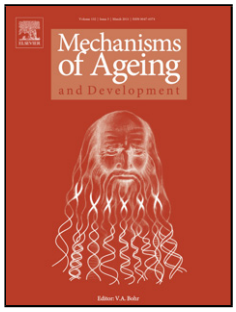

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THE ROLE OF THE TOR PATHWAY IN MEDIATING THE LINK BETWEEN NUTRITION AND LONGEVITY

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Highlights

- The conserved TOR signaling pathway links internal and external signals to various cellular processes
- Deprivation of nutrients is an important determinant of TOR activity
- Dietary interventions affect lifespan and activity of the TOR pathway
- Rapamycin and metformin realize anti-aging effect via regulation of TOR

Abstract

The target of rapamycin (TOR) pathway integrates signals from extracellular and intracellular agents, such as growth factors, nutrients, mediators of energy balance, oxygen availability and other environmental cues. It allows the regulation of multiple cellular processes including protein and lipid synthesis, ribosome biogenesis, autophagy and metabolic processes. Being conserved across different phyla, TOR regulates longevity of various organisms in response to dietary conditions. In this review we described the main components of the TOR pathway and its upstream effectors and downstream processes in relation to aging. The potential contribution of the TOR pathway in lifespan-extending effects of varied dietary interventions, and the anti-aging drugs rapamycin and metformin direct or indirect regulation of TOR activity in yeasts, worms, flies and mammals are also discussed.

Key words: *aging, lifespan, nutrition, rapamycin, TOR signaling pathway,*

Abbreviations: TOR, target of rapamycin; TSC, tuberous sclerosis complex; Rheb, Ras homologue enriched in brain; 4EBP, 4E binding protein; S6K, S6 kinase; PI3K, phosphatidylinositol-3-kinase; Raptor, regulatory-associated protein of mTOR; Deptor, DEP domain-containing mTOR-interacting protein; SREBP1/2, sterol regulatory element-binding protein 1/2; IGF, insulin-like growth factor; TNFα, tumor necrosis factor α; PDCD4, programmed cell death 4; HIF1α, hypoxia inducible factor α; DR, dietary restriction; YY1, Ying-Yang 1; SIRT1, silent information regulator 1; AMPK, adenosine monophosphate-activated protein kinase.

1. Introduction

Aging is a physiological process regulated by numerous signaling pathways and transcription factors. Among them, the target of rapamycin (TOR) signaling pathway is one of the most important controlling mechanisms. TOR-kinase, a key component of this pathway, was discovered as a target for antibiotic action of rapamycin. The TOR signaling network takes part in all eukaryotic metabolism and is essential for organism growth and development (Kennedy and Lamming, 2016; Markaki and Tavernarakis, 2013). The involvement of TOR signaling pathway in

controlling lifespan was firstly shown in the nematode *Caenorhabditis elegans* (Vellai et al., 2003). In this study, genetic inhibition of TOR activity led to two-fold extension of lifespan. Subsequently, the modulation of genes in the TOR signaling pathway also resulted in significant life extension in fruit fly *Drosophila melanogaster* (Kapahi et al., 2004). During the last decade, TOR-signaling inhibition was repeatedly shown to increase lifespan in most invertebrate models (Kaeberlein et al., 2005a), presenting evidence for the role of this pathway in aging processes over a broad spectrum of species, from single-celled eukaryotes to multicellular organisms.

Nutrition is an important endogenous factor that affects longevity. Various dietary interventions have shown to extend the lifespan in organisms from yeasts to mammals. Caloric restriction (CR) is an intervention when the total caloric density of the diet is limited or amount of food given is restricted. Dietary restriction, on the other hand, is when specific macronutrients such as protein of amino acids are manipulated, and may or may not include caloric restriction as well. Dietary interventions such as these are effective at extending lifespan and also affect the TOR pathway. Furthermore, there are some “anti-aging” drugs that can substantially affect the lifespan through different mechanisms, but the possibility of their use in humans is under debate (Vaiserman and Lushchak, 2017). Some of them like rapamycin or metformin have distinct intracellular targets. Others can decrease food consumption or extend the lifespan by mimicking CR (Lushchak and Gospodaryov, 2017).

In this review we describe the main components of the TOR signaling pathway, the mechanisms and upstream effectors of TOR activity, and downstream targets and processes that can affect longevity. We also explore dietary interventions that extend lifespan and their impact on TOR activity, and the effects of two well known and promising anti-aging drugs rapamycin and metformin with respect to this pathway.

2. Components of the TOR signaling pathway

The TOR signaling pathway receives signals, either directly or indirectly, from various extracellular and intracellular effectors such as growth factors, amino acids, mediators of energy balance, oxygen availability and other environmental cues. These effectors influence specific components of the TOR system leading to either activation or inhibition of the TOR-kinase to modulate lipid and protein synthesis, autophagy, lysosome biogenesis and cell survival (Laplante and Sabatini, 2012). Upstream effectors and downstream targets in mammals are shown on Figure 1.

