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Biogerontology

ISSN 1389-5729
Volume 12
Number 5

Biogerontology (2011) 12:377-386
DOI 10.1007/s10522-011-9324-9

Springer

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DOI 10.1007/s10522-011-9324-9

REVIEW ARTICLE

The role of autophagy in genetic pathways influencing ageing

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Received: 10 January 2011 / Accepted: 13 February 2011 / Published online: 24 February 2011

Abstract Autophagy is a conserved cellular degradation pathway for the breakdown of cytosolic macromolecules and organelles. Constitutive autophagy has a housekeeping role and is essential for survival, development and metabolic regulation. Autophagy is also responsive to stress and can degrade damaged proteins and organelles, oxidized lipids and intracellular pathogens. Defects in the autophagic degradation system are linked to disease pathogenesis and ageing. Different signalling pathways converge on autophagy to regulate lifespan in diverse organisms. We discuss recent findings that provide insight into the cross-talk between this critical regulator of metabolic homeostasis and molecular mechanisms that promote longevity.

Keywords Beclin · *Caenorhabditis elegans* · Caloric restriction · *Drosophila* · Homeodynamics · Homeostasis · Insulin · p53 · Rapamycin · Resveratrol · Spermidine · TOR · Tunicamycin

Abbreviations

AMPK	AMP-activated protein kinase
Atg	Autophagy-related genes
CaN	Calcineurin
FOXO	Forkhead transcription factor
HIF	Hypoxia inducible factor
Insulin/IGF-1 pathway	Insulin/Insulin-like Growth Factor-1
JNK1	c-Jun N-terminal kinase
LAMP-2A	Lysosome-associated membrane protein-2
MAP1LC3	Microtubule-asociated protein 1 light chain 3
NAD^+	Nicotinamide adenine dinucleotide
PNC-1	Pyrazinamidase/ Nicotinamidase
PtdIns3K	Phosphatidylinositol-3 kinase
ROS	Reactive oxygen species
TOR	Target of rapamycin

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Introduction

Macroautophagy (hereafter autophagy) is the main intracellular degradation process by which cytosolic macromolecules and organelles are engulfed in double-membrane vesicles called autophagosomes and delivered to lysosomes for digestion by specific acidic hydrolases. Autophagy has a housekeeping

function in recycling of cytoplasmic material under normal conditions and plays essential roles in normal development and physiology. Autophagy is also critical for adaptation to starvation providing nutrients and energy and thus enhancing survival. Although autophagy mostly serves a cytoprotective function against distinct kind of stress such as hypoxia, endoplasmic reticulum stress, DNA damage and invasive pathogens, it has also been linked to cell death (Cuervo et al. 2005; Samara and Tavernarakis 2008). It is now clear that the contribution of autophagy in protein and organelle quality control is important for both cellular and organismal homeodynamics. Cellular self-digestion, cellular housekeeping and/or recycling become essential particularly in non-dividing differentiated cells, such as neurons and myocytes or senescent cells (Hara et al. 2006; Komatsu et al. 2006; Komatsu et al. 2005; Komatsu et al. 2007; Nakai et al. 2007). Accumulating evidence suggests that different signalling pathways converge on autophagy to regulate ageing (Madeo et al. 2010).

The molecular machinery of autophagy

Understanding of the molecular mechanisms involved in autophagy was initially catalyzed by pioneering studies in yeast (Huang and Klionsky 2002), which identified more than 30 autophagy-related genes (ATG) needed for autophagy induction, autophagosome nucleation, expansion and completion and the retrieval of autophagic proteins from mature autophagosomes (Fig. 1). The core autophagic machinery includes five essential functional groups: (1) the Atg1-Atg13-Atg17 kinase complex that is required for autophagy induction, (2) the class III phosphatidylinositol 3-kinase (PtdIns3K) complex I, consisting of Vps34, Vps15, Atg6 and Atg14, implicated in autophagosome formation, (3) two ubiquitin-like protein conjugation systems (Atg12 and Atg8) which are involved in the expansion of autophagosome membranes, (4) Atg9 and its recycling system that potentially contributes to the delivery of membranes forming autophagosome and

