Can autophagy promote longevity?

Frank Madeo, Nektarios Tavernarakis and Guido Kroemer

Organismal lifespan can be extended by genetic manipulation of cellular processes such as histone acetylation, the insulin/IGF-1 (insulin-like growth factor 1) pathway or the p53 system. Longevity-promoting regimens, including caloric restriction and inhibition of TOR with rapamycin, resveratrol or the natural polyamine spermidine, have been associated with autophagy (a cytoprotective self-digestive process) and in some cases were reported to require autophagy for their effects. We summarize recent developments that outline these links and hypothesize that clearing cellular damage by autophagy is a common denominator of many lifespan-extending manipulations.

The most prominent and best-studied form of autophagy is macroautophagy (hitherto referred to as 'autophagy'), where portions of the cytoplasm are sequestered within autophagosomes and then targeted to lysosomes for digestion[1]. Autophagy has often been regarded as a self-destructive process, contributing to 'autophagic cell death'. However, most data indicate that autophagy rather serves a cytoprotective function. When dying cells display autophagic vacuolization most die with features of (rather than by) autophagy, which has been activated as an unsuccessful homeostatic counter-reaction against cell death[2]. For example, during metabolic stress triggered by nutrient depletion and hypoxia, autophagy improves cellular survival, and hence enhances cellular fitness, whereas its inhibition precipitates bioenergetic failure and cell death[3]. Suppression of autophagy by knockout or knockdown of essential Atg (autophagy-related) genes often leads to apoptotic or necrotic demise of cells that would otherwise survive in conditions of elevated stress[4–7]. Conversely, induction of autophagy can enhance resistance of cells to distinct kinds of stress. This can even be observed at the organismal level. Fasting induces autophagy in most somatic cells[8] of an organism. It also leads to an increased tolerance of flies against anoxia and of mice against the lethal side-effects of high-dose chemotherapies[9,10]. Intriguingly, caloric restriction (while maintaining adequate nutrition) induces autophagy in non-mammalian model organisms and it is the only food-based manipulation that extends lifespan in all species tested so far. Recent data indicate that the specific induction of autophagy can increase longevity in multiple animal species, as will be discussed in this article.

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Genetic manipulations that affect lifespan extension and autophagy

Numerous conserved genes known to increase lifespan were originally identified using the yeast, *Saccharomyces cerevisiae*, as a model[11–13]. Sirtuin 1, a phylogenetically conserved NAD^+-dependent histone deacetylase, was identified in a screen for genes that increase replicative lifespan in yeast[11]. Transgenic overexpression of *sirtuin 1* increases the longevity of *Drosophila melanogaster* and *Caenorhabditis elegans*[14,15], and inhibits apoptosis in mammalian cells[16]. Epigenetic deacetylation of histones has therefore been regarded as a key process during physiological ageing of various organisms[17,18], and genetic manipulation of histone acetylation status has been repeatedly shown to influence cellular and organismal lifespan[19,20]. Interestingly, transgenic expression of *sirtuin 1* induces autophagy in mammalian cells *in vitro*[21,22] and in *C. elegans in vivo*[22]. Although it has not been excluded that autophagy induction by *sirtuin 1* is mediated through deacetylation of histones, *sirtuin 1* overexpression also results in the deacetylation of several cytoplasmic proteins, including major regulators of autophagy (such as AMP-dependent kinase, AMPK) and autophagy-relevant gene products (such as Atg5, Atg7 and Atg8/LC3)[21]. Deletion or depletion of Beclin 1/ATG6 not only suppresses the induction of autophagy by *sirtuin 1* expression but also abrogates longevity in nematodes[23] (Fig. 1). Moreover, the knockdown, knockout or pharmacological inhibition of *sirtuin 1* prevents the induction of autophagy and the improvement of organismal or cellular survival by resveratrol (an indirect activator of *sirtuin 1*), nutrient starvation (in human cells) or caloric restriction (in *C. elegans*)[23]. Thus, *sirtuin 1* may be a major regulator of both autophagy and longevity, which seem to be interrelated.

Further implicating autophagy in lifespan regulation, *C. elegans* with a loss of function mutation in the insulin-like signalling pathway (*daf-2*) display extended lifespan and induction of autophagy[24,25]. The importance of this finding was later underscored by evidence that autophagy is required for caloric restriction effects on lifespan in *C. elegans*[26,27].

