Ageing Research Reviews 60 (2020) 101064

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Ancestral germen/soma distinction in microbes: Expanding the disposable soma theory of aging to all unicellular lineages

Jérôme Teulière[a], Debashish Bhattacharya[b], Eric Bapteste[a,*]

[a] Institut de Systématique, Evolution, Biodiversité (ISYEB), Sorbonne Université, CNRS, Museum National d'Histoire Naturelle, EPHE, Université des Antilles, Paris, France
[b] Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08901, USA

ARTICLE INFO

Keywords:
Unicellular
Germen/Soma
Aging
Rejuvenation
DNA replication
Asymmetric cell division
Disposable Soma Theory
Evolution
Epigenetics
Protists
Prokaryotes

ABSTRACT

Life has persisted for about 3.5 billion years (Gy) despite fluctuating environmental pressures and the aging and mortality of individuals. The disposable soma theory (DST) notoriously contributes to explain this persistence for lineages with a clear soma/germen distinction. Beyond such lineages however, the phylogenetic scope of application of the DST is less obvious. Typically, the DST is not expected to explain the survival of microbial species that comprise single-celled organisms apparently lacking a germen/soma distinction. Here, we present an evolutionary argument that generalizes the explanatory scope of DST to the entire microbial world and provides a novel characterization of the deep molecular and evolutionary roots supporting this expanded disposable soma theory of aging. Specifically, we argue that the germen/soma distinction arose early in evolution and identify DNA semi-conservative replication as a critical process through which two forms of rejuvenation could have evolved in the first microbes. Our hypothesis has fundamental and practical implications. First, whereas unicellular organisms were long thought of as potentially immortal, we suggest instead that all unicellular individuals (prokaryotes or protists alike) are very likely to age, either replicatively or physiologically, or both. Second, our theory introduces a profound reconsideration of microbial individuality, whereby, all microbial individuals, as seen by natural selection, present an obligate transient germen/soma distinction during their life cycles. Third, our work promotes the study of cellular division in prokaryotes and in protist mitosis to illuminate the evolutionary origin of the soma and germen division, traditionally studied in animals. These ideas set the stage for progress in the evolutionary theory of aging from a heretofore overlooked microbial perspective.

1. Introduction

Individuals of all species die, yet Life has persisted since it appeared about 3.5 Gy ago. Thus, organismal lineages survive beyond the lifespan of individuals. Consequently, Life has evolved processes (typically by introducing selectable genetic variation at each generation) that allows it to persist despite fluctuating environmental pressures and individual mortality. This fundamental persistence of Life has evolutionarily ancient and biologically deep roots. In animals, the general functional division between germen and soma potentiates such lineage immortality despite organismal mortality (Kirkwood and Holliday, 1979). Whereas the soma is disposable post-reproduction, the replicative ability of the germ-line is potentially unlimited (Kirkwood and Holliday, 1979). The nature of molecular mechanisms that render the germen replicatively immortal is however not well understood (Rando, 2006). Yet, the prominent disposable soma theory of aging (DST) can be invoked to explain why the soma physiologically ages and why ultimately all these multicellular individuals die (Kirkwood and Holliday, 1979). Genetic determinism is likely involved in the evolutionarily restricted fate of animal somatic lineages, because the number of cell divisions of these cells is limited (Hayflick, 1965). That all somatic cells have limited replicative abilities is also known as replicative aging. If the DST illuminates how animal lineages persist in the face of aging, the phylogenetic scope of application of the DST becomes less obvious beyond lineages with a clear soma/germen distinction, in particular for unicellular organisms, naively commonly assumed to not exhibit senescence (Bell, 1984; Kirkwood, 2005). Yet, in some very well studied protists and prokaryotes, replicative aging and physiological aging (a progressive functional decline, notably in the ability of the organism to resist stress, damage and disease, during its lifespan (Jazwinski, 2002) have been characterized. These results suggest that, for these lineages as well, processes evolved that allow them to persist in the face of individual aging. More generally, one can ask whether a form of replicative aging might not also affect all microbial species

* Corresponding author.
E-mail address: eric.bapteste@upmc.fr (E. Bapteste).

https://doi.org/10.1016/j.arr.2020.101064
Received 10 February 2020; Received in revised form 12 March 2020; Accepted 31 March 2020
Available online 05 April 2020

(Florea, 2017). Because, at first blush, microbial species have the quasi-definitional property to be composed of single-celled organisms, single cell individuals should lack a germen/soma distinction which would argue against using DST to explain the survival of microbial lineages in the face of microbial aging. For an alternative view, see Nyström, (Nyström, 2002) who considers a trade-off between reproduction and survival activities in *E. coli* as a form of DST, in the absence of a germen/soma distinction. Here, instead we present an evolutionary argument that generalizes the explanatory scope of DST by demonstrating that all unicellular individuals are very likely to age, either replicatively or physiologically, or both, and that: i) no microbial individual (prokaryotes or protists alike) is therefore potentially immortal, but that, ii) all such individuals present an obligate transient germen/soma distinction during their life cycles. Thus, our work suggests that the germen/soma distinction arose early in evolution and further proposes that DNA semi-conservative replication was a critical process through which two forms of rejuvenation could have evolved in microbes and resulted in the evolution of immortalized microbial lineages. Thereby, we offer a novel characterization of the deep molecular and evolutionary roots for the disposable theory of aging, with two testable hypotheses that expand the scope of DST to the entire microbial world.