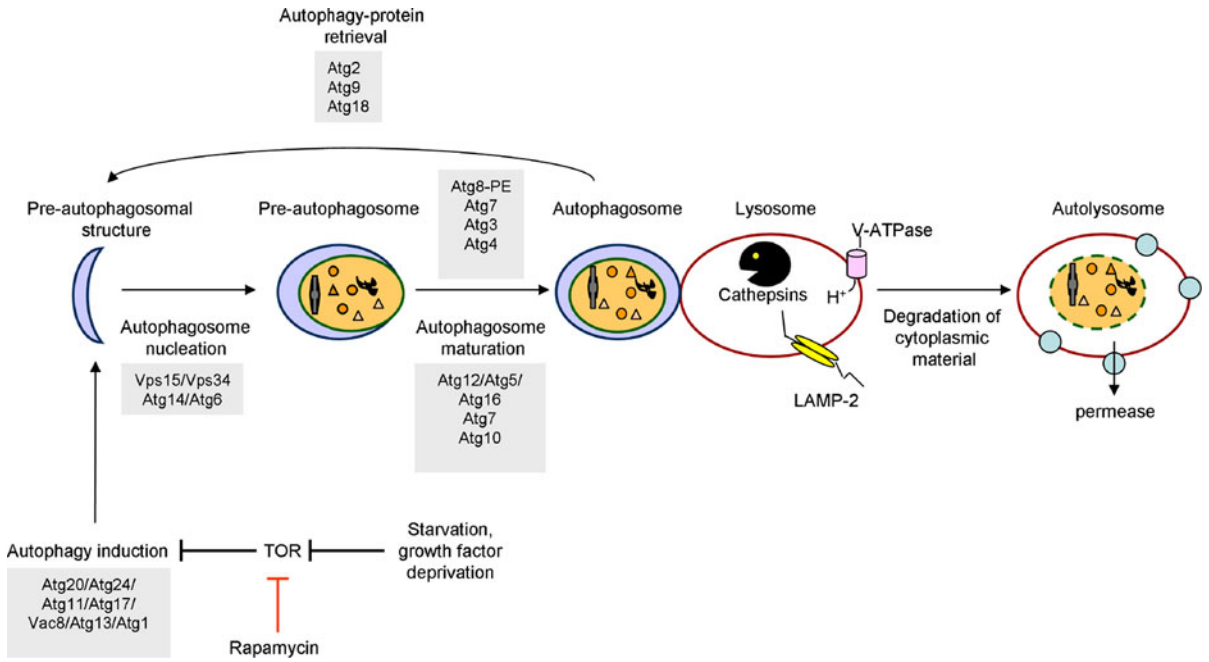

Fig. 1 The process of macroautophagy. Autophagy proceeds in distinct steps leading to the formation of double-membrane vesicles called autophagosomes. Autophagosomes engulf large portions of cytoplasm containing macromolecules and organelles or target cargos selectively. The outer autophagosome membrane then fuses with the lysosome (the resulting fusion organelle is known as the autolysosome) and the remaining autophagic body is released into the lumen for subsequent degradation by specific hydrolases. The resulting breakdown products are released through specific permeases into the cytosol for re-use

(5) the proteins needed for the last steps of the process (reviewed in (Yang and Klionsky 2010).

The functional conservation of yeast Atg proteins has greatly facilitated studies of autophagy in higher eukaryotes, such as *Caenorhabditis elegans*, *Drosophila* and mice (Levine and Klionsky 2004; Megalou and Tavernarakis 2009; Melendez and Neufeld, 2008; Mizushima 2005). Autophagy is typically monitored by means of the mammalian Atg8 orthologue, MAP1LC3, also known as LC3, (Kabeya et al. 2000) or LGG-1 in the worm, which associates with the autophagosomal membrane in a phosphatidylethanolamine-conjugated form and remains bound until after fusion of autophagic vesicles with lysosomes.

