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Metabolic Regulation, Mitochondria and the Life-Prolonging Effect of Rapamycin: A Mini-Review

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Abstract
The fungicide rapamycin increases lifespan in eukaryotes by interfering with the activity of a serine/threonine kinase called TOR (target of rapamycin). TOR complex 1 (TORC1) is an essential integrator of cellular nutrient cues, growth signals and cellular metabolism. Here, we review major components of TORC1, its downstream effectors and lifespan studies in various organisms involving these signaling components. In particular, we focus on the role of rapamycin in mitochondrial biogenesis, in metabolic regulation and in the control of reactive oxygen species production.

Introduction: Rapamycin, TOR and Downstream Signaling Components

Every eukaryotic organism ages, a process characterized by a progressive loss of cellular function that ultimately leads to death. Lifespan is regulated by a complex network of extrinsic and intrinsic factors. Extrinsic factors, such as nutrient levels and environmental stress exposure, represent a major determinant of lifespan. Organisms respond actively to extrinsic factors by triggering conserved signaling pathways that influence lifespan, as shown by extensive research in various model systems, such as mice, flies *(Drosophila melanogaster)* and budding yeast. The 'target of rapamycin' (TOR) pathway, which senses nutrient availability and environmental stress, has recently emerged as a major regulator of lifespan. Here, we focus on how the TOR complex 1 (TORC1) and its downstream effectors influence lifespan by regulating cellular metabolism and mitochondrial functions.

TOR is a Ser/Thr protein kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family [1]. TOR is comprised of a cluster of amino-terminal HEAT repeats (Huntingtin, Elongation factor 3, A subunit of protein phosphatase 2A and TOR1), a FAT domain (FRAP, ATM and TRRAP), a FKBP12-rapamycin binding (FRB) domain, a Ser/Thr kinase catalytic domain, and a FATC domain (FAT C-terminal). TOR exists in two functionally distinct multi-protein complexes named TOR complex 1 (TORC1) and TOR complex 2 (TORC2). In mammals, two accessory proteins named regulatory-associated protein of mTOR (RAPTOR) and rapamycin-insensitive companion of mTOR (RICTOR) distinguish TORC1 from TORC2, respectively [2, 3]. Rapamycin and the 12-kDa FK506-binding protein (FKBP12) form a complex that binds to and inhibits

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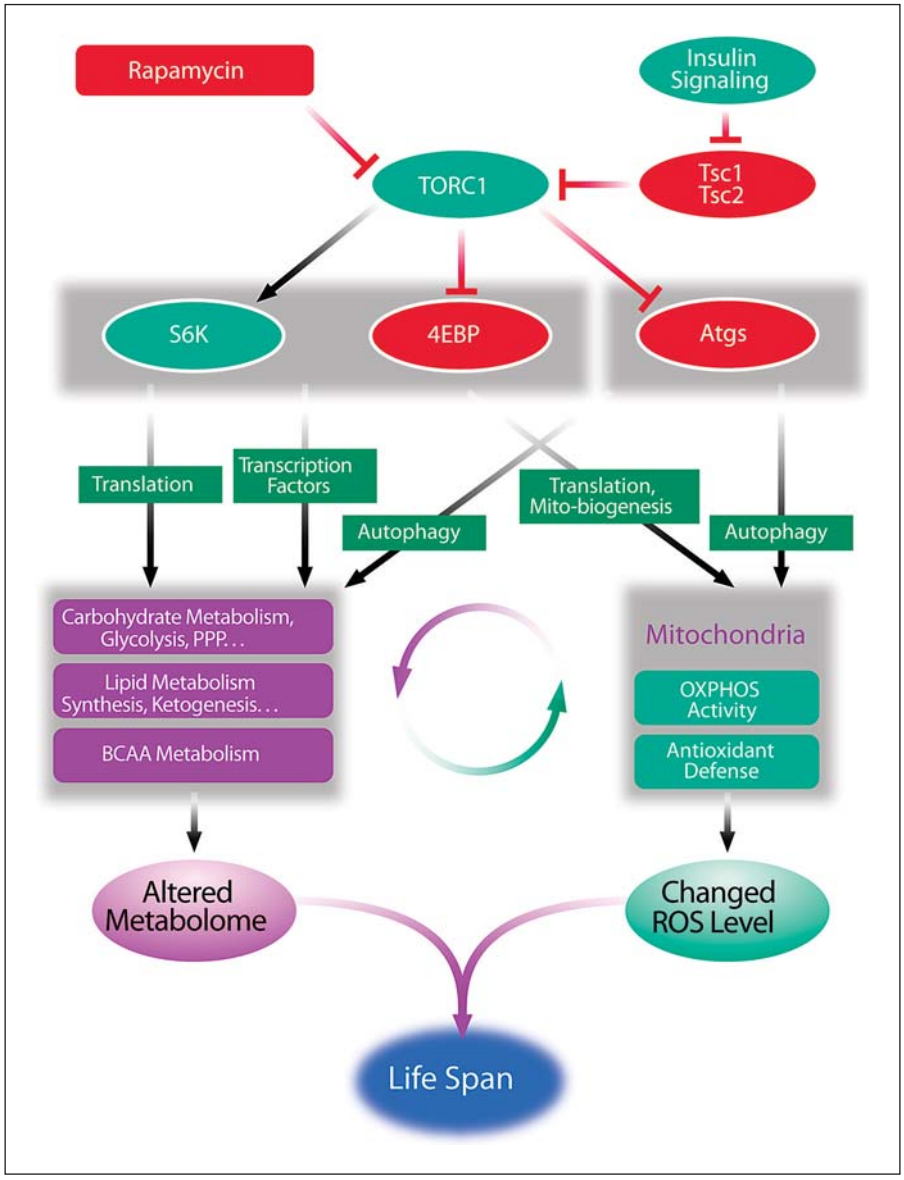

