Growth and aging: a common molecular mechanism

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Abstract: It is commonly assumed that growth and aging are somehow linked, but the nature of this link has been elusive. Here we review the aging process as a continuation of TOR-driven growth. TOR is absolutely essential for developmental growth, but upon completion of development it causes aging and age-related diseases. Thus, the nutrient-sensing and growth-promoting TOR signaling pathway may provide a molecular link between growth and aging that is universal from yeast to human.

INTRODUCTION

At first glance, growth and aging appear to be opposites. Growth is energy-driven synthesis of macromolecules from simple nutrients, an increase of order and a decrease of entropy. Aging is decay, a loss of order and a rise of entropy. Seemingly, growth and aging are mutually exclusive. Forever proliferating cells, such as legendary hydras, do not show signs of aging. In contrast, when an organism ceases to grow, aging follows. However, manipulations that decrease growth also decrease aging and prolong life span. For example, calorie restriction (reduced nutrient intake) reduces growth and increases longevity in diverse species from yeast to mice. Rapamycin, which inhibits growth in yeast, decelerates yeast aging. Inactivation of the growth-promoting insulin/IGF-1 signaling pathway increases life span, from worms to mice. Why do growth-inhibiting conditions slow down aging? Are growth and aging mechanistically similar? As we discuss here, growth and aging may not be opposites but rather a continuation of one another driven by the same molecular pathway. Aging and growth may be linked

in a way that growth produces aging. In other words, excessive growth is a driving force for aging. The molecular pathway that drives both growth and aging appears to be the evolutionarily conserved TOR (target of rapamycin) pathway.

The TOR pathway

TOR (Target Of Rapamycin), as its name indicates, was originally discovered, in yeast, as the target of the antifungal drug rapamycin. Rapamycin is a natural secondary metabolite produced by soil bacteria to inhibit growth of fungal competitors. Thus, it is a mirror image of penicillin that is produced by fungi to inhibit bacterial growth. Remarkably, TOR is structurally and functionally conserved from yeast to human (including worms, flies, plants and mice) as an essential, central controller of cell growth [1]. TOR in mammals (mTOR) controls cell growth and metabolism in response to nutrients (e.g., amino acids), growth factors (e.g., insulin, IGF-1, PDGF), and cellular energy status (ATP). Nutrients are the dominant TOR input as high levels of amino acids can compensate for an

absence of the other mTOR inputs but not vice versa [2], and only nutrients activate TOR in unicellular organisms. The growth factor signaling pathway, grafted onto the more ancestral nutrient sensitive TOR pathway, co-evolved with multicellularity. TOR activates cell growth by positively and negatively regulating several anabolic and catabolic processes, respectively, that collectively determine mass accumulation. The anabolic processes include transcription, protein synthesis, ribosome biogenesis, nutrient transport, and mitochondrial metabolism. Conversely, TOR negatively regulates catabolic processes such as mRNA degradation, ubiquitin-dependent proteolysis, and autophagy. TOR is an atypical serine/threonine kinase that is found in two functionally and structurally distinct multiprotein complexes, TORC1 and TORC2 (mTORC1 and mTORC2 in mammals), each of which signals via a different set of effector pathways. TORC1 is rapamycin sensitive whereas TORC2 is rapamycin insensitive. The best-characterized phosphorylation substrates of mTOR are S6K and 4E-BP1 via which mTORC1 controls translation, and Akt/PKB via which mTORC2 controls cell survival and other processes [3]. Like TOR itself, the two TOR complexes and the overall architecture of the TOR signaling network appear to be conserved from yeast to human [1, 4]. TOR and many of the processes it controls have also been shown to play a role in aging (in addition to growth) in a wide variety of organisms, as described below.

Aging in budding yeast

Budding yeast *S. cerevisiae* is a particularly useful model system to study aging because it can be used to study both replicative aging and chronological aging. Replicative aging is measured by the number of daughter cells (divisions) a mother cell can produce. Chronological aging, also referred to as postmitotic aging, is measured by the length of time a non-dividing cell can survive. Inhibition of TORC1 signaling in yeast extends both replicative [5] and chronological [6] life span. Thus, TOR appears to promote aging regardless of physiological context (mitotic or post-mitotic cells).

In yeast, the link between growth and aging has been known since the 1950's and is particularly spectacular. Yeast cells grow larger as they grow older [7, 8]. The yeast *S. cerevisiae* typically divides asymmetrically to give a large mother cell and a smaller daughter cell. As mother cells become old, they enlarge and produce daughter cells that are larger than daughters derived from young mother cells. Like large mothers, large daughter cells have shorter replicative life span [8].

The use of unicellular yeast to study aging is revealing also because here the cell is the organism. Therefore, it is a model for cell aging and organismal longevity. As we discuss below, TOR also controls longevity in multicellular organisms.