2. Microbial individuals aging and potential microbial lineage immortality

An initial, trivial explanation for the persistence of unicellular lineages is that all single-celled organisms are replicatively immortal and all microbes behave as typical germ-cells (Florea, 2017; Rose, 1994; Weismann, 1889; Williams, 1957). However, this analogy is difficult to support. Minimally, aging in unicellular organisms is expected to occur, in some cells at least, as a result of mutations, under the Mutation Accumulation hypothesis (Medawar, 1952; Partridge and Barton, 1993). The emblematic work by Medawar predicts that for purely demographic reasons, chronologically older individuals (here, cells) in a replicating population are irremediably subject to weaker natural selection than younger individuals. Therefore, the oldest cells accumulate detrimental, and ultimately evolutionary lethal mutations (Medawar, 1952). Therefore, to explore our idea that all microbial individuals age, we will first explore the extreme situation that all single cells are potentially limited in their replicative abilities or, in other words, that all single-celled organisms behave as somatic cells.

If universal replicative aging were the case for all microbes, all lineages of unicellular organisms would go extinct when the replicative limit of their constitutive cells was reached. A microbial lineage would only escape extinction if some of its cells could somehow rejuvenate; i.e., if some of these cells were able to reset their replicative age. In this extreme model of universal replicative aging ('all microbes are like somatic cells'), the additional possibility of physiological aging (heritable or not) would further imply that the terminal cells having undergone the last possible cellular division possible under replicative aging, not only could not evolve, but also could not persist forever. In this expanded model (replicative and physiological aging in early microbes), microbial cells would then have needed at the very least to counterbalance their physiological aging after the terminal division that produces these cells to counteract lineage extinction.

The idea that all unicellular organisms age physiologically has not yet been tested (Florea, 2017), but it is in principle testable, using time-series analyses of transcriptomes and of metabolomes from synchronized microbial populations, or by tracking the physiological fate of cells over time using cell sorting approaches. Physiological aging has already been reported for some unicellular taxa using these techniques (Egilmez and Jazwinski, 1989; Janssens et al., 2015; Lesur and Campbell, 2004; Lin et al., 2001; Yiu et al., 2008). For microbes, it may even be the case that physiological aging and replicative aging interfere with one another; e.g., with replicative aging leading to physiological aging (Kennedy et al., 1994), so that the more realistic starting point to investigate the evolution of microbial lineage immortalization would be the refined model in which all microbes age both replicatively and physiologically. Nonetheless, irrespectively of whether physiological aging and replicative aging interfere, replicative aging without rejuvenation is an obvious evolutionary dead end. Therefore, our argument will primarily focus on replicative aging, although we will specify when the analysis of our basic and refined models also introduces new knowledge with respect to the evolution of physiological aging.

3. An evolutionary connection between aging and rejuvenation

Assuming that all single-cells age replicatively (not only extant taxa, but also the first unicellular lineages on Earth), one must expect the evolution of some form of rejuvenation in at least some (if not all) of these cells to prevent lineage extinction. This view on rejuvenation is consistent with the idea that if aging is a byproduct of natural selection, longevity regulation, hereby rejuvenation, is a highly adaptive trait (Kirkwood, 1992).

In theory, rejuvenation could take place according to at least four temporalities during the lifespan of any single cell. First, rejuvenation could occur randomly at any time. Such a random process would be wasteful from an evolutionary point of view, because rejuvenating young, functional cells, early in their replicative or physiological age stages, seems to be a costly, unnecessary investment. Second, rejuvenation could occur constantly. Again, this possibility seems extremely costly, and not robust to cheaters. More fundamentally, a constant fight against replicative aging would necessarily involve some fortunate past clade selection (Doolittle, 2017) or some strong kin selection, because natural selection does not predict the future, beyond one individual generation. Yet, the imminent termination of replication by replicative aging, by definition, only concerns the last dividing generation of cells. Therefore, a strong selection for constant rejuvenation against replicative aging seems implausible. In contrast, physiological aging could be constantly counteracted (Gensler and Bernstein, 1981), but empirical evidence indicates that constant repair cannot fix all physiological issues arising during a cell lifespan (Łapińska et al., 2019). Thus, this constant fight could only be lost during an individual lifespan, which suggests that some trade-off would evolve to limit the investment to prevent or lessen physiological aging (Nyström, 2002). Third, rejuvenation could theoretically occur at a critical time during the life of cells, or at a critical time with respect to their population dynamics (if kin selection was involved). For example, cells could rejuvenate against replicative aging (or against physiological aging) upon reception of some inner signals reflecting their own physiological status or their own replicative age or, at a population level, upon reception of a density-dependent signal; e.g., prompting rejuvenation of the replicative or physiological age in case of critically low population density. Whereas well-coordinated and rapid periods of optimization for rejuvenation could be favored by natural selection at the individual level or by kin selection at the population level, such signal-based processes and trade-offs would more likely be secondary adaptations, in the sense that they would require the preliminary establishment of the lineage replicative immortality to be provided with sufficient time to evolve. And needless to say, initiating rejuvenation, when population size is low and cells physiologically weak, would be a risky evolutionary strategy. Therefore, physiologically/ecologically triggered rejuvenation programs are unlikely to be universally shared by prokaryotes and protists, and they do not provide a decisive argument for universal aging (replicative, physiological or both) in unicellular species. To make our argument, we therefore consider the fourth situation when rejuvenation would be meaningful from an evolutionary perspective. Rejuvenation could occur at a critical time shared across all unicellular life: cell division.