Fig. 1. A simplified model of TORC1-mediated regulation of the metabolome, mitochondrial function and lifespan. The green ovals designate TORC1 signaling components activated by nutrients and insulin. The red ovals designate TORC1 signaling components suppressed by nutrients. The rectangles indicate the downstream physiological consequences of altered TORC1 signaling. Rapamycin and upstream signaling components like the Tsc proteins modulate TORC1 activity. Active TORC1 signals through downstream effectors, such as S6K, 4EBP and Atgs, causing alterations in metabolism and mitochondrial functions. These alterations result in changes in metabolomic profiles and ROS levels, which in turn impacts lifespan.

RAPTOR-bound TORC1, but not RICTOR-bound TORC2 [4]. While TORC2 has critical regulatory functions in cytoskeletal polarity and cell survival [3], TORC1 is the major nutrient sensor and therefore the subject of this mini-review. TORC1 lies at the intersecting point of diverse cellular inputs that include hormones (insulin or insulin-like growth factor 1, IGF-1), nutrients (glucose and amino acid), energy (ATP levels) and stress (reactive oxygen species). In mammals and flies, the tuberous sclerosis complex (Tsc1-Tsc2) integrates hormonal signals, and negatively regulates mammalian TORC1 (mTORC1) and fly TORC1, respectively (fig. 1). IGF-1 and insulin signal to mTORC1 via the PI3K-Akt pathway, which phosphorylates Tsc2. Phosphorylated Tsc2 dissociates from Tsc1 resulting in mTORC1 signaling [5].

Key downstream mediators of TORC1 signaling are also conserved among eukaryotic organisms. These include S6 kinase (S6K), the translation inhibitory factor 4EBP and several autophagy-related genes (Atgs). S6K is an AGC (protein kinase A, G and C) family kinase. Mammalian S6K as well as its orthologs in fly (dS6K) and in yeast (Sch9) are all directly phosphorylated and activated by TORC1 [6–8]. S6K phosphorylates a wide range of targets including ribosomal protein S6, thereby enhancing

protein translation in the cytoplasm. In contrast, TORC1 phosphorylation of 4EBP disrupts its association with the translation factor eIF4E [6, 9, 10]. The release of eIF4E not only promotes global translation, but also preferentially enhances the translation of mRNAs with more complex 5′ UTR structures. TORC1 also negatively regulates autophagy, while inhibition of TORC1 activity by genetic deletion or by rapamycin exposure induces autophagy. In yeast, Atg13 is hyperphosphorylated by TORC1 under nutrient-rich conditions, but is rapidly dephosphorylated after nitrogen starvation or rapamycin treatment [11]. Hypophosphorylated Atg13 then interacts with Atg1 and Atg17, thereby activating the kinase activity of Atg1 and initiating autophagy [11]. The ULK1–Atg13–FIP200 complex represents the mammalian ortholog of the yeast Atg1–Atg13–Atg17 complex, and is regulated by mTORC1 in a similar manner [12].

