

## The Interplay between Autophagy and Aging

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Numerous studies have established a link between autophagy and aging; however, the relationship has not been clearly defined. Aging is a very complex process caused by the accumulation of various factors due to the gradual failure of cellular maintenance. Recent studies have shown that autophagy reduces the stress responses induced by starvation, reactive oxygen species, and the accumulation of intracellular proteins and organelles through cytoprotection, clearance of damaged mitochondria, and lysosomal degradation. Here, we summarize our current understanding of the relationship between autophagy and the aging process.

**Keywords:** Aging; Autophagy; Caloric restriction; Mitochondria; Stress

### INTRODUCTION

Autophagy is a catabolic process in eukaryotic cells that delivers cytosolic substrates to the lysosome for degradation. Autophagy consists of several steps: 1) induction and nucleation, 2) elongation of phagophores and sequestration of cytosolic components through autophagosome formation, 3) transport to the lysosome, 4) degradation, and 5) utilization of degradation products [1]. Despite the simplicity of this process, autophagy plays a role in various physiological processes, including cell growth, cell differentiation, cell survival, and immune responses, as well as in pathological diseases, including cancer, neurodegeneration, and metabolic syndrome [2]. Numerous studies also suggest that autophagy is closely related to aging through its housekeeping function in the degradation and recycling of cytoplasmic components and damaged organelles. Recent studies have emphasized the role of autophagy as a key regulator of the aging process, especially through the removal of damaged mitochondria. Autophagy is also associated with well-known longevity-promoting signals, including caloric restriction (CR), insulin/insulin-like growth factor (IGF)-1 signaling, and the p53 pathway [3]. Therefore, it is clear that au-

tophagy contributes to the overall health and lifespan of individual cells as well as the whole organism. Here, we describe and discuss the likely relationships between autophagy and aging, as well as the potential molecular mechanism underlying the regulation of aging by autophagy.

### AUTOPHAGY PROTECTS CELLS AGAINST STRESS

Cells are constantly exposed to internal and external stresses, which can cause problems for the maintenance of homeostasis and functional integrity and can eventually lead to cell death. Therefore, understanding the protective pathway against death induced by a variety of stresses is useful for deciphering the aging process [4]. An important function of autophagy is a self-limited survival strategy, which is a cytoprotective mechanism against starvation and oxidative stress due to mitochondrial dysfunction, aggregate-prone proteins, and pathogens [5]. Under prolonged starvation, autophagy plays a crucial role in maintaining an amino acid pool for survival [2]. Mice with a knockout of *atg5*, an essential gene for membrane elongation in autophagy, are normal at birth but show severe nutri-

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ent and energy insufficiency within 10 hours after birth and have significantly shorter survival times than wild-type mice under starvation conditions [6]. Moreover, *beclin-1* or *atg8* RNAi-mediated inhibition of autophagy in *Caenorhabditis elegans* causes a failure to survive during dauer diapause [7]. This implies that recycling via autophagy is critical for the maintenance of cellular energy homeostasis and survival.

Recent studies suggest that well-known aging regulators, such as SIRT1 and DAF-16/FOXO, are required to induce autophagy under starvation conditions. For example, expression of SIRT1, an NAD<sup>+</sup>-dependent deacetylase [8], is increased during starvation [9] and activates autophagy by deacetylating Atg5, Atg7, and Atg8 under starvation conditions in both nematodes and mammals [10,11]. Moreover, SIRT1 increases the median and maximum lifespan of *C. elegans*. The transcription factor DAF-16/FOXO, which is known to extend lifespan through its cytoprotective effects, is also involved in autophagy activation [12]. Deficiency of p53, a tumor suppressor that influences lifespan, can also induce autophagy in mammalian systems [13]. These findings point to a molecular connection between the aging process and the cytoprotective activity of autophagy.

Autophagy may contribute to lifespan expansion through regulation of reactive oxygen species (ROS) production. It is commonly known that even a tolerable level of ROS induces autophagy, which in turn reduces oxidative damage in cancer, cardiac, and neurodegenerative disease [14,15]. For example, starvation increases ROS, which regulates autophagy through adenosine monophosphate-activated protein kinase (AMPK) activation [16]. ROS itself accumulates in *Atg5*- and *Atg7*-knockout mice [17] and in *Atg5*- and *Atg10*-knockdown cells under starvation conditions [18]. Interestingly, ATG4, an essential protease in the autophagy process, was identified as a direct target for oxidation by hydrogen peroxide, which activates autophagy [19]. Numerous studies have shown direct evidence that continuous oxidative stress occurs during the aging process [20], which might disrupt protein turnover [21]. In fact, enhanced autophagy eliminates oxidative stress through enhanced lysosome activity and alteration of the luminal pH, and/or direct regulation of lysosomal enzymes [22] and removal of damaged mitochondria [23]. While the relationship between autophagy and ROS production is still a subject of debate, autophagy apparently functions to remove ROS generated by starvation and/or aging. Taken together, these studies clearly demonstrate that autophagy plays a critical role in the

aging process through the regulation of ROS.

