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The paths of mortality: How understanding the biology of aging can help explain systems behavior of single cells

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Abstract

Aging is a fundamental aspect of life, yet also one of the most confounding. In individual cells, aging results in a progressive decline which affects all organelles and reduces a cell's ability to maintain homeostasis. Because of the interconnected nature of cellular systems, the failure of even a single organelle can have cascading effects. We are just beginning to understand the dramatic physiological changes that occur during aging. Because most aging research has focused on population dynamics, or differences between wild-type and mutant populations, single-cell behavior has been largely overlooked. An open question is whether aging cells are defined by predictable sequences of physiological changes, or whether they proceed along divergent aging trajectories defined by whichever system begins to fail first. Can aging be best characterized by a cell-cycle like model with stereotyped states all cells progress through, or a Waddington landscape with divergent trajectories? Here we present work on understanding the changing physiological states of aging cells, why it will impact systems and synthetic biologists, and how the systems community can contribute significantly to the study of aging.

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> "Happy families are all alike; every unhappy family is unhappy in its own way."
>
> -Leo Tolstoy

Introduction

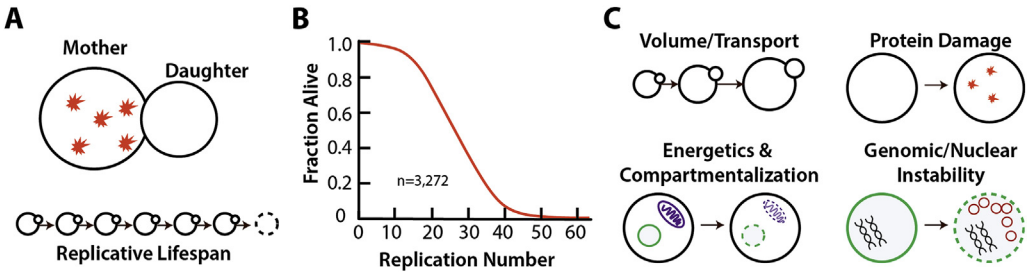
In a healthy, young cell, all systems operate properly, but aging has a multitude of targets to choose from, and the failure of even a single process can result in death. This abundance of opportunities to fail can be clearly seen in the widely varying lifespans of clonal organisms. Even for isogenic populations of single-celled budding yeast that are grown in identical environments, the lifespans will vary by over an order of magnitude [1]. The same is true for genetically and environmentally identical multicellular animals [2]. The fact that a population of seemingly identical individuals can have such disparate lifespans demonstrates the complexity of the aging process. Although cells have powerful networks to maintain homeostasis, the many ways cellular systems can fail means that any collection of young cells will be far more similar than any collection of middle-aged or old cells. With increasing age, the variation between cells is likely to increase significantly, suggesting that old cells will have more divergent responses to an environmental stimulus, and that the behavior of synthetic circuits will be more varied and unpredictable. Understanding how cells proceed through different physiological states will thus aid not just our understanding of the biology of aging — but how natural and synthetic circuits behave in an aging cellular chassis.

As systems begin to fail, how do homeostatic networks compensate for these failures, and do cells proceed through different physiological states based on which systems failed first? Detailed studies of individual aging cells are, for the first time, starting to grapple with the concept of divergent aging trajectories. Critically important aspects of this are the molecular identification of distinct subsets of aging cells and the penetrance within the population of different aging phenotypes. Here we will focus on the budding yeast *Saccharomyces cerevisiae*, as it is both one of the most widely studied aging models and extremely popular within the systems biology community. The majority of the physiological changes discussed here, however, represent hallmarks of aging that are present in widely divergent organisms [3]. Thus, it seems likely that much of what we are able to decipher about modes of failure during aging in yeast will be relevant in more complex animals and even people.