In this case, two possibilities for the modes of rejuvenation would be compatible with the repeated observation that after 3.5 Gy of evolution,

microbial lineages are still thriving on Earth. First, rejuvenation during cell division could equally affect both daughter cells, producing, without biases regarding their age status, two rejuvenated daughter cells. This would correspond to the scenario where 'all cells behave as germ-cells' that we suggest is unlikely. Second, rejuvenation during cell division could be biased, in terms of restored/restricted replicative aging abilities, and impact only one of the two daughter cells. At each cell division, this biased cell division would result in the production of one (replicatively or physiologically) rejuvenated daughter cell and one older cell (in which replicative or physiological aging would not be counteracted).

A third possibility for rejuvenation during cell division is more difficult to reconcile with the observation that microbial lineages persist on the planet. It is the intermediate situation between complete biased and unbiased rejuvenation, with partial rejuvenation (likewise, biased or unbiased) in both daughter cells, with respect to the replicative (or physiological) age of their mother cells. Partial rejuvenation in daughter cells would only delay lineage extinction, because the new generation of microbes, if not critically replicatively or physiologically old, would nonetheless be older than the former generation. Such a mode of rejuvenation (by degree) during cell division could only be transient, and seems unlikely to have lasted over eons. Therefore, to explain the 3.5 Gy history of microbes, we are left with two likely modes of rejuvenation: biased or unbiased during cell division. Is there any evolutionary or biological argument to favor one over the other?

4. An evolutionary connection between rejuvenation and semi-conservative DNA replication

At the outset, the odds may seem to favor unbiased rejuvenation during cell division. Unbiased rejuvenation 'of all daughter cells' would directly optimize cells' fitness, whereas the biased rejuvenation mode would entail an immediate fitness cost for one of the two cells, with possible additional long-term fitness consequences in terms of replicative aging. This is because cells descended from increasingly replicatively 'older' cells may eventually fail to divide earlier than cells born from younger cells (Kennedy et al., 1994), which could lead to some form of clade selection (Doolittle, 2017) against the biased mode of rejuvenation and in favor of the unbiased mode of rejuvenation during cell division. Furthermore, because cell division superficially looks symmetrical, it is commonly assumed that unicellular organisms tend to produce identical daughter cells, which would reinforce the notion that, mechanistically, cell division is a "fair" process that could result in equally rejuvenated daughter cells. Under this naive (yet self-contradictory) model, once rejuvenation would have become coupled to cell division, all replicatively or physiologically aging cells would be able to overcome replicative or physiological aging at every cell division.

Yet, such a naive view, in which a 100 % somatic-like microbial world would turn into a 100 % germ-line like microbial world, is clearly challenged by the data. It cannot be universal. There are clear examples in which protists and prokaryotes, such as the yeasts *S. cerevisiae* (Barton, 1950; Janssens et al., 2015; Müller, 1985; Sinclair and Guarente, 1997) and *S. pombe* (Barker and Walmsley, 1999), and the bacteria *E. coli* (Dukan and Nyström, 1998) and *Caulobacter crescentus* (Ackermann, 2003), divide asymmetrically and age, both morphologically (Gammoudi et al., 2016), physiologically, and replicatively (Florea, 2017). For instance, at each asymmetric cell division, *S. cerevisiae* mother cells bud off a smaller newly formed daughter cell (Sinclair, 2002), whose cell wall is newly synthesized (Kennedy et al., 1994), and themselves grow larger, accumulate bud scars, divide more slowly, for about 20 times on average before becoming sterile and dying. Many causes of aging have been identified in yeast, from damages by ROS (Longo, 1999) to cytoplasmic senescence factors (Egilmez and Jazwinski, 1989; Kennedy et al., 1994), and increased gene dysregulation (Jazwinski, 2002; Sinclair, 2002). Likewise, when an *E. coli* cell seemingly divides symmetrically, one daughter cell receives the old cell pole, while the other daughter cell receives a new cell pole. This demonstrably induces a physiological asymmetry between the old pole cell and the new pole cell, because components of the cell wall with a long half-life and a limited diffusion are predicted to accumulate at the old pole, where they were formed (Stewart et al., 2005). Consistently, old pole cells show various evidence of aging: cumulatively slowed growth, reduced offspring biomass production and an increased probability of death with respect to new pole cells (Stewart et al., 2005). These counter-examples to universal rejuvenation through supposedly symmetrical division are not minor evolutionary exceptions. Rather, they highlight a fundamental property of life: no cell division can be symmetric at the level of the genetic material and consequently at the physically related epigenetic level. This obvious observation immediately makes symmetric, unbiased modes of cellular division unlikely to have been an evolutionary ancestral trait, whereas asymmetric, biased modes of cellular divisions have a greater likelihood of having been ancestral states. The evolution of an unbiased rejuvenation mode would therefore require cells to evolve a symmetric process of rejuvenation within the context of a universal genetically and epigenetically asymmetric cell division.

