Trends in Cell Biology

CellPress OPEN ACCESS

Review

# Design principles of gene circuits for longevity

Paula Godoy[1] and Nan Hao [1,2,3,*]

**Aging is a dynamic process that is driven by cellular damage and disruption of homeostatic gene regulatory networks (GRNs). Traditional studies often focus on individual genes, but understanding their interplay is key to unraveling the mechanisms of aging. This review explores the gene circuits that influence longevity and highlights the role of feedback loops in maintaining cellular balance. The SIR2–HAP circuit in yeast serves as a model to explore how mutual inhibition between pathways influences aging trajectories and how engineering stable fixed points or oscillations within these circuits can extend lifespan. Feedback loops crucial for maintaining homeostasis are also reviewed, and we highlight how their destabilization accelerates aging. By leveraging systems and synthetic biology, strategies are proposed that may stabilize these loops within single cells, thereby enhancing their resilience to aging-related damage.**

## Highlights

Systems-level approaches complement static insights by revealing how gene regulatory networks (GRNs) dynamically regulate cellular aging and promote longevity.

Observing the dynamics of heme biosynthesis and nucleolar silencing in budding yeast reveals distinct aging modes, and synthetic biology can significantly extend lifespan.

Negative feedback loops in aging-related processes such as proteostasis, energy metabolism, and DNA damage response pathways maintain cellular homeostasis and counteract aging-related deterioration.

Synthetic biology offers potential interventions to reinforce feedback circuits, thereby mitigating age-related diseases and promoting healthy aging.

## Unraveling gene regulatory networks

Although human lifespan has increased over time, this has not necessarily been accompanied by a corresponding improvement in the health of the elderly population [1,2]. Reducing the healthcare burden of aging requires novel approaches that are rooted in a deeper understanding of the biology of aging [3,4]. The aging process is driven by the accumulation of cellular and genetic damage, stemming from a complex interplay of intrinsic biological mechanisms and external environmental influences [4,5]. Earlier aging studies focused on individual genes or pathways in isolation and measured lifespan as a static endpoint [6–11]. As a result, how aging-related genes interact with one another and how these **gene regulatory networks** (**GRNs,** see Glossary) operate dynamically to drive aging remain significant unanswered challenges.

GRNs consist of nodes, that symbolize genes or regulatory elements, and edges, that depict the interactions or regulatory connections between these nodes (Figure 1). Highly connected nodes at the center of a GRN are the major orchestrators of the response of a cell to stimuli. The **dynamics** of these nodes can often be explained by focusing on a few key local interactions, namely subgraphs. This simplification facilitates mathematical modeling and permits simulations of dynamics under different **parameter** regimes. Network motifs are recurrent sub-GRNs, typically including up to four nodes, that have characterized behaviors [12,13]. Network motifs can be as simple as positive autoregulation which ensures the sustained activity of a node (Figure 1, top right). By contrast, mutual inhibition between two nodes can lead to two distinct cell fates (Figure 1, center right) where the system stabilizes in one of two states based on initial conditions but can also be extended to support quadrastable states [14]. The negative feedback loop is a motif that is especially crucial for ensuring homeostasis, and is activated by deviations from a set point that trigger mechanisms to counteract those changes (Figure 1, bottom right). These motifs are observed in many GRNs and are reinforced by redundant and compensatory pathways to increase the **resilience of the system** to perturbations.

Decoding the emergent behavior of aging-related GRNs sets the stage for rational design of new interventional strategies to mitigate age-related diseases and promote healthy **longevity**.

[1]Department of Molecular Biology, School of Biological Sciences, University of California San Diego, La Jolla, CA 92093, USA
[2]Synthetic Biology Institute, University of California San Diego, La Jolla, CA 92093, USA
[3]Department of Bioengineering, University of California San Diego, La Jolla, CA 92093, USA

*Correspondence: nhao@ucsd.edu (N. Hao).

However, the intricate nature of aging-related processes cannot be fully understood through traditional reductionist methods. Instead, systems-level approaches designed to analyze the nonlinear dynamics of gene circuits are required. In addition, such network-based approaches can be naturally integrated with synthetic biology to reveal the design principles of prolongevity strategies. This review focuses on a series of recent studies in the model organism *Saccharomyces cerevisiae* (baker's yeast). *S. cerevisiae* is a powerful model organism for studying aging because it shares evolutionarily conserved molecular mechanisms that are associated with longevity, is highly amenable to genetic manipulation, and, owing to its relatively short generation time, can reveal aging-related processes over multiple generations in a relatively short period. Yeast display several of the canonical hallmarks of aging including dysregulation of nutrient sensing [15,16], mitochondrial dysfunction [17–19], loss of proteostasis [20–22], genomic instability [23–27], and others [28]. We review how these studies demonstrate that a dynamic systems approach can bring new insights to aging biology, followed by discussing the potential applications of this approach to different aging-related networks in yeast and mammals.

## An example of a pipeline for the assembly and verification of a GRN

In decoding the regulatory relationships of a GRN, researchers can infer not only the direct interactions between nodes but also speculate on their emergent and dynamic behaviors. For instance, a negative feedback loop might suggest oscillatory dynamics between nodes A and B, where the system alternates between an $A^{high}B^{low}$ state and the reverse (Figure 1, bottom right). Such insights are most accurately derived from a combination of methods. This section provides an example of a pipeline for constructing GRNs that incorporate both static and dynamic information.

Generating novel or utilizing publicly available bulk or single-cell RNA-sequencing (scRNA-seq) data is an excellent starting point owing to its high dimensionality. For example, bulk RNA-sequencing performed before and after a perturbation can be analyzed using weighted gene coexpression network analysis (WGCNA) to identify gene modules based on coexpression patterns that become differentially expressed in response to a perturbation [29]. These modules can then be input into pathway analysis algorithms such as gene set enrichment analysis (GSEA) [30] to determine enriched biological pathways and processes.

If scRNA-seq data are available, tools such as SCENIC and SCENIC+ add an additional layer of information by incorporating transcription factor (TF) binding and target gene interactions to generate coexpression modules specific to subpopulations within heterogeneous samples [31,32]. For example, by analyzing the covariability between *cis*-regulatory elements and gene expression, researchers identified GRNs that encode cell type-specific gene expression in fly brain [33]. Gene expression trajectories can be inferred using tools such as Monocle [34] and scVelo [35] to elucidate how dynamics differ during branching events that lead to the differentiation of distinct cell types. Pathway analysis and tools such as MetaFlux [36] can predict pertinent cellular processes for distinct cell types.

To summarize, at this point in the pipeline, the following have been identified: (i) differentially regulated genes and regulons at specific timepoints, (ii) key regulators of cell trajectories and gene expression dynamics, and (iii) pathways associated with these timepoints. This information can provide the starting point for generating ordinary differential equations (ODEs) that describe the system. For example, TFs identified through SCENIC+ as key regulators can be modeled as activators or repressors in ODEs, and their target gene expression levels described by production and degradation rates can be inferred from Monocle or scVelo. Additional nodes that represent larger metabolic pathways can be incorporated with the appropriate regulatory interactions

## Glossary

**Dynamic range:** the range of activity levels a gene or network can achieve, from its lowest (or baseline) level to its highest possible level in response to a stimulus or signal.
**Dynamics:** the changes in node activity over time. For example, a gene can turn on or off and have different magnitudes of its expression/activity. Dynamics can be nonlinear, for example oscillations or fluctuations in response to different conditions or signals.
**Gene regulatory networks (GRNs):** a 'wiring diagram' that illustrates the interactions between genes and their regulatory elements. In GRNs, genes are referred to as nodes and the interaction between two nodes is referred to as an edge. These interactions control the levels of gene expression or protein activity, determine how a cell responds to various stimuli, and coordinate its biological processes.
**Gompertz law:** a mathematical model describing the exponential increase in mortality rate with age, commonly used in aging research.
**Limit cycle:** a closed trajectory in phase space that represents sustained oscillations with neither decay nor growth. As an isolated trajectory, neighboring paths either spiral towards or diverge away from it. In biology, it often describes a pattern of behavior where the activity of particular genes or proteins repeatedly cycles over time in a stable and predictable way; for example, the expression of genes involved in the circadian rhythm oscillates in a 24 h cycle.
**Longevity:** the length of time an organism lives, as well as its ability to maintain good health and function during that time (i.e., healthspan).
**Parameter:** in mathematical modeling, a parameter is a fixed value used in the model to describe specific aspects of the system being studied. Parameters act like the settings or 'dials' that define how nodes behave and interact over time.
**Replicative aging:** in yeast, this refers to the number of times a mother cell can divide to produce daughter cells before it stops dividing and enters senescence. Yeast cells are asymmetrically dividing organisms, meaning the mother cell retains particular aging-related factors while the daughter cell starts 'fresh'.
**Resilience:** the ability of a node to maintain stable expression levels or

deduced from coexpression of genes within pathways. Once a GRN has been assembled, its dynamic behavior can be simulated with COPASI [37] or PySB [38] for ODE-based simulations, or by using stochastic frameworks such as StochPy [39] to capture cell-to-cell variability in gene expression.

