Integrating evolutionary theory into a framework for the mechanistic evaluation of candidate anti-aging interventions

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Abstract

Despite decades of research into the molecular hallmarks of aging, geroscience lacks a unifying framework to guide the development of effective anti-aging interventions. Here, we integrate two leading evolutionary theories—the Disposable Soma Theory and Hyperfunction Theory—into a layered model of aging biology, the “Aging Onion”. In this framework, aging arises both from persistent activity of growth pathways and from insufficient investment into somatic maintenance. We use principles from caloric restriction (CR), a conserved pro-longevity intervention, to test the Aging Onion’s predictive and explanatory power. Pitting CR against rapamycin, a proposed pharmacological mimetic, we review evidence from the nutrient-sensing pathway and disease-prone mouse models. Here, the Aging Onion helps explain CR’s broader efficacy, which derives from simultaneous hyperfunction suppression and maintenance upregulation. Conversely, rapamycin primarily ablates growth signalling and falls short of reproducing CR’s multi-layered impact. Building on this insight, we propose a transcriptomic screening strategy that classifies gene expression changes according to their mechanistic role in aging: hyperfunction suppression *vs.* maintenance activation. Our approach offers a route to towards mechanistically informed intervention discovery. The ‘Aging Onion’ may thus provide a conceptual and methodological framework to align future candidate interventions with the causal architecture of aging.

Keywords: Caloric Restriction; Disposable Soma Theory; Gene Expression Profiling; Geroprotectors; GLP-1 Receptor Agonists; Hyperfunction Theory; Rapamycin; Drug Discovery.

1. Introduction

Aging is the dominant risk factor for nearly all chronic human diseases[1]. Here, we refer to aging as both the passage of time and the functional decline that can accompany it. While the latter is most commonly termed senescence[2], this word has cell-specific connotations and can be inconsistently applied in biomedical contexts. We therefore use *aging* throughout. By 2050, over one in six people worldwide are projected to be over the age of 65[3]. All else being equal, the resulting rise in age-related disease will worsen existing strain on social, economic, and healthcare systems[4,5]. Aging is significant not only because it limits lifespan but more importantly because it constrains healthspan – the period of life spent in good health, free from chronic disease and functional decline[6].

In the late 20th century, key medical advances were made in the investigation of the pathophysiology of age-related disease[7]. Examples include the mechanistic underpinnings of cardiovascular disease and cancer[8,9]. In parallel, biology more broadly investigated conserved mechanisms of deterioration across the tree of life[10]. The different investigatory lenses of medicine and biology map onto the concepts of proximate and ultimate explanations of aging, respectively. Proximate explanations address the *how* – the immediate mechanisms precipitating a biological event. Ultimate explanations address the evolutionary *why* - asking why a trait evolves and how it persists through natural selection. Our thesis here is that biogerontology has much to gain by integrating both types of explanation.

In medicine, proximate explanations of phenomena are valued for their mechanistic clarity and actionable translational potential. However, the diverse pathophysiologies of age-related diseases do not readily converge on a common point of vulnerability. Recognition of this limitation[11] prompted investigation into the shared proximate mechanisms of aging across different tissues and pathologies, leading to the ever-expanding list of 'hallmarks of aging'[12]. While these hallmarks uncover common mechanistic processes driving multiple aging

phenotypes[12], they have yet to yield intervention points sufficiently robust to support clinical trials aimed at delaying aging or ameliorating age-related disease[13].

In contrast, ultimate explanations of aging offer the prospect of identifying a conserved regulatory network that evolution has already exploited to extend or constrain lifespan across species. If natural selection can act on this network to modulate longevity, then it may likewise represent a targetable vulnerability for therapeutic intervention[14]. Many ultimate explanations also recognise that this network can be modulated within organismal lifetime through adaptive physiological responses. Caloric restriction (CR) exemplifies this principle. CR is an evolved response to resource scarcity whose broadly conserved[15,16]anti-aging effect across taxa suggests direct action on the same network that regulates aging. However, we note that the practical implementation of CR in humans is likely challenging owing to behavioural, social, and physiological constraints. This translational barrier has motivated efforts to develop CR mimetics[15], *i.e.* pharmacological interventions that reproduce its benefits. The most extensively studied candidate is rapamycin[17], an mTORC1 inhibitor, yet its effects fall short of CR in many models[18]. If CR is an evolved, adaptive modulation of the core regulatory network identified by ultimate theories of aging, it is a benchmark against which mechanistic models of this network must be tested. A successful model ought to explain both the mechanistic basis of CR and the shortcomings of current mimetics.

We therefore need a conceptual framework that is evolutionarily grounded and mechanistically explicit; capable of explaining both the CR response and the differing efficacies of its mimetics. To this end, we synthesise two leading ultimate explanations – the Hyperfunction Theory[19] and the Disposable Soma Theory[14] - into a unified framework that addresses:

(i) the principles of the evolution of aging,

(ii) the full spectrum of aging phenotypes,

(iii) the mechanistic basis of adaptive anti-aging responses such as CR and, by extension

(iv) the diverging efficacies of CR mimetics.

We argue that the conserved regulatory network of aging likely comprises elements from both theories: namely the pathological persistence of growth pathways (Hyperfunction Theory) and insufficient investment into somatic maintenance (Disposable Soma Theory). This synthesis, termed the Aging Onion, provides a layered view of aging biology (**Figure 1**). We review the performance of CR relative to rapamycin and argue that the Aging Onion framework helps explain their divergent outcomes. Building on this insight, we propose the Aging Onion as a potential predictive paradigm for evaluating new and existing geroprotective drugs. In doing so, we aim to provide a conceptual and methodological basis for identifying compounds that engage conserved mechanisms of aging regulation.

2. Integrating evolutionary theories of aging: the 'aging onion' framework

In this section, we first summarise the prominent evolutionary thinking regarding aging before developing a conceptual synthesis that integrates two leading theories of aging: the Hyperfunction Theory[19] and the Disposable Soma Theory[14]. Evolutionary theories of aging seek to ultimately explain the progressive functional decline of organisms that leads to increasing risk of disease, loss of vitality, and eventual death[14]. These theories must not only account for the aging phenotype, but its occurrence in the first place. Early group-selection explanations suggested that an aging programme exists to secure a turnover of generation[20]. However, patterns of age-related mortality in wild populations suggest that organisms may succumb to extrinsic mortality before age-related deterioration can be identified[21 (but see16)]. Aging, therefore, occurs in a 'selective shadow'[22]; the deleterious effects of ageing arise only late in life, at which point individuals do not (at least directly – see the 'grandmother hypothesis'[23]) contribute to the performance of the population[22]. The phenotype of aging is thus not directly programmed by evolution. Accumulation of late-acting mutations has been proposed as an alternative explanation[22], but this hypothesis fails to explain the conserved proximate drivers of aging across species[24]. The most compelling existing explanations view aging as a byproduct of evolutionary compromises embedded into an organism's