## AUTOPHAGY CONTROLS THE AGING PROCESS THROUGH DEGRADATION OF MISFOLDED AND AGGREGATE-PRONE PROTEINS

Aging is characterized by inefficiency and even failure of cellular maintenance, repair, and turnover mechanisms, which results in the accumulation of damaged substances or organelles in aged cells [24]. The primary function of autophagy is protein and organelle turnover for the maintenance of homeostasis. During aging, protein turnover slows down, and aggregate-prone proteins, such as mutant  $\alpha$ -synuclein, tau, and mutant huntingtin which cause Parkinson, Alzheimer, and Huntington diseases, respectively, accumulate [25]. In fact, ATG5, ATG7, and BECN-1 expression is down-regulated in the aged human brain [26]; therefore, autophagy activity is likely down-regulated with aging. Rapamycin, an inhibitor of mammalian target of rapamycin (mTOR) and autophagy inducer, boosts the clearance of mutant huntingtin fragments and attenuates toxicity in cells [27]. Similarly, rapamycin reduces tau toxicity in *Drosophila melanogaster* as well as the appearance of aggregates and cell death associated with poly-glutamine expansion in *D. melanogaster* and mammals [28,29]. In contrast, inhibition of autophagy increases mutant huntingtin aggregates [30,31]. In Alzheimer disease (AD), an AD-causing mutation in presenilin disrupts autophagy activity [32], and deletion of *beclin-1* leads to neurodegeneration and amyloid  $\beta$  accumulation via decreased neuronal autophagy and disruption of lysosomes [33].

The proteolytic activity of the ubiquitin proteasome system (UPS), which is the major degradation pathway for short-lived and soluble proteins, declines during aging [34-36]. Similar to the early aging phenotype of animals with UPS impairment [36,37], a genetic model with reduced autophagy activity also exhibits an accelerated aging phenotype [38]. These observations led to the proposal that increased proteolytic activity of autophagy and UPS may delay aging [39,40]. Consistently, genetic ablation of *p62*, an ubiquitin- and LC3-binding protein that is involved in the clearance of ubiquitin-conjugates [41], enhances the formation of ubiquitin-positive protein aggregates, resulting in liver injury and neurodegeneration in autophagy-deficient mice [17] and accelerated presentation of ageing phenotypes [42]. More recently, Pyo et al. [43] present-

ed direct evidence that overexpression of Atg5 in mice activates autophagy and significantly extends lifespan. Therefore, it is generally believed that degradation of misfolded and aggregate-prone proteins—which are apparently responsible for aging and aging-related diseases when they accumulate—is accomplished through the modulation of autophagy. While impairment of UPS might induce compensatory activation of autophagy in cells [44,45], their crosstalk in the aging process of animal model is not yet clear.

### MITOPHAGY CONTROLS MITOCHONDRIA QUALITY AND TURNOVER

One of the major functions of autophagy is the degradation of excess or injured organelles [1]. Accumulated mitochondrial damage leads to mitochondrial dysfunction, a common feature of aging that involves changes in mitochondria membrane potential, ROS and adenosine triphosphate (ATP) production, and calcium homeostasis. Under such conditions, cells usually overcome the damage by inducing autophagy to remove the dysregulated mitochondria [46]. Mitophagy, a mitochondrial-selective type of autophagy, removes the damaged mitochondria for quality control. This process was first described in yeast, where ATG32 was discovered as a key receptor in mitophagy that interacts with ATG8/LC3. Extensive studies in mammalian systems revealed that mitophagy has unique machinery and is mediated by PINK1, an outer mitochondrial membrane (OMM) kinase, and PARKIN, a cytosolic E3 ubiquitin ligase [47]. Upon induction of mitophagy, cytosolic PARKIN is recruited to the damaged mitochondria destined for degradation by PINK1 on the OMM, which then induces mitophagy to remove the damaged mitochondria [48].