Simultaneous induction of lifespan extension and autophagy is also observed in *C. elegans* following knockdown of the p53 orthologue, CEP-1, and both effects are abolished by depleting Beclin 1/ATG6[28]. Further examples of longevity-promoting genetic manipulations that depend on autophagy are listed in Table 1. Consistent with the idea that

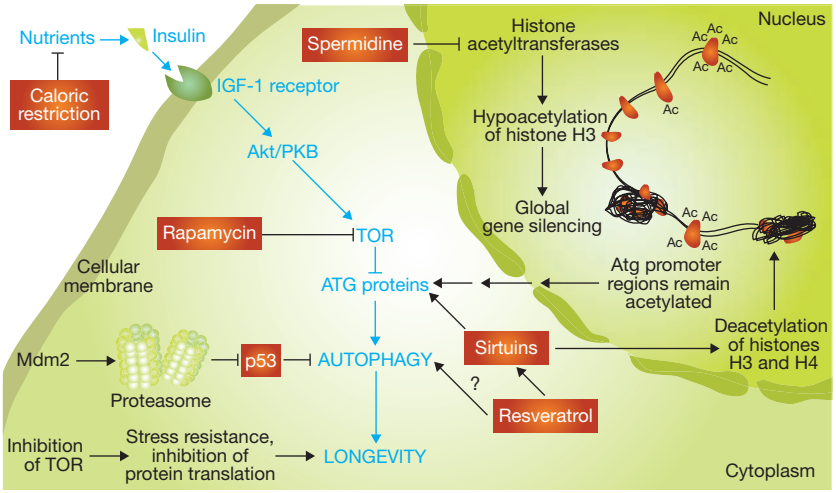

Figure 1 Linking different lifespan-prolonging treatments to autophagy. Summary of the genetic and pharmacological manipulations of autophagy that cause lifespan extension. Pharmacological treatment with spermidine, resveratrol or rapamycin, caloric restriction, depletion of p53 or overexpression of sirtuin 1 prolong life (red) and can be integrated in a single pathway, which controls autophagy (blue). Although spermidine probably functions through epigenetic (and hence nuclear) mechanisms, rapamycin and p53 depletion may act on cytoplasmic targets. Sirtuins may deacetylate both histones and cytoplasmic targets. Caloric restriction likewise acts by reducing the concentration of insulin-like growth factors and/or by activating sirtuin 1.

autophagy participates in longevity-extending processes, a screen for genes regulating clonogenic cell death during the chronological ageing process of yeast revealed that deletion of most autophagy-related genes enhances cell death and induces premature ageing[19,29].

As a caveat, it should be noted that in many cases autophagy has been causally linked to longevity using experimental evidence involving the deletion or depletion of essential regulators of autophagy, such as Beclin 1 (ref. 30), that may participate in diverse cellular functions. Such manipulations *per se* are sufficient to disturb cellular homeostasis, and thereby reduce cell survival and lifespan. Hence, even models where a treatment that prolongs the life of wild-type subjects but fails to do so in autophagy-defective subjects may not be the ultimate proof that longevity promotion depends solely on autophagy induction. For instance, defective autophagy may induce homeostatic feedback mechanisms that also have an impact on ageing, such as changes in protein translation or perhaps even through inhibition of TOR (target of rapamycin)[31].

Pharmacological manipulations that affect lifespan extension and autophagy

Rapamycin, the best-characterized pharmacological autophagy inducer, functions by inhibiting TORC1 (target of rapamycin complex 1) and prolongs life in all studied organisms[18,32,33], including mice[34]. In *C. elegans* and in yeast, rapamycin extends lifespan only under conditions in which autophagy can be induced[28,33,35]. Rapamycin-induced autophagy is independent of sirtuin 1 in human cells and in *C. elegans*[23], suggesting that rapamycin and sirtuin 1 promote autophagy through distinct, non-overlapping mechanisms (Fig. 1). In mice, whether rapamycin prolongs longevity by autophagy remains to be determined, as autophagy has not been monitored or blocked in these experiments[34]. Thus, it is possible that rapamycin-fed mice display lifespan extensions through anti-inflammatory functions that are beneficial under laboratory conditions. Promoting those functions might become deleterious in the wild.

Resveratrol, a polyphenol present in red wine and an indirect activator of sirtuin 1, prolongs the lifespan of yeast[36], worms and flies[37]. In mice, resveratrol extends lifespan only if animals are kept on a high-calorie diet (and hence become obese)[38]. Resveratrol is also a potent inducer of canonical and non-canonical autophagy[39]. It only prolongs *C. elegans* lifespan if the animals express a functional *sirtuin 1* gene[23]. Knockdown of Beclin 1/ATG6 abolished the beneficial effects of resveratrol on longevity[22]. Taken together, the data indicate that resveratrol increases lifespan of *C. elegans* through sirtuin 1-dependent induction of autophagy. However, it is noteworthy that sirtuin 1 overexpression or resveratrol fails to extend the lifespan of mice on a normal diet. The ability of sirtuins to increase lifespan in lower eukaryotes is controversial, as in yeast they can also shorten chronological lifespan[40]. Moreover, the inhibition of sirtuin 1 actually prevents features of ageing (decreased IGF-1 signalling and increased protection from oxidative stress in neurons) in mice, suggesting that the notion of sirtuins as solely anti-ageing molecules constitutes an oversimplification[41,42]. In addition, recent evidence indicates that resveratrol does not activate sirtuin 1 directly[43], but rather functions promiscuously on various targets including receptors, ion channels, enzymes and transporters[43,44].

