Cell
Leading Edge
Review

Autophagy and Aging

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DOI 10.1016/j.cell.2011.07.030

Genetic inhibition of autophagy induces degenerative changes in mammalian tissues that resemble those associated with aging, and normal and pathological aging are often associated with a reduced autophagic potential. Pharmacological or genetic manipulations that increase life span in model organisms often stimulate autophagy, and its inhibition compromises the longevity-promoting effects of caloric restriction, Sirtuin 1 activation, inhibition of insulin/insulin growth factor signaling, or the administration of rapamycin, resveratrol, or spermidine. Here, we discuss the probable cause and effect relationship between perturbed autophagy and aging, as well as possible molecular mechanisms that may mediate the anti-aging effects of autophagy.

Introduction

Autophagy encompasses the different routes that cells use to deliver cytoplasmic substrates to lysosomes for degradation. These include macroautophagy, chaperone-mediated autophagy, and microautophagy. Here, we focus on macroautophagy (which we will hereafter refer to as autophagy), a process of cellular self-cannibalism in which portions of the cytoplasm are sequestered within double- or multimembraned vesicles (autophagosomes) and then delivered to lysosomes for bulk degradation (Figure 1). The initial phases of macroautophagy consist of the formation of a phagophore (also called isolation membrane), the engulfment of cytoplasmic material by the phagophore, the elongation of the phagophore membrane, and fusion of its edges to close the autophagosome. The outer membrane of the autophagosome fuses with the lysosome to form the autolysosome (also called autophagolysosome) in which the luminal material including the internal membrane is degraded. The resulting breakdown products are released through permeases and are recycled in the cytosol (Ravikumar et al., 2010). Thus, autophagy is the mechanism through which the nonnuclear (cytoplasmic) parts of the cell can be renewed and through which cytoplasmic macromolecules can be mobilized to generate energy-rich compounds that can meet the bioenergetic demand of the cell in conditions of dwindling external or internal resources.

Autophagy is a predominantly cytoprotective (rather than a self-destructive) process (Kroemer and Levine, 2008). Accordingly, autophagy can mediate protective effects in multiple rodent models of organ damage affecting the liver (Zhang and Cuervo, 2008), heart (Gottlieb and Mentzer, 2010), nervous system (Ravikumar et al., 2010), and kidney (Jiang et al., 2010), just to mention a few examples.

Beyond its function in the adaptation of individual cells or organs to changing conditions, autophagy has a prominent role in determining the life span of many model organisms. Reduced autophagy has been associated with accelerated aging, whereas stimulation of autophagy might have potent anti-aging effects (Madeo et al., 2010). This Review focuses on several related questions. How can dysfunctional autophagy lead to accelerated aging? In which cases does increased autophagy counteract normal or pathological aging processes? And which are the molecular mechanisms that link autophagy, cytoprotection, longevity, and healthy aging?

Principal Functions of Autophagy

In yeast, autophagy is critical for survival during nutrient deprivation, as it enables recycling of macromolecules to provide new nutrients and energy. A key role for autophagy is as a degradative pathway. Its substrates include aggregate-prone intracytoplasmic proteins that are associated with neurodegenerative diseases such as mutant α-synuclein (which causes forms of Parkinson's disease), tau (implicated in Alzheimer's disease), and polyglutamine-expanded proteins like mutant huntingtin (that causes Huntington's disease). Indeed, when autophagy is inhibited in otherwise normal mice, protein aggregates form in the cytoplasm (Ravikumar et al., 2010).

Autophagy is also a critical regulator of organellar homeostasis, particularly of mitochondria. Dysfunctional mitochondria that have lost their membrane potential and are more likely to release toxic apoptotic mediators and reactive oxygen species are apparently selectively removed by autophagy relative to "healthy" mitochondria via phosphorylation reactions mediated by the kinase PINK1 and subsequent ubiquitination of

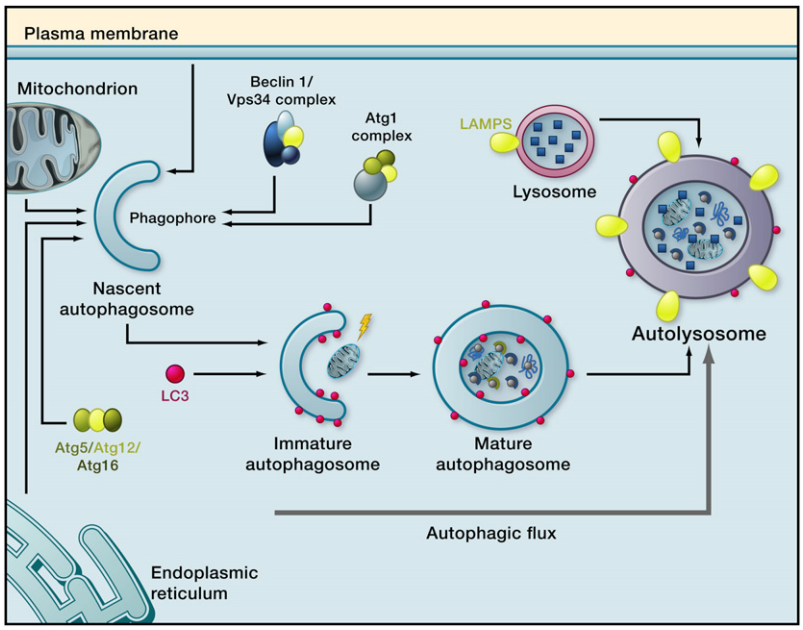

Figure 1. Macroautophagy: An Overview
Irrespective of the origins of their double membrane, autophagosomes engulf bulk portions of cytoplasm or selective cargo, thus closing to become mature autophagosomes. In this complex process, a series of autophagy-related proteins and protein complexes are sequentially involved. Autophagosomes eventually fuse with lysosomes to form autolysosomes, in which the autophagic cargo is degraded. The resulting biomolecules are finally recycled back to the cytoplasm.

mitochondrial membrane proteins by the E3 ligase Parkin. Autophagy can also enhance degradation of various bacteria and viruses and may play protective roles in numerous infectious diseases (Ravikumar et al., 2010).

Autophagy Machinery

Traditionally, it has been believed that autophagosome formation starts at phagophore assembly sites (Figure 1). Phagophore formation (autophagosome precursor/pre-autophagosomal structures) requires the class III phosphoinositide 3-kinase (PI3K) Vps34, which acts in a large macromolecular complex that also contains Atg6 (also called Beclin 1), Atg14, and Vps15 (p150). Other proteins involved in the early stages of autophagy include Atg5, Atg12, Atg16, focal adhesion kinase (FAK) family-interacting protein of 200 kD (FIP200), which interacts with Atg1 (also called ULK1), and the mammalian ortholog of Atg13 (Ravikumar et al., 2010).

