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11. CELLULAR SENESCENCE

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In all multicellular species, progressive deterioration of cellular features eventually gives rise to organismal breakdown, essentially guaranteeing mortality beyond a particular maximum lifespan. Although some forms of environmental modification, such as restricted feeding, can prolong longevity, in no case is a multicellular soma known to be immortal. Finite lifespan might be a necessary outcome of cell lineages that terminally differentiate and therefore have no capacity for producing rejuvenated progeny cells. However, senescence does not appear to be unique to multicellular organisms. Here, we focus on mortality features intrinsic to individual cells in unicellular species (i.e., independent of external factors such as predators and pathogens), exploring the degree to which individual cell lineages are subject to senescence (i.e., increased mortality rates with increasing age).

It can be difficult to contemplate the issue of senescence in unicellular species, as the concept normally applies to specific adult individuals. In the case of an asymmetrically reproducing cell, as in the budding yeast *S. cerevisiae,* it is natural to call the smaller product the daughter cell, although it need not always follow that the smallest member of a pair is the more rejuvenated of the two (in the case of *S. cerevisiae,* it is). Things become more ambiguous when reproduction involves symmetrical fission, but even cells that appear superficially to divide symmetrically may experience internal asymmetrical transmission of damage. One might then view the least damaged of the two cells following a division as the offspring, although quantifying such differences between two daughter cells is difficult. An alternative strategy is to base the age of a cell on some key inherited component, such as a specific pole of a parental cell.

When viewed in this way, quite possibly all cells eventually succumb to senescent decline, even if nothing more than a consequence of a series of unfortunate stochastic events. Because no cell division can be perfectly symmetric, this means that senescence is an inherent feature of life, present since the beginning of biology. There is nothing intrinsically beneficial about death, but as will be discussed below, the selective advantage of prolonging the life of individual cell lineages beyond extended periods of time may be negligible, in effect stalled by a drift barrier. What remains less unclear is the degree to which senescence proceeds more rapidly in some unicellular species than in others.

Physiological Load

In simplest terms, senescence is the gradual deterioration of the self-sustaining features of cells. The relevant determinants include: the preservation of membrane, messenger RNA, and protein integrity; clearance of cytotoxic features such as harmful metabolites and protein aggregates; and maintenance of large cellular complexes such as ribosomes and nuclear pore complexes. All biomolecules are subject to harmful chemical and physical modifications. For example, proteins can accumulate damage in a number of ways, such as deamidation of Asn to Asp and Gln to Glu, both of which lead to a negatively charged amino acid with possible effects on three-dimensional protein structure. The fundamental question is how the accumulation of damage over the life of a cell lineage proceeds at a manageable rate for maintaining a viable population, as opposed to overwhelming the capacity for repair, leading to population demise.

The two potential routes to avoid extinction by senescence at the population level are: 1) to have the rate of damage accrual in an average cellular lifetime be less than or equal to the rate at which such damage is diluted by cell division; and/or 2) to have daughter cells experience asymmetric inheritance of parental-cell damage, such that one of the two products of cell division acquires more damage than its own level at birth, while the other is rejuvenated (Figure 11.1).

Error catastrophe. Under the assumption that deteriorated proteins and macromolecular complexes accumulate during cell lifetimes, Medvedev (1962) and Orgel (1963) proposed an error-catastrophe hypothesis for aging, arguing that progressive decline in the accuracy of the transcription and translation machinery would lead to still further accumulation of erroneous proteins. The argument here is that because transcription and translation processes involve multiple proteins (Chapter 20), as these incur errors, the error-rate itself would be expected to increase, leading to a downward spiral of cell fitness. Some contribution to error catastrophe might result from genomic mutations (below), but the mechanisms envisioned here primarily concern phenotypic errors.

The vast majority of research on this matter has focused on the cells of multicellular species. Abnormal proteins are known to accumulate in the cells of old organisms (Holliday and Tarrant 1972; Rothstein 1975), although it is unclear whether this is a consequence of declining accuracy of transcription and/or translation. Ribosomes in rodents do not appear to decline in terms of translational accuracy with age, although the activity of ribosomes does decline (Mori et al. 1979; Butzow et al. 1981; Filion and Laughrea 1985). There is some suggestion that transcript errors accumulate in old *S. cerevisiae* cells, although these results were obtained in cell lines with error-prone RNA polymerase, and it also remains unclear whether the error rate of transcription itself increased (as expected under the error-catastrophe hypothesis) or whether older transcripts simply accumulate more damage while circulating within cells (Vermulst et al. 2015).

Other results yield a mixed perspective on the error-catastrophe hypothesis. For example, by applying the drug streptomycin, Edelmann and Gallant (1977) elevated the rate of translation error $50\times$ in *E. coli,* and found that although the misincorporation of amino acids into proteins increased substantially, cellular error levels reached a steady state, with growth potential reaching a plateau rather than exhibiting a downward spiral. Another *E. coli* strain engineered to be defective in

translation (by removing the editing capacity of a tRNA synthetase, which loads amino acids onto cognate tRNAs; Chapter 20) experienced an increase in the mutation rate, apparently as a consequence of the activation of an error-prone DNA repair pathway (Bacher and Schimmel 2007). Likewise, Krisko and Radman (2013) found that progressive oxidative damage in this species magnifies the genomic mutation rate. These latter two studies demonstrate that physiological damage may sometimes generate genomic damage in a positive feedback.

Orgel (1970) eventually realized that error catastrophe is not inevitable. As noted above, the key issue is whether the error rate grows sufficiently rapidly with the accumulation of damage to outpace the rate of removal. Let $D(t)$ be the amount of cellular damage at time t. If λ is the baseline rate of production of new cellular damage (assumed to be constant), and δ is the fractional rate of removal of prior damage, the expected amount of damage in the next time unit is $\lambda + (1 - \delta)D(t)$ (Foundations 11.1). Negative δ indicates that prior damage accelerates the rate of accumulation of future damage, ensuring error catastrophe. On the other hand, if $0 < \delta \leq 1$, a stable equilibrium level of damage equal to λ/δ is predicted, such that the input of new damage is exactly balanced by the loss of old damage.

