Review Article

Links Between Autophagy and Healthy Aging ☆

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Abstract

Several if not all manifestations of aging can be postponed by a healthy lifestyle involving a balanced diet coupled with regular exercise and sufficient sleep. Similarly, various genetic and pharmacological longevity interventions can exert beneficial effects across species in a conserved manner, extending both lifespan and healthspan. While all these interventions—ranging from genetic perturbations to pharmacological supplementation to lifestyle changes—affect diverse biological processes, a common candidate mechanism underpinning at least some of their benefits is autophagy, a cellular recycling process essential for maintaining cellular homeostasis. In this review, we summarize how autophagy is affected by various pharmacological and lifestyle factors, with a focus on studies in which autophagy have been shown to play a causal role in promoting healthy aging. Specifically, we review the molecular mechanisms through which pharmacological agents, dietary restriction, exercise, sleep adjustments, as well as temperature modulation affect autophagy to extend lifespan and often also healthspan in model organisms and humans. Still, major gaps remain in human research due to limited assays to monitor autophagy and the scarcity of longitudinal studies linking autophagy dynamics to health outcomes. Closing this gap is a key challenge in converting discoveries from model organisms into interventions that consistently enhance healthy aging in humans. By summarizing current findings and highlighting remaining uncertainties, this review aims to provide a roadmap for translating insights on autophagy from model organisms into strategies to promote healthy aging in humans.

Introduction

Autophagy is a cellular recycling process by which various cellular materials, referred to as cargo, are degraded in acidic, hydrolase-filled lysosomes. Autophagy can be divided into at least three different types, depending on how the intracellular cargo is delivered to lysosomes: macroautophagy [1], microautophagy [2,3], and chaperone-mediated autophagy (CMA, see abbreviation list) [4]. Macroautophagy (hereafter autophagy, Figure 1) is extensively studied in the context of genetic, pharmacological, and lifestyle factors relevant to human health, and is therefore the focus in this review.

Here we review literature in which autophagy (and CMA for a few relevant studies) has been shown to change and possibly causally promote healthy aging in response to different genetic longevity paradigms or lifestyle factors. Specifically, we summarize key studies that meet at least one of three criteria: (1) the study investigates links between lifestyle factors and autophagy in

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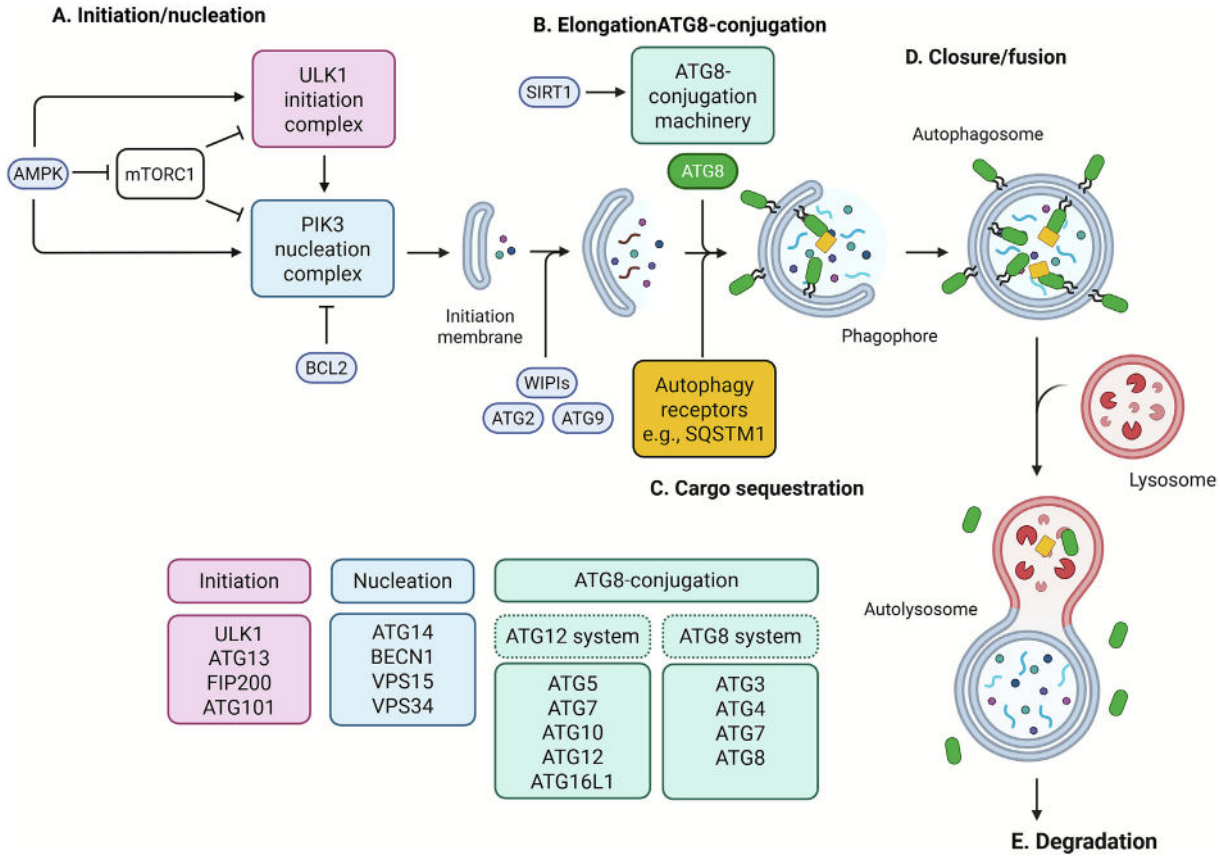

Figure 1. Overview of the (macro)autophagy process. During autophagy, multiple autophagy-related proteins facilitate the formation of autophagosomes, sequestration of cargo to autophagosomes, fusion of autophagosomes with lysosomes and degradation of cargo. **A.** Initiation/nucleation step. The ULK1 complex (ULK1–ATG13–ATG101–FIP200) activates the class III PI3K complex (BECN1–ATG14–VPS15–VPS34) to generate PI3P on the nascent isolation membrane, recruiting PI3P-binding proteins such as WIPIs and ATG9-containing vesicles to promote membrane expansion. **B.** Elongation/ATG8 conjugation. Two ubiquitin-like conjugation systems mediate ATG8 lipidation on the phagophore membrane. The ATG12–ATG5–ATG16L1 complex (E3-like) facilitates the covalent attachment of ATG8 proteins to PE, driving membrane elongation and autophagosome formation. **C.** Cargo sequestration. Autophagy receptors such as p62 recognize ubiquitinated cargo and link them to ATG8-conjugated membranes, enabling selective autophagy. **D.** The completed autophagosome seals and subsequently fuses with a lysosome, forming autolysosome. **E.** Degradation. Lysosomal hydrolases degrade the sequestered material and inner autophagosomal membrane, releasing amino acids, lipids and sugars back to the cytosol for reuse.

humans; (2) the study reveals causal links between lifestyle factors and autophagy in model organisms (i.e., the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, the mouse *Mus musculus* or the rat *Rattus norvegicus*) by conducting genetic or pharmacological manipulation of autophagy genes (knockdown, knockout or pharmacological treatment with autophagy activators/inhibitors); or (3) a correlational study links autophagy and lifestyle factors in the context of aging in model organisms. Studies from the third criterion, investigating emerging links between autophagy and lifestyle factors in aging, are summarized in Table 1. Given the breadth of the literature, the following sections are organized by individual lifestyle factors. Each subsection integrates findings on longevity, autophagy modulation, and links between autophagy and aging, while noting remaining open questions. Notably, while causal evidence for autophagy's role in aging comes from model organisms, human studies remain correlative or indirect. This emphasizes the need for reliable, non-invasive measures of autophagy in humans, and highlights the current knowledge gaps that this review summarizes.

Below, we first briefly summarize the molecular underpinnings of the autophagy process (Figure 1), then provide an overview of the various assays used in these studies to monitor autophagy in different model organisms

(Figure 2), as well as review the evidence linking autophagy and aging in genetic longevity paradigms from these model organisms.