Why is there no such thing as symmetric cell division? Not because of unavoidable stochastic variation in the volume, cytoplasmic or membrane contents of daughter cells, but because of a fundamental, systematic and universal asymmetrical biological process, associated with cell division. Namely, DNA replication is semi-conservative. Thus, during each DNA replication, two neo-synthesized ('young') DNA strands are produced, each from a pre-existing ('old') template DNA strand. The DNA molecules to be inherited by each daughter cell are thus always asymmetrical: one DNA molecule contains a 3′-5′ 'old' template strand with a complementary 5′-3′ neo-synthesized strand, whereas the other DNA molecule contains a 5′-3′ 'old' template strand with a complementary 3′-5′ neo-synthesized strand. Whereas the genetic information (in terms of what is encoded) is the same in the DNA of the two daughter cells (because the daughter cells are clonal), the relative age and the nature of genetic material physically inherited differs between the daughter cells. This major difference entails that any epigenetic marks associated with a template 'old' DNA strand will not be inherited symmetrically in the two daughter cells. DNA methylation is evolutionarily conserved (Johnson et al., 2012) and present in most unicellular organisms, to the notable exception of some yeasts, including *S. cerevisiae*, which unquestionably age, yet have secondarily lost DNA methylation during evolution, and whose epigenetics relies on heterochromatin and telomeric silencing (Casadesus and Low, 2006; O'Kane and Hyland, 2019; Zemach et al., 2010). If early epigenetics indeed involved currently known forms of DNA methylations on Cytosine or on Adenine, it is possible that the unavoidable asymmetry between old and young DNA strands was also associated (and possibly further amplified) by some additional nucleotide compositional asymmetry. In particular, a bias in CpG islands composition in some genes, or in their promoter regions could have evolved along with ageing mechanisms, since CpG islands are a mutation hotspot, especially when cytosine is methylated. But because our scenario focuses on early life, by epigenetic marks here, we will consider as relevant, not specifically the diverse set of methylations currently known in some extant prokaryotes and protists (Blum and Payne, 2019; Casadesús, 2016; Casadesus and Low, 2006; Iyer et al., 2011), but more generally any sort of modification associated with a DNA strand, with potential genetic regulatory effects, that would be altered by the process of semi-conservative DNA replication. By this definition, both daughter cells will differ from their mother cell because: i) one-half (but not the same half) of their DNA material is new, and ii) potentially, one-half (and not the same half) of their epigenetics marks are lost. Moreover, the two daughter cells will also differ from one another because they will have retained different template DNA strands and potentially different sets of epigenetics marks. From a genetic (material) and an epigenetic point of

view, cellular division is thus fundamentally an asymmetrical process. And for life as we know it, it has always been so.

Such an asymmetrical process, resulting in a fundamental distinction between any pair of daughter cells may have had two distinct evolutionary outcomes. As noted by Kirkwood, 'wherever there is asymmetry, it is possible for labour to be divided unequally" (Kirkwood, 2005). Thus, either there was some evolutionary advantage to the universality of semi-conservative DNA replication regarding the coupling of rejuvenation against replicative or physiological aging with cellular division, or there was none. In the instance that semi-conservative DNA replication neither eased, nor directly contributed to rejuvenation, populations of early cells affected by replicative (or possibly physiological) aging would have been able to divide, but they would not have been able to counteract replicative (or possibly physiological) aging. The net result would be that these asymmetrically dividing yet non-rejuvenating populations would have gone extinct, unless they had evolved a form of rejuvenation during cell division that was independent of DNA replication. This evolution of independent rejuvenation programs would have to occur before the end of the race against time for dividing cells at the end of their replicative potential. There was no selective pressure to evolve anti-replicative aging rejuvenation before cell division existed. Furthermore, there is no such selective pressure before the final fatal round of cell division for a lineage of replicatively limited cells. Therefore, if there existed no direct biological connection between semi-conservative DNA replication and rejuvenation against replicative aging, rejuvenation should then be invented *de novo*, and separately from cell division, to prevent early life extinction. In the case of unbiased rejuvenation, as we noted above, the rejuvenation process should affect equally both daughter cells, in spite of their fundamental differences. Therefore, a more likely scenario is that asymmetrical cell division induced by semi-conservative DNA replication involves mechanisms that contribute to the rejuvenation of some cells among the earliest populations of unicellular organisms, maybe if initially, by degree.