2.1. TSC1/TSC2

TSC (tuberous sclerosis complex) is a heterodimeric protein complex, consisting of TSC1 (hamartin) and TSC2 (tuberine), that indirectly inhibits TOR-kinase (Gao et al., 2002; Inoki et al., 2002; Manning et al., 2002; Menon et al., 2014; Tee et al., 2002). Structure of these polypeptides is yet to be determined; however, the presence of an activating GTP-domain on the C-terminus of TSC2 protein has been established. The active TSC1/TSC2 complex inactivates its main target – GTPase Rheb – by means of GTPase activity, and subsequently stimulates the formation of inactive GTP-bound Rheb (Dibble et al., 2012; Tee et al., 2002). TSC1/TSC2 complex integrates signals from numerous factors (Fig. 1), particularly from hormones and growth factors, i.e. insulin and insulin-like growth factor, acting through phosphatidylinositol-3-kinase (PI3K) and Ras pathways. PI3K phosphorylates phosphatidylinositol (PI) to form phosphatidylinositol-3,4,5-phosphate (PIP3), activating protein kinase B (Akt). The Ras pathway components activate ERK1/2-kinases (extracellular-signal-regulated kinase 1 and 2). Akt and ERK1/2 protein kinases inactivate TSC1/TSC2 by phosphorylation, which, in turn causes mTor-kinase activation (Inoki et al., 2003; Ma et al., 2005; Manning et al., 2002). Other growth factors (TNFα) inhibit TSC1/TSC2 as well, thus activating TOR-pathway (Laplante and Sabatini, 2012). Along with growth factors, signals from multiple stress factors such as hypoxia and DNA damage operate through TSC. DNA damage also causes TSC2 overexpression, inhibiting mTOR-kinase activity (Feng et al., 2005; Stambolic et al., 2001). In response to a lack of energy, AMP-kinase phosphorylates TSC2, thereby leading to TSC1/TSC2 complex inactivation (Feng et al., 2005).

2.2. Rheb

The Ras homologue enriched in brain (Rheb) GTP-ase was first detected in brain tissues due to *Rheb* gene overexpression following epileptic seizures (Yamagata et al., 1994). Afterwards it was established that Rheb is the target for TSC1/TSC2 complex (Zhang et al., 2003). TSCs act like GTP-activating proteins for Rheb GTP-ase, stimulating Rheb-bound GTP hydrolysis to GDP. This ultimately leads to inactivation of the GDP-bound Rheb. Genetic studies show that TSC1/TSC2 inhibit the TOR pathway through Rheb inactivation (Fig. 1) (Avruch et al., 2006). Active GTP-bound form of Rheb directly joins the mTORC1 complex and enhances mTOR kinase activity. Insulin activates PI3K/Akt signaling pathway, as a result, Akt phosphorylates GTP-ase activating proteins (GAP), which are TSC2 compounds. Phosphorylation inactivates TSC1/TSC2 complexes, and it subsequently loses the ability to inactivate Rheb (Takahara and Maeda, 2012). Active Rheb actualizes its functions after change of TSC activity and directly influences mTOR-kinase (Saucedo et al., 2003).

2.3. TOR kinase

TOR kinase functions through the formation of two distinct complexes: TORC1 and TORC2 (Fig. 1). In mammals, mTORC1 complex contains regulatory-associated protein of mTOR (Raptor), LST8 (mLst8, also known as GbL), Deptor domain (DEP, including mTOR-interacting protein) and proline-enriched substrate PRAS40 (proline-rich Akt substrate of 40-kDa). mTORC2 functions in complex with Rictor proteins (rapamycin-insensitive companion of mTOR – insensitive to rapamycin mTOR partner), mLst8, mSin1, Deptor and Protor1/2 protein (Takahara and Maeda, 2012). It was also shown that Deptor interacts with both TORC1 and TORC2 complexes (Peterson et al., 2009). Deptor, similarly to PRAS40, inhibits TORC1. TORC1 is sensitive to rapamycin and nutrient availability, whereas TORC2 is insensitive. Also, both complexes are activated in response to growth factor exposure. Structural compounds of these complexes are highly conservative from yeast to mammals.

TOR protein contains HEAT sequences (Huntington, EF3A, ATM, TOR) at the N-terminus, FAT (FRAP, ATM, and TRRAP), catalytic kinase and FATC domains (FRAP, ATM and TRRAP C-terminal) at the C-terminus. Rapamycin bound to FKBP12 cellular receptors (12-kDa FK506-binding protein), interact with FKBP12-rapamycin-binding domains which are situated between the FAT and catalytic kinase domains of TOR, thus allosterically inhibiting its activity (Wullschleger et al., 2006). The detailed mechanism of this inhibition is not clear, however, rapamycin likely deteriorates the TOR molecule structure (Yip et al., 2010), as it decreases kinase domain activity allosterically (Kaeberlein, 2014).

2.4. S6K

Two S6 kinases (S6K1 and S6K2), encoded by separate genes, have been discovered (Shima *et al.*, 1998). S6K2 has only recently been identified, and thus, the cell growth-related functions of S6K1 are characterized to a greater extent. S6K phosphorylation leads to increased gene expression by affecting translation initiation and elongation (Laplante and Sabatini, 2012). Specific S6 components of the 40S ribosome subunit are the substrate for S6K. Levels of S6 phosphorylation is tightly linked to TORC1; therefore, it is widely used to assess TORC1 complex activity *in vivo*. S6 phosphorylation takes place in five serine residues that are conserved in different species (Meyuhas and Dreazen, 2009).

An alternative target for S6K is the translation initiation factor elF4B (eukaryotic translation initiation factor 4B). Phosphorylated factor elF4B is responsible for facilitating mRNA binding to ribosomes (Hershey and Merrik, 2000), thus intensifying translation, activating cell growth and proliferation. Besides protein biosynthesis, S6K is involved in programmed cell death through PDCD4 (programmed cell death 4), regulation of protein synthesis through eEF2K (eukaryotic

elongation factor-2 kinase) and cell growth regulation through SKAR (S6K1 Aly/REF-like target) (Ma and Blenis, 2009).

2.5. 4E-BP

Ribosomal S6 protein kinase and translation initiation factor binding protein 4E (4E-binding protein), 4E-BP1, are two the most studied and important TORC1 substrates, and belong to translation repressor family of proteins (Fig. 1). Interesting, S6K became active when phosphorylated while phosphorylation makes 4E-BP1 inactive. In mammals, three subtypes of proteins were identified (4E-BP1, 4E-BP2, and 4E-BP3). When TORC1 is inhibited, dephosphorylated 4E-BP binds to the translation initiation factor eIF4E (eukaryotic translation initiation factor 4E) (Ma and Blenis, 2009). When bound with 4E-BP, eIF4E is unable to bind eIF4G (eukaryotic translation initiation factor 4 gamma 1), thus preventing eIF4F complex formation (Ma and Blenis, 2009). When activated, the TORC1 complex phosphorylates 4E-BP, which loses its capacity to interact with eIF4E in such form, thereby activating translation (Johnson et al., 2015; Thoreen et al., 2012).