In principle, however, an equilibrium level of damage might still be high enough that cell death is inevitable. An additional issue is that this simple computation is performed in a deterministic framework and assumes no variation among cells. As noted below, variation in damage inheritance is the expectation, and this can lead to a tail of extreme individuals with low fitness. Under some conditions, this might lead to a point of no return, i.e., a sort of ratchet effect towards lineage extinction. However, as noted in Foundations 11.1, among-individual variation can also lead to population immortality, as cells with exceptionally low damage loads are promoted at the expense of damage-laden cells.

Cellular vs. population immortality. Because all progeny inherit their initial cytoplasm from their mothers, asymmetric transmission of damage provides a powerful mechanism for avoiding population senescence – although maternal cells might progressively accumulate damage that reduces cell-division potential, at least one of their daughters might be rejuvenated (Ackermann et al. 2007; Erjavec et al. 2008; Chao 2010). Under this view, the gradual senescence that accrues in some cell lineages can be offset by rejuvenation in others (Figure 11.2; Foundations 11.1), such that lineage senescence need not lead to population demise. This is no different than a population of a multicellular species remaining viable despite the senescence of old individuals.

The stalked bacterium *Caulobacter crescentus,* whose swimming progeny eventually settle down themselves, provides a clear example of this kind of asymmetric effect. Cell division is asymmetrical in this taxon, with the maternal (sedentary, stalked) cells experiencing a progressive loss of cell-division potential, and near-complete loss of reproductive capacity after producing ~ 200 progeny (Ackermann et al. 2003, 2007). However, the species lives on as the physiological decline of parental cells is offset by improved states in progeny.

In contrast, something close to an equilibrium growth rate is demonstrable with *E. coli* cell lineages grown in a contraption within which mother cells are trapped and consecutive progeny are released. In this so-called "mother machine," maternal

cells retain one pole dating back to the onset of the experiment, with the opposite pole being created anew at each cell division. When treated in this way, the growth rates of individual maternal cells remain constant for up to 150 divisions, although there does appear to be an increase in the probability of mortality per division after ~ 100 generations (Wang et al. 2010). As discussed further below, other observations on the growth of single *E. coli* cells reveals that the cell-division times of descendant lineages eventually converge on equilibrium values (Chao 2010; Rang et al. 2011).

Molecular and Cellular Determinants

The previous section highlighted the basic determinants of whether some or all cells within a population will experience senescence associated with physiological decline, and also how populations can remain effectively immortal when individual cell lineages do not. Here, we more explicitly consider the physical aspects of damage inheritance observed in various unicellular organisms. As noted above, asymmetric inheritance provides a simple mechanism by which some sublineages of cells become rejuvenated (albeit at the expense of aging in others).

In *E. coli,* protein aggregates that accumulate with age, and are presumably harmful to cell health, are progressively moved to cell poles in an entirely passive manner (Lindner et al. 2008; Winkler et al. 2010). To see how this process can lead to lineage-specific senescence, consider an aggregate incapable of movement. If such a cellular inclusion develops at the pole of a cell, it will remain at that location in all descendants inheriting the pole (Figure 11.3). Likewise, if it appears near the midpoint of a symmetrically dividing cell, then it will also succumb to a permanent pole location in all future descendants. Finally, if the aggregate is near the 1/4 or 3/4 point in a rod-shaped cell, upon division at the midpoint, it will be present at the midpoint of one of the daughter cells, and hence will sequestered at the pole of one of the granddaughters. A similar mechanism appears to result in the differential sequestration of outer membrane proteins to the older poles of cells (Bergmiller et al. 2017).

In contrast to the situation in bacteria, which generally lack significant internal structure, aggregates in eukaryotic cells can be actively transported along the cytoskeleton to destinations associated with protein-management activities, including aggregation disassembly and protein refolding by chaperones (Kawaguchi et al. 2003; Kaganovich et al. 2008; Malmgren Hill and Nyström 2016). Despite such activities, however, the few eukaryotic species to be evaluated still exhibit patterns of senescence of individual lineages.

One of the more dramatic examples involves the budding yeast, *S. cerevisiae,* which has asymmetric cell division, with the daughter (the bud) being substantially reduced in size relative to the mother. The evidence supports the long-term sequestration of damage within maternal *S. cerevisiae* cells (Aguilaniu et al. 2003). Aging cells progressively experience higher mortality rates and higher degrees of sterility (inability to mate) (Kaeberlein 2010; Schlissel et al. 2017) (Figure 11.4). Although one of the most well-established methods for individual lifespan extension in animals is caloric restriction (imposition of a nutrient-sufficient diet low in calories) (Fontana et al. 2010), such treatment has essentially no effect on lifespan in *S. cere-*

visiae, which produce an average of 25 to 30 offspring per maternal cell regardless of conditions (Huberts et al. 2014).

Numerous mechanisms for aging have been suggested for yeast, including the accumulation of extrachromosomal circular DNAs, malfunctioning mitochondria, and damaged proteins (Macara and Mili 2008; Kaeberlein 2010; Denoth-Lippuner et al. 2014; Kaya et al. 2021). Older cells also progressively experience a genome-wide loss of nucleosomes, elevated rates of chromosomal breaks, and increases in translocations, insertions of mitochondrial DNA, and transposition of mobile elements (Hu et al. 2014). About 74 proteins are mother-enriched and 60 daughter-enriched, the former being biased toward those localized to plasma membranes and vacuoles, and the latter toward those involved in bud construction and genome maintenance (Yang et al. 2015). Such asymmetric inheritance must be due to active transport and/or exclusion mechanisms, which include a diffusion barrier at the bud neck preventing the passage of large complexes (Shcheprova et al. 2008). Individual molecules of ~ 135 proteins are retained within maternal *S. cerevisiae* cells for up to 28 generations, consistent with the potential for long-term accumulation of protein damage (Thayer et al. 2014).