If one wishes to speculate further on the origins of such an ancestral biased rejuvenation process, coupled with DNA replication, one might consider several alternative scenarios. For example, replicative or physiological aging may be reset by a dosage effect caused by the inheritance of a single template DNA strand in each daughter cell. Likewise, replicative or physiological aging may be prevented by the loss of 50 % of the epigenetic marks. More likely, we suggest that rather than a mere dosage effect, replicative or physiological aging may be reset by the inheritance of a specific template DNA strand, or by the loss of the epigenetic marks on a specific neo-synthetized DNA strand. Indeed, the asymmetry in daughter cells genetic and epigenetic content would then immediately translate into an asymmetry in replicative (or physiological) aging potential: one cell being rejuvenated, but not the other. It is even possible that the same asymmetrical division process, by introducing a distinction between the two daughter cells, may also have played an additional role in the evolution of rejuvenation to counteract physiological aging. The biased inheritance of dysfunctional proteins (Coelho et al., 2013; Lloyd-Price et al., 2012) or the biased segregation of mitochondria with damaged DNA (Lai et al., 2002; Okamoto et al., 1998; Takizawa et al., 2000), and/or of less functional (old) cellular poles (Shapiro et al., 2002; Stewart et al., 2005). during cell division in the non-rejuvenated daughter cell (the one holding the replicatively doomed DNA strand) would further improve the fitness of the other daughter cell, spared from replicative aging.

5. Early evolution of the germen/soma distinction in microbes

If this exaptive model in which semi-conservative DNA replication mechanistically combined with early rejuvenation is correct, three predictions can be made. First, a form of replicative or physiological aging and a corresponding rejuvenation process (likely based on asymmetrical DNA inheritance, possibly interfering with epigenetic regulation) is ancestral to all life. Because these two processes are so deeply engrained, and selection against rejuvenation is unlikely, derived forms of these ancestral processes likely still exist in unicellular organisms. If so, all unicellular species age replicatively, or potentially physiologically, or both, and they rejuvenate. The converse scenario, considering the Eden-like ancestral state where unicellular populations were comprised only of replicatively immortal cells dividing symmetrically (Partridge and Barton, 1993) seems highly unlikely. As noted above, symmetric cell division does not exist, at least for the genetic material, and its epigenetic marks. Ancestral cellular replicative immortality would mean that the yeast and *E. coli* lineages would have secondarily acquired replicative aging. Thus, 50 % of their cells would relinquish replicative immortality at every cell division, whereas the other 50 % would retain the ancestral ability, a major fitness loss that seems difficult to explain by natural selection. Moreover, physiological aging would also be expected in such immortal populations, due to mutation accumulation for purely demographic reasons (Medawar, 1952).

The second prediction of our model is that the distinction between cells with limited replicative abilities and rejuvenated, hence immortalized, cells evolved early in biological evolution and was coupled with cell division. This distinction in the fate of two daughter cells evokes the germen/soma distinction investigated in animals, in the sense that for two genetically clonal cells, one is doomed to decay (it acquires reduced replicative or physiological abilities), whereas the other thrives (it is rejuvenated). Consequently, the germen/soma distinction would be an extremely ancient way to guarantee lineage immortality. Yet, this prediction invites a profound rethink of the nature of microbial individuals (the ones seen by natural selection). From our perspective, the unbiased rejuvenation model, *a priori* considering the two daughter cells as *bona fide* independently selectable individuals, operates under a naive ontology of microbial individuals. This naive view ignores the possibility that microbes have a germen/soma division, because, although clonal within a population, each cell is *a priori* considered a *bona fide* evolutionary individual. Instead, in our model, the two clonal daughter cells constitute a transient temporal stage in the life cycle of a spatio-temporally broader microbial individual.

Such an ontological claim has already been considered. Guarente and Kenyon (Guarente and Kenyon, 2000), later followed by Lai et al. (Lai et al., 2002), proposed that, although yeasts seem 'to violate the rule of demarcation of soma and germ-line', their cell division resembles 'a renewal process in the budding of the daughter cells, from an aging, soma-like lineage of mother cells'. Our model generalizes this claim, because any microbe lifecycle critically involves at least one clonal reproduction, a stage at which, under our hypothesis, the real microbial individual seen by natural selection is transiently yet obligately composed of two functionally integrated clonal cells. The real microbial individual is thus in fact transiently multi-cellular, composed by the two daughter cells, one of which is acting as the germ-line, whereas the other acts as the somatic-line.