2.6. TOR pathway is evolutionary conserved in different organisms

TOR kinase, belonging to the group of serine/threonine kinases, was discovered in *Saccharomyces cerevisiae* in 1991 as a target of the antibiotic activity of rapamycin (Hartford and Ratain, 2007; Heitman et al., 1991). Later, two genes, *Tor1* and *Tor2,* encoding proteins Tor1 and Tor2 were identified in yeast (Heitman et al., 1991). Tor1 and Tor2 are constituents of two independent oligomeric complexes – TORC1 and TORC2. Mammalian TOR (mTOR), homologous to those in yeast, mediates its functions through forming two complexes - mTORC1 and mTORC2. These complexes perceive signals from different environmental factors and control different cellular processes (Laplante and Sabatini, 2012; Wullschleger et al., 2006). In both yeast and mammals, only TORC1 is rapamycin-sensitive, but prolonged rapamycin action can inhibit activity of TORC2 as well (Sarbassov et al., 2006).

The TOR pathway has also been found in other fungi, nematodes, flies, plants and mammals, suggesting a conserved function of this signaling pathway in eukaryotes. Comparative analysis of the compounds of this signaling pathway and oligomeric TOR-complexes composition points towards their similarity in different organisms. In yeast, TORC1 complex is composed of Tor1 and Tor2 proteins, Kog1, Tco89 and Lst8, while TORC2 consists of Tor2, Lst8, bit61, Avo1, Avo2 and Avo3 (McCormick et al., 2011). The TORC1 complex of *C. elegans* contains LET363 and DAF15 proteins. TORC2 contains LET363, SINH1 (Avo1 homologue in yeast) and RICT-1 (Avo3 homologue in yeast) proteins. In fruit flies, TORC1 complex consists of Tor and Raptor proteins

(Kog1 homologue in yeast and DAF15 in nematode), second complex contains Tor, SIN1 proteins (Avo1 homologue in yeast and SINH1 in nematode) and Rictor (Avo3 homologue in yeast and RICT-1 in nematode). Similar to *S. cerevisiae*, mammals have mTOR, Raptor (Kog1 homologue in yeast), and mLST8 proteins in TORC1 complex. TORC2 complex comprises mTOR, mLST8, Rictor (Avo3 homologue in yeast) and SIN1 (Avo1 homologue in yeast) (McCormick et al., 2011).

3. TOR pathway regulation under environmental cues and its relation to aging

The TOR signaling pathway is known to activate and regulate physiological processes in eukaryotic organisms responding to nutrient availability (Wullschleger et al., 2006). Due to the multicomponent structures of this pathway, it might be affected by many environmental cues, both directly and indirectly, modifying various components of this pathway. Since negative relationship between lifespan and TOR pathway functioning has been demonstrated (Bonawitz et al., 2007; Garratt et al., 2016), it is suggested that environmental effects on longevity can be mediated by affecting TOR signaling pathway activity (Fig. 2).

Consumed nutrients are basic regulators of this signaling pathway. Identically in yeast and mammalian cells, amino acids and glucose transporters take part in TOR activity regulation (Gobardhan et al., 2009). Reduction of carbohydrate consumption was shown to extend lifespan in *S. cerevisiae*, and their excess led to lifespan shortening (Weinberger et al., 2010). This regulation is mediated by signaling proteins Sch9, Tor1 and Ras.

Insulin and IGFs (insulin-like growth factors) take part in TORC1 complex activation through protein kinase B (Fig. 2). At the same time, protein kinase B acts as the TOR kinase activity enhancer directly or by inhibiting TSC, which is responsible for inhibition of the direct TORC1 activator, Rheb (Markaki and Tavernarakis, 2013).

TSC transmits signals to mTORC1 not just in response to growth factor action. Proinflammatory cytokines such as TNFα (tumor necrosis factor-α) activate mTORC1 in a way identical to growth factors. IkB kinase b (IKKb) phosphorylates TSC1 causing TSC inhibition (Lee et al., 2007). Wnt signaling pathway regulating cell growth, proliferation and differentiation, also activates TORC1 through TSC1/TSC2 activity reduction. This reduction is possible due to glycogensynathase kinase 3b inhibition (Lapalante and Sabatini, 2012). GSK3 kinase phosphorylates TSC2, which leads to TSC1/TSC2 activation and TOR-pathway inhibition. Wnt inhibits GSK3, and TSC is consequently inhibited (Inoki et al.*et al.*, 2006). The presence of nutrients determines cell energy balance, which in turn modulates mTORC1 activity through TSC1/TSC2 (Kapahi et al., 2010). Cell energy reduction causes an increase in AMP/ATP ratio, thus

inducing AMP-dependent protein kinase (AMPK) activation (Hardie et al., 2007). AMPK inhibits TOR signaling pathway through direct TSC2 phosphorylation (Gwinn et al., 2008).