Although evolutionary conserved, the process of autophagy is more complex in higher organisms. A genetic screen in *C. elegans* identified four metazoan-specific autophagy genes, *epg-2, -3, -4* and *-5* that function in discrete steps of the autophagic pathway. The mammalian EPG-3, -4 and -5 homologs are essential for starvation-induced autophagy (Tian et al. 2010). Recently, a systematic proteomic analysis in human cells provided mechanistic and functional insights of the autophagy interaction network (Behrends et al. 2010).

The complex interplay between autophagy and signalling mechanisms that modulate ageing

Autophagy can be both non-selective and selective. Bulk degradation of cytoplasm constituents is non-selective, whereas the degradation of defective cellular proteins or organelles is highly selective. Selective and non-selective, as well as, basal and induced autophagy, are tightly regulated processes. Autophagy primarily serves as a cell survival mechanism, providing a source of nutrients in starving cells, or clearing defective proteins, damaged organelles and invasive pathogens that cause disease. The presence of autophagic structures in dying cells of diverse organisms, indicates that autophagy also functions in cell death. Autophagy is often called "type 2 programmed cell death" in contrast to apoptosis (type 1 programmed cell death) or necrotic cell death (type 3). Autophagic cell death occurs not only during development (Berry and Baehrecke 2007; McPhee and Baehrecke 2009), but also in various pathological conditions such as cancer, neurodegeneration and pathogen infection (Yang and Klionsky 2010). Direct induction of autophagy through Atg1 overexpression in *Drosophila*, is sufficient to inhibit cell growth and induce caspase-dependent cell death with apoptotic features (Scott et al. 2007).

Autophagy and apoptosis share common stimuli such as starvation, ER-stress and anti-apoptotic factor antagonists. Nematodes lacking *bec-1* show increased caspase-dependent apoptosis and an elevated number of apoptotic cell corpses in embryonic tissues. These observations indicate that *bec-1* coordinates the involvement of autophagy in cell death. However, the interplay between these two processes is complex and not fully understood. In addition to apoptosis, autophagy is also up-regulated by necrosis-inducing stimuli (Diskin et al. 2005; Erlich et al. 2006). In *C. elegans*, genetic or pharmacological impairment of autophagy partially suppresses necrotic neuronal death induced by diverse genetic (hyperactive MEC-4, DEG-1 and DEG-3 ion channels) and environmental insults (prolonged hypoxia). In contrast, autophagy upregulation by inhibition of the negative autophagy regulator CeTOR or under nutrient deprivation contributes to neuron necrosis. Autophagy and lysosomal proteolytic mechanisms synergize to facilitate necrotic cell death (Samara et al. 2008). These studies, in their totality indicate that excessive, runaway autophagy can be detrimental to cell viability and cause undesirable cell death (Kourtis and Tavernarakis 2009; Samara et al. 2008; Samara and Tavernarakis 2008).

The target of rapamycin (TOR) is considered a central node for integrating signalling pathways that regulate autophagy (Efeyan and Sabatini 2010). Other, TOR independent, regulators of autophagy are the FOXO transcription factors (Zhao et al. 2007) and the hypoxia-inducible factors (HIFs) (Zhang et al. 2008).

Recent studies have produced compelling genetic evidence implicating autophagy genes in ageing. Ageing is a complex process characterized by the progressive accumulation of damage in molecules, cells and tissues. Autophagic activity decreases with age, leading to poor response to stress and inefficient clearance of damaged proteins and organelles in cells. The decline in autophagy, may contribute to the functional deterioration of ageing organisms (Cuervo and Dice 2000). Studies in *C. elegans* (Hars et al.