The elongation of membranes that is thought to be critical for autophagosome formation is associated with two ubiquitination-like reactions. In the first of these, Atg12 is conjugated to Atg5 by Atg7, which is similar to an E1 ubiquitin-activating enzyme, and Atg10, which is similar to an E2 ubiquitin-conjugating enzyme. The Atg5-Atg12 conjugates interact noncovalently with Atg16L1 and this complex associates with phagophores but dissociates from completed autophagosomes. In the second of the ubiquitin-like reactions, microtubule-associated protein 1 light chain 3 (MAP-LC3/Atg8/LC3) is conjugated to the lipid phosphatidylethanolamine (PE) by Atg7 (E1-like) and Atg3 (E2-like) to form LC3-II (Ravikumar et al., 2010). Multiple LC3-positive autophagosomes form randomly in the cytoplasm, after which they are trafficked along microtubules in a dynein-dependent manner to lysosomes, which cluster close to the microtubule-organizing center (MTOC) near the nucleus. Autophagosome-lysosome fusion appears to be mediated by the SNARE proteins VAMP8 and Vti1B (Furuta et al., 2010).

Signals for Autophagosome Formation

The (mammalian) target of rapamycin (mTOR) is a primordial negative regulator of autophagy in organisms from yeast to man. mTOR is inhibited under starvation conditions, and this contributes to starvation-induced autophagy via activation of mTOR targets Atg13, ULK1, and ULK2. This inhibition can be mimicked by mTOR inhibitory drugs like rapamycin (Ravikumar et al., 2010).

One of the important pathways regulating mTOR is initiated when growth factors like insulin-like growth factor bind to insulin-like growth factor receptors (IGF1R) (Figure 2). These receptors signal, via their tyrosine kinase activities, to effectors like the insulin receptor substrates (IRS1 and IRS2), which in turn activate Akt. Akt inhibits the activity of the TSC1/TSC2 (proteins mutated in tuberous sclerosis) complex, a negative regulator of mTOR. In this way, IGF1R signaling activates mTOR and inhibits autophagy, and the converse occurs when nutrients are depleted.

mTOR activity can also be regulated by GTPases that influence its lysosomal localization during extreme starvation. Under these circumstances, mTOR complex 1 (which regulates autophagy) dissociates from lysosomes where its direct activators reside (Sancak et al., 2010). Under milder starvation conditions, mTOR activity is also inhibited, but the kinase remains associated with lysosomes, which cluster around the MTOC. This brings more lysosomes in the travel path of autophagosomes and enhances autophagosome-lysosome fusion. When nutrients are replenished, the lysosomes with the associated mTOR move toward the plasma membrane. This is essential for mTOR activation resulting in decreased autophagosome formation (Korolchuk et al., 2011).

p53, which is commonly mutated in human cancers, can positively and negatively regulate autophagy via the mTOR pathway. Oncogenic or genotoxic stress stabilizes and activates p53 and may stimulate autophagy by activating AMPK or by upregulating the phosphatase and tensin homology (PTEN), a negative regulator of Akt, and TSC1. On the other hand, genetic or chemical inhibition of p53 also activates autophagy (Fleming et al., 2011; Tasdemir et al., 2008).

Another pathway involved in starvation-induced autophagy is mediated by inhibition of interactions between Beclin-1 (Atg6) and the apoptosis-related proteins B cell lymphoma 2 (Bcl-2) or basal-cell lymphoma-extra large (Bcl-X_L). Starvation activates c-Jun N-terminal kinase-1 (Jnk-1), which phosphorylates Bcl-2, thereby disrupting the interaction between Beclin-1 and Bcl-2

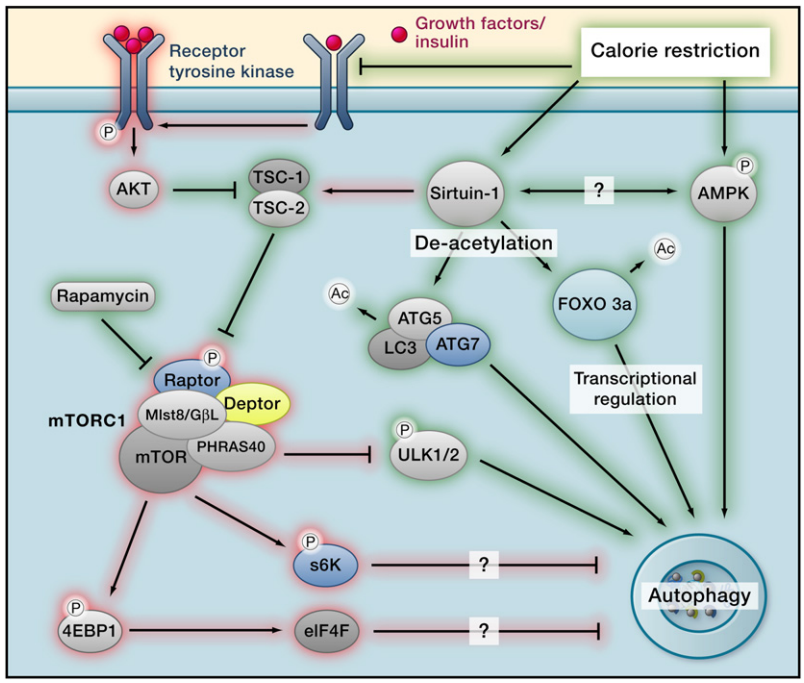

Figure 2. The Regulation of Autophagy and Life Span

The schematic represents a simplified overview of the signaling pathways regulating autophagy and life span. Green marks proteins/signaling circuitry that increase longevity, whereas life span-reducing signals are marked in red. Note that autophagy is only one of the consequences of the depicted signal transduction pathways.

and inducing autophagy. This mechanism may also contribute to autophagy upregulation by endoplasmic reticulum (ER) stress (Ravikumar et al., 2010).

Autophagy can also be directly activated by adenosine monophosphate-activated protein kinase (AMPK), which is induced when nutrients are scarce or when AMP/ATP ratios rise, leading to direct Ulk1 activation (Egan et al., 2011; Kim et al., 2011). In addition, AMPK activation can also induce autophagy by inhibiting mTOR.

Another mTOR-independent autophagy pathway is mediated by the inhibition of inositol monophosphatase (IMPase), which reduces free inositol and myoinositiol-1,4,5-triphosphate (IP3) levels. Lithium, carbemazepine, and valproate—drugs that are used to treat a range of neurological and psychiatric conditions—induce autophagy via this pathway (Fleming et al., 2011). IP3 may regulate autophagy negatively by binding to ER IP3 receptors, which releases Ca^{2+} from stores in the ER, resulting in the activation of proteases of the calpain family that inhibit autophagy by cleaving and activating Gsα, which produces excess cAMP (Williams et al., 2008). However, other mechanisms also appear to contribute. For example, lowering IP3 levels decreases ER Ca^{2+} influx into mitochondria and mildly impairs mitochondrial respiration, leading to autophagy upregulation via AMPK (Cárdenas et al., 2010). Alternatively, IP3 receptors may also serve as negative regulators of autophagy by binding Beclin-1 (Vicencio et al., 2009).

Premature Aging Due to Autophagy Inhibition

Multiple reports indicate that ATG proteins or other proteins required for autophagy induction, such as Sirtuin 1, have reduced expression in aged tissues and that autophagy diminishes with aging. This applies, for instance, to normal human brain aging (in which Atg5, Atg7, and Beclin 1 are downregulated) (Lipinski et al., 2010), insulin resistance and metabolic syndrome (in which Sirtuin1 is downregulated) (de Kreutzenberg et al., 2010), or osteoarthritis (in which ULK1, Beclin1, and LC3 are downregulated) (Caramés et al., 2010). Likewise, IP3 receptor signaling is increased in age-related neurodegenerative diseases such as Alzheimer's disease, as well as in other conditions like cardiac hypertrophy, suggesting a possible decrease in autophagy in these conditions (Decuypere et al., 2011). Though merely phenomenological and correlative, these findings suggest that the enfeeblement of the autophagic response may contribute to the aging phenotype.

