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# Perspective: Modulating the integrated stress response to slow aging and ameliorate age-related pathology

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## Abstract

Healthy aging requires the coordination of numerous stress signaling pathways that converge on the protein homeostasis network. The Integrated Stress Response (ISR) is activated by diverse stimuli, leading to phosphorylation of the eukaryotic translation initiation factor elF2 in its α-subunit. Under replete conditions, elF2 orchestrates 5' cap-dependent mRNA translation and is thus responsible for general protein synthesis. elF2α phosphorylation, the key event of the ISR, reduces global mRNA translation while enhancing the expression of a signature set of stress response genes. Despite the critical role of protein quality control in healthy aging and in numerous longevity pathways, the role of the ISR in longevity remains largely unexplored. ISR activity increases with age, suggesting a potential link with the aging process. Although decreased protein biosynthesis, which occurs during ISR activation, have been linked to lifespan extension, recent data show that lifespan is limited by the ISR as its inhibition extends survival in nematodes and enhances cognitive function in aged mice. Here we survey how aging affects the ISR, the role of the ISR in modulating aging, and pharmacological interventions to tune the ISR. Finally, we will explore the ISR as a plausible target for clinical interventions in aging and age-related disease.

## Abbreviations

| | |
|---|---|
| **AD** | Alzheimer's disease |
| **ALS** | Amyotrophic lateral sclerosis |
| **ATF4** | activating transcription factor 4 |
| **BiP** | Endoplasmic reticulum chaperone BiP |

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| **CHOP/DDIT-3** | C/EBP-homologous protein, DNA damage-inducible transcript 3 |
| **eIF2** | eukaryotic translation initiation factor 2 |
| **GCN2** | general control nonderepressible 2 (EIF2AK4) |
| **HRI** | Heme-regulated inhibitor (EIF2AK1) |
| **ISR** | integrated stress response |
| **ISRIB** | integrated stress response inhibitor |
| **MEHMO** | mental deficiency, epilepsy, hypogenitalism, microcephaly and obesity) |
| **PERK** | PKR-like endoplasmic reticulum kinase (EIF2AK3) |
| **PKR** | protein kinase R (EIF2AK2) |
| **PPP1R15A/PP1** | protein phosphatase 1 |
| **PPP1R15B/GADD34** | Growth Arrest and DNA Damage-Inducible Protein |
| **CReP** | Constitutive Repressor of eIF2a Phosphorylation |
| **TM** | tunicamycin |
| **TOR** | target of rapamycin |
| **uORF** | upstream open reading frame |
| **UPR** | unfolded protein response |
| **VWM** | vanishing white matter disease |

## Introduction

Aging predisposes to numerous diseases and confronts us with major economic and social challenges. Defined as a pathological process [1], aging leads to a progressive loss of physiological integrity, accompanied by reduced cellular, organ, and systemic performance. Aging is characterized by cellular hallmarks including genomic instability, deregulated nutrient sensing, and loss of protein homeostasis [2]. During aging, stress signaling pathways such as the Heat Shock Response (HSR) or the ER Unfolded Protein Response (UPR) become dysfunctional leading to an imbalance of the protein homeostasis network [3–5]. The ISR responds to internal and external stimuli and controls numerous outputs including amino acid metabolism, apoptosis, and protein homeostasis via the regulation of the eukaryotic initiation factor 2 (eIF2) and mRNA translation. Thus, the ISR plays a critical role in organismal resilience.

## The ISR controls mRNA translation initiation

The ISR is an evolutionarily conserved pathway in eukaryotic cells whose function is to restore cellular homeostasis in response to stress [6–8] (Figure 1). The key node of the ISR is the GTPase eIF2 that controls global protein synthesis as the central regulator of mRNA translation initiation [9] . During translation initiation, the ternary complex composed of eIF2, GTP, and the initiator Met-tRNA, binds to the 40S ribosomal subunit with eIF1, eIF1A, eIF3, and eIF5 to form the 43S preinitiation complex [10] . The preinitiation complex scans an mRNA and a stable mRNA codon-tRNA anticodon interaction upon reaching an AUG triggers GTP hydrolysis and Pi release. eIF2·GDP has a lower affinity to Met-tRNA, releasing eIF2·GDP. Next, eIF5B mediates binding of the large 60S subunit to form the 80S ribosome ready to start translation elongation. eIF2B is the guanine nucleotide exchange factor of eIF2, contributing to ternary complex recycling for a new round of translation initiation [11] .