Researchers can then follow up on central regulators by single-cell imaging. By using endogenous tagging, fluorescent reporters, dyes, or biosensors, the dynamics of these nodes can be revealed through single-cell time-lapse imaging [40]. For example, to capture the dynamics of a TF identified by SCENIC+, one can use as a proxy the expression of a fluorescent protein driven from a promoter recognized by that TF. For nodes that encompass multiprotein kinases that are largely regulated through post-translational modifications, biosensors are a good option [41]. Researchers can also use dyes that report on metabolic activity, such as tetramethylrhodamine (TMRM or TMRE) which exhibits increased fluorescence with higher mitochondrial membrane potential. The data collected from imaging the dynamics of these processes in response to a perturbation can be used to constrain the parameters of the ODE, thereby refining the model. Once the model has been optimized it can reveal crucial information about the stability of cellular states – for example, whether oscillations within the GRN represent a stable **limit cycle** or transient fluctuations between unstable points, and which specific cell states are maintained over time.

## Dynamic systems analysis to gene circuits in cellular aging

The **replicative aging** of the budding yeast *S. cerevisiae* has proved to be a genetically tractable model for the aging of mitotic cell types in mammals and has led to identification of many conserved genes that influence longevity [42]. By contrast, the study of chronological aging in *S. cerevisiae,* namely the lifespan of a non-dividing cell, provides complementary insights, especially regarding postmitotic cells such as neurons [43]. This paper focuses on gene circuits that extend replicative aging to explore its contributions to mitotic cell aging.

Over the past 7 years the integration of high-throughput dynamic measurements with computational modeling has been used to investigate the network-driven dynamics of yeast aging [44–50]. This work revealed that genetically identical yeast cells age through two different trajectories that display distinct phenotypic changes – one with ribosomal DNA (rDNA) silencing loss and nucleolar decline (Figure 2, designated as mode 1 aging), and the other with heme depletion and mitochondrial decline (Figure 2, mode 2 aging). The divergent progression towards mode 1

recover its normal patterns of activity following disruptions caused by environmental changes, stress, or damage. This resilience is supported by multiple direct and indirect interactions with other nodes in the network which help to buffer against disturbances and restore balance.

**Robustness:** the ability of a GRN to maintain stable and proper functioning despite challenges such as cellular stressors, genetic mutations, or noise in gene expression. For example, a GRN may be considered to be robust if removal of a node within that GRN does not alter its ability to respond to a stimulus.

**Senescence:** a state in which a cell stops dividing and growing but remains alive and metabolically active.

**Senescence-associated secretory phenotype (SASP) factors:** the molecules that senescent cells release into their surroundings, including proteins that induce inflammatory signals.

**Stable fixed point:** a state where the activity levels of genes or proteins settle into a constant value and remain there, no matter the initial conditions or small disturbances.

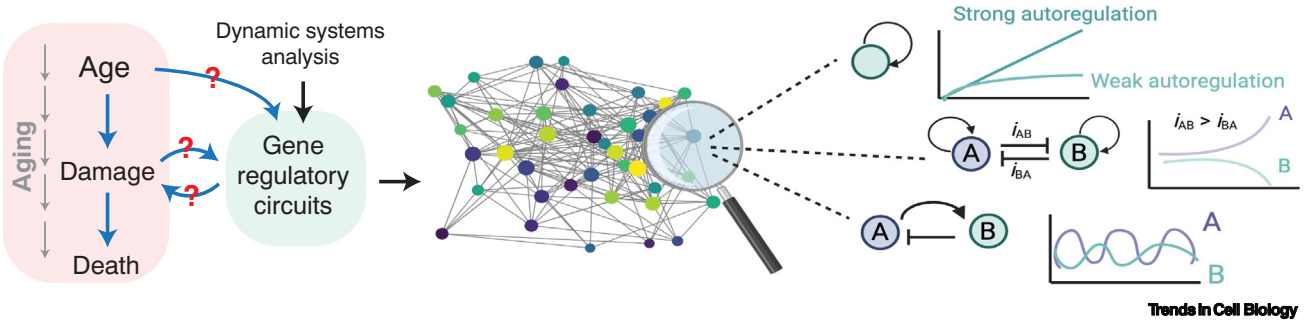


Figure 1. Representation of the aging process through subnetworks of gene regulatory networks (GRNs). Dynamic systems analysis can be used to model the complex interplay between GRNs in the aging process. GRNs are composed of nodes (genes or regulatory elements, depicted as circles, e.g., node A and node B) and edges (interactions or regulatory relationships between these nodes, depicted as lines, e.g., $i_{AB}$ is the regulation of node A onto node B). Damage accumulation and changes in gene regulation are key factors that drive aging, and the GRNs that respond to such perturbations can be broken down into smaller and more manageable subnetworks (sub-GRNs). These sub-GRNs typically consist of 1–4 nodes (circles) and their relationships (arrows) with other nodes. Sub-GRNs that occur consistently within GRNs are termed network motifs, which give rise to important temporal behaviors such as sustained activity, bimodality, and oscillatory behavior. These dynamical behaviors then lead to a specific response or change in the cellular state.

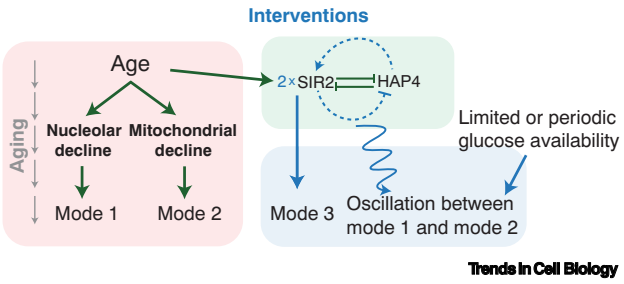


Figure 2. Dual aging trajectories and engineered longevity interventions in *Saccharomyces cerevisiae*. Replicative aging of *S. cerevisiae* proceeds through two distinct aging trajectories: mode 1, characterized by nucleolar decline and ribosomal DNA (rDNA) silencing loss, and mode 2, marked by mitochondrial decline and heme depletion. The balance between these two aging modes is governed by a mutual inhibition circuit between SIR2, a lysine deacetylase involved in rDNA silencing, and HAP, a complex that regulates heme biosynthesis and mitochondrial function. Genetic interventions designed to overexpress SIR2 result in a new aging mode (mode 3). Genetically rewiring the SIR2–HAP circuit into a negative feedback loop led to longevity with intermediate SIR2 and HAP levels (oscillating between mode 1 and mode 2) and thus to significant lifespan extension. This was also evident in cases of limited or periodic glucose availability. These findings demonstrate how genetic manipulations can modulate aging trajectories in yeast, and offer a framework for studying longevity interventions that could be further investigated in other organisms.

versus mode 2 aging is governed by a mutual inhibition circuit of sirtuin 2 (SIR2) and heme-activated protein (HAP) in which the lysine deacetylase SIR2 mediates rDNA silencing to maintain nucleolar stability [25,51], whereas the HAP complex controls the expression of genes required for heme biogenesis and mitochondrial function [52].

A mathematical model composed of two ordinary differential equations revealed that mutual inhibition between SIR2 and HAP gives rise to multiple different **stable fixed points** (or steady states) on the SIR2–HAP landscape [46]. The aging process of each single cell can thus be viewed as divergent progression towards these steady states – progression towards the low-SIR2 high-HAP state corresponds to mode 1 aging with rDNA silencing loss and nucleolar decline, whereas trajectories towards the low-HAP state correspond to mode 2 aging featuring mitochondrial deterioration [46].

This dynamical model not only serves to understand the natural aging processes but can also guide the design of interventional strategies for longevity. The main objective of such interventions is to prevent or at least delay progression towards the detrimental ending states of either mode 1 or mode 2 aging that are linked to rapid damage accumulation and cell death. This task is particularly challenging because elevating either SIR2 or HAP, the two opposing longevity factors, tends to push the cell towards the alternative aging path, which ultimately leads to cellular decline and death. Therefore, effective interventions must navigate this delicate balance to avoid inadvertently accelerating the aging process by tipping the system too far in either direction. From a dynamical systems perspective, this requires the creation of a new stable fixed point or a limit cycle around the healthy, young state with intermediate SIR2 and HAP levels. Under the guidance of modeling, this has been realized experimentally by genetic engineering and synthetic biology [46,49].