Accordingly, an unbiased screen searching for chronological aging factors in the yeast *Saccharomyces cerevisiae* led to the identification of multiple short-lived mutants with autophagy defects (including 10 ATG mutations among a total of 117 short-lived mutants) (Matecic et al., 2010). Loss-of-function mutations in Atg1 (Unc-51), Atg7, Atg18, and Beclin 1 (Bec-1) also decrease the life span of the nematode *Caenorhabditis elegans* (Tóth et al., 2008). Similarly, deficient expression in Atg1, Atg8, and Sestrin1 (which is also required for basic autophagy) reduces the life span of the fruit fly *Drosophila melanogaster* (Lee et al., 2010a; Simonsen et al., 2008). This is linked to age-associated pathologies, including triglyceride accumulation, mitochondrial dysfunction, muscle degeneration, and cardiac malfunction (Lee et al., 2010a).

In mice, the knockout of essential ATG proteins is lethal during the early postnatal period because autophagy is indispensable for mobilizing intracellular energy reserves during the transition from intrauterine metabolism to weaning (Levine and Kroemer, 2008). The tissue-specific knockout of ATG genes has a less-dramatic phenotype and instead precipitates the manifestation of multiple age-associated stigmata, including the accumulation of intracellular inclusion bodies containing ubiquitinylated proteins, accumulation of lysosomes containing the aging pigment lipofuscin, disorganized mitochondria, and oxidation of proteins leading to their carbonylation, carboxymethylation, or nitrosylation (Table 1).

Few studies have tested whether one can affect age-related phenotypes by correcting aging-associated defects in autophagy. Zhang and Cuervo (2008) noted that the expression level of LAMP2a dropped off with aging in the mouse liver and expressed an inducible hepatocyte-specific LAMP2a transgene to correct levels of this protein to those seen in younger mice. Reinstatement of normal LAMP2a levels averts the aging-associated

defect in chaperone-mediated autophagy (CMA) and macroautophagy, and it decreases the abundance of oxidized proteins, polyubiquitinated protein aggregates, and apoptotic cells within the liver (Zhang and Cuervo, 2008). Liver autophagy is also decreased in obesity and diabetes induced either by high-fat diet or genetic deficiencies in leptin. Adenovirus-directed overexpression of Atg7 can correct this hepatic autophagic defect, diminish ER stress, and counteract insulin resistance (Yang et al., 2010). These results suggest that aging is associated with insufficient autophagy, which can explain at least part of the aging phenotype.

Increased Autophagy Delays Aging and Extends Longevity

The first indication that increased autophagy may contribute to longevity comes from the seminal observation that inhibition of the insulin-like growth factor pathway causes autophagy in *C. elegans* and that inhibition of autophagy by mutation of essential Atg genes prevents the gain of longevity (Meléndez et al., 2003). Caloric restriction (CR), that is reduced food intake without malnutrition, is the key anti-aging intervention that extends life span in most animals so far tested, including in rhesus monkeys, in which it reduces the incidence of diabetes, cardiovascular disease, cancer, and brain atrophy (Colman et al., 2009). Epidemiological studies suggest that CR is also beneficial to human health. CR is the most physiological inducer of autophagy (Levine and Kroemer, 2008), and inhibition of autophagy prevents the anti-aging effects of CR in all species investigated in this respect (Table 2).

Depending on the way that CR is imposed—by intermittent feeding versus chronically reduced intake, its onset (juvenile versus adult), or its intensity—CR induces autophagy through the activation of either of two energy sensors, AMPK (Egan et al., 2011; Kim et al., 2011) and Sirtuin 1 (SIRT1), which engage in a positive forward loop of mutual activation (Cantó et al., 2010). Moreover, CR can induce autophagy through the inhibition of insulin/insulin-like growth (IGF) factor signaling, which also results in TOR inhibition (Kenyon, 2010).

CR does not further increase life span when TOR signaling is already reduced in yeast, worms, or flies, suggesting that common mechanisms mediate both of these anti-aging interventions (Grandison et al., 2009). One such common mechanism is autophagy because knockout or knockdown of *atg* genes abolishes the life span-extending effects of rapamycin in all species investigated (Bjedov et al., 2010) (Table 2). Inhibition of TOR, either pharmacologically (with rapamycin) or genetically, extends life span in yeast, *C. elegans* (Kenyon, 2010), *D. melanogaster* (Bjedov et al., 2010), and mice (Harrison et al., 2009). Although rapamycin potently induces autophagy, it may impact on aging of mice by virtue of its capacity to suppress inflammatory and autoimmune processes (Kapahi et al., 2010) that negatively affect longevity. Moreover, TORC1 inhibition has major effects on protein translation (Kapahi et al., 2010), raising the question of whether mTOR inhibition enhances longevity due to the induction of autophagy or whether this effect also involves autophagy-unrelated effects.

The effects of mTOR inhibition on translation are explained by the hypophosphorylation of its substrates S6K (which loses its kinase activity upon dephosphorylation) or that of 4E-BP (which upon hypophosphorylation becomes active as a translational repressor to block the activity of the eIF4F complex). Rapamycin fails to extend the life span of flies that overexpress a constitutively active form of S6K (Bjedov et al., 2010). Impaired expression of S6K extends the life span of *C. elegans*, *D. melanogaster*, and mice (Hands et al., 2009; Selman et al., 2009). S6K deletion leads to the activation of AMPK and fails to increase longevity in AMPK-deficient nematodes (Selman et al., 2009). Because AMPK is a potent activator of autophagy (Ravikumar et al., 2010), it should be clarified whether S6K deletion causes autophagy and whether autophagy may contribute to the life span extension of S6K-deficient animals.

Inactivation of eIF4F by rapamycin reduces the translation of transcripts with extensive secondary structure in their 5′ untranslated regions, and downregulation of various components of the eIF4F cap-binding complex extends life span of *C. elegans* (Hands et al., 2009; Syntichaki et al., 2007). In *Drosophila*, 4E-BP extends life span upon dietary restriction, presumably because mitochondrial proteins involved in oxidative phosphorylation that are encoded by nuclear genes are translated more efficiently in a context of global decrease of translation, hence improving mitochondrial function (Zid et al., 2009). Similarly, reduced cytosolic protein synthesis suppresses the age-associated mitochondrial degeneration in yeast cells (Wang et al., 2008). However, it has not been excluded yet that increased autophagy resulting from 4E-BP overexpression (or perhaps other methods to reduce translation) might increase the turnover and hence improve the quality control of mitochondria. Moreover, it is formally possible that normal levels of autophagy may be sufficient to guarantee for optimal selection of functional mitochondria (and removal of dysfunctional ones) if the number of organelles that are newly formed is reduced and/or their initial quality is improved. Hence, the impact of invalidated autophagy pathways on the longevity conferred by the activation of 4E-BP or the inactivation of S6K or eIF4F should be studied in the future.