In contrast to *S. cerevisiae,* the fission yeast *S. pombe* has morphologically symmetrical division. Yet, there is still some asymmetric inheritance of damage, with "maternal" cells (those retaining bud scars) inheriting more damaged proteins (Erjavec et al. 2008). Despite this partitioning, senescence appears to occur primarily under stressful conditions, with only cells accumulating large aggregates suffering higher mortality rates (Coelho et al. 2013). Under well-nourished conditions, aging appears not to occur (Spivey et al. 2017) (Figure 11.4). Whether such behavior reflects the predicted behavior of the model outlined in Foundations 11.1, wherein both sublineages of daughter cells evolve to a finite cell-division time and hence immortality, remains unclear.

Finally, an issue of special interest with respect to senescence in eukaryotes is the biogenesis of complex macromolecular structures and organelles during cell division. Are pre-existing assemblies retained intact across cell divisions, with entirely new complexes being assembled from newly synthesized subcomponents as daughter cells grow? Or are such features frequently disassembled and reconstructed from mixtures of old and newly synthesized components?

For many organelles (endoplasmic reticulum, mitochondria, vacuoles, and peroxisomes) in yeast, preexisting structures and their protein constituents are symmetrically inherited, with new membrane complexes (consist of mixtures of components of similar ages) being incorporated with organelle growth. For example, old nuclear-pore complexes (consisting of dozens of proteins) are inherited as a unit in yeast and remain separate from those constructed from newly synthesized components (Shcheprova et al. 2008; Menendez-Benito et al. 2013). In post-mitotic rat brain cells, proteins in large complexes appear to remain in place for extended periods of time, with $\sim 25\%$ of proteins associated with nuclear pores remaining intact after an entire year (Toyama et al. 2013). In *C. elegans,* age-related deterioration of nuclear pore complexes leads to increasingly leaky nuclei enabling the entry of increased numbers of inappropriate cytoplasmic proteins (D'Angelo et al. 2009).

Evolution of Senescence

The preceding discussion establishes the fact that unicellular species do experience senescence, with sublineages stochastically incurring enough consecutive generations of excess damage accumulation eventually succumbing to a physiological load. Unclear, however, is the extent to which the rate of senescent decline is molded by the forces of natural selection, as opposed to being a simple passive response to selection on other cellular features.

Evolutionary theories of senescence have been developed primarily from the standpoint of multicellular organisms, focusing on the separation of the largely quiescent germline and the soma, which represents the manifestation of nearly all gene expression. As the latter is disposed after each sexual generation, its accumulated damage is expendable, whereas the expectation is that the germline will be effectively immortal.

Starting from this premise, the evolution of senescence is thought to be molded by two mechanisms (Charlesworth 2000). First, the pleiotropy hypothesis embraces the idea of tradeoffs between alternative fitness traits, e.g., selection for energetic investment in early reproduction subtracting from what can be invested in survival (Williams 1957). A second hypothesis focuses on the diminishing fitness payoffs of progeny produced late in life (Medawar 1952; Hamilton 1966). Like compound interest applied to a bank account, the payoffs of which grow exponentially, progeny successfully produced at a young age enter the gene pool earlier and contribute to elevated rates of expansion of the genes contributing to such a life-history strategy. In the extreme situation in which individuals late in life produce no offspring at all, there can be no selection on survival-enhancing traits expressed only beyond that point (except in the case of cross-generational care, as in social animals). In this case, deleterious late-acting mutations will tend to accumulate by a combination of mutation pressure and random genetic drift.

These two hypotheses are not mutually exclusive, as there is no reason that both mechanisms cannot be operating simultaneously. Moreover, as the number of genes influencing survival and reproductive rates must constitute nearly the entire genome, it is likely that the genetic mechanisms of senescence will be diverse among phylogenetic lineages, owing to the stochastic nature of the mutation process and the targets hit.

Because comparative studies of senescence in unicellular species are nearly nonexistent, the extent to which either of these hypotheses is relevant to microbes remains unclear, although they are, in principle, testable. For example, the antagonistic-pleiotropy hypothesis predicts that species with higher cell-division rates would exhibit more rapid rates of demise in sublineages of cells that progressively inherit the most damage from their parental cells. The mutation-accumulation hypothesis predicts that alleles with deleterious effects on fitness will be enriched in classes of genes whose expression is confined to old mother cells. In this case, selection for more rapidly dividing cells or life in an environment nonconducive to old age should ultimately lead to the accumulation of deleterious mutations in late-acting genes being expressed at chronologically earlier ages. Whether genes with the postulated age-specific expression properties actually ever exist in unicellular species is an open question, but data from animal species support this argument (Rodriguez et al. 2017; Cui et al. 2019; Cheng and Kirkpatrick 2021), and there is clear evidence of genetic variation for lifespan in yeast (Kaya et al. 2021).

Mutational Meltdown

One of the more dramatic observations from experimental gerontology is that isolated lineages of vertebrate cells generally have finite lifespans, regardless of the population size of cells. After a series of cell divisions of apparently unimpaired growth, culture loss often becomes certain. Fibroblast cell lines from long-lived vertebrates are capable of more divisions than those from short-lived species, and cells taken from older individuals have diminished numbers of remaining cell divisions (Martin et al. 1970; Hayflick 1977). As these results arise in cultures containing large numbers of cells, they suggest some kind of near-deterministic mechanism of sudden breakdown in cellular integrity, e.g., programmed cell death. However, observations of single-cell lineages suggest that this is not the case – the loss of cell-division potential is not abrupt but continuous throughout the lifespan of the extended lineage (Raes and Remacle 1983; Smith and Pereira-Smith 1977; Karatza and Shall 1984; Angello and Prothero 1985) (Figure 11.5).