Genetic perturbations of SIR2 and HAP were performed to create a new stable fixed point for longevity within the SIR2–HAP space. It was found that twofold overexpression of SIR2 generated a new long-lived aging mode that concluded at a state characterized by intermediate levels of SIR2 and HAP (Figure 2, mode 3 aging). This observation was reproduced by the model in which twofold elevation in SIR2 level can partially counteract the inhibition from HAP, leading to the emergence of a new stable fixed point which corresponds to experimentally observed mode 3 aging. The model further predicted that an additional elevation in HAP under these conditions can stabilize this new stable fixed point and enrich mode 3 cells in an aging population. This prediction was validated experimentally, and resulted in a ~60% increase in lifespan [46].

In a separate study, to create a limit cycle for longevity, the endogenous SIR2–HAP circuit was genetically rewired into a negative feedback loop which can theoretically produce sustained oscillations in SIR2 and HAP levels. To accomplish this experimentally, the native promoter of *SIR2* was replaced by the HAP-inducible *CYC1* promoter to introduce positive transcriptional regulation of *SIR2* by HAP. To enable transcriptional inhibition of HAP by SIR2, a construct containing the *HAP4* gene (encoding a major component of the HAP complex) was inserted under a constitutive promoter into the rDNA region which is subject to transcriptional silencing mediated by SIR2. The rewired strain exhibited oscillations in SIR2 and periodic cycling of rDNA silencing and heme biogenesis during aging, without a prolonged commitment to either rDNA silencing loss (mode 1) or heme depletion (mode 2). This resulted in an 82% increase in lifespan – a record for yeast lifespan extension by interventions [49] (Figure 2). This work established, for the first time, the causal connection between gene network architecture and cellular longevity.

The stable fixed point for longevity can also be created by modulating environmental glucose levels. Previous studies showed that glucose limitation can increase the activities of both SIR2 and HAP [53–58] and can extend lifespan in yeast and other model organisms [59], raising the possibility that such environmental alterations could give rise to a longevity stable fixed point similar to the one created by gene overexpression. To test this, the effect on yeast aging of glucose concentrations ranging from 5% to 0.02% was systematically evaluated. Changes in glucose levels were found to modulate SIR2 and HAP activities with different dose–response relationships and thereby influence the balance of the two factors and the fate decision of aging – decreasing glucose level biases the fate commitment towards mode 1 aging. Intriguingly, 0.1% glucose leads to a subtle equilibrium between SIR2 and HAP at intermediate levels, resulting in the emergence of a longevity stable fixed point and therefore an optimal lifespan extension effect compared to other glucose levels tested. Moreover, it was shown both by theory and experiment that periodic oscillations of external glucose levels can also enable a dynamic stabilization of the system around intermediate SIR2 and HAP levels, leading to an extended lifespan without creating the longevity stable fixed point [50]. Efforts are ongoing to expand the SIR2–HAP circuit to more effectively simulate yeast aging trajectories, including the identification of novel modes of aging. This includes leveraging sequencing and imaging approaches, as discussed in the previous section, and elucidating whether there is bifurcation in the dynamics of additional pathways associated with aging (see Outstanding questions). The lifespan-extending effects of SIRT1, the mammalian homolog of SIR2, have also been observed in mice when SIRT1 is activated either pharmacologically [60] or through brain-specific overexpression [61] in mice on a standard diet, suggesting that it is relevant to mammalian aging models.

In addition to work on the SIR2–HAP circuit in aging, there a growing number of studies have used a systems approach to unravel the emergent quantitative properties of aging. For example, the group of Uri Alon developed a paradigmatic model to simulate the dynamics of damage accumulation and cell death. In their model, the aging process can be described as a competition between accelerating damage accumulation and saturating damage removal in which cell death occurs when the damage level exceeds a particular threshold. This model can help to quantitatively explain the characteristic dynamic features of aging, such as the **Gompertz law** [62], and can recapitulate aging dynamics and survival curves across different organisms [63–65]. The group of Murat Acar used microfluidics-based time-lapse imaging to monitor replicative aging in yeast cells and revealed emergent single-cell properties during aging, such as transcriptional noise reduction [66] and lifespan scalability ([67], reviewed in [68,69]). A more comprehensive review on systems analysis of aging across different scales from single cells to human physiology is provided by Cohen *et al.* [70].

## A general design principle for gene circuits related to longevity

A general theme from the studies discussed in the preceding text can be summarized as follows: aging drives a deviation of important functional proteins from their 'healthy' ranges, leading to cell deterioration and eventually cell death. Gene circuits embedded around these key factors can either accelerate or decelerate this process. Among these circuits, negative feedback loops function to dynamically counteract the effects of aging and thereby could serve as a general design principle for longevity. In many cases, such feedback loops can give rise to oscillations in the level of key functional factors, thereby enabling dynamic homeostasis around the healthy state. This section discusses different pathways that contain endogenous negative feedback loops with a potential prolongevity function. Speculation is offered on how aging may disrupt these feedback circuits, leading to functional decline in aged cells. Strategies are proposed to enhance the **robustness** and resilience of these circuits that may mitigate the effects of aging.

### Proteasome feedback circuit

Loss of protein homeostasis is a conserved hallmark of aging [4,71] and is associated with neurological age-related pathologies such as Alzheimer's disease and Parkinson's disease [72]. Studies in model organisms have revealed that damaged/misfolded proteins accumulate in aging cells, causing cell deterioration and age-related pathologies [73,74]. The proteasome is a multisubunit proteolytic complex that degrades proteins marked by the attachment of small ubiquitin peptides which functions as the primary intracellular machinery for removing damaged proteins in aging [75].

Proteasomal activity is a conserved mechanism for protein degradation in eukaryotes, and its capacity is modulated in response to the level of damaged proteins through intricate feedback mechanisms. For example, in yeast, the abundance of the proteasome is regulated by a negative feedback loop [76]. In this case, RPN4 is a transcriptional activator required for expression of the genes encoding proteasomal components. However, it is also a target for degradation by the assembled, active proteasome, and is therefore extremely short-lived (Figure 3). Under normal

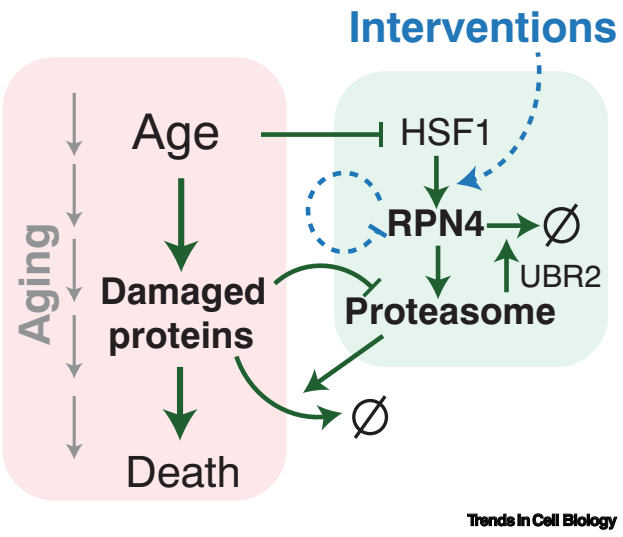


Figure 3. Regulation of the proteasome feedback loop and its deterioration with age. The proteasome is a multisubunit complex responsible for degrading damaged proteins to maintain protein homeostasis. RPN4, a key transcriptional activator, upregulates the expression of proteasome genes and is degraded by the active proteasome, thereby forming a negative feedback loop. As damaged proteins accumulate, RPN4 degradation decreases, which in turn increases proteasome expression to restore balance. However, during aging, the transcriptional capacity of proteasome genes decreases owing to disruptions of regulatory elements such as RPN4 and transcriptional activators such as HSF1. This breakdown leads to reduced proteasome activity, causing further protein damage accumulation and ultimately cell death. Interventions are proposed to enhance the resilience of the proteasome feedback loop. These include engineering *RPN4* and proteasome gene promoters to increase their transcriptional capacity, thereby introducing an alternative negative feedback loop (blue dashed lines) that is less sensitive to age-related transcriptional declines, potentially leading to sustained proteostasis which may delay aging. Ø denotes degradation of protein.

physiological conditions this feedback circuit maintains homeostasis between the proteasome and damaged proteins. An increase in the amount of damaged proteins, which compete with RPN4 for the existing proteasome pool, can reduce RPN4 degradation and thereby elevate RPN4 protein levels. RPN4, in turn, upregulates the amount of the proteasomes to remove excess damaged proteins. Once damaged proteins are cleared, RPN4 is rapidly degraded by the proteasomes, bringing the proteasome pool back to its basal level.