TOR and aging from worm to mammals

Inhibition of TOR signaling enhances life span in worms, flies and possibly mammals. The nematode *C. elegans* contains a constant number of post-mitotic cells and lives about twenty days. The first demonstration that TOR controls life span was by Vellai et al [9] who showed that knocking down TOR in -*C. elegans* more than doubles this worm's normal life span. They examined specifically the role of TOR in aging based on the prior knowledge that both TOR and life span are regulated by nutrients and insulin. Indeed, a large body of earlier, groundbreaking work showing that calorie restriction [10, 11] or down regulation of insulin/IGF-1 signaling [12, 13] extends life span is consistent with the observation that down regulation of TOR also extends life span. Interestingly, inhibition of TOR starting either during development or on the first day of adulthood gives comparable life span extension, suggesting that TOR controls longevity mainly, if not exclusively, during adulthood [9]. Jia et al. [14] subsequently demonstrated that worms deficient in raptor, a TORC1-specific subunit, also have an extended life span, indicating that TOR promotes aging via at least TORC1, if not via both TORCs.

 In *Drosophila*, TOR is required for growth during larval development, and for increases in cellular growth caused by growth factor signaling and nutrient availability [15]. Genetic inhibition of the *Drosophila* TOR pathway, either upstream or downstream of TOR, extends life span [16, 17]. Furthermore, reducing the function of *Drosophila* TOR results in decreased lipid stores and glucose levels, and prevents metabolic syndrome [18]. The life span extension is observed upon down regulation of TOR signaling in the fat body [17], underscoring the importance of fat in aging [19-21, 12]. Downregulating TOR signaling in the fat body not only extends the life of the fly, it also reduces the size of the entire organism [22], providing yet another link between growth and aging. The apparently central role of TOR signaling in the fly fat body in regulating life span may be recapitulated by mammalian TOR in adipose tissue. In mice, decreased insulin/IGF-1 signaling in adipose tissue, and consequently less active downstream mTOR signaling, increases life span [23, 20]. Furthermore, adult-onset growth hormone or IGF-1 deficiency increases life span in rodents [24]. Also, mice deficient for mTORC1 or the mTORC1 effector

S6K are protected against age- and diet-induced obesity [25, 26]. Rapamycin is currently being tested for its ability to extend lifespan in mice in the National Interventions Testing Program.

Finally, calorie restriction, in which nutrient intake is restricted to 60-70% that of voluntary levels, increases life span in most species including mammals [10-12]. Although anti-aging mechanisms of CR are still disputed, one of the mechanisms is likely inactivation of the TOR pathway. Indeed, taking into account that i) inhibition of TOR extends life span and ii) nutrients activate TOR, the mechanism of how restriction of nutrients can increase life span seems apparent.

Downstream of TOR

How does TOR promote aging in response to nutrients? In other words, which of the many TORC1-controlled processes that are either up regulated or down regulated upon nutrient deprivation (TORC1 inactivation) leads to longer life? Recent evidence suggests that TORC1 controls aging via several of its downstream processes, including autophagy, ribosome biogenesis and protein synthesis, transcription, and mitochondrial activity. Indeed, there is a remarkable correlation between TOR-controlled processes and processes in aging. It is also important to note that these processes constitute the normal program via which TOR controls cell growth, suggesting that TOR control of aging is an extension or continuation of its control of growth.

TORC1 inhibits autophagy, a process of bulk degradation of proteins and organelles by lysosomes [27]. Autophagy is inhibited in aging and age-related diseases [28]. Restoration of autophagy depletes mitochondria with deleterious mtDNA mutations but spares their normal counterparts [29]. Furthermore, autophagy is essential for life span extension at least in worms [30]. This suggests that TORC1 promotes aging in part via inhibition of autophagy.

TORC1 activates ribosome biogenesis and protein synthesis. Recent studies show that inhibition of ribosome biogenesis and global protein synthesis extends life span [31-34]. Reducing the levels of ribosomal proteins and translation initiation factors extends life span in both yeast and worms. Thus, this is consistent with the notion that TORC1 may promote aging via activation of ribosome biogenesis and protein synthesis.

TORC1 in yeast negatively regulates the stress-activated transcription factors GIS1 and MSN2/4. Both transcription factors are required for life span extension

upon down regulation of TOR [35, 36]. A longevity-related gene up regulated by MSN2/4 upon TOR inhibition is the nicotinamidase gene *PCN1*. Interestingly, nicotinamidase converts nicotinamide to NAD^+ which in turn activates SIR2, suggesting that TOR and sirtuins are part of the same longevity pathway [35]. Furthermore, as discussed below, TOR negatively regulates mitochondrial gene expression to limit life span [37].

