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The role of autophagy in the regulation of yeast life span

Jessica K. Tyler[1] and **Jay E. Johnson**[2]

[1]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, New York

[2]Orentreich Foundation for the Advancement of Science, Cold Spring, New York

Abstract

The goal of the aging field is to develop novel therapeutic interventions that extend human health span and reduce the burden of age-related disease. While organismal aging is a complex, multifactorial process, a popular theory is that cellular aging is a significant contributor to the progressive decline inherent to all multicellular organisms. To explore the molecular determinants that drive cellular aging, as well as how to retard them, researchers have utilized the highly genetically tractable budding yeast *Saccharomyces cerevisiae.* Indeed, every intervention known to extend both cellular and organismal health span was identified in yeast, underlining the power of this approach. Importantly, a growing body of work has implicated the process of autophagy as playing a critical role in the delay of aging. This review summarizes recent reports that have identified a role for autophagy or autophagy factors in the extension of yeast life span. These studies demonstrate (1) that yeast remains an invaluable tool for the identification and characterization of conserved mechanisms that promote cellular longevity and are likely to be relevant to humans and (2) that the process of autophagy has been implicated in nearly all known longevity-promoting manipulations and thus represents an ideal target for interventions aimed at improving human health span.

Keywords

life span; aging; autophagy; budding yeast; *Saccharomyces cerevisiae*; health span

Introduction

Aging is a complex, multifactorial process that is driven by the progressive accumulation of several types of cellular damage. At critical levels, age-related damage leads to cellular dysfunction, senescence, and/or death. A popular model of aging in multicellular organisms posits that cellular aging is a key contributor to organismal senescence.[1] That is, functional impairment at the cellular level underlies observed defects in tissues and organ systems, thereby causing a multitude of pathologies and disorders in aged individuals. Thus, if this theory were true, then any effort to promote healthy aging in mammals would necessitate a

Address for correspondence: Jay E. Johnson, Ph.D., Senior Scientist, Department of Biology, Orentreich Foundation for the Advancement of Science, 855 Route 301, Cold Spring, NY 10516. jjohnson@orentreich.org.

Competing interests

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comprehensive understanding of the molecular mechanisms underlying aging at the cellular level. In recent years, such studies have been performed in a wide array of model organisms, from *in vivo* vertebrate and invertebrate animal studies to *in vitro* donor-derived cultured cell studies, as well as experiments in the highly genetically tractable budding yeast *Saccharomyces cerevisiae*. Strikingly, many of the mechanisms that contribute to cellular aging appear to be conserved from yeast to humans.[2,3] Consistent with this finding, nearly all dietary, genetic, or chemical interventions that are currently known to extend mammalian health span were first identified in yeast.[4] Each of these manipulations was found to extend the life span of yeast in at least one of the two most common yeast assays of cellular aging, which assess the chronological life span (CLS) or replicative life span (RLS) of yeast, respectively.

The CLS assay measures the period of time in which a non-dividing population of yeast can remain viable and able to re-enter the cell cycle.[5] This assay is thus intended to serve as a model of aging in quiescent eukaryotic cells. In contrast, the RLS assay assesses the number of divisions that each individual yeast cell can undergo[6,7] and is thus used to explore the pathways that inform the proliferative capacity of mitotic cells. While the types of cellular aging observed using these two assays often have different molecular and genetic determinants, they are not entirely independent. For example, cells that have undergone chronological aging have been demonstrated to have a reduced RLS once they re-enter the cell cycle.[8–10] Together with the observation that certain longevity-promoting interventions extend both CLS and RLS, it is therefore likely that certain types of age-related damage accumulate during both assays and are detrimental both to the cell's proliferative capacity and its ability to survive quiescence. Indeed, there is evidence to suggest that dysfunctional proteins present just such a type of damage, as defects in protein quality control contribute to decreased survival in both the CLS and RLS assays.[11] While the cell has multiple stress-responsive mechanisms to combat the proteotoxic damage associated with cellular aging, a major player in the maintenance of the proteome is the degradative process of autophagy.

Autophagy refers to a variety of mechanisms by which aged and/or damaged cytoplasmic material, including proteins, lipids, and membrane-bound organelles, is translocated to the vacuole and catabolized.[12,13] Both amino acids and fatty acids are acquired in this way and are used to synthesize new proteins and ATP, respectively. Collectively, the processes underlying autophagy are both highly complex and beyond the scope of this study, but they have been extensively reviewed elsewhere.[12,13] However, we will briefly overview the basics of autophagy here to allow readers to appreciate how autophagy and life span extension functionally intersect in yeast. There are two main modes of autophagy in yeast, differing on the basis of the mechanism by which intracellular cargo is transported to the vacuole: macroautophagy and microautophagy. Macroautophagy is a multistep process by which portions of the cytoplasm and/or organelles are sequestered in a double-membrane structure (autophagosome) and delivered to the lysosome for degradation. Although macroautophagy is generally a nonspecific process, there are instances in which organelles, such as mitochondria, are preferentially targeted for autophagic degradation. Microautophagy is similar to macroautophagy, but involves the direct transfer of cytoplasm into the vacuole via invaginations of the vacuolar membrane. In addition, the cytoplasm-to-vacuole targeting (Cvt) pathway is a constitutive biosynthetic process whereby two specific proteases are

delivered to the vacuole and may thus be considered a specialized form of autophagy. For the purpose of this review, the term “autophagy” will be used to describe both macroautophagy and most forms of selective autophagy.