In the early phases of aging, this feedback circuit functions to remove age-induced damaged proteins and maintain proteasome homeostasis. However, during aging, reduced transcriptional capacity of proteasome genes compromises this GRN [77,78]. scRNA-seq over the lifespan of *C. elegans* revealed that the most commonly downregulated genes across cell types were those involved in proteostasis within the endoplasmic reticulum (ER), including the chaperone HSP4/BiP [78]. An age-dependent decline in the activity of heat shock factor 1 (HSF1), a conserved transcriptional activator of the proteasome genes [79], has also been observed in various tissues and organisms [80–82]. These age-induced changes take effect together and push the feedback system out of its homeostatic regime, leading to further reduced proteolytic activity and uncontrolled protein damage accumulation and eventually cell death, as observed in various types of organisms [73,83–90] In support of this scenario, deletion of *RPN4*, leading to a reduced proteasome pool, shortens yeast lifespan, whereas deletion of *UBR2*, encoding a ubiquitin ligase that mediates RPN4 degradation by the proteasome, leads to elevated proteasome capacity [91] and extends lifespan [92].

A brute-force strategy to overcome the effects of aging is to simply increase the proteasome pool by gene deletion or overexpression, which can indeed lead to lifespan extension to some extent [92]. However, constitutive overexpression or overactivation of the proteasome has detrimental side effects and impairs cell growth and viability [93,94]. Therefore, a more delicate and effective strategy to enhance the resilience of the proteasome feedback circuit can be achieved by engineering the promoters of key factors in the circuit. For example, increasing the transcriptional capacity and **dynamic range** of RPN4 and proteasome genes may endow a more robust response against age-induced alterations (Figure 3, blue dashed lines). Alternatively, an orthogonal negative feedback loop that is less sensitive to the effects of aging can be introduced to drive the expression of proteasome genes. Such efforts can be built upon previous work that created synthetic gene oscillators using standardized transcriptional control elements [95–97].

### Energy homeostasis circuit

Metabolic reprogramming is associated with aging, and modulating energy intake or metabolic rate can dramatically influence longevity [98–100]. For example, mTORC1 is well known to be negatively associated with lifespan [101]. Deletion of *TOR1* in yeast extends lifespan [102]. Depleting mTORC1 activity either through deletion of the gene encoding its downstream effector S6 kinase or by introducing a hypomorph [103,104] leads to increased survival in mice. Pharmacological inhibition of mTORC1 by rapamycin has shown increase in lifespan in yeast, worms, flies, and mouse [105].

It was recently found that the divergent aging trajectories of single yeast cells are associated with distinct metabolic changes – mode 1 aging features a transition from fermentation to respiration, whereas mode 2 aging features enhanced glycolysis and suppressed respiration which may result in changes to intracellular ATP [50]. Importantly, many studies showed that ATP levels decline with age in various organisms, indicating that loss of energy homeostasis is a major hallmark of aging [50,106,107].

Maintaining energy homeostasis requires a balance between ATP production and consumption. AMP-activated protein kinase (AMPK) is a primary energy sensor that is conserved throughout eukaryotes [108–110]. AMPK senses increases in the intracellular ratio of AMP/ATP and promotes catabolic pathways to generate ATP. Under normal physiological conditions when ATP is sufficient, AMPK is inactive. However, when the intracellular ATP level goes down, AMPK becomes active and phosphorylates metabolic enzymes and TFs to promote ATP-producing catabolic pathways and inhibit ATP-consuming biosynthetic pathways [111] (Figure 4). AMPK is switched off when the ATP level is restored to its normal state.

In the early phases of aging, the AMPK pathway is sufficient to counteract age-induced ATP changes and maintain energy homeostasis of the cell. During aging, mitochondrial dysfunction causes a substantial decrease in ATP production. However, at the same time, the responsiveness of AMPK declines with age [112–116]. Although the mechanisms underlying this reduction remain unclear, they may relate to age-dependent elevation in the expression or activity of protein phosphatases that function to turn off AMPK [116]. The decreased sensitivity of AMPK breaks the balance between ATP sensing and production, resulting in uncontrolled energy deficiency in aged cells. In agreement, boosting AMPK activity slows aging and extends lifespan [117–119], whereas deletion or inhibition of AMPK shortens lifespan [120].

Similarly to SIR2, HAP, and proteasomes, although AMPK is generally considered to be a prolongevity factor, constitutive overexpression or overactivation of AMPK can elicit negative effects on various physiological processes [108,121] and lifespan [120]. A potential strategy against the effects of aging could be to engineer a robust negative feedback loop to drive the expression of AMPK. This could be achieved by introducing an additional copy of the *AMPK* gene under an the control of an AMPK-repressible promoter (Figure 4). Governed by this circuit, the expression of AMPK can be dynamically adjusted based on its activity and can counteract its reduced responsiveness in aged cells.

### p53 feedback circuit

p53 (also known as TP53) is a central TF within the GRN that responds to DNA damage, and has therefore been dubbed the guardian of the genome. The major regulator of p53 dynamics is its transcriptional target, *MDM2,* encoding the repressor MDM2, yielding a negative feedback loop

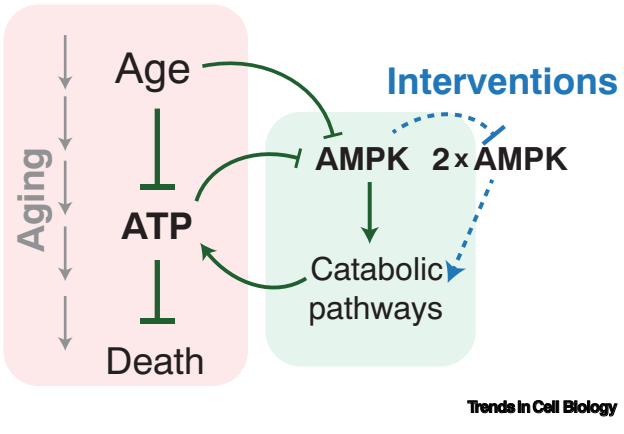


Figure 4. Regulation of AMPK and its role in maintaining energy homeostasis during aging. When intracellular ATP levels drop, AMPK becomes active and promotes ATP-producing catabolic pathways while inhibiting ATP-consuming biosynthetic pathways. During early aging, this system remains effective. However, as aging proceeds, mitochondrial dysfunction reduces ATP production, and AMPK responsiveness declines, leading to energy imbalances and cell death. Aging drives the dysfunction of energy production, whereas AMPK promotes catabolic pathways to restore ATP levels. Decreased AMPK sensitivity disrupts energy balance, pushing the cell towards death. A proposed intervention (blue dashed lines) includes introducing an additional *AMPK* gene copy under a negative feedback loop to dynamically regulate AMPK expression and counteract its age-related decline in activity. This intervention aims to restore energy balance while preventing the detrimental effects of AMPK overexpression on longevity.

(Figure 5). p53 was predicted and later experimentally verified to oscillate in response to DNA damage [122–126]. When a DNA double-stranded break (DSB) occurs, the MRN complex (including MRE11, RAD50, and NBS1) recognizes the break and recruits ATM to the site [127–129]. Upon recruitment, ATM becomes autophosphorylated and activated as a serine/threonine kinase [130,131]. Phosphorylated ATM then upregulates p53 levels by inhibiting MDM2, thereby preventing p53 ubiquitination and degradation, and, by directly phosphorylating p53, enhances its stability and activity [132,133]. p53 subsequently promotes the transcription of hundreds of genes in a cell type-dependent manner [134], including the gene encoding its negative regulator MDM2. The promoter of the *MDM2* gene has a relatively low activation threshold that makes it highly sensitive to p53 pulse duration [135], thereby enforcing p53 homeostasis. If there is continual stabilization of p53 by ATM, the cycle occurs again, yielding the observed p53 oscillatory behavior. These oscillations enable repetitive surveillance and maintenance of DNA integrity in the form of G1 and G2 cell-cycle arrest without surpassing the thresholds that would trigger apoptosis or **senescence** [136] (Figure 5). In mathematical models, the parameters that influence the rates of protein production and degradation, particularly of MDM2, can explain the differences in pulse amplitudes [123,124]. Experimentally, p53 pulses can be modulated by the addition of nutlin 3, an inhibitor of MDM2, demonstrating that MDM2 is the major influencer of p53 oscillatory dynamics [135].