Although the relevance of cells having an "out-of-body" experience to natural populations of unicellular species is unclear, there are also cases of cultures of unicellular species having finite numbers of cell divisions. Most notable is the situation in ciliates, many of which have characteristic culture lifespans. A substantial body of literature focused on these kinds of observations early in the 20th century has been extensively reviewed (Nanney 1974; Smith-Sonneborn 1981; Bell 1988). For a number of species, extinction occurs in a predictable number of cell divisions (usually in the range of 150-1,500 cell divisions, depending on the isolate, and with low intrastrain variance). Moreover, this can be true both for lineages maintained by single-cell descent and in moderately large cultures. The results do not appear to be a consequence of deterioration of the lab environment, and nuclear transplantation experiments indicate that clonal decline is caused by nuclear rather than cytoplasmic factors (Aufderheide 1984, 1987). As in the case of fibroblast cultures, close observations of the fitness features of individual *Paramecium* cells are inconsistent with a threshold reduction in cell-division potential (Figure 11.5). Rather, there is a steady decline in cell reproductive capacity with time ($\sim 0.1\%$ per generation) (Smith-Sonneborn 1979; Takagi and Yoshida 1980; Lynch and Gabriel 1990).

Whereas the exact mechanisms underlying such behavior are not clear, the observed patterns are consistent with some sort of cumulative genomic deterioration. As first pointed out by Muller (1964), obligately asexual lineages are subject to a ratchet-like mechanism of mutation accumulation. With a high rate of genome-wide input of deleterious mutation (Chapter 4), populations of clonally reproducing cells are expected to contain a distribution of fitness classes defined by recurrent mutation pressure and selection against highly loaded individuals. This distribution is expected to be unimodal (and under many genetic models, close to Poisson in form; Chapter 23), with only a small fraction of the population contained within the best fitness class. The smaller the population size, the smaller the number of individuals in the best class, and each generation, the possibility exists that individuals in this class either produce no progeny at all or only progeny acquiring a new deleterious mutation. Either way, the best class will have been irreversibly lost, as in the absence of sexual reproduction, there is no way to recover the prior fitness class except in the extremely rare case of a precise back-mutation. Once the best

class is lost, the prior second-best class advances to be the best in the population, but it too will eventually suffer the same fate, and so on.

The natural culmination of Muller's ratchet is extinction by a process known as the mutational meltdown (Lynch and Gabriel 1990; Lynch et al. 1993; Gabriel et al. 1994). Initially, a population will typically have excess reproductive capacity, but as mean individual fitness declines, a point will eventually be reached at which each individual is just able to replace itself on a per-generation basis. At that point, any further increase in the mutation load will lead to a reduction in population size, but with fewer individuals, natural selection is less efficient (Chapter 8). This then increases the rate at which the fitness ratchet clicks, continuously driving the population to a still smaller size, in a downward spiral that leads to extinction via deleterious-mutation accumulation.

As this process can operate in populations containing many thousands of asexual individuals, yielding fairly deterministic extinction times despite the stochastic accumulation of mutations, it may provide an explanation for the above-noted results. The situation for ciliates likely relates to their unique binuclear form of genetic organization, involving a germline micronucleus and a somatic macronucleus. The micronucleus replicates by mitosis during clonal propagation, but is transcriptionally inactive. It undergoes meiosis during sexual reproduction, and following daughter-cell formation divides by mitosis, one product remaining as the germline and another becoming the macronucleus. During macronuclear maturation, its chromosomes undergo many rounds of replication until all are duplicated to very high ploidy levels (typically, several hundreds), and it is from this genome that all gene expression occurs. Although the mechanism of macronuclear division is poorly understood, it is more along the lines of random fragmentation than symmetric mitosis.

Given this genetic system, loss of viability in a ciliate population during clonal propagation must be a consequence of deleterious changes in the macronuclear genome. However, such deterioration is unlikely to involve genomic mutations. In ciliates, point mutations involving single base-pair substitutions arise at a rate of $\sim 10^{-11}$/nucleotide site/cell division in the micronucleus (Sung et al. 2012; Long et al. 2016), the lowest known rate of any organism. Assuming the error rate is the same in the macronucleus, then because the macronuclear genome size is $\sim 10^8$ bp, only one mutation is expected to arise per haploid genome every 1000 cell divisions. Because there can be ~ 1000 copies of each chromosome in the macronucleus, the total mutation load would be $\sim 1000\times$ higher than this, but each mutation would be complemented by the ~ 1000 unmutated copies, raising significant questions as to whether conventional mutation accumulation is responsible for loss of reproductive capacity on a time scale of hundreds of cell divisions.

The more likely path to cellular senescence in ciliates involves the amitotic (fission-like) nature of macronuclear propagation, which can lead to random drift in chromosome copy numbers, stoichiometric imbalance, and eventual loss of entire chromosomes. Assuming simple random sampling of chromosomes following macronuclear genome duplication, Kimura (1957) produced a stochastic model of chromosome loss that yields extinction dynamics that are reasonably consistent with the existing data.

Less clear is whether mutation can account for the behavior of vertebrate fibroblast cultures. Although the mutation rate of such cells may be as high as 10^{-9}

per bp per cell division (Lynch 2010) and the haploid genome size is of order 10^9 bp, at most 10% of the vertebrate genome is under significant selection, so it is likely that < 1 functionally relevant mutation (and maybe considerably less) arises per cell division. More significantly, immortalized mammalian cell lines are readily obtained from cancer cells with substantial genomic rearrangements, raising questions about the adequacy of mutation accumulation as an extinction mechanism.

Summary

- The molecular constituents of cells naturally deteriorate over time, raising the possibility of cellular senescence analogous to aging in multicellular species. However, the asymmetric inheritance of damage by daughter cells, whether programmed or simply stochastic, can lead to the rejuvenation of one member of the pair at the expense of the other. This then ensures indefinite population survival, as in multicellular species that discard the senescing somas of aging individuals.