The autophagy process at the molecular level

During the autophagy process, autophagy proteins (ATGs) regulate a complex, multi-step process (Figure 1). At least five different steps are involved: (A) the initiation/nucleation step, in which a double-membrane sheet called the phagophore is formed; (B) the ATG8-conjugation step, in which ATG8 proteins are lipidated and conjugated to the phagophore membrane; (C) the cargo sequestration step, in which so called autophagy receptors bind to ATG8 proteins on the phagophore membrane while sequestering specific cargo; (D) the closure/fusion step, in which the autophagosome closes to form a double-membrane vesicle and fuses with the acidic, hydrolase-containing lysosome to form autolysosomes; and (E) the degradation step, in which autolysosomal cargo is degraded for subsequent reuse as building blocks in the cell. Because the closure/fusion and degradation steps are not specific to autophagy, i.e., they are shared with other non-autophagic, intracellular pathways such as endocytosis, we summarize the autophagy-specific steps before closure/fusion below.

Upstream regulators of autophagy initiation. While basal levels of autophagy maintain cellular homeostasis during normal conditions, it is often further induced by stressors that can damage cellular components. Decreased nutrient availability, particularly of amino acids and glucose, is a well-characterized stimulus regulating autophagy that is also linked to aging. Autophagy initiation is controlled by a complex network of signaling pathways that sense cellular energy and nutrient status. Among the key regulators are several conserved proteins that coordinate growth, metabolism, and stress responses. These include: **(1) mTORC1:** Under nutrient-rich conditions, the nutrient-sensing serine/threonine kinase mechanistic target of rapamycin (mTOR) is activated and forms two pro-growth protein complexes: mTOR complex 1 (mTORC1) and mTORC2. mTORC1 regulates cell growth and metabolism, whereas mTORC2 is involved in proliferation and cell survival [5]. mTORC1 is especially well-studied for its role in suppressing autophagy (Figure 1A). mTORC1directly phosphorylates key components of the autophagy initiation complex (UNC-51-like kinase 1 (ULK1) and ATG13), as well as the nucleation complex (ATG14), thereby inhibiting the formations of these complexes. In contrast, mTORC1 is inhibited during nutrient deprivation, allowing these complexes to become active and initiate autophagy. **(2) TFEB:** The helix-loop-helix transcription factor EB (TFEB) functions as a central transcriptional activator of autophagy and lysosomal biogenesis, promoting the transcription of numerous autophagy-related and lysosomal genes by binding to the coordinated lysosomal expression and regulation (CLEAR) motifs in their promoters [6]. mTORC1 phosphorylates and inhibits TFEB by sequestering it in the cytoplasm. Phosphorylated TFEB is thought to be targeted for degradation via the ubiquitin–proteasome system or retained in the cytoplasm through binding to 14-3-3 protein, which masks its nuclear localization signal and thereby prevents its transcriptional activity [6]. By contrast, inhibition of mTORC1 allows TFEB to translocate to the nucleus and activate the TFEB-regulated gene network [6]. **(3) AMPK:** Another key autophagy regulator is AMP-activated protein kinase (AMPK), which is activated under low-energy conditions such as starvation (Figure 1A) [7]. AMPK promotes autophagy by phosphorylating ULK1 and Beclin1 (BECN1), enhancing initiation and nucleation complex formation. It also indirectly activates autophagy by inhibiting mTORC1 through phosphorylation. **(4) SIRT1:** A further important regulator, also linked to aging, is the NAD^+-dependent protein deacetylase sirtuin 1 (SIRT1). SIRT1 expression increases during starvation and promotes autophagy by deacetylating components of ATG8-conjugation machinery such as ATG5, ATG7 and ATG8, thereby facilitating autophagosome formation [8].

Initiation/nucleation step. Autophagy initiation begins with the assembly of two key protein complexes (Figure 1A). The initiation complex, composed of ULK1, ATG13, ATG101 and FAK family-interacting protein of 200 kDa (FIP200), phosphorylates downstream targets to promote autophagosome formation. One of its main roles is to phosphorylate and activate the nucleation complex, a class III phosphoinositide 3-kinase (PI3K) complex including BECN1, ATG14, vacuolar protein sorting 15 (VPS15), and VPS34. The nucleation complex generates isolation membranes (or phagophore precursors), which serve as platforms for autophagosome formation [5], and the activity of the nucleation complex is tightly regulated to prevent inappropriate autophagy activation. BCL2, an anti-apoptotic protein, binds to BECN1/Beclin1 and inhibits the assembly of the nucleation complex. Phosphorylation of BCL2 disrupts its interaction with BECN1/Beclin1, thereby relieving inhibition and promoting autophagy initiation. A key function of the nucleation complex is to generate PI3-phosphate (PI3P) in the nascent isolation membrane, which in turn recruits PI3P-binding proteins such as WD-repeat protein interacting with phosphoinositides (WIPIs). WIPIs help expand the isolation membrane, facilitating its growth into a mature phagophore, through interacting with the lipid-transfer protein ATG2 and with

Table 1 Studies linking autophagy and aging influenced by lifestyle factors.

Lifestyle factors	Key findings	Causal/correlational	Organisms	References
Pharmacology				
Rapamycin	*Atg1*, *Atg7* and *Atg11* are required for lifespan extension by rapamycin	→	Yeast	[20]
	Atg1 and *Atg5* are required for lifespan extension by rapamycin	→	Flies	[21,22]
	Rapamycin treatment induces autophagy and reduces aging-associated senescent cells	→	Mice	[151]
Metformin	Metformin treatment in late middle-aged animals improves cognitive function and enhances autophagy in the hippocampus	→	Mice	[26]
	Metformin treatment in aged animals reduces age-related inflammation and boosts autophagy in the liver and intestine	∝	Rats	[25]
Spermidine	*Atg7* is required for lifespan extension by spermidine	→	Yeast	[27]
	bec-1/BECN1 is required for lifespan extension by spermidine	→	Worms	[27]
	Atg7 is required for lifespan extension by spermidine	→	Flies	[27]
	Spermidine treatment induces autophagy, reduces aging-associated senescent cells and liver fibrosis, and delays cardiac aging. *Atg5* is required for the delay of cardiac aging by spermidine	∝	Mice	[151–153]
	Spermidine treatment in T cells and B cells from old individuals suppresses age-dependent decline in autophagy	∝	Humans	[154,155]
Resveratrol	*bec-1/BECN1* is required for lifespan extension by resveratrol	→	Worms	[30]
	Resveratrol treatment prevents aging-associated alterations and autophagy decline in skeletal muscle and heart tissue	∝	Mice	[31]
Urolithin A	*bec-1/BECN1*, *vps-34*, *sqst-1/p62*, *dct-1/BNIP3, pink-1* and *prk-1/Parkin* are required for lifespan extension by urolithin A	→	Worms	[35]
	Urolithin A treatment in middle-aged individuals improves muscle strength and exercise performance, and upregulates mitophagy	∝	Humans	[36]
Dietary restriction (DR)				
	Atg5, Atg7, Atg8, Atg15, Vam3/STX7, Vam7, and *Vma7/ATG6V1F* are required for lifespan extension by DR	→	Yeast	[48]
	unc-51/Atg1/ULK1, *bec-1/BECN1, vps-34*, *atg-7,* and *atg-18/WIPI2* are required for lifespan extension by DR	→	Worms	[41,43,49,50]
	Atg1/ULK1 and *Atg8a/GABARAP* are required for lifespan extension by intermittent time-restricted feeding (iTRF)	→	Flies	[51]
Exercise				
	unc-51/ULK1, *epg-1/ATG13*, *bec-1/BECN1*, *let-512/VPS34*, *pink-1* and *dct-1/BNIP3* are required for lifespan extension by swimming	→	Worms	[79]
	Endurance exercise prevents age-related reductions in autophagy in skeletal muscle	∝	Mice	[80]
	Age influences autophagy response in plasma after endurance exercise	∝	Humans	[82]
Sleep				
	Atg1/ULK1 and *Atg8a/GABARAP* are required for lifespan extension by iTRF	→	Flies	[51]
	Sleep fragmentation disrupts autophagy in the brain and causes cognitive decline and neurodegeneration-associated protein accumulation	∝	Mice	[102]

Table 1 (*continued*)

Lifestyle factors	Key findings	Causal/correlational	Organisms	References
Temperature stress				
Heat stress	*unc-51/ULK1*, *bec-1/BECN1*, *lgg-1/GABARAP* and *sqst-1/p62* are required for lifespan extension by hormetic heat stress	→	Worms	[11,127]
	Heat shock induces autophagy stronger in older animals	∝	Rats	[128]
	Heat shock induces autophagy in older individuals	∝	Humans	[82]
Cold stress	Older individuals show impaired cold-induced autophagy	∝	Humans	[129]

→: causal evidence for lifestyle-induced autophagy's role in aging/longevity; ∝: correlational evidence with aging-related phenotypes by lifestyle-induced autophagy. For studies linking autophagy and genetic longevity paradigms, see a previous review [17].

