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Senescence: Even Bacteria Get Old

Cellular senescence, even in the presence of abundant nutrients, has now been demonstrated in several microbes, including most recently the bacterium *Escherichia coli*, suggesting that it may be a universal adaptive response to the inevitable damage to cell constituents that accumulates with time.

Craig Stephens

“Must not all things at the last be
swallowed up in death?”
Plato, *Dialogues*

The process of aging, and the apparent certainty of death, is as fascinating to scientists as to philosophers. Why do we grow old? We humans tend to view aging as a regrettable, but inevitable, process. Within the microcosm of our bodies, the vast majority of somatic cells divide infrequently and age fairly rapidly. The ‘disposable soma’ theory of aging postulates that senescence — the progressive, age-related decline in critical biological functions — is evolutionarily linked to the high cost of maintenance of functional cellular systems [1,2]. Only for relatively rare stem cells and the reproductive germ line are sufficient resources invested to avoid senescence while continuing to grow and divide. Unicellular microbes that reproduce solely through binary fission are analogous to the germ line of metazoans. It is perhaps surprising, therefore, that recent research [3,4] has revealed subpopulations of microbial cells that routinely age and die, even in the presence of abundant nutrients. Although mechanisms of microbial senescence are not yet clear, further investigation might yield insights relevant to aging in all life forms.

In the context of biology, the terms ‘aging’ and ‘senescence’ imply more than just the passage of time in the lifespan of an organism. With respect to microbes, the critical measure of aging would be a reduction in reproductive capacity — longer generation intervals resulting in fewer offspring produced as a function of time. Most work on microbial aging has been done with the eukaryotic budding yeast, *Saccharomyces cerevisiae* [5]. Cell division in budding yeast is asymmetric, with the larger mother cell easily distinguishable from the budded daughter cell. In 1959, Mortimer and Johnson [6] first examined the reproductive life span of individual *S. cerevisiae* cells. Using a micromanipulator, they physically removed the newly budded daughters after each division, recording the number and timing of each division event. The average mother cell underwent 24 divisions; senescence was evident during the last few divisions, which typically took considerably longer and were followed in most cases by the cells becoming granular and/or lysing (death).

Improvements in imaging technology now allow aging to be addressed experimentally in prokaryotes, which tend to be considerably smaller than yeast cells. *Caulobacter crescentus*, a bacterium studied intensively as a model for cellular development, is unusual (but not unique) among prokaryotes in that it undergoes asymmetric division [7]. One of the

cells possesses a stalk, a thin, tubular extension of the cell envelope that is adhesive, and serves to attach the cell to surfaces (Figure 1A). The anchored stalked cell acts like a stem cell, producing a slightly smaller ‘swarmer’ cell each generation that bears a polar flagellum, rather than a stalk. The motile swarmer leaves behind the stalked cell to search for new nutrients, but eventually sheds its flagellum and grows its own stalk, allowing it to attach to a new home and continue through the cell cycle.

Caulobacter offers an ideal opportunity to observe senescence, as the stalk cell naturally immobilizes itself, and the swarmer swims away on its own after every division. Ackermann *et al.* [4] carried out a simple experiment in which a flow chamber was prepared on the surface of a microscope slide. Stalked cells were allowed to attach to the glass surface, where they were fed and oxygenated by a gentle flow of nutrient medium. The cells were photographed at 10 minute intervals for several days. Time intervals between cell divisions were calculated and analyzed as a function of the age of the cell, to determine whether generation time increased — so that reproductive output decreased — as the stalked cells aged.

Over the course of roughly 100 generations, Ackermann *et al.* [4] observed that the mean generation time nearly doubled, from approximately 2.6 hours per generation, to over 5 hours per generation. To control for possible deleterious effects of the lengthy observation period, stalked cells arising during the experiment — from swarmers that occasionally persisted in the chamber long enough to differentiate — were also observed. Despite

descending from geriatric stalk cells whose reproductive output was already in decline, these new stalk cells divided as rapidly as the cells at the beginning of the experiment. Passing through the swarmer stage had apparently rejuvenated these cells!

For both *S. cerevisiae* and *C. crescentus*, division is overtly asymmetric. Could asymmetry actually be *necessary* for aging and senescence to occur, as some have argued [8]? To address this question, Stewart *et al.* [3] applied sophisticated cell imaging and tracking technology to determine whether senescence occurs in *Escherichia coli*, a rod-shaped bacterium that reproduces by binary fission. Underlying this experiment was the suspicion that even a superficially symmetrical division produces two distinct cell poles — an ‘old’ pole that is already present in the mother cell, and the ‘new’ pole that is formed during the division process (Figure 1B). When these offspring in turn divide, two of the second generation progeny inherit a pole that is now two generations old, along with a new pole. The other two second generation progeny will have a one-generation old pole, along with their new poles.

