CHAPTER

2

The Role of Autophagy in Aging: Molecular Mechanisms

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OUTLINE

Abstract

Autophagy is a conserved, subtly regulated process responsible for the lysosomal degradation of cytoplasmic components, such as long-lived proteins and unnecessary or dysfunctional organelles. It has a well-established housekeeping role under physiological conditions, and a cytoprotective and adaptive role under stress. The aging process is associated with marked decrease of autophagic activity and deregulated autophagy often correlates with accelerated aging and age-related pathologies. Conversely, genetic or pharmacological interventions that extend lifespan, such as reduced insulin/insulin-like growth factor-1 (IGF-1) signaling, target of rapamycin inhibition, deacetylation of histones and nonhistone cytoplasmic proteins, caloric restriction (CR), rapamycin, resveratrol, or spermidine supplementation, have been associated with autophagy and in some cases their beneficial effects on health and longevity require autophagy. Interestingly, the recently gained knowledge about autophagy regulation has revealed that several transcription factors required for lifespan extension have an

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important role in the upregulation of autophagy-related genes. Moreover, acetylation of histones and autophagy proteins has emerged as an essential regulatory mechanism of both autophagy and longevity, at least in yeast and mammals. Further studies are needed to clarify the antiaging role of autophagy and the conditions under which autophagy upregulation can sufficiently improve health and longevity. However, the existence of a large arsenal of drugs that induce autophagy, sometimes by mimicking the effects of long-term CR or short-term starvation, is a valuable tool for the development of future antiaging treatments.

INTRODUCTION

Autophagy (from the Greek word *αυτό*, meaning "self," and *φαγία*, meaning "to eat") is a cellular regulated process through which cells dismantle unnecessary or dysfunctional cytoplasmic components, such as misfolded proteins and damaged organelles. This phenomenon was at first observed by Keith R. Porter and Thomas Ashford at the Rockefeller Institute in 1962 (Ashford and Porter, 1962), when they found that glucagon addition in rat liver cells triggered an increase in the number of lysosomes, some of which contained other organelles, such as mitochondria. However, the term "autophagy" was introduced by Christian de Duve, the discoverer of lysosomes, in 1963, in London. It was then, when he distinguished the heterophagic and autophagic functions of lysosomes and suggested the name "autophagic vacuoles". Subsequently, he established lysosomes as the sites of intracellular autophagy (Deter et al., 1967). Despite the impressive findings of 1970s, it took more than 20 years for the next boost in the field of autophagy. Research in yeast revealed the first autophagy defective mutant, *apg*1, which failed to complete starvation-induced autophagy (Tsukada and Ohsumi, 1993). This finding opened the route for the discovery of more autophagy deficient mutants (Harding et al., 1995), which were later defined as defective in *ATG* genes (autophagy-related genes). Characterization of such mutations affecting proteins involved in the autophagic machinery led to the understanding of the autophagy process.

There are three different types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy (hereafter, referred to as autophagy) has a major role in damaged cell organelles and proteins clearance, as also in intracellular pathogens degradation (Klionsky, 2005). It involves the formation of the phagophore or isolation membrane (vehicle nucleation), its expansion (vehicle elongation) to engulf the substrate marked for destruction and fusion of its edges resulting in the formation of a double-membraned structure, the autophagosome (vehicle completion). Then, the outer membrane of the autophagosome fuses with a lysosome to form an autolysosome (docking and fusion steps), in which the enclosed material including the inner autophagosomal membrane are degraded by resident acidic hydrolases (vehicle breakdown and degradation steps). The resulting breakdown products are released back into the cytosol through the activity of specific membrane permeases for reuse (Fig. 2.1) (Klionsky, 2005; Ravikumar et al., 2010). On the other hand, microautophagy involves the direct invagination of substrates by lysosomes and it is essential for survival under starvation. Chaperone-mediated autophagy is highly selective for soluble cytosolic proteins, which are selected through a chaperone-dependent mechanism, targeted to lysosomes and finally degraded, in a process that vesicles formation is not required.