- Two hypotheses have been suggested for the evolution of senescence: 1) a trade-off associated with pleiotropic effects, whereby investment in early reproduction imposes costs on future survival; and 2) diminishing payoffs resulting from the compound-interest effect of early contributions to the gene pool enhancing fitness more than progeny produced later in life. Both hypotheses were constructed with multicellular species in mind, and the extent to which they apply to unicellular species remains unexplored.

- Numerous examples exist in which single-cell lineages have well-defined finite life-spans, most notably in ciliates and vertebrate cell cultures. The mechanisms in these cases likely involve large-scale problems with chromosomal integrity and imbalance, such as telomere and/or chromosome loss, rather than the accumulation of point mutations. In such cases, rejuvenation can be accomplished by sexual outcrossing and/or inactivation of genes involved in telomere erosion.

Foundations 11.1. The physiological damage load in a cell lineage. Over time, whether stationary or in a period of active growth, a cell can be expected to acquire some reduction in physiological capacities owing to the accumulation of damaged molecules (here, assumed to accumulate at rate λ per unit time). Prior damage might also be fractionally eliminated at rate δ, such that with a starting level of damage at birth D_0, the amount of damage accumulated over time can be expressed with the recursion equation

$$D(t+1) = \lambda + (1-\delta)D(t). \tag{11.1.1a}$$

Note that provided $0 < \delta \leq 1$, for large t, an equilibrium level of damage is asymptotically approached. This is obtained by setting $D(t+1) = D(t)$,

$$\widehat{D} = \frac{\lambda}{\delta}. \tag{11.1.1b}$$

A special case was considered by Chao (2010), who assumed $\delta t \ll 1$, so that damage simply accumulates linearly with time before cell division, and the above-noted equilibrium is never reached. Letting D_0 denote the damage of a particular cell at birth, its damage at the time of cell division T is then

$$D(T) \simeq D_0 + \lambda T. \tag{11.1.2}$$

Suppose that some critical cell feature has to achieve a critical value P_c prior to cell division (e.g., time to achieve a threshold amount of some key cellular component), with cellular damage detracting from the rate of accumulation of P, such that

$$\frac{dP}{dt} = 1 - D(t). \tag{11.1.3}$$

Equation 11.1.3 equates the rate of product accumulation to 1.0 in the absence of damage. The solution to Equation 11.1.3, obtained after substituting Equation 11.1.2, leads to a quadratic equation defining the time to cell division,

$$P_c = P(T) = (1-D_0)T - (\lambda/2)T^2. \tag{11.1.4}$$

Given D_0 at birth and P_c at division (a fixed parameter), Equation 11.1.4 yields the cell-division time T as a function of λ. With no damage accumulation, $\lambda = D_0 = 0$, and Equation 11.1.4 leads to the definition $P_c = T$, i.e., P_c denotes the time required to linearly build up to the checkpoint in the absence of any damage accumulation.

The previous expressions assume cells growing deterministically, with each daughter cell sharing exactly half the damage in the maternal cell at the time of fission. However, with asymmetrical cell division, the population will be heterogeneous. Letting $a \leq 1/2$ and $1-a \geq 1/2$ be the fractions of damage transmitted to the two offspring cells (indexed as 1 and 2, with the maternal cell denoted as 0), from Equation 11.1.2 the damage transmitted to the two newborns becomes

$$D_1 = a(D_0 + \lambda T_0), \tag{11.1.5a}$$
$$D_2 = (1-a)(D_0 + \lambda T_0). \tag{11.1.5b}$$

Substituting these expressions for D_1 and D_2 for D_0 in Equation 11.1.4, and solving yields the times to cell division for the two daughter cells as a function of the features of the maternal cell (D_0, T_0),

$$T_i = \frac{1 - D_i - \sqrt{(1-D_i)^2 - 2P_c\lambda}}{\lambda}, \tag{11.1.6}$$

where $i = 1$ or 2.

This overall system of equations allows one to start with a given value of T_0 for a parental cell, and then obtain the cell division times for all subsequent cells (Figure 11.6). The critical issue with respect to senescence is whether T_1 and/or T_2 increase without limits. If this were true for both daughter-cell types, runaway damage and aging of the entire population would ensue. In contrast, whereas population survival in spite of some cell senescence would occur if T_2 stabilized, i.e., the least loaded daughters do not experience ever-increasing damage.

Equilibrium requires that the reduction in damage by dilution from the mother cell be balanced by the buildup of new damage in the daughter. The equilibrium conditions are obtained by substituting $D_i = D_0$ into Equations 11.1.5a,b, so that $D_i(1-a) = a\lambda T_i$. Letting $\alpha = a/(1-a)$ and writing $T_0 = \widehat{T}_1, \widehat{T}_2$, yields the equilibrium physiological loads,

$$\widehat{D}_1 = \widehat{T}_1 \lambda \alpha, \tag{11.1.7a}$$

$$\widehat{D}_2 = \widehat{T}_2 \lambda / \alpha. \tag{11.1.7b}$$

Finally, substituting into Equation 11.1.4 yields the equilibrium solutions,

$$\widehat{T}_1 = \frac{1 - \sqrt{1 - 2P_c\lambda(1 + 2\alpha)}}{\lambda(1 + 2\alpha)}, \tag{11.1.8a}$$

$$\widehat{T}_2 = \frac{1 - \sqrt{1 - 2P_c\lambda[1 + (2/\alpha)]}}{\lambda[1 + (2/\alpha)]}. \tag{11.1.8b}$$

The conditions for equilibrium are that the terms within the square roots in the previous equations be nonnegative, which requires

$$\alpha \leq \frac{1 - 2P_c\lambda}{4P_c\lambda}, \tag{11.1.9a}$$

$$1/\alpha \leq \frac{1 - 2P_c\lambda}{4P_c\lambda}. \tag{11.1.9b}$$

Thus, a key for the immortality of a cell line is that the composite quantity $P_c\lambda$ not be too large. Given the definition of P_c noted above, $P_c\lambda$ can be thought of as the total amount of damage that would be built up in a cell with minimum possible division time. With symmetric division ($\alpha = 1$), the criterion for equilibrium reduces to $P_c\lambda \leq 1/6$. With asymmetric inheritance and $P_c\lambda$ sufficiently small, both $\widehat{T}_1$ and $\widehat{T}_2$ reach real equilibria, but with increasing levels of damage, at most one of the cell classes can reach an equilibrium. Eventually, with high enough $P_c\lambda$, neither daughter is capable of sustained reproduction, and senescence of the entire population would ensue without some intervening rejuvenation mechanism.