The *E. coli* cells were grown in microcolonies on an agarose surface so that they were relatively immobile. Under these conditions, cells with defined orientations — and hence poles with known ages — were tracked for up to nine generations. Time intervals were calculated between every division event, and correlated with the age of the cell poles. The striking conclusion was that aging of cell poles had significant deleterious consequences [3]. Cells inheriting two-generation old poles grew roughly 2% more slowly than the cells inheriting one-generation old poles, and the effects of polar aging were roughly additive each generation. During the time course captured in these experiments (6 hours), old pole cells as a group produced less offspring biomass (3%) than their brethren inheriting newer poles, and cells inheriting the oldest poles were more likely to completely stop growing. (This experiment did not attempt to

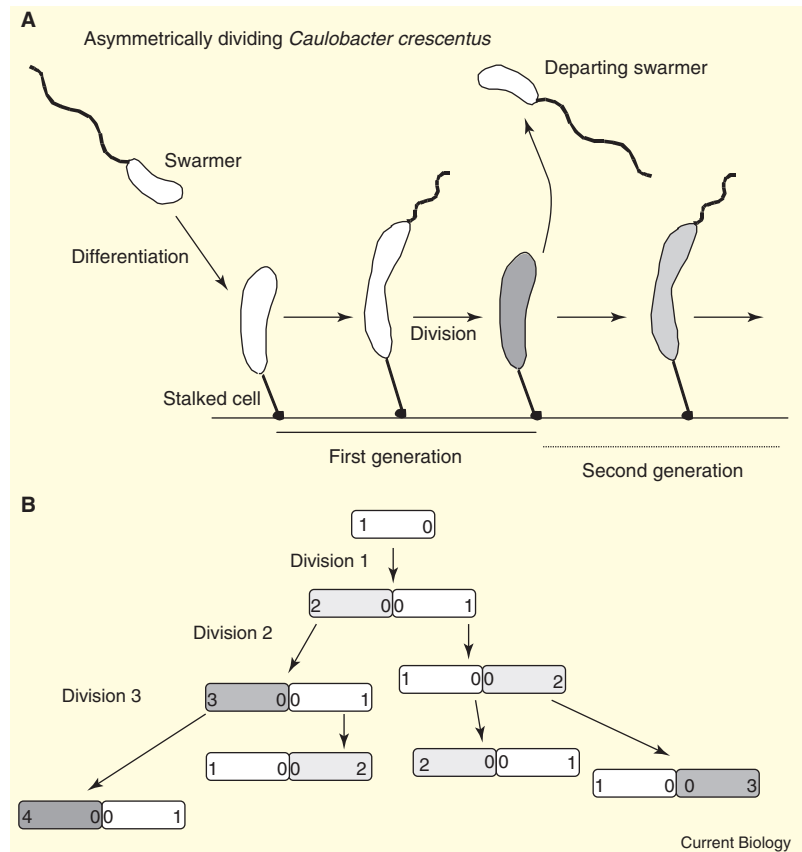


Figure 1. Systems for studying senescence in prokaryotes.

(A) Aging of attached *Caulobacter crescentus* stalked cells, which undergo asymmetric division, has been examined by Ackermann *et al.* [4]. Stalked cells produce a motile swarmer cell each cell cycle. The second generation stalked cell is shaded, indicating aging. See [4] for more details. (B) Stewart *et al.* [3] followed *Escherichia coli* cell division, which is symmetric with respect to cell size and mass, but generates cell poles of unequal age. The age of cell poles (in generations) is shown for a sequence of three division events. Increasingly shaded cells possess older poles; increasing length of arrows between generations reflects the longer time to division for these cells. See [3] for more details.

determine whether non-growing cells were dead.)

The magnitude of the loss of reproductive potential was similar to that observed in *Caulobacter*. Stewart *et al.* [3] calculated the cost of the aging process to the *E. coli* population under these conditions, in terms of lost growth, to be approximately 2%. They further noted that “in competition, these cells would be rapidly displaced by competitors that did not age, but only if the cost of avoiding senescence were not equal or greater” [3]. These costs are presumably in the form of repair or replacement costs for damaged components, including DNA, proteins, lipids or cell wall components [1–3]. Depending on how expensive it is to evolve and operate ‘perfect’ repair systems, a

more effective solution might be to partition damaged materials to just one of the progeny cells. Supporting this possibility are observations that oxidatively damaged proteins are selectively retained in *S. cerevisiae* mother cells after division [9].