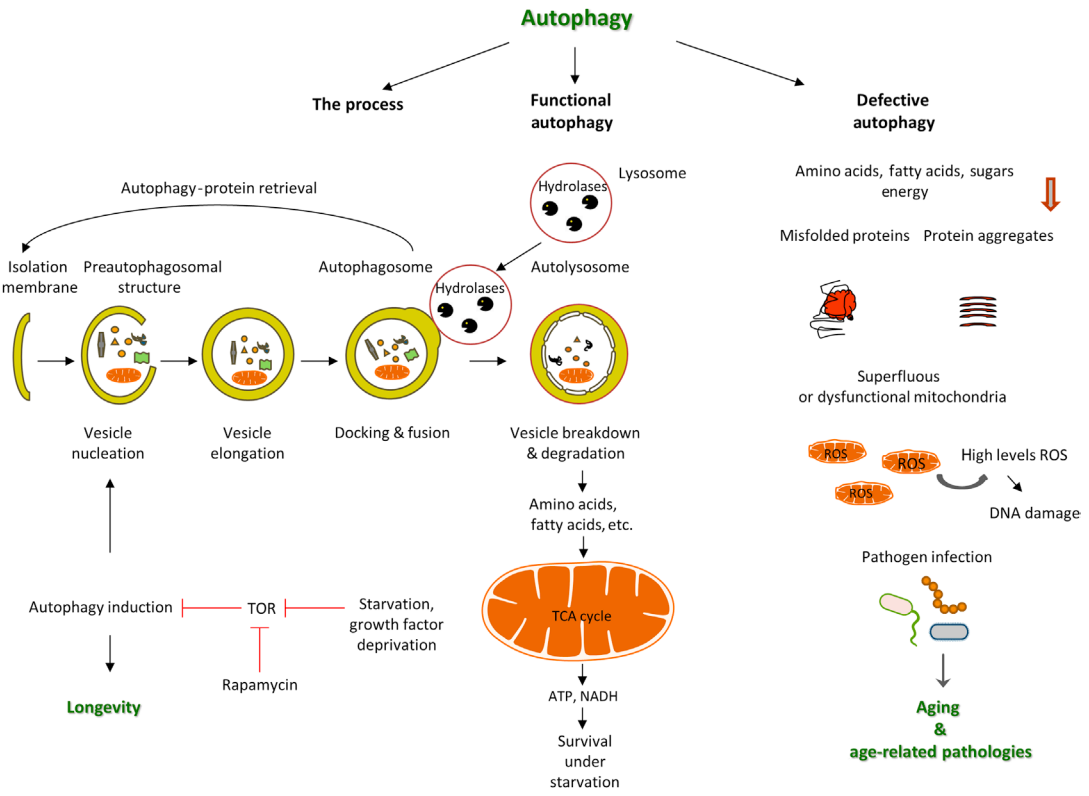

FIGURE 2.1 Autophagy begins with the formation of the isolation membrane. Subsequent phases involve the expansion of the isolation membrane (vehicle elongation) to engulf cytoplasmic constituents and organelles and fusion of its edges, resulting in the formation of a double-membraned structure, the autophagosome (vehicle completion). The outer membrane of the autophagosome fuses with a lysosome to form an autolysosome (docking and fusion steps), in which the sequestered material including the inner autophagosomal membrane are degraded by resident acidic hydrolases (vehicle breakdown and degradation steps). The resulting breakdown products are released back into the cytosol through permeases and are recycled. Free amino acids and fatty acids are used as building blocks for new protein synthesis and ATP production, promoting homeostasis and survival at both the cellular and whole organism level. Starvation or growth factor deprivation activates autophagy through TOR inhibition, promoting longevity. In contrast, autophagy defects lead to reduced energy and building blocks supply for biosynthetic pathways, loss of proteostasis, accumulation of dysfunctional organelles, including mitochondria and increased susceptibility to infection. Thus, age-related decline of autophagic activity affects cell function and tissue homeostasis, contributing to organismal aging and age-associated diseases. *NADH*, reduced form of nicotinamide adenine dinucleotide; *ROS*, reactive oxygen species; *TCA*, tricarboxylic acid; *TOR*, target of rapamycin.

Animals are constantly subjected to environmental and endogenous stressors. In nature, organisms often undergo pressure from lack of food and shortage of the necessary nutrients that endanger their existence. Under such unfavorable conditions, autophagy assures survival, through recycling intracellular materials and thus providing energy and nutrients. A typical example exemplifying the importance of autophagy under starvation conditions is that 48 h of starvation are enough to trigger degradation of 30–40% of liver proteins

(Pfeifer and Strauss, 1981). A growing body of evidence has revealed a clear connection of autophagy with aging and a wide range of age-related human pathologies (Cuervo, 2008). Here, we discuss the link between autophagy and senescent decline, highlighting recent discoveries that shed light on the molecular mechanisms underlying the effects of autophagy on aging and the pathogenesis of major human diseases.

CYTOPROTECTIVE AND HOMEOSTATIC ROLE OF AUTOPHAGY

Exposure to oxidative stress and xenobiotics is another major and continuous threat for cellular functionality and survival. Autophagy serves as a major homeostatic mechanism that counteracts intracellular accumulation of deleterious chemicals and harmful or unnecessary molecules, mainly protein aggregates. These can damage cellular homeostasis threatening cellular functionality. Therefore autophagy has a major cytoprotective role in cells, contributing to the maintenance of organelle homeostasis and the avoidance of proteotoxicity (Rubinsztein et al., 2011).

Several examples from human pathology studies verify a beneficial role for autophagy on health. Neurodegeneration, cancer, stroke, metabolic syndromes, infections, and heart diseases are only some examples of pathologies associated with autophagy (Cuervo and Macian, 2014). Certain aspects of aging, as well, are linked to autophagy, in model organisms (Lionaki et al., 2013), such as the decline in protein synthesis and degradation through aging (Makrides, 1983). In mammals, in particular, a decline in protein synthesis rates of 4–70% has been reported with age. Moreover, several studies suggest that proteostasis severely declines during aging (Koga et al., 2011). Taken that lysosomal proteolytic activity declines with age, which coincides with a simultaneous reduction in activity of autophagy-related genes (Cuervo, 2008), one could easily speculate that age-related changes in autophagy might underlie, at least in part, age-associated deterioration and disease. Indeed, common aging features in animal models are the intracellular accumulation of damaged DNA, proteins, lipids, and organelles, as also an increased sensitivity to oxidative damage.

Neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases (AD/PD), affect mainly aged individuals and are associated with the gradual accumulation of protein aggregates, causing neuronal impairment and loss of performance. Although autophagy is dispensable for the clearance of small proteins, it is necessary for the clearance of bigger protein aggregates. Interestingly, brain aging coincides with reduced expression of the Atg5, Atg7, and beclin1 genes, coding for major autophagy components (Lipinski et al., 2010). Hence, the role of autophagic machinery as a cellular scavenger of protein aggregates and damaged organelles is an important proteostatic mechanism that can protect nerve cells from proteotoxicity.

Except from proteins, autophagy also degrades lipids, in a process called lipophagy. It is an alternative way for organisms to mobilize fat and regulate lipids homeostasis. Triglycerides and cholesterol are trapped in autophagosomes and degraded by acidic hydrolases of lysosomes. Lipophagy regulates lipid metabolism and impaired lipophagy causes lipid accumulation that can lead to hepatic steatosis and other related lipids accumulation diseases, altered body mass and altered sensitization to death stimuli (Ward et al., 2016).

Autophagy is known to inhibit programmed cell death through interacting with apoptosis. Although this interaction is not well understood, the Beclin1-Bcl-2 complex appears to have a crucial role in autophagy regulation. It acts as a rheostat that keeps autophagy levels within a physiological range, thus ensuring cell survival in response to stress (Pattingre et al., 2005). Also, autophagy scavenges damaged organelles, which can trigger cell death through the release of pro-apoptotic factors and reactive oxygen species. Moreover, autophagy is indispensable for muscle mass maintenance in mice and it has been suggested to protect against age-related muscle atrophy (Masiero et al., 2009). On the other hand, proteins of the autophagic machinery are also suspected to participate in the induction of apoptosis or necrosis and cause autophagic cell death (Marino et al., 2014).

Autophagy has also been suggested to support adaptive responses to stress in mammals. Conversely, autophagy blockage impairs resistance of cells against several stressors (Kroemer et al., 2010). Low levels of a certain stressor can enhance organismal reaction against high levels of the same stressor, which could be otherwise deleterious. This phenomenon is called *hormesis* and the molecular mechanisms that govern it remain largely unknown. Recent evidence indicates that autophagy is one of the main hormetic mechanisms triggered by nonlethal levels of endoplasmic reticulum (ER) stress. By interacting directly with the ER unfolded protein response (UPR^{ER}) pathway, autophagy confers neuroprotection against experimental PD (Matus et al., 2012).

AUTOPHAGY AND AGING

Autophagy and the Aging Phenotype: An Intricate Relationship

The autophagic machinery progressively losses activity during aging. This raises the possibility that the aging process could be accelerated by the gradual reduction of cellular cytoprotection that autophagy confers. Genetic analyses, combined with pharmacological and longevity studies in several animal models have clearly associated defective autophagy with impaired healthspan and reduced lifespan (Pyo et al., 2013). In yeast, a large-scale screen for genes associated with chronological lifespan, identified short-lived mutants that had autophagy defects. Interestingly, these mutants did not respond to lifespan extension induced by limitation of amino acids in the growth media (Matecic et al., 2010), indicating that the longevity effect of dietary restriction is autophagy dependent in yeast. Similarly, in *Caenorhabditis elegans*, loss-of-function mutations in several genes coding for essential autophagy proteins are short lived (Melendez et al., 2003). In mice, tissue-specific knockout of essential autophagy genes causes accumulation of inclusion bodies containing ubiquitinated proteins, lipofuscin-containing lysosomes and disorganized mitochondria, accompanied by an accumulation of oxidized and ubiquitinated proteins and increased ER stress (Hartleben et al., 2010). In *Drosophila melanogaster*, reduced expression of the ATG1 protein kinase and sestrin1, a negative feedback regulator of target of rapamycin (TOR), shortens lifespan and causes triglyceride accumulation, mitochondrial dysfunction, muscle degeneration, and cardiac malfunction, phenocopying the inhibition of autophagy (Lee et al., 2010). These are some of the studies showing that reduced autophagy accelerates aging in yeasts, nematodes, flies

and mammals, the basic animal model systems. In some of these studies, loss of autophagy caused impairments in heart function and muscles degeneration, among others.

Taken together, these observations show that functional autophagy is a prerequisite for animal models to sustain normal lifespan and healthspan, but they do not support a longevity-promoting effect of autophagy. Nevertheless, recent experiments studying lifespan in animals with increased expression of essential autophagic machinery components, through genetically driven upregulation or, more convincingly, through pharmacological treatment, indicate a positive association between autophagy activity and lifespan.

Perhaps the most convincing experiment indicating a lifespan extending role for autophagy after a single autophagy-related gene upregulation took place in 2013 (Pyo et al., 2013). In this experiment, ubiquitous overexpression of an essential for the autophagosome formation gene (Atg5) extended median lifespan of mice by 17.2%. Atg5 overexpressing mice showed antiaging phenotypes, including leanness, increased insulin sensitivity, and improved motor function. Interestingly, increased autophagy enhanced oxidative stress resistance in embryonic fibroblasts cultured from these transgenic mice. Also in *Drosophila*, induction of Atg8a expression in brains of aged flies extended the mean lifespan by 56%, promoted resistance to oxidative stress and suppressed the accumulation of ubiquitinated and oxidized proteins (Simonsen et al., 2008). These are the most solid proofs showing that overexpression of a single autophagic machinery component can sufficiently enhance lifespan.