In *C. elegans*, the longevity-enhancing effect of inactivating TOR or the S6K homolog *rsks-1* (but not that of inactivating the eIF4F component eIF4E/*ife-2*) requires *pha-4* (Sheaffer et al., 2008), which is the ortholog of the transcription factor FoxA. PHA4 (FoxA) is also required for induction of autophagy by CR (Hansen et al., 2008), suggesting yet another link between CR, TOR, autophagy, and longevity. That said, the relationship between CR and TOR is not completely linear, and rapamycin can increase the longevity of weak *insulin/Igf* signaling (IIS) pathway mutants and of flies with life span maximized by dietary restriction, indicating additional mechanisms (Bjedov et al., 2010).

Sirtuin 1 (SIRT1) and its orthologs (Sir2 in yeast and *Drosophila*, *sir-2.1* in *C. elegans*) reduce aging and prolong life span upon their transgenic overexpression. CR extends life span, in part, by increasing SIRT1 expression (or by activating its enzymatic activity), and in yeast, worms, and flies, the lack of Sir2 abrogates the effects of CR on life span. Resveratrol, which directly or indirectly activates SIRT1, can prolong life span, and this longevity-increasing effect is lost in yeast, worms, and flies lacking Sir2. Similarly, mice that are deficient for SIRT1 do not show some of the beneficial effects of CR related to longevity (Haigis and Sinclair, 2010). CR and resveratrol induce

Table 1. Selected Aging-Related Phenotypes of Autophagy-Deficient Mice

Autophagy Deficiency and Genotype	Phenotype	Disease Relevance	Reference
Heterozygous knockout of Beclin 1	Enhanced inflammation, steatohepatitis, enhanced frequency of hepatocellular carcinomas, lung adenocarcinomas, and lymphomas in aging mice; enhanced accumulation of intraneuronal amyloid ß upon transgenic expression of human amyloid precursor protein	Aging-related malignancies; neurodegeneration	Mathew et al., 2009; Pickford et al., 2008
Bcn1$^{+/-}$			
Central nervous system-specific knockout of Atg5 or Atg7	Progressive accumulation of ubiquitinylated proteins as inclusion bodies in neurons, followed by cortical and cerebellar neuronal loss and premature death	Neurodegeneration	Komatsu et al., 2006; Hara et al., 2006
Atg5$^{F/F}$;*Nestin-Cre* or *Atg7*$^{F/F}$; *Nestin-Cre*			
Purkinje cell-specific knockout of Atg5 or Atg7	Axonal dystrophy and degeneration of axon terminals; subsequent Purkinje cell death and cerebellar ataxia		Komatsu et al., 2007
atg7$^{flox/flox}$;Pcp2-Cre			
Central nervous system-specific knockout of FIP200	Accumulation of ubiquitinylated proteins as inclusion bodies in Purkinje neurons, cerebellar cell loss, and cortical spongiosis		Liang et al., 2010
FIP200$^{F/F}$;*Nestin-Cre*			
General knockout of HDAC6	Intracellular ubiquitin^{+} aggregates in the brain from 6 months of age	Neurodegeneration	Lee et al., 2010b
HDAC6$^{-/-}$			
General knockout of PINK1	Selective mitochondrial defect (complex I + II) in the striatum	Parkinson's disease	Gautier et al., 2008
PINK1$^{-/-}$			
Dynein mutations (fly and mouse models)	Premature aggregate formation of mutant huntingtin with increased autophagosome formation and impaired autophagosome-lysosome fusion	Huntington's disease, motor neuron diseases	Ravikumar et al., 2005
Liver- and spleen-specific interferon-γ-inducible knockout of Atg7	Hepatomegaly, accumulation of peroxisomes, deformed mitochondria and ubiquitin-positive inclusions in hepatocytes, hepatocyte death (increased serum level of alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase); accumulation of p62/STQM and consequent NRF2 activation	Hepatopathy	Komatsu et al., 2010
Atg7$^{flox/flox}$;*Mx1-Cre*$^{+}$			
Hepatocyte-specific knockout of Atg7	Lipid droplets containing triglycerides and cholesterol accumulate in liver cells; ER stress in hepatocytes and insulin resistance	Metabolic syndrome, steatohepatitis	Singh et al., 2009; Yang et al., 2010
Atg7$^{F/F}$;*Alb-Cre*			
Skeleton-muscle specific knockout of Atg7	Muscle atrophy and age-dependent decrease in force; accumulation of abnormal mitochondria, sarcoplasmic reticulum distension, disorganization of sarcomere; enhanced muscle loss after fasting or denervation	Sarcopenia	Masiero et al., 2009

Table 1. *Continued*

Autophagy Deficiency and Genotype	Phenotype	Disease Relevance	Reference
Atg7$^{flox/flox}$;*Myosin light chain-Cre*$^{+}$			
Islet ß cell-specific knockout of Atg7	Accumulation of ubiquitinated protein aggregates colocalized with p62, mitochondrial swelling, endoplasmic reticulum distension, and vacuolar changes; degeneration of islets and impaired glucose tolerance with reduced insulin secretion; enhanced susceptibility to high-fat diet-induced diabetes; this phenotype can be improved by feeding the antioxidant N-acetylcysteine	Insulin-dependent diabetes	Jung et al., 2008; Ebato et al., 2008; Wu et al., 2009
Atg7$^{flox/flox}$;*RIP2-Cre*$^{+}$			
Cardiomycyte-specific constitutive knockout of Atg5	No cardiac phenotype in baseline conditions but enhanced susceptibility to acute pressure overload-induced cardiac dysfunction and left ventricular dilatation	Dilated cardiopathy	Nakai et al., 2007
Atg5$^{flox/flox}$;MLC2v-*Cre*$^{+}$			
Podocyte-spccific constitutive knockout of Atg5	Spontaneous age-dependent late onset glomerulosclerosis with accumulation of oxidized and ubiquitinylated proteins, compensatory proteasome activation, lipofusin, damaged mitochondria, ER stress, albuminuria, podocyte loss	Glomerulosclerosis	Hartleben et al., 2010
Atg5$^{flox/flox}$;Podocin-Cre^{+}	Enhanced susceptibility to glomerulopathy induced by injections of doxorubicin or puromycin aminonucleoside		

autophagy in a Sir2-dependent fashion in yeast and *C. elegans*, and the knockout or knockdown of *atg* genes abolishes the life span-prolonging effect of CR, resveratrol, and Sir2 overexpression (Morselli et al., 2010). Thus, autophagy is required for the life span-prolonging effect of SIRT1.