The p53 network includes positive and negative feedback mechanisms to precisely regulate p53 activity; negative feedback prevents p53 hyperactivity, whereas positive feedback supports the crucial role of p53 in activating DNA damage response pathways. Therefore, disrupting members of the p53 network that regulate its activity has significant consequences on health and lifespan. Deletion or inactivation of *TP53* is observed in more than half of all cancer types, and mice and zebrafish with genetic deletion of *Tp53* often develop various types of tumors [137,138]. By contrast, mutations that result in hyperactivity of p53, such as truncations at the C-terminus, can lead to a significant reduction in lifespan and are associated with aging-related characteristics [139–141]. p53 therefore has a Goldilocks effect in which its activity must be precisely regulated – not too little, to prevent uncontrolled cell proliferation and cancer, and not too much, to avoid excessive cell death and accelerated aging. This is in part exemplified by mouse models with mutations that weaken p53 because the mice that do not develop tumors tend to have longer lifespans [142].

Germline mutations in *MDM2* can result in premature aging as a result of increased p53 activity [143]. Specifically, targeted *Mdm2* deletion in the epidermis has been shown to cause premature

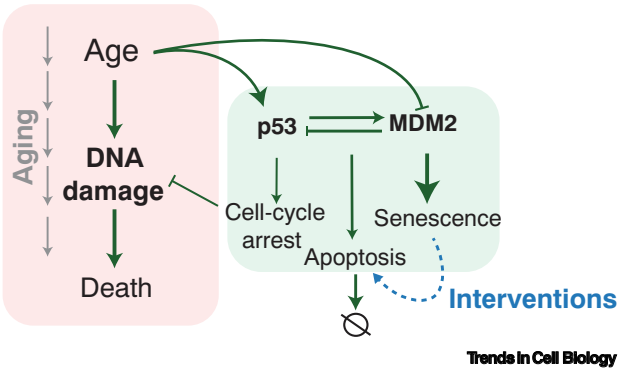


Figure 5. The p53–MDM2 negative feedback loop displays an aging-dependent bias towards senescence. The central role of the p53–MDM2 negative feedback loop is in maintaining genomic stability and regulating cell fate decisions in response to DNA damage. p53 activates the transcription of *MDM2*, thereby forming a feedback loop that limits p53 activity and prevents its overactivation. Oscillatory p53 dynamics enable repeated surveillance of damaged DNA, thus promoting cell-cycle arrest to allow repair or initiating apoptosis if the damage is irreparable. Prolonged or excessive p53 activation due to persistent or excessive DNA damage can trigger apoptosis or senescence. However, aging appears to bias cell fate towards senescence, leading to the senescence-associated secretory phenotype (SASP) and inflammaging. Targeting the p53–MDM2 interaction to modulate senescence and apoptosis presents a potential strategy to mitigate the increase of senescent cells observed throughout aging. Ø denotes cell death.

skin aging [144]. Pharmacological inhibition of MDM2 decreases the activity of senescent cells in primary human fibroblasts and reduces the production of **senescence-associated secretory phenotype (SASP) factors** [145]. Mice with *Mdm2* mutations that reduce the ability of MDM2 to inhibit p53 exhibit premature aging phenotypes [146]. Disrupting the ability of p53 to bind to MDM2 reduced the number of senescent cells in skeletal muscle and enhanced its regeneration and repair [147]. In human, protein produced from a common splice variant of *MDM2, MDM2-a*, that was identified in both tumors and normal tissues, can bind to full-length MDM2 and inhibit the p53–MDM2 interaction, leading to a reduced lifespan of ~20% [148–150].

In the event of weakened negative feedback or stronger positive feedback, p53 displays dynamics that may induce senescence. Although senescence is an important safeguard to prevent tumor formation, the SASP has detrimental consequences [151]. Senescent cells are themselves one of the 12 hallmarks of aging because the number of senescent cells increases throughout lifespan [4,152–154]. This increase is accompanied by chronic inflammation due to the SASP and can lead to various chronic disorders and tissue dysfunction.

RNA sequencing of cells that enter senescence exposed to ionizing radiation revealed that upregulation of *FOXO4* sustains the survival of senescent cells despite the presence of proapoptotic genes such as *PUMA* and *BIM* [155]. Interestingly, FOXO4 has been shown to colocalize with promyelocytic leukemia (PML) bodies, nuclear structures that are a hallmark of senescence and are known to restrain p53 activity [155–157]. Inhibiting FOXO4 allows p53 to be released from the nucleus, enabling its translocation to the mitochondria, where it can interact with apoptotic machinery and trigger cell death. Therefore, downregulation of factors such as FOXO4 in senescent cells, which are necessary for inducing and maintaining senescence, is a strategy which may enable apoptosis (Figure 5). FOXOs are a family of TFs that activate GRNs associated with stress responses [158]. In yeast, overexpression of FOXO TFs extends lifespan whereas deletion shortens lifespan [159]. Overexpression of FOXO1 in mice does not significantly alter lifespan; however, because FOXO activity is suppressed by insulin/IGF-1 signaling, many lifespan-extending interventions in mice, including a brain-specific mutation and nutrient limitation, target this pathway [160].

Another approach could be to modulate cell–cell communication between senescent cells and neighboring cells, an approach that uses agents known as senomorphics [161]. In this approach, instead of promoting apoptosis, the goal is to prevent the secretion of soluble factors, including proinflammatory cytokines (e.g., IL-6, IL-8, and IL-11) [162–164]. IL-11, for example, can activate the ERK–mTOR pathway in cells to induce senescence. Repressing the expression of these cytokines specifically in senescent cells could be programmed by utilizing the promoters of genes that specifically respond to sustained p53 signaling such as *PML* [136] or *CDKN1A* [135] to drive the expression of IL-10, which may aid in attenuating the inflammatory response of senescent cells [165].

## Concluding remarks

GRNs orchestrate highly tuned, evolutionarily conserved responses to a variety of stimuli. These networks are dynamic, and respond not only to binary signals but also to more nuanced factors such as the amplitude, duration, and gradients of stimuli or TFs. Aging, that is often characterized by a breakdown of these regulatory systems, provides a unique opportunity to understand and potentially optimize these dynamics for improving health.

Throughout the lifespan, regulatory circuits such as the SIR2–HAP circuit in yeast govern crucial cellular processes to maintain a balance between cellular states such as rDNA silencing and

## Outstanding questions

As illustrated by the divergence between nucleolar silencing and heme biosynthesis in yeast, how do the interactions between the aging-related GRNs discussed here and other networks shape distinct aging trajectories, and what regulatory mechanisms underlie these interactions?

What is the cell type-specific resilience of negative feedback loops, and which compensatory pathways are the first to deteriorate during aging across different tissues?

How does cellular heterogeneity within tissues contribute to aging, and can targeting specific cell populations slow down or reverse tissue aging?

For longer-lived organisms, how can we integrate single-cell technologies to predict the dynamic activities of GRNs and their eventual breakdown during aging?

mitochondrial function. However, as cells age, this balance deteriorates, pushing cells into detrimental modes of regulation, and ultimately leading to death. By studying these breakdowns in regulatory circuits it is possible to identify points of intervention, thereby enabling synthetic engineering of prolonged lifespan through targeted regulation of these circuits.

Aging-related negative feedback circuits maintain cellular homeostasis through push-and-pull mechanisms, and failure to remain resilient can accelerate aging (see Outstanding questions). Approaches to enhance the robustness of these circuits, either through synthetic biology or nutrient limitation, can extend the resilience of cells to the accumulation of age-related damage. Notably, these circuits do not act in isolation; they are interconnected, as seen in the SIR2–HAP system, where shifts in one pathway influence others. In addition, pathways do not only affect one another in a cell-intrinsic manner but also affect neighboring cells, including stem cells and cell types from other tissues. These cell-intrinsic pathways can collectively contribute to aging in a cell-extrinsic manner.

For example, hematopoietic stem cells (HSCs) are a well-studied population that undergo cell-intrinsic aging while also exerting systemic effects on overall organismal aging, including reduced immune responses and changes in HSC fitness [166]. HSCs can be genetically modified, thereby enabling researchers to perturb these systems and examine their emergent effects on lifespan [167]. By focusing on these stem cell populations, researchers can model aging trajectories and test interventions without introducing excessive complexity generated by the multitude of cell types, interactions, and tissues in multicellular organisms. These approaches provide a scalable way to study aging while linking cellular and systemic perspectives.

## Acknowledgments

We thank Dr Lorraine Pillus for carefully reading the manuscript and providing insightful comments. This work was supported by National Institutes of Health (NIH) grants R01AG056440, R01GM144595, R01AG068112, and R01AG086348.

## Declaration of interests

The authors declare no competing interests.