In two more cases, indirect increase of autophagy led to improved longevity. In one case, induced damage in mitochondria of human umbilical vein endothelial cells led to increased lifespan (Mai et al., 2012). Authors suggested that enhanced lifespan was due to the upregulation of LC3B, ATG5, and ATG12. Along similar lines, helix-loop-helix (HLH)-30, the predicted *C. elegans* ortholog of the mammalian HLH transcription factor EB (TFEB), a key autophagy regulator, is additionally required for the enhanced longevity of multiple long-lived mutants, while its overexpression sufficiently extends the lifespan of otherwise wild type animals. The finding that TFEB expression is increased in the livers of dietary-restricted mice, a well-known lifespan-prolonging intervention, suggests a conserved role for HLH-30/TFEB in longevity regulation (Lapierre et al., 2013).

Conversely, there are several examples showing that general induction of autophagy through pharmacological treatment can sufficiently increase longevity in different animal models. TOR is an evolutionarily conserved serine/threonine kinase that integrates signals from nutrient, mainly amino acids, and energy, growth factors, and stress to regulate cell growth and proliferation, development, metabolism, and aging (Wullschleger et al., 2006). TOR is consisted of two branches, the TORC1 and TORC2. TORC1 regulates translation through the phosphorylation of eukaryotic initiation factor 4E-binding protein (4E-BP) and ribosomal protein S6 kinase (S6K) and autophagy (TORC1 inhibits autophagy) through *atg* genes. TOR can be downregulated by the TOR kinase-specific inhibitor rapamycin. This pharmacological perturbation of TOR increases lifespan in all organisms tested, thus making rapamycin the first experimental elixir of life we know so far (Kapahi et al., 2010). Longevity extension by rapamycin in *Drosophila* is mediated through either 4E-BP1 or Atg5 (Bjedov et al., 2010), thus making autophagy a candidate universal antiaging mechanism.

Another autophagy inducer that promotes lifespan is the natural polyphenol resveratrol. It is a deacetylase activator; its main target is Sirtuin 1 (SIRT1), the mammalian ortholog

of yeast Sir2. SIRT1 is a conserved NAD^+-dependent protein deacetylase that reportedly extends lifespan in several organisms (Wood et al., 2004). Notably, resveratrol is shown to induce autophagy by decreasing protein acetylation (Morselli et al., 2011). Death-associated protein kinase 1 (DAPK1) has been suggested to be another mediator of the stimulatory effect of resveratrol on autophagy (Choi et al., 2013), though recent evidence suggests that resveratrol induces autophagy through TOR inhibition (Park et al., 2016).

On the other hand, the polyamine spermidine that inhibits protein acetylation also extends the lifespan of yeast, worms, and flies and the survival of human cancer cells through increased autophagy (Eisenberg et al., 2009). Furthermore, spermidine administration ameliorates age-related memory impairment in flies, through Atg7 (Gupta et al., 2013). Interestingly, spermidine exerts its beneficial effects on lifespan and health by directly inhibiting the EP300 (E1A-binding protein p300) acetyltransferase that acts as a negative regulator of autophagy (Pietrocola et al., 2015). Together, these findings indicate that both resveratrol and spermidine increase autophagy via inhibition of proteins acetylation, thus most likely by modifying the activity of autophagy core proteins through direct or indirect interactions that alter their acetylation state. It is therefore reasonable to assume that balance between acetylation and deacetylation seems to be an important coordinator of autophagy.

Autophagy and Aging Influencing Pathways

The discovery of the first single genetic mutations shown to increase lifespan in animals has boosted aging research in the last three decades. These mutations reduce the activity of insulin–insulin like growth factor signaling pathway (IIS) and following studies verified the evolutionarily conserved antiaging effect of lowered IIS in various animal model systems (Kenyon, 2010). Since then, several genes, signaling pathways, dietary interventions, and drugs have been shown to affect aging (Fig. 2.2) (Lopez-Otin et al., 2013). Their properly orchestrated manipulation could held promise to delay aging and increase healthy lifespan in humans. IIS is a major longevity regulator in animals and its reduction extends lifespan through activation of forkhead box O (FOXO) transcription factors. Active FOXO proteins translocate from the cytoplasm to the nucleus where they activate several genes that affect oxidative stress, xenobiotics responses, innate immunity, metabolism and autophagy. In particular, FOXO3 is required for the transcriptional activation of autophagy-related genes, including *LC3* and *Bnip3* during fasting. Furthermore, FOXO3-induced autophagy appears to require Bnip3 (Mammucari et al., 2007). Besides its role in longevity of IIS defective animals, FOXO is also essential for lifespan extension through activation of the stress-responsive c-Jun N-terminal kinase (JNK) pathway in worms and flies (Wang et al., 2005). In these cases, JNK antagonizes IIS, causing nuclear localization of FOXO, which in turn upregulates its targets, including genes involved in stress response and growth control. A recent study in *Drosophila* has shown that JNK activates the transcription of multiple *ATG* genes in response to oxidative stress. It is therefore reasonable to assume that dFoxO may activate transcription of *ATG* genes in response to JNK signaling (Wu et al., 2009).