Reduced TOR Activity Is Associated with Increased Lifespan

There is growing evidence that the TORC1 pathway also regulates lifespan in mammals. Dietary supplementation of rapamycin at various growth stages extends lifespan in mice. Remarkably, initiation of rapamycin feeding late in life (day 600) in mice extends average lifespan without a change in body weight [13]. Treatment with rapamycin prolongs mean lifespan in both male and female mice by 9 and 13%, respectively. Rapamycin feeding also significantly extends maximum lifespan, as shown by the proportion of treated mice living beyond the 90th percentile of mortality observed in the control population (5.9% of the controls vs. 20.2% of the rapamycin-treated males, and 4.8% of the control vs. 21.5% of the rapamycin-treated females) [13]. Genetic models of reduced TORC1 signaling recapitulate these results. For example, female mice heterozygous for both mTOR and mLst8 ($mtor^{+/-}mlst8^{+/-}$) exhibit an increase in mean lifespan by 14.4% relative to their wild-type counterparts [14]. The heterozygous males, surprisingly, do not exhibit longer lifespan with reduced TORC1 signaling [14]. TORC1 signaling also modulates lifespan in other genetic models. The Ames dwarf mouse, a long-lived mutant line with pituitary hormone deficiencies, shows reduced TORC1-mediated signaling levels in liver and muscle [15].

TORC1 controls lifespan in invertebrates as well as in eukaryotic microorganisms. Rapamycin feeding extends lifespan in several lines of *D. melanogaster* [16]. Expression of a dominant negative form of TOR or overexpression of Tsc1 or Tsc2, both upstream inhibitors of TORC1, also extends fly lifespan [17]. In yeast, TORC1 regulates both replicative lifespan (as measured by the number of budding events by a given mother cell) and chronological lifespan (as measured by the duration of post-mitotic culture in stationary phase) [18–20]. Rapamycin treatment of yeast during various growth stages extends lifespan, as does reduction of TORC1 in mutants like the *TOR1*-knockout yeast strain *(tor1Δ)* [18–20].

TORC1's effects on lifespan across species are mediated by several of its downstream effectors. Deletion of S6K1, an isoform of mammalian S6K, extends the lifespan of mice, particularly in females [21]. In flies, overexpression of a dominant negative form of *Drosophila* S6K (dS6K) increases lifespan, while the constitutively active form decreases lifespan [17]. In yeast, deletion of the S6K ortholog *SCH9* extends both replicative and chronological lifespan [18, 22]. Because the knockout of many mTORC1 signaling components results in embryonic lethality, epistasis analyses have been primarily attempted in flies and yeast. Overexpression of S6K or suppression of 4EBP negate the lifespan extension induced by rapamycin treatment [16]. Similarly, yeast *sch9* knockout *(sch9Δ)* extends lifespan, but the *tor1-sch9* double knockout *(tor1Δsch9Δ)* does not prolong lifespan beyond what is observed in the *sch9Δ* mutant alone [23]. These observations show that suppression of cytoplasmic protein translation is a conserved mechanism by which TORC1 prolongs lifespan [10, 24]. Probing the effects of translation in mammalian lifespan continues to be a challenge, but results from mice lacking the translational regulator S6K1 are consistent with experimental data from yeast and flies [21]. Regulation of autophagy is also critical in TORC1 regulation of lifespan. Rapamycin-induced lifespan extension in yeast and flies is abrogated if autophagy is inhibited [16, 25]. Multiple autophagy-defective yeast mutants are short-lived, a phenotype that cannot be rescued by rapamycin [25]. Similarly, downregulation of Atg5 reduces the lifespan of the fruit fly treated with rapamycin [16].