ATG9-containing vesicles; these are small, mobile membrane carriers are enriched in the transmembrane scramblase ATG9 and supply additional membrane and phospholipids to the isolation membrane.

Elongation & ATG8-conjugation steps. During the subsequent elongation step of the autophagy process, the growing double-membrane phagophore engulfs cytosolic material, referred to as cargo, eventually sealing to form a closed double-membrane vesicle, called an autophagosome. A key event in this process is the conjugation of ATG8 proteins to membrane lipids (Figure 1B), also called ATG8 lipidation, which facilitates cargo selection. In humans, ATG8 family proteins are divided into two subfamilies: microtubule-associated proteins 1A/1B light chain 3 (LC3) and gamma-aminobutyric acid type A receptor-associated proteins (GABARAPs). ATG8 proteins are covalently conjugated to phosphatidylethanolamine (PE), a lipid enriched on phagophore membranes, through ubiquitin-like conjugation steps. These steps depend on two interconnected enzymatic systems: (1) the ATG8 system, which includes ATG4 (a cysteine protease that primes ATG8 via cleaving off the ATG8 C-terminus), ATG7 (E1-like enzyme), and ATG3 (E2-like enzyme), directly lipidates ATG8, and (2) the ATG12 system, which includes ATG12, ATG5, and ATG16-like 1 (ATG16L1) in a complex, acts as an E3-like ligase, and targets the lipidation of ATG8 to the phagophore membrane. Nonlipidated ATG8, before its conjugation to PE, is referred to as ATG8-I, whereas lipidated ATG8 is referred to as ATG8-II. While ATG8-II on the phagophore membrane is thought to facilitate its elongation and maturation to an autophagosome, the detailed molecular mechanisms remain unclear. Because ATG8-II remains membrane-bound until removed by ATG4 or degraded in the autolysosome, it is widely used as a marker of autophagosome formation (see Section 'Assays used to monitor autophagy in different models' below).

Cargo-sequestration step. Following ATG8 conjugation, proteins called autophagy receptors are recruited to the expanding autophagosomal membrane (Figure 1C) [9]. Autophagy receptors contain domains such as LC3-interacting region (LIR) motifs that enable them to bind to ATG8 family proteins embedded in the membrane [10]. Through this interaction, autophagy receptors sequester specific intracellular targets (so-called cargo) to the autophagosome in a process known as selective autophagy. Multiple forms of selective autophagy have been characterized, each targeting distinct cargo. For example, mitophagy targets mitochondria, and lipophagy targets lipid droplets, both of which are discussed further below (see Sec-

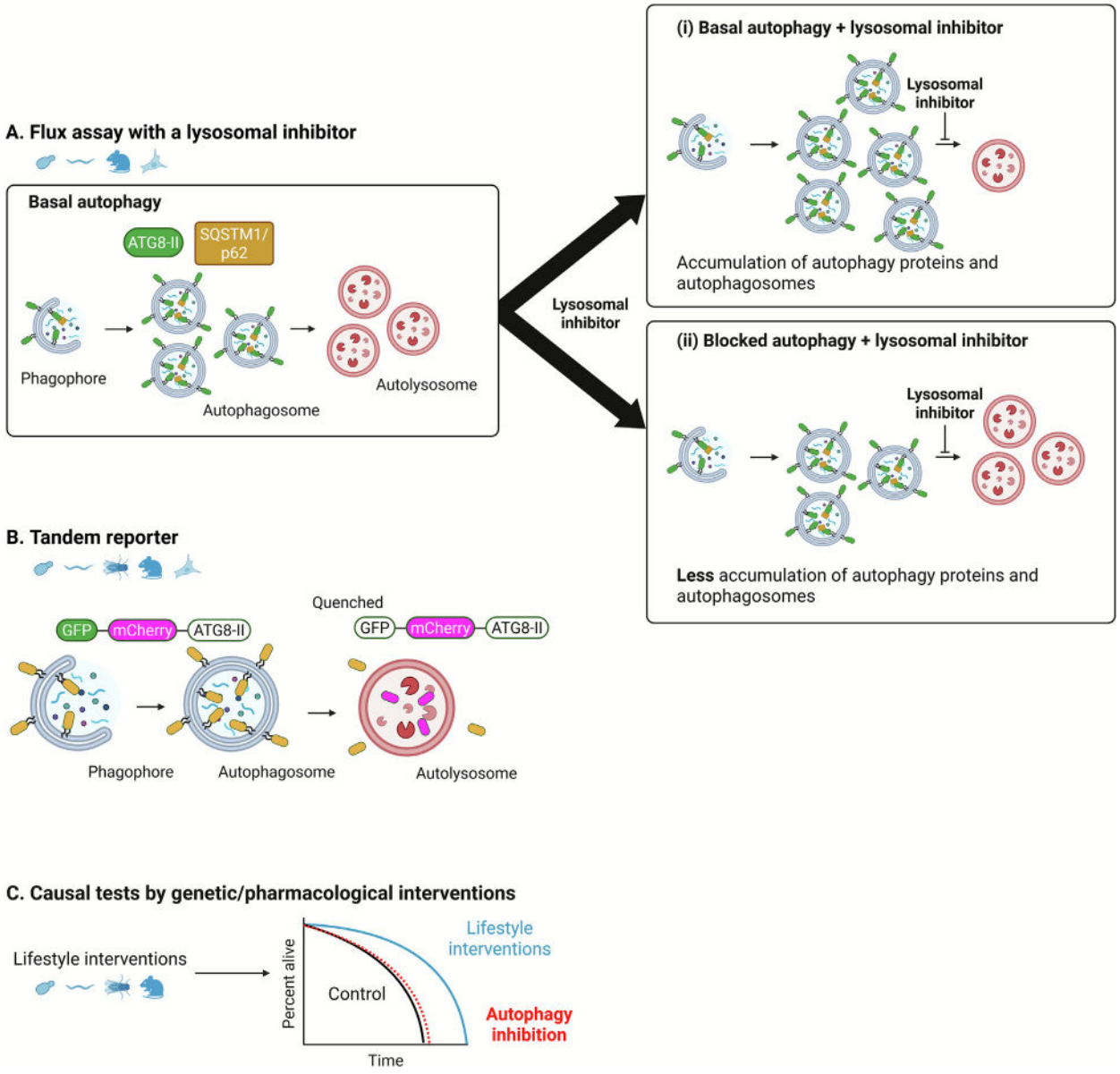

Figure 2. Experimental approaches to assess how lifestyle interventions modulate autophagy and aging. **A.** Autophagy flux assays using lysosomal inhibitors (including Bafilomycin A1 and chloroquine) distinguish between increased autophagosome formation and impaired degradation by quantifying autophagy protein levels (e.g., ATG8-II/ATG8-I ratio or p62/SQSTM1) or autophagosome numbers. **B.** Tandem ATG8 reporters tagged by a combination of pH-sensitive and insensitive fluorophores can differentiate autophagosomes from autolysosomes. The acidic environment of autolysosomes quenches pH-sensitive fluorophores such as GFP, while it does not affect pH-insensitive fluorophores such as mCherry. **C.** Causal testing of lifestyle–autophagy relationships can be performed by combining lifestyle interventions with genetic knockdown/knockout of autophagy genes or pharmacological modulation (autophagy activators or inhibitors) in model organisms ranging from yeast to mammals. See text for details.

tions 'Urolithin A' and 'Temperature stress and autophagy' below). Among known autophagy receptors, sequestosome 1 (SQSTM1, also called p62) is well characterized and is known to play a role in both mitophagy and aggrephagy (selective autophagy targeting ubiquitinated protein aggregates). Notably, p62 overexpression in simpler organisms (*sqst-1* in *C. elegans* and *dp62*/*Ref(2)p* in *Drosophila*) extends lifespan in association with increased autophagy, underscoring the functional importance of this cargo sequestration step [11,12]. Because of its association with autophagosomes, p62 is often used as another autophagosome marker in addition to ATG8-II (see

Section 'Assays used to monitor autophagy in different models' below).