How SIRT1 triggers autophagy is not entirely clear. SIRT1 is an NAD^{+}-dependent deacetylase that acts both in the nucleus and in the cytoplasm (Haigis and Sinclair, 2010). A cytoplasmic variant of SIRT1 is as efficient as wild-type SIRT1 in inducing autophagy, and resveratrol induces autophagy in enucleated cells, suggesting that SIRT1 can induce autophagy through nonnuclear effects (Morselli et al., 2011). Accordingly, SIRT1 deacetylates several *atg* gene products (ATG5, ATG7, and ATG8/LC3) (Lee et al., 2008), and resveratrol induces the deacetylation of dozens of cytoplasmic proteins (Morselli et al., 2011). SIRT1 also deacetylates the transcription factors p53, NF-κB, HSF1, FOXO1, -3, -4, and PGC1α, all of which have widespread effects in life span regulation and simultaneously influence SIRT1 expression (Saunders and Verdin, 2009). In *C. elegans*, life span extension by *sir-2.1* requires the worm forkhead protein DAF-16/FOXO (which is directly activated by *sir-2.1*) but may not require an intact insulin signaling pathway (Wang and Tissenbaum, 2006).

These results underscore the importance of DAF-16/FOXO for determining life span. Indeed, genetic variations in one of the human four *FOXO* orthologs, *FOXO3A*, have been linked to longevity in multiple population studies (Kenyon, 2010). In nematodes, the longevity of *daf-2* mutants (*daf-2* encodes a hormone receptor similar to the insulin and IGF-1 receptors) is abolished by inactivation of *daf-16* as well as by genetic inhibition of autophagy. However, DAF-16/FOXO is not required for the induction of autophagy by *daf2* (Hansen et al., 2008). These results have been interpreted to mean that autophagy frees up new resources for the cell but that transcription factors like the DAF-16/FOXO protein must channel this raw material into new cell-protective proteins in order for life span to be increased (Hansen et al., 2008) or, alternatively, that DAF-16/FOXO regulates the expression of proteins that affect autophagy-independent aspects of longevity.

Nonetheless, transgenic overexpression of DAF-16/FOXO enhances autophagy in *C. elegans* (Jia et al., 2009), suggesting that FOXO transcription factor may have a direct impact on autophagy. There are also data in mammals suggesting that FOXO3a can stimulate autophagy in response to starvation or SIRT1 activation. In primary mouse renal cells, SIRT1 is activated by CR and deacetylates FOXO3a, thereby favoring the transactivation of *bnip3*, which codes for a potent autophagy inducer (Kume et al., 2010). Similarly, FOXO3a inhibition or depletion prevents autophagy induction by starvation in vivo in the mouse muscle (Mammucari et al., 2007), confirming a strong link between transcription factors of the FOXO family and autophagy. It has not been investigated whether deacetylation (and consequent inactivation?) of the p53 ortholog CEP1 is required for longevity extension by *sir-2.1* in *C. elegans*. However,

Table 2. Associations between Autophagy and Aging in Animals

Longevity-increasing/Anti-aging Manipulations	Phenotype	Relationship to Autophagy	Reference
daf-2 (Insulin/IGF-1 receptor) loss-of-function mutant in *C. elegans*	Enhanced autophagy and life span	Abolishment of longevity phenotype in animals with loss-of-function mutations of *bec-1* or upon *atg-7* or *atg-12* RNAi	Melendez et al., 2003
Dietary restriction mutant eat-2 (ad1113) in *C. elegans*.		Loss of longevity phenotype after *bec-1* and *atg7* RNAi	Jia and Levine, 2007
Calcineurin (*cnb-1)* null mutant (jh103) in *C. elegans*		Loss of longevity phenotype after *bec-1* and *atg7* RNAi	Dwivedi et al., 2009
Administration of rapamycin to *C. elegans*		Loss-of-function mutations of Atg1 or Atg7 abolish life span extension	Alvers et al., 2009
Administration of spermidine to *C. elegans*		Loss of longevity phenotype after *bec-1* RNAi	Eisenberg et al., 2009
Administration of resveratrol to *C. elegans*		Loss of longevity phenotype after *bec-1* RNAi	Morselli et al., 2010
Transgenic expression of Atg8a in the brain of *D. melanogaster*	Counteracts the age-associated loss of Atg8a expression; increased life span (up to 56% in females), reduced accumulation of insoluble ubiquitinated proteins and carbonylated proteins, increased resistance against H_20_2	Direct increase in autophagy within neurons	Simonsen et al., 2008
Transgenic expression of FOXO in the muscles of *D. melanogaster*	Improved proteostasis and muscle function during aging; enhanced longevity	Enhanced muscle autophagy; knockdown of Atg7 enhances the deposition of polyubiquitinated protein aggregates	Demontis and Perrimon, 2010
Administration of rapamycin to *Drosophila*	Enhanced autophagy and life span	Loss of longevity phenotype after *atg-5* RNAi	Bjedov et al., 2010
Administration of spermidine to *Drosophila*	Enhanced autophagy and life span	Loss-of-function mutations of *atg-7* abolish life span extension	Eisenberg et al., 2009
Increase of LAMP-2A abundance by means of an inducible, hepatocyte-specific transgene in mice; LAMP2a-Alb-Tet-off	Reduced abundance of oxidized proteins, polyubiquinated protein aggregates, and $TUNEL^+$ cells	Restoration of chaperone-mediated autophagy and macroautophagy in livers from aged animals	Zhang and Cuervo, 2008
Cardiomyocyte-specific expression of a dominant-negative PI3Kα (p110α)	Enhanced autophagy, reduced lipofuscin levels in the heart, and increased longevity	Same as above	Inuzuka et al., 2009
Knockin mutation of huntingtin, causing the deletion of the polyglutamine stretch (ΔQ); $Htt^{\Delta Q/\Delta Q}$	Increased longevity of mice; the ΔQ Htt protein induces autophagy in vitro	Same as above	Zheng et al., 2010
Administration of rapamycin to mice	Extension of maximum life span by up to 14% in males and females accompanied by mTOR inhibition	Same as above	Harrison et al., 2009

knockdown of CEP1 is sufficient to induce massive autophagy in adult nematodes (Tasdemir et al., 2008) and to increase their life span. Both of these effects are lost upon simultaneous knockdown of *atg* genes (Tavernarakis et al., 2008).

In yeast, sir2p catalyzes histone H4 lysine 16 deacetylation. An H4 K16Q mutation (which mimics deacetylation) suffices to increase life span (Dang et al., 2009). Spermidine, which acts as a histone acetylase inhibitor, reduces histone acetylation while it upregulates the expression of *atg* genes, induces autophagy, and extends longevity. The spermidine-induced increase in longevity is lost in yeast, nematodes, and flies when *atg* genes are knocked out or knocked down (Eisenberg et al., 2009) yet is not affected by the depletion of SIRT1 orthologs (Morselli et al., 2011). Thus, enzymes with opposing functions on protein acetylation, the deacetylase SIRT1 (and its orthologs), and a range of histone acetylases increase autophagy-dependent longevity when they are activated or inhibited, respectively. Although it remains to be determined which acetylation reactions are the most relevant for the regulation of autophagy, it is noteworthy that both resveratrol and spermidine stimulate overlapping deacetylation reactions of cytoplasmic proteins (Morselli et al., 2011).