The insulin signaling pathway was shown to be able to work autonomously, apart from TOR activation by amino acids (Avruch et al., 2006). The mTOR kinase is an intersection point between both of pathways, although the signal from amino acids is dominant and mTOR activation by amino acids does not require the insulin signaling pathway involvement. On the other hand, short-term experiments in cell cultures showed that insulin pathway is ineffective in cases where amino acid signaling is absent. It is also known that amino acids act independently from TSC1/TSC2 complexes (Smith et al., 2005). In 2008, two research groups revealed that amino acid-dependent TOR activation requires presence of Rag GTP-ase (Groenewoud et al., 2013; Kim et al., 2008; Powis and De Virgilio, 2016). The Rag-mediated TORC1 translocation to lysosomal membranes has been argued to be a crucial event in amino acid signaling to TORC1 (Laplante and Sabatini, 2012; Sancak et al., 2010). Environmental non-nutritional factors also can influence the TORC1 activity. Experiments with *S. cerevisiae* have shown that high temperature, hydrogen peroxide and osmotic stress inhibit TORC1 (Urban et al., 2007). Hypoxia is the most studied stress factor influencing TOR signaling pathway activity. Low oxygen concentration inside the cell leads to HIF1α stabilization, promoting complex formation with aryl hydrocarbon receptor nuclear translocator (ARNT). The formed complex activates the transcription of genes responsible for survival in hypoxic conditions (Brugarolas et al., 2004). One example of a gene activated by this complex is REDD1, which activates TSC, and in turn inhibits mTORC1 (Bernardi et al., 2006).

4. Dietary interventions, longevity and TOR activity

Dietary composition and ratio between specific macronutrients regulate longevity in many organisms. Specific protocols have been developed and extensively used in different model organisms, however, the terms used for different dietary interventions vary between laboratories. Here we define Caloric Restriction (CR) as dietary manipulation to decrease total caloric intake. CR includes interventions like glucose dilution for yeast, dilution of bacterial density for worms, simple food dilution for fruit fly or food restriction for mammals. Dietary restriction (DR), on the other hand, involves manipulations of specific nutrients like casein, yeast, yeast extract, autolyzed yeast or methionine. Since all mentioned nutrient have caloric value, DR is a partial example of CR. Furthermore, the ratio between macronutrients changes under DR. For example, manipulation of protein-containing nutrients at constant carbohydrate concentration causes the change in protein-to-carbohydrate ratio that is believed by many researchers, including us, as a primary factor to regulate longevity. Below we describe how varied dietary interventions affect longevity and activity of the TOR pathway.

4.1. Effects of dietary interventions on longevity

Replicative and chronological senescence are studied in unicellular budding yeast *Saccharomyces cerevisiae*. However, dietary interventions have been shown to extend lifespan in, worms, fruit flies and mammals as well (Table 1). In yeast, extended lifespan was observed when medium glucose concentration was reduced to 0.5 or 0.05% (Lin et al., 2002; Smith et al., 2007; Longo et al., 2012). Restriction of amino acids, specifically methionine and tryptophan, also slowed senescence (Jiang et al., 2000; Johnson and Johnson, 2014). *Cenorabditis elegans* also displayed a longer lifespan when fed diluted bacterial cultures of $2x10^7$ or $5x10^8$ bacteria/ml (Lenaerts et al., 2008; Greer et al., 2007; Smith et al., 2008) or without bacteria altogether (Kaeberlein et al., 2006; Lee et al., 2006). Similar to dilute bacterial cultures, complete reduction of peptone as a source of peptides and amino acids was also effective to extend worm lifespan (Chen et al., 2009; Stastna et al., 2015). Various dietary interventions have also been applied to the fruit fly *Drosophila melanogaster* (Table 1). Caloric restriction induced by food dilution, or restriction of casein or yeast, extended their lifespan (Banerjee et al., 2012; Mair et al., 2005; Min at al., 2006; Min and Tatar, 2006; Rodina and Helfand, 2004; Van Herrewege, 1974) as well as when fed diets with low P:C ratio (Bruce et al., 2013; Lee et al., 2008; Lushchak et al., 2012). Long-living mice were observed when given restricted amount food by 40% (Liao et al., 2010; Swindell, 2012; Villian et al., 2016) or at restriction of amino acid methionine. Again, mice lived longer when they were fed diets with low P:C ratio (Solon-Biet et al., 2014). CR by food restriction (Swindell, 2012) and decreases of methionine content extended the lifespan in rats (Zimmerman et al., 2003). Finally, restriction by 23-30% from ad libitum level extended lifespan in monkeys together with beneficial health effects (Colman et al., 2009; Colman and Anderson, 2011; Kemnitz, 2011).

4.2. Dietary interventions and TOR activity

The activity of the TOR pathway has been extensively studied under different dietary interventions (Table 2). Shortly, caloric restriction by dilution of dietary glucose inhibit TOR activity in yeast (Fontana et al., 2010; Kaeberlein et al., 2005b; Medvedik et al., 2007; Wei et al., 2008). The decrease of TOR activity was also found in worms and fruit flies in response to food or dietary restriction (Emran et al., 2014; Hansen et al., 2007; Zid et al., 2009), and interestingly, the activity of TOR in mice decreases on diets with low protein and high carbohydrate content (Solon-Biet at al., 2014). Also TOR activity is influenced in mice on food restricted diets (Dogan et al., 2011; Lamming et al., 2014; 2015; Schloesser et al., 2015).

4.3. The role of TOR pathway in mediating the link between nutrition and longevity

Known to be nutrient-sensitive, the TOR pathway is suggested to play a key role in mediating beneficial effects of DR on aging and longevity (Ehninger et al., 2014). TORC1 is mainly involved in lifespan control by regulating important cellular processes such as transcription, translation and autophagy (Inoki et al., 2012; Kaeberlein, 2014). If the amounts of nutrients, as well as growth factors and energy availability are sufficient, TOR switches off the stress resistance and autophagy pathways, and switches on cell growth pathways, including translation and ribosomal biogenesis (Kapahi et al., 2010). Since it is well known that decreased protein synthesis may increase longevity (Hipkiss, 2007), the inhibition of TOR pathway and, accordingly, the suppression of protein synthesis, can likely be implicated in life-extending effects across species. In some studies, it has been demonstrated that TOR may be activated by single amino acids (Bar-Peled and Sabatini, 2013). It can also provide a causal explanation of recent data showing that reduction in the proportion of protein in the diet, rather than DR *per se*, could promote longevity in various animal models (Lee, 2015; Solon-Biet et al., 2014, 2015). TOR signaling responds not only to the nutrient concentration but also to the nutrient proportion (Simpson and Raubenheimer, 2009). Moreover, both carbohydrate and protein influence aging through TOR signaling. Previous findings demonstrated that glucose robustly activates mTOR in an amino acid-dependent manner in rodent and human islets (Kwon et al., 2004). Flies were longest-lived at a relatively low P:C ratio of 1:16 (Lee et al., 2008) or even 1:57 (Lushchak et al., 2012). But the role of TOR signaling in mediating the effect from P:C proportion on longevity is unknown. However, it is known that diet balance realized its effect on aging through TOR/AMPK interaction (Simpson and Raubenheimer, 2009). Low protein diet can drive overconsumption, metabolic disorders and shortened lifespan unless excess ingested energy is dissipated (Simpson and Raubenheimer, 2009). Otherwise, low percent protein diets are life extending via the normal actions of AMPK, whereas high percent protein diets shorten lifespan and encourage aging via the TOR pathway (Simpson and Raubenheimer, 2009).