IIS interacts with TOR, which inhibits the autophagy-promoting Unc-51 like kinase (ULK1) complex (the mammalian homolog of yeast Atg1), the most upstream component of the autophagic machinery (Fig. 2.2). In yeast, TOR phosphorylates Atg13 and decreases its binding to another autophagy-promoting factor, Atg1. Starvation or rapamycin treatment

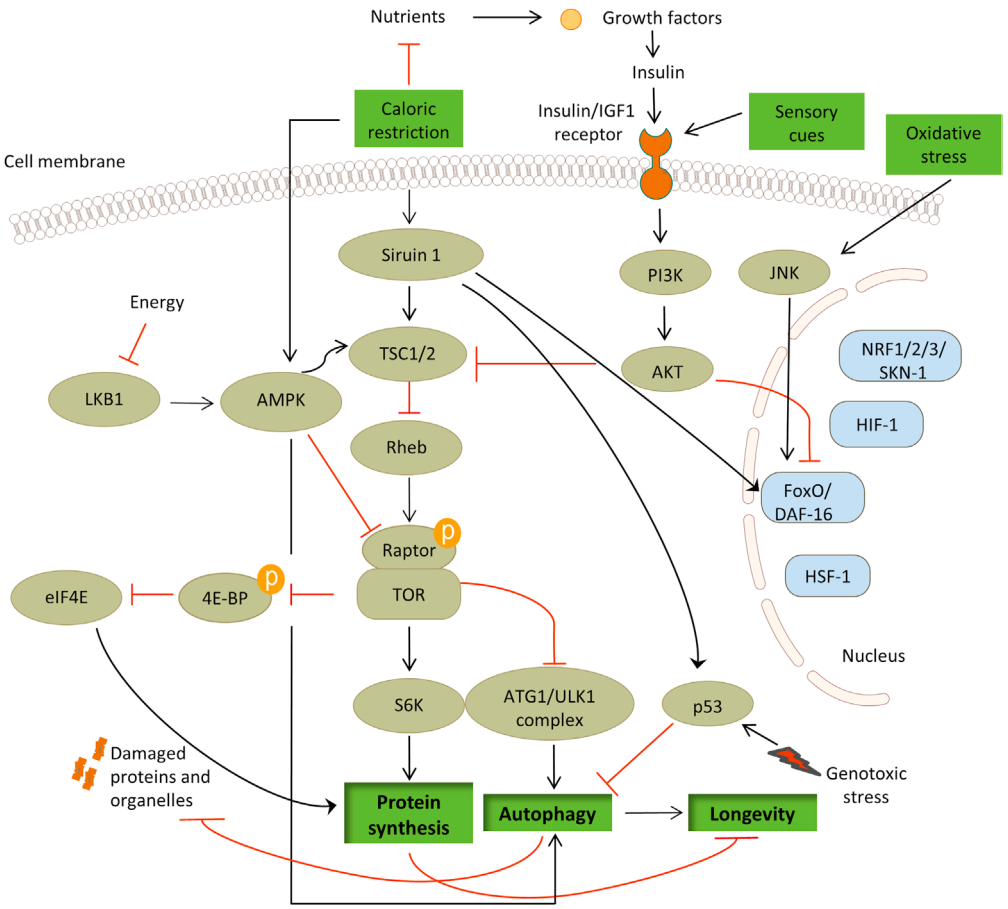

FIGURE 2.2 Signal transduction pathways that influence aging cross-talk with autophagy regulatory mechanisms. Reduced insulin/IGF-1 or TOR kinase signaling, depletion of p53 and activation of sirtuin 1, among others, extend lifespan in a wide variety of species, including yeast, worms, flies, and mice. Similarly, limitation for nutrients, energy and growth factors, also promote longevity. Many of these pathways associate with autophagy regulatory mechanisms. TOR plays a central role in the control of autophagy. Under nutrient rich conditions and growth factor signaling, TOR promotes cell growth/metabolic activity and inhibits the Atg1 complex, inhibiting autophagy. Under nutrient deprivation or stress, various signaling pathways inactivate TOR kinase activity, and thus suppress cell growth, while inducing autophagy. Energy depletion activates the adenosine monophosphate-activated protein kinase (AMPK), which inhibits TOR and activates autophagy. Black arrows indicate stimulatory inputs. Red bars indicate inhibitory interactions. For clarity, some of the signaling connections between longevity pathways and autophagy are not shown. See text for details. *AKT*, AKT8 virus proto-oncogene; *4E-BP*, eukaryotic initiation factor 4E-binding protein; *eIF4E*, eukaryotic translation initiation factor 4E; *FOXO/DAF-16*, a forkhead box O(FOXO) transcription factor; *HSF-1*, heat shock response transcription factor-1; *JNK1*, c-Jun N-terminal kinase; *LKB1*, serine/threonine protein kinase; *NRF1/2/3/SKN-1*, NF-E2-related factor/SKiN head transcription factor-1; *PI3K*, phosphatidylinositol-3 kinase; *Rheb*, Ras homologue enriched in brain; *S6K*, S6 kinase; *TSC1/2*, tuberosclerosis complexes 1 and 2.

dephosphorylates Atg13, resulting in a subsequent activation of Atg1 (Kamada et al., 2000). Hence, reduced IIS, but also depletion of nutrients under starvation, induce autophagy through a common mechanism (Fig. 2.2). Starvation also affects TORC1-lysosomes positioning, which in turn enhances autophagosome-lysosome fusion (Korolchuk et al., 2011). In contrast, when nutrients are sufficient, mTORC1 complex phosphorylates ULK1, preventing its association and activation by adenosine monophosphate-activated protein kinase (AMPK), a kinase that is activated by starvation and reduced IIS (Fig. 2.2). These studies have revealed a molecular mechanism of ULK1 regulation that coordinates nutrient and growth factor signaling, including insulin, via the actions of AMPK and mTORC1.