2007) and *Drosophila* (McPhee and Baehrecke 2009) indicate that autophagy may function downstream of different longevity pathways, such as insulin/IGF-1 signalling, TOR signalling, caloric restriction and mitochondrial activity to regulate animal ageing (Table 1).

The evolutionary conserved insulin/IGF-1 signalling pathway influences lifespan in organisms ranging from yeast to mammals (Fontana et al. 2010; Kenyon 2010). Reduced insulin/IGF-like signalling extends lifespan in *C. elegans* and other multicellular organisms through the Forkhead transcription factor DAF-16/FoxO, the heat-shock transcription factor HSF-1 and the ortholog of mammalian Nrf1/2/3 SKN-1 (Johnson 2008; Tullet et al. 2008). DAF-16/FoxO homologues also regulate lifespan in *Drosophila* and mice (Holzenberger et al. 2003; Tatar et al. 2001). Depletion of several autophagy genes, including Atg6/Beclin 1/*bec-1* suppresses the long-lived phenotype of nematodes with a loss of function mutation in the insulin/IGF-1 receptor homolog DAF-2, indicating that autophagy is required for lifespan extension by *daf-2* mutations (Hars et al. 2007; Melendez et al. 2003). Indeed, long-lived *daf-2* insulin/IGF-1 receptor mutants require both autophagy and the DAF-16 for their longevity, but DAF-16 itself is not required for autophagy (Hansen et al. 2008).

The TOR kinase, a major amino acid and nutrient sensor, also modulates lifespan. Inhibition of TOR increases lifespan in many diverse species by inducing autophagy (Hansen et al. 2008; Jia et al. 2004; Juhasz et al. 2003; Kaeberlein et al. 2005; Kapahi et al. 2004; Vellai et al. 2003) (Fig. 2). Stimulation of autophagy in *C. elegans* TOR mutants requires the PHA-4/FOXA transcription factor (Hansen et al. 2008). Rapamycin, an inhibitor of the TOR pathway extends lifespan in yeast (Kaeberlein et al. 2005; Powers et al. 2006), nematodes (Vellai et al. 2003), fruit flies (Kapahi et al. 2004) and mice (Harrison et al. 2009). In yeast and in *C. elegans*, autophagy is required for rapamycin to increase chronological lifespan (Alvers et al. 2009; Hansen et al. 2008; Johnson 2008). Likewise, down-regulation of TOR activity by rapamycin extends lifespan in *Drosophila* through translational changes and autophagy (Bjedov et al. 2010). In human cells and in *C. elegans*, rapamycin-induced autophagy is independent of sirtuin 1 (Morselli et al. 2010a) (Fig. 2). In mice, the mechanisms that underlie the effects of rapamycin on longevity remain unclear. Rapamycin may increase survival through a combination of anti-neoplastic activities and effects on stress resistance and response to nutrient dynamics (Harrison et al. 2009).

Caloric restriction (limiting of dietary energy intake without malnutrition) extends lifespan of diverse organisms, including yeast, worms, flies, fish and mammals (Fontana et al. 2010; Mair and Dillin 2008). Lifespan extension is observed even under complete starvation in yeast and worms. Although altered diet *per se* triggers the longevity response, in many cases chemosensation may play an important role in the response to caloric restriction (Libert et al. 2007; Poon et al. 2010; Smith et al. 2008). In worms, flies and mice, the insulin/IGF-1 nutrient-sensing pathway mediates the longevity response to dietary restriction, at least under certain conditions (Fig. 2). Of all the nutrient-sensing pathways, the TOR signalling has been most consistently linked to caloric restriction, and may slow ageing by preventing the decline of autophagic activity (Hansen et al. 2008; Jia et al. 2007; Koubova and Guarente 2003).