## References

1. Partridge, L. *et al.* (2018) Facing up to the global challenges of ageing. *Nature* 561, 45–56
2. Campisi, J. *et al.* (2019) From discoveries in ageing research to therapeutics for healthy ageing. *Nature* 571, 183–192
3. Kennedy, B.K. *et al.* (2014) Geroscience: linking aging to chronic disease. *Cell* 159, 709–713
4. López-Otín, C. *et al.* (2023) Hallmarks of aging: an expanding universe. *Cell* 186, 243–278
5. Melzer, D. *et al.* (2020) The genetics of human ageing. *Nat. Rev. Genet.* 21, 88–101
6. Guarente, L. and Kenyon, C. (2000) Genetic pathways that regulate ageing in model organisms. *Nature* 408, 255–262
7. Kaeberlein, M. and Kennedy, B.K. (2005) Large-scale identification in yeast of conserved ageing genes. *Mech. Ageing Dev.* 126, 17–21
8. Kuningas, M. *et al.* (2008) Genes encoding longevity: from model organisms to humans. *Aging Cell* 7, 270–280
9. McCormick, M.A. *et al.* (2015) A comprehensive analysis of replicative lifespan in 4,698 single-gene deletion strains uncovers conserved mechanisms of aging. *Cell Metab.* 22, 895–906
10. Bin-Jumah, M.N. *et al.* (2022) Genes and longevity of lifespan. *Int. J. Mol. Sci.* 23, 1499
11. Uno, M. and Nishida, E. (2016) Lifespan-regulating genes in *C. elegans*. *npj Aging Mech. Dis.* 2, 16010
12. Mercatelli, D. *et al.* (2020) Gene regulatory network inference resources: a practical overview. *Biochim. Biophys. Acta (BBA) Gene Regul. Mech.* 1863, 194430
13. Jiang, Y. and Hao, N. (2021) Memorizing environmental signals through feedback and feedforward loops. *Curr. Opin. Cell Biol.* 69, 96–102
14. Wu, F. *et al.* (2017) Engineering of a synthetic quadrastable gene network to approach Waddington landscape and cell fate determination. *eLife* 6, e23702
15. Campos, S.E. and DeLuna, A. (2019) Functional genomics of dietary restriction and longevity in yeast. *Mech. Ageing Dev.* 179, 36–43
16. Mohammad, K. *et al.* (2018) Some metabolites act as second messengers in yeast chronological aging. *Int. J. Mol. Sci.* 19, 860
17. Ruetenik, A. and Barrientos, A. (2015) Dietary restriction, mitochondrial function and aging: from yeast to humans. *Biochim. Biophys. Acta (BBA) Bioenerg.* 1847, 1434–1447
18. Aerts, A.M. *et al.* (2009) Mitochondrial dysfunction leads to reduced chronological lifespan and increased apoptosis in yeast. *FEBS Lett.* 583, 113–117
19. Zuin, A. *et al.* (2008) Mitochondrial dysfunction increases oxidative stress and decreases chronological life span in fission yeast. *PLoS ONE* 3, e2842
20. Stein, K.C. *et al.* (2022) Ageing exacerbates ribosome pausing to disrupt cotranslational proteostasis. *Nature* 601, 637–642
21. Sampaio-Marques, B. and Ludovico, P. (2018) Linking cellular proteostasis to yeast longevity. *FEMS Yeast Res.* 18, foy043
22. Sun, Y. *et al.* (2021) A quantitative yeast aging proteomics analysis reveals novel aging regulators. *GeroScience* 43, 2573–2593

23. Lee, J.W. and Ong, E.B.B. (2021) Genomic instability and cellular senescence: lessons from the budding yeast. *Front. Cell Dev. Biol.* 8, 619126
24. Novarina, D. *et al.* (2017) Increased genome instability is not accompanied by sensitivity to DNA damaging agents in aged yeast cells. *DNA Repair* 54, 1–7
25. Saka, K. *et al.* (2013) Cellular senescence in yeast is regulated by rDNA noncoding transcription. *Curr. Biol.* 23, 1794–1798
26. Saka, K. *et al.* (2016) More than 10% of yeast genes are related to genome stability and influence cellular senescence via rDNA maintenance. *Nucleic Acids Res.* 44, 4211–4221
27. Mojumdar, A. *et al.* (2022) Changes in DNA double-strand break repair during aging correlate with an increase in genomic mutations. *J. Mol. Biol.* 434, 167798
28. Janssens, G.E. and Veenhoff, L.M. (2016) Evidence for the hallmarks of human aging in replicatively aging yeast. *Microb. Cell* 3, 263
29. Langfelder, P. and Horvath, S. (2008) WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform.* 9, 559
30. Subramanian, A. *et al.* (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci.* 102, 15545–15550
31. Aibar, S. *et al.* (2017) SCENIC: single-cell regulatory network inference and clustering. *Nat. Methods* 14, 1083–1086
32. González-Blas, C.B. *et al.* (2023) SCENIC+: single-cell multiomic inference of enhancers and gene regulatory networks. *Nat. Methods* 20, 1355–1367
33. Janssens, J. *et al.* (2022) Decoding gene regulation in the fly brain. *Nature* 601, 630–636
34. Trapnell, C. *et al.* (2014) The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat. Biotechnol.* 32, 381–386
35. Bergen, V. *et al.* (2020) Generalizing RNA velocity to transient cell states through dynamical modeling. *Nat. Biotechnol.* 38, 1408–1414
36. Huang, Y. *et al.* (2023) Characterizing cancer metabolism from bulk and single-cell RNA-seq data using METAFlux. *Nat. Commun.* 14, 4883
37. Mendes, P. *et al.* (2009) Systems biology. *Methods Mol. Biol.* 500, 17–59
38. Lopez, C.F. *et al.* (2013) Programming biological models in Python using PySB. *Mol. Syst. Biol.* 9, MSB20131
39. Maarleveld, T.R. *et al.* (2013) StochPy: a comprehensive, user-friendly tool for simulating stochastic biological processes. *PLoS ONE* 8, e79345
40. Skylaki, S. *et al.* (2016) Challenges in long-term imaging and quantification of single-cell dynamics. *Nat. Biotechnol.* 34, 1137–1144
41. Frei, M.S. *et al.* (2024) Next-generation genetically encoded fluorescent biosensors illuminate cell signaling and metabolism. *Annu. Rev. Biophys.* 53, 275–297
42. He, C. *et al.* (2018) The yeast replicative aging model. *Biochim. Biophys. Acta (BBA) Mol. Basis Dis.* 1864, 2690–2696
43. Ruetenik, A. and Barrientos, A. (2018) Exploiting post-mitotic yeast cultures to model neurodegeneration. *Front. Mol. Neurosci.* 11, 400
44. Li, Y. *et al.* (2017) Multigenerational silencing dynamics control cell aging. *Proc. Natl. Acad. Sci.* 114, 11253–11258
45. Jin, M. *et al.* (2019) Divergent aging of isogenic yeast cells revealed through single-cell phenotypic dynamics. *Cell Syst.* 8, 242–253
46. Li, Y. *et al.* (2020) A programmable fate decision landscape underlies single-cell aging in yeast. *Science* 369, 325–329
47. O'Laughlin, R. *et al.* (2020) Advances in quantitative biology methods for studying replicative aging in *Saccharomyces cerevisiae*. *Transl. Med. Aging* 4, 151–160
48. Paxman, J. *et al.* (2022) Age-dependent aggregation of ribosomal RNA-binding proteins links deterioration in chromatin stability with challenges to proteostasis. *eLife* 11, e75978
49. Zhou, Z. *et al.* (2023) Engineering longevity – design of a synthetic gene oscillator to slow cellular aging. *Science* 380, 376–381
50. Liu, Y. *et al.* (2024) Enhanced cellular longevity arising from environmental fluctuations. *Cell Syst.* 15, 738–752
51. Gartenberg, M.R. and Smith, J.S. (2016) The nuts and bolts of transcriptionally silent chromatin in *Saccharomyces cerevisiae*. *Genetics* 203, 1563–1599
52. Buschlen, S. *et al.* (2003) The *S. cerevisiae* HAP complex, a key regulator of mitochondrial function, coordinates nuclear and mitochondrial gene expression. *Comp. Funct. Genomics* 4, 37–46
53. DeRisi, J.L. *et al.* (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278, 680–686
54. Lin, S.-J. *et al.* (2002) Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418, 344–348
55. Anderson, R.M. *et al.* (2003) Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. *Nature* 423, 181–185
56. Medvedik, O. *et al.* (2007) MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*. *PLoS Biol.* 5, e261
57. Westholm, J.O. *et al.* (2008) Combinatorial control of gene expression by the three yeast repressors Mig1, Mig2 and Mig3. *BMC Genomics* 9, 601
58. Wang, J. *et al.* (2010) Gene regulatory changes in yeast during life extension by nutrient limitation. *Exp. Gerontol.* 45, 621–631
59. Hwangbo, D.-S. *et al.* (2020) Mechanisms of lifespan regulation by calorie restriction and intermittent fasting in model organisms. *Nutrients* 12, 1194
60. Mitchell, S.J. *et al.* (2014) The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. *Cell Rep.* 6, 836–843
61. Satoh, A. *et al.* (2013) Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab.* 18, 416–430
62. Kirkwood, T.B.L. (2015) Deciphering death: a commentary on Gompertz (1825) 'On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies'. *Philos. Trans. R. Soc. B Biol. Sci.* 370, 20140379
63. Karin, O. *et al.* (2019) Senescent cell turnover slows with age providing an explanation for the Gompertz law. *Nat. Commun.* 10, 5495
64. Karin, O. and Alon, U. (2020) Senescent cell accumulation mechanisms inferred from parabiosis. *GeroScience* 43, 329–341
65. Yang, Y. *et al.* (2023) Damage dynamics and the role of chance in the timing of E. coli cell death. *Nat. Commun.* 14, 2209
66. Liu, P. *et al.* (2017) Noise reduction as an emergent property of single-cell aging. *Nat. Commun.* 8, 680
67. Liu, P. and Acar, M. (2018) The generational scalability of single-cell replicative aging. *Sci. Adv.* 4, eaao4666
68. Santiago, E. *et al.* (2021) Modeling aging and its impact on cellular function and organismal behavior. *Exp. Gerontol.* 155, 111577
69. Song, R. *et al.* (2018) The systems biology of single-cell aging. *iScience* 7, 154–169
70. Cohen, A.A. *et al.* (2022) A complex systems approach to aging biology. *Nat. Aging* 2, 580–591
71. Hipp, M.S. *et al.* (2019) The proteostasis network and its decline in ageing. *Nat. Rev. Mol. Cell Biol.* 20, 421–435
72. Kurtishi, A. *et al.* (2019) Cellular proteostasis in neurodegeneration. *Mol. Neurobiol.* 56, 3676–3689
73. Santra, M. *et al.* (2019) Proteostasis collapse is a driver of cell aging and death. *Proc. Natl. Acad. Sci.* 116, 22173–22178
74. Krisko, A. and Radman, M. (2019) Protein damage, ageing and age-related diseases. *Open Biol.* 9, 180249
75. Pohl, C. and Dikic, I. (2019) Cellular quality control by the ubiquitin-proteasome system and autophagy. *Science* 366, 818–822
76. Finley, D. and Prado, M.A. (2020) The proteasome and its network: engineering for adaptability. *Cold Spring Harb. Perspect. Biol.* 12, a033985
77. Sacramento, E.K. *et al.* (2020) Reduced proteasome activity in the aging brain results in ribosome stoichiometry loss and aggregation. *Mol. Syst. Biol.* 16, e9596
78. Roux, A.E. *et al.* (2023) Individual cell types in *C. elegans* age differently and activate distinct cell-protective responses. *Cell Rep.* 42, 112902

