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Metabolic Control by Target of Rapamycin and Autophagy during Ageing – A Mini-Review

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Abstract
The conserved target of rapamycin (TOR) pathway integrates signals from nutrient and energy availability, growth factors and stress to regulate cell growth and proliferation, development and metabolism. Growing evidence suggests that TOR signalling controls the rate at which cells and tissues age, thereby contributing to whole-organism ageing. Although significant progress has been made in the last decades towards understanding fundamental aspects of the ageing process, the precise mechanisms underlying the age-related effects of TOR are still not fully understood. TOR interfaces with several cellular processes, such as DNA transcription, mRNA translation, protein turnover and autophagy, among others. Interestingly, TOR regulates various aspects of metabolism including mitochondrial function and lipid metabolism. Inhibition of TOR activity stimulates autophagy, a conserved lysosomal catabolic pathway that controls the degradation and turnover of macromolecules and organelles. Autophagy also has an important role in maintaining metabolic homeostasis at both the cellular and whole-organism level. Ageing in diverse organisms ranging from yeast to mammals appears to be associated with insufficient autophagy. Here, we summarize recent developments that outline how TOR and autophagy modulate the ageing process, with special emphasis on their role in the regulation of metabolism. A better understanding of the complex interplay between TOR, autophagy and ageing will pave the way for the development of novel therapeutic strategies to treat age-related pathologies.

Introduction

Ageing, the lifelong accumulation of damage to molecules, cells and tissues, is often associated with increased vulnerability to disease, such as cancer, cardiovascular and neurodegenerative diseases. During the past 20 years, extensive studies in model organisms have revealed that ageing is a process subjected to regulation by classical signalling pathways and transcription factors [1]. Most of these studies also suggest that nutrient and stress sensors or reduced availability of growth factors alarm eukaryotic cells to reduce their metabolic activity in order to survive. One of the central regulators of cellular and organismal me-

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tabolism in eukaryotes is the target of rapamycin (TOR). TOR is an evolutionarily conserved nutrient-sensing Ser/Thr kinase which functions in 2 multiprotein complexes (TORC1 and TORC2) composed of distinct as well as overlapping components. TORC1 is rapamycin sensitive and controls temporal aspects of cell growth. TORC2 is involved in organization of the actin cytoskeleton and regulation of cell polarity, thereby affecting spatial aspects of cell growth. TORC2 is thought to be rapamycin insensitive, although recent work indicates that prolonged rapamycin treatment suppresses the assembly of mTORC2 and inhibits AKT/PKB (AKT8 virus proto-oncogene akt, also known as protein kinase B; Akt/PKB) signalling in many cell types [2]. The TOR pathway regulates cell growth and proliferation, development, metabolism and ageing in response to 4 major signalling cues, i.e. growth factors, nutrients, energy and stress. All these stimuli, except amino acids, signal to TORC1 through the tuberous sclerosis TSC1-TSC2 complex and the small GTPase Ras homologue enriched in brain (Rheb). Growth factors such as insulin or insulin-like growth factors (IGFs) activate TORC1 through the insulin receptor/phosphoinositide 3-kinase/AKT signalling pathway. AKT/protein kinase B functions to activate the TOR kinase, either directly or through inhibition of the TSC2 tumour suppressor, a negative regulator of Rheb, which activates TOR (fig. 1).

Recent findings indicate that amino acid signalling promotes mTORC1 translocation to the lysosomal membrane, where it becomes activated upon conversion of RagA or RagB GTPases from a GDP- to GTP-bound state [3]. Studies in model organisms have shown that TOR regulates organism growth during early development and lifespan during adulthood [4]. The mechanisms through which TOR influences ageing are not entirely understood. In most cell types, TOR activity is necessary and sufficient to suppress autophagy when food is plentiful.