Four stress sensors, the eIF2 kinases, activate the ISR: heme-regulated inhibitor (HRI) [7] , protein kinase R (PKR) [12] , general control nonderepressible 2 (GCN2) [6] , and protein kinase R-like endoplasmic reticulum kinase (PERK) [13] . The kinases respond to distinct inputs such as iron deficiency and mitochondrial stress (HRI) [14,15] , viral infection (PKR), amino acid deprivation (GCN2), and accumulation of misfolded protein in the ER (PERK). Activation of the ISR first requires autophosphorylation of the kinases [16] . The substrate of the four ISR kinases is serine 51 of eIF2α, providing an elegant mechanism that integrates the various upstream stress signals through one specific chemical modification. Phosphorylated eIF2α (p-eIF2α) is a potent noncompetitive inhibitor of its own activator eIF2B, leading to reduced ternary complex formation and attenuated global protein synthesis [17–19] . Paradoxically, several mRNAs that contain short upstream open reading frames (uORFs), such as the activating transcription factors 4 (ATF4) and 5 (ATF5), C/EBP homologous protein (CHOP), and PPP1R15A/GADD34, are selectively translated when eIF2α is phosphorylated [20–22] . eIF2α phosphorylation is further controlled by protein phosphatase 1 (PP1), as a catalytic core, in complex with either the constitutively expressed PPP1R15B/CReP [23] or the ISR-inducible PPP1R15A/GADD34 [24] . GADD34 contributes to a feedback loop to antagonize the ISR and is responsible for termination of the ISR by promoting p-eIF2α dephosphorylation. Depending on stress intensity and duration, ISR signaling is adaptive to promote homeostasis, or it can lead to apoptotic cell death [25] .

It is important to note that the ISR kinases directly act on the key node of the translation initiation machinery without the involvement of a *bona fide* signal transduction pathway. Thus, the ISR acts through the eIF2 pathway and the activity of the ISR kinases favors certain states of translation initiation: the ISR is a tuning mechanism of translation initiation. In line with this, the ISR contributes to maintaining tight regulation of global translation rates. Forced expression of ATF4 and CHOP, for instance, leads to increased protein synthesis, ATP depletion, oxidative stress, and cell death [26] . In turn, loss of the CHOP-GADD34 feedback loop promotes eIF2α phosphorylation, reduces ER protein aggregation and counters ER stress-induced apoptosis *in vivo* [27] .

There is extensive cross talk between the ISR and other signaling pathways. The UPR specifically monitors the luminal ER milieu and PERK is one of its stress sensors, along with IRE1 and ATF6 [28] . Thus, PERK-induced signaling represents an overlap between the ISR and the UPR. Additionally, the transcription factor NRF2 is a PERK substrate and critical for PERK-mediated cell survival during stress [29] . Downstream ISR effectors also cross-talk with other pathways. ATF4, for example, mediates metabolic effects of mTORC1 signaling without eIF2α phosphorylation [30,31] . Given the function of the mTORC1 pathway in growth, this appears counter-intuitive, but ATF4 controls amino acid synthesis and uptake which are downstream of both mTORC and ISR signaling.

Deregulation of the ISR is observed in a wide variety of diseases such as cancer, neurodegenerative disorders, and metabolic syndrome [32–42] . Mutations in any eIF2B subunit can cause vanishing white matter (VWM) disease, an autosomal recessive leukoencephalopathy characterized by myelin loss and ataxia [43–45] . Mutations in HRI and PKR have been detected in individuals presenting developmental delay and leukoencephalopathy, supporting the role of eIF2 in neuronal homeostasis [46] . eIF2 mutations have been linked to MEHMO syndrome, a rare X-linked intellectual disability [47–49] . Moreover, mutations in the eIF2α phosphatase regulator CReP causes diabetes, short stature, and microcephaly [50,51] . Mutations in PERK result in the rare Wolcott-Rallison syndrome that is characterized by insulin-requiring diabetes and impaired neuro-physiological development [52,53] . Mutations in GCN2 are linked to pulmonary veno-occlusive disease (PVOD), a form of pulmonary hypertension [54] . These diseases demonstrate the essential role of the ISR in cellular resilience and health.

## Does aging affect the ISR?

Pathways that affect aging often undergo dramatic changes with age, and the causes need to be carefully disentangled from consequences. To first delineate a possible link between aging and the ISR, here we refer to studies that are sufficiently powered and that use truly aged animals that, in the case of mice and rats, are over 18 months of age. The eIF2α kinases constitute the first layer of ISR regulation and several studies using rodents report age-related changes in their expression. PERK phosphorylation is increased in the pancreas of old male mice compared to young adult animals [55] . A comparison of adult and old male mice demonstrated an increase of PKR protein levels in all tested tissues, including kidney, liver, colon, brain, testes, pancreas, lung, and heart [56] . Human muscle biopsies from donors ranging between 20 and more than 80 years of age also show increasing PKR abundance with age [56,57] . Further studies analyzed the phosphorylation status of GCN2, finding that it is increased in the brain of old (aged 19 months, n=3) male mice compared to mature adults (3-6 months of age, n=5) [58] . To our knowledge, no studies reported results on HRI activity during aging.

At the level of eIF2α phosphorylation, there is evidence for age-related ISR induction in multiple species. In very old male mice, the p-eIF2α:eIF2 ratio in skeletal muscle is 2-fold higher compared to mature adult animals [59] . Mice also show an increase in eIF2α phosphorylation in aged liver and kidney, albeit only two animals were used per condition in this study [56] . In male rats, the p-eIF2α:eIF2 ratio is increased in the aged cortex [60] .

Clearly, rodent studies using females are yet lacking to delineate possible sex differences in age-dependent ISR changes. In the fruit fly, eIF2α phosphorylation is increased when comparing animals aged 9 to 12 days with animals aged 8 weeks [61] . In *Caenorhabditis elegans*, we demonstrated a strong increase in eIF2α phosphorylation beginning early in adulthood [62] .