79. Schmidt, R.M. *et al.* (2019) The proteasome biogenesis regulator Rpn4 cooperates with the unfolded protein response to promote ER stress resistance. *eLife* 8, e43244
80. Jurivich, D.A. *et al.* (2019) Multifactorial attenuation of the murine heat shock response with age. *J. Gerontol. Ser. A* 75, 1846–1852
81. Apostolopoulos, A. *et al.* (2018) Nuclear accumulation of HSP70 in mouse skeletal muscles in response to heat stress, aging, and unloading with or without reloading. *Front. Genet.* 9, 617
82. Trivedi, R. and Jurivich, D.A. (2020) A molecular perspective on age-dependent changes to the heat shock axis. *Exp. Gerontol.* 137, 110969
83. Chondrogianni, N. *et al.* (2003) Central role of the proteasome in senescence and survival of human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation. *J. Biol. Chem.* 278, 28026–28037
84. Carrard, G. *et al.* (2003) Impact of ageing on proteasome structure and function in human lymphocytes. *Int. J. Biochem. Cell Biol.* 35, 728–739
85. Carrard, G. *et al.* (2002) Impairment of proteasome structure and function in aging. *Int. J. Biochem. Cell Biol.* 34, 1461–1474
86. Saez, I. and Vilchez, D. (2014) The mechanistic links between proteasome activity, aging and age-related diseases. *Curr. Genomics* 15, 38–51
87. Petropoulos, I. *et al.* (2000) Increase of oxidatively modified protein is associated with a decrease of proteasome activity and content in aging epidermal cells. *J. Gerontol. Ser. A Biol. Sci. Méd. Sci.* 55, B220–B227
88. Bulteau, A.-L. *et al.* (2002) Age-dependent declines in proteasome activity in the heart. *Arch. Biochem. Biophys.* 397, 298–304
89. Keele, G.R. *et al.* (2023) Global and tissue-specific aging effects on murine proteomes. *Cell Rep.* 42, 112715
90. Wood, N.B. *et al.* (2024) Examination of aging-related loss of proteostasis in yeast with limited proteolysis mass spectrometry. *Biophys. J.* 123, 203a
91. Wang, L. *et al.* (2004) Rpn4 is a physiological substrate of the Ubr2 ubiquitin ligase. *J. Biol. Chem.* 279, 55218–55223
92. Kruegel, U. *et al.* (2011) Elevated proteasome capacity extends replicative lifespan in *Saccharomyces cerevisiae*. *PLoS Genet.* 7, e1002253
93. Ju, D. *et al.* (2010) Inhibition of proteasomal degradation of Rpn4 umpairs nonhomologous end-joining repair of DNA double-strand breaks. *PLoS ONE* 5, e9877
94. Liew, H.-K. *et al.* (2019) Over-activated proteasome mediates neuroinflammation on acute intracerebral hemorrhage in rats. *Cells* 8, 1326
95. Stricker, J. *et al.* (2008) A fast, robust and tunable synthetic gene oscillator. *Nature* 456, 516–519
96. Danino, T. *et al.* (2010) A synchronized quorum of genetic clocks. *Nature* 463, 326–330
97. Elowitz, M.B. and Leibler, S. (2000) A synthetic oscillatory network of transcriptional regulators. *Nature* 403, 335–338
98. Ravera, S. *et al.* (2019) Discrete changes in glucose metabolism define aging. *Sci. Rep.* 9, 10347
99. Azzu, V. and Valencak, T.G. (2017) Energy metabolism and ageing in the mouse: a mini-review. *Gerontology* 63, 327–336
100. Petr, M.A. *et al.* (2021) A cross-sectional study of functional and metabolic changes during aging through the lifespan in male mice. *eLife* 10, e62952
101. Papadopoli, D. *et al.* (2019) mTOR as a central regulator of lifespan and aging. *F1000Research* 8, 998
102. Kaeberlein, M. *et al.* (2005) Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* 310, 1193–1196
103. Wu, J.J. *et al.* (2013) Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Rep.* 4, 913–920
104. Selman, C. *et al.* (2009) Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science* 326, 140–144
105. Mannick, J.B. and Lamming, D.W. (2023) Targeting the biology of aging with mTOR inhibitors. *Nat. Aging* 3, 642–660
106. Chaudhari, S.N. and Kipreos, E.T. (2018) The energy maintenance theory of aging: maintaining energy metabolism to allow longevity. *BioEssays* 40, e1800005
107. Błaszczyk, J.W. (2020) Energy metabolism decline in the aging brain – pathogenesis of neurodegenerative disorders. *Metabolites* 10, 450
108. Burkewitz, K. *et al.* (2014) AMPK at the nexus of energetics and aging. *Cell Metab.* 20, 10–25
109. Shaw, R.J. (2018) AMPK: guardian of metabolism and mitochondrial homeostasis. *FASEB J.* 32, 379.3
110. Herzig, S. and Shaw, R.J. (2018) AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell Biol.* 19, 121–135
111. Hardie, D.G. (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* 8, 774–785
112. Reznick, R.M. *et al.* (2007) Aging-associated reductions in AMP-Activated protein kinase activity and mitochondrial biogenesis. *Cell Metab.* 5, 151–156
113. Ljubicic, V. and Hood, D.A. (2009) Diminished contraction-induced intracellular signaling towards mitochondrial biogenesis in aged skeletal muscle. *Aging Cell* 8, 394–404
114. Qiang, W. *et al.* (2007) Aging impairs insulin-stimulated glucose uptake in rat skeletal muscle via suppressing AMPKalpha. *Exp. Mol. Med.* 39, 535–543
115. Liu, F. *et al.* (2012) Age-related changes in AMP-activated protein kinase after stroke. *AGE* 34, 157–168
116. Salminen, A. and Kaarniranta, K. (2012) AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing Res. Rev.* 11, 230–241
117. Ulgherait, M. *et al.* (2014) AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. *Cell Rep.* 8, 1767–1780
118. Greer, E.L. *et al.* (2009) AMP-activated protein kinase and FoxO transcription factors in dietary restriction–induced longevity. *Ann. N. Y. Acad. Sci.* 1170, 688–692
119. Stancu, A.L. (2015) AMPK activation can delay aging. *Discoveries* 3, e53
120. Ashrafi, K. *et al.* (2000) Sip2p and its partner Snf1p kinase affect aging in *S. cerevisiae*. *Genes Dev.* 14, 1872–1885
121. Steinberg, G.R. and Kemp, B.E. (2009) AMPK in health and disease. *Physiol. Rev.* 89, 1025–1078
122. Bar-Or, R.L. *et al.* (2000) Generation of oscillations by the p53–Mdm2 feedback loop: a theoretical and experimental study. *Proc. Natl. Acad. Sci.* 97, 11250–11255
123. Geva-Zatorsky, N. *et al.* (2006) Oscillations and variability in the p53 system. *Mol. Syst. Biol.* 2, 2006.0033
124. Lahav, G. *et al.* (2004) Dynamics of the p53–Mdm2 feedback loop in individual cells. *Nat. Genet.* 36, 147–150
125. Hamstra, D.A. *et al.* (2006) Real-time evaluation of p53 oscillatory behavior in vivo using bioluminescent imaging. *Cancer Res.* 66, 7482–7489
126. Zhang, X.-P. *et al.* (2009) Cell fate decision mediated by p53 pulses. *Proc. Natl. Acad. Sci.* 106, 12245–12250
127. Lee, J.-H. and Paull, T.T. (2005) ATM activation by DNA double-strand breaks through the Mre11–Rad50–Nbs1 complex. *Science* 308, 551–554
128. Lee, J.-H. and Paull, T.T. (2004) Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science* 304, 93–96
129. Uziel, T. *et al.* (2003) Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* 22, 5612–5621
130. Bakkenist, C.J. and Kastan, M.B. (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 421, 499–506
131. So, S. *et al.* (2009) Autophosphorylation at serine 1981 stabilizes ATM at DNA damage sites. *J. Cell Biol.* 187, 977–990
132. Khosravi, R. *et al.* (1999) Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage. *Proc. Natl. Acad. Sci.* 96, 14973–14977
133. Saito, S. *et al.* (2002) ATM mediates phosphorylation at multiple p53 sites, including Ser46, in response to ionizing radiation. *J. Biol. Chem.* 277, 12491–12494
134. Moyer, S.M. *et al.* (2020) p53 drives a transcriptional program that elicits a non-cell-autonomous response and alters cell state in vivo. *Proc. Natl. Acad. Sci.* 117, 23663–23673
135. Harton, M.D. *et al.* (2019) p53 pulse modulation differentially regulates target gene promoters to regulate cell fate decisions. *Mol. Syst. Biol.* 15, e8685