Autophagy encompasses the different pathways by which cytoplasmic components are delivered to lysosomes in animal cells or vacuoles in plant and yeast cells for degradation [5, 6]. Three main types of autophagy have been defined, namely macroautophagy, microautophagy and chaperon-mediated autophagy. The predominant form, macroautophagy (hereafter referred to as autophagy), involves the engulfment of organelles, proteins or portions of the cytoplasm within double-membrane vesicles called autophagosomes. The sequestered contents are then delivered to lysosomes for degradation [7]. In physiological conditions, a low level of constitutive autophagy is important for normal turnover of cytoplasmic contents especially in post-mitotic differentiated cells. In the absence of nutrients, such as amino acids and glucose, autophagy is induced, providing the nutrients required for survival. Autophagy can also be stimulated by other forms of cellular stress, including reactive oxygen species, DNA damage, protein aggregates, damaged organelles or intracellular pathogens. Autophagy is a tightly regulated process, and TOR is one of the most important autophagy regulators [8]. In deprivation or stress, the activity of TOR is inhibited and autophagy is induced.

Early observations established that both TOR and autophagy act as crucial regulators of a network controlling metabolic homeostasis at both the cellular and the organismal level [4, 9, 10]. Moreover, the TOR pathway has been shown to regulate the lifespan of many species, including yeast, worms, flies and mice [1]. Likewise, accumulating findings indicate that various signalling pathways and environmental factors may converge on autophagy to regulate ageing [11]. Here, we briefly summarize recent evidence from model organisms in support of close connections between TOR, autophagy and ageing. We specially highlight the importance of TOR and autophagy in regulating cellular and organismal metabolism and thereby preventing ageing and illness.

TOR Plays a Conserved Role in the Regulation of Ageing

TOR, which is essential for growth during early development, contributes to cellular senescence and organismal ageing during adulthood. Aberrant TOR signalling has been implicated in a large number of age-related diseases including cancer, cardiovascular disease and metabolic disorders [4, 10]. It is noteworthy that post-developmental inhibition of TOR signalling extends lifespan in diverse organisms [1]. In *Saccharomyces cerevisiae,* deletion of *tor1* or inhibition of TORC1 by rapamycin extends the replicative and chronological lifespan, indicating that TOR can promote ageing of both mitotic and post-mitotic cells [12]. Similarly, mutations that decrease the activity of TORC1 extend the *Caenorhabditis elegans* lifespan. RNAi knockdown of *CeTor (let-363), rheb-1* or heterozygosity for *daf-15*/raptor, which encodes an essential TORC1 component, increase the lifespan of the worm. DAF-16/forkhead box O (FoxO) activity is required for increased *daf-15*/+ longevity. DAF-16 has been shown to negatively regulate *daf-15* transcription. Thus, insulin/IGF-1 signalling and TOR signalling converge on DAF-15 to regulate *C. elegans* larval development, metabolism and lifespan [13]. Interestingly, *let-363 (RNAi)* animals share