Another longevity-promoting intervention that activates autophagy is caloric restriction (CR), defined as reduced food intake without malnutrition. CR induces autophagy via AMPK, which directly activates ULK1 (Egan et al., 2011), and SIRT1, which activates autophagy through deacetylating its core components (Canto et al., 2010), but also indirectly through inhibiting IIS and TOR. Additionally, CR can modulate autophagy by activating MEC-17, an acetyltransferase that stimulates the essential for autophagy microtubules transport machinery (Mackeh et al., 2014). In MEC-17 mutants, microtubule hyperacetylation is inhibited and this impairs cell survival under stress and starvation-induced autophagy, suggesting that microtubule hyperacetylation promotes cell survival under stress by inducing autophagy.

As mentioned before, the balance between acetylation/deacetylation is a major regulator of the autophagy machinery. In this regard, resveratrol and spermidine activate autophagy through opposing mechanisms converging on the acetylproteome (Morselli et al., 2011). Specifically, resveratrol has been suggested to induce autophagy by directly activating SIRT1 (Baur et al., 2006; Lagouge et al., 2006), which in turn modulates cellular senescence and lifespan via the activation of autophagy (Morselli et al., 2010). Likewise, spermidine has been shown to increase the lifespan of animal models in an autophagy-dependent fashion (Eisenberg et al., 2009). Lack of endogenous polyamines causes hyperacetylation, increase of reactive oxygen species, premature necrotic death, and shortened lifespan (Minois et al., 2011). In support of findings linking deacetylation to autophagy levels, a connection between levels of acetate, acetyl-coenzyme A (acetyl-CoA), and autophagy inactivation has been recently reported. Acetyl-CoA donates acetyl groups for epigenetic and posttranslational modifications and can lead to proteins inactivation. High acetylation levels cause histones hyperacetylation, reduced expression of ATG genes, autophagy inhibition, and a shortened lifespan (Eisenberg et al., 2014). Hence, a clear association between factors regulating cellular acetylation levels, autophagy and longevity is emerged.

Further supporting a key role for autophagy in lifespan extension, several experiments demonstrate not only that longevity pathways interact with autophagy but also that their lifespan effects are mediated through autophagy. For example, inhibition of IIS pathway by mutations in the gene encoding the insulin-IGF-1-like receptor abnormal DAuer Formation 2 (DAF-2) or the phosphatidylinositol-3 kinase (PI3K) AGing alteration 1 (AGE-1) remarkably increases lifespan in *C. elegans*. This gain in longevity is abrogated upon depletion of the Beclin1 ortholog BEC-1 and knockdown of the *atg-7* and *atg-12* genes (Melendez et al., 2003). The same effect has been reported on *eat-2* (eating defective-2) mutants, which have a CR phenotype due to defects in pharyngeal pumping, upon expression of *bec-1* and *atg-7* RNAi (Jia and Levine, 2007). Also, rapamycin- and spermidine-mediated lifespan

extension is abolished by silencing of *atg1*, *atg7*, and *bec-1*, respectively (Alvers et al., 2009; Eisenberg et al., 2009). Along similar lines, RNAi knockdown of *bec-1* has a similar effect on resveratrol-mediated lifespan extension (Morselli et al., 2010). In *Drosophila*, rapamycin and spermidine administration enhances lifespan, but not in *Atg5* RNAi expressing flies or *Atg7* mutants (Bjedov et al., 2010; Eisenberg et al., 2009). In mice, CR prolongs lifespan through induction of autophagy and increase in SIRT1 expression (Mercken et al., 2014). Also in yeast, worms and flies, the beneficial effects of CR and resveratrol on lifespan involve the SIRT1-dependent induction of autophagy (Morselli et al., 2010). Moreover, SIRT1 deacetylates the transcription factors p53, NF-kB, HSF-1, and FOXO, which feedback to regulate SIRT1 expression levels in a stress-dependent manner. Therefore, SIRT1 may influence cell and whole organism survival through the activation of these factors (Saunders and Verdin, 2009).

After several years of research and work on different animal models, it is now clear that the major pathways and interventions that extend lifespan in an evolutionarily conserved way, involve autophagy upregulation. In addition, loss or downregulation of autophagy components severely abrogate lifespan extending effects gained by their proper manipulation. An interesting and novel theory that might explain, at least in part, the beneficial autophagy effect on longevity is the participation of autophagy in hormetic responses. According to this, low levels of oxidative stress through reactive oxygen species (ROS) production in mitochondria protect organisms from severe oxidative damage that accumulates through aging (Ristow and Zarse, 2010). In support, ischemic preconditioning, a typical example of hormesis, has protective effects for organisms, and autophagy is shown to actively participate in this hormetic reaction (Gottlieb and Mentzer, 2010).