Transgenic overexpression of sirtuins (NAD^+-dependent protein deacetylases), has been reported to extend lifespan in yeast, worms and flies (Kenyon 2005). The mechanisms by which sirtuins influence longevity are not clear. In yeast, extra copies of SIR2 extend lifespan by mimicking caloric restriction (Kaeberlein et al. 1999; Lin et al. 2000). In *C. elegans,* transgenic overexpression of the pyrazinamidase/nicotinamidase PNC-1 increases lifespan by activating the sirtuin 1 orthologue SIR-2.1, via depleting its inhibitor nicotinamide (Morselli et al. 2010b) (Fig. 2). In cells lacking sirtuins, caloric restriction can extend lifespan by means of TOR inhibition (Kaeberlein et al. 2005).

A loss of function mutation in the p53 orthologue, *cep-1*, extends lifespan in *C. elegans* by inducing autophagy, further implicating autophagy in lifespan regulation (Tavernarakis et al. 2008). However, p53 deficiency does not further enhance the lifespan extension conferred by *sir-2.1* overexpression. The lack of an additive effect suggests that SIR-2.1 activation and CEP-1 depletion affect longevity through a common pathway, involving autophagy. Autophagy triggered by the p53 inhibitor pifithrin-α, or the endoplasmic reticulum stress inducer tunicamycin is sirtuin 1-independent (Morselli et al. 2010a).

Table 1 Autophagy and pathways influencing ageing

Pathway	Genetic variation	Phenotype	Autophagy dependence	Species	References
Insulin/IGF-1 signalling	IGF-1 receptor/DAF-2 (loss of function)	Lifespan increase	Atg6/*bec-1*, *atg-7*, Atg8/*lgg-1*, *atg-12*, *atg-18*, *vps-34*	*C. elegans*	(Hansen et al. 2008; Hars et al. 2007; Melendez et al. 2003; Toth et al. 2008)
	FOXO/DAF-16 (overexpression)	Lifespan increase	Atg6/*bec-1* or Atg8/*lgg-1* or *atg-7*	*C. elegans* *D. discoideum*	(Jia et al., 2009)
JNK	Overexpression	Increased stress-resistance	ATG1, ATG6	*D. melanogaster*	(Biteau et al. 2010)
Caloric restriction	*eat-2* (loss of function)	Lifespan increase	Atg1/*unc-51*,Atg6/*bec-1*, *atg-7*, *vps-34*	*C. elegans*	(Hansen et al. 2008; Jia et al. 2007; Toth et al. 2008)
AMPK	Overexpression	Lifespan increase	Not determined	*C. elegans*	(Apfeld et al. 2004)
	Depletion by RNAi	Lifespan decrease	Not determined	*D. melanogaster*	(Toth et al. 2008)
TOR	TOR/*let-363* (loss of function)	Lifespan increase	Atg1/*unc-51* or Atg6/*bec-1* or *atg-18*	*C. elegans*	(Hansen et al. 2008; Toth et al. 2008)
Sirtuins	SIRT1/*sir-2.1* activation (by transgenic overexpression, nicotinamide depletion, resveratrol treatment)	Lifespan increase	Atg6/*bec-1*	*C. elegans*	(Morselli et al. 2010a; Morselli et al. 2010b)
p53/*cep-1*	Loss of function	Lifespan increase	Atg6/*bec-1*	*C. elegans*	(Tavernarakis et al. 2008)
Mitochondrial respiratory chain	*atp-3* (loss of function)	Lifespan increase	Atg1/*unc-51* or *atg-18* or Atg6/*bec-1*	*C. elegans*	(Toth et al. 2008)
	clk-1 (loss of function)	Lifespan increase	Atg1/*unc-51* or *atg-18* or Atg6/*bec-1*	*C. elegans*	(Toth et al. 2008)
	isp-1 (loss of function)	Lifespan increase	Atg6/*bec*-1 is not required	*C. elegans*	(Hansen et al., 2008)
Calcineurin (CaN)	*tax-6* (loss of function)	Lifespan increase	Atg6/*bec-1* or *atg-7*	*C. elegans*	(Dwivedi et al. 2009)
LAMP-2A	Increase of hepatic LAMP-2 abundance	Restoration of liver chaperone-mediated autophagy in aged animals	*lamp2* (LAMP-2A; hepatic expression)	*M. musculus*	(Zhang and Cuervo 2008)