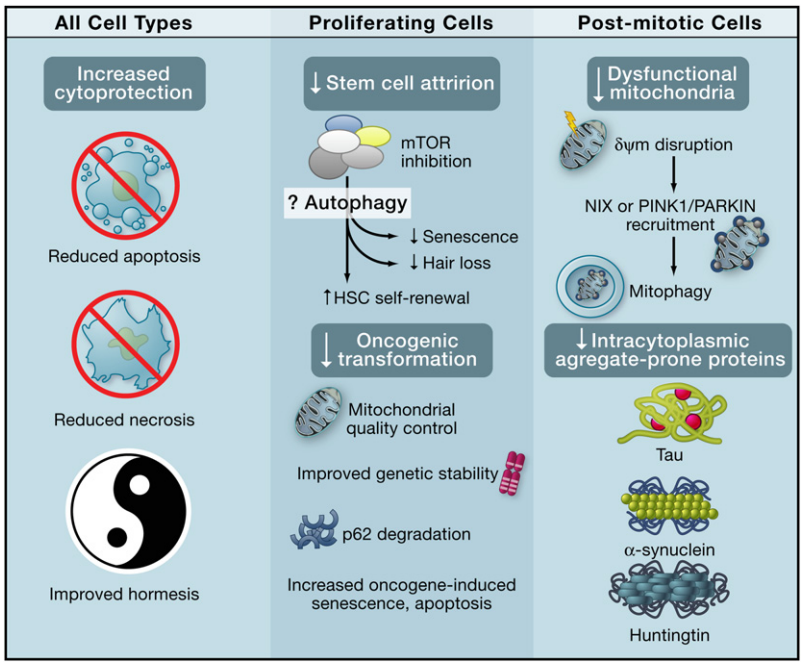

Figure 3. Cell-Autonomous Anti-Aging Effects of Autophagy

Autophagy may increase organismal fitness by inhibiting cell death, reducing oncogenic transformation, or increasing hormesis, both in quiescent and dividing cells (left). In addition, autophagy may contribute to life span extension through distinct mechanisms in postmitotic (middle) and proliferating cells (right). HCS, hematopoietic stem cell.

As mentioned earlier, Huntington's disease (HD) is caused by a polyglutamine expansion in the *huntingtin* gene. Normal mice have seven glutamines in this gene. Mice that are homozygous for an artificial mutation in *huntingtin* (*htt*) from which the polyglutamine tract has been completely removed (ΔQ) demonstrate an extension in life span that is associated with an increased level of autophagy (Zheng et al., 2010). Although ΔQ htt induces autophagosome formation in an mTOR-independent manner and this is not seen with the wild-type protein (Zheng et al., 2010), it is possible that this mutant protein may have other functions that contribute to the longevity of these mice.

Altogether, accumulating yet fragmentary evidence indicates that genetic or pharmacological manipulations that result in an increase in longevity stimulate autophagy and that autophagy is often, if not always, required for mediating the increase in life expectancy.

Anti-Aging Effects of Autophagy in Postmitotic Cells

Autophagy plays a major role in protein homeostasis (proteastasis) and organelle turnover. This role is particularly important in nonproliferating cells because there is no cell division-mediated "dilution" of intracellular debris. Moreover, the anti-aging effects of cytoprotection are particularly relevant for cells that are not (or are poorly) replaced from stem cell niches (Figure 3).

Reduced Accumulation of Toxic Protein Aggregates

Intracellular protein misfolding and aggregation are the cardinal features of many neurodegenerative diseases called proteinopathies. These include Alzheimer's disease, Parkinson's disease, tauopathies, and polyglutamine expansion diseases such as HD. A number of experimental autophagy inducers, including rapamycin, rapalogs, valproate, and lithium, can attenuate the accumulation of mutated huntingtin protein and cell death in HD models (Ravikumar et al., 2010). Rapamycin-stimulated autophagy may be instrumental for the treatment of other intracellular proteinopathies, including those induced by mutant and wild-type Tau (Ravikumar et al., 2010). Inhibition of TOR by transgenic overexpression of TSC1 and TSC2 also stimulates the clearance of a human polyglutamine-expanded huntingtin protein in vivo in photoreceptor neurons from *Drosophila*, and a similar effect can be obtained by overexpressing ATG1 (Wang et al., 2009). Overexpression of TSC1 and TSC2 can avoid the accumulation of cytosolic rhodopsin-arresting complexes that result from the neurodegeneration-inducing mutation of the phosphilase C gene *norpA* (Wang et al., 2009), further supporting the notion that autophagy can attenuate proteotoxicity.

Misfolded, aggregated, and ubiquitinylated proteins also accumulate in nondividing cells, in particular, neurons of patients during normal aging. However, it has not been formally demonstrated that such protein aggregates are causally involved in the aging process (instead of merely constituting a sign of aging) and whether the anti-aging effects of autophagy may be explained by (rather than correlated with) the removal of such inclusion bodies.

Improved Function of Mitochondria

Reportedly, mitochondrial fission can occur in an asymmetric fashion, yielding one functional mitochondrion (that undergoes successive rounds of fusion and fission) and one dysfunctional one with a low membrane potential ($\Delta\Psi_m$) that is targeted for autophagic destruction (Twig et al., 2008). This mechanism may contribute to autophagy-dependent mitochondrial quality control. One prominent hypothesis of aging postulates an accumulating mitochondrial damage leading to progressive mitochondrial uncoupling with a consequent bioenergetic insufficiency and increased production of reactive oxygen species (ROS) (Vijg and Campisi, 2008). As a common leitmotif, autophagy inhibition results in deteriorated mitochondrial function in nonmammalian model organisms and in mice. For instance, isolated mitochondria from ATG-deficient postmitotic cells (e.g., in skeletal muscles lacking expression of Atg7) exhibit a defective oxidative phosphorylation tied to a switch of cellular metabolism from respiration to glycolysis (Wu et al., 2009). Similarly, mitochondria from the striatum of PINK1 knockout mice are deficient in complexes I and II (Gautier et al., 2008).

These examples illustrate the concept that the deficient quality control of dysfunctional mitochondria may lead to their

pathogenic accumulation and that autophagy is responsible for this quality control.

Reduced Cell Death and Improved Hormesis

Autophagy is a potent mechanism that reduces unwarranted apoptotic or necrotic death of postmitotic cells. The induction of autophagy is coupled to the liberation of Bcl-2 from the Beclin 1 complex, as well as that of FLIP from Atg3, potentially allowing Bcl-2 and FLIP to become available to acutely block the intrinsic and extrinsic pathways of apoptosis, respectively (Lee et al., 2009; Pattingre et al., 2005). It remains to be determined to what extent this putative mechanism might tie autophagy induction to acute cytoprotection.

Beyond its involvement in organellar quality control and the removal of potential proteotoxins, autophagy plays an essential role in the recovery of acutely injured cells with damaged mitochondria that otherwise would undergo apoptosis or necrosis (Colell et al., 2007). Mitochondrial membrane permeabilization (MMP) is (one of) the rate-limiting steps of cell death, and the threshold beyond which MMP affecting a variable portion of mitochondria kills cells is likewise determined by the autophagic removal of permeabilized organelles (Kroemer et al., 2007). If the most damaged mitochondria are selectively removed by autophagy, the remaining mitochondria will likely have a higher threshold for MMP, greater resistance to cytochrome *c* release, and diminished ROS production.