136. Purvis, J.E. *et al.* (2012) p53 dynamics control cell fate. *Science* 336, 1440–1444
137. Chen, X. *et al.* (2022) Mutant p53 in cancer: from molecular mechanism to therapeutic modulation. *Cell Death Dis.* 13, 974
138. Ignatius, M.S. *et al.* (2018) tp53 deficiency causes a wide tumor spectrum and increases embryonal rhabdomyosarcoma metastasis in zebrafish. *eLife* 7, e37202
139. Tyner, S.D. *et al.* (2002) p53 mutant mice that display early ageing-associated phenotypes. *Nature* 415, 45–53
140. Moore, L. *et al.* (2007) Aging-associated truncated form of p53 interacts with wild-type p53 and alters p53 stability, localization, and activity. *Mech. Ageing Dev.* 128, 717–730
141. Maier, B. *et al.* (2004) Modulation of mammalian life span by the short isoform of p53. *Genes Dev.* 18, 306–319
142. Zhao, Y. *et al.* (2018) A polymorphism in the tumor suppressor p53 affects aging and longevity in mouse models. *eLife* 7, e34701
143. Lessel, D. *et al.* (2017) Dysfunction of the MDM2/p53 axis is linked to premature aging. *J. Clin. Invest.* 127, 3598–3608
144. Gannon, H.S. *et al.* (2011) Mdm2–p53 signaling regulates epidermal stem cell senescence and premature aging phenotypes in mouse skin. *Dev. Biol.* 353, 1–9
145. Wiley, C.D. *et al.* (2018) Small-molecule MDM2 antagonists attenuate the senescence-associated secretory phenotype. *Sci. Rep.* 8, 2410
146. Pant, V. *et al.* (2013) The p53–Mdm2 feedback loop protects against DNA damage by inhibiting p53 activity but is dispensable for p53 stability, development, and longevity. *Genes Dev.* 27, 1857–1867
147. Nolt, G.L. *et al.* (2024) Inhibition of p53–MDM2 binding reduces senescent cell abundance and improves the adaptive responses of skeletal muscle from aged mice. *GeroScience* 46, 2153–2176
148. Bartel, F. *et al.* (2002) Alternative and aberrant splicing of MDM2 mRNA in human cancer. *Cancer Cell* 2, 9–15
149. Bartel, F. *et al.* (2004) Expression of alternatively and aberrantly spliced transcripts of the MDM2 mRNA is not tumor-specific. *Int. J. Oncol.* 24, 143–151
150. Volk, E.L. *et al.* (2009) MDM2-A, a common Mdm2 splice variant, causes perinatal lethality, reduced longevity and enhanced senescence. *Dis. Model. Mech.* 2, 47–55
151. Micco, R.D. *et al.* (2021) Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat. Rev. Mol. Cell Biol.* 22, 75–95
152. Dimri, G.P. *et al.* (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci.* 92, 9363–9367
153. Wang, C. *et al.* (2009) DNA damage response and cellular senescence in tissues of aging mice. *Aging Cell* 8, 311–323
154. Biran, A. *et al.* (2017) Quantitative identification of senescent cells in aging and disease. *Aging Cell* 16, 661–671
155. Baar, M.P. *et al.* (2017) Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell* 169, 132–147
156. Simoni, M. *et al.* (2024) PML restrains p53 activity and cellular senescence in clear cell renal cell carcinoma. *EMBO Mol. Med.* 16, 1324–1351
157. Wang, M. *et al.* (2020) PML2-mediated thread-like nuclear bodies mark late senescence in Hutchinson–Gilford progeria syndrome. *Aging Cell* 19, e13147
158. Webb, A.E. and Brunet, A. (2014) FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem. Sci.* 39, 159–169
159. Postnikoff, S.D.L. *et al.* (2012) The yeast forkhead transcription factors Fkh1 and Fkh2 regulate lifespan and stress response together with the anaphase-promoting complex. *PLoS Genet.* 8, e1002583
160. Vitale, G. *et al.* (2019) ROLE of IGF-1 system in the modulation of longevity: controversies and new insights from a centenarians' perspective. *Front. Endocrinol.* 10, 27
161. Zhang, L. *et al.* (2023) Targeting cellular senescence with senotherapeutics: senolytics and senomorphics. *FEBS J.* 290, 1362–1383
162. Herbstein, F. *et al.* (2024) The SASP factor IL-6 sustains cell-autonomous senescent cells via a cGAS-STING-NFκB intracrine senescent noncanonical pathway. *Aging Cell* 23, e14258
163. Zheng, Y. *et al.* (2023) Secretion of IL-6 and IL-8 in the senescence of bone marrow mesenchymal stem cells is regulated by autophagy via FoxO3a. *Exp. Gerontol.* 172, 112062
164. Widjaja, A.A. *et al.* (2024) Inhibition of IL-11 signalling extends mammalian healthspan and lifespan. *Nature* 632, 157–165
165. Li, Y. *et al.* (2024) IL-10 deficiency aggravates cell senescence and accelerates BLM-induced pulmonary fibrosis in aged mice via PTEN/AKT/ERK pathway. *BMC Pulm. Med.* 24, 443
166. Mansell, E. *et al.* (2023) New insight into the causes, consequences, and correction of hematopoietic stem cell aging. *Exp. Hematol.* 125, 1–5
167. Ferrari, S. *et al.* (2023) Genetic engineering meets hematopoietic stem cell biology for next-generation gene therapy. *Cell Stem Cell* 30, 549–570