Regulation of the aging process by autophagy

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Autophagy is involved in cellular protein and organelle degradation, which is mediated by the lysosomal pathway. Autophagocytosis has a key role in cellular housekeeping by removing damaged organelles. During aging, the efficiency of autophagic degradation declines and intracellular waste products accumulate. In Caenorhabditis elegans, there is clear evidence that lifespan is linked to the capacity to regulate autophagy. Recent studies have revealed that the same signaling factors regulate both aging and autophagocytosis, thus highlighting the role of autophagy in the regulation of aging and age-related degenerative diseases. Here, we examine in detail the interactions of the signaling network involving longevity factors SIRT1, mTOR, FoxO3, NF-κB and p53 in the regulation of autophagy. We discuss the possibility that these well-known stress resistance and longevity factors regulate the aging process via autophagy.

Introduction

Autophagy (see Glossary) is an intracellular catabolic process involved in protein and organelle degradation via the lysosomal pathway. The autophagosome is the hallmark of autophagic uptake and degradation, and the mechanism underlying its formation has been extensively studied and is described in detail elsewhere [1–4]. Autophagosomes fuse with lysosomal vesicles and form digestive autolysosomes. Cytoplasmic material can be delivered for lysosomal degradation along three pathways: (i) macroautophagy, in which organelles such as mitochondria are isolated from cytoplasm and taken up into autophagosomes; (ii) microautophagy, which involves the phagocytic uptake of cytoplasmic material into the lysosome itself; and (iii) chaperone-mediated autophagy (CMA), which involves distinct cellular chaperones assisting in the uptake of specific proteins into lysosomes via the lysosomal-membrane protein type 2A (LAMP-2A) receptor [3,4]. Autophagy acts in conjunction with proteasomes in the basic turnover of material within the cell but, during cellular stress, autophagic processes can be strongly induced to support energy balance via cytoplasmic self-digestion, for example, during starvation. Furthermore, autophagic degradation is involved in several human diseases, such as Alzheimer’s and Huntington’s diseases, X-linked myopathy, and several inflammatory diseases [2,4]. Autophagy is also involved in host defense, linking it with the functions of innate and adaptive immunity [5].

Housekeeping and quality control of proteins and organelles seem to be the main functions of autophagy [2–4]. Autophagocytosis can clear damaged or defective organelles, mainly mitochondria, but in some cases can also remove peroxisomes and membranes of the endoplasmic reticulum. CMA uses the KFERQ-like protein sequence to recognize either misfolded or unfolded proteins. It can also identify important regulatory proteins, such as inhibitory-κB kinase (IKK), and thereby participate in the regulation

Glossary

Aging: the aging process involves the progressive accumulation of damaged and defective cellular components, which induce a decline in the physiological function of tissues and cause organismal aging.

Atg proteins: autophagy-related genes are called ATG genes (named for ‘autophagy’). Over 30 ATG genes, and corresponding Atg proteins, have been found in yeast. Several human homologues have been identified. Atg proteins are involved in the induction and formation of the autophagosome [1,45].

Autophagy: autophagy or autophagocytosis is a cellular self-digestion process, which involves the uptake of cellular components for the degradation in lysosomal system.

Lifespan: an average lifespan refers to the life expectancy of a particular organism. Maximum lifespan corresponds to the age of the most long-lived member of species.

Chaperone-mediated autophagy (CMA): in this process, particular cytoplasmic proteins containing the consensus sequence bind first to hsc70 chaperone, forming a complex that is recognized by the LAMP-2A receptor at the lysosomal membrane. The protein is unfolded and translocated into the lysosomal lumen for degradation.

FoxO: mammalian FoxO genes and daf-16 homolog of C. elegans belong to the forkhead family of transcription factors. FoxO proteins regulate cellular metabolism and stress resistance, for example.

Longevity: longevity refers to long life and, in particular, to extended lifespan.

Lysosome: lysosmes are cellular organelles containing digestive enzymes called acid hydrolases. Lysosomes fuse with vacuoles which might originate from autophagic, phagocytic or endocytic uptake.