117 developmental programming[14,19]. These compromises are moulded by trade-offs in metabolic
118 resource allocation that prioritise reproduction and growth over long-term maintenance.
119
120 Kirkwood's Disposable Soma theory suggests that, because somatic maintenance must only
121 sustain an organism's physiological condition for as long as survival in the wild is likely, its
122 allocated investment is inherently suboptimal[14]. Stochastic molecular damage (*i.e.*, somatic
123 mutation[25]) can thus accumulate over time, leading to degenerative phenotypes in the post-
124 reproductive period[14]. **Figure 2** illustrates how fixed levels of investment into maintenance and
125 repair functions (MRFs) (**Box 1**) ensure survival within an organism's expected wild lifespan
126 but become life-limiting when extrinsic mortality is reduced. In this vain, large-scale
127 mutagenesis screens have identified many maintenance-associated genes regulating aging[26].
128 However, a major limitation of these approaches is that mutations shortening lifespan or those
129 linked to progeroid syndromes (rare genetic disorders that mimic features of accelerated
130 aging) may not correspond to life-limiting MRFs in wild-type aging.
131
132 A key assumption of the Disposable Soma theory is that aging and its pathologies result
133 entirely from suboptimal buffering of stochastic molecular damage[14]. The burden of
134 accumulating stochastic molecular damage can account for cell-autonomous aging and clearly
135 drives pathophysiology in certain age-related pathologies, particularly the neurodegenerative
136 conditions [27,28]. In Parkinson's disease, the aggregation of amyloid-like α-synuclein in
137 dopaminergic neurons of the substantia nigra pars compacta leads to an accumulation of
138 neurotoxic oligomers, cell death, and loss of inhibitory tone on the basal ganglia[29].
139 Mitochondrial dysfunction also drives dopaminergic cell death in idiopathic and familial
140 Parkinson's[29]. In familial forms of the disease, key implicated genes such as PINK1 and Parkin
141 are components of the mitophagy pathway, and their loss-of-function leads to impaired
142 mitochondrial quality control with resultant cell death[29]. These damage driven processes,
143 which are readily apparent in the pathophysiology other neurodegenerative conditions such
144 as ALS (reviewed succinctly here[30]), exemplify the Disposable Soma framework; pathology

arises from defective or overwhelmed somatic-maintenance processes and accumulated cellular damage.

In contrast, stochastic molecular damage is harder to implicate as the primary driver of pathogenesis in other age-related conditions. This is particularly evident where pathology manifests at a system-wide level, as in cardiovascular aging and disease. An illustrative example is left-ventricular hypertrophy, an independent risk factor for stroke, heart failure, sudden cardiac death, and all-cause mortality[31]. Left-ventricular hypertrophy begins as an adaptive increase in cardiomyocyte size and protein synthesis in response to chronic pressure or volume overload, mediated by neurohumoral and growth-signalling pathways[32]. Only when these programmes remain chronically engaged does the phenotype progress to cell death, fibrosis, mitochondrial dysfunction, impaired protein and mitochondrial quality control, metabolic remodelling and, ultimately, heart failure, arrhythmias and death[32]. Thus, in left-ventricular hypertrophy the primary lesion is the sustained activation of otherwise physiological growth and remodelling pathways. Molecular damage emerges as a downstream consequence, illustrating the limits of a purely Disposable-Soma-like account for cardiovascular aging. More broadly, such cases highlight the need for an explanation of why age-related disease processes can be initiated independently of stochastic molecular damage.

The insufficiency of damage-based explanations in accounting for system-level age-related diseases is the impetus for Blagosklonny's thesis[19]. The author aims to provide an ultimate explanation for aging that maintains a central claim: aging and age-related pathology exist along the same continuum, driven by the same underlying mechanisms[33]. On this view, lifespan limitation and morbidity are not separate phenomena but reflect different manifestations of a single aging process. A satisfactory theory of aging must therefore explain all the diverse pathologies that accompany aging. Blagosklonny notes that many aging pathologies, such as cancer, atherosclerosis, and diabetes, are in fact diseases of

'hyperfunction'[34]. Though focused on TOR (target-of-rapamycin), a conserved master regulator of growth/proliferation[35], Blagosklonny's arguments are applicable to the broader endocrine network regulating development and growth, including insulin and insulin-like growth factor signalling (IIS)[19,36]. These pathways drive pathology through promotion of hyperplasia, dysplasia, hypertrophy, and hypersecretion[33]. As pathophysiological mechanisms, these processes are distinct from the stochastic damage accumulation predicted by the Disposable Soma theory. Crucially, as illustrated by left-ventricular hypertrophy, disease initiation can occur in cells whose core functions remain largely intact. In the hyperfunction model, the continued action of growth and developmental programmes, signalling, and effectors beyond when they are required drives aging[19]. Further support for the hyperfunction model comes from observations on the correlation between pace-of-life and longevity. Pace-of-life, refers to the overall tempo at which an organism develops, grows, and metabolise. This tempo, itself determined by activity of growth and developmental programmes, remains one of the strongest predictors of lifespan and healthspan both between and within species[37]. The strong correlation between pace-of-life and longevity therefore strongly implicates the mediators of growth and development in longevity determination.

The evidence-based success of the Disposable Soma Theory's predictions may stem from the fact that many hyperfunction mediators, such as TOR, are themselves negative regulators of MRFs. Inhibition of TOR disinhibits autophagy, an essential MRF, and many studies of life- and healthspan link ablation of the TOR pathway to longevity through autophagy upregulation[38]. This conclusion, however, overlooks a key confounder: the simultaneous loss of TOR's positive effectors. Surprisingly few studies have directly tested whether autophagy is required for the protective effects attributed to TOR suppression. Where this question has been examined, the findings indicate that autophagy is not always required for the protective effects of TOR inhibition. For example, in a myocardial ischaemia–reperfusion injury model in mice, rapamycin confers robust cardioprotection even when autophagy is pharmacologically blocked with 3-methyladenine[39]. Such autophagy-independent effects are rarely interrogated

experimentally, leaving a gap in our understanding of how TOR inhibition mediates protection across tissues and pathologies independently of its canonical effect of autophagy disinhibition.

Neither the Disposable Soma Theory nor the Hyperfunction Theory alone can account for the full spectrum of aging phenotypes. We argue, however, that their shared evolutionary logic provides a compelling basis for synthesis into a more comprehensive model of the core regulatory network of aging – one that integrates and extends the explanatory scope of both theories. As in the Disposable Soma theory, the maladaptive continuation of hyperfunction programmes exists because of the selective shadow that occurs beyond the expected lifespan of wild organisms. In other words, an “off-switch” would provide no fitness benefit. Moreover, hyperfunction aligns with the same life-history trade-offs in resource allocation that are central to the Disposable Soma theory. As shown in **Figure 3**, many positive mediators of hyperfunction concurrently supress MRFs. It follows that the trade-off between growth and maintenance is embedded into the evolved mediators of development and growth.

The Disposable Soma and Hyperfunction theories differ in the proximate mechanisms emphasised. In the case of the Disposable Soma theory, the mechanism is a deficit in somatic maintenance, while in the Hyperfunction theory, the mechanism is persistent activation of growth and developmental regulators. We propose that the integration of both theories can be conceptualised as an 'Aging Onion' (**Figure 1**). According to this framework, aging becomes a series of stacked biological processes whereby removal of one limiting factor (*e.g.,* TOR driven hyperfunction) reveals another beneath (*e.g.,* DNA damage accumulation). A comprehensive anti-aging strategy should aim to target multiple layers of this Aging Onion - addressing both hyperfunction and MRF deficiencies.