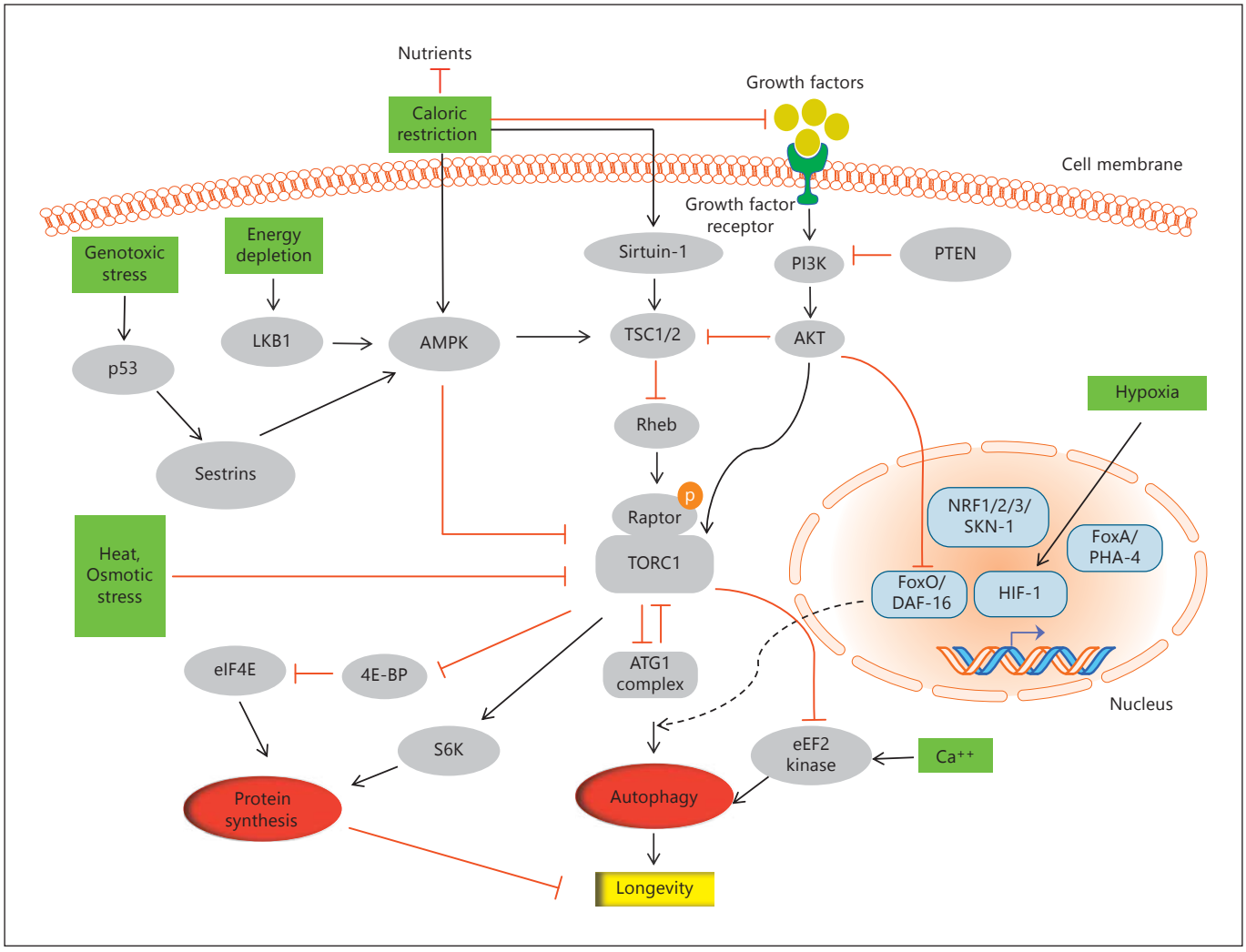

Fig. 1. The TOR signalling pathway and the autophagy pathway are tightly linked to control ageing. The TOR kinase functions in 2 distinct, evolutionarily conserved multiprotein complexes, called TORC1 and TORC2. Nutrients, growth factors, energy status and stress signals regulate the activity of TORC1. When nutrients and other growth stimuli are present, TOR upregulates translation and promotes cell growth and metabolic activity, whereas it blocks autophagy by inhibiting the Atg1 complex. Under nutrient deprivation, energy depletion or stress, various signalling pathways inactivate TOR kinase activity and thus suppress cell growth, while inducing autophagy. Limitation of nutrients, energy and growth factors also augments longevity. Thus, TOR signalling stands at the crossroads of metabolism, protein homeostasis and ageing. Many longevity-promoting regimens are associated with autophagy regulation mechanisms, several of which converge on the AMPK-TORC1 axis. For clarity, some of the signalling connections between longevity pathways and autophagy are not shown. In addition, some of the information presented in this figure is not included in the main text. Black arrows indicate stimulatory inputs. Red bars indicate inhibitory interactions. AKT = AKT8 virus proto-oncogene; AMPK = AMP-activated protein kinase; eEF2 = translation elongation factor 2; FoxO/DAF-16 = a forkhead box O (FoxO) transcription factor; FoxA/PHA-4 = a forkhead box A transcription factor; HIF-1 = hypoxia-inducible factor 1; LKB1 = serine/threonine protein kinase; NRF1/2/3 = NF-E2-related factor; PI3K = phosphatidylinositol-3 kinase; PTEN = phosphatase and tensin homologue; TSC1/2 = tuberosclerosis complexes 1 and 2.