The majority of proteins that mediate autophagy are encoded by two groups of genes, known as either autophagy–related (ATG) or vacuolar protein sorting (VPS) genes. A key control point in the initiation of all autophagy is the Atg1 complex, formed by association of the Atg1 protein kinase, the regulatory protein Atg13, and the scaffold protein Atg17 (which, in turn, is complexed with Atg31 and Atg29) (Fig. 1). In response to nutritional or endoplasmic reticulum (ER) stress, Atg1 binds to Atg13 and Atg17 to form a multiprotein complex (Fig. 2), which subsequently recruits multiple autophagy proteins to a site called the pre-autophagosomal structure (PAS). In addition to the induction of autophagy by Atg1, additional steps in the autophagic process include vesicle nucleation, vesicle expansion, vesicle completion, and fusion of the completed vesicle with the vacuole. Vesicle nucleation involves assembly of proteins at the PAS and is dependent on the class III phosphatidylinositol 3 kinase (PtdIns3K) Vps34, as well as Vps15, Vps30/Atg6, and Atg14. Expansion of the autophagosome requires conjugation of Atg12 to Atg5 and conjugation of Atg8 to phosphatidylethanolamine (Atg8-PE), during which both Atg5 and Atg12 are activated for conjugation by the E1-like enzyme Atg7. Upon autophagosome formation, Atg8-PE follows the autophagosome to the vacuole, where Atg4 cleaves the complex, releasing Atg8 to the lysosomal lumen for degradation. After fusion of the autophagosome to the vacuole, the former is broken down, and its cellular macromolecules are recycled. The Atg15 lipase is involved in degradation of the inner vesicle, while Atg22 transports amino acids and other small molecules back to the cytosol for protein synthesis and other cellular functions. In this review, we summarize recent studies that have identified novel roles for autophagy factors in the regulation of yeast CLS and RLS. As the activation of autophagy has been observed (or the pathway implicated) in the action of many longevity-promoting interventions, we anticipate that at least some of the aforementioned autophagy factors may present promising targets for therapeutic interventions that activate autophagy to improve health span in mammals.

Nutrient and energy sensors regulate CLS and RLS in part through effects on autophagy

The effects of autophagy on yeast life span were last reviewed by Sampaio-Marques *et al.*[11] While the current review focuses on more recent studies that have been published in the interim, a brief summary of the key points from the Sampaio-Marques *et al.* review is necessary to provide the requisite framework in which to place new findings. The primary focus of the Sampaio-Marques *et al.* review was that four overlapping nutrient- and energy-sensing pathways, mediated by (1) target of rapamycin (TOR)/S6 kinase homologue Sch9, (2) Ras/cAMP-dependent protein kinase A (PKA), (3) AMPK/Snf1, and (4) sirtuins, regulate both CLS and RLS and are known to affect autophagy. TOR is a central controller of cell growth that stimulates many effector pathways, including ribosome biogenesis and translation induction. TOR activity, and therefore growth, is inhibited by reduced levels of specific amino acids.[14,15] Similarly, genetic studies have indicated that the decrease in

carbon levels associated with calorie restriction (CR) extends yeast life span, at least in part through reduced TOR signaling.[16] Consistent with this finding, a preponderance of studies demonstrated that TOR inactivation significantly extends both CLS and RLS.[16–19] Despite this, the mechanistic basis for these extensions remains unclear. Toward an answer, a growing body of evidence suggests that the induction of autophagy may be involved. Reduced TOR signaling is well known to induce autophagy in yeast,[20] and the pathway is thus considered a central regulator of autophagy. In rich growth media, active Tor1 phosphorylates Atg13 and reduces its interaction with Atg1.[21] However, under starvation, when Tor1 is inactive, Atg13 phosphorylation is suppressed and the hypophosphorylated form of Atg13 both induces localization of Atg1, Atg17 and other essential autophagy factors to the PAS[22] and enhances the kinase activity of Atg1, which is essential for autophagosome formation (Figs. 1 and 2). Notably, both the induction of autophagy and associated life span extension conferred by TOR inhibition are mediated by the activation of the Msn2/4 stress resistance transcription factors.[17,23–25]

The Sch9 kinase is one of the main TOR effectors in yeast, although it can also function independently of TOR.[26,27] Deletion of *SCH9* results in a marked extension of both CLS and RLS.[16,17,19] While *SCH9* deletion causes only a minor increase in basal and starvation-induced autophagic flux,[28] it may affect other downstream aspects of autophagy. For example, Sch9 functions to disassemble the vacuolar proton pump (V-ATPase) upon glucose starvation, and thus its deletion helps maintain vacuolar acidification.[28] Maintenance of vacuolar acidification upon *SCH9* deletion and its accompanying RLS extension calls to mind the loss of acidification of the vacuole that occurs during aging.[29] The loss of vacuolar acidity observed during normal aging has been shown to contribute to aging via reduced storage of neutral amino acids in the vacuole.[29] As such, deletion of *SCH9* could extend RLS and CLS by helping to maintain the acidic pH of the vacuole in aged cells. Notably, autophagy has been implicated in vacuolar acidification, which in turn has been associated with life span extension, at least for CLS.[30] Whether this is also the case for RLS remains to be determined. In addition, we do not yet know whether the induction of autophagy caused by inactivation of the TOR pathway by CR, deletion of *TOR1* or *SCH9*, or drugs, such as rapamycin, is required for RLS extension. If this were the case, the next step would be to determine whether induction of autophagy is sufficient for yeast life span extension.