Through this and related mechanisms, autophagy might participate in hormesis, the phenomenon whereby cells, organs, and organisms resist usually lethal conditions after they have been exposed to a sublethal damage. One of the best-explored examples of hormesis is ischemic preconditioning, the exposure of the brain or heart to short episodes of ischemia that renders these organs (relatively) resistant against subsequent stroke or infarction, respectively. Autophagy has been shown to participate in the beneficial effects of preconditioning (Gottlieb and Mentzer, 2010; Sheng et al., 2010). If hormesis determined the pace of aging and death, as has been postulated (Le Bourg, 2009), and if autophagy were one of the effector mechanisms of hormesis, for instance as a cleansing mechanism of damaged organelles (Ristow and Zarse, 2010), then improved hormesis might constitute one of the links between autophagy and longevity.

Anti-Aging Effects of Autophagy in Proliferating Cells

In proliferating cells, autophagy not only mediates cytoprotective effects as those evoked above for postmitotic cells, but probably also plays a major role in the avoidance of malignant transformation, as well as possibly in the maintenance of stem cell characteristics (Figure 3).

Avoidance of Stem Cell Attrition

Progressive numerical or functional decline of tissue stem cells may contribute to the aging phenotype. In old mice, rapamycin increases life span (Harrison et al., 2009) and simultaneously restores the self-renewal of hematopoietic stem cells (HSCs), thus improving the function of the immune system (Chen et al., 2009). Similarly, rapamycin can reverse the stem cell loss in hair follicles that overexpress Wnt1 under the control of an inducible promoter, as well as the consequent alopecia (Castilho et al., 2009). Conversely, mTOR activation through conditional deletion of Tsc1 in the HSCs of young mice mimics the phenotype of HSCs from aged mice, leading to a relative decrease in lymphopoiesis, an impaired capacity to reconstitute the hematopoietic system, as well as an increased expression of the CDK inhibitors p16(Ink4a), p19(Arf), and p21(Cip1), which are potential markers of cellular senescence (Chen et al., 2009). Though other mTOR-related functions may contribute, at least part of this phenotype may be mediated by deficient autophagy because *atg7* knockout leads to a severe attrition of the hematopoietic stem cell and progenitor cell compartments (Mortensen et al., 2011).

Similarly, inactivation of the tumor suppressor PTEN elicits a senescence response that is different than the oncogene-elicited DNA damage-dependent program and has been termed PTEN loss-induced cellular senescence (PICS). PICS depends on the activation or mTOR and can be potentiated by inhibition of MDM2, which leads to an increase in p53 expression (Alimonti et al., 2010) and can be expected to inhibit autophagy (Tasdemir et al., 2008).

Rapamycin can avoid the permanent cell-cycle arrest (senescence) that is artificially induced by inducible p21 expression without affecting the acute block in cell-cycle advancement (Blagosklonny, 2009). Thus, mTOR is likely to restrain proliferative potential (competence) and to mediate stem cell attrition through senescence, an effect that can be suppressed by rapamycin. Whether this effect of TORC1 is mediated by autophagy or alternative rapamycin-elicited effects awaits urgent clarification, and thus far no studies have addressed the importance of autophagy for the maintenance of stem cell characteristics.

Reduced Oncogenic Transformation

Several oncogenes (for instance, activated Akt1, PI3K, and anti-apoptotic proteins from the Bcl-2 family) suppress autophagy. In addition, multiple tumor suppressor proteins (such as BH3-only proteins, death-associated protein kinase-1 [DAPK1], PTEN, tuberous sclerosis complex 1 and 2 [TSC1 and TSC2], and LKB1/STK11) stimulate autophagy, meaning that their loss reduces autophagy. Beclin-1, which is required for autophagy induction, acts as a haploinsufficient tumor suppressor protein, and other essential autophagy mediators (such as Atg4c, AMBRA1, UVRAG, and Bif-1) are also bona fide oncosuppressors because their genetic invalidation increases the frequency of tumors in mice (Maiuri et al., 2009).

Autophagy can suppress oncogenesis through cell-autonomous effects such as improved quality control of mitochondria, ameliorated genetic instability, and/or the autophagic removal of the potentially oncogenic protein p62/SQTM1 (Mathew et al., 2009). According to yet-to-be confirmed reports, autophagy is upregulated during and partially required for oncogene-(H-*ras*V12)-induced senescence (Young et al., 2009) and apoptosis (Elgendy et al., 2011), suggesting that autophagy can contribute to tumor suppression at this level.

Cell-Nonautonomous Anti-Aging Effects of Autophagy

Beyond its action on proliferating or postmitotic cells, autophagy may also mediate anti-aging effects by cell-nonautonomous effects, including the defense against infectious microorganisms, innate immune and inflammatory responses, and neuroendocrine regulation (Figure 4).

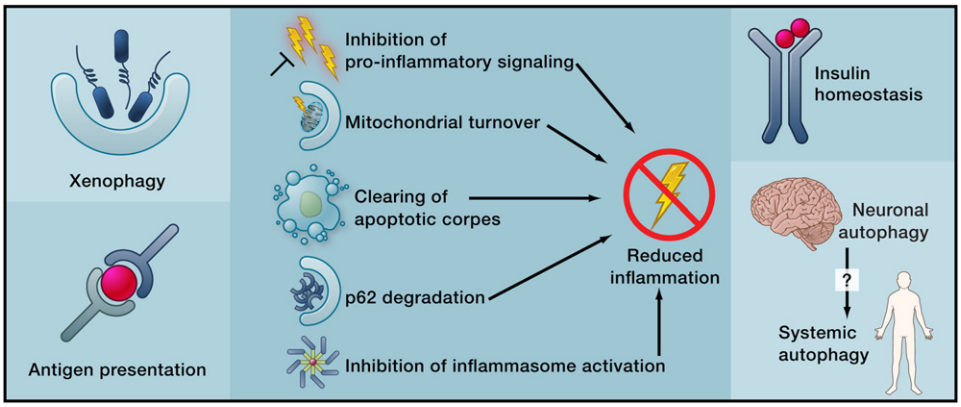

Figure 4. Systemic Anti-Aging Effects of Autophagy
Beyond its cell-autonomous action, autophagy can reduce age-related dysfunctions through systemic effects. Autophagy may contribute to the clearance of intracellular pathogens and the function of antigen-presenting cells (left), reduce inflammation by several mechanisms (middle), or improve the function of neuroendocrine circuits (right).

Improved Innate Immune and Reduced Inflammatory Responses

The molecules and processes involved in autophagy play a major role in the control of intracellular microorganisms by "xenophagy," the cell-autonomous response against pathogen invasion (Levine et al., 2011). Autophagy within dying antigen donor cells can improve the cross-presentation of dead-cell antigens by dendritic cells, perhaps because autophagosomes ferry antigens to dendritic cells via an as yet unknown mechanism (Li et al., 2008) or because higher amounts of type I interferon are induced (Uhl et al., 2009). Moreover, in dendritic cells, autophagy induction improves antigen presentation (Jagannath et al., 2009), and several major immune effectors, including T lymphocytes, rely on autophagy for the maintenance of their function (Levine et al., 2011).