3. Why caloric restriction matters: a test case for the aging onion

Having outlined two major evolutionary theories of aging and their integration into the ‘Aging Onion’ on conceptual grounds, we explicitly consider how this synthesis can be tested. Below,

we summarise the evolution of the CR response, and why it serves as a natural case study for assessing the explanatory power of the Aging Onion.

Evolution optimises the trade-off in the investment of limited resources to growth, reproduction, and somatic maintenance, thus resulting in species-specific life histories that determine lifespan[14,37]. In many species this optimised trade-off is, to varying degrees, plastic during the life of an organism. Nutrient scarcity tends to trigger a conserved pro-longevity response across taxa: yeast, roundworms, rodents, and increasingly in primates show life- and healthspan extension under CR[15 (but see16)]. Although the Disposable Soma and Hyperfunction theories offer different mechanistic rationales for this response[14,19], its consistent empirical effects suggest that the core regulatory network of aging is environmentally modulable. This capacity for environmental adaptation underpins the interest in CR as a longevity intervention. The nutrient sensing pathway acts as a central hub in this process, integrating environmental signals and directing them into coordinated, pro-longevity outputs within the conserved aging network.

Although caloric restriction robustly extends lifespan across taxa, its effects can be highly context-dependent, varying markedly with species, sex, and genetic background. Indeed, a 2012 meta-analysis of 145 studies across 36 species found that CR reduces mortality risk by ~60% on average, with maximal benefit at 50% calorie reduction[40]. Effects of CR were 20% greater in females compared to males and 100% greater in in model organisms such as mice and roundworms compared to less-studied species like fish and primates. Therefore, beneath the striking reduction in mortality lies substantial intra- and inter-species heterogeneity with regards to CR's effects. CR in *Caenorhabditis elegans* can increase lifespan by upwards of 40%[41], and meta-analysis reports a median lifespan increase of 30.4% in rats and 14.6% in mice[42]. Even then, mice show large variation depending on strain and genetic background[43]. The power of CR to regulate aging thus has multiple confounders, the most pronounced being species itself. In *C. elegans*, CR induces a state of extreme metabolic dormancy with

drastically enhanced longevity[44] whereas more complex and long-lived organisms – mice, monkeys, humans - appear to exhibit smaller, but still meaningful lifespan extension.

In humans, fasting has long been associated with health benefits anecdotally, but rigorous clinical data remains limited. The CALERIE 2 trial, the first long-term CR study in non-obese humans, showed improvements in biomarkers of health and morbidity risk, but the relatively young cohort limits conclusions on morbidity or lifespan outcomes[45]. Turning to primates, two major studies have evaluated chronic CR in Rhesus Monkeys[46], which share 93% sequence identity with the human genome. One study found a significant increase in survival[47],while the other did not[48], although methodological differences likely explain the negative result[49]. Both, however, reported reductions in disease incidence and age-related pathologies[47–49].

The apparent inverse relationship between organismal complexity and the magnitude of CR's benefits should not be interpreted as a limitation. *Caenorhabditis elegans*' ability to dramatically extend its lifespan is a product of its life-history strategy[44]. Rapid development, early reproduction, and strong selection for plastic responses to environmental stress allow lifespan extension through profound metabolic and developmental suppression. Likewise, the more modest extensions seen in mice and higher organisms likely speaks to some evolved developmental restrictions on the upper-limits of physiologically tolerable lifespan extension in more complex, longer-lived organisms[14]. If CR reflects an upper limit of attainable life- and healthspan extension, then even incremental improvements in human healthspan and morbidity risk would carry substantial clinical and public-health significance. More importantly, CR's mechanistic underpinnings represent an inroad to the architecture of conserved biological processes that regulate aging[15].

CR poses a promising avenue of exploration. This is so because, should CR simultaneously regulate multiple aging processes, it may represent a single, evolutionarily conserved point of intervention, and a mechanistic benchmark for drug development. Currently however, targeted

pathway inhibition prevails in the literature[17]. The most prominent example is rapamycin, a selective mTORC1 inhibitor, which extends lifespan in several model organisms and ameliorates age-associated phenotypes in mammals[17,50]. However, if aging is truly multi-layered, targeting a single pathway like TOR may be insufficient to engage the full spectrum of mechanisms required for robust geroprotection.

In the section that follows, we use CR and its leading pharmacological mimetic, rapamycin[17], to evaluate whether the Aging Onion model can map these interventions onto different mechanistic layers of aging and thereby explain their differential performance. We review three lines of evidence to substantiate this discussion: the structure of the nutrient-sensing pathway, outcomes in model systems, and transcriptomic data.

4. Comparative evaluation of caloric restriction and rapamycin

4.1 The nutrient sensing pathway

The nutrient sensing pathway integrates multiple metabolic signals to determine whether an organism prioritises growth or maintenance and is illustrated in **Figure 3**. Rather than mapping every interaction of the nutrient sensing pathway, the goal of this discussion is to highlight principles distinguishing CR from TOR inhibition, and their relevance to aging.

A key distinction in CR and Rapamycin's effects lies in how IIS and TOR engage downstream pathways. Akt activation simultaneously promotes TOR signalling and suppresses FOXO transcription factors via phosphorylation, effectively shutting down stress response pathways[51]. Therefore, IIS inhibition not only downregulates TOR but also relieves FOXO suppression, allowing for an enhanced somatic maintenance response[52]. Conversely, TOR inhibition independently of Akt, whether through amino acid (AA) deprivation or rapamycin, reduces growth signalling but fails to restore FOXO-mediated longevity pathways[53]. TOR's failure to engage FOXO pathways is a key mechanistic distinction which could confer CR with

broader effects. Nevertheless, mutant studies in *C. elegans* suggest that inhibiting either IIS or TOR alone extends lifespan, but their combination does not provide an additive effect[54]. Notably however, in *C. elegans*, TOR can directly inhibit FOXO, integrating a canonical IIS effector into the TOR pathway[55] - an interaction not observed in mammalian models to our knowledge. TOR–FOXO coupling in C. *elegans* may reflect selection for a tightly coordinated nutrient-sensing system capable of driving the organism's binary dauer (dormancy) response to scarcity[44]. Longer-lived species have likely evolved more modular nutrient-sensing architectures to allow for nuanced longevity modulation which may limit the efficacy of targeted inhibitions such as rapamycin.

Beyond IIS and TOR, AMPK (AMP-activated protein kinase) and sirtuins mediate energetic stress pathways which play crucial roles in longevity-regulation. Supporting TOR inhibition, AMPK phosphorylates ULK1 (Unc-51-like kinase 1) to initiate autophagy and activates TFEB (Transcription Factor EB), a master regulator of lysosomal biogenesis[56]. While TOR controls TFEB's cytosolic retention[57], its transcriptional activation is mediated specifically by AMPK-dependent phosphorylation[58]. Concordantly, the longevity phenotype of *C. elegans* TOR mutants depends on intact AAK-2[59], its ortholog of the AMPK α-subunit, underscoring AMPK's essential role in mediating the benefits of TOR inhibition.