some characteristics of mutants with reduced activity of the insulin/IGF-1 receptor orthologue DAF-2. Unlike *daf-2* mutants, which require DAF-16 for lifespan extension, *let-363 (RNAi)* worms are not dependent on DAF-16 for longevity, suggesting that TOR may be acting downstream or independently of DAF-16 [14]. Nevertheless, a recent study revealed a novel link between TOR and insulin/IGF-1 signalling. Intriguingly, genetic interference with either TORC1 or TORC2, or rapamycin treatment, extends the worm lifespan in a skin in excess (SKN)-1-dependent

manner. DAF-16 is also required for the stress resistance and longevity that result from genetic inhibition of TORC1, but not TORC2 or rapamycin. These data imply that the insulin/IGF-1 and TOR pathways each influence ageing by regulating SKN-1/Nrf and DAF-16/FoxO, and that these transcription factors may be important in mediating the effects of TORC1 in vivo. Collectively, SKN-1/Nrf and DAF-16/FoxO might mediate an opposing relationship between growth signals and longevity [15]. Together, these findings suggest that there is a complex cross-talk between the two signalling pathways. In *Drosophila,* inactivation of dTOR or activation of the upstream negative regulators dTSC1 and dTSC2 extends lifespan. Similarly, feeding rapamycin to adult *Drosophila* consistently extends lifespan in diverse genetic and cytoplasmic backgrounds and in both sexes. Moreover, rapamycin confers increased resistance to both starvation and paraquat. However, rapamycin-treated flies still respond to dietary restriction (DR), defined as a reduction in particular or total food intake without malnutrition. Clearly, these data indicate that additional longevity assurance pathways may underlie the beneficial effects of rapamycin on ageing [16]. In mice, rapamycin fed late in life extends the median and maximal lifespan of both female and male animals, suggesting that pharmacological inhibition of TOR signalling might be a promising anti-ageing intervention [17].

Inhibition of the TOR pathway in yeast, worms or flies provides no additional benefit over DR, a well-known strategy for extending lifespan in many species [18]. These findings suggest that common mechanisms may mediate the anti-ageing effects of both TOR and DR. It has been suggested that animals change their energy investment strategy during periods of food deprivation. The diversion of limited energy resources from reproduction to cellular repair and maintenance mechanisms would extend lifespan under DR [19]. In mice, deletion of S6 kinase (S6K1), a major downstream effector of TOR, leads to increased lifespan and resistance to age-related pathologies. Notably, deletion of S6K1 induces gene expression patterns similar to those seen in DR, further supporting the view that DR acts, at least in part, through inhibition of the TOR pathway in mammals as well [1, 10].

TOR-Regulated Cellular Processes That Influence Longevity

TOR interfaces with several biological processes, such as mRNA translation, autophagy, stress response and metabolism, among others. These downstream targets of TOR signalling most likely mediate the effects of TOR on longevity. Here, we more closely examine the mechanisms by which TOR regulates metabolism and present data on the cross-talk between TOR and autophagy and how they may affect longevity and age-related diseases.

TOR and Metabolism

Accumulating findings suggest that metabolic function declines with age. Comparative transcriptional profiling of ageing in human, mouse and fly reveals that the electron transport chain pathway decreases expression with age. Interestingly, in worms and flies, the expression of genes involved in mitochondrial oxidative respiration is repressed early in adulthood before the onset of functional decline [18]. Given the key role of TOR in regulation of growth, development and ageing, it is perhaps not surprising that TOR regulates cellular metabolic capabilities, including rates of protein turnover, mitochondrial function and lipid metabolism, and there is increasing evidence that TOR signalling also impacts glucose metabolism in vivo [20]. Therefore, TOR is an important regulator of metabolic homeostasis at both the cellular and the organismal level and as such promotes healthspan.