Simply put, microbial individuals are not really and strictly unicellular, one brief stage of their lifecycle is always transiently comprised of two clonal cells. Consequently, our third prediction is that the study of cellular division in prokaryotes and in mitotic protists should elucidate the evolutionary origin of the more canonical soma and germen division, traditionally studied in animals. For example, within unicellular species, the systematic single cell sequencing and comparison of the transcriptomes and of the epigenomes of each daughter cells, or potentially, of these single cell metabolomes, could unravel key aspects of what makes the two daughter cells distinct. Some of these transcriptomic and epigenetic differences between daughter cells may even be conserved across unicellular species, and be evolutionary related to the processes that make the germen distinct from soma in animals. Studying the germen/soma distinction with microbes also conveys additional advantages: it is likely technically less difficult to perform single cell studies on unicellular organisms, separated by

microfluidics, than it is to separately compare somatic and germ cells from animal lineages, which are difficult to isolate from one another.

Of note, in their life cycles, many protists experience an alternative life stage to mitotic cell division when they differentiate into gametes, as a result of meiosis. Although meiosis evolved later than mitosis, a temporality which may have allowed for possible additional evolutionary refinements regarding the interconnected processes of rejuvenation and aging in protists, the same fundamental universal pattern of asymmetric division also occurs in this process. Namely, meiosis involves semi-conservative DNA replication (during its first phase). Therefore, all four gametes produced during meiosis have their chromatids essentially composed of a template ('old') DNA strand and of a neo-synthesized DNA strand (to the possible exception of recombination caused by crossing-overs). Therefore, under our hypothesis that couples early forms of rejuvenation to semi-conservative DNA replication, some rejuvenation of physiological or of replicative aging would also be very likely to occur in the gametes of early meiotic protists. The fraction of the gametes rejuvenated in this manner would however depend on whether the rejuvenation of microbial individuals operates mostly by dosage effects (then all gametes would be rejuvenated by the presence of one neo-synthesized DNA strand and of one template strand) or by strand-specific mechanisms (in which case, more subtle outcomes could occur). Systematic epigenetic and transcriptomic studies of single protist gametes thus also appear as another research avenue where new data could contribute fundamental knowledge with respect to past and extant rejuvenation processes in unicellular eukaryotes.

6. Conclusion

Our goal was to explain the persistence of microbial life over billions of years, in the face of aging. Starting from a model where all cells do not replicatively, nor physiologically age (hence would be by all means immortal, since early Life) seemed contradicted by the data, and therefore was refuted as a plausible evolutionary path. Instead, we elaborated upon an initial model, where all cells were considered as replicatively aging (and potentially as physiologically aging). This first basic model led us to conclude that the evolution of transient microbial individuals with a necessary germen/soma distinction during cell division allows a form of rejuvenation that targets replicative aging. Microbial life would have then evolved by a contingent yet necessary process, selecting for individuals in which replicative aging, countered by a form of rejuvenation associated with semi-conservative DNA replication, would become confined to 'somatic' lineages while being reset in 'germen' cells. Other early microbial clades lacking this biological property would have simply gone extinct. But starting from a different model, where all cells do not necessarily replicatively age, yet would potentially all physiologically age, led to an analogous conclusion. Namely, this situation would also lead to the evolution of transient microbial individuals with a germen/soma distinction during cell division, except here, rejuvenation would target physiological (not replicative) aging. Physiologically older cells would be confined to the 'somatic' lineage, whereas physiological age would be reset in the germen cells. This second type of rejuvenation is all the more likely because it can be directly favored by natural selection at every generation of microbial individuals. In both cases, the universal asymmetry in DNA replication entailed a fundamental asymmetry in cell division, that appears as the fundamental biological process, as ancient as the first cells, that was critical for the survival of unicellular species, through an early germen/soma distinction. Therefore, with the possibility of a universal soma/germen distinction early in the history of Life comes the plausible hypothesis that all microbial species which descended from these early populations; i.e., Life, age, either replicatively, or physiologically, or both.

Our conclusions give rise to two testable hypotheses. The first and most fundamental hypothesis from an evolutionary perspective is that all extant microbial species age replicatively. For instance, this can be tested by sorting individual cells, after each division, and quantifying the proportion of these individual cells that fail to replicate after a certain number of divisions. If sorted cells from a given microbial species continue to replicate, the hypothesis of universal replicative aging in microbial species would be falsified. The second likely hypothesis supported by our model is that all microbial species physiologically age. This can also be tested, for example either by tracking the physiological and metabolic potential of individual microbial cells between cell divisions. Although it may still be currently technically challenging to achieve this at the scale of single cells, an approximation can be obtained by measuring the physiological and metabolic potential of synchronized populations of microbial cells between cell divisions, eventually by artificially blocking cell division. If some microbial cells or synchronized populations would maintain their physiologic and metabolic potential under such a protocol, the hypothesis of universal physiological aging across microbial species would be falsified. In summary, we hope our work will encourage such systematic analyses and set the stage for novel progress in the evolutionary theory of aging, from a microbial perspective.