Moreover, AMPK and sirtuins independently activate MRFs that are not regulated by TOR/IIS, thereby broadening the effector repertoire of CR beyond those of the TOR/IIS axis. Both AMPK and sirtuins enhance the activity of Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha (PGC-1a), a central regulator of mitochondrial biogenesis[60]. Sirtuins, particularly SIRT1 and SIRT6, play critical roles in DNA repair and maintenance of genomic stability[61,62]. SIRT6 for example activates PARP1 to promote DNA repair in conditions of oxidative stress[62]. Thus, unlike IIS/TOR inhibition, which primarily removes constraints on MRF activity, AMPK and sirtuins actively upregulate MRFs that are suboptimally expressed rather than suppressed. Through these regulators, CR may modulate distinct, non-

hyperfunction layers of the Aging Onion - targeting aging processes that arise not from unchecked growth, but from sub-optimal activation of MRFs.

4.2 Outcomes in model systems

4.2.1 *Lifespan effects and sex specificity*

A natural metric to take under consideration when looking at the anti-aging effects of CR and rapamycin is of course lifespan itself. A comparative review of multiple studies assessing rapamycin and CR treatments in mice found that CR consistently produced a more robust extension of lifespan[18]. Notably however, rapamycin exhibited significant sex-specific variation, with average lifespan extension of approximately 19% in female mice compared to only 10% in males. In contrast, CR yielded more substantial and balanced effects, extending lifespan by roughly 30% in females and 28% in males[18]. These findings suggest that the architecture of the Aging Onion may differ between sexes, with the relative contribution of different components varying accordingly. The broader impact of CR relative to rapamycin likely reflects its engagement of multiple longevity effectors beyond TOR inhibition (see section **4.1**), which may help mitigate the pronounced sex differences seen in rapamycin-treated cohorts. CR thus produces greater and more uniform lifespan extension across both sexes.

4.2.2 *Healthspan effects – Mouse models of disease*

Because lifespan alone is an incomplete endpoint, disease-prone mouse models provide a complementary way to assess whether candidate interventions improve healthspan-relevant pathology. Indeed, Blagosklonny emphasised that evaluating anti-aging interventions requires modelling of specific age-related pathologies, rather than lifespan in isolation[33]. Moreover, disease-prone mouse models allow a more granular test of intervention mechanisms, by asking whether benefits arise through suppression of hyperfunction, enhanced damage

buffering, or both. Both CR and rapamycin improve outcomes in multiple disease-prone mouse models[18], yet in some contexts their effects diverge[63–69]. Building on a hypothesis that CR and rapamycin's diverging efficacies can be mapped to differential engagement of Aging Onion layers, we next examine whether this hypothesis is testable in the context of disease-prone mouse models.

While organismal aging is a multilayered process, the pathophysiology of individual age-related pathologies can be driven predominantly by one component of the Aging Onion. For example, stochastic damage accumulation appears to be the dominant force driving the neurodegenerative conditions[70,71]. Here, we use an exploratory categorisation of disease processes - as hyperfunction-dominated, stochastic damage–dominated, or mixed - to ask whether CR and rapamycin's outcomes vary systematically with the type of pathological mechanism driving each disease. While not intended as definitive labels, any consistent trends arising from this approach would strengthen the case for the categorisation itself and for the Aging Onion framework more broadly. **Table 1** presents five disease models in which both CR and rapamycin have been tested under comparable conditions, listing outcomes alongside the putative dominant pathological driver.

In models where pathology is driven primarily by stochastic damage accumulation, such as in *p53*$^{-/-}$, SOD1$^{H46R/H48Q}$, or Ercc1$^{\Delta/-}$ mice, CR extends lifespan while rapamycin does not[63–66]. Rapamycin's poorer performance here suggests that its efficacy depends on intact damage surveillance systems, whereas CR can upregulate MRFs more broadly. Conversely, in *Rb*$^{+/-}$ mice, where tumorigenesis is driven by mitogenic hyperfunction, rapamycin outperforms CR[67,68], highlighting its effectiveness in suppressing overactive growth signalling when somatic maintenance remains intact. Interestingly, in a study of cardiac aging in senile mice, a condition involving both hyperfunction[72] and stochastic damage elements[73], CR again outperformed rapamycin[69]. Even on metrics typically driven by unchecked growth, such as left-ventricular hypertrophy[72], CR had greater impact[69]. This finding suggests that in such

mixed-pathology contexts, CR may suppress as many, or more, drivers of hyperfunction as rapamycin.

Taken together, these comparisons demonstrate the explanatory power of the Aging Onion framework. CR proves effective in conditions where pathology stems from stochastic damage or mixed mechanisms, whereas rapamycin is most effective when pathology is driven primarily by hyperfunctional signalling. This pattern of differential efficacy supports the view that anti-aging interventions can be assessed through the range and type of 'layers' of aging they engage. By extension, this putative categorisation of age-related pathologies according to their dominant pathophysiological mechanism - hyperfunction, maintenance failure, or mixed - may offer two practical benefits:

Firstly, such categorisation offers a pragmatic basis for the evaluation of candidate geroprotective interventions. If an intervention's efficacy derives from its differential engagement of the Aging Onion's layers, then the dominant pathophysiological mechanism of a disease becomes a relevant organising principle. Stratifying pathologies as hyperfunction-, damage-, or mixed-driven enables retrospective evaluation of existing pharmacological agents for which effects on specific age-related pathologies have been recorded. Particularly strong candidates would be those that show efficacy across multiple pathologies driven by the same underlying mechanism (*e.g.*, hyperfunction or MRF failure), for they likely act on conserved regulatory nodes within that layer of the Aging Onion. Even more promising agents would be effective across both hyperfunction and maintenance failure layers, offering broader modulation of aging biology

Secondly, the hyperfunction/stochastic-damage/mixed categorisation could have more short-term implications for clinical translation. In practice, the clinical application of aging biology is likely to emerge first in the management of age-related diseases rather than in primary prevention. This trajectory reflects the position of aging outside formal disease classification,

and the correspondingly high evidentiary and regulatory burden that would be required for interventions that seek to modify aging rather than treat established pathology[74]. Therefore, stratifying diseases of aging by their dominant pathophysiological mechanism (hyperfunction/stochastic damage/mixed) may help guide treatment choice from drugs that are already available. Hyperfunction-dominated conditions may respond best to targeted pathway inhibitors, while diseases involving damage accumulation or mixed mechanisms may require CR-like interventions with broader reach.

4.3 Gene expression analysis

Across this paper, we have suggested that aging at its core arises from both hyperfunction and MRF deficiencies, and that CR engages both mechanisms more broadly than its widely studied mimetic, rapamycin. This conclusion is supported by the structure of the nutrient-sensing pathway and by differential outcomes across disease models. The Aging Onion framework thus offers a compelling and mechanistically grounded evolutionary rationale for the differential efficacy of interventions across model systems. However, the framework's translational value hinges on whether the mechanistic distinctions it proposes are reflected in quantitative outputs such as transcriptomic signatures.