There is plenty of evidence that the TOR signaling pathway plays a crucial role in mediating aging and age-related pathological conditions, such as type 2 diabetes, obesity and cancer (Albert and Hall, 2015). Its inhibition has been repeatedly demonstrated to provide protection against various aging-related pathologies and to promote longevity in different animal models (Johnson et al., 2013). Genetic inhibition of the TOR signaling resulted in life extension in nematode (Jia et al., 2004; Vellai et al., 2003), fruit fly (Kapahi et al., 2004), and rodents (Lamming et al., 2012). Recent transcriptional analysis revealed that the TOR pathway is strongly related to human health and lifespan (Passtoors et al., 2013). Therefore, TOR is believed to be a most promising target for

pharmacological interventions to modulate the nutrient-sensitive pathways and for slowing the aging process.

However, there is also alternative theory on the aging regulation. According to M. Blagosklonny aging is a "quasi-program" and is continuation of the development program. Continuous activity of genes, that are absolutely necessary for organism development, cause aging. Therefore, aging is quasi-programmed into the genome. TOR plays a key role as nutrient-sensing pathway and growth promoter. Inhibition of mTOR can prolong lifespan but continued operation of mTOR pushes cells into the senescent state (Blagosklonny et al., 2015).

5. Lifespan modulation by rapamycin and metformin

Rapamycin (also termed as sirolimus), appears to be the most effective lifespan-extending drug. This natural compound was originally isolated from the bacterium *Streptomyces hygroscopicus* taken from the Easter Island soil sample around 1970 (Arriola Apelo and Lamming, 2016). Rapamycin was initially developed as an antifungal agent; however, it was subsequently shown to have significant regulatory effects on fundamental biological processes such as cellular growth, proliferation, as well as inflammation through the inhibitory action on TOR (Lamming et al., 2013). Since this agent was found to inhibit the immune response, it is widely used in immunosuppressive therapy to prevent graft rejection and to treat different autoimmune disorders (Ingle et al., 2000). Presently, rapamycin and its analogues (rapalogs), such as everolimus and temsirolimus, are believed to be among the most promising anti-aging medications (Blagosklonny, 2007; Longo et al., 2015).

In a mice model, therapy with rapamycin has been shown to delay aging-associated changes, such as liver degeneration, accumulation of sub-cellular alterations in the myocardium, tendon stiffening, endometrial hyperplasia, and decline of physical activity (Wilkinson et al., 2012). Furthermore, in the rodent models, experimental evidence shows that it can be effective against various aging-related disorders including cardiac hypertrophy, retinopathy, neurodegenerative disorders, cognitive decline, and loss of stem cell function (Chen et al., 2009; Halloran et al., 2012; Kolosova et al., 2012; Shioi et al., 2003; Spilman et al., 2010). The life-extending ability of rapamycin has been studied in a number of studies from yeast to mammals. The main findings from these studies are summarized in the Table 3.

Metformin as the most widely used biguanide drug to treat type 2 diabetes mellitus (T2DM) with over 120 millions prescriptions worldwide. This biguanide drug reduces hyperglycemia, inhibits lipolysis and affects levels of circulating free fatty acids. The actual mechanism of action

remains unclear, however the majority of metabolic actions are mediated through AMPK activation and modulation of the incretin axis (Violett et al., 2012). Metformin acts as a phosphorylation inhibitor for of the S6K1 and 4E-BP1 substrates for mTORC1 and decreases translation (Dowling et al., 2007). Phosphorylation inhibition was initially described as an AMPK-dependent mechanism, however it was shown that metformin inhibits Ras-related GTP binding (Rag) GTPases, thus directly regulating mTORC1 activity (Kalender et al., 2010). Metformin also has an indirect mechanism for mTORC1 inhibition via upregulation of REDD1, consequently enhancing activity of TSC2 (Ben Sahra et al., 2011).

The mechanisms potentially involved in rapamycin-induced life extension include stem cell guidance (Maiese, 2015), anticancer effects (Blagosklonny, 2012; Saran et al., 2015), induction of autophagy (Perluigi et al., 2015), as well as anti-inflammatory and immune-modulating effects (Araki et al., 2011). In addition, these effects are suggested to be mediated by reducing the rate of protein synthesis, since the decrease of overall translation rate can prevent the accumulation of misfolded or damaged proteins that may, in turn, affect longevity (Hipkiss, 2007). In addition, it must also take into account that TOR is negatively regulated by other nutrient-sensing pathways known to be significantly implicated in the control of longevity, such as silent information regulator 1 (SIRT1) and adenosine monophosphate-activated protein kinase (AMPK) pathways (Cetrullo et al., 2015). Rapamycin treatment was shown to inhibit both TORC1 and TORC2, but its life-extending effect is mediated by TORC1 inhibition only. Importantly, the treatment with rapamycin starting in later life can be sufficient to promote longevity; similar benefits are also achieved by transient treatments in late life (Kaeberlein, 2014). For example, in mice, rapamycin supplementation which was initiated late in life (20 months of age) was nearly as effective as if beginning at 9 months of age (Harrison et al., 2009; Miller et al., 2011). Transient treatment with rapamycin in late life has been also found to reverse the age-related heart dysfunction, and also resulted in beneficial skeletal, motor and behavioral changes in 24-month-old C57BL/6J females (Flynn et al., 2013). The mid- or late-life rapamycin therapy seems especially promising since such intervention is clearly preferable when considering the potential for the translation of anti-aging therapies to human beings (Kaeberlein, 2014).