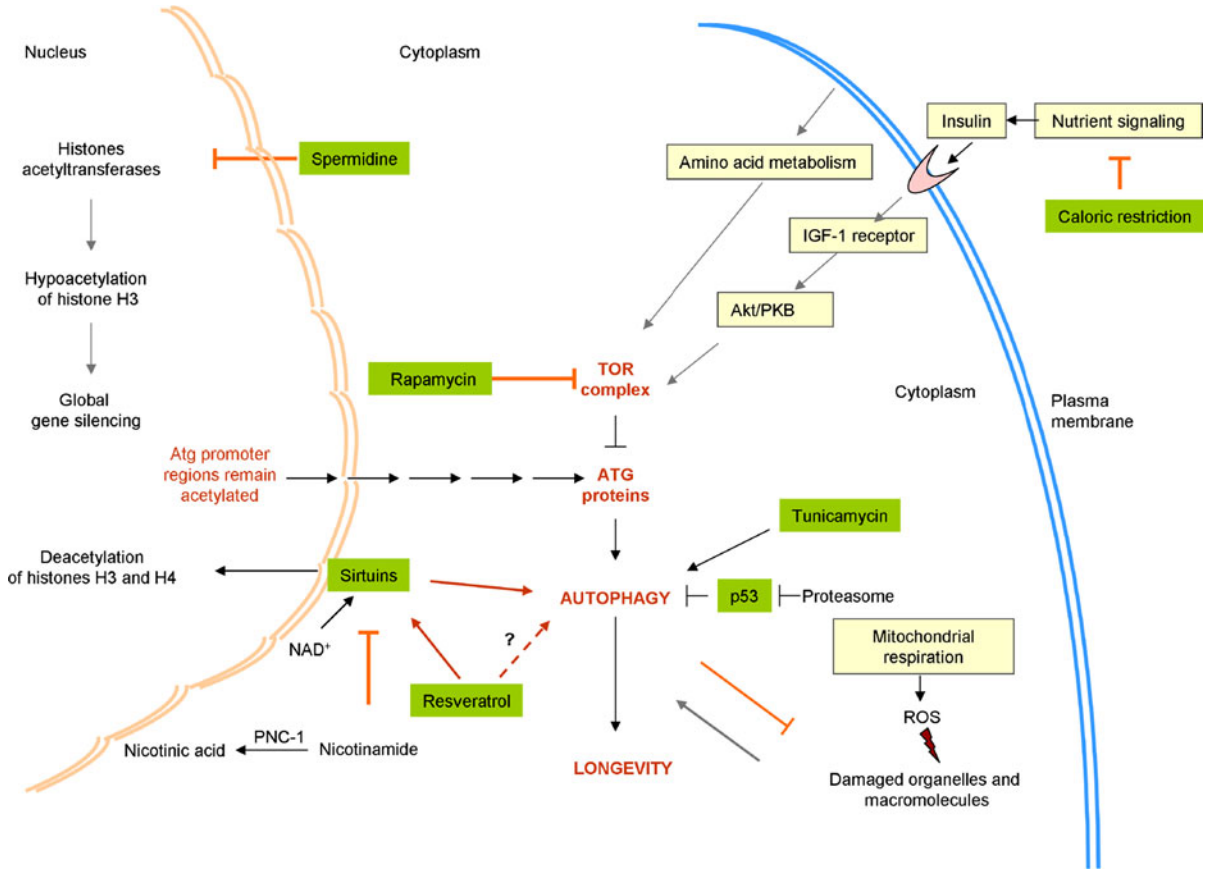

Fig. 2 Signalling pathways and environmental factors, such as nutrient availability, converge on autophagy to regulate lifespan. Inhibition of the insulin/IGF-1 pathway, the TOR kinase pathway, respiration and food intake, as well as, overexpression of sirtuin 1 or depletion of p53 promote longevity in diverse organisms through the induction of autophagy. Caloric restriction acts by reducing the concentration of insulin-like growth factors and/or by activating sirtuin 1. Sirtuins deacetylate both histones and cytoplasmic targets. Pharmacological treatment with spermidine, resveratrol or rapamycin increases cellular and organismal survival and also stimulates autophagy, which is required for lifespan extension. Resveratrol requires sirtuin 1/*sir-2.1* activity to extend lifespan, at least in some cases in worms and flies but the relationship between resveratrol and sirtuins is unclear. Spermidine may function by inhibiting histone acetyltranserases, while rapamycin inhibits TOR and p53 may act on cytoplasmic targets

Resveratrol, the plant polyphenolic compound which has been reported to activate sirtuins, slows metazoan ageing by mechanisms that may be related to caloric restriction (Wood et al. 2004). In mice, resveratrol extends lifespan of animals fed high-fat diet but not that of mice fed a normal diet (Pearson et al. 2008). Recent studies have shown that caloric restriction and resveratrol enhance the resistance of human cells to metabolic stress and extend lifespan in worms by inducing autophagy. Knockdown of Beclin 1/*bec-1* abolished these beneficial effects. Likewise, knockout or pharmacological inhibition of sirtuin 1 prevents induction of autophagy and the improvement of cellular/organismal survival by resveratrol and nutrient starvation (human cells) or caloric restriction (*C. elegans*). Therefore, caloric restriction and resveratrol require functional sirtuin 1 to stimulate autophagy and enhance longevity (Morselli et al. 2010a). Together, these findings indicate that activation of sirtuin 1, either by genetic or pharmacological interventions, enhances cellular and organismal survival by inducing autophagy.