In contrast to the global knockout of TORC1 signaling components, tissue-specific knockouts yield intriguing insights into how the local regulation of TORC1 signaling can impact global physiology and lifespan. In flies, expression of the dominant negative form of dTOR or dS6K, or overexpression of dTsc2 in fat tissue alone, is sufficient to increase lifespan [17]. In mice, fat-specific deletion of RAPTOR expression causes a global increase in insulin sensitivity and protects against weight gain (in mice fed either a high-fat diet or normal chow), which are two

common aging-associated metabolic abnormalities [26]. While a high-fat diet decreases insulin sensitivity, induces S6K hyperphosphorylation and AKT hypophosphorylation, RAPTOR knockout restores the loss of insulin-induced AKT phosphorylation [26]. No aging study has yet been reported on mice with a fat-specific reduction of TORC1, but mice with reduced insulin signaling via fat-specific knockout of the insulin receptor (FIRKO mice) do live longer [27]. Interestingly, FIRKO and fat-specific RAPTOR knockout mice both exhibit lower body weight, lower fat mass and better glucose tolerance compared to wild-type controls, even though their fat tissues do not respond to insulin. In contrast, skeletal muscle-specific knockout of RAPTOR (RAmKO) shortens lifespan [28]. Compared to controls, RAmKO mice develop kyphosis, muscle dystrophy and other aging-related phenotypes, thereby revealing a tissue-specific role for TORC1 in lifespan regulation [28].

TORC1 has been implicated as a key mediator of the effects of dietary restriction (DR). DR, defined as a decrease in calorie intake (10–50%) without malnutrition, increases lifespan in multiple species from yeast to mammals [29]. Importantly, in yeast and fruit flies, DR does not increase the lifespan of organisms carrying a TOR deletion [17, 18]. DR also extends lifespan in wild-type mice, and their gene expression profiles resemble those of S6K1-knockout mice, suggesting that DR acts through mTORC1 inhibition [21]. While the evidence supporting a role for TORC1 in DR-induced lifespan extension is compelling, caveats must also be noted. First, it still remains unclear whether tissue-specific TORC1 signaling is sufficient, or whether global TORC1 downregulation is required for the beneficial effects of DR. Whether and how TORC1 interacts with other known lifespan modulators like the sirtuins remains unknown. Furthermore, interactions between TORC1 and DR may be regulated by genetic background, as DR has drastically different effects on lifespan in different mouse strains [30].

Metabolome: You Are Not Only What You Eat

Metabolomic analysis, defined as the unbiased measurement of a large number of cellular metabolites, revealed that active TORC1 stimulates carbohydrate metabolism and that this effect is suppressed by rapamycin [31]. Knockout of the negative TORC1 regulator Tsc2 ($T2^{-/-}$) leads to TORC1 activation and the upregulation of gene expression for glycolytic and oxidative pentose phosphate proteins, resulting in accumulation of the intermediate metabolites glucose-6-phosphate, dihydroxyacetone phosphate, lactate and ribose-5-phosphate [31]. TORC1 signals to several carbohydrate metabolic pathways: S6K activation signals to increase transcription of the oxidative arm of the pentose phosphate pathway, while 4EBP modulates glycolysis via translational control of the hypoxia-inducible factor 1α (Hif1α) [31].

TORC1 also regulates lipid metabolism and ketogenesis. TORC1 increases de novo lipid synthesis in a S6K-dependent fashion [31], and suppresses lipid breakdown and ketogenesis. Under fasting conditions, TORC1 activity is decreased and total serum ketone levels are increased [32]. Constitutively active hepatic TORC1 in the absence of Tsc1 attenuates fasting-induced ketogenesis: lipid breakdown and total ketones are both reduced in the liver [32]. Cell culture studies confirm that the knockdown of Tsc1 suppresses ketogenesis, which is restored by rapamycin treatment [32].

TORC1 signaling is implicated in amino acid metabolism. Branched chain amino acids (BCAAs), i.e. leucine, isoleucine and valine, induce the phosphorylation of mTOR and activation of the TORC1 pathway in mice and rats [33, 34]. A high-fat diet combined with BCAAs induces insulin resistance, which is reversed by rapamycin treatment [33]. Unexpectedly, feeding dietary BCAAs alone to middle-aged mice is associated with increased median lifespan, although not maximum lifespan [34]. The mechanism responsible for these effects is poorly understood, but these results highlight the importance of defining the effect of TORC1 on lifespan in a tissue-specific context. Intriguingly, BCAAs activate TORC1-regulated mitochondrial biogenesis selectively in skeletal muscle and not in fat or liver [34]. Therefore, the beneficial effects of BCAAs are likely to be mediated in a cell-type-specific manner by active muscle TORC1 signaling.