Similar to TOR/Sch9, inhibition or abrogation of Ras1, Ras2, or cAMP-PKA can have profound implications for life span, as assessed by both aging assays.[31–34] The Ras/cAMP-PKA pathway plays a central role in regulating growth in response to the quality and quantity of the available extracellular carbon source by stimulating mass accumulation and inhibiting the stress response.[35] When glucose and essential nutrients are abundant, active PKA contributes to the inhibition of autophagy via its phosphorylation of Atg1, which helps maintain the Atg1 kinase in an inactive state (Fig. 2). Once Atg1 phosphorylation is lost upon PKA inactivation, Atg1 associates with Atg13 and localizes to the PAS. Atg13 and Atg18 also have predicted PKA sites.[36] In response to glucose or nutrient limitation, as well as various deletions, inactivation of PKA extends both CLS and RLS[33] and induces autophagy, albeit less efficiently than inactivation of TOR.[35] Abrogation of Ras1 and Ras2 has been reported to have opposing effects on life span, depending upon the assay. For example, deletion of *RAS1* increases RLS but causes a slight decrease in CLS, whereas

deletion of *RAS2* extends CLS but compromises RLS.[31,32,34] In addition, there may be cross talk between TOR- and PKA-dependent effects on autophagy and life span regulation, as simultaneous inactivation of Sch9 and PKA leads to greater stimulation of autophagy, suggesting that these factors function in parallel pathways to cooperatively regulate autophagy.[27] Additionally, similar to the case for TOR, suppression of autophagy by PKA depends on its ability to suppress translocation of the Msn2/4 transcription factors and the Rim15 kinase to the nucleus, where they mediate transcription of stress-response genes.[17,25,37] Yet further support for a common mechanism of life span extension by TOR and PKA inhibition is the observation that these pathways both exert their inhibitory effects on autophagy through phosphorylation of autophagy factors, such as Atg1 and Atg13 and potentially Atg18.[21,36,38] Finally, it is possible that autophagy might, in turn, modulate PKA activity via feedback regulation, as recent work found that various autophagy factors (Pho80, Atg10, Atg14, Atg15, and Atg16) were themselves putative negative regulators of PKA.[39,40]

Snf1 (sucrose non-fermentable protein kinase 1) is a metabolic sensor and the yeast orthologue of mammalian AMPK, is regulated by PKA and Tor1, and targets Sch9.[41–43] Intriguingly, the presence of Snf1 is deleterious to RLS, whereas this factor is necessary for maintaining cellular fitness during chronological aging, as its genetic deletion shortens CLS.[44–46] Furthermore, it has been suggested that *SNF1* deletion results in a reduced CLS owing to the role of Snf1 in promoting autophagy.[47]

Sirtuins are a family of protein deacetylases that have important roles in a number of biological processes, and are considered to be master regulators of cellular aging. Deletion of *SIR2* decreases yeast RLS, whereas its overexpression significantly extends it.[48] In addition, it is possible that Sir2 activation might partially underlie the extension of RLS by TOR inhibition. As discussed above, the Msn2/4 transcription factors are required for the extended RLS that results from decreased TOR signaling.[25] Upon TOR inhibition, Msn2/4 increases the expression of the nicotinamidase gene *PCN1*, which in turn stimulates the activity of Sir2 and other sirtuins.[25] The beneficial effect of increased Sir2 activity on yeast RLS upon TOR inhibition may also be mediated via the autophagy pathway, because overexpression of Sir2 counterparts in worms upregulates autophagy.[49] However, future experiments will be required to determine if autophagy is indeed required for the extension of RLS by *SIR2* activation, particularly as it remains unclear how relevant the mechanism by which Sir2 extends yeast RLS is to the longevity-promoting benefits of mammalian sirtuins.[50]

The role of Sir2 in the regulation of CLS is even more complex. Deletion of *SIR2* does not adversely affect the survival of wild-type cells in the CLS assay but does compromise the extension of CLS observed in *sch9Δ* cells, as well as cells treated with resveratrol, a polyphenolic, life span–extending CR mimetic.[51,52] As a result, Sir2 is clearly important for maintaining the fitness of cells undergoing extension of chronological life span. Consistent with this idea, Sampaio-Marques *et al.* demonstrated that, during chronological aging, Sir2 activates both macroautophagy and mitophagy, the latter being a selective form of autophagy that degrades mitochondria.[53] Specifically, Sir2 is required for the transcriptional upregulation of both *ATG8* and *ATG32,* a gene encoding a factor essential for mitophagy.[54] Thus, Sir2 appears to support the extension of CLS produced by other autophagy-promoting

interventions (i.e., *SCH9* deletion and resveratrol treatment) by maintaining high levels of both a core autophagy factor and a mitophagy-specific protein, respectively.

Autophagy is required for maximal CLS and has been implicated in the extension of CLS by multiple life span–promoting interventions

While the studies described above strongly suggested a role for autophagy in the regulation of yeast life span, a complementary body of work sought to ascertain whether an intact autophagy system was required for the CLS observed for wild-type cells, as well as the extension of CLS promoted by various dietary interventions. Toward this goal, multiple groups assessed the life spans of mutant cells lacking various autophagy genes under different conditions, and observed that deletion of *ATG1*, *ATG2*, *ATG7*, *ATG8*, *ATG16*, or *VPS21* shortened the CLS of otherwise wild-type cells.[5,46,55,56] Interestingly, Matecic *et al.* also demonstrated that the extension of CLS produced by incubation in an amino acid–limited media formulation required Vps30, a member of the PtdIns3K complex I that is involved in autophagosome nucleation.[46] Consistent with the above observations, treatment with resveratrol, as well as administration of the natural polyamine spermidine, robustly extended both CLS and RLS, and both interventions have been shown to activate autophagy.[57,58] Furthermore, in the case of CLS, autophagy is required for the life span extension induced by spermidine.