Acknowledgements

The authors thanks F. Papale, C. Bernard, G. Bernard, P. Lopez and C. Lane for critical comments.

References

Ackermann, M., 2003. Senescence in a bacterium with asymmetric division. Science 300https://doi.org/10.1126/science.1083532. 1920–1920.
Barker, M.G., Walmsley, R.M., 1999. Replicative ageing in the fission yeast Schizosaccharomyces pombe. Yeast 15, 1511–1518 https://doi.org/10/d5phfv.
Barton, A.A., 1950. Some aspects of cell division in saccharomyces cerevisiae. J. Gen. Microbiol. 4, 84–86 https://doi.org/10/ddw26q.
Bell, G., 1984. Evolutionary and nonevolutionary theories of senescence. Am. Nat. 124, 600–603 https://doi.org/10/bz2mxx.
Blum, P., Payne, S., 2019. Evidence of an epigenetics system in Archaea. Genet. Epigenet. 12, 251686571986528 https://doi.org/10/gf6mpb.
Casadesús, J., 2016. Bacterial DNA methylation and methylomes. In: Jeltsch, A., Jurkowska, R.Z. (Eds.), DNA Methyltransferases - Role and Function. Springer International Publishing, Cham, pp. 35–61. https://doi.org/10.1007/978-3-319-43624-1_3.
Casadesus, J., Low, D., 2006. Epigenetic gene regulation in the bacterial world. Microbiol. Mol. Biol. Rev. 70, 830–856 https://doi.org/10/cj8bt7.
Coelho, M., Dereli, A., Haese, A., Kühn, S., Malinovska, L., DeSantis, M.E., Shorter, J., Alberti, S., Gross, T., Tolić-Nørrelykke, I.M., 2013. Fission yeast does not age under favorable conditions, but does so after stress. Curr. Biol. 23, 1844–1852 https://doi.org/10/f5f3qh.
Doolittle, W.F., 2017. Making the most of clade selection. Philos. Sci. 84, 275–295 https://doi.org/10/ggjbr4.
Dukan, S., Nyström, T., 1998. Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. Genes Dev. 12, 3431–3441 https://doi.org/10/b74mkx.
Egilmez, N.K., Jazwinski, S.M., 1989. Evidence for the involvement of a cytoplasmic factor in the aging of the yeast Saccharomyces cerevisiae. J. Bacteriol. 171, 37–42 https://doi.org/10/gfv99d.
Florea, M., 2017. Aging and immortality in unicellular species. Mech. Ageing Dev. 167, 5–15 https://doi.org/10/gcjhv7.
Gammoudi, I., Mathelie-guinlet, M., Morote, F., Beven, L., Moynet, D., Grauby-heywang, C., Cohen-bouhacina, T., 2016. Morphological and nanostructural surface changes in Escherichia coli over time, monitored by atomic force microscopy. Colloids Surf. B Biointerfaces 141, 355–364 https://doi.org/10/f8jbvj.
Gensler, H.L., Bernstein, H., 1981. DNA damage as the primary cause of aging. Q. Rev. Biol. 56, 279–303.
Guarente, L., Kenyon, C., 2000. Genetic pathways that regulate ageing in model organisms. Nature 408, 255–262 https://doi.org/10/c8srqs.
Hayflick, L., 1965. The limited in vitro lifetime of human diploid cell strains. Exp. Cell Res. 37, 614–636 https://doi.org/10/bfsfxn.
Iyer, L.M., Abhiman, S., Aravind, L., 2011. Natural history of eukaryotic DNA methylation systems. Progress in Molecular Biology and Translational Science. Elsevier, pp. 25–104. https://doi.org/10.1016/B978-0-12-387685-0.00002-0.
Janssens, G.E., Meinema, A.C., González, J., Wolters, J.C., Schmidt, A., Guryev, V., Bischoff, R., Wit, E.C., Veenhoff, L.M., Heinemann, M., 2015. Protein biogenesis machinery is a driver of replicative aging in yeast. eLife 4, e08527 https://doi.org/10/gfwc38.
Jazwinski, S.M., 2002. Growing old: metabolic control and yeast aging. Annu. Rev. Microbiol. 56, 769–792 https://doi.org/10/fjpmvm.