Despite the expectation of a connection between mitophagy and aging, there is little evidence showing a direct correlation. However, a limited number of studies have demonstrated an association between mitophagy and aging. A defect in mitophagy is apparently associated with Parkinson disease caused by loss of function mutations in *PINK1* or *PARK2*. Several mutations in parkin and *PINK1* in Parkinson disease display defects in parkin-induced mitophagy [49,50]. Interestingly, parkin-knockout mice have reduced lifespan and receive less neuroprotection against aging [51]. In addition, Uth1p, one of the four youth proteins on the OMM, participates in mitophagy and prolongs the lifespan of yeast under starvation through regulation of oxidative stress [52]. Deficiency in Cisd2, which

is involved in mammalian lifespan control and is a causative gene for Wolfram syndrome 2, leads to mitochondrial degeneration, which appears to induce autophagy [53]. In addition to damaged mitochondria quality control, mitophagy is also involved in the steady-state turnover of mitochondria which removes undamaged mitochondria during developmental processes. In most mammals, mature red blood cells lack mitochondria, which are removed by the mitophagy-mediated action of NIP3-like protein X during maturation [54]. Therefore, the effect of mitophagy on aging should be determined through distinct studies.

Although there are many sites of ROS production in the cell, mitochondria are a major source of ROS. Because mitochondrial proteins and mitochondrial DNA are easily exposed to oxidative damage, and mitochondrial damage in turn stimulates ROS generation, this continuous process eventually allows cells to age and die [55]. The murine lifespan is extended by the overexpression of catalase in mitochondria [56] and by superoxide dismutase/catalase mimetics and detoxification of mitochondrial ROS [57]. Although it is becoming clear that mitophagy is critical for the quality control of mitochondria, a clear connection among mitophagy, mitochondrial quality control, turnover, and the aging process remains to be fully addressed.

### AUTOPHAGY IS CONNECTED TO AGING VIA ENERGY CONTROL

Among the many factors that influence lifespan, such as oxidative stress, DNA damage, genetic programming, and the environment, CR is the best-known factor that extends lifespan in various organisms and reduces the pathogenesis of age-related diseases, including diabetes, cardiovascular disease, cancer, and neurodegeneration [58,59]. It has been reported that CR induces a maximum rate of autophagy proteolysis in rat liver [60]. Interestingly, CR can extend lifespan in model organisms by inducing autophagy and reducing mTOR and protein kinase A (PKA) activity, and protein kinase B (PKB)/Sch9 signaling [61]. Autophagy is required for lifespan extension of an *eat-2* deletion mutant, a CR model in *C. elegans* [62], and knockdown of *beclin-1* and *atg7* suppresses the longevity associated with CR [63]. Conversely, forced activation of autophagy in *Atg5* transgenic mice is accompanied by less obesity than their age-matched littermates [43]. These observations illustrate that the *atg* genes, which are essential in autophagy, are

also critical for lifespan extension by CR, indicating a strong correlation between CR and autophagy.

In addition, more evidence has shown a functional correlation between autophagy and energy levels. SIRT1, which is essential for lifespan extension through CR via the insulin/IGF pathway in mice [64], is also a potent autophagy inducer [9]. AMPK, another energy sensor, functions to extend lifespan in *C. elegans* and induces autophagy in human cell lines [65]. Sch9 serine/threonine protein kinase, a nutrient sensor, is also involved in lifespan extension and modulation of autophagy in yeast [66]. Based on these findings, we know better than before that the established signals regulating the aging process and energy level can also coordinate autophagy activity, drawing more attention to the role of autophagy in lifespan control.

## CONCLUSIONS

In this review, we have elucidated the functional relationship between autophagy and aging while encompassing the recent discoveries. Autophagy is now believed to be a pathway regulating healthy aging and lifespan, although the relationship between the subordinate and the superior is not clear. Increasing evidence illustrates that many aging pathways can control autophagy activity and that autophagy can regulate aging in various organisms. Although a detailed molecular mechanism linking autophagy to aging is not yet clear, the crucial roles of autophagy in aging seem to be associated with the removal of factors affecting the aging process: for example, cytoprotection against stresses, such as ROS, and the clearance of misfolded or aggregate prone-proteins and damaged subcellular organelles, such as the mitochondria. In particular, the understanding that the regulation of aging by CR is apparently associated with autophagy is a considerable advancement. There are also accumulating reports demonstrating autophagy control through genetic or pharmacological manipulation. Nonetheless, we currently lack a suitable chemical or genetic modulator that selectively activates autophagy that could be used as a lifespan extender in mammals. Therefore, the discovery of an autophagy regulator is the best strategy to pursue in healthy aging and improve lifespan.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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