Macroautophagy: macroautophagy involves the uptake of specific cellular targets, such as organelles and invasive microbes, or any non-specific bulk cytoplasm by wrapping the target into autophagosomes with a double-membrane structure.

Mammalian target of rapamycin (mTOR): mTOR is a serine/threonine protein kinase which integrates several upstream signaling pathways. mTOR is a sensor of cellular energy and redox status and a major inhibitor of autophagy.

NF-κB system: the NF-κB transcription factor is a multiprotein complex that establishes a host defense mechanism in cellular and environmental stress insults. The cytoplasmic NF-κB complex is activated by several upstream signaling links and, subsequently, translocates to nuclei and transactivates a set of target genes.

p53: p53 protein is a transcription factor that regulates the cell cycle. It is a major suppressor oncogene, maintaining genome stability, and is therefore called the ‘guardian of the genome’.

Sirtuin: the seven mammalian sirtuins (SIRT1-7) are homologs of Sir2 in budding yeast. Sirtuins are NAD+-dependent protein deacetylases and important regulators of cellular metabolism and homeostasis.
of nuclear factor (NF)-κB function (see later) [6,7]. Tissue-specific knockout of ATG genes (named for ‘autophagy’), which are crucial to autophagosome formation, induces the accumulation of ubiquitin-positive protein aggregates in the cytoplasm [2–4]. Similar aggregates are present in several age-related degenerative diseases [2]. Moreover, irregular mitochondria and endoplasmic reticulum structures are known to accumulate in Atg7-deficient mouse hepatocytes, pointing to a defect in macroautophagy [8]. Interestingly, inhibition of the CMA pathway increases the extent of macroautophagy [9], whereas activation of CMA impairs macroautophagy [10], suggesting that there must be considerable flexibility between the different uptake pathways. Recently, Levine and Kroemer [2] have reviewed the phenotypes of mice with mutations in genes associated with autophagy.

Several studies have also demonstrated that enhancing autophagic degradation, for example with rapamycin and trehalose, can have therapeutic potential in experimental models of the protein aggregation diseases, such as Huntington’s and Parkinson’s diseases [11,12]. Recent studies have shown that p62 protein, which is also known as SQSTM1, has a key role in the autophagic uptake of ubiquitylated protein aggregates [13]. The p62 protein can bind to polyubiquitylated proteins and form aggregates, but can also interact with autophagic effector protein LC3 and thus mediate the autophagic uptake of aggregated proteins. These studies indicate that signaling factors regulating the expression of p62 protein have an important role in housekeeping, which is mediated by the autophagic system. Proteasomal inhibitors and apoptosis activators (such as serum deprivation or okadaic acid) have also been observed to be powerful inducers of p62 expression in neuronal cells [14]. Accordingly, deficiency of p62 protein disturbs housekeeping activities and enhances the formation of protein aggregates in mouse hepatocytes and neurons [15].

Studies of the aging process have revealed a plethora of dysfunctions in physiological systems during aging [16–18]. One common feature of all age-related changes at the tissue level is the accumulation of damage and harmful modifications in DNA, proteins and lipids, and in cellular organelles. The origin of this damage is still a matter of debate, although certain mechanisms, such as free radical attack, have been known for >50 years [19]. There is also accumulation of lipofuscin, a hallmark of senesced cells, in post-mitotic cells (e.g. muscle cells and neurons), which also disturbs the function of the cellular cleaning system [20]. It seems that housekeeping mechanisms become compromised during aging and, therefore, there has been intense interest focused on research into age-related changes in the autophagic system [21–23].

**Autophagic degradation declines during aging**

The quality of cellular housekeeping is dependent on efficient protein synthesis and degradation systems. The accumulation of waste products in post-mitotic cells during aging is evidence of problems in the cleaning systems. Several different approaches have confirmed that the proteolytic efficiency of cells decreases with aging [21,22,24–26]. The activity of proteasomal degradation is reduced during aging, although the exact molecular aspects of this regulation need to be clarified. Reduced turnover rates can expose proteins to age-related modifications, such as protein carbonyl formation and aggregation, which can increase the pressure on the autophagic system. The functional efficiency of the autophagic–lysosomal system also clearly declines during aging but the effects are more diverse than those found in proteasomal degradation owing to the inducible nature of autophagic function and the presence of several compensatory adaptations within the system [21–26].