Johnson, A.A., Akman, K., Calimport, S.R.G., Wuttke, D., Stolzing, A., de Magalhães, J.P., 2012. The role of DNA methylation in aging, rejuvenation, and age-related disease. Rejuvenation Res. 15, 483–494 https://doi.org/10/f4dj9d.
Kennedy, B.K., Austriaco, N.R., Guarente, L., 1994. Daughter cells of Saccharomyces cerevisiae from old mothers display a reduced life span. J. Cell Biol. 127, 1985–1993 https://doi.org/10/bsjzb5.
Kirkwood, T.B., 1992. Comparative life spans of species: why do species have the life spans they do? Am. J. Clin. Nutr. 55, 1191S–1195S https://doi.org/10/ggh77j.
Kirkwood, T.B.L., 2005. Asymmetry and the origins of ageing. Mech. Ageing Dev. 126, 533–534 https://doi.org/10/bdj4mp.
Kirkwood, T., Holliday, R., 1979. The evolution of ageing and longevity. Proceedings of the Royal Society of London. Series B. Biol. Sci. 205, 531–546 https://doi.org/10/c7p9sb.
Lai, C.-Y., Jaruga, E., Borghouts, C., Jazwinski, S.M., 2002. A mutation in the ATP2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae. Genetics 162, 73.
Łapińska, U., Glover, G., Capilla-Lasheras, P., Young, A.J., Pagliara, S., 2019. Bacterial ageing in the absence of external stressors. Philos. Trans. R. Soc. Lond. B Biol. Sci. 374, 20180442 https://doi.org/10/gf9t85.
Lesur, I., Campbell, J.L., 2004. The transcriptome of prematurely aging yeast cells is similar to that of telomerase-deficient cells. Mol. Biol. Cell 15, 1297–1312 https://doi.org/10/fg6k79.
Lin, S.S., Manchester, J.K., Gordon, J.I., 2001. Enhanced gluconeogenesis and increased energy storage as hallmarks of aging in Saccharomyces cerevisiae. J. Biol. Chem. 276, 36000–36007 https://doi.org/10/frfx2v.
Lloyd-Price, J., Hakkinen, A., Kandhavelu, M., Marques, I.J., Chowdhury, S., Lihavainen, E., Yli-Harja, O., Ribeiro, A.S., 2012. Asymmetric disposal of individual protein aggregates in Escherichia coli, one aggregate at a time. J. Bacteriol. 194, 1747–1752 https://doi.org/10/fxknzm.
Longo, V.D., 1999. Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells. Neurobiol. Aging 20, 479–486 https://doi.org/10/fwrf9x.
Medawar, P.B., 1952. An unsolved problem of biology. College.
Müller, I., 1985. Parental age and the life-span of zygotes of Saccharomyces cerevisiae. Antonie Van Leeuwenhoek 51, 1–10 https://doi.org/10/dpskxx.
Nyström, T., 2002. Aging in bacteria. Curr. Opin. Microbiol. 5, 596–601 https://doi.org/10/btmmgx.
O'Kane, C.J., Hyland, E.M., 2019. Yeast epigenetics: the inheritance of histone modification states. Biosci. Rep. 39, BSR20182006 https://doi.org/10/ggnnsh.
Okamoto, K., Perlman, P.S., Butow, R.A., 1998. The sorting of mitochondrial DNA and mitochondrial proteins in zygotes: preferential transmission of mitochondrial DNA to the medial bud. J. Cell Biol. 142, 613–623 https://doi.org/10/chkm6b.
Partridge, L., Barton, N.H., 1993. Optimally, mutation and the evolution of ageing. Nature 362, 305–311 https://doi.org/10/fjd77g.
Rando, T.A., 2006. Stem cells, ageing and the quest for immortality. Nature 441, 1080–1086 https://doi.org/10/db5mz6.
Rose, M.R., 1994. Evolutionary Biology of Aging, 1. Paperback Issue. Ed. Oxford Univ. Press, New York.
Shapiro, L., McAdams, H.H., Losick, R., 2002. Generating and exploiting polarity in bacteria. Science 298, 1942–1946 https://doi.org/10/d3r7th.
Sinclair, D.A., 2002. Paradigms and pitfalls of yeast longevity research. Mech. Ageing Dev. 123, 857–867 https://doi.org/10/dm2nx7.
Sinclair, D.A., Guarente, L., 1997. Extrachromosomal rDNA circles— a cause of aging in yeast. Cell 91, 1033–1042 https://doi.org/10/dh7pxs.
Stewart, E.J., Madden, R., Paul, G., Taddei, F., 2005. Aging and death in an organism that reproduces by morphologically symmetric division. PLoS Biol. 3, e45 https://doi.org/10/dh77wt.
Takizawa, P.A., DeRisi, J.L., Wilhelm, J.E., Vale, R.D., 2000. Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. Science 290, 341–344 https://doi.org/10/dnbvrm.
Weismann, A.T., 1889. The Duration of Life (a Paper Presented in 1881). Essays upon Heredity and Kindred Biological Problems by Dr. August Weismann.
Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11, 398–411 https://doi.org/10/gft2dg.
Yiu, G., McCord, A., Wise, A., Jindal, R., Hardee, J., Kuo, A., Shimogawa, M.Y., Cahoon, L., Wu, M., Kloke, J., Hardin, J., Mays Hoopes, L.L., 2008. Pathways change in expression during replicative aging in Saccharomyces cerevisiae. J. Gerontol. A Biol. Sci. Med. Sci. 63, 21–34 https://doi.org/10/bkn8ht.
Zemach, A., McDaniel, I.E., Silva, P., Zilberman, D., 2010. Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science 328, 916–919 https://doi.org/10/cttqcv.