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Preview

Rationally reprogramming single-cell aging trajectories and lifespan through dynamic modulation of environmental inputs

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How do variations in nutrient levels influence cellular lifespan? A dynamical systems model of a core circuit involved in yeast aging suggests principles underlying lifespan extension observed at static and alternating glucose levels that are reminiscent of intermittent fasting regimens.

Aging is a complex and multifaceted process driven by the accumulation of damage in cells through both cell intrinsic and extrinsic factors. While specific drivers of aging have been identified in broad biological contexts spanning epigenetic regulation, metabolism, and stress response, these findings are complicated by the fact that said pathways are often intricately coupled.[1] At the same time, significant effort in the aging field has focused on the identification of individual genes that affect lifespan when deleted in model organisms.[2] Despite important findings, such approaches tend to overlook the interactions between multiple genes and pathways and thus fail to capture the complexity of aging. A systems perspective is necessary to understand how aging-associated phenotypes are realized over time within cells[3]; such an understanding could pave the way for novel interventions aimed at extending healthy lifespan or healthspan.

The development and application of dynamical systems models offers a path toward tackling the aforementioned complexity associated with aging.[4] Furthermore, the extensibility of such models—particularly their capacity to integrate stochastic and nonlinear dynamic elements—makes them well-suited to the inherently noisy and nonlinear features of the associated biological processes. Accordingly, such approaches could be instrumental for decoding the intricacies of cellular aging and identifying lifespan determinants through mechanistic modeling of datasets obtained from aging cells.

Previous studies in model organisms have predominantly focused on aging and lifespan in static environments. However, environmental factors, such as nutrients and stress, impose constraints on cells almost exclusively in a dynamic fashion, making it imperative to understand how naturally changing environments affect genetic networks regulating cellular aging and lifespan.

The paper by Liu et al. demonstrates the utility of combining these perspectives and approaches.[5] It shows how a stochastic model of nonlinear gene regulatory dynamics can provide testable predictions of aging dynamics and lifespan outcomes in both static and fluctuating environmental conditions. Using a microfluidics-coupled time-lapse microscopy setup, the authors quantitatively tracked individual yeast *Saccharomyces cerevisiae* cells throughout their entire replicative lifespan, spanning multiple days. Replicative lifespan is a frequently used metric that measures the total number of mitotic division events—or generations—for a newborn yeast cell until its death. For example, wild-type yeast cells of the type used in the current study live, on average, 22–23 generations. Until recently, it was unknown whether yeast aging was associated with multiple distinct phenotypes. In a previous landmark study, the same UCSD collaboration that participated in the Liu et al. study discovered that two distinct morphologies (named "Mode 1" and "Mode 2") were associated with aging yeast cells; driven by the loss of ribosomal DNA (rDNA) silencing and nucleolar decline, Mode 1 aging was characterized by the continuous production of elongated daughter cells.[6] On the other hand, driven by heme depletion and mitochondrial deterioration, Mode 2 aging was characterized by the production of small and round daughter cells.[6] The researchers further showed that the Sir2 proteins take a representative role in Mode 1 aging through mediating chromatin silencing at rDNA, while the heme-activated protein (HAP) complex takes a key role in Mode 2 aging through regulating genes important for heme biogenesis and mitochondrial function.

The current study is built on the same Sir2-HAP circuit, but this time it focuses on the impact of varying glucose levels on this aging circuit and single-cell lifespan. In addition to uncovering that such variations affected the metabolic states associated with Mode 1 and Mode 2 aging, Liu et al. develop a stochastic model to investigate the effects of varying glucose levels on yeast cells microfluidically tracked over the course of aging. The investigations led to the discovery of two new lifespan extension mechanisms, with the first one acting through stabilization of the healthy state of a cell by establishing a balance between Sir2 and HAP, and the second one acting by causing dynamic stabilization of the system around the healthy state on the Sir2-HAP state space.

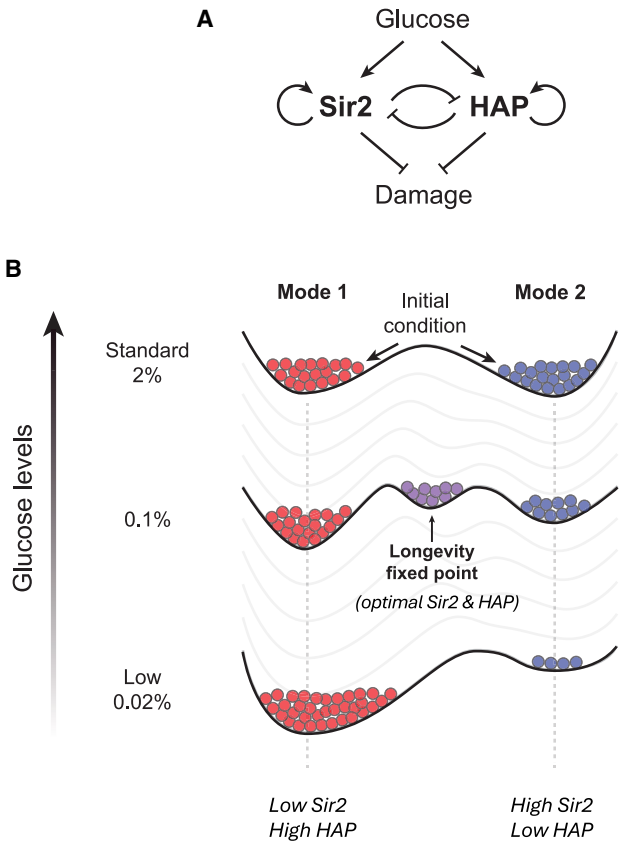

Figure 1. Cellular aging trajectories influenced by an environmentally modulated gene network

(A) Schematic of the toggle-switch network.