Recent experiments have clearly shown the existence of a homeostatic feedback loop associating metabolic signals with biogenesis and turnover of mitochondria. In this model, mitophagy (a selective type of autophagy targeting mitochondria for degradation) is required for longevity under low IIS or impaired mitochondrial function and it is indispensable for stress resistance, through DCT-1 (DAF-16/FOXO Controlled, germline Tumor affecting-1). SKiNhead-1 (SKN-1) the nematode homologue of nuclear factor-erythroid 2-related factor 2 (NRF2) transcription factor, regulates both mitochondrial biogenesis and degradation under stress. Deregulation of these processes during aging causes overproliferation of damaged mitochondria and decline of cellular function (Palikaras et al., 2015). These experiments sufficiently associate longevity, autophagy, and stressors and, at least in part, explain the fundamental role of functional autophagy in cellular and whole organism homeostasis.

AUTOPHAGY AND AGE-RELATED DISEASES

Basal activity of autophagy machinery is essential for protein and organelle turnover, as also for adaptation to acute stress conditions. Defective autophagy leads to intracellular accumulation of toxic macromolecules, mainly protein aggregates and impaired cellular homeostasis that accelerates entry into senescence and tissues malfunction with pathophysiologic implications (Mizushima and Komatsu, 2011). Postmitotic cells are particularly susceptible to toxic materials accumulation, since they cannot dilute them through cell

division. According to a simplistic, but solid theory, toxic aggregates accumulate intracellularly with age due to a progressive reduction in autophagy, which has been associated with devastating human pathologies, such as AD, PD, cancer, cardiovascular diseases, atherosclerosis, age-related muscles degeneration, infectious diseases, among others (Schneider and Cuervo, 2014). The incidence of all of these diseases increases rapidly with aging and severely affect longevity of human populations. We will briefly refer to the most life threatening age-related pathologies, which are neurodegeneration, cancer, and heart diseases.

The most common neurodegenerative disease is AD. Intracellular accumulation of beta-amyloids (Aβ), derived from amyloid precursor protein, causes accumulation of toxic aggregates that impair cellular function and kill cells. Mitochondrial dysfunction and impaired mitophagy have been linked to the development of the disease (Boland et al., 2008). Mitochondrial damage upon elevated ROS production has been suspected for enhanced tau toxicity and Aβ accumulation, though the existence of autophagic vacuoles in neurons of AD patients links autophagy to the disease development. Moreover, impaired mitochondrial bioenergetics is one of the early manifestations of the disease (Caldwell et al., 2015). Interestingly, AD-causing mutations have been associated with autophagy malfunction, and beclin1 mutations cause neurodegeneration and amyloid β accumulation, through defective neuronal autophagy and disruption of lysosomes (Pickford et al., 2008). Furthermore, presenilin1 (PS1), which is commonly mutated in early-onset familial AD, is required for the acidification of lysosomes/autolysosomes and the clearance of autophagosomes (Lee et al., 2010). However, experimental results regarding the role of autophagy in AD treatment are contradictory. Specifically, in an AD mouse model, long-term rapamycin administration prevents symptoms and decreases levels of $A\beta_{42}$, a main component of the amyloid plaques found in the brains of Alzheimer patients. It has also been reported to reduce amyloid plaques and cognitive deficits, and induction of autophagy following rapamycin supplementation ameliorates amyloid pathology. However, other experiments show that autophagy induction after the formation of mature plaques and tangles has no effect on AD-like pathology or cognitive deficits (Majumder et al., 2011) and rapamycin treatment of flies expressing $A\beta_{1–42}$ shortens lifespan. Thus, it appears that despite the obvious role of defective autophagy in the development of AD, the benefit of autophagy induction is context dependent (Liang and Jia, 2014).

Another major age-related disease linked to autophagy is PD. In PD, dopamine neurons are characterized by intra cytoplasmic inclusions containing α-synuclein and ubiquitin (Lewy bodies) and they selectively die prematurely. Mitophagy seems to have a central role in the development of the disease and deregulation of the autophagy pathway has been observed in the brains of PD patients and in animal models of PD (Lynch-Day et al., 2012). Mutations in the genes coding for the PINK1 protein kinase and the E3 ubiquitin ligase PARKIN, which act in a common pathway to mediate mitophagy under physiological conditions in vivo (Vincow et al., 2013), underlie the early onset of autosomal recessive PD. Excessive mitochondrial damage has been linked to PD (Schapira, 2008) and other studies show that PARKIN also mediates proteasome-dependent degradation of outer membrane proteins of depolarized mitochondria. Although the etiology of PD is still obscure, there is an obvious correlation between dopaminergic neuron loss and accumulation of mitochondrial damage in the development and progression of the disease. Several findings suggest that impaired ubiquitination of mitochondrial substrates or PARKIN could cause

mitochondrial dysfunction and subsequent death of dopaminergic cells (Lionaki et al., 2013). Interestingly, neuronal upregulation of *Parkin* in flies increases lifespan and mutations in core autophagy components in both flies and mice lead to PD-like phenotypes (Knuppertz and Osiewacz, 2016). In *C. elegans*, mitophagy interferes with mitochondrial biogenesis to regulate mitochondrial content and longevity. Coordination of mitochondrial biogenesis and degradation is therefore an integral aspect of mitochondria quality control. As such, it regulates both cellular adaptation in response to various intrinsic and extrinsic stimuli and longevity (Palikaras et al., 2015). Disturbances in this regulation contribute to accumulation of damaged mitochondria and decline of cellular function. These findings highlight the importance of mitophagy in maintaining cellular homeostasis, further supporting the notion that defects in mitophagy might underlie, at least some of the neurodegenerative diseases, such as familiar PDs.

