AGING

Genetic circuitry boosts cell longevity

Reprogramming cellular dynamics is used to study and delay the onset of aging in yeast

By **Howard M. Salis**

Over the past decade, cellular aging research has been accelerated by the identification of pathways that control the onset of age-associated cell states (the so-called hallmarks of aging) alongside the development of candidate therapeutics that attempt to delay or reverse the onset of aging (*1*). But what if cells were preprogrammed to undergo cellular aging? Cellular aging in yeast (*Saccharomyces cerevisiae*) was shown to be controlled by a genetic circuit that forces cells to either slow down heme biosynthesis, leading to mitochondrial dysfunction, or lose their ability to engage in chromatin silencing, leading to ribosomal DNA (rDNA) instability and fragmented nucleoli (*2*). Simple interventions to this evolutionarily conserved genetic circuit (e.g., overexpressing the key regulators) increased the cell's longevity by modest amounts. On page 376 of this issue, Zhou *et al.* (*3*) reveal that introducing designed genetic circuitry to rewire these dynamics increased cellular longevity by 80%.

The current paradigm for slowing or reversing aging is to develop therapeutics that restore natural pathway functions, push cells back to healthy states, or kill senescent (aged) cells (*4*, *5*). Such pathways combine gene regulatory, signaling, and metabolic interactions to control essential processes for maintaining healthy cell states, such as epigenetic silencing, mitochondrial function, protein homeostasis, telomerase activity, and autophagy. When these processes become dysregulated or disrupted, the effects can be widespread, increasing the risk and morbidity of several age-associated diseases (e.g., cancer, type 2 diabetes, arthritis, and Alzheimer's disease).

Zhou *et al.* controlled aging in yeast cells by manipulating the expression levels of two conserved transcriptional regulators [silent information regulator 2 (Sir2) and heme activator protein 4 (Hap4)]. Sir2 removes the acetyl group from acetylated lysines in histone H3 and H4, causing chromatin compaction and gene silencing (*6*). Sir2 has more specific silencing activity at the rDNA locus, where more than 100 copies of rDNA encode the genes for manufacturing ribosomes. Without Sir2, the loss of silencing causes disruption of the rDNA locus by triggering recombination, eventually creating fragmented nucleoli. By contrast, overexpression of Sir2 causes widespread gene silencing and cell toxicity. Hap4 is a transcriptional activator that increases heme biosynthesis and mitochondrial biogenesis (*7*). Without Hap4, yeast cells do not carry out respiration and exhibit widespread cell toxicity, whereas overexpression of Hap4 causes cells to have too many mitochondria, which wastes electrons and energy (*8*). The expression levels of Sir2 and Hap4 are coregulated by a genetic circuit such that Hap4 and Sir2 indirectly activate their own expression while also cross-repressing each other's expression, creating mutual inhibition (a toggle switch) (*2*). This natural genetic circuit causes aging yeast cells to commit to either mitochondrial dysfunction or rDNA instability, subject to random perturbations inside the cell and its environment.

"...rationally rewiring cellular dynamics is a potent way to delay cellular aging..."

To increase cell longevity, Zhou *et al.* applied dynamical systems theory and synthetic biology to engineer a new genetic circuit. Dynamical systems theory helped them understand how systems change over time and how small perturbations can have substantial effects, and tools from synthetic biology enabled them to rationally engineer the genetic circuit with the desired function. As a result, they engineered a circuit that causes cells to oscillate between high Sir2 or high Hap4 expression, preventing cells from committing to either dysfunctional state for an extended period. In this synthetic oscillator circuit, Hap4 activates Sir2 expression, whereas Sir2 represses Hap4 expression. They used fluorescent biomarkers and single-cell, time-lapsed microscopy to quantify genetic circuit function and measure longevity, comparing the effects of their engineered genetic circuitry with those of simpler genetic interventions. Yeast cells using their synthetic oscillator circuit had faster cell cycles and longer life spans than cells subject to other interventions, demonstrating that rationally rewiring cellular dynamics is a potent way to delay cellular aging and increase longevity.

How do these results affect the study of cellular aging in humans and the development of therapeutics? The many pathways that control cellular maintenance and aging are often depicted using static schematics, although they generate and in turn are controlled by emergent dynamical behaviors. Therapeutics perturb these dynamics, according to their binding activities and pharmacokinetics, in ways that remain challenging to understand, which is perhaps one reason why candidate antiaging therapeutics remain controversial. As Zhou *et al.* have demonstrated, a road to understanding and controlling cellular aging is to measure the dynamics of these pathways, develop system-wide models, and apply mathematical analysis to pinpoint the tunable knobs and swappable wires that can be manipulated to redirect a cell's natural dynamics away from aging and toward the maintenance of healthy cell states. By combining system-wide models with engineered genetic systems (*9*–*12*), candidate therapeutics could be developed—for example, a small-molecule inhibitor that pushes cell dynamics away from dysfunctional states or a combination strategy that removes senescent cells and replaces them with improved cells through ex vivo therapy. System-wide models will also help clarify how the myriad environmental perturbations (such as circadian rhythms, diet, and stressors) and genetic backgrounds contribute to outcomes and off-target effects. If the collective objective of these interventions is to maintain healthier cell states, then the risk and morbidity of age-associated diseases will be reduced. Boosting cellular longevity and healthy life span might simply become a beneficial by-product. ■

Departments of Agricultural and Biological Engineering, Chemical Engineering, and Biomedical Engineering, Bioinformatics and Genomics Program, Pennsylvania State University, University Park, PA, USA. Email: salis@psu.edu

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