One of the best-characterized functions of TOR is its ability to regulate protein synthesis. Two key downstream targets of TOR are the ribosomal subunit S6K and the eukaryotic initiation factor 4E-binding protein (4E-BP). TORC1 regulates translation in part by activating S6K, which phosphorylates the 40S ribosomal protein S6 and selectively promotes the translation of 5′TOP mRNAs, a specific subset of mRNAs containing a terminal oligopyrimidine tract [21]. Upon activation, TOR also phosphorylates 4E-BP, which then releases the eukaryotic translation initiation factor 4E (eIF4E), a key regulator of mRNA translation initiation, thereby stimulating protein synthesis. When the TOR pathway is inhibited, the hypophosphorylated form of 4E-BP inhibits protein synthesis by binding eIF4E and thus blocking the activity of the eIF4F complex [22]. Reduction of global protein synthesis under unfavourable stressed conditions would result in notable energy saving. This energy could then be diverted to cellular repair and maintenance processes, thus contributing to longevity. Surprisingly, TOR inhibition leads to a selective increase in the translation of various nuclear encoded electron transport chain components and mitochondrial ribosomal proteins under nutrient-limited conditions that are known to reduce bulk protein synthesis rates. 4E-BP is upregulated upon DR and mediates the selective translation of nuclear en-

coded mitochondrial genes and lifespan extension under DR conditions in *Drosophila*. Thus, modulation of mitochondrial activity via the TOR effector 4E-BP contributes, at least in part, to the longevity conferred by DR. 4E-BP, being involved in translational regulation of mitochondrial gene expression, can induce a metabolic shift towards increased mitochondrial efficiency in order to ensure sustained ATP production and enhance survival under nutrient deprivation [23]. Consistent with this idea, increased mitochondrial activity has been reported to be essential for DR-induced lifespan extension in many organisms [24].

Further supporting the role of TOR signalling in the regulation of cellular metabolism, recent studies have revealed that mTORC1 activation induces the expression of genes involved in glucose uptake and glycolysis through hypoxia-inducible factor 1 and of genes functioning in the pentose phosphate pathway and lipid and sterol biosynthesis, through sterol regulatory element-binding protein. All these metabolic changes are frequently detected in human cancers [25].

It is becoming increasingly apparent that ageing affects the ability of the organism to withstand extrinsic and intrinsic stressors. Accumulating findings indicate that signal transduction pathways influencing ageing associate with stress response mechanisms [26]. Given that TOR integrates various extracellular and intracellular signals to regulate ageing, it is not surprising that TOR inhibition increases resistance to environmental stress. In yeast, long-lived mutants lacking *SCH9* (a homologue of the mammalian kinases AKT and S6K), *TOR1* or *RAS2* show decreased expression of genes encoding proteins that function in the tricarboxylic acid (TCA) cycle and respiration, but enhanced expression of glycerol biosynthetic genes. The homologues of SCH9, TOR1 and RAS2 are also known regulators of lifespan in worms, flies and mammals. Both TOR and Ras/cAMP-protein kinase A pathways have been reported to regulate stress-responsive (STRE) genes. The metabolic switch from the TCA cycle and respiration to glycolysis and glycerol production together with the direct regulation of stress resistance systems leads to enhanced cellular protection and lifespan extension. In this way, TOR1/SCH9-regulated glycerol biosynthesis may lead to a carbon source substitution that is as effective as caloric restriction in lifespan extension [27]. Moreover, TORC1 negatively regulates the stress-activated transcription factors GIS1 and MSN2/4, both of which are required for lifespan extension upon TOR inhibition in yeast. TOR inhibition also increases the expression of the nicotinamidase gene PNC1 through the relocalization of the transcription factors MSN2p and MSN4p from the cytoplasm to the nucleus. PNC1 converts nicotinamide, a natural SIR2 inhibitor, to nicotinic acid, thus activating SIR2. Being regulated by the cofactor nicotinamide adenine dinucleotide, sirtuins may serve as sensors of the metabolic state of the cell and organism [28]. These findings suggest that both TOR and sirtuins act as crucial regulators of the network that controls metabolic homeostasis at both the cellular and the organismal level (fig. 1) [12].