**Autophagy in Caenorhabditis elegans**

*Caenorhabditis elegans* is one of the most commonly used model organisms in aging and longevity studies. *C. elegans* is a nematode that can enter a diapause state of arrested development, called the dauer larva, in unsuitable environmental conditions [27]. Loss-of-function mutations of genes in the dauer formation (DAF)-2 pathway can also induce diapause-like metabolic changes in the adult larva that considerably extend the lifespan of *C. elegans* [28]. Inhibition of the DAF-2 pathway activates the DAF-16 protein, which is encoded by one of the *daf* genes in *C. elegans* [28]. The DAF-2 pathway is analogous to the mammalian insulin–phosphoinositide-3 kinase (PI3K) pathway and the *daf-16* gene is a homolog of the mammalian FoxO genes [29]. In mammals, forkhead box O (FoxO) transcription factors have been associated with similar activities to DAF-16 protein in *C. elegans* (i.e. changes in the regulation of stress resistance and longevity networks) [29].

Melendez et al. [30] were the first to demonstrate that an autophagy gene called *bec-1*, an ortholog of *Beclin1*, is required for dauer development and lifespan extension in DAF-2 mutants. Dauer formation and lifespan extension were clearly associated with increased rates of autophagy. Hars et al. [31] used RNAi knockdown to demonstrate that the loss of function of two other autophagy genes, Atg-7 and Atg-12, also shortened the lifespan of wild-type and DAF-2 mutants. These studies clearly emphasize that autophagy is an important factor in longevity regulation in *C. elegans*.

Caloric restriction is commonly used to extend lifespan experimentally, both in *C. elegans* and mammals. Hansen et al. [32] observed that autophagy is a transcriptionally regulated response to caloric deficiency but lifespan extension is also dependent on the expression of transcription factor DAF-16 (also known as FOXO). Moreover, it seems that the activation level of autophagy is important because the physiological level promotes survival, whereas either insufficient or excessive levels of autophagy trigger death in *C. elegans* [33]. Increased autophagy in DAF-2 mutants also potentiates the autophagic degradation of amyloid-β peptides, which opposes the proteotoxicity mediated by age-onset amyloid aggregation in *C. elegans* [34].

**Autophagy in mammals**

The role of autophagy and the lysosomal system in the mammalian aging process has been a topic of intensive research over the past 20 years. There is a general consensus that the function of both macroautophagy and CMA decline during aging [21,22,35]. Cuervo and Dice [35]...
demonstrated that the efficiency of CMA decreases in rat liver during aging. The binding of damaged proteins to the lysosomal membrane and their transport into lysosomes was clearly impaired with aging. Interestingly, these studies were able to establish that the reduced efficiency was due to the progressive age-related decrease in the expression of LAMP-2A protein, the receptor protein in CMA uptake. By contrast, the expression of HSC70 protein, the targeting protein in CMA, was largely unaffected by aging. The Cuervo laboratory has extended these studies, demonstrating that preventing the age-related decline in LAMP-2A protein in transgenic mice can maintain an active and functioning CMA until advanced ages and, importantly, the preservation of CMA was associated with lower accumulation of damaged proteins and improved liver function [36].

The mechanism of the LAMP-2A decline in lysosomes during aging seems to be post-transcriptional because aging does not affect the transcription efficiency of LAMP-2A [37]. Instead, there are age-related changes in the trafficking and stability of LAMP-2A protein in the lysosomal membrane [37]. This indicates that aging can affect the assembly and function of the LAMP-2A complex in lysosomal membranes and impair CMA properties during aging. The Cuervo laboratory has recently elucidated the structural and functional properties of the LAMP-2 complex [38]. Heat shock protein HSP90 is the key component in the assembly of the LAMP-2A complex [38]. Studies have shown that the availability of chaperones decreases during aging owing to the increased requirements for the suppression of proteotoxic attack, as reviewed by Soti and Csermely [39]. Furthermore, the level of HSP90 protein declines during aging in rat liver [40]. Given that HSP90 protein is also an important regulator of proteasomal degradation [41], it seems that chaperones can affect aging and longevity by their ability to maintain the function of proteolytic pathways.