Assays used to monitor autophagy in different models

Reliable measurement of autophagy is essential for interpreting how lifestyle factors modulate autophagy and influence aging. Because autophagy is a dynamic, multi-step process as outlined above, assessing its activity requires careful selection of assays that reflect not only static levels of autophagy-related proteins, but also the flux of the pathway (Figure 2). Moreover, the feasibility of performing certain assays varies between model systems, making the methodological context critical for interpreting experimental results.

As mentioned above, among the most widely used autophagy markers are the lipidated form of ATG8-II and the autophagy receptor p62. In both cellular and animal models, these proteins can be quantified by immunoblotting: an elevated ATG8-II/ATG8-I protein ratio indicates increased autophagosome formation, and decreased p62 protein levels reflect higher turnover of p62 by autophagy. Autophagosomes can also be visualized as ATG8 puncta by immunostaining or fluorescence microscopy of transgenic, fluorophore-tagged ATG8 (with GFP being the most common), when tissues or cells of interest are accessible. However, interpretation of these autophagy markers such as ATG8-II/ATG8-I ratio, ATG8 foci, and p62 protein levels, requires caution. An increase in protein levels or autophagosome number does not necessarily indicate autophagy upregulation: when lysosomal degradation is inhibited, i.e., turnover by autophagy is blocked, autophagy proteins and autophagosomal vesicles can accumulate. To resolve this ambiguity, autophagy flux assays are used to assess the dynamic progression of the process, specifically, the fusion of autophagosomes with lysosomes and subsequent degradation of autophagosomal contents, by treating cells or animals with lysosomal inhibitors that block lysosomal hydrolase activity or lysosomal acidification, which is essential for optimal hydrolase activity (Figure 2A) [13]. Commonly used lysosomal inhibitors are bafilomycin A1 (BafA1), which inhibits lysosomal acidification by blocking lysosomal V-ATPase activity, and chloroquine (CQ), which is a weak base disrupting lysosomal acidification [13]. When autophagy is active, such inhibition leads to accumulation of autophagy markers and cargo proteins, which are proteins degraded by autophagy (Figure 2Ai); when autophagy is already blocked, no further accumulation occurs (Figure 2Aii). Flux assays are commonly used in cell culture, yeast, nematodes, and mice, and increasingly in human studies, but are less common in flies due to reported secondary effects of lysosomal inhibitors [14].

An alternative approach is the GFP-mCherry-ATG8 tandem reporter, which takes advantage of GFP quenching in acidic lysosomes to distinguish autophagosomes (GFP^+ $mCherry^+$) and autolysosomes ($mCherry^+$ only) (Figure 2B). This method is especially valuable in systems where pharmacological inhibition of lysosomes is not feasible and are used in cells, nematodes, flies, and mice [13]. Because it requires genetic modification to express fluorescently tagged proteins, it is not applicable to human studies. Given these limitations, recent efforts, particularly in human research, have emphasized the need for robust, non-invasive assays to assess autophagy status [15]. Throughout this review, we note the model systems used, and the specific assays applied when interpreting experimental findings.

Autophagy and aging in genetic longevity paradigms

Autophagy plays key roles in cellular homeostasis, and consequently, it has been found to be tightly linked to many basic biological processes, including aging. Autophagy is considered a hallmark of aging [16] since it fulfills the following three premises: (i) it declines over time, (ii) decreases in autophagy shorten lifespan, and (iii) increases in autophagy, by extrinsic interventions, delay aging.

The evidence for autophagy declining over time comes from multiple model organisms, including the reduction in lysosomal protease activity in both the yeast *S. cerevisiae* and the nematode *C. elegans*, and the expression of several autophagy genes and their proteins levels decline in the fruit fly *Drosophila*, mice, and in select human tissues. Consistently, tissue-specific autophagy flux analyses in *C. elegans* injected with autophagy inhibitors at different time points show an age-dependent decrease in activity (reviewed in [17]). *S. cerevisiae, C. elegans*, and *Drosophila* have been especially instrumental in defining different genetic pathways and extrinsic interventions that can extend lifespan, including reduced mTOR signaling, reduced insulin/IGF-1 signaling, dietary restriction, and germline loss [17]. While several of these paradigms were first shown to extend lifespan in *C. elegans*, perturbations in mice and other model systems also confer an extended lifespan, emphasizing that these longevity paradigms are conserved.

In each of these settings, combining the longevity paradigm with inhibition of multiple autophagy genes, either by genetic mutation or pharmacological treatments, suppresses the lifespan extension of the organism [17], making a strong genetic argument for the process of autophagy at least in part being functionally involved,

i.e., causal to the longer lifespan (Figure 2C). In addition, these longevity paradigms generally appear to boost autophagy, as shown by Atg8 autophagy markers in combination with flux assays [17]. Moreover, activation of autophagy by genetic overexpression of individual autophagy-related genes such as the transcription factor HLH-30/TFEB in *C. elegans* or pharmacological induction (see Section 'Pharmacological interventions' below) are sufficient to extend organismal lifespan [17]. Collectively, many molecular links have been established between autophagy and aging, especially in simpler model organisms, supporting the view that decreased autophagy is a hallmark of aging [18].

Autophagy, aging and lifestyle factors

Recent research highlights a growing interest in not just increasing lifespan but particularly in enhancing healthspan, an endeavor that seeks to improve the quality of life in later years with little to no functional decline or aging-related pathologies. Interventions targeting mechanisms that delay aging at the cellular and molecular level hold promises for achieving these goals. To this end, it is of interest to understand how lifestyle factors, i.e., beneficial changes to human living conditions, can improve healthspan. Below we summarize current evidence for a role of autophagy in the physiological response to pharmacological agents, dietary restriction, exercise, sleep, and temperature control in different model systems; these interventions may all be possible to do in humans when such paradigms have been appropriately investigated in double-blind human trials.

Pharmacological interventions

Several small compounds or drugs have been found to extend lifespan in different organisms. While the downstream molecular mechanisms are still under investigation for how such interventions affect lifespan and healthspan, induction of autophagy has been shown to be a common culprit for several of these when tested, at least in simpler model organisms (Figure 3, Table 1). These small molecules include rapamycin, metformin, spermidine, resveratrol, NAD^+ precursors, and urolithin A, which will be discussed below.

Rapamycin. Rapamycin, an mTOR inhibitor isolated from a bacterium identified on the Pacific Island Rapa Nui, has emerged as an impactful compound for extending lifespan and modulating aging from yeast to rodents, as well as in human cellular aging models. Its effects on autophagy are well-documented, as it induces autophagic processes by inhibiting mTORC1, a key negative regulator of autophagy described above [17,19]. In model organisms from yeast to mice (both males and females), rapamycin treatment has been shown to extend lifespan, enhance survival under stress, and increase autophagy biomarkers [17]; however, methods of assessing autophagy flux to formally prove autophagy induction are still lacking. Autophagy mutants fed rapamycin do not show lifespan extension, as demonstrated in both yeast, where *Atg1, Atg11*, and *Atg7* are required for lifespan extension [20] and in female flies (males not investigated), where *Atg1* and *Atg5* are required [21,22]. However, the chronic use of rapamycin in humans carries significant risks, particularly because of its immunosuppressive effects and potential inhibition of mTORC2, which could negatively impact metabolic processes and immune function [1]. These limitations highlight the need for careful consideration in therapeutic contexts, especially in populations with compromised immune systems or metabolic disorders. Nonetheless, the conserved longevity effects of rapamycin across species suggest it could be a valuable pharmacological tool in aging interventions, provided that its application is closely monitored.

