the lifespan. Since some of these aging genes (Sir2, Tor1, Sch9) are related to cell growth and stress, it is likely, but remains to be tested in detail, that pH, oxidative and temperature stresses affect the rate of aging in this organism.

Growth stress drives cellular aging: common principles and mechanisms?

The extent to which the environment can influence aging depends on the cell division strategy: (1) cells that divide morphologically asymmetrically and constitutively segregate damage to the aging "mother" cell (Figure 1C) and (2) cells that divide morphologically symmetrically and segregate damage stochastically (Figure 1A). Strategy (1) seems to concentrate damage in a few cells in the population and ensure the progeny is clear of damage, creating heterogeneity in damage content and strategy (2) simply distributes the damage approximately equally among all individuals, which all have the same age. While for (1) the growth environment can delay or accelerate aging, it cannot preclude it – the presence of a pre-determined aging program is defined by the asymmetry in division. However, this strategy might be better suited to resist stress. For (2) it is possible that the environment prevents aging, or slows it down to undetectable rates, which would be optimal in favourable environments.

A role for stress in modulating asymmetries at cell division

Stress can increase the total amount of damage, but also its segregation. In S. cerevisiae, following heat-shock, there is an accumulation of aggregated proteins in the

Division type	Organism	Ageing marker	Environment/lifespan	Ageing phenotype	References
Budding/ asymmetric	*C. crescentus* (prokaryote)	Stalked cell	Liquid media in microfluidic device	Decrease in reproductive output 6.7×10^{-4} gen/h, 2003 3.3×10^{-4} gen/h, 2007	Ackermann, 2003[2]; Ackermann 2007[12]
	S. cerevisiae (eukaryote)	Mother cell, scars, ERCs, protein aggregates, old mitochondria	Solid media	Sharp decrease in division time before death (24 gen, diploid)	Mortimer & Johnston, 1959[7]
			Liquid media in microfluidic device	Decrease in division time before death (23-26 gen, haploid)	Zhang et al, 2012[11]
Binary fission/ symmetric	*E. coli* (prokaryote)	Old pole, aggregate	Solid media	Decrease in normalized growth rate	Stewart et al, 2005[3]
			Liquid media in microfluidic device	No decrease in division time	Wang et al, 2010[4]
	Bacillus subtilis (prokaryote)	Old pole	Solid media	Decrease in normalized growth rate	Veening et al, 2008[13]
	S. pombe (eukaryote)	Protein aggregates	Favorable growth in solid media	No decrease in division time	Coelho et al, 2013[6]
		Old cell pole/ Protein aggregates	Stress in solid media	Decrease in division time and increase in death	Barker & Walmsley, 1999[14]; Coelho et al, 2013[6]

Table 1: Aging in different unicellular organisms and its dependency on the growth environment

mother and also in the daughter cell, and simultaneously the disruption of structures, such as the polarisome and actin cables, or mitochondria, that tether these aggregates and prevent their passage to daughter cells[9] (Figure 1D). This alone can contribute to an acceleration of the aging of the mother cell, but also to the leakage of damaged molecules that will create an "older" daughter (Figure 1D). In fact, similar to what occurs during stress and in mutants that fail to segregate damage, very old mother cells are unable to generate daughter cells that have the full replicative potential. In *S. pombe* cells, the stress generates a large amount of protein aggregates, which over the course of two cell divisions, fuse together into a single large aggregate, generating a cell clean of damage[10] (Figure 1B). Depending on the intensity or type of the stress and the total number of aggregates generated, this asymmetry in the distribution of damage is more pronounced. The cells that inherit the larger portion of the aggregated proteins usually exhibit aging and die. A similar conserved mechanism might justify different aging rates in *E. coli* – under stress, the rapid accumulation of protein aggregates at the cell pole might contribute to aging[3,5], while under more favourable growth conditions, these aggregates are successfully diluted between sister cells, precluding aging[4]. Other unicellular organisms might even change their division morphology in response to stress. The investigation of the mechanisms of damage segregation in other cell types and under different growth conditions might reveal a more general relationship between damage segregation, aging and stress (Table 1).

A role for aging in establishing different strategies to respond to stress

It is hard to conceive aging evolving as a beneficial trait, especially at the single cell-level. If a cell divides twice before it ages and dies, it will successfully grow as a population to colonize an environment, supporting evolutionary antagonistic pleiotropy. Nonetheless, aging contributes to heterogeneity: age-stratified populations, such as *S. cerevisiae* cells, explore a heterogeneous phenotypic space in a specific environment. Besides age-related differences in cell cycle duration, and amount or biochemical composition of cell components, very young cells are sensitive to pH stress, while young mothers are stress resistant and older cells more competent to become quiescent, or perform meiosis and sporulate. In a similar way, old or filamentous growing and slowly dividing *E. coli* cells can resist antibiotics and re-colonize environments, a phenotype that is clinically relevant. It is also likely that, even in non-aging cells growing under favourable conditions, such as *S. pombe*, differences in the amount of damage contribute to stress resistance or survival in the absence of nutrients. Therefore, aging might have evolved as a stress-response at the population level.

Future directions in aging–environment research

To better understand the relationship between aging and the environment (temperature, pH, nutrients, osmotic pressure) one should combine advanced microfluidic-imaging and automated image analysis[3,11], which allow us to track aging cells for many generations. In these experiments, we could also compare the effect of transient, versus prolonged stress, by measuring the decline in normalized growth rate/division time, the average lifespan and the increase in death probability in aging cells in a robust way. Comparing these variables we can understand how aging changes with the environment composition for a given organism and which changes are conserved between species. The objective of such an experiment is to define evolutionarily conserved rules for how the rate of aging changes with stress – is there a hill with an optimum in all three variables, or different aspects of aging are maximized in distinct environments? It would also be interesting to see how long-lived mutants respond to stress – is there a trade-off between stress response and longevity? Complementarily, to go from microscopy to genetics and biochemistry of aged cells, we need to expand the scale of purification methods, such as the mother-enrichment program or biotin-label separation. Also, cultivating organisms from distinct ecological niches in laboratory conditions might reveal the influence of ecology on aging, and how plastic aging in these organisms is once the growth conditions change. ■

Miguel Coelho studied Biochemistry at the University of Lisbon, conducting his diploma work on ciliary biogenesis in Tetrahymena at the Gulbenkian Institute for Science. During his PhD at the Max-Planck Institute in Germany, Miguel discovered that fission yeast cells switch from a non-aging to an aging life-cycle depending on the growth environment, due to damage fusion and segregation at cell division. He is currently at the Molecular and Cellular Biology department at Harvard University studying the evolution of genetic instability in yeast. For more information on the author, please visit mcc150.wix.com/miguelcoelho or e-mail costacoelho@fas.harvard.edu.

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