Autophagy plays a role in the extension of CLS by methionine restriction

Methionine restriction (MR) is a dietary intervention that has been demonstrated to extend the life spans of a variety of multicellular model organisms, from nematodes to rodents.[59–62] Furthermore, this intervention is known to extend cellular life span. Specifically, there are benefits of MR to yeast CLS, as well as to the replicative capacity of cultured mammalian fibroblasts.[30,63–66] Exploration of the genetic requirements for CLS extension by MR revealed several genes required for the benefits of this intervention, including the retrograde stress response factor gene *RTG3*, the amino acid sensor gene *GCN2*, and the autophagy-related genes *ATG5*, *ATG7*, and *ATG8*.[30,63,66] A requirement for an intact *RTG3* gene is interesting, as the retrograde stress response was previously implicated in the control of RLS.[67] In addition, a recent study found that methionine levels can modulate the activity of PKA, which, as described above, regulates both autophagy and CLS.[39] Together, these studies demonstrate that retrograde stress signaling, amino acid and nutrient sensing, and autophagy are crucial for the extension of yeast CLS by MR. Work by Ruckenstuhl *et al.* suggested that CLS extension by MR is likely mediated, at least partially, by vacuolar acidification that occurs as a result of autophagy, although it remains unclear exactly how vacuolar acidification promotes extended life span.[30]

To further investigate the mechanistic basis of CLS extension by MR, we previously subjected methionine-restricted yeast to gene expression profiling and found that this intervention resulted in the altered expression of 1625 gene probes, while 313 of these changes (19%) were dependent on an intact retrograde response.[63] Of those factors found to be differentially expressed in response to MR, several (particularly those that were upregulated) had roles in metabolism, stress response, and protein quality control. In fact,

there was a preponderance of autophagy genes among the differentially-expressed factors (*IML1*, *VPS33*, *ATG2*, *ATG7*, *ATG9*, *ATG17*, *ATG22*, *ATG27*, *ATG29*, *ATG31*, *ATG33*, *ATG34*, *ATG14*, *VPS15*, *VPS30*, and *VPS34*). Notably, the last of these represent all four subunits of the PtdIns3K complex I (PtdIns3K-I), which is responsible for localizing autophagy proteins to the PAS and promoting the autophagic process.[68–70] As all four PtdIns3K-I members were found to be transcriptionally upregulated in methionine-restricted yeast, it is an intriguing possibility that this intervention activates autophagy by upregulating the activity of PtdIns3K-I. We also obtained data implicating tRNA metabolism in the regulation of autophagy and life span through effects on the retrograde stress response. Specifically, we found that both methionine restriction and a genetic intervention that results in hypomethylation of tRNAs (deletion of *NCL1*, encoding a tRNA methyltransferase) resulted in an increased abundance of multiple tRNA species.[63] In addition, the extension of CLS produced by these manipulations required the retrograde stress response, as the extended life spans of methionine-restricted and *ncl1Δ* cells were completely dependent on the transcription factor Rtg3 and partially dependent on Rtg2 (J.E.J., unpublished data), a protein required for the canonical form of retrograde signaling.[71,72] We hypothesized that an increased abundance of tRNAs might activate retrograde signaling to upregulate various stress responses, including autophagy, thereby promoting improved survival during chronological aging. However, another possibility is that elevated levels of tRNAs might extend life span cooperatively through activation of both retrograde signaling and the integrated stress response.[73] In either case, a putative role for tRNAs in yeast life span regulation is notable given a recent report describing how abrogation of MAFR-1 (a repressor of RNA polymerase III) in *Caenorhabditis elegans* alleviates the inhibition of tRNA synthesis that normally accompanies CR, thereby upregulating various stress responses, activating autophagy, and extending life span.[74] These findings thus provide a tantalizing suggestion that tRNA-related mechanisms that regulate longevity in response to dietary interventions may be conserved from yeast to more complex organisms.

Epigenetic regulation of CLS and RLS through effects on autophagy

As described above, the nutritional status of yeast can have profound implications for the activation of autophagy and cellular longevity. Eisenberg *et al.* explored the role of acetyl-coenzyme A (AcCoA) production on the regulation of CLS.[75] They found that accumulation of AcCoA repressed the transcription of a number of autophagy genes (*ATG7*, *ATG11*, and *ATG14*) and thus negatively regulated autophagic flux during aging. Through a series of elegant experiments, the authors obtained data suggesting that accumulation of AcCoA results in hyperacetylation of histones, which in turn reduces expression of the aforementioned autophagy genes, inhibiting autophagy, with deleterious consequences for chronologically aging cells. Consistent with their proposed mechanism, the authors also observed that several histone gene point mutations rendered cells unresponsive to AcCoA levels (with respect to autophagy gene repression) and constitutively activated autophagy during aging. To explore the implications of these findings in a multicellular organism, Eisenberg *et al.* generated a brain-specific gene knockout of the *Drosophila melanogaster* AcCoA synthetase and found that the resulting flies were long-lived. As AcCoA production is affected by a variety of nutritional signals, the authors argue that the above epigenetic

mechanism might, in fact, underlie the autophagy-dependent life span extension observed for a number of longevity-promoting dietary interventions.