Early observations suggest that TOR also controls fat metabolism. Increasing 4E-BP activity within the context of a whole animal increases fat accumulation in *Drosophila*. Conversely, reduced 4E-BP activity leads to an increased rate of fat burning and impaired survival under unfavourable conditions. These findings provide evidence that 4E-BP plays an important role in the regulation of fat metabolism. 4E-BP is assumed to act as a metabolic brake that is activated under conditions of environmental stress to control the rate of fat metabolism [29]. Of note, mTOR kinase activity is required for adipocyte differentiation. It is shown that rapamycin treatment directly inhibits the activity of peroxisome proliferator-activated receptor-γ, thus blocking adipogenesis and lipid accumulation [4]. Despite growing evidence suggesting a role for TOR in the regulation of fat metabolism, whether this function of TOR is required for delaying the ageing process is still elusive.

Autophagy: TOR and More

In addition to controlling cell growth and metabolism, TOR negatively regulates autophagy in yeast and higher eukaryotes when nutrients and growth factors are abundant. One of the earliest steps in autophagy induction is mediated by the Atg1/ULK1 complex (Atg1 in yeast and ULK1 in mammals), which is composed of the Ser/Thr kinase Atg1 and two scaffolding proteins, Atg13 and Atg17 (FIP200 in mammals). Under nutrient-rich conditions, TOR-dependent phosphorylation of Atg13 suppresses the complex, thus inhibiting autophagy. Under nutrient deprivation or stress, multiple signalling pathways inactivate TORC1, leading to an increase in Atg1 activity, which mediates an early activation step in the autophagic process, autophagosome nucleation and elongation [8, 9]. It has been shown that starvation induces autophagy in the *Drosophila* larval fat body, a functional homologue of vertebrate liver and adipose tissue, through inactivation of TOR and its upstream regulators phosphoinositide 3-kinase and Rheb. Surprisingly, the activity of S6K is required for starvation-induced autophagy,

contradicting the prevailing view that S6K acts as a suppressor of autophagy. The finding that genetic disruption of autophagy in TOR mutants is detrimental supports the notion that autophagy plays a protective role in fat body cells lacking TOR [30].

Notably, recent findings suggest that TOR signalling not only regulates autophagy but is also influenced by the cellular rates of autophagy [8]. In some cases, autophagy is also regulated by the activation of FoxO transcription factors, which act in parallel to TOR downstream of AKT, leading to transcription of *atg* genes (fig. 1). FoxO3 transcription factor, which plays a critical role in muscle atrophy, is both necessary and sufficient to induce autophagy in skeletal muscle in vivo. The effect of FoxO3 on autophagy is mediated by the upregulation of BNIP3 that is induced by hypoxia in cultured cardiomyocytes. Notably, overexpression of BNIP3 also increases autophagy in hypoxic tumour cells. It has been shown that FoxO3 controls the two major systems of protein breakdown in skeletal muscle, the ubiquitin-proteasomal and autophagic/lysosomal pathways, independently. Thus, it seems reasonable to assume that this enables skeletal muscle to meet their unique metabolic demands [31].

A recent study revealed a novel transcriptional, mTORC1-independent, mechanism that regulates autophagy. This mechanism relies on the transcription factor EB (TFEB), which is a master regulator of lysosomal biogenesis. Data suggest that TFEB also controls crucial steps of the autophagic pathway during starvation. Under nutrient deprivation, TFEB translocates to the nucleus, where it activates a transcription program that coordinates lysosome formation and autophagy. TFEB nuclear translocation and activity is regulated by the mitogen-activated protein kinase ERK2. Thus, TFEB links autophagy to lysosome biogenesis [32].