How TORC1-induced metabolomic alterations influence aging is still poorly understood, but it is likely that both autophagy and translation control represent two critical pathways. Autophagy, which is negatively regulated by TORC1, plays a critical role in amino acid homeostasis. Plasma amino acid concentrations, especially for the essential amino acids and BCAAs, rapidly decrease in autophagy-defective compared to wild-type mice [35]. In wild-type yeast cultured in nitrogen-deficient media, amino acid pools initially decrease but are partially replenished thereafter. By comparison, in autophagy mutants this replenishment does not occur, indicating that TORC1-regulated autophagy is critical for maintaining cellular viability during nitrogen starvation [36]. Autophagy also plays a role in lipid metabolism:

macrolipophagy, defined as the sequestration of cellular lipid droplets inside autophagosomes for lysosomal degradation, releases free fatty acids for mitochondrial β-oxidation and energy production [37]. Inhibition of autophagy by TORC1 in hepatocytes leads to suppressed β-oxidation and lipid accumulation [37]. Like autophagy, protein translation is closely associated with nutrient availability. Suppressed translation signals amino acid biosynthesis regulator Gcn4, which is required for lifespan extension in *tor1Δ* or *sch9Δ* [24]. Therefore, by sensing the abundance of various nutrients and regulating the activity of critical processes such as autophagy and translation, the TORC1 signaling pathway lies at the intersection between environmental and innate mechanisms of aging.

Mitochondria: An Intersection for TORC1 and Lifespan

Mitochondria play key roles in energy production, intermediary metabolism and cellular signaling. They contain enzymes for critical metabolic pathways, including the tricarboxylic acid (TCA) cycle, fatty acid β-oxidation and oxidative phosphorylation (OXPHOS). OXPHOS is the major cellular source of ATP, reactive oxygen species (ROS), reactive nitrogen species (RNS) and other strong oxidants. To counteract the damage to macromolecules and cellular components caused by reactive species, especially ROS, mitochondria deploy antioxidant defense mechanisms such as superoxide dismutase 2 (SOD2). Denham Harman first proposed that the accumulation of mitochondrial ROS induces cellular damage and thereby decrease lifespan. Harman's simple emphasis on the damaging effects of mitochondrial ROS to cells and the negative impact on lifespan has since been challenged by many studies [reviewed in 38]. Not recognized by Harman's initial theory, mitochondrial ROS significantly influence cellular signaling by activating key metabolic regulators such as Hif-1α [reviewed in 39]. Although the current understanding of the mitochondrial impact on lifespan is far from perfect, a growing body of evidence supports the concept that mitochondrial function critically influences lifespan through its effects on OXPHOS activity and ROS levels.

Importantly, TORC1 signaling regulates mitochondrial biogenesis and turnover in mammals as well as lower model organisms. In mice, the effect of TORC1 on mitochondrial function heavily depends on the tissue being studied. In skeletal muscle, rapamycin reduces the expression of genes encoding OXPHOS components in a PGC1α-dependent fashion [40]. In agreement with these observations, RAmKO mice show lower OXPHOS levels and abnormal mitochondrial biogenesis [28], while TORC1 activation by BCAAs in muscle cells promotes mitochondrial biogenesis [34]. In contrast, mice with fatty tissue-specific knockout of RAPTOR expression have higher rates of respiration [26]. This protects the mice against weight gain on both regular and high-fat diets because increased mitochondrial uncoupling results in higher energy expenditure [26]. FIRKO mice, like the RAPTOR knockout mice, exhibit increased respiration and higher energy expenditure because they have an elevated expression of genes involved in OXPHOS and β-oxidation [41]. Intriguingly, the greatest difference in OXPHOS expression occurs in older mice [41]. The inverse relationship between TORC1 activity and respiration in fat tissues is similar to what is observed in yeast, whereby rapamycin treatment or deletion of TORC1 components increases oxygen consumption [20]. The expression of proteins from respiratory complexes, encoded by both the nuclear and mitochondrial genomes, is globally upregulated [23].