In a contrasting example of histone acetylation-mediated regulation of autophagy and life span, Agustin Aranda's group demonstrated that the SAGA complex positively regulates autophagy, with benefits to CLS.[76,77] The yeast SAGA complex is a multi-subunit complex that mediates nucleosomal histone acetyltransferase (HAT) activity and includes, among many other factors, Gcn5, Spt20, and Ubp8. Aranda's group demonstrated that both Gcn5 and Spt20 are required for the efficient activation of autophagy, and that deletion of their encoding genes negatively affected CLS.[76,77] Additionally, they demonstrated a similar but much less robust requirement for Ubp8. Interestingly, genetic deletion of this factor, which provides the deubiquitinase activity of the complex, actually extends RLS.[3] While additional work will be necessary to determine exactly how the HAT activity of the SAGA complex regulates autophagy and life span, it is clear from the above studies that AcCoA levels and histone acetylation can have profound effects on the activation of autophagy and, consequently, cellular longevity. This is further supported by a 2014 study by Orlandi *et al.* that demonstrated that impairment of the yeast mitochondrial pyruvate carrier (Mpc1) reduced autophagy and shortened CLS.[78] In *mpc1Δ* mutant cells, decreased pyruvate import into mitochondria resulted in depletion of TCA cycle intermediates, with a concomitant reduction in the generation of nucleocytosolic AcCoA. Reduced AcCoA levels were associated with decreased autophagic flux, possibly through a decrease in SAGA-mediated histone acetylation, as described above.[76,77] Interestingly, the authors found that supplying carnitine to *mpc1Δ* cells was sufficient to bolster the levels of TCA cycle intermediates, increase AcCoA production, and rescue the short-lived phenotype of *mpc1Δ* cells (presumably via the restoration of normal autophagic activity).

There are also data that implicate the epigenetic regulation of autophagy as playing a role in the determination of yeast RLS. In an impressively exhaustive study, Yi *et al.* identified Esa1 and Rpd3 as an antagonistic acetyltransferase–deacetylase pair that regulates autophagy through posttranslational modification of Atg3.[79] Specifically, in response to starvation, Esa1 acetylates K19–K48 of Atg3, promoting interaction of Atg3 with Atg8, lipidation of the latter factor, and autophagosome expansion. The authors also found that Rpd3 is responsible for the inactivation of Atg3 by hypoacetylation at K19–K48, as deletion of *RPD3* resulted in increased acetylation at these residues, as well as the activation of autophagy. Strikingly, Esa1 and Rpd3 also control the acetylation status of Sip2, an AMPK regulatory subunit.[42] Acetylation of Sip2 by Esa1 inactivates AMPK, whereas deacetylation of Sip2 by Rpd3 promotes the activity of the holoenzyme. Thus, the action of Esa1 on both Atg3 and Sip2 would be predicted to robustly activate autophagy, whereas the Rpd3-dependent deacetylation of Atg3 and Sip2 should, conversely, result in the potent inhibition of autophagy. Consistent with such predictions, as well as the benefit of autophagy to replicative aging, impairment of Esa1 has been observed to reduce RLS,[42] whereas deletion of *RPD3* extends RLS.[80] Taken together, the above studies clearly demonstrate a bona fide role for epigenetics in the regulation of autophagy and, by proxy, both types of yeast life span. It will be interesting to confirm whether, as suggested by the Eisenberg *et al.* results in *Drosophila*, such epigenetic regulatory mechanisms are indeed conserved in higher organisms.

Lipid homeostasis, autophagy, and CLS

Recently, Garay *et al.* performed a genome-wide screen to identify novel factors involved in the regulation of CLS.[81] One of the factors identified as important for maximal CLS was Arv1, a protein involved in sterol and sphingolipid metabolism.[82,83] Deletion of *ARV1* negatively affected autophagy and reduced CLS. Furthermore, genetic epistasis experiments comparing the life spans of various autophagy mutants, *arv1Δ* cells, and combinations thereof, demonstrated that the benefits of Arv1 to CLS depended on an intact autophagic system. A complementary study was published the following year, demonstrating that supplementation of cultures with a lipid precursor of phosphotidylethanolamine (PE) or genetic interventions that result in increased PE production are capable of activating autophagy and extending CLS.[84] However, in contrast to the Arv1 observation described above, there is a clear connection (literally and figuratively) between PE and autophagy, as PE is covalently attached to Atg8 through a series of ubiquitination-like reactions that ultimately lead to autophagosome expansion. Consistent with these observations, deletion of any yeast phosphotidylserine decarboxylase (which participates in PE synthesis) resulted in accelerated chronological aging. Strikingly, the authors demonstrated that administration of the PE precursor ethanolamine also conferred longevity-enhancing benefits to multicellular organisms, as this intervention extended the life spans of both cultured mammalian cells and flies. Yet another study, aimed at exploring the role of Isc1 in yeast, found that *isc1Δ* cells exhibited mitochondrial dysfunction, increased mitophagy, decreased stress tolerance, and decreased CLS.[85] Isc1 is an inositol phosphosphingolipid phospholipase that hydrolyzes sphingolipids to produce the waxy lipid molecule ceramide. The authors found that Isc1-mediated ceramide production directly affected mitochondrial dynamics, with obvious consequences for cellular life span. Thus, in total, the above studies demonstrate that lipid metabolism can engage the autophagy machinery to positively regulate longevity in a variety of model organisms, from yeast to mammals.