Using such a partitioning strategy, some cells would accumulate irreparably damaged components, compromising their own reproductive potential in order to create fitter, rejuvenated offspring with maximal reproductive capacity. The evidence so far implicates older cell poles as potential ‘garbage dumps’ where damaged components accumulate. Poles are important sites in bacterial cells, with relevance to chromosome partitioning, cytoskeletal

organization and dynamics, synthesis of cell surface structures and organelles, and motility [10]. Perhaps cumulative damage to non-diffusible peptidoglycan, protein or lipid complexes at bacterial cell poles eventually compromises their ability to anchor chromosome segregation or critical cytoskeleton-dependent processes. Newly synthesized poles, by contrast, are built from 'fresh' components that do not begin to show wear and tear until subsequent generations.

Our understanding of aging in bacteria is clearly rudimentary at present, but new insights could be just around the corner. Tremendous advances in fluorescence-based imaging of proteins and chromosomes in live bacterial cells provide optimism that structural differences between

senescent cells and their rejuvenated brethren, and between old poles and new poles, will be detectable. Furthermore, both *E. coli* and *Caulobacter* are attractive systems for genetic analysis of aging, as has been so valuable in dissecting molecular determinants of aging and lifespan in yeast, worms, and flies [11]. Time will tell whether molecular mechanisms of aging are as conserved in the prokaryotic world as they seem to be among eukaryotes.

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Cytokinesis: New Roles for Myosin

Myosin II is the motor for cytokinesis, an event at the end of cell division during which the animal cell uses a contractile ring to pinch itself in half. New and surprising research shows that myosin, either through light chain phosphorylation or through its ATPase activity, also plays an important role in both the assembly and disassembly of the actin contractile ring.

David R. Burgess

Cytokinesis is the final act in cell division during which the cytoplasm in animal cells is pinched in half by a circumferential constricting 'purse string', termed the contractile ring [1]. The contractile ring is composed of actin and myosin II and was first identified in marine eggs nearly four decades ago [2]. The analogies to a muscle sarcomere have led to the long-held, conventional view that myosin's role in the contractile ring is to provide the force of contraction within a static actin assembly [1]. Two papers published in this issue of *Current Biology* [3,4] provide compelling evidence that myosin is doing much more than simply acting as the motor for contraction. Myosin is now shown to play key roles in both the assembly and the disassembly of the contractile ring

via its interactions with actin. Moreover, these new studies confirm that actin itself is highly dynamic in the contractile ring.

Myosin II's involvement as an ATPase motor in cytokinesis has been known for some time [5,6] and, as in other non-muscle forms of cell motility, myosin II's activity during cytokinesis is regulated by phosphorylation of its regulatory light chain [7]. Several studies have suggested that myosin may have a role in the assembly of the actin contractile ring and that such movements are regulated by its phosphorylation state [8,9]. The new studies [3,4] dissect the role of myosin light chain phosphorylation and of myosin II ATPase activity, thereby delineating the distinct effects of these two properties of myosin on cytokinesis.

The two groups used different techniques to label actin in the contractile ring of mammalian cells in culture and then used

fluorescence recovery after photobleaching (FRAP) to monitor actin dynamics. Murthy and Wadsworth [4] generated cells stably expressing GFP-actin allowing for easier and more reproducible analysis, and Wang and colleagues [3] traced actin by microinjection of fluorescently labeled phalloidin. Murthy and Wadsworth [4] found that actin in the forming contractile ring turns over at a much faster rate than that in stress fibers or in other cortical regions. In fact, the numbers generated for actin turnover in the mammalian contractile ring are similar to those previously determined for actin turnover in the contractile ring of fission yeast [10]. Furthermore, Murthy and Wadsworth [4] demonstrate the occurrence of dramatic cortical flow of short actin filaments and show that this cortical flow is responsible for fluorescence recovery during cytokinesis. Such flow of actin has been noted during wound closure in *Xenopus* eggs [11]. Similarly, earlier studies from Wang's group documented such movement of labeled actin filaments toward the contractile ring of mammalian cells in culture [12].

So, what is the role of myosin II in the actin dynamics of the