The polyamine spermidine has been recently discovered as a molecule that induces longevity and promotes autophagy. External administration of spermidine prolongs the lifespan of yeast, flies and worms in an autophagy-dependent fashion[19]. A diet enriched in physiologically relevant polyamines (that is, putrescine, spermidine and spermine) also increases lifespan and health span in mice, although the requirement for autophagy in this case has not yet been investigated[45]. In yeast, spermidine causes global hypo-acetylation of histone H3, but selective histone acetylation at the promoter region of the *ATG7* gene[19]. Thus, transcription of *ATG7* is enabled in the context of general gene silencing resulting in a relative upregulation of *ATG7* (and also of other *ATG* genes). Spermidine-mediated transcription of autophagy-relevant genes may account for the observed induction of autophagy. Spermidine was shown to inhibit histone acetyltransferases in cell-free assays. In support of a role for histone acetyltransferases in the lifespan of yeast, deletion of essential subunits in two histone acetyltransferases increased lifespan, and although treatment of this strain with polyamines further increased longevity, it was not to the same extent as that seen in wild-type cells[19]. However, by comparison to sirtuin 1, which affects autophagy through deacetylation of essential autophagy-relevant proteins[21], spermidine may induce autophagy through a combination of nuclear (transcriptional) and cytoplasmic (transcription-independent) mechanisms (Fig. 1). An exciting speculation is that inhibition of protein acetyltransferases (by spermidine) and activation of deacetylases (by resveratrol) might converge on similar autophagy regulators or effectors. However, the precise identity of these targets remains elusive and should thus constitute a focus of future research.

Table 1 Examples of longevity-promoting manipulations that depend on autophagy g manipulations that depend on autophagy

Age-protective autophagy			
Lifespan-prolonging measure	**Dependency on autophagy gene[1]**	**Species**	**Ref.**
Activation of sirtuin 1 by three alternative methods: transgenic overexpression of sirtuin 1, treatment with resveratrol, an allosteric activator of sirtuin 1 and depletion of nicotinamide, a negative regulator of sirtuin 1, by transgenic overexpression of the pyrazinamidase/ nicotinamidase *PNC-1*	*BEC-1*/*ATG-6* (RNAi)	*C. elegans*	22,23
Depletion of *p53*/*CEP-1* by RNA interference (RNAi)	*BEC-1*/*ATG-6* (RNAi)	*C. elegans*	28
Pharmacological treatment with rapamycin	*Atg7* (deletion) or *Atg1* (deletion) *ATG-5* (RNAi) *Atg5* (RNAi) Not determined	*S. cerevisiae* *C. elegans* *D. melanogaster* *M. musculus*	35 33 33 34
TOR deficiency	*UNC-51*/*ATG-1* or *BEC-1*/*ATG-6* or *ATG-18* (deletion)	*C. elegans*	27
External supply of spermidine	*ATG7* (deletion) Atg7 (deletion) BEC-1/ATG-6 (RNAi)	*S. cerevisiae* *D. melanogaster* *C. elegans*	19
Restoration of liver chaperone-mediated autophagy[2] and macroautophagy achieved by LAMP-2A maintenance in aged animals. Reduced abundance of oxidized proteins, polyubiquinated protein aggregates and TUNEL$^+$ cells.	*Lamp2* (LAMP-2A; hepatocytic expression)	*M. musculus*	71
RNAi knockdown of genes required for optimal mitochondrial activity, such as *atp-3* and *clk-1*	*UNC-51*/*ATG-1* or *BEC-1*/*ATG-6* or *ATG-18* (deletion)	*C. elegans*	27
Brain-specific overexpression of *Atg8a*	*Atg8a*	*D. melanogaster*	68
Dietary restricted mutant animals (*eat-2(ad1113)*)	*BEC-1*/*ATG-6* or *Ce-atg7* (RNAi) *UNC-51*/*ATG-1* or *BEC-1*/*ATG-6* (deletion)	*C. elegans*	26 27
Defective insulin/IGF-1 signalling mutants	*ATG-18* (RNAi) *BEC-1*/*ATG-6* (RNAi) *ATG-7* and *ATG-12* (RNAi)	*C. elegans*	27 24 72

[1]Text in parentheses indicates the mode of genetic manipulation.
[2]Chaperone-mediated autophagy is responsible for lysosomal degradation of a specific pool of cytosolic proteins.