Delayed chronological aging in yeast models of Parkinson's disease through effects on autophagy

α-Synuclein is one of the major components of the insoluble protein aggregates, known as Lewy bodies, that accumulate in the brains of patients with neurodegenerative disorders like Parkinson's disease.[86] To model Lewy body production in yeast, constructs were generated allowing for the ectopic expression of α-synuclein, which is toxic to yeast and dramatically shortens CLS.[87] In a recent study, Guedes *et al.* explored whether the improved protein quality control associated with CR might rescue the cytotoxicity of α-synuclein expression in aging yeast.[88] They found that α-synuclein toxicity was alleviated by CR, restoring normal chronological longevity. In addition, TOR inhibition (via deletion of *TOR1*) similarly rescued the shortened CLS of α-synuclein–expressing cells. Somewhat surprisingly, however, each intervention was associated with a decrease in autophagic flux. Indeed, the level of autophagy is nearly 500% higher than normal in cells expressing α-synuclein, as assessed by the alkaline phosphatase assay. Both the authors and others previously demonstrated that the cytotoxicity of α-synuclein expression is associated with an aberrantly high activation of autophagy.[53,89–91] The authors argue that both CR and TOR

inhibition rescue the lethality and reduced CLS of alpha-synuclein–expressing cells by reducing autophagy to normal levels. This study thus suggests that it is important to maintain autophagy within an ideal homeostatic window, as too much activity (at least under certain conditions) can also have disastrous consequences for cellular survival.

In a related study exploring the consequences of ectopic expression of the Parkinson's disease-related protein Parkin in budding yeast, it was noted that Parkin extends CLS and confers increased oxidative stress resistance to cells, dependent on the presence of autophagy factors Atg1, Atg5, and Atg11.[92] Parkin was also observed to translocate to mitochondria, and its expression resulted in increased mitochondrial turnover. The mechanisms underlying the benefits associated with Parkin expression remain unclear, yet one possible explanation is that they are engendered by a stress hormetic effect. That is, Parkin might cause mitochondrial stress, which in turn would activate a generalized stress response that renders cells both long-lived and more tolerant to oxidative damage. Future studies will ultimately determine whether or not the proposed explanation is, in fact, the mechanism by which Parkin expression extends CLS.

Hormetic stress extends RLS and activates autophagy

Autophagy does not appear to contribute to RLS under normal growth conditions. This is suggested by the fact that deletion of most *ATG* genes has minimal effects on RLS, and in some cases their deletion even extends yeast RLS, such as deletions of *ATG1, ATG8*, and *ATG10*.[3,93] However, many of the interventions that have been discovered to extend RLS induce low levels of cellular stress that would otherwise be lethal at higher levels. These so-called hormetic stresses also often induce autophagy. We propose that these stress-associated RLS-extending regimens extend life span via autophagy induction and discuss the data supporting this proposal below. Notably, many of the genetic, therapeutic, or environmental interventions that extend yeast RLS constitute either a decrease of nutrients per se or they genetically or chemically decrease the activity of the nutrient signaling pathways. As discussed above, two of the major nutrient signaling pathways are proaging during yeast RLS: the target of rapamycin (TOR)/S6 kinase homologue Sch9 pathway[16] and the Ras/cAMP–PKA pathway.[33] In general, when nutrients are abundant, these nutrient-sensing pathways are active and inhibit autophagy (Figs. 1 and 2). However, when nutrients are scarce, the nutrient-sensing pathways are inactivated, their inhibitory influence on autophagy is released, and RLS is extended. Also, other interventions, such as the induction of ER stress, reduced mitochondrial function or mRNA translation, and spermidine administration, do not directly impinge on the above nutrient-sensing pathways, but extend RLS and often require autophagy for life span extension, as discussed in detail below. The integrated stress response, mediated by activation of the Gcn2 kinase and its effector, the Gcn4 transcriptional activator of numerous stress response pathway genes, is also induced by hormetic stress (Fig. 2). One output of the integrated stress response is autophagy induction. The role of the integrated stress response in RLS extension has been reviewed elsewhere recently[73] and will not be discussed further here.

ER–Golgi apparatus–mediated RLS extension requires autophagy

Disruptions of the ER–Golgi apparatus can promote the RLS of yeast. Specifically, gene deletions that compromise ER–Golgi function and induce ER stress were shown to induce autophagy and extend RLS.[93] Indeed, this is the strongest evidence to date for a role for autophagy in the extension of yeast RLS. One of the genes that promote RLS extension when deleted, *RER1*, encodes a receptor that maintains ER compartmentalization. Deletion of *RER1* resulted in ER stress and activated both the unfolded protein response (UPR) and autophagy. The life span extension associated with *RER1* deletion requires autophagy, as deletion of *ATG5* or *ATG8* (which are required for autophagosome expansion; Fig. 1) abrogated the extended RLS of *rer1Δ* cells. The authors also reported that deletion of *MNT3* (which encodes a Golgi glycosylase) similarly led to activation of the UPR, elevated autophagy, and extended RLS. Thus, the observed induction of autophagy and the concomitant increase in RLS in yeast lacking either *RER1* or *MNT3* appears to be a generalized adaptive response elicited by impaired protein modification or trafficking across the ER–Golgi network.

A role for autophagy in the extension of RLS by CR?