While the ISR attenuates global protein synthesis, it enhances translation of select uORF-regulated mRNAs. GCN4/ATF4 is conserved from yeast to mammals and it is considered one of the main ISR effectors [20] . A study in rat substantia nigra neurons showed elevated ATF4 in aged females [63] . Furthermore, ATF4 was found increased in the brain of old male mice [58] . Long-lived mice subjected to caloric or methionine restriction, or after treatment with the lifespan extending drugs rapamycin and acarbose, showed elevated ATF4 and CHOP. This suggests an adaptive ISR in long-lived animals [64,65] .

Together, these observations suggest that the ISR is activated in aged animals, which goes hand in hand with the reduction in protein synthesis observed during aging [66–69] (Table 1). However, from these studies it remains open if the ISR might promote health in old animals or if it might instead contribute to age-related dysfunction. There likely exists cell type heterogeneity regarding ISR kinase expression and ISR tuning with age in different tissues. Studying this relationship at the single-cell level and using existing ATF4 reporter mice [70] , or the recently engineered *in vivo* ISR reporter [71] , would constitute an important next step.

Beyond its function as a central hub of protein homeostasis, the ISR is implicated in other hallmarks of aging as well [2] . UV irradiation leads to ribosome collisions that activate a GCN2-mediated stress response [72] , an elegant mechanism to resolve the stress through translational attenuation. DNA damage also leads to eIF2 phosphorylation through PKR, inducing cell death [73] . Furthermore, altered nutrient sensing and nutritional stress have been linked to ISR activation. Dietary deficiency of essential amino acids activates the neuronal GCN2-p-eIF2α axis in mice, affecting foraging behavior and food selection for complementary amino acid sources [74] . Obesity is characterized by chronically elevated glucose, free fatty acids, and inflammatory cytokines, which trigger ER stress [75] . ATF4 null mice resist diet- or age-induced obesity, they are hypoglycemic, and have increased energy expenditure, mimicking some effects of mTORC inhibition, again linking downstream effects of ISR and TOR signaling [76] . Finally, nutrient limitation in solid tumors is linked to the GCN2-p-eIF2α-ATF4 axis promoting cancer cell survival and proliferation through expression of asparagine synthase [77] whereas the PERK-p-eIF2α-ATF4 axis is critical for hypoxia adaptation of tumor cells [78] . In conclusion, a variety of cellular stimuli mediate ISR activation during aging or age-related pathologies, contributing to physiological changes at the organismal level.

## Genetic manipulation of the ISR affects aging

As the previously mentioned studies suggest a link between aging and ISR signaling, we will next discuss a causal involvement of the ISR in the aging process. In yeast, translation rates decrease during replicative aging as a consequence of GCN2 activation and eIF2α phosphorylation but without induction of the ATF4 homolog GCN4. Experimental activation

of GCN4, however, extends yeast lifespan by activating autophagy [79] . An excellent recent review further covers the links between ISR activation and lifespan extension in *S. cerevisiae* [80] .

The use of *C. elegans* to investigate the ISR has been fairly limited although it is optimally suited to study fundamental aspects of aging and longevity. The genome of *C. elegans* contains orthologues of key ISR genes including two eIF2α kinases *pek-1*/PERK and *gcn-2*/GCN2 [81,82] . At the amino acid level, eIF2α shares 50% identity with the human protein and the residues surrounding Ser51 are completely conserved. Knock-down eIF2Bδ reduces overall mRNA translation and extends lifespan [83] . Genetic analysis of the 5'UTR of *C. elegans atf-4* demonstrated the presence of two uORFs. A translational reporter containing the *atf-4* 5'UTR and uORFs GFP expression was built in David Ron's lab, and ER stress increases its expression. Thus, as in mammals, worm ATF-4 is induced by cellular stress [84] . Similarly, as in yeast or mammals, amino acid limitation increases ATF-4, and this is *gcn-2*-dependent, demonstrating the conservation of key players of the ISR [85] .

*pek-1* deletion mutants are hypersensitive to TM, functionally supporting the role of *pek-1* as the PERK orthologue [79] . Several studies highlight the role of *pek-1* in nematode survival during regular or stressed conditions. *pek-1* is partially required to ensure normal larval development [86] . In the absence of ER stress, the UPR is an important regulator of ER homeostasis and *pek-1* is required to promote larval survival upon bacterial infection [81] . In response to crowding, elevated temperature, or starvation, *C. elegans* enter into an alternative developmental dauer state that is protected from the effects of aging [87] . The phosphorylation of eIF2α by PEK-1 in specific chemosensory neurons is a key mediator of the transition into the dauer state to promote survival [88,89] .