Autophagy Plays Essential Roles in Metabolism and Longevity

Under normal physiological conditions, basal autophagy enables recycling of macromolecules and energy production by supplying amino acids, glucose and free fatty acids. This bulk form of degradation is particularly important under metabolic stress such as nutrient deprivation, growth factor depletion and hypoxia. Presumably, amino acids derived from autophagy can be essential for biosynthetic functions. In fact, several amino acids such as histidine, methionine and glutamine/glutamate fall below a critical value, thus becoming limiting for protein synthesis in starved ATG mutant yeast cells. Defective autophagy renders yeast cells unable to synthesize proteins essential for adaptation to starvation [33]. Recently, it has been shown that autophagy plays an important role in maintaining mitochondria function under starvation conditions in yeast. The inability of autophagy-defective mutants to upregulate components of the respiratory pathway and reactive oxygen species-scavenging enzymes is a major cause of cell death during nutrient starvation [34]. Autophagy is essential not only for maintaining TCA cycle metabolites in mitochondria but also for mitochondria quality control.

Consistent with a role in cellular metabolic homeostasis, autophagy also regulates lipid content, especially in the liver, contributing to constitutive breakdown of lipid droplets and triglyceride. Inhibition of autophagy increases lipid storage both in vitro and in vivo, and loss of autophagy decreases triglyceride breakdown in cultured rat hepatocytes [35]. However, knockdown of autophagy-related genes 5 (Atg5) or 7 (Atg7) in 3T3-L1 preadipocytes inhibits lipid accumulation and adipose differentiation. Adipocyte-specific *Atg7* mice are lean and have decreased white adipose mass and enhanced insulin sensitivity, indicating that autophagy regulates body lipid accumulation by controlling adipocyte differentiation and determining the balance between white and brown fat [36]. Further implicating starvation-induced autophagy in metabolic compensation, liver-specific autophagy has been reported to play an important role in the regulation of blood glucose by converting amino acids to glucose via gluconeogenesis. Consistent with this idea, liver-specific Atg7-deficient mice show a significant reduction in blood glucose levels during starvation, most probably due to their inability to release amino acids through autophagic proteolysis [37].

Considering the importance of autophagy as a dynamic recycling system contributing to cellular renovation and homeostasis, it is not surprising that autophagy has been assigned an anti-ageing function. Numerous studies suggest that autophagy declines with age in a variety of organisms. In *S. cerevisiae,* a microarray-based screen for genes involved in the regulation of chronological lifespan identified short-lived mutants defective for autophagy, indicating that autophagy is required for longevity. In addition, defects in autophagy prevented lifespan extension induced by restriction of amino acids in the growth media, consistent with a role for autophagy in starvation-induced longevity. Other examples come from studies in *C. elegans* showing that loss of

function mutations in the essential autophagy genes *unc-51*, *bec-1*, *atg-18* and *atg-7* shorten *C. elegans* lifespan by accelerating the rate at which the tissues age [6]. Moreover, RNAi knockdown of either *bec-1* or *vps-34* during adulthood significantly shortens the long lifespan of *eat-2* mutants experiencing chronic DR. Stimulation of autophagy by DR requires the PHA-4/forkhead box A transcription factor, which is also necessary for the lifespan extension elicited by DR [38]. Several recent studies have revealed a novel and intriguing link between lipophagy, a process through which autophagy hydrolyzes lipids, and ageing. Evidence suggests that autophagy and lipase LIPL-4-dependent lipolysis act interdependently to extend lifespan in germline-deficient worms. TOR appears to play an important role in longevity conferred through the link between autophagy and lipid metabolism [39]. *Drosophila* flies with compromised autophagy are also short-lived. Interestingly, recent findings indicate that inhibition of autophagy phenocopies *Drosophila* sestrin loss of function. Sestrins are highly conserved proteins that accumulate in cells exposed to stress and appear to act as negative feedback regulators of TOR function in *Drosophila*. Enhanced expression of *Drosophila* sestrin protects flies from age-associated pathologies including triglyceride accumulation, mitochondrial dysfunction, muscle degeneration and cardiac malfunction that may result from diminished autophagic clearance of damaged mitochondria, protein aggregates or lipids [40]. Mutations in the *Drosophila* Atg8a gene result in accumulation of insoluble ubiquitinated proteins, increased sensitivity to oxidative stress and reduced lifespan, supporting a key role for autophagy in determining healthspan and longevity. In contrast, enhanced Atg8a expression in older fly brains extends lifespan and promotes resistance to oxidative stress and the accumulation of ubiquitinated and oxidized proteins [6]. These findings indicate that autophagy plays a prominent role in determining healthspan and lifespan.