CR is a popular RLS extension regimen in which a role for autophagy is not yet apparent. An analysis to identify *ATG* gene deletions that prevent RLS extension resulting from CR (growth in 0.5% glucose) found that only Atg15, which promotes the disintegration of autophagic vesicles within vacuoles, was required.[94] The authors of this study also found a role for proteins involved in vacuole-to-vacuole fusion in CR-dependent RLS extension. While they did not find a role for most of the *ATG* genes in CR-dependent RLS extension, it may be noteworthy that the replicative life spans of their 2% glucose controls were unusually short.[94] This phenomenon of shortened life span of the control strain has been reported for other studies that report CR-mediated RLS extension, suggesting that RLS extension by CR might not be as robust as once believed.[95]

Summary and remaining questions

The yeast CLS and RLS cellular aging assays have been instrumental in supporting the identification of dietary, genetic, and chemical interventions that delay both cellular aging and the aging of multicellular organisms. While there are some differences with respect to the effects that these manipulations have on either CLS or RLS, many of them provide benefits to both aging paradigms. Most likely, the observed commonalities (as well as differences) are due to the specific nuances of each assay, as well as the types of age-related damage that may accumulate during each type of aging. For example, when yeast are chronologically aged in media containing a fermentable carbon source, acetic acid is produced as a by-product of glycolysis, a cell-extrinsic cytotoxic intermediate that kills yeast over time and at high concentrations.[96] However, acetic acid toxicity has not been demonstrated to play a role in limiting RLS. Therefore, those interventions that either reduce the production of the nutritional acetic acid stress or render cells more tolerant to it would be predicted to extend CLS. Indeed, this has been found to be the case.[96] However, it would not be expected that manipulations that specifically affect acetic acid accumulation (or the

tolerance of cells to its effects) would affect RLS. Conversely, the production of extrachromosomal rDNA circles (ERCs) is associated with the loss of viability during the replicative aging of yeast[97] but has not been implicated in the regulation of CLS. Thus, any manipulations that specifically affect the production of ERCs would not necessarily be expected to affect CLS. Nevertheless, those interventions that affect sources of age-related damage that are held in common between these assays or that activate generalized stress responses with far-reaching effects, such as the integrated stress response and autophagy, might be expected to extend both CLS and RLS. To date, the best examples of such manipulations are alterations of the major nutrient- and energy-sensing pathways (1) TOR/Sch9, (2) Ras/cAMP–PKA, (3) AMPK/Snf1, and (4) sirtuins. In these cases, it is likely that altered signaling through these pathways leads to a slowing of aging via increased stress resistance and the upregulation of proteostatic mechanisms like autophagy.

Given the overwhelming amount of evidence suggesting that autophagy contributes to the extension of yeast life span conferred by a variety of interventions, an obvious question is whether autophagy activation is sufficient to extend yeast life span. Such a possibility is supported by a study in mice where overexpression of Atg5 increased autophagic flux and extended life span.[98] A similar study found that the administration to mice of a small autophagy-inducing peptide derived from the mammalian Vps30 homologue Beclin1 resulted in the stimulation of immune function, the decline of which over time represents one of the hallmarks of aging.[99] To date, however, no such studies have been described for yeast. A further complication is that many selective types of autophagy have been described, including but not limited to mitophagy,[100] lipophagy,[101] nucleophagy,[102] ER-phagy,[102] and pexophagy,[103] the activation of any one of which has the potential to extend yeast life span, although this has not yet been investigated. Mitophagy, in particular, might be beneficial for longevity because it degrades damaged mitochondria that are prone to release proapoptotic factors and reactive oxygen species, while lipophagy recycles lipid droplets and lipid storage organelles to allow the use of fat as an alternative energy source. Yeast replicative aging is accompanied by the degradation of specific mitochondrial proteins via a novel autophagy-dependent protein degradation pathway,[104] but whether this influences longevity is currently unknown. A better understanding of the aging process would be gained by knowledge of which selective autophagy pathways, if any, need to be activated to extend yeast life span.

Going forward, yeast remains an ideal model organism for aging studies for a number of reasons. Principal among them is the facility with which yeast can be genetically manipulated, as well as the fact that its genome has been well characterized. These features, combined with ease of growth and a short doubling time, conspire to make yeast highly amenable to a variety of high-throughput screening procedures. The use of yeast as an experimental system also allows for incomparably rapid life span experiments, as the RLS of yeast is measured on a day time scale (median RLS of wild-type yeast ≈ 20 divisions or ~ 3 days), and CLS, while longer, is still measured only on a week time scale (median CLS of wild-type yeast ≈ 2 weeks). Furthermore, budding yeast is the only eukaryote for which single-cell RLS can be accurately determined, and the recent development of microfluidic devices makes high-throughput RLS determination possible.[105] Perhaps most importantly, since many of the pathways regulating longevity are conserved from yeast to more complex eukaryotes, including mammals, novel pharmaceutical or nutritional regulators of these

processes that are identified in yeast will likely be translatable to the ultimate goal of delaying aging and improving the health span of humans.