Metformin. Metformin, a widely used drug for managing Type II diabetes in humans, also has potential pro-longevity effects in multiple organisms, from yeast to mammals. Studies are ongoing in primates and in humans (TAME, Targeting Aging with Metformin), and although there is no conclusive evidence for lifespan extension in humans at this point, other ongoing studies are testing for protective roles against age-related diseases [23]. Metformin activates autophagy through multiple mechanisms, including activation of AMPK, which is required for metformin-induced lifespan extension in *C. elegans* [24]. Metformin has been demonstrated to boost autophagy in tissues like the liver and intestine in older male rats [25], and it has also been associated with improved cognitive function through enhanced autophagy in the hippocampus of middle-aged male mice [26]. Direct tests of the requirement of autophagy genes in metformin-induced longevity are still needed. Unlike rapamycin, metformin has a more favorable safety profile for humans with fewer immunosuppressive effects. This makes it a potentially safer option for long-term use in human aging interventions. However, as with any pharmacological intervention, its effects may vary among individuals, and careful monitoring is needed to ensure its efficacy and safety, particularly in older populations or those with pre-existing health conditions.

Spermidine. Spermidine, a naturally occurring polyamine, has been shown to induce autophagy and promote longevity across multiple species, including yeast, worms, flies, and mice (both males and females), as well as some human immune cells [27]. The lifespan

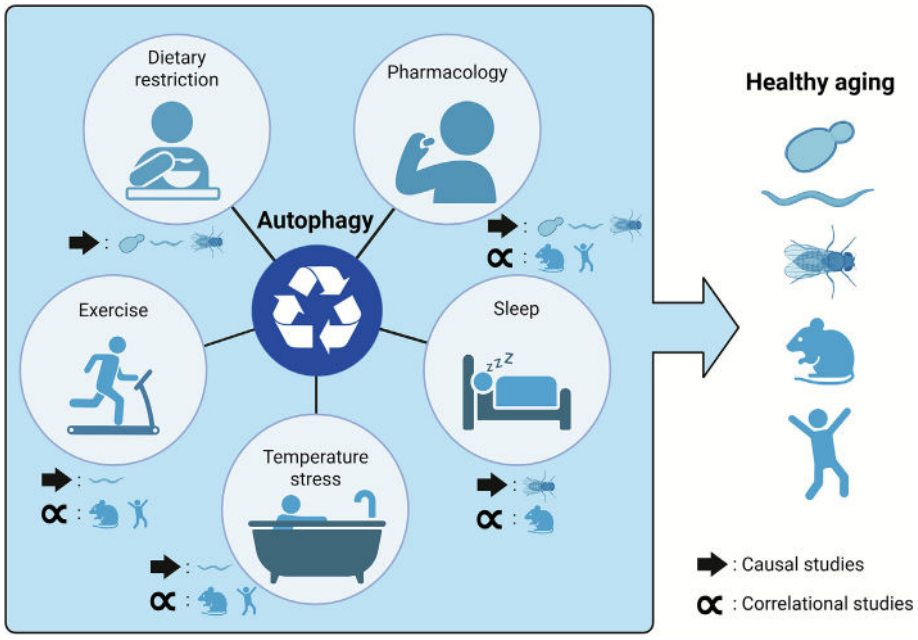

Figure 3. Autophagy as a potential driver to achieve healthy aging in different lifestyle interventions. Various lifestyle factors induce autophagy and have beneficial effects on healthspan and lifespan. See text and Table 1 for causal and correlative links between lifestyle factors and autophagy.

extension observed in yeast and flies is dependent on *atg-7*, and in *C. elegans* it is dependent on *bec-1/BECN1* [27]. Spermidine activates autophagy, at least in part, by promoting hypusination of the eukaryotic translational initiation factor (eIF5A), a post-translational modification essential for autophagy initiation [28]. In addition to its beneficial effects on both male and female lifespans, spermidine has been linked to a number of other physiological improvements, such as improved cardiovascular health and cognitive function in male rodents [29]. While human trials have indicated a correlation between high dietary spermidine and improved mortality, more research is needed to understand if spermidine can promote human healthspan by boosting autophagy [23].

Resveratrol. Resveratrol, a plant phenol known for its activation of the sirtuin SIRT1, has garnered attention for its ability to extend lifespan in multiple organisms, which is largely attributed to its ability to induce autophagy [1]. In *C. elegans*, resveratrol requires both *sir-2.1/SIRT1* and *bec-1/BECN1* for lifespan extension [30]. Resveratrol has been shown to attenuate aging-associated alterations in skeletal muscle and heart tissue in male mice suggesting a protective role against several age-related diseases [31]. However, variability in lifespan experiments in flies and rodents as well as in other human trials, combined with its limited bioavailability, are some of the practical challenges associated with this compound as a human lifespan intervention [31,32].

NAD⁺ precursors. Nicotinamide adenine dinucleotide (NAD^+) is a critical coenzyme involved in various cellular processes, including energy production, DNA repair, and the regulation of sirtuins, which are NAD^+-dependent histone deacetylases that modulate autophagy [33]. Increasing NAD^+ levels through supplementation with precursors such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) activates autophagy and enhances mitochondrial function in multiple organisms, offering potential therapeutic benefits for aging and age-related diseases [34]. NAD^+ precursors can extend longevity and improve metabolic health in animal models, including male mice and *C. elegans,* although it remains to be directly tested if such lifespan extensions are dependent on autophagy genes. Human clinical trials with NR and NMN have shown promising results, with improvements in kidney and cardiovascular disease, as well as in hearing loss [23]. However, the mechanism of action of NAD^+ precursors, remains to be fully investigated.

Urolithin A. Urolithin A is a gut metabolite derived from ellagic acid which is commonly present in fruits like pomegranate, and it has gained attention for its ability to induce mitophagy—a selective form of autophagy targeting damaged mitochondria—and promote longevity in multiple model organisms. Specifically, in *C. elegans* and rodent models, Urolithin A has been shown to improve mitochondrial function, increase muscle strength, and extend lifespan [35,36]. Urolithin A has been

well studied in *C. elegans*, where its lifespan effects depend on multiple autophagy genes, including *bec-1/BECN1*, *vps-34*, the autophagy receptor *sqst-1/p62*, as well as the mitophagy genes *dct-1/BNIP3, pink-1* and *prk-1/Parkin* [35]. In humans (both men and women of age 40–65 years), Urolithin A has demonstrated improvements in muscle strength and possibly exercise performance [36–38], further supporting its role in enhancing physical health and function in aging individuals; however, the intersection with autophagy has yet to be directly explored. Urolithin A's ability to specifically induce mitophagy, at least in simpler organisms, suggests its potential to target mitochondrial dysfunction, another key molecular mechanism of aging, making it a promising compound for age-related diseases such as frailty and neurodegeneration. However, more clinical trials are necessary to better understand its long-term effects and therapeutic potential in human aging.

Dietary restriction

Dietary restriction (DR), defined as reduced nutrient intake without malnutrition, encompasses caloric restriction (CR), which specifically refers to limiting total calorie intake regardless of food type. In this review, we use DR as the more inclusive term including restriction of one or more components of macronutrients without reduction in total caloric intake.

Dietary restriction and longevity. DR has been shown to extend both lifespan and healthspan in simpler organisms like yeast, nematodes, flies, and rodents [39,40]. In these species, DR triggers biological pathways that lead to slower aging and increased longevity, including a reduction in oxidative stress and improvements in cellular protein homeostasis and metabolic efficiency. These benefits are linked to several mechanisms including the inhibition of the nutrient sensor mTOR, as well as activation of sirtuins and autophagy, as reviewed below (Figure 3, Table 1). While direct evidence is still being explored in humans, studies suggest that moderate DR or intermittent fasting (IF, dietary regimens characterized by cycles of alternating periods of eating and fasting) may improve metabolic health and reduce insulin sensitivity and inflammation [39,40]. These changes by DR or IF ultimately can lead to physiological improvements at the organ and organismal level, including reduced muscle wasting (sarcopenia) [39,40]. Though the exact impact on human lifespan remains uncertain, DR appears to contribute to at a minimum to a better healthspan by potentially reducing the risk of chronic diseases like diabetes, heart disease, and neurodegenerative conditions [39].