Several studies focused on the role of *gcn-2* in *C. elegans* physiology. Increased lifespan mediated by mitochondrial dysfunction depends on *gcn-2* and eIF2α phosphorylation, suggesting that in *C. elegans*, the kinase plays a role as a mediator of the mitochondrial stress response pathway [90] . In mammalian cells, in contrast, HRI links mitochondrial stress with the ISR [14,15] . Translation attenuation and protein aggregation caused by hypertonic stress are mediated by *gcn-2* and eIF2α phosphorylation [91,92] . Moreover, *gcn-2* plays an important role in the lifespan extension mediated by dietary restriction or TOR inhibition [85] . GCN1 is a scaffold protein required for GCN2 activation [93] and this interaction is modulated by IMPACT that prevents GCN2 from interacting with GCN1 [94] . IMPACT suppression thus activates the ISR and increases worm lifespan and stress resistance [95] . Of note, a partial *atf-4* deletion does not affect *C. elegans* lifespan [85] . Recently, we showed that pharmacological or genetic inhibition of the ISR leads to longevity [62] . In an unbiased point mutagenesis screen in *C. elegans* for longevity, we found dominant mutations in eIF2Bγ as well as loss-of-function mutations in PEK-1 and GCN-2. An eIF2αS51A mutant confirmed that ISR inhibition leads to longevity. In line with ISR inhibition this occurred *without* reduction in global mRNA translation, but through translational changes of selected mRNAs [62] . This work revealed a novel longevity mechanism triggered by ISR inhibition in addition to known longevity paradigms that require GCN-2 and ATF-4 activation. Longevity caused by ISR inhibition suggests that ISR modulation and translational reprogramming, in addition to a role in specific diseases, impinges on the aging process itself.

In *D. melanogaster*, as in *C. elegans*, the main actors of the ISR including GCN2 (dGCN2), PERK (dPERK), and eIF2B are conserved and they are essential for development and homeostasis [96–98]. The fruit fly also possesses an eIF2α phosphatase regulatory subunit dPPP1R15, which is functionally homologous to the mammalian CReP, and, as in mammals, its expression is controlled by uORFs [99]. Over-expression of the eIF2α kinase dPERK in photoreceptor neurons leads to eye defects and this phenotype can be rescued by over-expressing the eIF2α phosphatase [100]. dPERK is a crucial regulator of intestinal homeostasis, promoting regeneration [101]. Prolonged ISR activation, however, is detrimental in later age and a partial dPERK knockdown in intestinal stem cells improves gut homeostasis, barrier function, and extends lifespan [101]. This strikingly mirrors our findings in the worm that link ISR inhibition to prolonged survival. As in worms and yeast, dietary restriction mediated longevity in *D. melanogaster* is dGCN2 dependent [102]. Concluding, genetic manipulation of the ISR in yeast, worms, and flies has provided considerable knowledge about its role in development and health, but more studies are required to understand how the ISR controls aging. One possible direction is an exploration of tissue-specific effects of ISR manipulation.

As detailed above, the ISR plays a crucial role in mammalian development and physiology. The homozygous eIF2αS51A substitution, which disrupts phosphorylation, is lethal in neonatal mice [103]. Similarly, deletion of PERK leads to prenatal death in 40% of pups and surviving newborns display severe growth retardation and hyperglycemia [104]. Constant ISR activation mediated by the genetic deletion of both GADD34 and CReP is lethal as well [105]. Whether genetic manipulation of the ISR affects mammalian aging remains unknown, but potent pharmacological ISR modulators have emerged, as detailed next.

## Pharmacological ISR modulation in aging and age-related diseases

Genetic and pharmacological ISR inhibition enhance memory in aged mice and increase survival in *C. elegans* [58,62]. It is thus tempting to speculate that small-molecule ISR inhibitors could increase health- and lifespan. ISR inhibition ameliorates pathology in mouse models of the progeroid Down syndrome [38] and Alzheimer's disease (AD) [40]. However, beneficial effects of ISR activation have been demonstrated in Huntington's disease [106] ,ALS [42,107,108] and multiple sclerosis [109]. Thus, depending on disease context, the ISR appears to be protective or maladaptive and during aging the consequences of chronic ISR modulation remain unclear. We will next discuss ISR activator and inhibitor compounds and their role in age-related diseases (summarized in Table 2).

The HRI-activating compounds 1-((1,4-trans)-4-aryloxycyclohexyl)-3-arylureas (cHAUs) increase p-eIF2α, inhibit mRNA translation and slow cancer cell proliferation [110]. At this point, they have not been widely explored in further disease contexts. Salubrinal was first identified as an inhibitor of CreP and GADD34 [111] but direct evidence supporting this conclusion is missing. Nonetheless, it is a potent activator of the ISR [112] and is successfully used for *in vivo* studies [40]. In a mouse model of Parkinson's disease, salubrinal attenuates motor dysfunction and extends lifespan [41]. Guanabenz and its derivative Sephin1 protect against toxic protein misfolding and ER stress [113]. Guanabenz and Sephin1 bind to GADD34 to counter p-eIF2α dephosphorylation [107]. *In vitro* evidence supports the role of