Possible mechanisms linking autophagy to cytoprotection and longevity

Given that autophagy has some longevity-promoting effects and autophagy acts to promote removal of intracellular material, what are the targets responsible for these effects? As they age, organisms accumulate intracellular and macromolecular damage resulting from the action of multiple exogenous or endogenous agents. In the face of these 'ravages of time', all species have developed intricate protective strategies and repair mechanisms. Protein misfolding is thought to constitute a major driving force of age-associated disorders[46] and heat shock proteins together with the unfolded protein response (UPR) serve as effective safeguards against the deleterious consequences of relatively small protein aggregates[46, 47]. However, the destruction and recycling of larger protein aggregates (that is, inclusion bodies) and damaged organelles is accomplished through autophagy[1, 48]. Connections between autophagy, cell death and the ageing process (Fig. 2) are apparent in diverse diseases, including neurodegeneration, infections, inflammatory diseases and cancer.

Many reports indicate that the pharmacological stimulation of autophagy reduces the accumulation of toxic protein aggregates (such as mutated huntingtin, α-synuclein and β-amyloid, which are associated with Huntington's, Parkinson's and Alzheimer's disease, respectively)[46,47]. Inhibition of autophagy through brain-specific knockout of essential autophagy genes (such as *atg5* and *atg7*) in mice leads to the accumulation of neuronal inclusion bodies containing polyubiquitylated proteins. The appearance of these inclusion bodies correlates with premature neurodegeneration[47,49,50], though it is unclear whether the inclusion bodies are protective or promote the disease. Autophagy thus seems to reduce the accumulation of potentially toxic aggregates and, if proteotoxicity partly accounts for the 'normal' (non-disease-associated) ageing process, autophagy could mitigate the effects of ageing through this effect. Damaged mitochondria containing mutated mitochondrial DNA accumulate during ageing and are a production site of harmful reactive oxygen species; autophagy targets mitochondria that have lost their transmembrane potential[4,51], which may facilitate the removal of such damaged mitochondria.

Autophagy directly facilitates the destruction of intracellular infectious pathogens, a process referred to as 'xenophagy', wherein autophagy invigorates the innate immune response against microbial agents[1]. Autophagy is also required at multiple levels for the normal function of the immune system and for avoiding unwarranted inflammatory reactions. For example, pre-apoptotic autophagy in antigen donor cells — either virus-infected or cancer cells — is necessary for the optimal transfer of antigens to dendritic cells and/or their subsequent presentation to T lymphocytes[52,53]. Autophagy is also indispensable for dying cells to emit the 'find-me' signal, lysophosphatidylcholine (a chemo-attractant for macrophages), as well as to efficiently expose the apoptotic 'eat-me' signal, phosphatidylserine, on the plasma membrane surface (which facilitates the engulfment of dying cells). Insufficient autophagy thus reduces the efficacy of corpse removal: this causes cells that would otherwise be engulfed during apoptosis to undergo secondary necrosis[1,54,55], which subsequently triggers inflammatory responses. In this context, it seems particularly intriguing that spermidine was first identified by its ability to alleviate programmed, age-associated necrosis in yeast cells, as well as in human peripheral blood mononuclear cells[19]. Whether this 'anti-necrotic' action contributes to the anti-ageing effects of autophagy inducers remains an open possibility.

Autophagy can prevent apoptotic and necrotic cell death in multiple cellular models; we have yet to understand whether this cytoprotection is

linked to autophagy-dependent longevity. The inhibition of apoptotic or necrotic cell death can extend longevity in various lower eukaryotes. For example, deletion of the sole caspase-like protease prolongs lifespan of both *Podospora anserina* and *S. cerevisiae*[56,57]. However, whether apoptosis is a pro- or anti-ageing mechanism in higher organisms is a subject of considerable debate. The Bax protein is a central regulator of apoptosis that causes mitochondrial outer membrane permeabilization, a likely 'point of no return' in apoptosis execution. Age-induced increase in Bax levels could contribute to age-associated atrophy in skeletal and heart muscle of rats[58,59]. Also, an intriguing link between anti-ageing mechanisms and apoptosis inhibition may connect Bax activation to Sirtuins. Sirtuin 1 deacetylates the DNA repair factor Ku70 causing dissociation of Bax from mitochondria, thus inhibiting apoptosis[16]. However, the effects of autophagy on Bax have not been studied in detail.