A direct way to interrogate the Aging Onion hypothesis is to ask whether CR and rapamycin produce distinct transcriptional programmes consistent with differential engagement of hyperfunction and MRF-related biology. For instance, to evaluate the biological programs engaged by CR *vs.* rapamycin, Fok and colleagues [75] conducted comprehensive transcriptomic analysis of liver tissue from male C57BL/6 mice treated with CR, rapamycin, or both for six months. Among 25,600 transcripts analysed, ~2,500 were significantly altered by either intervention but only 20% of these overlapped between groups. Notably, CR had a greater impact on upregulated genes while rapamycin more strongly affected downregulated genes. This distinction may reflect their underlying mechanisms: CR may activate suboptimal MRFs whereas rapamycin more narrowly suppresses overactive hyperfunctional

pathways. The authors did not test for alignment into hyperfunction or MRF-like categories. However, upon qualitative inspection of the top 15 IPA-enriched terms, we found that rapamycin predominantly affects canonical growth and biosynthesis pathways, consistent with hyperfunction suppression. Additionally, several CR-associated pathways (protein ubiquitination, mitochondrial dysfunction, epithelial adherens junction signalling, remodelling of epithelial adherens junctions, and FXR/RXR activation) may plausibly constitute MRF upregulation. However, without a formal, conceptually grounded classification of MRF gene programs as pertains to the logic of Disposable Soma theory, it would be premature to interpret these as definitive MRF activation. Rather, the data serves to highlight the need for hypothesis-driven annotation frameworks capable of testing this axis more rigorously.

Interpretation of transcriptomic data in other head-to-head studies of CR and rapamycin is similarly difficult. Zhang and colleagues[76] demonstrated in yeast that CR and rapamycin affect largely distinct gene sets in both mitotic and postmitotic phases. However, the published gene-ontology term summary is restricted to the most enriched pathways rather than the full functional spectrum. In these top hits, a bias towards hyperfunction suppression or MRF activation in either treatment is not readily evident. Likewise, in Ham and colleagues'[77] study of skeletal muscle in geriatric mice treated with either CR or rapamycin, top pathways are not clearly interpretable within our framework and mainly show divergences in metabolic reprogramming. This is not to say that Aging Onion's categorisation does not have explanatory power, but rather that, in this tissue context and given the selective representation of pathways in figure panels, the data are not readily interpretable. The hypothesis of the Aging Onion is that across organisms and tissue types, intervention success could be mechanistically and quantitatively interpretable through differential engagement of hyperfunction and MRF programs. Validating this hypothesis would require omic datasets to be screened using this axis explicitly, with careful, biologically justified *a priori* classification of genes into either category.

Mechanistic clarity can improve when model systems are designed to probe a specific limiting process. A compelling insight arises from Ham's[77] use of a constitutively active TORC1 mouse model in which proteostasis is compromised, as evidenced by p62 accumulation. In this setting, CR alleviates sarcopenia via upregulation of Xbp1 (a UPR transcription factor), Keap1 (a regulator of the NRF2 oxidative stress response), and the resulting reduction in p62 burden. Because the model renders maintenance failure overt, the experimental design becomes a functional screen for interventions that restore somatic resilience. The success of CR at alleviating sarcopenia under these conditions, despite persistent TORC1 signalling, demonstrates its capacity to engage MRFs independently of TOR effectors[77]. Thus, when maintenance failure is experimentally foregrounded, CR's engagement of MRFs becomes both detectable and relevant. This observation underscores a broader methodological point: full transcriptomic datasets from CR- or rapamycin-treated models may harbour testable signals of mechanistic divergence but require hypothesis-driven gene categorisation to be meaningfully interpreted.

5. Towards an evolutionarily-grounded drug development workflow

Evidence from the nutrient-sensing pathway and disease models suggests that CR engages more layers of aging biology than rapamycin, particularly by activating MRFs. Above, we highlighted the potential for the Aging Onion model to be tested against transcriptomic datasets to determine whether it can explain or predict intervention performance. To assess whether such a methodology could be of use in anti-aging drug discovery, it is helpful to first take a brief tour of the current landscape.

The ideal geroprotector should not only increase lifespan but also attenuate biomarkers of aging and improve healthspan[78]. Several pharmacological candidates have been advanced on the grounds that they reproduce elements of CR's input into the nutrient-sensing pathway. These include rapamycin, metformin, NAD^+ boosters, and more recently the blockbuster GLP-1 receptor agonists (GLP-1RAs)[79]. Against this backdrop, the National Institute on Aging's

Interventions Testing Program provides an important benchmark[80]. Screening a broad panel of candidate geroprotectors in genetically heterogeneous mice, the Interventions Testing Program has consistently found CR to outperform other interventions, whether alone or in combination.

Notably, newer candidates such as the Glucagon-like peptide-1 receptor agonists (GLP-1RAs) have not yet been included in Interventions Testing Program analyses, yet their widespread clinical use and emerging preclinical data make them especially important to contextualise. GLP-1RAs are incretin-based therapies that have rapidly gained popularity in the treatment of obesity and type 2 diabetes. GLP-1RAs act centrally to delay gastric emptying, suppress appetite, enhance glucose-dependent insulin secretion, and reduce glucagon release[81]. The resulting reduction in energy intake promotes weight loss and can correct metabolic dysregulation[81]. Because appetite suppression is a primary mechanism of GLP-1RA action, and weight loss a common outcome, they naturally serve as clinical probes for CR-like responses in humans. Their documented cardio- and reno-protective effects in obese and diabetic patients[82–85] strengthen this impression, although pre-existing morbidity in these cohorts makes it difficult to distinguish genuine modulation of aging biology from disease modification. Post-hoc analyses also struggle to disentangle whether benefits are mediated directly or secondary to weight loss[86,87]. Preclinical work, however, points to important weight-loss-independent effects. Notably, low dose exenatide reverses omic aging signatures across multiple tissues in aged mice, even without changes in food intake or body weight[88]. These effects are mediated solely by central, hypothalamic GLP-1 signalling[88]. If sustainable use in otherwise healthy individuals ultimately requires such low doses, the key question becomes whether hypothalamic GLP-1R agonism, in the absence of reduced energy intake, can recapitulate the breadth of nutrient-sensing inputs engaged by true CR. Whether central GLP-1 signalling engages both hyperfunctional and maintenance-repair layers of aging biology, or instead acts more narrowly, remains to be established.

These uncertainties reinforce the need for discovery workflows that can capture the full breadth of CR's effects while also situating emerging candidates such as GLP-1RAs within a mechanistically grounded framework. One widely used drug discovery workflow is high-throughput transcriptomic screening. A popular approach focuses on reversing age-associated transcriptional changes without explicitly imposing a mechanistic framework on those changes. For instance, Donertas and colleagues[89] compiled gene expression profiles from multiple datasets of aged human brain tissue to identified genes whose expression changed with age. They then queried CMap to identify drugs that induce opposing transcriptional effects. This approach, which focuses on global reversal of age-associated gene expression signatures, identified 24 candidate compounds, including several known pro-longevity agents. However, the authors note that this strategy cannot distinguish between adaptive *vs.* maladaptive age-related expression changes. A refinement came with the ANDRU pipeline[90] which begun by constructing co-expression networks from transcriptomes of young and old human adipose tissue. The workflow then identified age-perturbed subnetworks that converge with age-related disease signatures. Drug perturbation databases were subsequently queried to find compounds that reverse these specific subnetwork level changes. The workflow thus narrows the scope from global differentially expressed genes to those which are likely relevant to the mechanisms of age-related disease in the tissue in question. While these methods have produced plausible leads, both rely on (high-dimensional) statistical correlation between transcriptomic states without explicit integration of *what kinds* of biological processes are being reversed or engaged.