Spermidine is a natural polyamine whose concentration declines with age. It has been shown that external supplementation of spermidine prolongs lifespan in yeast, worms and flies, increases the survival of human immune cells and reduces age-related oxidative stress in mice. In yeast, spermidine

treatment triggers the global hypoacetylation of histone H3, but the selective acetylation at the promoter region of Atg7 gene and therefore causes the transcriptional upregulation of ATG (Fig. 2). Depletion of essential autophagy genes (Atg7, Beclin 1) abrogates the lifespan-extending effect of spermidine. These findings suggest that autophagy mediates the longevity response to spermidine (Eisenberg et al. 2009). Therefore autophagy appears to be a common denominator of molecular mechanisms that promote longevity.

Perspectives

Autophagy plays a primary role in the baseline turnover of intracellular proteins and organelles under normal growth conditions. This function underlies dynamic processes such as cellular remodeling/metamorphosis during the development of multicellular organisms. In *C. elegans*, the inability of Beclin-1-deficient larvae to complete dauer morphogenesis argues that autophagy is required for tissue remodeling that accompanies normal dauer formation. Furthermore, depletion of several autophagy genes, including *bec-1* can result in lethality in wild type *C. elegans* suggesting that autophagy is required for normal nematode development (Melendez et al. 2003). In *Drosophila*, autophagy is essential for metamorphosis. Larvae lacking the function of Draut1, the *Drosophila* homolog of yeast Aut1/Apg3, are unable to induce autophagy in fat body cells before pupariation and animals die during metamorphosis (Juhasz et al. 2003). Loss of function analysis of autophagy genes (Atg) in mice has revealed the important roles of the autophagy pathway in mammalian differentiation and development (Mizushima and Levine 2010).