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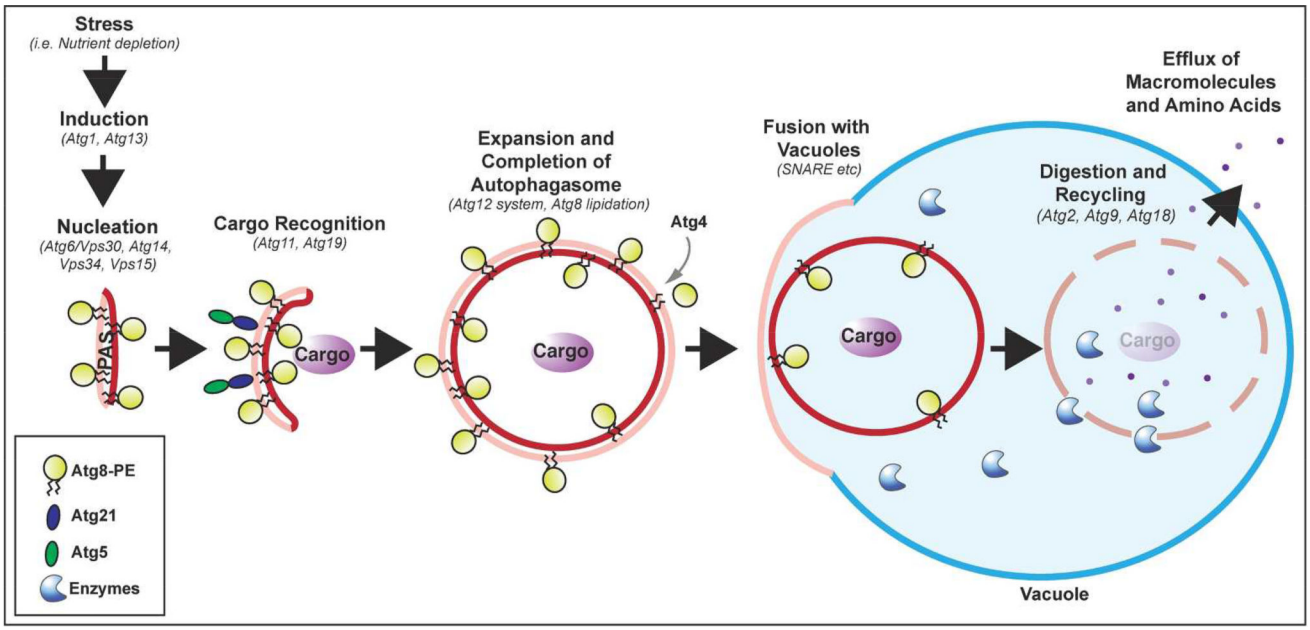

Figure 1.

Schematic of the key stages in the macroautophagy process with some of the gene products involved indicated, as described in the text.

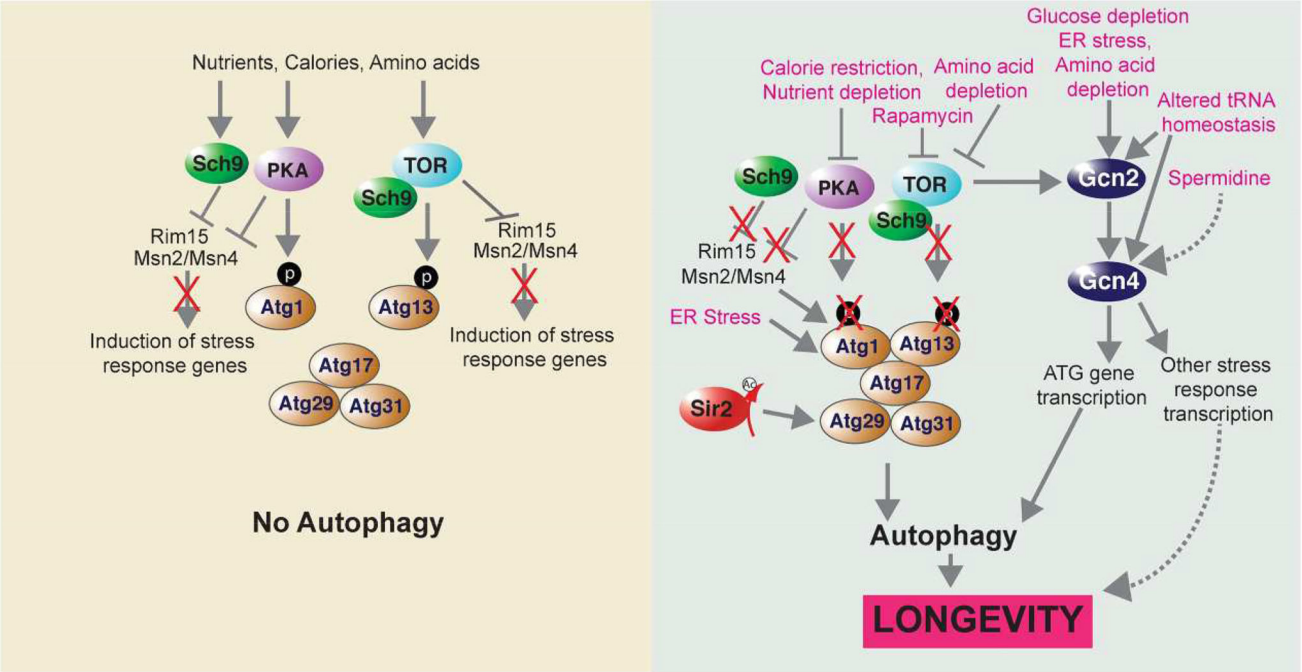

Figure 2.

Schematic of how life span expansion interventions activate autophagy. On the right side, ample nutrients, nitrogen, and calories stimulate the nutrient-sensing kinases to inhibit autophagy at multiple steps, as discussed in the text. Various regimens, indicated in pink, extend yeast life span by inhibiting the nutrient-sensing pathways and/or via stimulating the integrated stress response through Gcn2/Gcn4. Dotted lines indicate likely functional interactions that have not yet been conclusively demonstrated.