Some efforts have used the transcriptional signature of CR as a benchmark to screen for drugs with similar profiles. This approach leverages CR's well-established effects on conserved regulators of ageing. This approach avoids some pitfalls of simple signature reversal by anchoring candidate selection to an empirically validated pro-longevity intervention rather than to general aging-associated change. However, similarity to CR at the level of the whole-transcriptome does not reveal *which aspects* of CR biology are being mimicked. Calvert and

colleagues[91] provide an instructive example of this limitation. The group used a curated transcriptional signature derived from CR treated rat and monkey cells to query for drugs with similar profiles. Their strongest match was rapamycin, a drug that, as discussed before, affects largely distinct gene sets[75–77], engage divergent pathways[75–77], and which ultimately fails to recapitulate CR's effects in models[18]. Without dissecting which aspects of CR biology are being mimicked allows compounds with very different mechanisms, and inferior performance, to score highly if their net transcriptomic effects align with CR on aggregate.

Transcriptomic screening for new drugs may thus benefit from integration of the Aging Onion's mechanistic granularity. In it, aging arises from the dual forces of (i) continued or excessive activity of developmental and growth programs (hyperfunction), and (ii) inadequate activation of MRFs. This framework enables the *a priori* classification of genes into functionally meaningful categories. Those promoting biosynthesis, cell cycle activity, and nutrient-driven growth would be labelled hyperfunction genes. Those that contribute to genomic stability, proteostasis, autophagy, detoxification, and damage repair would be labelled MRF genes. **Figure 4** demonstrates what a subsequent workflow could look like.

The first testable hypothesis of framework is that in existing transcriptomic data from head-to-head CR *vs.* rapamycin studies, this Aging Onion-guided workflow may mechanistically trace CR's superior efficacy to broader modulation of both aging axes: downregulating hyperfunction and upregulating maintenance. In contrast we would expect rapamycin to predominantly suppress TOR-mediated hyperfunction. Subsequently, the workflow could be applied to tissues or cells treated with candidate interventions in high throughput, with CR and rapamycin scores used as benchmark.

This framework does not seek to mimic the CR signature wholesale, but rather to resolve its effect into interpretable biological dimensions. Unlike CR-mimicry, which treats all DEGs as equal regardless of function, this approach might distinguish between transcriptional changes

that are likely causal in aging *vs.* those that are correlative or downstream. A compound that replicates a CR-like expression profile but fails to promote maintenance gene expression, for example, would not score highly under this schema. Critically, for this method to yield insight beyond existing reverse-signature approaches, several conditions must be met:

1) gene classification must be rigorous, reproducible, and ideally supported by GO, KEGG, or manually curated mechanistic literature,

2) the approach must demonstrate that stratifying gene responses by hyperfunction/MRF categories provides additional discriminative power in compound ranking or outcome prediction beyond traditional metrics, and

3) transcriptomic comparisons (*e.g.* CR *vs.* RM *vs*. aging) must be controlled across tissue, timepoint, and cell type, given the contextual specificity of gene function and age-related deterioration.

If these requirements are met, this strategy could provide a significant advance over correlation-based aging transcriptomic pipelines. Interventions could be screened not just for their ability to reverse age-related gene expression or mimic CR's transcriptome, but for their capacity to rebalance core biological processes implicated in the causal architecture of aging.

6. Conclusion

Aging research has long lacked a unifying framework to guide intervention assessment and discovery[92]. The 'Aging Onion' model, a layered synthesis of two major evolutionary theories, highlights two core domains that drive aging: hyperfunctional growth signalling and suboptimal MRFs. Evidence from the nutrient-sensing pathway and disease-prone animal models suggests that simultaneous engagement of both domains explains CR's broader efficacy compared to rapamycin. We therefore propose the 'Aging Onion' as a useful predictive paradigm for the assessment of candidate interventions. Initial validation requires retrospective analysis of transcriptomic datasets comparing CR and rapamycin, before subsequent analysis of novel candidates through a lens which distinguishes whether

618 interventions suppress hyperfunction, upregulate maintenance, or both. We argue that this
619 methodological shift, in contrast to current correlative approaches, is better placed to select
620 for interventions which truly regulate the core drivers of aging.
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Table 1. Differential effects of caloric restriction (CR) and Rapamycin in disease-prone mouse models. Five mouse models of age-related disease are shown in which both CR- and rapamycin-treated cohorts have been evaluated under comparable conditions. For each model, the dominant pathological mechanism was categorised, using the 'Aging Onion' framework, as either hyperfunction-driven, stochastic damage-driven, or mixed. Outcomes of CR and rapamycin interventions are summarised as reported in the cited studies. Across models dominated by stochastic damage, CR outperforms rapamycin. In contrast, in the $Rb1^{+/-}$ cancer model, driven by hyperfunctional mitogenic signalling, rapamycin outperformed CR. In the mixed pathology cardiac aging model, CR has stronger restorative effects on myocardial performance than rapamycin. Together, these comparisons suggest that intervention outcomes align with the dominant mechanistic driver of pathology: rapamycin is most effective against hyperfunction, whereas CR exerts broader benefits by also engaging maintenance-and-repair processes.

Disease Model	Dominant Pathology Mechanism	CR Effect	Rapamycin Effect	Reference
Amyotrophic Lateral Sclerosis ($SOD1^{H46R/H48Q}$)	*Primarily stochastic molecular damage through (oxidative stress, mitochondrial dysfunction, protein aggregation)*	*- Delayed disease onset* *- extended lifespan by ~14%, prolonged motor function*	*- No impact on disease onset, duration, or survival*	64

Cancer ($Rb1^{+/-}$)	*Hyperfunction through loss of checkpoint for mitogenic signalling and resultant neuroendocrine tumorigenesis*	*- No significant effect on lifespan or tumour growth*	*- Substantial lifespan extension* *- Suppression of pituitary and thyroid tumours* *- Reduced metastasis*	67,68
Cancer ($p53^{-/-}$)	*Mixed, loss of mitogenic signalling checkpoint but also substantial stochastic damage accumulation through loss of DNA damage sensing and apoptotic regulation*	*- Delays tumour onset, increases median survival by ~56%*	*- No lifespan benefit at standard dosing; dependent on intact p53 for tumour suppression*	63,65
Cardiac Aging (26 months)	*Primarily hyperfunction through sustained TOR-driven myocardial growth signalling and metabolic remodelling. Secondary contributions from stochastic protein damage and proteostatic decline*	*- Strongly decreased left ventricular hypertrophy to youthful levels* *- Reversed myocardial performance to youthful levels* *- Restored diastolic function to youthful levels*	*- Moderately decreased left ventricular hypertrophy* *- Moderately restored myocardial performance* *- Restored diastolic function to youthful levels*	69
Progeroid DNA repair deficiency ($Ercc1^{\Delta/-}$)	*Stochastic damage accumulation due to defective nucleotide excision repair leading to transcriptional stress, chronic p53 and interferon signalling. Results in premature senescence, stem cell depletion, neurodegeneration, systemic failure.*	*- Triples lifespan* *- improves neuromuscular function, delays onset of age-related symptoms* *- reduces oxidative DNA damage, senescence markers, inflammatory gene expression*	*- No extension of lifespan* *- No improvement in function or reduction of pathology* *- No impact on DNA damage burden* *- No effects despite effective mTORC1 inhibition*	66

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873 **Box 1**

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BOX 1: KEY DEFINITIONS: EVOLUTIONARY THEORIES AND THEIR MECHANISTIC MEDIATORS

For the purposes of reconciling Hyperfunction Theory and Disposable Soma Theory into an integrated model of aging biology, we provide here a succinct summary of the conceptual proposition of each theory and their mechanistic mediators. It is these mechanistic mediators that drive the aging phenotype, and which through modulation may allow extension of life- and health-span.