TORC1 controls mitochondrial activity, but in different ways depending on the organism. In yeast, TORC1 inhibits mitochondrial respiration whereas in mammals (at least in muscle) it stimulates respiration [37-42]. This divergence in regulation is probably related to the fact that glucose, a nutrient sensed by TORC1, triggers anaerobic fermentation in yeast. A similar glucose-dependent shift in respiration does not occur in mammals. Consistent with the above, increased mito-chondrial respiration extends life span in yeast whereas in mammalian cells life span extension correlates with reduced respiration [37, 39, 43]. However, the role of mitochondria in life span extension remains elusive, particularly with the recent demonstration that TORC1 in mammalian adipose tissue, like in yeast, negatively controls respiration [26].

Accumulation of aggregation-prone proteins is involved in neurodegeneration. TOR causes neurodegeneration in a *Drosophila* tauopathy model [44]. The TOR pathway is involved in Alzheimer's disease by increasing Tau protein synthesis [45]. Furthermore, rapamycin en-hances clearance of pathologic proteins and thereby reduces their toxicity [46].

As we discuss below, overactive TOR seems to be involved in the hypertrophic phenotype of aging mammalian cells, thus linking TOR mediated cell hypertrophy to organismal aging. In contrast, a replicative limit has never been shown to be important *in vivo* [47]. It is a hyperthropic, secretory phenotype of aging cells that can be linked to organismal aging [48-50].

Hypertrophic phenotype of aging cells

If growth and aging are mechanistically linked, are older cells larger? In yeast, old cells are large and cell size predicts replicative life span [51, 52]. This also appears to be the case for senescent mammalian cells. An increase in cell size is a hallmark of senescent fibroblasts [53]. Their cell volume is several fold greater compared with proliferating cells. Cell size is progressively increased in cell culture as cells progress toward senescence [54-56]. Furthermore, it was

suggested 20 years ago that cell size is a marker of cell senescence [54, 57]. Ironically, TOR had not been discovered at that time and the significance of this phenomenological observation was unclear. The notion that TOR is involved in both growth and aging now provides a mechanistic explanation for an old observation.

Cell growth is an increase in cell volume, or mass, due to metabolic activity including synthesis of macromolecules (RNA, protein, lipid) and organelles. If a cell grows without division, it becomes hypertrophic. In other words, when the cell cycle is blocked in the presence of growth-promoting signaling, then cells increase in size [56, 58, 59].

Thus, cell growth is counterbalanced by cell division such that cells maintain a characteristic size. The simplest way to cause both cell hypertrophy and cell senescence is to prevent cell division without inhibiting cell growth. Inhibition of mTOR with rapamycin decreases the hypertrophic cell phenotype caused by induction of the CDK inhibitor p21 [58-60].

All these observations suggest that mTOR signaling plays a role in aging of single cells. How is this related to aging of multicellular organisms? As discussed elsewhere [1, 61], TOR-driven alterations can be linked to metazoan aging and, in particular, diseases of aging such as cancer, metabolic syndrome, atherosclerosis, hypertension and hypertrophic heart.

Rapamycin in humans

Rapamycin is given to renal transplant patients everyday for several years to prevent organ rejection. We view this as an unintentional clinical trial of a potentially anti-aging drug. First, in such patients, rapamycin unexpectedly turned out to prevent cancer [62-64] and even cured some types of pre-existing tumors [65, 66]. Second, 2 years after transplantation, body-mass index was significantly lower in the rapamycin-based treatment arm compared to cyclosporine, indicating that rapamycin prevents obesity [67].

Rapamycin is safe enough to be used in healthy volunteers to study its pharmacokinetics [68-70]. In healthy volunteers, a single dose was not associated with side effects. In 11 healthy men (29 years old, BMI 23 kg/m^2), 6 mg of rapamycin decreased S6K phosphorylation, preventing insulin resistance caused by nutrients. Thus, the activity state of the mTOR pathway can modulate insulin sensitivity in humans and mTOR inhibitors prevent nutrient-induced insulin resistance

[70].

Why TOR?

Cell growth and division are the two most fundamental features of life. Using simple compounds and energy, living organisms build macromolecules according to their own plan, transforming non-self to self. Not surprisingly, the growth-controlling TOR signaling pathway is conserved from yeast to human. In unicellular organisms, it maximizes growth as long as nutrients are available. However, life-promoting TOR signaling seems also to contain seeds of death. Aging and its manifestations such as age-related diseases appear with excessive growth-promoting signaling, when actual growth is not longer possible. Aging is not programmed, of course, but is an aimless continuation of the same process that drives developmental growth. Since aging does not limit life span in the wild, switch-off of this 'growth program' cannot evolve. Growth should be robust and not be slowed down to avoid aging. Furthermore, the aging-growth program cannot be switched off by an accidental mutation, because such a mutation would be lethal or at least reduce fitness during development. Yet, TOR can be inhibited pharmacologically.

ACKNOWLEDGEMENTS

CONFLICT OF INTERESTS STATEMENT

The authors declare no conflict of interests.

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