(B) Landscape representation of the distribution of cellular states realized at varying glucose concentrations. Red and blue filled circles represent yeast cells occupying states associated with Mode 1 and Mode 2 aging trajectories, respectively. Purple filled circles represent cells in the vicinity of a "longevity fixed point" characterized by optimal Sir2 and HAP levels.

Most yeast studies use 2% glucose as the carbon source to be consistent with previous studies. Reducing the glucose concentration to 0.05%, which corresponds to a calorie-restriction (CR) regimen for yeast, is known to extend yeast lifespan. The positive lifespan impact of CR is not specific to yeast; CR extends lifespan and/or promises to extend healthspan in all known model organisms and humans,[7] although the exact mechanisms through which it causes lifespan/healthspan extension are not fully understood.

Liu et al. systematically characterized how yeast lifespan was affected by static glucose concentrations ranging from 0.02% to 5% and showed that 0.1% glucose gave rise to maximal lifespan extension for microfluidically grown yeast. It is important to highlight the microfluidic nature of their experimental platform, as it has recently been shown[8] that the type of aging platform used (microdissection on solid media vs. microfluidic) leads to significant differences in quantitative lifespan outcomes.

Interestingly, compared to 0.1% glucose, yeast lifespan was lower at 0.02% glucose despite normal growth rate and physiology mediated by 0.02% glucose, indicating the optimal nature of CR observed at 0.1% glucose. To analyze this phenomenon, the authors study a simple two-species model of the Sir2-HAP toggle-switch network: autoregulated Sir2 and HAP levels are dependent on external glucose, they mutually inhibit each other, and the dynamics of an abstract damage variable are in turn dependent on Sir2 and HAP levels (Figure 1A). The cell dies once this damage variable crosses a threshold. Cells starting from a "healthy" intermediate state of both Sir2 and HAP will ultimately converge toward one of two stable fixed points, as is characteristic of toggle-switch networks. The Mode 1 aging trajectory is defined by a terminal state of high HAP and low Sir2, whereas Mode 2 aging instead features high Sir2 and low HAP at cell death. Intriguingly, Liu et al. associate the increased longevity at 0.1% glucose to the emergence of a stable third state at intermediate levels of Sir2 and HAP, which they refer to as a "longevity fixed point" (LFP).

The longer cells occupy this third state before noise pushes them to one of the two terminal states, the better they avoid damage accumulation and death. Stochastic fluctuations of cells among the associated basins of attraction at a given level of external glucose, combined with accumulation of a damage variable in a manner that depends on Sir2 and HAP levels, thus provide a concise framework for interpreting their experimental data (Figure 1B).

To characterize the impact of dynamic glucose levels on the underlying Sir2-HAP state space, Liu et al. first ran stochastic simulations with glucose concentrations switching between 0.02% and 2%. With sufficiently high switching frequency, one observes a distribution of cell states with three peaks; the form of this "seascape" relates to a superposition of the 0.02% and 2.0% landscapes and resembles the static one found for 0.1% glucose. In this dynamic setting, Liu et al. refer to cells occupying the third state as being dynamically stabilized (DS). These predictions were supported by results from aging experiments performed under glucose oscillations, where roughly one-third of cells were found to be in a DS state. These DS cells displayed a much longer average lifespan (31 generations) compared to the Mode 1 and Mode 2 cells. The fraction of DS cells would be expected to increase and then plateau with increasing glucose switching frequency, and that was indeed the case.

Along the same lines, but without the need to augment the environmental conditions, a negative feedback loop synthetically introduced and tuned between Sir2 and HAP can induce limit-cycle dynamics in the Sir2-HAP state space, thus facilitating balanced oscillations between optimal Sir2 and HAP levels during aging. Indeed, a previous study published[9] by the same researchers has shown that extraordinarily long yeast lifespan could

be achieved through this negative feedback circuit by avoiding commitment to the terminal states.

There are noteworthy similarities between the current study's design focus on glucose fluctuations and intermittent fasting (IF) regimens frequently adhered to by humans due to their proven health benefits.[10] IF may involve an extended period of no or very low calorie intake followed by an unrestricted eating period. Properly adapted to the relevant timescales, dynamical systems modeling of the healthspan-affecting pathways operational in human cells can provide testable insights into the effects of varying IF frequencies and calorie levels on human healthspan.

A natural direction for improvement to the current study is the need for integration of the full set of lifespan extension pathways. For example, extending the current Sir2-HAP network by the TOR pathway could potentially help some of the experimental results better align with the computational predictions. For example, experimentally observed lifespan extension by Sir2 overexpression in 0.1% glucose was not as much as what was predicted by the model. Also, more comprehensive experimental characterizations are needed to understand why the average lifespan (24 generations) of the aging population containing DS cells under glucose fluctuations was slightly shorter than the average lifespan (26 generations) at the static 0.1% glucose condition.

Results from this study underscore the importance of considering environmental fluctuations in the study of aging. The findings reveal that dynamic environmental inputs can significantly influence the aging process and that it is possible to rationally reprogram aging trajectories by modulating these inputs. The study also provides valuable biological insights into the metabolic shifts associated with different aging phenotypes in fluctuating environments. Future research could build on these findings by exploring other environmental factors and their interactions with genetic networks. Additionally, studying the effects of environmental fluctuations in other model organisms and in more complex systems could provide further insights into the universality of these mechanisms. By continuing to integrate systems-level approaches and dynamical systems modeling applied to genetic circuits, researchers can uncover new strategies to promote healthy aging and extend lifespan across different species.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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