HYPERFUNCTION THEORY:

- **Proposition:** persistent activity of growth and developmental programmes beyond their adaptive window promotes aging. These states reflect evolutionarily unopposed programmatic activity in the post-reproductive period – aging as a continuation of development.
- **Mechanistic Mediators:** Molecular processes driving hyperplasia, hypertrophy, metabolic imbalance, and chronic secretory phenotypes. These states arise from unopposed signalling through growth pathways (*e.g.,* TOR, IGF, PI3K/AKT) and often suppress somatic maintenance processes.

DISPOSABLE SOMA THEORY:

- **Proposition:** organisms allocate finite resources to maximise reproductive success rather than indefinitely maintaining the soma. Activity of maintenance and repair functions (MRFs) is calibrated to support a health soma through the organism's expected reproductive lifespan. Insufficient investment into MRFs permits accumulation of stochastic molecular damage and degradation of cellular integrity that contributes to aging and its pathologies in later life.
- **Mechanistic Mediators:** (suboptimal activity of) MRFs – includes DNA repair, proteostasis, autophagy, detoxification, and antioxidant defence.

Figure captions

Figure 1. The Aging Onion Model: integrating evolutionary theories of aging into a layered framework of pathophysiology and therapeutic strategy. The model depicts aging as arising from two interacting processes derived from evolutionary theory: persistent activity of growth-promoting pathways beyond their adaptive window (*Hyperfunction Theory*) and suboptimal allocation of resources to somatic maintenance (*Disposable Soma Theory*). Each concentric layer of the "onion" represents a mechanistic component of aging biology. The 'Pathophysiological Role in Aging' column summarises how each layer contributes to organismal decline: either through pathological overactivity (hyperfunction) or through insufficient somatic maintenance (maintenance and repair function, MRF, failure). Corresponding therapeutic strategies map onto each layer: suppression of hyperfunctional signalling to prevent pathological overactivity, and upregulation of maintenance programmes to buffer stochastic damage. The "onion" metaphor emphasis that interventions can act on single or multiple layers, and that more comprehensive strategies ought to engage both hyperfunction and MRF layers. Order and relative size of layers are schematic only and do not reflect biological importance.

Figure 2. Survival as a function of age under wild and protected conditions, illustrating the role of maintenance and repair functions (MRFs)[14] Maintenance and repair functions (MRFs) refer to cellular and molecular processes – such as DNA repair, proteostasis, and autophagy – that sustain somatic integrity and delay physiological decline. The length of each arrow indicates the functional lifespan of a given MRF – the duration of time for which it can protect the organism from a certain type of damage. The staggered lifespans of different MRFs reflect how individual

maintenance systems fail at different times during organismal life. This figure contrasts survival dynamics under two ecological contexts. **A.** Under wild conditions, survival (sold gold line) declines rapidly with age due to extrinsic hazards such as predation, infection, and starvation. Few individuals live long enough for MRF failure, an intrinsic aging mechanism, to influence mortality. **B.** Under protected conditions (*e.g.* laboratory conditions), extrinsic mortality is reduced, and the resultant survival curve (dashed gold line) allows intrinsic aging to become the dominant life-limiting factor. Longevity is then constrained by MRF failure, leading to stochastic molecular damage accumulation, physiological deterioration, and eventual death. Longevity limits are thus set by the capacity for somatic maintenance. Adapted from [14].

Figure 3. The nutrient-sensing pathway as a mediator of caloric restriction (CR) and rapamycin in aging. CR influences multiple upstream metabolic inputs, including reduced amino acids, and increased AMP/ATP and NAD+/NADH ratios. These signals converge on key nodes such as mTORC1, AMPK, Akt, and sirtuins, which in turn regulate downstream effectors including autophagy, FOXO transcription factors, and maintenance-and-repair functions (MRFs). CR extends life- and healthspan by simultaneously suppressing pro-growth signals and activating protective effectors. Rapamycin is shown for comparison: it inhibits mTORC1 directly but does not replicate CR's broader engagement of multiple inputs and pathways. These differences highlight CR's broader mechanistic reach relative to targeted pharmacological inhibition. **CR differentially modulates these inputs depending on nutrient composition of the diet.*

Figure 4. Workflow for the mechanistic evaluation of candidate anti-aging interventions within the Aging Onion framework. This workflow outlines a method for translating transcriptomic responses to interventions into a mechanistically interpretable measure of engagement with conserved aging processes. The approach proceeds through three stages: (1) extraction of intervention-induced transcriptional signals, (2) functional categorisation of genes into hyperfunction-associated, maintenance-associated, or neutral classes in line with the Aging Onion framework (**Fig. 2)** and (3) generation of a composite mechanistic score that reflects the extent to which an intervention suppresses hyperfunctional pathways and activates maintenance and repair functions (MRFs). By weighting transcriptional changes according to biological role rather than overall similarity, this framework enables causal interpretation of intervention effects and allows systematic comparison of compounds according to their predicted influence on the core regulatory axes of aging.

939 **Figure 1**

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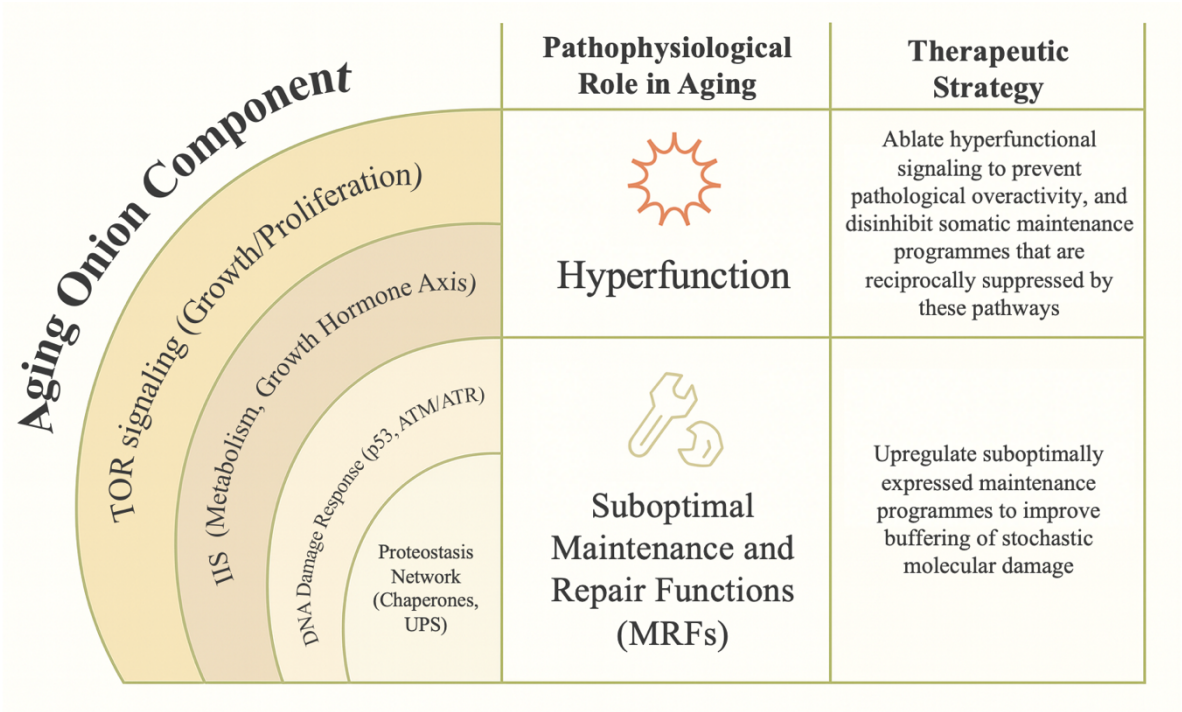