Presently, some inhibitors of the TOR pathway are already clinically approved, and others are under the development. There are, however, several concerns on the applicability of rapamycin and its derivatives (rapalogs) in anti-aging medicine. In the aforementioned research by Wilkinson et al. (2012), the rapamycin supplementation starting at nine months of age caused significantly higher incidence of cataract and testicular degeneration than in control mice. It was also revealed that rapamycin-induced suppression of TORC2 may result in impaired glucose homeostasis and insulin resistance due to their adverse effect on hepatic gluconeogenesis (Lamming et al., 2012). Side

effects of chronic rapamycin intake, including glucose intolerance, hyperglycemia and insulin resistance, have been shown in several other rodent studies as well (Deblon et al., 2012; Houde et al., 2010). Some unfavorable effects have been also reported in clinical trials with cancer patients treated with rapamycin monotherapy (Richardson, 2013). Among these effects, there were metabolic abnormalities including glucose intolerance, decreased insulin sensitivity, hypertension, hyperlipidemia, and increased incidence of new-onset diabetes (Lamming et al., 2013), and also diarrhoea, anaemia, thrombocytopenia, stomatitis, skin rash, and certain malignancies (namely, lymphoma and skin cancers) (Lamming et al., 2012; Zafar et al., 2009). Such rapamycin-induced metabolic impairments were shown to be completely reversible. In both lean and obese mice, these effects were almost lost after a few weeks of cessation of rapamycin treatment (Liu et al., 2014). Most serious concerns on the clinical applicability of rapamycin are currently linked to its immunosuppressive properties. Even though rapamycin and its rapalogs are widely used now in the cancer prevention and therapy, the fear of cancer is the main concern about their clinical application (Blagosklonny, 2013). Therefore, these medications should be applied with caution to avoid the potentially dangerous effects.

Metformin promotes longevity in worms, flies and rodents (Table 4). In *C. elegans* model metformin extended life span and youthful locomotory activity into later adulthood. These effects were not related to insulin signaling pathway; metformin engaged both DR and oxidative stress pathways to prolong life and healthspan in this model. Increase in lifespan and healthspan requires AMPK activity, as well as its catalytic subunit AAK-2, its upstream kinase LKB1/PAR-4 and transcription factor SKN-1/Nrf (Onken et al., 2010).

Quite conflicting evidence about potential benefits of metformin treatment was obtained in different strains of rodents. MF-treated B6C3F1 and C57BL/6 male mice showed slight extension in mean lifespan and better characteristics of glucose and lipid metabolism, compared to controls (Martin-Montalvo et al., 2013). In short-lived, tumor-prone HER2/neu mice metformin treatment promoted longer lifespan and inhibited carcinogenesis (Anisimov et al., 2005). In female SHR mice MF-supplementation increased mean lifespan, but had no influence on maliganancy incidence (Anisimov et al., 2008). In neonatally MF-exposed 129/Sv mice treatment resulted in larger lifespan extension in males, while in females it produced decreased rate of malignancies (Anisimov et al., 2015).

However, in male Fischer-344 rats MF supplementation did not show any benefits in lifespan prolongation (Smith *et al.*, 2010). In multi-centered randomized controlled trial UKPDS, metformin decreased the risk of diabetes-related mortality and myocardial infarction in overweight patients

with type 2 diabetes mellitus (T2DM) (Anon 2013). However, potential molecular mechanisms of metformin-induced longevity in humans are yet to be elucidated (Lamming et al, 2013).

In T2DM patients, continuous use of metformin has been associated with significant decrease in risk of colorectal, liver, pancreatic, stomach, and oesophagus cancer, with metformin use associated with a 35% reduction in the risk of cancer mortality, and a 31% reduction in the risk of any cancer (Franciosi et al., 2013). Potential anticarcinogenic effects of metformin seems to be regulated by several mechanisms, involving LKB1/AMPK pathway activation, induction of cell cycle arrest and/or apoptosis, and inhibition of protein synthesis. The activation of LKB1/AMPK pathway inhibits mammalian target of rapamycin (mTOR), thus inhibiting protein synthesis in malignant cells (Kourelis et al., 2012).

Conclusions and perspectives

During the last few decades, the molecular mechanisms of TOR signaling regulation and its downstream target processes have been clarified in detail. Findings from the research conducted on different model organisms allow concluding that these genetic pathways in lifespan regulation are conserved from single-cell organisms to humans. This review summarizes current knowledge and recent findings suggesting a crucial role of TOR signaling pathway in cellular processes related to metabolism, immune function, and tumorigenesis, as well as in the control of aging and longevity. Our recent study discussed that TOR is also involved in pharmaceutical targeting of lifespan and healthspan in animal models (Vaiserman et al., 2016). Numerous cellular processes described are presumably involved in life extension through the TOR signaling pathway inhibition in various species. Genetic interventions that modulate the activity of TOR signaling cascade, thereby affecting aging process, are reported. Some inhibitors of TOR pathway are already clinically approved, and others are under development. Considering the fact that this signaling pathway is highly conserved, it could be suggested that inhibition of TORC1 signaling by pharmacological or biotechnological interventions may be highly promising approach for the extension of human healthspan and longevity.