Some insight into the level of fitness reduction that results from damage accumulation can be acquired by noting that for the case of symmetrical division, a damage-free cell would reproduce at age P_c, doubling the population size in that interval. In contrast, in the presence of damage accumulation, cell doubling will require time T_1 (as defined by Equation 11.1.8a with $\alpha = 1$), so that at time P_c the population would have increased by a factor of $2^{P_c/\widehat{T}_1}$. Thus, relative to a damage-free cell, the reproductive rate become $(2^{P_c/\widehat{T}_1})/2$. The fractional reduction in fitness, which can be viewed as the equilibrium physiological load of damage accumulation, is then

$$L = 1 - 2^{(P_c/\widehat{T}_1)-1}, \tag{11.1.10a}$$

which for moderate amounts of damage, $P_c/\widehat{T}_1$, simplifies to

$$L \simeq \frac{1 - (P_c/\widehat{T}_1)}{\sqrt{2}}.$$

(11.1.10b)

The damage load can be viewed as the amount of improvement in cell fitness that could be achieved by establishing mechanisms that completely eliminate damage, i.e., $\lambda = 0$, although this view ignores any bioenergetic cost of producing and maintaining damage-control mechanisms.

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Figure 11.1. Serial buildup and dilution of cellular damage. Depending on the stochastic patterns of asymmetric damage inheritance among sublineages, the entire population of cells may remain effectively immortal, while some portions of the extended pedigree may experience runaway damage and go extinct. The intensity of blue denotes the physiological load carried by a cell, with the top sublineage being one in which regular dilution of parental-cell damage leads to periodic rejuvenation and persistence, and the bottom sublineage representing the opposite extreme – a series of consecutive generations of inheritance of excess parental cell damage, leading to cumulative physiological deterioration.
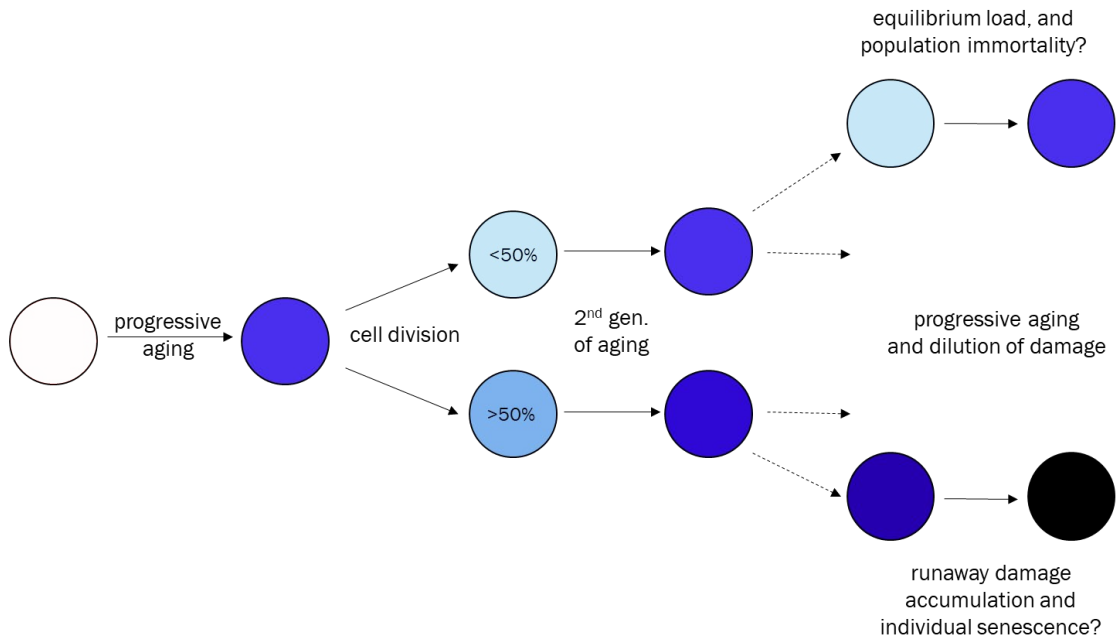
Figure 11.2. The distribution of damage inheritance in pedigrees of cells. **Left**) Transmission of parental damage is perfectly symmetrical, leading to invariance in the level of damage among descendent cells. In principle, the descendant cells can reach an equilibrium level of nonlethal damage if the rate of damage removal is not overwhelmed by damage accumulation each generation. **Right**) Asymmetric inheritance leads to phenotypic variance in the damage levels in populations of cells, with some nearly damage-free cells being maintained at the expense of damage-laden cells. Two extended single-cell lineages with low levels of damage (blue) are highlighted with bold connecting lines.
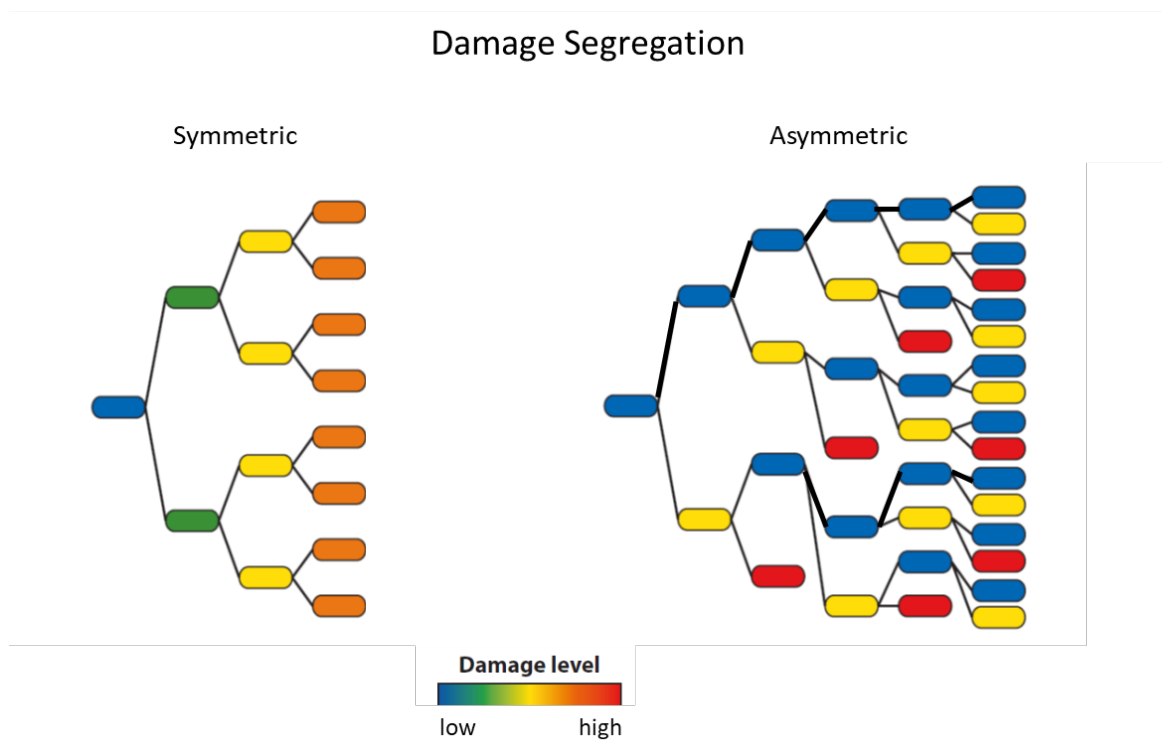