Autophagy can mitigate inflammatory reactions through several mechanisms. Autophagy in dying cells is required for optimal macrophage-mediated clearing of apoptotic corpses, thus reducing inflammatory reactions (Levine and Kroemer, 2008). As cells succumb to apoptosis, autophagy is often induced until the ultimate state of the apoptotic process that is usually accompanied by caspase activation. Autophagy is essential for the maintenance of intracellular ATP levels, which in turn are required for the secretion of the "find-me" signal lysophosphatidylcholine by, as well as the efficient exposure of the "eat-me" signal phosphatidylserine on, dying cells. This explains how insufficient autophagy can stimulate inflammatory responses secondary to insufficient clearance of dead cells (Orvedahl et al., 2007). Moreover, disabled autophagy provokes the accumulation of p62/STQM1, which can activate the proinflammatory transcription factor NF-κB and the stress-responsive transcription factor NRF2, thereby favoring inflammation and tissue injury (Levine et al., 2011).

Autophagy can inhibit proinflammatory signaling via RIG-I-like receptors, via direct conjugation of the receptors with Atg12-Atg5 complexes, and through elimination of dysfunctional mitochondria (Saitoh and Akira, 2010). Autophagy can also inhibit the activation of the NLRP3 inflammasome, the caspase-1 activation complex required for the production of interleukin-1β, by removing permeabilizing or ROS-producing mitochondria (Nakahira et al., 2011; Zhou et al., 2011). Because neurodegenerative processes and pathological aging in general are frequently accompanied by chronic inflammation, these anti-inflammatory effects of autophagy may mediate additional health benefits.

Altogether, the available data suggest that autophagy may contribute to improving the efficacy of pathogen recognition by immune effectors while reducing inflammatory reactions.

Neuroendocrine Parameters

The absence or presence of autophagy does not only affect cellular physiology but likely can affect intercellular communication and hence affect aging-relevant processes in a noncell-autonomous manner.

Beyond the fact that the function of endocrine cells, including insulin-producing β cells, depends on autophagy (Wu et al., 2009), deficient autophagy can also compromise the hormonal response of tissues. Thus, age-associated defects in autophagy might be implicated in type-2 diabetes, in which peripheral tissues fail to respond to insulin. Thus, hepatic suppression of the *Atg7* gene results in ER stress and insulin resistance (Yang et al., 2010). Overexpression of FOXO or its target 4E-BP in the muscles of flies abolishes the age-associated decline in autophagy and increases longevity while it reduces food intake and insulin release from neurosecretory cells (Demontis and Perrimon, 2010), suggesting that maintenance of a normal autophagy level in one organ may positively affect whole-body metabolism.

Induction of autophagy in *Drosophila* neurons (by overexpressing of an ATG8 transgene under the control of a neuron-specific promoter) is sufficient to increase life span (Simonsen et al., 2008). Similarly, overexpression of dSIR2 in neurons is sufficient to increase the longevity in flies as much as the expression of dSIR2 under the control of an ubiquitous promoter (Rogina and Helfand, 2004). This suggests that autophagy induction in specific neuronal populations may be sufficient to reduce pathological aging. How this effect is achieved is not known, as whether the manipulation of autophagy in neurons might affect

autophagy in peripheral tissues has not been investigated. However, in *C. elegans*, in *daf-16/daf-2* double mutants, re-expression of *daf-16* in only one tissue (such as neurons or the intestinal/adipose tissue) can extend the life span of the whole animal (Murphy et al., 2007), presumably through feedback regulation of insulin gene expression in other tissues.

Concluding Remarks

As outlined in this Review, the level of macroautophagy may play a major role in determining health span and life span, although two major and presently near-unresolvable caveats apply. First, autophagy is not simply one uniform bulk degradation process mediated by a single set of stimuli, as it may also involve the selective removal of organelles, such as dysfunctional (mitochondria) and various infectious agents using additional machinery and signals. This raises the potential challenge of identifying specific pathways and substrates of autophagy with anti-aging properties. Second, all pharmacological and genetic manipulations of autophagy have off-target effects. Thus, even the products of *Atg* genes have multiple functions outside of the autophagic pathway, meaning that their overexpression or ablation may influence the aging process through autophagy-unrelated effects that must be studied in the future. Notwithstanding these limitations, it is possible that the aging process involves one or several vicious cycles that cause a progressive deterioration of the autophagic system. Thus, at the cellular level, insufficient autophagy compromises the degradation of ubiquitin-proteasome pathway substrates due to the accumulation of p62/SQSTM, resulting in higher levels of short-lived regulatory proteins like p53 (Korolchuk et al., 2009), which in turn can inhibit autophagy (Tasdemir et al., 2008) and induce cellular senescence. Similarly, defective autophagy in the brain may stimulate a systemic breakdown in neuroendocrine homeostasis that accelerates the physiological and neurological decline. Defective autophagy may also favor the accumulation of lipofuscin and lipid droplets, which in turn can inhibit autophagy (Singh et al., 2009).

We acknowledge that much of the data supporting the anti-aging effect of autophagy are from model organisms in which the causes of death/longevity may differ from what impacts human life span and health span. Ideally, one would like to be able to test the effects of possible genetic or environmental influences regulating autophagy with differences in human life span in carefully designed epidemiological studies. One current limitation in this context is the lack of suitable assays for measuring autophagic flux in living humans (Mizushima et al., 2010). However, it is likely that the physiological processes associated with longevity of model organisms may protect against diseases that afflict people. Indeed, it is worth noting that major causes of death in developed countries, such as heart disease, dementias, and cancers, may be enhanced by autophagy inhibition and hence perhaps attenuated by autophagy upregulation. As discussed here, even interventions aimed at stimulating autophagy at a relatively mature age may have beneficial effects on aging in mice. Thus, rapamycin treatment of middle-aged mice (starting at 600 days) still can extend their life span (Harrison et al., 2009), and a 3 month period of caloric restriction can improve verbal memory in healthy aged humans (Witte et al., 2009), suggesting that pro-autophagic strategies may reduce the time-dependent deterioration and even provide a transient rejuvenation if applied to the healthy elderly.

It is then the question of what the optimal strategy would be to improve healthy aging by enhancing autophagy without deleterious side effects. Intermittent fasting, for instance by alternating days of starvation with days of ad libidum feeding, can increase the life span of rodents as much as chronic caloric restriction without a concomitant major decrease in body mass—a measure that may avoid the negative effects of caloric restriction on bone density and consequent pathological fractures (Mattson and Wan, 2005). Moreover, it is tempting to speculate, though it remains to be demonstrated, that a diet enriched in natural pro-autophagic components including spermidine and polyphenols (such as resveratrol or curcumin) might promote good health and postpone our inevitable fate.

ACKNOWLEDGMENTS

We apologize to all colleagues whose work we could not cite owing to space limitations. G.K. is supported by the Ligue Nationale contre le Cancer (Equipes labellisée), Agence Nationale pour la Recherche, AXA Chair for Longevity Research, European Commission (Apo-Sys, ArtForce, ChemoRes, Apop-Train), Fondation Bettencourt-Schueller, Fondation pour la Recherche Médicale, the LabEx Immuno-Oncology, Institut National du Cancer, and Cancéropôle Ile-de-France. D.C.R. is supported by the Wellcome Trust (Senior Fellowship), a Wellcome Trust/Medical Research Council Strategic Award on Neurodegenerative Diseases, Medical Research Council, and the National Institute for Health Research Biomedical Research Centre at Addenbrooke's Hospital.

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