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Figure 1. The TOR signaling pathway in mammals. Key factors regulating the TOR signaling pathway activity are shown, as well as main targets and modified cellular processes. The TOR pathway responds to growth factors, amino acids, oxygen concentration, stresses and cell energy state. Direct or indirect (with certain signaling pathway compounds) TOR kinase activation leads to increased protein, lipid biosynthesis and autophagy suppression.

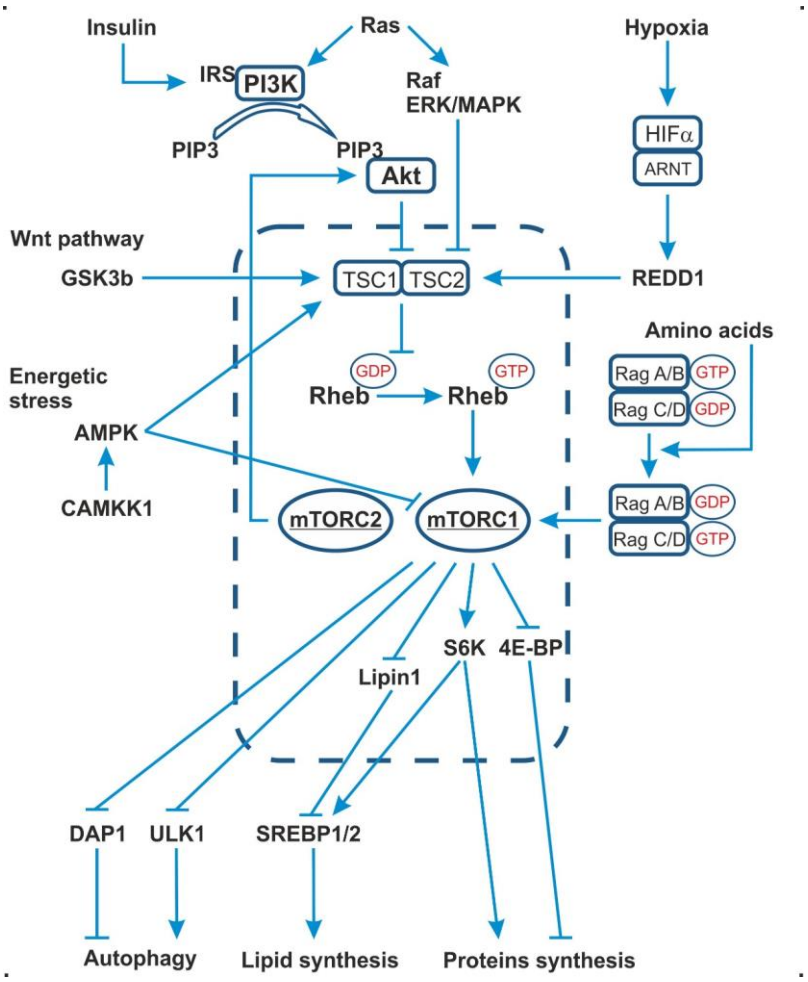

Figure 2. The role of TOR signaling pathway in lifespan regulation. Various environmental factors can affect components of the TOR signaling pathway (indicated in red) and regulate the ageing process and longevity. Activation of protein biosynthesis, regulated by S6K and 4E-BP, promotes ageing. TOR activation by temperature accelerates the rate of aging, while its inhibition by rapamycin slows down this process. Inhibition of TSC by insulin, TNFα and the Wnt-pathway reduces lifespan, while the activation of the p53 response by hypoxia has the opposite effect on life expectancy. Ageing is intensified in the case of Rheb activation by specific amino acids. TOR also blocks autophagy, which slows down ageing processes (Johnson et al., 2015; Kennedy and Lamming, 2016; Huang and Fingar, 2014).