During ageing, a growing number of haematopoietic (including immune) cells lose their capacity to proliferate and function properly due to senescence. Conditional deletion of the mTOR negative regulator, Tsc1, from haematopoietic stem cells accelerates senescence and thus mimics the phenotype of aged mice. Conversely, in old mice mTOR inhibition (for example, by rapamycin) can restore the self-renewal of haematopoietic stem cells, as well as their immunocompetence, as evidenced by restored haematopoiesis and effective responses against potentially lethal infection with influenza virus[60,61]. *In vitro*, rapamycin administration prompts cell cycle re-entry of human and rodent arrested cells[62]. Whether this anti-senescence action of rapamycin is mediated by autophagy remains to be investigated. Moreover, it should be noted that studies performed in cancer cells suggest that autophagy is required for the acquisition of the senescent phenotype[63,64]. Senescence and programmed cell death, both of which can be modulated by autophagy, constitute the major endogenous barriers against oncogenesis. Depletion of several autophagy-relevant genes (such as Beclin 1 and its interacting partners, UVRAG and Bmf1) enhance carcinogenesis[1,65]. At present, it can be conceived that autophagy mediates its tumour suppressive action by several non-exclusive mechanisms such as improved genomic stability, reduced inflammation and improved presentation of tumour-specific antigens.

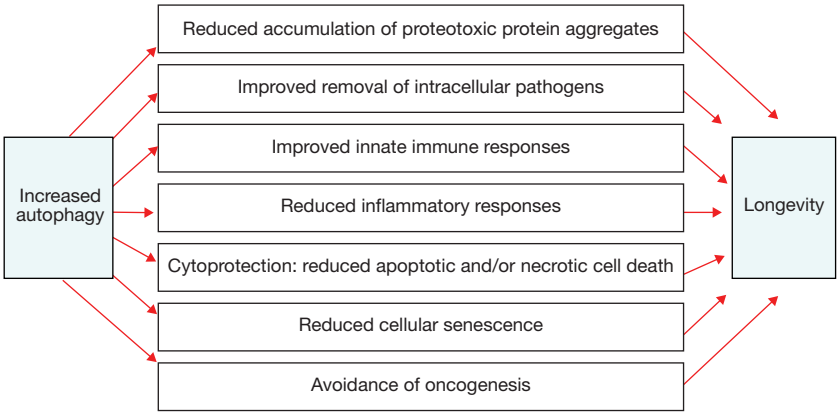

Figure 2 Linking autophagy-mediated lifespan extension to cytoprotection. Putative mechanisms linking autophagy to the inhibition of cell death (either apoptosis or necrosis) and to the induction of longevity.

Perspectives

Autophagy may be regarded as a mechanism of cellular 'cleansing' that could be a molecular correlate for the catharsis that is provoked by voluntary fasting[66]. This 'cleansing' action — combined with its recycling effects and bioenergetic advantages — might participate in the mechanisms protecting against degenerative, infectious, inflammatory and neoplastic diseases, all of which become more frequent as organisms age. The abundance of autophagy-relevant proteins declines with ageing, as does the efficacy of the autophagic process[67], suggesting that genetic, pharmacological or nutritional strategies designed to restore autophagy may constitute a strategy of choice to avoid or delay ageing-associated pathologies.

Although it is tempting to establish a simple equation in which autophagy protects cells against damage and death to promote longevity, there are some intriguing observations that suggest further refinement of the model is needed. For instance, brain-specific overexpression of *Atg8* in *Drosophila* extends longevity by 50%[68]. Unless such tissue-specific expression of *Atg8* can stimulate autophagy all over the body — perhaps through neuroendocrine mechanisms — these results suggest that autophagy processes within the central nervous system are particularly important for determining longevity. Similarly, improving protein homeostasis in neurons of *C. elegans* through expression of a heat shock factor (HSF-1) is sufficient to promote longevity[69] and in mice deletion of the huntingtin polyglutamine stretch enhances both neuronal autophagy and longevity[70]. Thus, it remains to be determined whether longevity-extending drugs should be designed as agents that mimic the natural pattern of autophagy induction by fasting (affecting most somatic cells) or that preferentially induce autophagy in the central nervous system. Future investigations will settle the question of whether such drugs could be effective or whether fasting should be promoted as the sole strategy for increasing health and lifespan. Regular periods of fasting could become a burgeoning trend of modern civilization, perhaps resetting our eating behaviour to our ancient roots, to times when nature, not culture, forced us to eat less.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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