Synthetic biology

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Engineering an oscillating gene circuit to delay cellular ageing

The engineering of gene circuits to modulate cellular functions based on known genetic factors is hindered by the complexity of regulation of many biological systems. Zhou et al. provide proof-of-concept for reprogramming the complex process of ageing in yeast by engineering an oscillating gene circuit that prevents commitment to either of two terminal cell fates.

The lysine deacetylase Sir2 and the haem-activated protein (HAP) complex have been identified in *Saccharomyces cerevisiae* as well-conserved transcriptional regulators of ageing in eukaryotic cells. Sir2 mediates chromatin silencing at ribosomal DNA (rDNA), which maintains nucleolar integrity. HAP is required for haem biogenesis and mitochondrial function. Wild-type yeast cells progress to one of two terminal states as they age, involving either decreased rDNA silencing and nucleolar disruption or decreased haem abundance and mitochondrial dysfunction. Sir2 and HAP positively regulate their own expression and repress each other's expression, creating a switch that drives these mutually exclusive cell fates.

The authors proposed that introducing a stronger negative feedback loop between HAP and Sir2 could lead to cyclical oscillations of the two regulators and thus delay cell fate commitment. They used a computational model to predict that oscillations would be favoured by three criteria and engineered a synthetic gene circuit to fulfil these criteria. One, strong positive transcriptional regulation of *SIR2* by HAP was engineered by replacing the native promoter of *SIR2* with a *CYC1* promoter, which is activated by HAP. Two, to ensure the capacity for high levels of HAP transcription, endogenous *HAP4* was deleted and replaced with *HAP4* expressed under a strong, constitutive promoter (*TDH3*). Three, to enable transcriptional repression of HAP by Sir2, *HAP4* was integrated in rDNA.

Microfluidics coupled with time-lapse microscopy of single cells showed that engineered cells have oscillations of Sir2 protein (tagged with a fluorescent reporter) that are of much greater amplitude than Sir2 fluctuations in wild-type yeast. Of the engineered cells, 65% had Sir2 oscillations throughout their lifespan, whereas 35% of the engineered cells lost oscillatory behaviour later in their lifespan. Synthetic circuits that were engineered to lack one of the three criteria did not produce Sir2 oscillations.

Yeast with the synthetic oscillator had an 82% increase in lifespan overall compared with wild-type yeast, comprising a 105% increase in cells with oscillations throughout their lifespan and a 45% increase in cells that lose oscillations late in life. The synthetic oscillator strain had a faster cell cycle rate that was retained throughout the lifespan of cells with sustained Sir2 oscillations. Thus, maintaining Sir2 oscillation reduces ageing-associated cell cycle elongation and extends lifespan. Cells with twofold constitutive overexpression of Sir2 (to approximate the lifetime average abundance of Sir2 in the synthetic oscillator cells) together with Hap4 overexpression had a ~42% increase in lifespan over wild-type cells, which shows that oscillation of Sir2 not just its increased expression is an important factor contributing to prolonged lifespan.

To analyse the mechanism of delayed ageing, the authors monitored rDNA silencing using a green fluorescent reporter inserted at the rDNA locus (rDNA–GFP reporter), and tracked haem abundance using a protein whose fluorescence depends on haem catabolism (iRFP reporter). Half of wild-type cells had increased GFP fluorescence at late stages of ageing, indicating a loss of rDNA silencing; the other half had decreased iRFP fluorescence at late stages, indicating low haem abundance. In contrast to this bifurcation of cell states in wild-type yeast, synthetic oscillator cells had intermittent GFP and iRFP signals throughout their lifespan. Synthetic circuits without one of the three main feedback criteria resembled wild-type cells in that they were not able to maintain both Sir2-mediated rDNA silencing and HAP-mediated haem biogenesis with ageing.

Thus, the synthetic *SIR2–HAP4* gene oscillator extends cell longevity by maintaining homeostasis of nucleolar and mitochondrial functions. Of note, the synthetic oscillator strain had a longer lifespan than the longest-lived single and double mutants previously identified from genetic screens. This work lays the foundation for engineering increased longevity in more complex organisms as well as other complex biological processes.

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