Guanabenz and Sephin1 as selective inhibitors of GADD34 [114,115] . Other studies, however, did not detect a direct effect of Guanabenz or Sephin1 on p-eIF2α dephosphorylation and cells lacking GADD34 or carrying the phospho-defective eIF2αS51A mutation remain responsive to Sephin1 [116,117] . In rodents, Guanabenz improves memory and reduces cortical tissue loss after traumatic brain injury [118] and it ameliorates myelin pathology caused by a VMW disease mutation [119] . Sephin1 is protective in a mouse model for multiple sclerosis and this effect depends on GADD34 [120] . Despite the debate regarding their molecular mechanism, Guanabenz and Sephin1 have therapeutic potential and they are tested in clinical trials in the context of Charcot-Marie-Tooth disease and ALS, where Guanabenz is showing encouraging results [121] . Raphin1, also derived from Guanabenz, binds the constitutive PP1 regulatory subunit CReP [106] . In HeLa cells, Raphin1 induces a transient increase in eIF2α phosphorylation with a concomitant increase in ATF4, GADD34, and translational attenuation; effects that are lost in the $CReP^{-/-}$ genetic background [106] . Administered *in vivo*, Raphin1 does not show any measurable adverse effects on body weight, pancreatic and liver function, or memory [106] . Instead, it attenuates neurological decline in a mouse model for Huntington's Disease [106] , making it an attractive candidate to study consequences of ISR activation in aging and other age-related diseases.

ISR inhibitors target the eIF2 kinases or desensitize cells to the effects of eIF2α phosphorylation. GSK2606414 and derivatives inhibit PERK activation [122] , they are effective in cultured cells [123] but have not been used in aging experiments. However, they might not be suitable due to side effects: GSK2606414 can lead to pancreatic toxicity [124] , weight loss, and mild hyperglycemia [125] , as a possible consequence of on target toxicity through pancreatic PERK inhibition. ISRIB (ISR inhibitor) decreases ATF4 translation during ER stress [126] and cells treated with ISRIB are resistant to eIF2α phosphorylation. ISRIB counters mRNA translation changes caused by TM stress and eIF2α phosphorylation, it exacerbates TM toxicity, and it attenuates the formation of stress granules [21,126] . In striking similarity to $eIF2^{+/S51A}$ heterozygous mice, ISRIB treatment increases long-term memory [126] . eIF2B is the target of ISRIB [112,113] that acts as a molecular staple promoting assembly of the hetero-octameric eIF2B$(\beta\delta\gamma\varepsilon)_2$ structure and enhancing its affinity to the eIF2B(α2) dimer [124,125] . On the eIF2B surface, the binding sites for p-eIF2 and ISRIB are ~50 Å apart and **i**ndeed ISRIB allosterically antagonizes the inhibitory binding of p-eIF2 to eIF2B [127,128] . ISRIB antagonizes the inhibitory binding of p-eIF2 to eIF2B. On the eIF2B surface, the binding sites for p-eIF2 and ISRIB are ~50 Å apart, suggesting allosteric interaction between ISRIB and p-eIF2 binding [121,122] . Although there is no evidence indicating that ISRIB might affect lifespan, a recent study showed that ISRIB reverses age-related memory decline in mice: ISRIB reverses age-related deficits in spatial, working, and episodic memory by improving both neuronal structure and function [58] . 2BAct was developed to enhance pharmacokinetic properties and solubility of ISRIB [129] . Trazodone and dibenzoylmethane (DBM) inhibit the ISR as measured using a CHOP::luciferase reporter [130] . Trazodone and DBM do not prevent eIF2α phosphorylation but decrease ATF4 protein levels and partially rescue translation, placing these compounds downstream of p-eIF2α but unlike ISRIB, trazodone or DBM do not affect eIF2B dimerization [130] .

ISR inhibition by compounds that desensitize cells to the inhibitory effects of p-eIF2α is beneficial in the context of multiple diseases. ISRIB and 2BAct ameliorate defects in mouse models of human disorders affecting protein synthesis through eIF2B mutations, for example in (VWM) [123,125], Down syndrome [38], and in traumatic brain injury [37]. Similarly, various ISR inhibitors have been successfully used in the context of age-related diseases. Administration of GSK2606414 in a mouse model for frontotemporal dementia restores protein synthesis and reduces the levels of p-PERK, p-eIF2α, and ATF4 that are increased in the vehicle controls [131]. On the other hand, the use of ISRIB did not alleviate the behavioral impairments or neuropathology observed in the PS19 tauopathy mouse model [132].

In a cellular ALS model, ISRIB decreases SOD1G93A-dependent neuronal death and ER-stress [39]. In prion disease, ISR inhibition by ISRIB, trazodone, or DBM prevent neurological symptoms and increase survival [130] oral administration of GSK2606414 to prion-infected mice limits p-PERK, p-eIF2α, ATF4, and CHOP levels, and eliminates the signs of prion disease, preventing neurodegeneration [125]. Additionally, ISRIB provides a valuable therapeutic approach in a mouse model of AD reversing memory impairment, dendritic spine loss and defective hippocampal protein synthesis induced by Aβ oligomers [40]. In contrast, ISRIB did not rescue spatial learning nor memory in the hAPP-J20 mouse model of AD [133].

In a mouse model of prostate cancer, characterized by PERK activation and translational attenuation, ISRIB not only restores protein synthesis but also induces tumor regression after a 3-week treatment: ISRIB extends survival and exhibits tumor regression in xenograft studies [35], making ISRIB and ISR inhibitors promising approaches in cancer therapy.