Dietary restriction and autophagy. Studies in multiple organisms from yeast to male rats have shown that DR generally induces markers of the autophagy process, including increased numbers of autophagosomes as assessed by electron microscopy (EM) and Atg8 and/or lysosomal reporters [40–43]. Human skeletal muscle (predominantly analyzed in ~50-year-old men) also shows an increase in several autophagy genes, including *BECN1* and *LC3B/ATG8*, by ~30% CR diet for 3–15 years [44], consistent with an induction of autophagy. Beyond the inhibition of mTOR, the downstream mechanisms employed by mTOR and possibly AMPK via DR are still to be elucidated, but they may involve several transcription factors including HLH-30/TFEB, as observed in dietary-restricted *C. elegans* and mice (both males and females) [45]. However, while the moderate autophagy induction resulting from most dietary regimens is beneficial, excessive autophagy due to prolonged or extreme DR can be harmful [40,46], as overactivation of starvation signal leads to autophagy-dependent organismal death in *C. elegans* [47].

Links between autophagy and aging during dietary restriction. Direct links between autophagy genes and their role in DR-induced longevity have been firmly established using genetic mutants of autophagy genes in yeast (*Atg7, Atg5, Atg8, Vam3/Syntaxin 7 (STX7), Vam7, Vma7/ATG6V1F,* and *Atg15*) [48], and in *C. elegans* (*unc-51/Atg1/ULK1*, *bec-1/BECN1, vps-34*, *atg-7,* and *atg-18/WIPI2)* [41,43,49,50], where autophagy mutants do not show a lifespan extension when subjected to DR. In addition to the standard DR protocol in which food intake is restricted, IF interventions, particularly intermittent time-restricted feeding (iTRF) protocols, have shown that circadian regulation of autophagy is critical for DR to extend lifespan in *Drosophila* [51]. An iTRF protocol with a night-biased iTRF extends lifespan in both male and female flies, while a day-biased iTRF does not. Importantly, the night-biased iTRF protocol that normally extends lifespan in wild-type female flies (males not tested) fails to do so upon knockdown of *Atg1/ULK1* and *Atg8a/GABARAP*. This suggests that aligning feeding windows with circadian rhythms can optimize the beneficial effects of autophagy on metabolic health (see Section 'Sleep'). In humans, early time-restricted feeding (eTRF) has been linked to enhanced autophagy, but it also improved glucose levels and optimal regulation of circadian clock markers, all of which contribute to health benefits like reduced inflammation and improved metabolic profiles [52]. While strong correlative evidence exists, it remains to be directly tested if the beneficial effects of DR in humans are mediated, at least in part, by an enhancement of autophagy.

Additionally, the impact of DR on autophagy is not limited to (macro)autophagy but also extends to chaperone-mediated autophagy (CMA) [4]. Briefly, in CMA, soluble proteins containing KFERQ-like motifs (i.e., a five amino acid motif) are recognized

by a cytosolic chaperone called heat-shock cognate protein of 71 kDa (HSC70) and its co-chaperones. HSC70 delivers cargo directly to the lysosomal membrane via interaction with lysosomal-associated membrane protein type 2A (LAMP2A). Thus, HSC70 and LAMP2A are used as markers for CMA. In a recent study in male rodents (mice and rats), Jafari et al. (2024) demonstrated that CR and CR 'mimetics' (CRMs) including spermidine activate CMA by stabilizing LAMP2A at the lysosome membrane, likely enhancing the degradation of dysfunctional proteins [53]. Importantly, beneficial effects of CRMs on aged mouse livers were diminished by *Lamp2a* knockout [53], suggesting that CMA is required for these benefits. This comprehensive modulation of different types of autophagy by DR highlights the multifaceted roles of autophagy in DR and IF interventions, making them promising strategies for aging interventions and healthspan extension in humans.

Exercise

Exercise refers to physical activity performed to improve or maintain fitness and can be broadly categorized into two main types: endurance (aerobic) exercise, such as running or swimming, which involves repetitive, cyclical movements that primarily enhance cardiovascular function and metabolic adaptations in muscle; and resistance (strength) exercise, such as weight training, which involves working against an external load and primarily promotes increases in muscle mass and strength [54].

Exercise and longevity. Regular physical activity is one of the most potent lifestyle factors for promoting longevity and healthspan across species. Exercise paradigms differ by organismal context: in *C. elegans*, swimming in liquid culture induces endurance-like activity [55,56]; in *Drosophila*, repeated upward walking through negative geotaxis behavior models endurance exercise [57]; and in rodents (mice and rats), treadmill running is widely used to simulate endurance training [58–60]. In humans, both endurance and resistance exercise consistently associates with reduced morbidity and mortality [61]. Exercise mitigates aging-related physiological decline by improving cardiovascular health [62], enhancing mitochondrial function [55,56,63,64], maintaining muscle mass [65], and reducing inflammation [59,66,67]. These benefits are mediated through key regulators such as AMPK [38,64] and sirtuins [68–70] as well as autophagy (Figure 3, Table 1), not only in muscle but also in other tissues. Additionally, systemic effects may be driven by exerkines, bioactive molecules secreted from multiples organs during exercise [71]. Given the broad, systemic benefit of exercise, elucidating the molecular mechanisms underlying these effects could inform therapeutic strategies to promote healthy aging.

Exercise and autophagy. Accumulating evidence shows that exercise can induce autophagy. Most studies linking exercise to autophagy have focused on endurance protocols. In male mice, acute endurance exercise increased the number of GFP-LC3B autophagosomes and increased LC3-II/LC3-I ratio along with AMPK activation and reduced p62 protein levels in skeletal and cardiac muscle [72], indicating enhanced autophagy. Notably, in the same study, male mice with impaired autophagy regulation (*Bcl2*^{AAA} and *Becn1*$^{+/-}$ mice) reduced muscle glucose uptake and maximal running distance, suggesting a contribution of autophagy to glucose metabolism and exercise performance, although other BCL2-related functions may also play a role. Moreover, acute endurance exercise also induced autophagy in non-muscle tissues including the brain, indicating a systemic response.

One potential mechanism for this systemic effect is inter-tissue communication via circulating factors. In a follow-up study, serum from exercised mice (both males and females) increased autophagy flux in cultured human cells, as measured by the number of GFP-LC3B positive autophagosomes in the presence of the lysosomal inhibitor CQ, pointing to systemic autophagy-modulating factors in serum by exercise [73]. In the same study, fibronectin 1 (FN1), an extracellular matrix protein, was identified as a major serum factor enriched post-exercise. Tissue-specific knockout of FN1 and its receptor showed that FN1 secreted from muscle acts as an inter-tissue autophagy inducer, at least in liver. Whether FN1 also contributes to autophagy activation in other tissues such as the brain, and what downstream pathways mediate its effects, remain to be determined. If conserved in humans, modulating muscle-derived secretory factors such as FN1 may offer therapeutic avenues to replicate some exercise benefits in individuals unable to perform physical activity.

Beyond acute exercise, chronic endurance training has also been shown to modulate autophagy. In mice (males), regular voluntary endurance exercise increased expression of autophagy proteins (LC3A/B-II, ATG7, Beclin1) and reduced p62 protein levels in skeletal muscle [74], indicating enhanced basal autophagy. These adaptations, along with improved maximal running distance, were diminished in *Becn1*$^{+/-}$ male mice, supporting a role for autophagy in mediating exercise benefits. Overall, exercise-induced autophagy has been shown to be required for some benefits conferred by exercise.