In addition to CMA, macroautophagy also seems to be repressed during aging in mammalian tissues. There is now abundant evidence, especially from the laboratories of Ettore Bergamini and Ulf Brunk, that aging can impair the function of macroautophagy, in particular the degradation of defective mitochondria along the macroautophagic pathway [17,21,24–26,42]. Macroautophagy of cellular organelles is an inducible, complex process involving the activation of Beclin 1 and other Atg genes [1–4]. Macroautophagy is activated during several stress conditions, such as starvation, and in response to hormone treatments. Interestingly, both caloric restriction and the suppression of the growth hormone–insulin-like growth factor (GH–IGF-1) axis stimulate macroautophagy and can reverse age-related changes [21,22,42]. The increase in stress resistance seems to be a characteristic feature of long-lived mouse models [43], and of hortencic lifespan extension in C. elegans [44].

**Signaling networks regulating autophagy, stress resistance and longevity**

It seems likely that autophagy can regulate longevity at a cellular level (see earlier) but it is yet to be established whether or not the signaling pathways regulating autophagy and the aging process are linked to each other at the molecular level. Several studies have recently identified the signaling pathways that regulate autophagic degradation [1,45]. In particular, the repressive effects of mammalian target of rapamycin (mTOR) and the role of Beclin 1, a repressor or activator of autophagy, have been described [1,46]. Interestingly, recent studies have revealed that distinct stress resistance and longevity signaling pathways, such as FoxO3 [47], NF-κB [48], p53 [49] and SIRT1 [50], are also potent regulators of autophagic degradation (Figure 1).

**mTOR signaling represses autophagy**

The formation of the autophagosome is regulated by a core machinery of ATG proteins [1,45]. The assembly and function of ATG complexes are the targets of several cellular signaling cascades. mTOR is a serine/threonine protein kinase that integrates the input of upstream signaling to different downstream effectors, for example to the Atg1 complex in autophagosome formation [45,46,51]. mTOR kinase assemblies complexes (mTORC1 and mTORC2), which receive several different upstream inputs, for example from growth factor receptors such as the insulin–PI3K–Akt pathway and adenosine monophosphate (AMP) kinase (AMPK); these regulate the activity of the mTOR complex. Rapamycin is a well-known inhibitor of mTOR function and a potent inducer of macroautophagy [46]. There is an abundance of literature demonstrating that the signaling pathways activating the mTOR complex can inhibit autophagy, whereas the signals that inhibit mTOR stimulate autophagic degradation (Figure 1).
The downstream target of mTOR is the Atg1 kinase complex, which also contains Atg11, Atg13 and Atg17 proteins [46]. In particular, this complex triggers autophagosomal vesicle formation. mTOR can also stimulate autophagocytosis by activating S6 kinase (S6K), which acts as a feedback signal to inhibit insulin signaling [46], and thereby activates the FoxO3 pathway (Figure 1; see later). mTOR signaling via the Atg1 complex transmits signals from cellular stress induced by starvation (amino acid level), energy deficiency (AMPK) and excessive Ca\(^{2+}\)-concentration [46]. Stress-induced signals in the endoplasmic reticulum (ER) can also activate the Atg1 kinase [52]. Recent studies have revealed that autophagosomal membranes originate from the secretory pathway and that autophagocytosis can regulate the quality of secreted proteins, along with ubiquitin–proteasome-mediated ER-associated protein degradation (ERAD) [53].