Constitutive autophagy is important for intracellular quality control particularly in non-dividing differentiated cells, such as neurons that cannot dilute and eliminate waste by cell division. Autophagy is also stress responsive and can degrade damaged proteins and organelles, oxidized lipids or intracellular pathogens. Growing evidence reveals that autophagy is implicated in a wide range of human disorders including neurodegeneration, microbial infection and cancer (Mizushima et al. 2008). Defects in autophagy have been associated with age-related cellular changes, even in the absence of any disease-associated mutant protein (Cuervo et al. 2005; Hara et al. 2006; Komatsu et al. 2006). However, activation of autophagy can also be harmful: Although autophagy can prevent the initiation of some cancers, it also may allow tumour cells to become resistant to chemotherapy (Degenhardt et al. 2006). Excessive autophagy contributes to cellular destruction during necrosis. Thus, preventing aberrant induction of autophagy may reduce cell damage during acute neurodegenerative episodes such as ischemic stroke (Samara et al. 2008).

Recent genetic studies indicate that autophagy genes are required for lifespan extension in various long-lived nematode mutants (Megalou and Tavernarakis 2009). In addition, inhibition of autophagy shortens lifespan in *Drosophila* (Bjedov et al. 2010; Kapahi et al. 2004). Because suppression of autophagy blocks lifespan extension in worms and flies, autophagy is considered a longevity determinant. However, several aspects of the complex interplay between autophagy and ageing remain enigmatic. How does autophagy function to regulate lifespan? Findings in diverse organisms indicate that autophagy plays a key role in cellular defence against cellular damage caused by reactive oxygen species (ROS), which are generated primarily during respiration. Accumulation of these radicals in mitochondria leads to mitochondrial dysfunction, a common feature in ageing cells (Wallace 2005). Loss of mitochondrial activity induces autophagy, which may slow ageing by controlling mitochondrial quality and quantity (reviewed in (Jia and Levine 2010)). In addition, autophagy serves an important cytoprotective function by mediating the clearance of aberrant cytosolic macromolecules, defective proteins, aggregates and toxins (Vellai 2009), promoting metabolic homeostasis both at the cellular and whole organism level (reviewed in (Rabinowitz and White 2010)). Moreover, autophagy may contribute to the removal of injured or damaged cells by facilitating cell death (Kourtis and Tavernarakis 2009; Samara et al. 2008; Samara and Tavernarakis 2008).

Although it is widely accepted that autophagy is required for lifespan extension by a broad set of genetic manipulations, environmental conditions and pharmacological treatments, it remains unclear whether autophagy is sufficient to increase longevity. In *C. elegans*, insulin/IGF-1 pathway mutants require

both autophagy and the DAF-16/FOXO transcription factor for their longevity. But although *daf-16(null);-daf-2(-)* double mutants exhibit the same high level of autophagy as do the long-lived *daf-2(-)* single mutants, *daf-16;daf-2* double mutants are not long-lived. This suggests that autophagy is not sufficient to extend lifespan in *C. elegans*. Perhaps the role of autophagy is to provide raw material to be recycled into new cell protective proteins by DAF-16 and other transcription factors (Hansen et al. 2008).

Finally, the pleiotropic effects of autophagy on organism physiology confound the influence on ageing. For example, a null mutation in *bec-1* (Beclin 1) gene causes embryonic lethality in *C. elegans* and a loss of function mutation in *unc-51* (Atg1) results in abnormal movement due to neuronal defect. Thus, the ageing phenotypes of autophagy gene inactivation could be secondary to the effects of autophagy on development. Despite its potential importance in the regulation of ageing, it is still unclear whether autophagy impairment accelerates directly ageing. Moreover, it is difficult to provide direct evidence of a causal role of autophagy in ageing, due to the pleiotropic nature of signalling pathways that regulate autophagy. For example, in addition to inhibiting autophagy, TOR signalling regulates a range of essential cellular functions, the best understood of these being protein synthesis, which also affects ageing (Hands et al. 2009; Tavernarakis 2008). Further studies are required to dissect the impact of elevated autophagic degradation on ageing. In addition, a better understanding of the regulatory mechanisms that control autophagy will provide potential sites for interventions aiming to ameliorate age-related pathologies.

Acknowledgments The authors wish to acknowledge funding from EMBO, the European Research Council (ERC) and the European Commission Framework Programmes.

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