Downstream of TORC1, S6K, 4EBP and Atg-family proteins all modulate mitochondrial biogenesis. Gene expression of PGC1α- and AMPK-regulated pathways is upregulated in the liver, muscle or white adipose tissue of S6K knockout mice [21]. However, the mechanism underlying these changes is not clear, while mRNAs encoding mitochondrial components do not significantly increase, and translational control may be involved, similar to what is observed for 4EBP (discussed below). In contrast to mammalian S6K, the role of the yeast ortholog Sch9 in regulating OXPHOS is better defined. Sch9 is a critical downstream effector of TOR and is involved in regulating mitochondrial oxygen consumption. Like *tor1Δ*, *sch9Δ* strains show increased levels of nuclear and mitochondria-encoded OXPHOS proteins [23]. In fruit flies, 4EBP is a major translational regulator of OXPHOS components. DR alters the mRNA translational profile in a 4EBP-dependent fashion [10]. mRNAs encoding OXPHOS proteins possess a short 5′ UTR and weak secondary structure. Translation of OXPHOS-encoding mRNA is therefore less dependent on eIF4E than translation of other mRNA species. Consequently, the expression of OXPHOS is maintained during DR despite reductions in global translation [10]. TORC1 not only regulates mitochondrial biogenesis through S6K and 4EBP, but also regulates mitochondrial turnover through the Atgs. Asymmetrical fission separates functional and dysfunc-

tional mitochondria, which are subsequently degraded by autophagy [42]. Autophagy therefore participates in mitochondria 'quality control'.

As a consequence of its ability to regulate mitochondrial biogenesis and turnover, TORC1 influences the levels of mitochondrial ROS. Defects in the clearance of damaged mitochondria by TORC1-regulated autophagy also contribute to ROS accumulation [43]. Isolated mitochondria obtained from mouse skeletal muscle lacking Atg7 (Atg7$^{-/-}$) exhibit a significant defect in mitochondrial respiration as well as increased levels of ROS [43]. The positive correlation between TORC1 signaling and ROS is also observed in lower organisms. In yeast, reduced oxidative damage was observed in the stationary phase culture of *tor1Δ* and *sch9Δ* strains [20, 22]. Interestingly, both rapamycin treatment and *tor1* knockout in yeast cause a temporary increase in superoxide levels during the log growth phase because these manipulations promote OXPHOS biogenesis [20]. A transient peak of mitochondrial ROS likely serves as a hormetic stimulus, which induces the expression of proteins involved in oxidative stress resistance and thereby extends lifespan [reviewed in 39]. This data raises the intriguing possibility that mitochondrial hormesis (or *mitohormesis*) mediates the effect of TORC1 on lifespan in mammals and in lower organisms.

It is therefore likely that mitochondrial OXPHOS function and changes in oxidative stress responses collaborate with metabolomic alterations to regulate lifespan (fig. 1). TCA cycle intermediates, such as α-ketoglutarate and mitochondrial ROS, regulate the activity of transcription factors such as Hif1α to change metabolism [reviewed in 39]. On the other hand, key metabolic pathways also influence ROS levels. For example, BCAAs reduce tissue hydrogen peroxide and oxidative damage levels and increase antioxidant defense signaling [34]. Although BCAAs induce TORC1 signaling in both liver and muscle [33], only active TORC1 enhances mitochondrial biogenesis and antioxidant defense mechanisms in muscle. Therefore, tissue-specific signaling processes heavily regulate the interactions between metabolome and mitochondrial activities, thereby influencing lifespan.

Aging: Above and beyond Rapamycin

Numerous studies have documented the role of rapamycin and TORC1 signaling in the determination of lifespan in various organisms. TORC1 signaling influences lifespan via its ability to regulate both metabolic pathways and oxidative damage (fig. 1). Such pathways are non-linear, since different signaling components and phenotypic outputs interact with each other in a network. Manipulation of TORC1 activity, either via DR or rapamycin treatment, can regulate lifespan. Much remains to be learned about the molecular mechanisms of these important effects. While there is no clinical evidence showing that rapamycin extends human lifespan, the evolutionary conservation of the TORC1 pathway and its effects in murine models warrant further study of this pathway in the control of human lifespan.

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