Exercise and CASA. While many studies linking exercise to autophagy have focused on endurance training, emerging evidence points to resistance exercise engaging a specialized, mechanosensitive form of selective autophagy in human skeletal muscle [13,75]. This mechanosen-

sitive autophagy pathway, called the chaperone-assisted selective autophagy (CASA), involves specific protein complexes that appear to mediate selective autophagic turnover of force-bearing, cytoskeletal components such as filamins under mechanical stress [75]. CASA requires a ternary complex of BCL2-associated athanogene 3 (BAG3), heat shock protein family A (HSPA) including HSC70 and heat shock protein family B member 8 (HSPB8). Even though HSC70 functions in CASA, CASA is mediated by autophagosome and considered not to require KFERQ motifs for their cargo [4]. By observing changes in protein and transcription levels of these CASA-related proteins and their colocalization with LC3B, human thigh muscle biopsies (from 25 to 32-year-old men) showed CASA induction after resistance exercise by leg extension [76]. In the same study, CASA activation by regular resistance exercise was also shown, with stronger activation by progressive-load training than constant-load training. The mechanism of CASA induced by resistance exercise has been investigated in some detail [77]. Specifically, BAG3 dephosphorylation appears critical for CASA complex assembly in human thigh muscle biopsies (from mainly men in their late 20 s) after resistance exercise. In the same study, BAG3 phosphorylation status affected its interaction with RAB GTPases, which specifically regulated CASA flux measured by a lysosomal inhibitor BafA1 in rat smooth muscle cell cultures. Collectively, these studies describe an exercise-responsive, chaperone-dependent form of selective autophagy in human muscle that may serve to preserve cytoskeletal integrity under mechanical strain. Because CASA seems to be regulated in parallel to other autophagy pathways known to be activated by endurance exercise [77], combining endurance exercise and resistance exercise may yield additive benefits for overall health.

Links between autophagy and aging during exercise. Exercise has been shown to counteract age-related declines in autophagy, though the causal links to longevity remain underexplored in mammals. In *C. elegans*, both acute and regular endurance exercise by swimming attenuate age-related decreases in autophagy, with autophagosome numbers assessed by fluorophore-tagged LGG-1/Atg8 reporters (preprinted in [78]). Importantly, a causal requirement for autophagy in exercise-induced benefits has been demonstrated in *C. elegans*: swimming-induced extensions of lifespan and healthspan—assessed by age-dependent declines in pharyngeal pumping and body thrashing—were abolished by RNAi knockdown of core autophagy genes, including *unc-51/ULK1*, *epg-1/ATG13*, *bec-1/BECN1*, and *let-512/VPS34* [79]. In addition, RNAi against mitophagy regulators *pink-1* and *dct-1/BNIP3* similarly suppressed exercise-dependent lifespan extension [79], indicating that selective autophagy is key to the pro-longevity effects of exercise in *C. elegans*. Similarly, regular endurance exercise in old mice (male) prevented age-related reductions of autophagy proteins (LC3B-II, ATG7 and BECN1) in skeletal muscle [80]. Interestingly, the autophagy response varied across muscle fiber types: endurance exercise preserved autophagy more effectively in type II (fast-twitch, glycolytic) fibers than in mixed fibers (fast- and slow-twitch), consistent with a previous study showing that type II fibers respond better to starvation-induced autophagy than oxidative type I fibers in young male mice [81]. Thus, age-related autophagy response to exercise may be specific to muscle fiber types. In humans (males), age appears to influence autophagy-related proteins in plasma after endurance exercise (men in early 20 s vs. early 70 s) [82]. Plasma from younger individuals showed increased BECN2 protein levels, but no change in protein levels of ULK1, p62 and LC3B-II after exercise. On the other hand, plasma from older individuals showed increased LC3B-II protein levels without changes in the other markers after exercise. Although these studies relied on static protein measurements rather than direct autophagy flux assays, they collectively suggest potentially conserved, age-dependent alterations of autophagy activation by exercise across species, including humans. Of note, an ongoing clinical study (PROFASTA trial) aims to clarify how exercise influences autophagy flux across age groups in healthy humans, particularly in combination with fasting [83]. Such work, alongside mechanistic animal studies, will be essential to define the role of exercise-induced autophagy in healthy aging and to determine whether targeted modulation of this pathway can extend healthspan.

Sleep

Sleep is a fundamental biological process that allows the body and mind to rest, recover, and maintain overall health. It is closely regulated by the circadian rhythm, the body's internal clock that aligns sleep–wake patterns with the day–night cycle. Disrupted or insufficient sleep, or misalignment of this rhythm, can impair restoration and cognitive functioning.

Sleep and longevity. Sleep quality and duration decline with age, and disrupted sleep is strongly associated with aging [84]. Sleep contributes to healthy aging by maintaining brain and systemic homeostasis through processes such as DNA repair, neurodevelopment, and immune regulation [85,86]. At the molecular level, sleep deprivation reduces sirtuin activity (e.g., SIRT1) [87] and activates Toll signaling [88,89], both of which influence cellular maintenance and immune responses. The regulation of sleep is tightly intertwined with circadian rhythms, which coordinate daily oscillations in

metabolism, hormone secretion, and cellular processes across tissues [90]. Disruption of circadian rhythms, through irregular sleep, feeding, or light exposure, has been shown to accelerate aging [90]. Many longevity-related pathways, including insulin/IGF-1 signaling (protein kinase B and forkhead box protein O1), mTOR, and AMPK, are under circadian control [91–93], and circadian misalignment can dysregulate autophagy (Table 1) [94]. Thus, maintaining regular sleep and meal timing may promote healthy aging by stabilizing circadian rhythms and supporting cellular homeostasis.

Sleep and autophagy. Sleep plays an essential role in brain waste clearance [94–96]. In particular, sleep is linked to the glymphatic system, which removes extracellular waste from the brain [94]. Recently, slow vasomotion, the rhythmic contraction and relaxation of cerebral blood vessel walls, was identified as a key driver of cerebrospinal fluid flow during the sleep, facilitating glymphatic waste clearance [97]. However, how intracellular waste is cleared during sleep remains unclear. As a major mechanism for maintaining proteostasis, autophagy may contribute to these restorative effects [94]. While autophagy dysregulation in various brain regions by sleep disruption has been tested in mice [98,99], mechanistic links remain incomplete. Genetic evidence from *Drosophila* provides more direct insight [100]. In Bedont et al., sleep-deprived female *Drosophila* (*Aus* mutants) showed suppressed autophagy in the brain. Specifically, *Aus* short-sleeping mutants accumulated GFP-mCherry-Atg8a/LC3 autophagosomes (GFP^+ $mCherry^+$) and reduced autolysosomes ($mCherry^+$ only) in the brain, consistent with a block in autophagosome degradation. In wild-type female flies, knockdown of autophagy genes *Atg1/ULK*1 and *Atg8b/GABARAP* lengthened sleep. By combining mechanical sleep deprivation, chemical sleep induction, and temporal analysis of autophagosomes, Bedont et al., demonstrated that autophagosome number in the brain is negatively correlated with sleep length. Together, these findings suggest that increased autophagosome accumulation in the brain may prolong sleep, possibly because additional time is needed to complete autophagosome degradation during sleep. This raises the possibility that, at least in *Drosophila*, autophagy dynamics may help set sleep/wake timing.

The effects of sleep on autophagy in humans remain largely unexplored, but a recent human study examined effects of sleep deprivation on autophagy [101]. In this study, participants (18–40-year-old men) slept only 4 h per night for 5 consecutive days, and autophagy levels were measured from thigh muscle biopsies. No significant changes were detected in p62 mRNA or LC3B-II/I protein levels in thigh muscle biopsies, although the sample size was small ($n = 8$ per condition), limiting statistical power, and this pilot study did not detect measurable effects. Clearly, more rigorous and larger human studies are needed to clarify how sleep and sleep deprivation influence autophagy in humans.

Links between autophagy and aging during sleep. Chronic sleep disruption may impair autophagy and contribute to aging-related pathologies such as neurodegeneration. Long-term sleep fragmentation disrupted autophagy in young male mouse brains, producing phenotypes reminiscent of aging-associated neurodegenerative disease [102]. Specifically, in the cortex from sleep-fragmented male mice, the levels of autophagy proteins LC3B and Beclin1 were increased, and EM revealed enlarged lysosomes. Furthermore, in the same study, sleep-fragmented male mice displayed cognitive decline and amyloid-β accumulation in the cortex and hippocampus, a hallmark of Alzheimer's disease. These findings suggest that persistent sleep disturbance dysregulates autophagy and may accelerate brain aging and neurodegeneration.