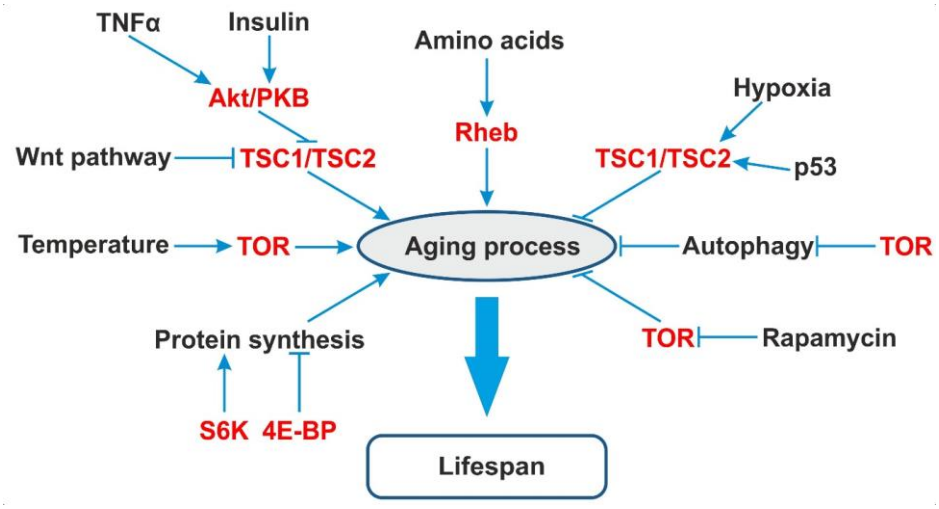

Table 1. Dietary interventions leading to maximal lifespan in various organisms

Model	**Dietary intervention**	**Conditions for maximum LS**	**Reference**
Yeast	CR by glucose dilution	Glucose 0.5% Glucose 0.5% or 0.05% Glucose 0.5%	Lin et al., 2002 Longo et al., 2012 Smith et al., 2007
	DR by restriction of amino acids	No amino acids	Jiang et al., 2000
	DR by restriction of methionine		Johnson and Johnson, 2014
Worm	DR by dilution of bacteria	$5X10^8$ bacteria/ml $2X10^7$ bacteria/ml No bacteria	Greer et al., 2007 Smith et al., 2008 Lenaerts et al., 2008 Kaeberlein et al., 2006; Lee et al., 2006
	DR by peptone dilution	0% peptone	Chen et al., 2009 Stastna et al., 2015
	Varying casein and sucrose	1% casein	Van Herrewege, 1974
Fruit fly	Different P:C by varying sucrose	P:C 1:16 P:C 1:57	Lee et al., 2008 Lushchak et al., 2012
	Varying carbohydrate and yeast	P:C 1:16	Bruce et al., 2013
	CR by food dilution	5% sucrose and 5% yeast 6.5% sucrose and 6.5% yeast 4% sucrose and 4% yeast	Rogina and Helfand, 2004 Mair et al., 2005 Min et al., 2006
	DR by varied yeast	0.25% yeast	Banerjee et al., 2012
	DR by varied casein	1% casein	Min and Tatar, 2006
	Varied carbohydrates	Fructose and glucose	Lushchak et al., 2014
	CR by food restriction	40% restriction	Liao et al., 2010 Swindell, 2012 Villian et al., 2016
Mouse	Varying carbohydrate and protein	low P:C	Solon-Biet , 2014
	DR by restriction of methionine	0.1% methionine	Miller et al., 2005

	DR by restriction of methionine	0.17% methionine	Zimmerman et al., 2003
Rat	CR by food restriction	40% restriction	Swindell, 2012
Monkey	CR by food restriction	30% restriction 23-24% restriction	Colman and Anderson, 2011 Colman et al., 2009 Kemnitz, 2011

Table 2. The effects of dietary interventions on the activity of TOR pathway in various organisms

Model	**Dietary intervention**	**TOR activity**	**Reference**
Yeast	CR by glucose dilution	inhibition	Fontana et al., 2010 Kaeberlein et al., 2005b Medvedik et al., 2007 Wei et al., 2008
Worms	Food restriction	inhibition	Hansen et al., 2007
Fruit fly	Food dilution	inhibition	Zid et al., 2009
Mouse	Food restriction	inhibition	Dogan et al., 2011 Fok et al., 2013 Lamming, 2014
	Manipulating the ratio of macronutrients	inhibition at diets with low P:C	Solon-Biet et al., 2014

Table 3. Summary of lifespan-modulating effects of rapamycin in different model organisms

Model	Impact on lifespan	Impact on longevity-associated traits	Reference
Saccharomyces cerevisiae	54% extension of chronological lifespan	ND	Powers et al., 2006
	15% increase of replicative lifespan	ND	Medvedik et al., 2007
	19% increase of replicative lifespan	ND	Ha and Huh, 2011
Cenorhabditis elegans	19 % increase of mean lifespan	ND	Robida-Stubbs et al., 2012
Drosophila melanogaster	Extension of female median and maximum lifespan: on rich media: 17% and 23%, respectively; during starvation: 54% and 36%, respectively.	Increased resistance to starvation and paraquat stress	Bjedov et al., 2010
	Standard diet: increase of male mean lifespan on 6%; increase of female mean lifespan on 26%; low-calorie diet: decrease of male mean lifespan on 12%; increase of male mean lifespan on 57%.	ND	Sun et al., 2012
Mus musculus	14% increase in 90% mortality in female and 9% in male genetically heterogeneous mice fed rapamycin beginning at 600 days of age	ND	Harrison et al., 2009
	Increased mean lifespan (male: 10%; female: 18%) in genetically heterogeneous mice fed rapamycin from 9 months of age.	Attenuating age-associated decline in spontaneous activity in males but not in females	Miller et al., 2011
	Increase of mean lifespan in genetically heterogeneous males by 23% and females by 26%.	ND	Miller et al., 2014
	11% increase in maximum lifespan in females.	Decreased rate of aging and suppressed carcinogenesis in transgenic HER-2/neucancer-prone mice. Prevention of age-related weight gain.	Anisimov et al., 2010a
	8% increase in maximum lifespan in inbred females	Inhibited age-related weight gain, decreased aging rate, and delayed spontaneous cancer	Anisimov et al., 2011b
	Improved survival in C57BL/6 mice after the rapamycin feeding in late life	Reduced total and resting metabolic rate	Zhang et al., 2014
	Increased median lifespan in male C57BL/6J mice	ND	Neff et al., 2013

ND: not determined.

Table 4. Summary of lifespan-modulating effects of metformin in different model organisms

Model	**Impact on lifespan**	**Impact on longevity-associated traits**	**Reference**
Cenorhabditis elegans	40% increase in median survival	Extended mobility; both reduction and delay in reproduction	Onken et al., 2010
Drosophila melanogaster	No effect	ND	Jafari et al., 2007
	No effect	Increased egg-laying in young females in intermediate concentrations	Slack et al., 2012
Mus musculus	Extension in male B6C3F1 mice by 4% and in male C57BL/6 mice by 6%	Lower HbA1c levels, reduction of serum insulin, total cholesterol, LDL-cholesterol and HOMA-IR index; level of antioxidant and stress response proteins: SOD2, TrxR1, NQO1 and NQO2	Martin-Montalvo et al., 2013
	Increased medium LS by 38% and maximum LS by 21% in SHR mice	No effect on total incidence of maliganacies	Anisimov et al., 2008
	Extended mean LS by 8%, and maximum LS by 13% in HER-2/neu mice	Inhibited development of mammary adenocarcinomas and increased their latency; slightly decreased food consumption	Anisimov et al., 2005
	Decreased in male mice by 13.4% and increased in female mice by 4.4%	No influence on tumor incidence in male mice, in female mice decreased the incidence of malignancies by 3.5 times	Anisimov et al., 2015
Rattus norvegicus	No effect in male Fischer-344 rats	No effect on insulin, glucose, or core body temperature with	Smith et al., 2010

ND: not determined.