Replicative aging

Budding yeast cells go through asymmetric divisions where the mother cell produces a newborn daughter cell [4,5]. Because this is a morphologically asymmetric division unlike fission yeast or bacteria, there is a clearly distinguished mother cell, which can be tracked as it divides (Figure 1A). During each cell cycle the mother

Figure 1



Replicative aging and physiological failures. A) Budding yeast undergo asymmetric divisions in which mother cells retain the majority of damaged proteins and organelles. After a number of divisions, a mother cell will stop dividing and die. B) A replicative survival curve of budding yeast (BY4742) from the Kaeberlein lab showing the enormous variation in lifespan. Isogenic cells, grown in an identical environment, may only bud 3–4 times, or 50–60. C) Categories of physiological changes that cells undergo as they age. Cells grow continuously in volume, experience increased protein damage, organelles like the mitochondria become less effective (purple), and in the nucleus there is increasing instability with increased rDNA circles (red) and reduced silencing.

cell retains damaged proteins, dysfunctional mitochondria and aberrant genomic material which allows daughter cells to be rejuvenated and begin life healthy [6]. As mother cells continue to divide, this accumulated damage takes a toll, and the cell cycle slows leading to senescence and death (Figure 1A). The number of daughter cells that each individual mother is able to produce before reaching senescence is defined as that cell's replicative lifespan (Figure 1B). Although a fascinating system in its own right, studies on the replicative aging of budding yeast have uncovered many genetic modifiers of lifespan that are conserved even up through mammals [3,6–9]. Furthermore, although we know that complex systems such as stem cells undergo cell intrinsic changes during aging that affect cell behavior [10–12], there are limited *in vivo* models of stem cell aging. Thus, understanding the principles of how cell physiology changes and affects cellular behavior in a simple, unicellular model organism can provide valuable insight that might not otherwise be possible.

Changes and physiological states during aging

Aging drives a large number of changes of key physiological parameters within cells (Figure 1C). Fundamental cell properties that are reported to change with age in yeast include increasing cell size and cell division time [13,14], alkalization of cytoplasmic and organelle pH [15], increasing oxidation and oxidative damage [16,17], loss of mitochondrial respiratory capacity [18] and selective destruction of mitochondria [19], loss of mating competency [20], accumulation of damaged or misfolded proteins [17,21], fragmentation of the nucleolus [22], and increasing genomic instability particularly near the ribosomal DNA [23]. These changes are highly interlinked and have been suggested to act in a causal fashion driving successive failures in related systems. Within a single cell, any of these changes will be mediated by homeostatic networks, but could also cause failures in interacting components as the cell attempts to compensate [24]. For example, a reduction in mitochondrial membrane potential that leads to a reduction of available ATP, would in turn affect the ability of proton pumps to function and result in a change to cellular pH. Thus, within these physiological variables, a number of them are likely to be highly correlated [24,25]. A physiological aging state would therefore be defined by the specific changes in a subset of physiological parameters that occur together.

Until recently, technological limitations have prevented the study of the ways in which related systems fail within single cells [26]. Although many experiments have assessed phenotypic changes in aging cells, they have largely been performed in a cross-sectional fashion where aging cells are isolated from a population [15,18,19,21,27–33]. Thus, the relationship between these spontaneous physiological changes and lifespan has remained cloudy. Only with the development of microfluidic systems in recent years has it become possible to watch individual cells throughout their entire lifespans, and observe whether changes in one network are linked to failures in another [16,34–38].

The physiological changes that occur during aging can be broadly categorized into four groups: 1) volume and transport, 2) energetics and compartmentalization, 3) protein damage, and 4) genomic/nuclear stability (Figure 1C). The first category, volume, is one of the most distinctive changes that occurs, as budding yeast cells will grow continuously, in a near-linear volumetric fashion, over their whole life. Although regulation of cell size in budding and division has been extensively explored in young cells [39], how this volumetric growth of 2–3 fold affects cell physiology – and whether it is actively regulated or a passive by-product of aging – has yet to be explored. Simplistically, this continuous growth changes the surface to volume ratio, likely