Numerous studies in model organisms suggest that signal transduction pathways that influence ageing and other longevity-promoting regimens, including caloric restriction and inhibition of TOR with rapamycin, resveratrol or the natural polyamine spermidine, often stimulate autophagy (fig. 1), and in some cases, inhibition of autophagy compromises their lifespan-extending effects [11, 41]. Loss of function mutations in the insulin/IGF-1 receptor homologue DAF-2 more than doubles the lifespan of *C. elegans,* and this effect requires the conserved transcription factor DAF-16/FoxO and autophagy. However, the fact that *daf-16/daf-2* double mutants have the same high level of autophagy as do *daf-2* single mutants, yet they are not long-lived, suggests that autophagy may be insufficient to extend lifespan or that autophagy may act downstream of DAF-16 [11]. Interestingly, it was recently reported that transgenic expression of sirtuin-1, a key regulator of metabolic homeostasis, induces autophagy in human cultured cells in vitro and in *C. elegans* in vivo. Moreover, sirtuin-1 is required for the autophagic response to nutrient deprivation in both human and nematode cells. Importantly, knockdown of *bec-1* suppresses the induction of autophagy by sirtuin-1 and abrogates the beneficial effects of DR on longevity in worms. Overall, these findings indicate that autophagy is universally required for lifespan extension by DR [42]. Similarly, recent published data suggest that autophagy mediates the lifespan-promoting effects of spermidine and resveratrol in yeast, nematodes and flies. These compounds ignite autophagy through distinct mechanisms converging on the acetylproteome [43]. Further implicating autophagy in longevity, mutations of the p53 orthologue CEP-1 extend the lifespan of *C. elegans* by increasing baseline autophagy [44].

Conclusion

The TOR signalling pathway integrates nutrient and anabolic signals to promote growth and block autophagy. Several recent studies support a key role for TOR in the modulation of lifespan and healthspan in diverse species ranging from yeast to mammals [18, 45]. Autophagy has also been implicated in ageing. Genetic or pharmacological manipulations that increase lifespan have been associated with autophagy, and in some cases they require autophagy for their longevity-promoting effects (fig. 1). However, the extent of the contribution of autophagy to lifespan extension is not known. Furthermore, how can autophagy prolong lifespan? One of the main causes of age-related accumulation of damage is the limited capacity of the cellular maintenance, repair and turnover pathways [46]. Increasing evidence suggests that autophagy declines with age [47]. A common feature of all ageing cells is a progressive accumulation of misfolded or aggregation-prone proteins and damaged organelles (e.g. defective mitochondria). Being a predominantly cytoprotective process, autophagy may thus function as a critical regulator of metabolic homeostasis at both the cellular and whole-organism level.

It is becoming increasingly apparent that autophagy regulation interfaces with TOR signalling, among others

(fig. 1). It is also known that in addition to inducing autophagy, reduced TOR activity downregulates mRNA translation under nutrient deprivation or environmental stress. Importantly, manipulations that lower the rate of mRNA translation increase the lifespan of different organisms [48]. Consequently, it will be important to investigate to what extent each of these two processes contributes to the lifespan extension associated with TOR inhibition. An important challenge for the future is to achieve a better understanding of the complex cross-talk between TOR and other signalling cascades known to be involved in senescent decline and ageing. This would facilitate the identification of specific targets for therapeutic interventions against age-related pathologies, thereby promoting healthspan.

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