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946 **Figure 2**

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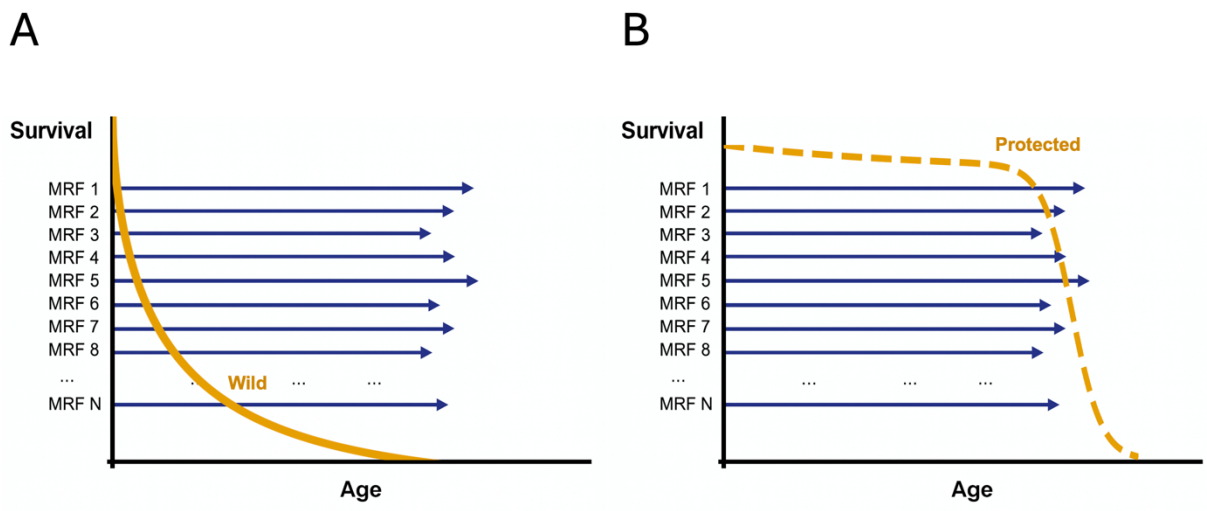

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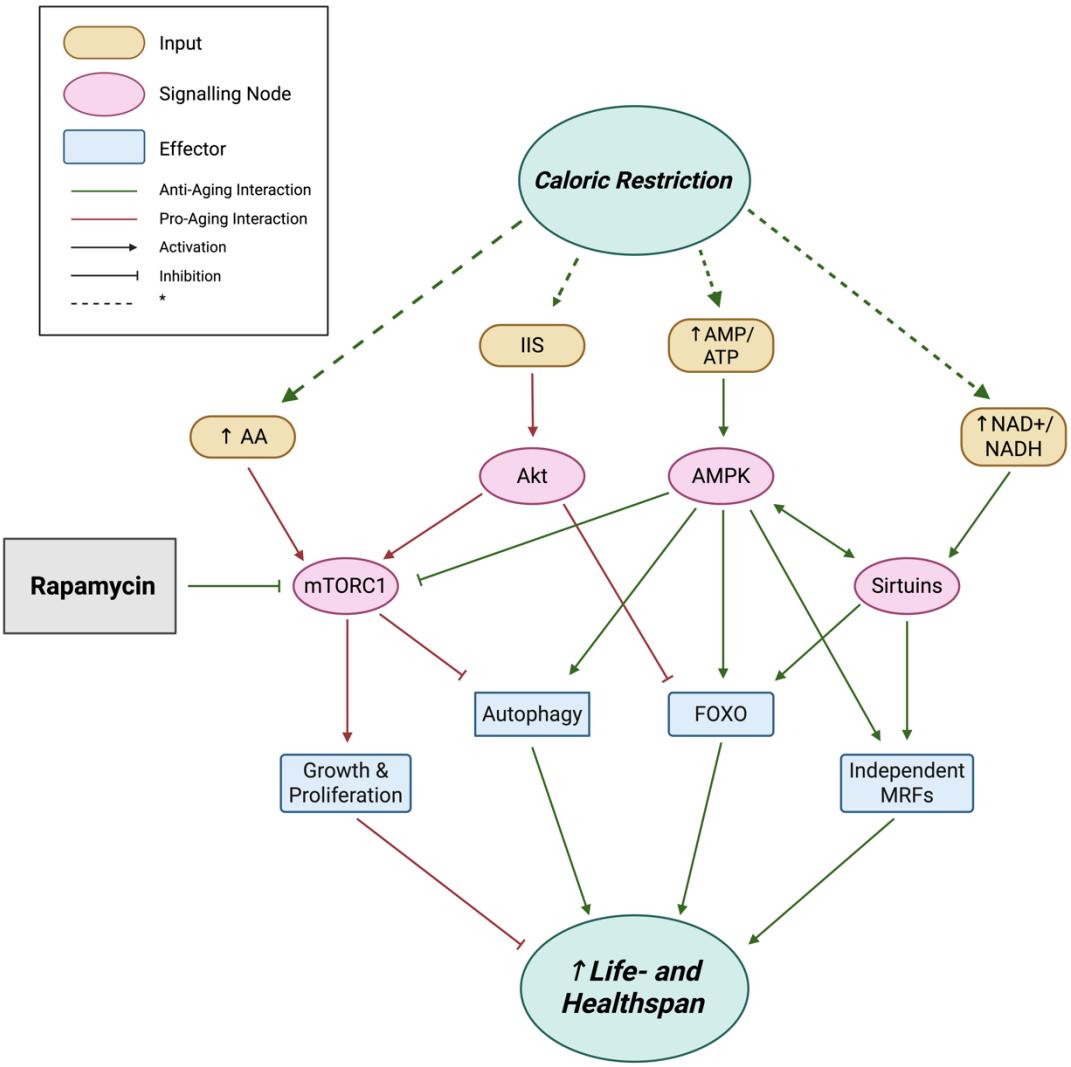
Figure 3


Input
Signalling Node
Effector
Anti-Aging Interaction
Pro-Aging Interaction
Activation
Inhibition
*
Caloric Restriction
IIS
↑AMP/ ATP
↑ AA
↑NAD+/ NADH
Akt
AMPK
Rapamycin
mTORC1
Sirtuins
Autophagy
FOXO
Growth & Proliferation
Independent MRFs
↑Life- and Healthspan

955 **Figure 4**

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