Autophagy has also been linked to cancer development and progression, but research in this field is still premature. It looks like autophagy has a general preventive, anticancer function, through suppressing genomic instability and inflammation, which can lead to tumor initiation, promotion, and progression (Chen and White, 2011). In support of this, several mutations in autophagy genes are associated with cancer in humans (Jiang and Mizushima, 2014). However, once tumor develops it might use autophagic machinery for its own cytoprotection. For example, mitophagy is proposed to mediate hypoxia adaptation responses and cancer cells might survive under hypoxia through mitophagy-related adaptive mechanisms (Zhang et al., 2008).

Moreover, autophagy is involved in cardiac diseases. Its deregulation is linked to multiple cardiovascular pathologies associated with aging, and its role is essential for ischemic preconditioning. Preconditioning or fatal ischemia is reported to induce autophagy via ER stress, AMPK/TSC/mTOR, Beclin1/BNIP3/SPK2, and FoxO/NF-κB transcription factors. Activated autophagic machinery then regulates cell death through activation or inactivation of apoptotic and necrotic signaling pathways (Sheng and Qin, 2015). Furthermore, defective autophagy is associated to cardiac diseases and autophagy activation through *Atg7* upregulation improves cardiac performance under proteotoxic conditions (Bhuiyan et al., 2013). Also, exercise is shown to reduce protein aggregates and extend survival through autophagy (Leon and Gustafsson, 2016). However, the actual role of autophagy, as in the case of cancer, is still obscure and more studies are required to establish its antiaging role in heart diseases.

CONCLUSIONS

Autophagy plays a well-established role in longevity and age-related diseases. Extensive research on the basic animal models, such as worms, flies, and mice, have clearly shown that defective autophagy harms healthspan and lifespan expectancy. Furthermore, an increase in beclin1 levels, a key regulator of autophagy, has been recently reported in healthy centenarians. Thus, autophagy activity might be linked to human exceptional longevity (Emanuele et al., 2014). Major factors accelerating aging are the accumulation of various forms of cellular damage, caused by malfunctioning organelles, proteins aggregates, and general proteostatic deregulation. Autophagy is the main system for clearing intracellular malfunctioning organelles and aberrant protein aggregates, but also pathogens; hence it has an essential

cytoprotective role in the cells. Several studies show that autophagy declines during aging and a simplistic, but solid, speculation leads to the assumption that this decline might have a causal link to physiological deterioration observed in older individuals.

In support, the main longevity promoting pathways and interventions, such as IIS, TOR, proteins deacetylation and CR, exert their action on longevity through the enhanced autophagic activity and well-established molecular interactions. However, this mediation does not necessarily mean that autophagy upregulation is sufficient for promoting longevity. One could presume that these antiaging factors are physiologically stressful for animals and basal levels of autophagy are a prerequisite to maintain cellular homeostasis and functionality. Indeed, several studies fail to prove that upregulation of single autophagic components can extend lifespan. On the other hand a few studies have clearly shown that autophagy upregulation can sufficiently prolong lifespan in different animal models. A possible answer to this controversy is that upregulation of autophagic components could lead to lifespan extension through counteracting deficiencies in proteostasis or immunity of laboratory animals. Alternatively, lifespan extending effects are achievable only through a well-orchestrated upregulation of autophagic components, which can be performed through manipulating factors that enhance autophagy as a whole, such as CR and protein deacetylation.

On the other hand, excessive autophagy induction could harm organismal health. For example, excessive over-activation of muscarinic acetylcholine signaling in *C. elegans* overactivates autophagy and leads to early mortality (Kang et al., 2007). Along the same lines, autophagosome formation is dramatically enhanced during early necrotic cell death, and autophagy is required for necrotic breakdown of nematodes neurons (Samara et al., 2008). Also, in *D. melanogaster*, strong overexpression of *Atg1* causes apoptosis induction (Scott et al., 2007). Therefore insufficient or excessive autophagy induction can have similar, deleterious effects for animals.

Despite the necessity for further studies to clarify the antiaging role of autophagy and the conditions under which autophagy upregulation can sufficiently improve health and longevity, the existence of a large arsenal of drugs that increase autophagy is a valuable tool for the development of future antiaging treatments. For example, rapamycin, resveratrol, and metformin are drugs that have been licensed for administration in humans and they not only induce autophagy, but also they have a known anticancer effect. Furthermore, CR is a well-established autophagy inducer with a strong antiaging effect. Hence, although still at a premature stage, carefully designed autophagy enhancing interventions might be used as antiaging treatments in humans in the near future.

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