Damage Segregation

Figure 11.3. Passive relocation of a cellular protein aggregate (red sphere) to the poles of symmetrically dividing cells. Because the aggregate is immobile, regardless of the starting point, it will eventually be located near the poles of descendant cells. The numbers at the ends of cells denote the ages of poles relative to the base individual. This schematic only shows the potential distribution of aggregates descendant from the single ancestral cell, but in reality, new damage aggregates will arise in each descendant and be apportioned into its descendants in the same manner. Not shown is the possibility that aggregates may also dissipate over time. Modified from Lindner et al. (2008).
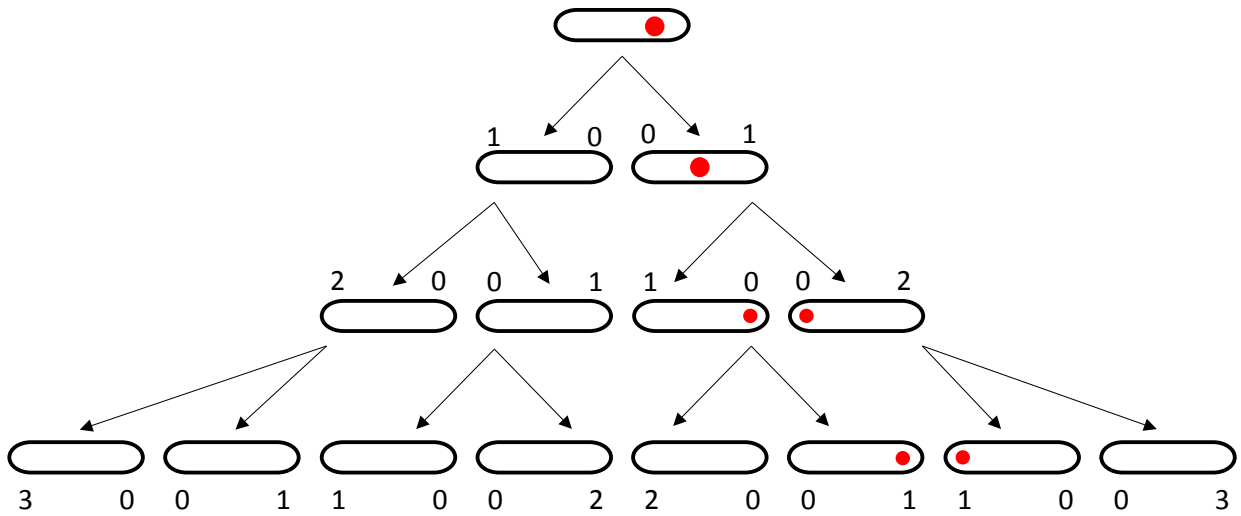
Figure 11.4. Individual cells of the budding yeast *S. cerevisiae* experience progressive decline in cell-division potential, and therefore an accelerated rate of mortality with age, whereas those of the fission yeast *S. pombe* experience a constant mortality rate, as revealed by the diagonal survivorship curve (on a logarithmic scale). From Spivey et al. (2017).
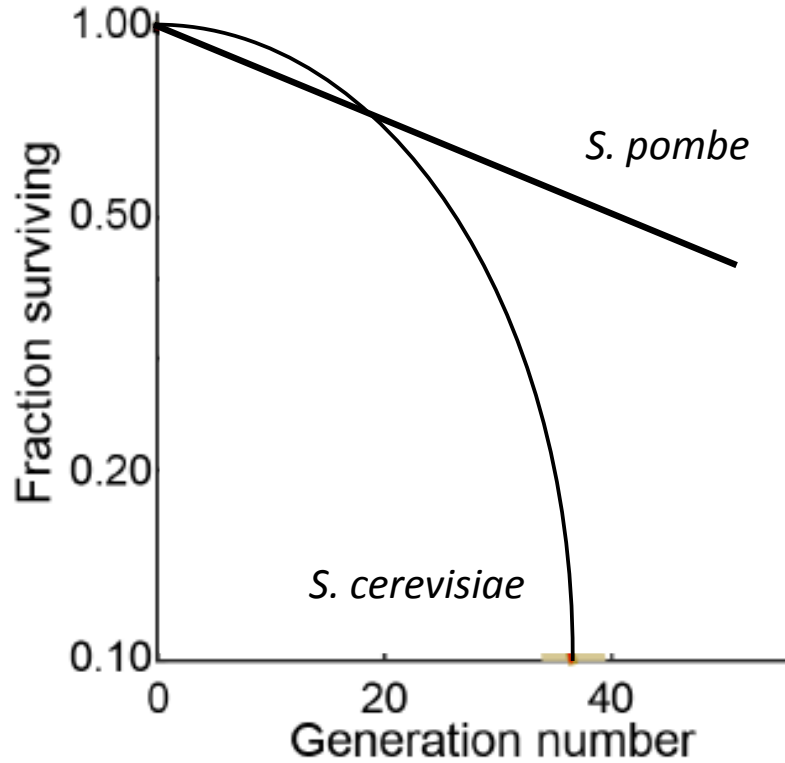
Figure 11.5. **Left)** Continuous decline of cell-division potential in single-cell lines of the ciliate *Paramecium caudatum* (Takagi and Yoshida 1980). **Right)** Similar plots for fibroblasts cultured from various vertebrates: human (Smith and Pereira-Smith 1977); hamster (Raes and Remacle 1973); chicken (Angello and Prothero 1985); and mouse (Karatza and Shall 1984). The lines are least-squares regressions scaled to give a time-zero fitness equal to 1.0, except in the case of hamster. From Lynch and Gabriel (1990).
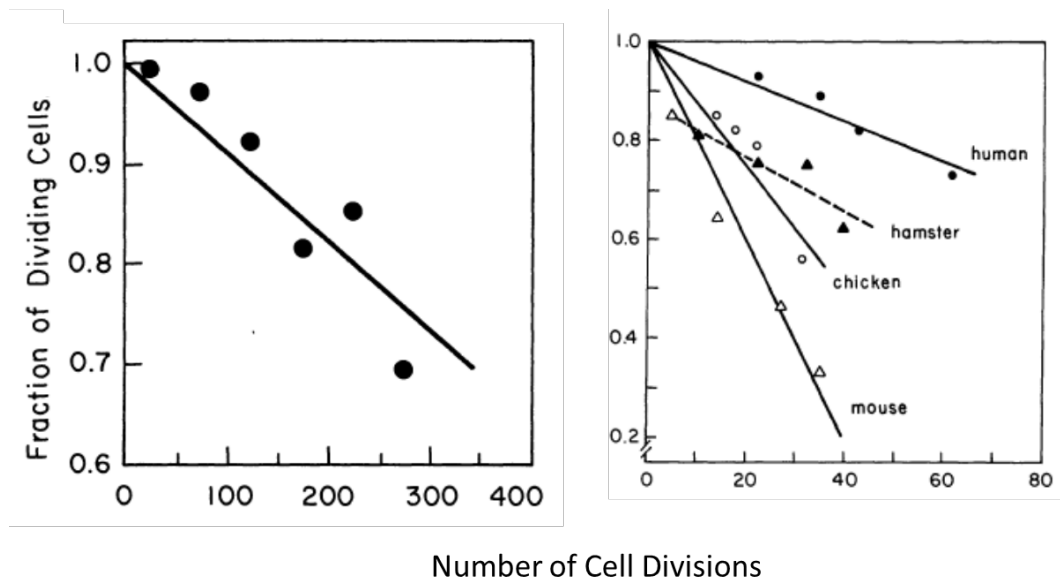

Number of Cell Divisions

Figure 11.6. Left) Recursion plots for the relationship between cell-division times (T) in mother and daughter cells (the x and y axes, respectively) for the case of symmetrical cell division. Diagonal dashed lines denote potential points of equilibrium, with daughter and parental-cell division times being equal to each other. Starting