## Concluding remarks

ISR inhibition or activation have the potential to counter pathology, depending on the disease context (Table 2), suggesting that ISR manipulation might be a therapeutic way forward [39,42,134,135]. How should we approach future studies addressing the ISR in disease and aging? We propose the parallel experimental use of ISR activators and inhibitors with known molecular mechanisms. This will reduce potential biases stemming from strains, genotypes, timing and dosing of interventions, analyzed tissues, age, and enhance reproducibility.

While many experiments have been performed to explore the consequences of ISR modulation in various diseases, it remains unknown whether pharmacological interventions modulating the ISR could affect aging. Our recent data indicate that pharmacological and genetic inhibition of the ISR extend lifespan in worms [62]. Of note, ISR inhibition initiated in mid-life had the same beneficial effect on survival as treatment throughout adult life, suggesting that relatively late intervention might increase survival also in other species. Leveraging the deep knowledge about molecular targets and their mechanisms of action, the small molecule ISR modulators Raphin1, ISRIB, and 2Bact should be tested for their potential to modulate the aging process. The clinical molecule Sephin1 as well as Salubrinal should also be tested for their effect on survival in higher organisms while more work is needed to understand the molecular mechanisms of action the latter. ISRIB

was found in screens using mammalian cells and the drug-target interaction is extremely specific [136], hence its use in lifespan experiments in non-vertebrates is likely to fail. We did not detect any biochemical or physiological effects of ISRIB in *C. elegans*; consequently, although lifespan experiments in rodents are time-consuming, they are needed to study how these compounds might impact the aging process. Lifespan studies in mice supplemented with ISRIB/2BAct, in parallel with ISR activators like Raphin1, will provide definitive data regarding the role of ISR tuning in mammalian aging. It would further be important to include late-life interventions. Given the complex nature of ISR signaling and outputs, a combination of proteomic, phosphoproteomic, and (single-cell) RNA sequencing experiments should be done to define the regulation of the ISR with aging.

The canonical view in geroscience predicts that longevity can emerge when stress response pathways are activated, or by inhibition of growth signals. The observation that translational reprogramming mediated by ISR inhibition leads to lifespan extension surprisingly goes against this concept. Nematode longevity occurs in eIF2Bγ mutants that are likely to mimic the effect of ISRIB [35]. Together with the beneficial effects of ISR inhibition in numerous age-related diseases, this strongly suggests that ISR inhibition modulates the aging process itself. With this, it does not only expand the theoretical framework of geroscience but provides a promising avenue in the prevention and treatment of aging and age-related diseases.

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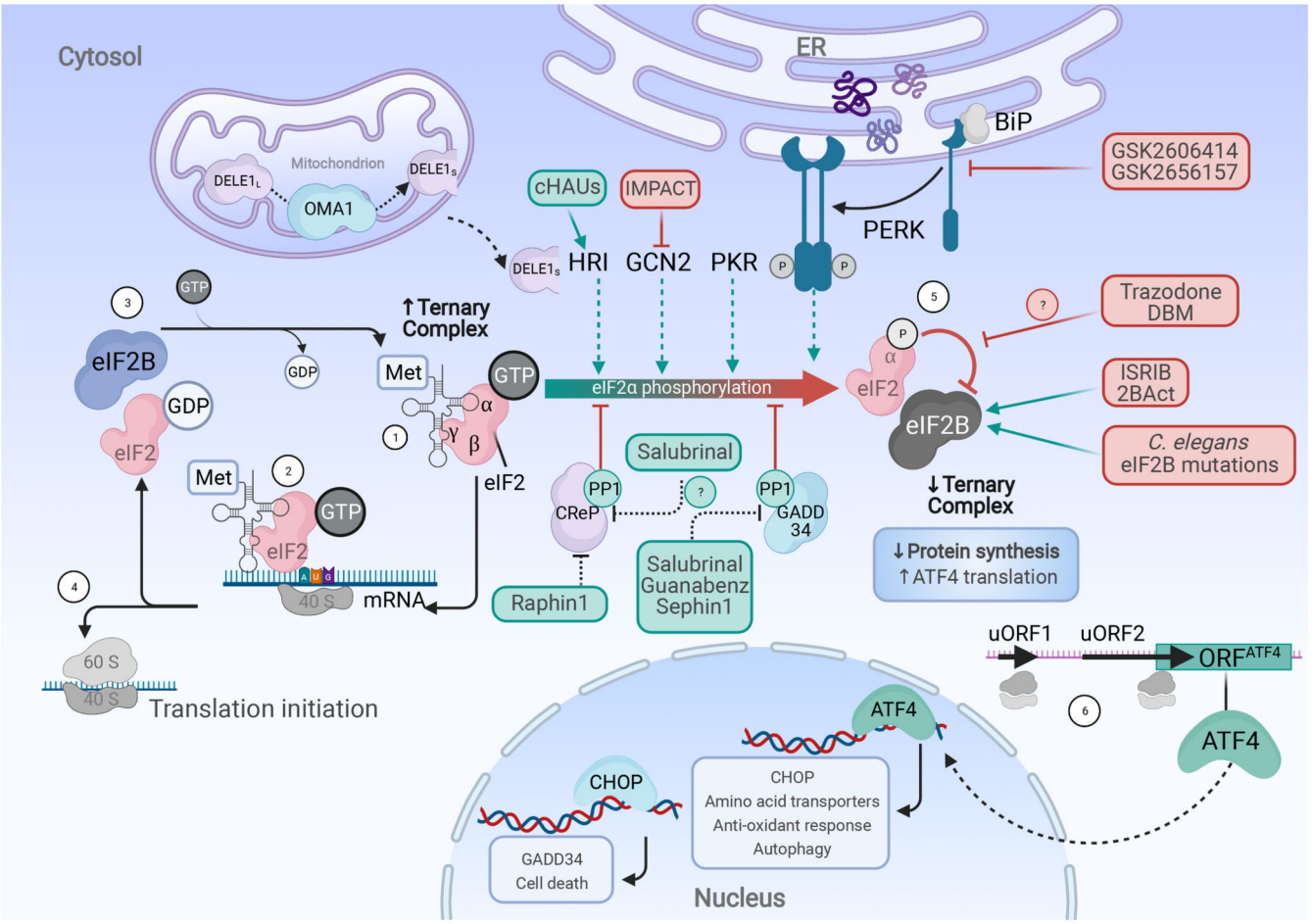