**Beclin 1: a platform of dual function**

Beclin 1 is a platform protein in the multiprotein complex that regulates the early phase of autophagosome formation [1,46,54]. The Beclin 1 protein interacts with several proteins either to activate or to suppress autophagocytosis. Beclin 1 is normally bound to anti-apoptotic B-cell lymphoma (Bcl)-2 or Bcl-xL proteins such that autophagocytosis. Beclin 1 is normally bound to anti-apoptotic proteins either to activate or to suppress apoptosis [1,46,54]. The Beclin 1 protein interacts with several proteins that regulate the early phase of autophagosome formation [52].

**IKK and NF-κB signaling can repress autophagy**

The NF-κB system is the most important signaling pathway that is induced in the defense of cells against cell damage and environmental danger [58]. However, the role of NF-κB signaling in autophagic degradation is largely unknown, although autophagy is known to be involved in several cellular functions regulated by NF-κB system, such as cellular homeostasis, immunity, cancer, development and aging. Recently, Djavaheri-Mergny et al. [59] demonstrated that NF-κB signaling represses TNFα-induced autophagy. They observed that the repression was linked to NF-κB-dependent activation of mTOR kinase, an inhibitor of autophagocytosis. Schöttmann et al. [60] have shown that the classical NF-κB signaling pathway can inhibit autophagy in macrophages by downregulating Atg5 and Beclin 1 expression, which promotes apoptosis and resolution of inflammation. The NF-κB pathway involves the IKK complex, containing IKKα and IKKβ kinases. These activate NF-κB signaling but they can also regulate other signaling targets, which are independent of the NF-κB pathway [61]. Dan and Baldwin [62] have demonstrated that IKKα and IKKβ are involved in the activation of the mTOR–Raptor complex in response to TNFα and insulin exposure. They observed that the activation of the mTOR/Raptor complex by IKKα was induced by Akt kinase, whereas IKKβ repressed the tuberous sclerosis complex (TSC), a well-known suppressor of mTOR/Raptor, and thus could activate mTOR kinase. Lee et al. [63] have also demonstrated that TNFα-activated IKKβ can suppress TSC1 and hence trigger the mTOR pathway. Because IKKβ is a partner in the IKK complex linked to inflammatory signaling, these observations indicate that inflammation is a potent inhibitor of autophagy (Figures 1, 2).

![Figure 1](image1.png)

**Figure 1.** A scenario depicting the interactions of SIRT1, NF-κB and FoxOs in the regulation of the appearance of senescent organismal phenotypes. Green arrows show the activating signaling routes and red arrows represent the inhibitory interactions. Senescent phenotypes involve the inflammatory disorders, protein depositions and tissue atrophy in several tissues. The major causes of these age-related changes are the activation of innate immunity and the decline in autophagocytosis along with changes in proteasomal degradation. SIRT1, NF-κB and FoxOs form an interacting signaling network that regulates innate immunity and autophagic and proteasomal degradation. Several factors, such as environmental insults and cellular stress, affect the function of this signaling network.

![Figure 2](image2.png)

**Figure 2.** A scenario depicting the interactions of SIRT1, NF-κB and FoxOs in the regulation of the appearance of senescent organismal phenotypes. Green arrows show the activating signaling routes and red arrows represent the inhibitory interactions. Senescent phenotypes involve the inflammatory disorders, protein depositions and tissue atrophy in several tissues. The major causes of these age-related changes are the activation of innate immunity and the decline in autophagocytosis along with changes in proteasomal degradation. SIRT1, NF-κB and FoxOs form an interacting signaling network that regulates innate immunity and autophagic and proteasomal degradation. Several factors, such as environmental insults and cellular stress, affect the function of this signaling network.
There is an interesting mutual crosstalk between NF-κB signaling and autophagocytosis, because the upstream NF-κB-activating kinases, IKKβ and NF-κB-inducing kinase (NIK) can be selectively degraded by autophagy [7,48]. Moreover, the IKK and NIK kinases are HSP90 client proteins; for example, the inhibition of HSP90 with geldanamycin disrupts the interaction and induces the autophagic degradation of these kinase proteins. Subsequently, mTOR can be inactivated and, in general, the autophagic process will be enhanced (Figure 1). By contrast, acute stress resistance via the upregulation of HSP90 level would be anticipated to reduce the extent of autophagy. However, HSP90 protein is the stabilizing factor for CMA and, hence, is an important regulator of CMA. It seems that CMA uptake and macroautophagy with the assembly of ATG proteins are differently regulated. For instance, in nutrient deprivation, the activation of macroautophagy is the early response, whereas CMA is induced later [64].