with a particular value of T for a mother cell, Equation 11.1.4 defines the starting damage in that cell, which when entered into Equation 11.1.5 generates the damage in the first-generation daughter cell at the time of its division; substitution of the latter into Equation 11.1.6 then leads to the cell division time of this daughter cell; and this sequence of calculations can be done anew to yield the cell-division time in the next generation. The solid line denotes the expected relationship between mother and offspring cell-division times, which must intersect the dashed line for an equilibrium to exist, and moreover must intersect from above to the left for this equilibrium to be stable (upper left panel). The solid lines with arrows denote the successive projections of cell-division times for mothers and daughters from one generation to the next – starting with a particular maternal T_0, the offspring T_1 is determined by reading the appropriate value for the solid line off the y axis; this time value then becomes T_0 for the next generation, and so on. In the upper panel, $P_c = 2$ and $\lambda = 0.005$, so the key parameter $P_c\lambda = 0.10$, which according to Equation 11.1.9 is sufficient to allow a stable equilibrium, as designated by the intersection of the solid and dashed lines; in this case, if T starts below the equilibrium, it progressively increases until reaching this point, and vice versa, if T starts above the equilibrium. In the lower panel, the rate of damage accumulation is too high for an equilibrium to be achieved; regardless of the starting point, T eventually increases without bound as descendants progressively accumulate more and more damage. **Right**) Actual observations on the cell-division times for parent and offspring pairs of *E. coli* cells, with the solid and open points designating the times for the daughter cells with the longest and shortest division times. Although the data are quite noisy, the regression lines for both sets of points intersect the diagonal, consistent with a non-senescing population. From Chao (2010).