**Figure 1. The ISR signaling pathway.**

Translation initiation is controlled by the abundance of the eIF2· GTP· Met-tRNAi ternary complex (1). The ternary complex and the 40S ribosome scan along mRNAs until AUG start codon recognition (2). GTP hydrolysis releases eIF2 and other initiation factors from the mRNA-40S-complex (3), allowing the 60S ribosomal subunit to bind and proceed to elongation (4). The guanine nucleotide exchange factor eIF2B catalyzes the GTP exchange to recycle the ternary complex ensuring further rounds of translation initiation. Several stimuli (ER and mitochondrial stress, heme deprivation, viral infection, and amino acid starvation) activate the eIF2 kinases PERK, HRI, PKR and GCN2, respectively, leading to eIF2α phosphorylation at Ser51 (5). p-eIF2 inhibits its own activator eIF2B, leading to ternary complex depletion, decreasing cap-dependent translation an elevating the selective translation of uORF regulated genes, such as ATF4 (6). ISR termination is accomplished by the dephosphorylation of p-eIF2α by protein phosphatase 1 (PP1) complexed with either the constitutively expressed CReP or the ISR-inducible GADD34 regulatory subunits. Pharmacological modulation of the ISR occurs at different levels: ISR activators (in green boxes) target the PP1:CReP complex or HRI while ISR inhibitors (in red boxes) target eIF2B, GCN2 or PERK.

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## Table 1

### Changes of ISR activity during aging.

Effects of aging on the ISR in indicated organisms and tissues. y: year, m: months, w: week, d: day. Data represent protein level changes.

| Name | Organism | Age | n | Sex | Tissue | Change with aging | Reference |
|---|---|---|---|---|---|---|---|
| **p-PERK** | Mouse | 22-27 m vs 10 w | 8 | M | Pancreas | Increased | Naidoo 2014 |
| **PKR** | Mouse | 20 m vs 8 w | 5 | M | Kidney, liver, colon, brain, testes, pancreas, lung and heart | | Ladiges 2000 |
| | Human | 20 y to >80 y | n≥10 | M/F | Muscle (biopsies) | | Ubaida Mohien 2019 |
| **p-GCN2** | Mouse | 19 m vs 3-6 m | 3/5 | M | Brain lysate | | Krukowski 2020 |
| **p-eIF2a** | Mouse | 20 m vs 8 w | 2 | M | Kidney and liver | | Ladiges 2000 |
| | Mouse | 26 m vs 8 m | 8 | M | Skeletal muscle | | Chalil 2015 |
| | Rat | 26 m vs 8 w | 4 | M | Brain cortex | | Segev 2013 |
| | Fruit fly | 8 w vs 9 d | 4 | F | Fly heads | | Brown 2014 |
| | Worm | d 6 vs d 1 | 4 | - | Worm lysate | | Derisbourg 2021 |
| **ATF4** | Rat | 24 m vs 2 m | 10 | M/F | Substantia nigra neurons | | Salganik 2015 |
| | Mouse | 19 m vs 3-6 m | 3/5 | M | Brain lysate | | Krukowski 2020 |

## Table 2

### Consequences of pharmacological ISR modulation in age-related diseases.

ISR activators and inhibitors used in rodents or cell culture models of neurodegeneration with the main observations reported in the cited studies. y: year, m: months, w: week, d: day, M: male, F: female, doses are per body weight.