**SIRT1 and FoxO3 signaling enhance autophagy**

SIRT1 is one of seven mammalian sirtuins, which are homologs of silent information regulator 2 (Sir2), a protein first found in budding yeast [65]. Sir2 protein is a well-known stress resistance and longevity factor in yeast biology. SIRT1 is a class III protein deacetylase and it regulates several functions of cellular metabolism and survival, and the induction of cancer and senescence. The FoxO family of transcription factors is also an important regulator of cellular metabolism, proliferation and stress resistance [29]. In C. elegans, the DAF-16 homolog of FoxOs is the major factor in dauer formation and lifespan regulation [27,28]. Interestingly, recent studies have demonstrated that SIRT1 and FoxO3-related signaling pathways are involved in the regulation of autophagy in mammals.

Lee et al. [50] demonstrated that SIRT1 is an activator of autophagy, both in cultured cells and in vivo in transgenic mice. Some characteristics in the phenotype of SIRT1−/− transgenic mice resemble those of Atg5−/− mice. For example, there is an accumulation of damaged organelles, which might be evidence for inhibition of autophagy. Furthermore, they observed that SIRT1 can interact with and deacetylate several components in the complexes of forming autophagosomes, such as Atg5, Atg7 and Atg8 proteins [50]. These observations clearly indicate that protein acetylation regulates macroautophagic processes. It is known that deacetylases and deacetylases can modulate protein stability and in that way they can alter the turnover of these target proteins [66]. The results of Lee et al. [50] are particularly interesting because they link together the Sir2 longevity factor with autophagic degradation (Figures 1,2).

Several studies have demonstrated that the impairment of the ubiquitin–proteasome system leads to the activation of autophagy via histone deacetylase 6 (HDAC6), which recognizes ubiquitylated cellular aggregates and enhances the rate of autophagy [67]. Iwata et al. [68] have demonstrated that HDAC6 and microtubules are required for the trafficking of aggregated proteins towards autophagic degradation. HDAC6 also can trigger the activation of heat-shock factor 1 (HSF1) and increase the expression of major cellular chaperones [58], such as HSP90, which is an important component in CMA. It seems that SIRT1 and HDAC6 regulate different targets in the autophagic pathway and are activated in distinct ways. SIRT1 regulation could be more important during starvation and aging when the expression of SIRT1 is increased [65].

FoxO transcription factors are major regulators of muscle atrophy and are induced by fasting, cachexia, diabetes and muscle disuse, for example [47,69,70]. FoxO3 protein increases the expression of atrogin-1 gene, a muscle-specific ubiquitin E3 ligase, which potentiates protein degradation via the ubiquitin–proteasomal pathway [69]. Recently, it was observed that FoxO3 transcription factor also activates autophagic–lysosomal degradation in muscle tissue [70]. FoxO3 protein can increase the expression of autophagy-associated proteins, such as LC3 and Bnip3. It seems that FoxO3 transcription factor enhances protein degradation by inducing the expression of genes related to the activation of autophagy and proteasomes and thereby supporting the organismal energy metabolism (Figures 1,2).