| Compound | Disease | Animal model | Main outcomes | Dose/Age/Time intervention/Sex | ISR manipulation | Reference |
|---|---|---|---|---|---|---|
| Salubrinal | **Parkinson's disease** | Mice over-expressing a-synuclein with PD-associated mutation A53T | • Extends lifespan and attenuates motor dysfunction | Presymptomatic mice (10-14 m), Salubrinal concentration not indicated | Activation is beneficial | Colla 2012 |
| Guanabenz | **TBI** | Injured rats | • Improves memory<br>• Reduces cortical tissue loss | 30 min post-injury, treatment with 5 mg/kg. Rats of 275-300 g (M) | Activation is beneficial | Dash 2015 |
| Guanabenz | **VWM** | VWM eIF2Be-R191H mice | • Improves memory<br>• Reduces cortical tissue loss | 8 m treatment (2-10 m of age) with 10 mg/kg (F) | Activation is beneficial | Dooves 2018 |
| Guanabenz | **ALS** | SOD1G93A mice | • Delays disease onset<br>• Prevents motor neuron loss | 4 mg/kg starting at 40 d of age until disease onset (F) | Activation is beneficial | Jiang 2014 |
| Guanabenz | **ALS** | SOD1G93A mice | • Delays disease onset | 8 mg/kg starting at 60 d of age until disease onset (F) | Activation is beneficial | Wang 2014 |
| Guanabenz | **Multiple Sclerosis** | GFAP/tTA;TRE/IFN-γ mice | • Protects myelin loss | 20 d treatment with 4.8 and 16 mg/kg (F) | Activation is beneficial | Way 2015 |
| Raphin1 | **Huntington's disease** | HD82Q mice | • Attenuates neurological decline | 4 w treatment with 2 mg/kg, starti ng at 4-10 w of age (M) | Activation is beneficial | Krzyzosiak 2018 |
| Sephin1 | **CMT1** | Myelin protein zero mutant mice | • Recovers myelination<br>• Normalizes of ER-stress gene expression | 28 d old mice, treatment bi-daily with 1 mg/kg Sehpin1 for 150 d (M) | Activation is beneficial | Das 2015 |
| Sephin1 | **Multiple Sclerosis** | Subcutaneous administration of myelin oligodendrocyte glycoprotein (MOG35-55) peptide | • Delays onset of clinical symptoms<br>• Interferon-β slows disease | Daily treatment 7 d after immunization (F) | Activation is beneficial | Chen 2019 |
| Sephin1 | **ALS** | SOD1G93A mice | • Prevents motor neuron loss<br>• Prevents motor deficits | 7 w treatment with 5 mg/kg, daily from 4 w of age (M) | Activation is beneficial | Das 2015 |

| Compound | Disease | Animal model | Main outcomes | Dose/Age/Time intervention/Sex | ISR manipulation | Reference |
|---|---|---|---|---|---|---|
| GSK2606414 | **Dementia** | rTg4510 mice, that expresses the tauP301L mutant in forebrain | • Prevents neuronal loss<br>• Reduces brain atrophy | 2 m treatment with 50 mg/kg starting at ~6 m of age (M) | Inhibition is beneficial | Radford 2015 |
| ISRIB | **Tauopathy** | PS19 mice | • No effect | 9 w treatment with 5 mg/kg starting at 8-9 m of age (M) | Inhibition has no effect | Briggs 2017 |
| 2BAct | **VWM** | VWM eIF2Be-R191H mice | • Prevents weight loss<br>• Prevents motor deficits<br>• Prevents myelin loss •Normlizes transcriptome and proteome | 21 w treatment with 30 mg/kg starting at 6-11 w of age (M/F) | Inhibition is beneficial | Wong 2019 |
| ISRIB | **TBI** | Injured mice | • Rescues cognition | Mice injured at ~12 w of age, ISRIB 2.5 mg/kg treated during training period 27 days post injury (M) | Inhibition is beneficial | Chou 2017 |
| ISRIB | **Down syndrome** | Ts65Dn mice | • Rescues synaptic plasticity<br>• Rescues deficit in long-term memory | 1 w treatment (once every 2 d) with 2.5 mg/kg, starting ~3-5 m of age | Inhibition is beneficial | Zhu 2019 |
| ISRIB | **Prostate cancer** | Mice over-expressing MYC and down-regulating PTEN | • Restores protein syntheis<br>• Induces tumor regression<br>• Decreases xenografts growth | 6 w treatment with 2.5 mg/kg ISRIB, starting ~6 m of age | Inhibition is beneficial | Nguyen 2018 |
| Trazodone and DBM | **Prion disease** | Prion-infected mice | • Rescues behavioral deficits<br>• Increases survival | T reatment with 40 mg/kg trazodone or diet containing 0.5% DBM, starting ~8 m of age | Inhibition is beneficial | Halliday 2017 |
| GSK2606414 | | | • Prevents spongiosis, gliosis and neurodegeneration | Treatment with 50 mg/kg twice daily, from 7 and 9 w post infection | Inhibition is beneficial | Moreno 2013 |
| ISRIB | | | • Prevents neuronal loss<br>• Increases survival | ISRIB was administered at 0.25 mg/kg once daily from 7 w post infection | Inhibition is beneficial | Halliday 2015 |
| ISRIB | **Alzheimer's disease** | Intracerebroventricular infusion of β-amyloid oligomers | • Reverses memory impairment and | 0.25 mg/kg ISRIB, 3 m of age (M/F) | Inhibition is beneficial | Oliveira 2021 |

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| Compound | Disease | Animal model | Main outcomes | Dose/Age/Time intervention/Sex | ISR manipulation | Reference |
|---|---|---|---|---|---|---|
| | | | dendritic spine loss | | | |
| | | Mice overexpressing amyloid precursor protein (APP) together with deleted presinilin-1 gene in exon 9 | • Reverses impaired long-term potentiation and memory | 0.25 mg/kg ISRIB, 10-13 m of age (M/F) | Inhibition is beneficial | Oliveira 2021 |