**p53: a context-dependent regulator**

Cancerous growth is the cell fate that involves escape from the organismal aging process. In cancer cells, the role of autophagy is still a matter of debate. Autophagic degradation seems to be suppressed because anti-cancer therapies can induce autophagy-related cell death. Autophagy-related cell death has been termed programmed cell death type II, with apoptosis being termed type I [55]. Cancer cells are refractory to apoptosis owing to at least three changes: (i) enhanced growth factor signaling; (ii) increased NF-κB signaling, which activates anti-apoptotic genes, such as Bel-2 and Bel-xL; and (iii) dysfunction of tumor suppressor proteins, such as the p53 protein. All these changes can inhibit cellular autophagy: growth factors are linked to mTOR activation, and Bel-2 and Bel-xL inhibit the Beclin 1 complex (see earlier), but the effect of p53 protein is dependent on the cellular context. Feng et al. [71] demonstrated that p53 protein can inhibit mTOR, either via AMPK or PTEN, and in this way it could induce autophagy. On the contrary, Tasdemir et al. [49] observed that a downregulation of p53 expression can trigger autophagy. They also demonstrated that only cytoplasmic p53 protein, but not nuclear p53, was capable of inhibiting autophagy (Figure 1).

The availability of transgenic mouse models that over-express p53 can shed light on this paradox, as described by Matheu et al. [72]. Mouse models carrying a truncated form of p53, insensitive to Mdm-2 inhibition and showing constitutive p53 activation, displayed a premature aging phenotype, probably as a result of the repression of autophagy and induction of apoptosis. Instead, transgenic mice containing an intact p53 gene along with transgenic alleles of p53 and Arf, an activator of p53 expression, showed a modest increase in p53 expression but exhibited a delay in the aging process [73]. The Arf–p53 pathway also induced a heightened level of cancer resistance and a decreased level of aging-associated oxidative damage. Interestingly, Feng et al. [74] have demonstrated a decline with aging in the function of p53, both in the inducible expression of p53
itself and its target genes. Given that the Arf–p53 pathway inhibits mTOR [71], it is probable that even a modest increase in p53 level if present at the right location can affect mTOR signalling, whereas constitutive hyperactivity with increased p53 acetylation, as observed in SIRT1 knockout mice [75], can lead to premature aging syndrome.

**Autophagy and delaying of the aging process**

Caloric restriction (CR), which is also called dietary restriction, is the only strategy known to extend lifespan universally in a wide range of organisms from yeast to non-human primates. The mechanism of lifespan extension is still unclear. However, several studies have demonstrated that autophagic degradation increases during caloric restriction [32,76,77]. Commonly, this is linked to the downregulation of the mTOR pathway, both in CR and during times of limited energy availability, but several indirect mechanisms could be involved. The effects of CR are highly tissue- and species-specific and vary according to treatment modality, as noted in microarray studies [78]. However, gene expression studies also confirm the phenotypic observations that CR can retard age-related changes, although microarray studies have not identified any one distinct mechanism. Teleologically, autophagy, which is a self-digestion mechanism, would represent a better way to maintain energy balance during CR, but it is also possible that CR is only an inducer of metabolic or genetic responses that retard the aging process [32,79].

Several studies have supported the role of SIRT1 in CR-induced effects, for example in lifespan extension. Transgenic mice overexpressing SIRT1 display several characteristics that are similar to those found in caloric-restricted mice, such as reduction in blood levels of cholesterol, adipokines and insulin and better glucose tolerance [80]. However, Chen et al. [81] recently demonstrated that the effects of CR on the expression levels of SIRT1 are tissue-specific; for instance, CR decreases the expression of SIRT1 in mouse liver. Also, microarray studies do not support a major role of SIRT1 in CR-induced changes during the aging process [78]. However, SIRT1 enzyme can be activated by NAD+, and the NAD+ concentration is increased during CR and energy deficiency. SIRT1 has several verified targets, which could potentially translate activation of SIRT1 into the enhancement of autophagy. SIRT1 inhibits the function of the NF-κB system and thereby can suppress several age-related stress and inflammatory responses and, in this way, enhance autophagocytosis (Figures 1,2). The effects of SIRT1 on the responses driven by NF-κB signaling have recently been reviewed [82]. Activation of the innate immunity system is a characteristic age-related change [83] and is also observed in microarray studies [78]. CR reduces the pro-inflammatory profile and thereby can inhibit the signaling to NF-κB and subsequently induce autophagy (Figure 1).

**Therapeutic implications**

The accumulation of protein aggregates in the cytoplasm and nuclei is a pathological hallmark in several degenerative diseases such as Alzheimer’s disease and other tauopathies, Huntington’s disease, and X-linked myopathy [2,84]. Extracellular depositions, such as amyloid plaques in Alzheimer’s disease, are also common in neurodegenerative diseases. Several observations imply that autophagocytosis is defective in many protein aggregation diseases and, therefore, the enhancement of autophagic degradation could have therapeutic potential. Confirming that assumption, recent studies have demonstrated that inducers of autophagy, for example rapamycin and trehalose, can clearly potentiate the clearance of aggregates and reduce the toxicity of mutant proteins in Huntington’s disease [11,12]. However, inefficient housekeeping in protein aggregation diseases can be caused by impaired clearance of autophagic vacuoles rather than by their induction capacity, as the results indicate in Alzheimer’s disease [85] and also in the aging process. For instance, lipofuscin material is indigestible by lysosomal enzymes and aggregates in lysosomal compartment in the cytoplasm [20,24,25]. However, the excessive induction of autophagic vacuoles and delay in their clearance can expose cells to autophagic stress, and the release of proteolytic enzymes can activate apoptotic cell death [57,84] (Box 1).

Recently, there has been considerable interest in designing different CR mimetics based on the activation of SIRT1. Resveratrol is one of the polyphenolic compounds that activates SIRT1 enzyme and is a popular model compound in the development of CR mimetics [86]. Several other targets have been proposed, such as inhibition of glycolysis and mTOR along with hormonal mimetics. They might possess important therapeutic benefits if they can induce autophagy (i.e. by enhancing the ‘cleaning out’ of protein aggregates that accumulate in age-related degenerative diseases). However, it is not yet clear whether or not these CR mimetics will be able to extend lifespan.

**Concluding remarks**

Autophagocytosis was discovered >40 years ago [87] but it is only during the past ten years that its impact on cellular physiology and pathology have been appreciated. Simultaneously, molecular studies with different models of aging have revealed a unified signaling network regulating the aging process. Interestingly, there is a clear overlap between the signaling networks regulating both aging and autophagocytosis, which emphasizes the important role of autophagy in the regulation of aging and age-related degenerative diseases (Figure 2). It is evident that increase in autophagy can extend lifespan. Therefore, it is not surprising that the well-known longevity factors, such as SIRT1, FoxOs and p53, can enhance autophagic degradation.
(Figure 1). By contrast, mTOR and NF-κB can inhibit autophagy and elicit the aging process. The role of NF-κB signaling is interesting because it can inhibit autophagy but can activate proteasomal degradation and support inflammatory responses (Figure 2). These cellular responses comprise the basic elements of the senescent phenotype, that is, protein deposition, tissue atrophy and inflammatory disorders (Figure 2). It seems that NF-κB has a central role in these detrimental tissue alterations because SIRT1 signaling [88] and directly enhance autophagic degradation (Figure 1). These signaling connections indicate that the decline of autophagocytosis might be involved in the inflammation-related aging process driven by NF-κB signaling [82, 83] (Box 1).

A wide range of literature shows that oxidative stress and DNA damage are involved in the aging process [17–19] and both of these cellular stresses can activate NF-κB signaling [89, 90]. In particular, DNA damage is the major defect in progeroid syndromes [90]. Activation of the NF-κB system induced by genotoxic and oxidative stress supports the inflammation-related aging model with a decline in autophagic degradation (Figure 2). However, oxidative stress and DNA damage can also regulate mTOR signaling or directly affect autophagosome formation [91–93]. Scherz-Shouval et al. [92] demonstrated that reactive oxygen species (ROS) can inhibit Atg4, a cysteine protease, and subsequently enhance Atg8–LC3-driven autophagosome formation. However, it is unknown if this kind of regulation is present in vivo. Studies clearly show that ROS can activate mTOR signaling via different pathways and thereby repress autophagic degradation, reviewed by Blagosklonny [91]. A TOR-centric model was also proposed, in which activators of mTOR reduce lifespan, and subsequently enhance Atg8

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