One potential mechanism linking sleep, autophagy, and aging is circadian regulation, which has bidirectional relationship with sleep: sleep timing and quality are regulated by the circadian system, while sleep itself helps maintain circadian rhythmicity [90]. A recent study directly showed a link between autophagy and the circadian clock to modulate aging in *Drosophila* [51]. Longevity conferred by iTRF (see Section 'Exercise' Dietary restriction) required both an intact circadian clock (*Period* and *Timeless*) and autophagy (*Atg1/ULK1* and *Atg8a/GABARAP*) in female *Drosophila*. Importantly, this lifespan extension was independent of caloric restriction, suggesting that circadian regulation of autophagy many promote longevity through mechanisms distinct from those of CR and CRMs [1] discussed above. In addition to autophagy, CMA has been linked to circadian rhythm and aging in male mice [103]. Loss of CMA via a *Lamp2a* knockout disrupted circadian rhythms in young mice, producing molecular and behavioral profiles resembling those of aged wild-type male mice. These findings suggest that CMA helps preserve circadian function and may thereby delay age-associated circadian dysregulation. Whether similar mechanisms operate in humans remains unknown, but maintaining circadian health through regular sleep patterns may be a practical lifestyle strategy to promote autophagy and healthy aging (Figure 3).

Temperature stress

Temperature stress refers to the physiological and cellular responses that occur when an organism encounters environmental temperatures outside its optimal range. As a fundamental

environmental factor, temperature influences a number of metabolic activities and stress signaling that help maintain cellular homeostasis.

Temperature stress and longevity. Temperature is a well-established environmental factor influencing lifespan across species [104]. In poikilotherms like *C. elegans* and *Drosophila,* whose body temperature depends on the outside temperature, lifespan decreases as ambient temperature increases [105–107]. In homeotherms like mammals and humans, which regulate their own temperature, a lower core body temperature is similarly associated with increased longevity [108–110]. This effect was traditionally attributed to passive thermodynamic slowing of biochemical reactions. However, short-term, mild environmental stressors, such as heat or oxidative stress, trigger adaptive responses that enhance cellular repair and stress resistance, a phenomenon termed hormesis [111]. Hormetic heat shock has been shown to extend lifespan in simple organisms such as *C. elegans* (30°C) [112] and *Drosophila* (36°C) [113], with beneficial effects reported in humans (in middle-aged, 42–61-year-old males by sauna bathing with 80–100°C at the level of the bather's head), including improvements in cardiovascular health and possible reductions in mortality risk [114]. While hormetic heat stress has been extensively studied, hormetic cold exposure, for example via cold showers and baths, also provides health benefits [115]. Because heat and cold exposure can be applied without intensive physical activity (e.g., sauna, bathing or cold showers), these treatments are gaining attention as accessible therapies promoting healthy human aging [115,116].

Temperature stress and autophagy. Temperature shifts can denature proteins and disrupt mRNA translation inside the cell, necessitating autophagy to remove damaged or misfolded proteins. Accordingly, increased autophagy may mediate some beneficial effects of temperature stress (Figure 3, Table 1). Studies in mammalian cell cultures demonstrate that acute heat stress (39–47°C) modulates autophagy [117]. For example, protein degradation in hepatocytes from male rats (age not mentioned) shows a positive correlation with temperature (15–40°C), and this process is partially blocked by a lysosomal inhibitor propylamine, implicating a role for autophagy [118]. Similarly, heat stress (43°C) increases autophagy flux in human cervical cancer cells and human embryonic kidney cells, measured by LC3-II protein levels in the presence of lysosomal protease inhibitors E64d and pepstatin A [119,120]. In contrast, heat stress (40°C) reduced autophagy flux in mouse differentiated myotubes, assessed by LC3A/B-II protein levels with or without the lysosomal inhibitor BafA1 [121]. These results suggest that autophagy response to heat stress may be cell type-specific.

Cold stress (4°C) has also been shown to induce autophagy, particularly in mice [122–124]. Many studies focus on brown adipose tissue (BAT), a key regulator of thermogenesis in mammals, which maintains core body temperature by breaking down lipids from the liver during cold exposure [125,126]. Cold shock increases the LC3B-II protein level in BAT from young mice (sex not mentioned), an effect absent in $Atg5^{\Delta BAT}$ mice [122]. It also enhances autophagy flux in BAT (from young males), as shown by LC3B and p62 levels in the presence of a lysosomal inhibitor CQ [123]. In another study, cold exposure elevated autophagy flux in BAT and liver (from both young males and young females), as measured by LC3B-II protein level with or without the lysosomal protease inhibitor leupeptin [124]. Notably, $Atg5^{\Delta BAT}$ mice [122] and $Becn1^{+/-}$ male mice [123], as well as wild-type mice treated with the lysosomal inhibitor CQ, displayed lower body temperature after cold stress [123], suggesting that autophagy activation in BAT is required for effective thermogenesis, at least in rodents.

A potential mechanism by which autophagy may regulate thermogenesis after cold exposure in BAT is lipid degradation by lipophagy. Cold stress promotes LC3B accumulation on lipid droplets (LDs) in BAT of mice (both males and females aged 5–6 months), whereas $Atg7^{\Delta BAT}$ mice lacked this response and showed bigger and more LDs [124], supporting the notion that cold exposure induces lipophagy. Cold stress also elevated autophagy flux in the hypothalamus, as indicated by increased LC3B-II protein levels in the presence of the lysosomal protease inhibitor leupeptin. Furthermore, administration of the mTOR inhibitor rapamycin to the hypothalamus was sufficient to induce autophagy flux and decrease LDs in BAT without cold exposure. Therefore, the study by Martinez-Lopez et al. suggests that hypothalamic activation of autophagy regulates peripheral lipophagy. Importantly, this hypothalamic activation of BAT lipophagy is abolished by denervation of BAT, demonstrating that neuronal signaling is important for this inter-tissue regulation of lipophagy under cold stress in mice. However, detailed mechanisms linking hypothalamic signaling, BAT autophagy, thermogenesis, and aging remain to be elucidated, including in humans.

Links between autophagy and aging during temperature stress. Direct genetic evidence links autophagy to heat stress-induced longevity in *C. elegans*. Short-term heat stress (36°C) is hermetic and extends lifespan in *C. elegans* and increases autophagy flux across tissues, as measured by GFP::LGG-1/GABARAP autophagosome numbers in the presence of the lysosomal inhibitor BafA1 [127]. This longevity effect is abolished by knockdown of autophagy genes (*unc-51/ULK1, bec-1/BECN1* and *lgg-1/GABARAP*), indicating that autophagy is required for the health benefit of heat

stress [127]. In particular, *C. elegans* animals lacking the autophagy receptor *sqst-1/p62* failed to exhibit both lifespan extension and autophagy induction after heat shock [11]. Although the mechanism by which SQST-1/p62 promotes autophagy under heat shock remains unknown, these findings demonstrate that, at least in *C. elegans*, autophagic machinery is essential for the longevity effects of hormetic heat stress.

The autophagy response to heat stress appears to change with age in mammals. EM revealed that heat stress (41°C) increases autophagosome numbers in male rat hepatocytes, with a stronger effect in older male rats [128]. While autophagy flux was not directly measured, this accumulation in older rats may reflect increased autophagosome formation coupled with impaired degradation. Similarly in humans, warm bathing (40°C) increases plasma p62 levels in older but not in younger men [82], consistent with reduced autophagosome clearance in aging. However, no significant changes were observed in other autophagy markers (LC3B-II/LC3-I ratio, LC3B-II, ULK1, or Beclin2 protein levels) in either age group, suggesting that heat exposure may not engage the full autophagy machinery. Whether regular heat exposure earlier in life can mitigate this apparent age-related decline remains unknown.

Cold stress also shows age-dependent differences in autophagy in men [129]. Specifically, moderate cold bathing (0.5°C decrease in core body temperature) increased LC3B-II protein level in peripheral blood mononuclear cells (PBMCs) of young men (20 s) but not older men (60 s). Older participants showed lower LC3B-II/LC3-I ratio after high cold stress and higher p62 protein levels after both moderate and high cold stresses in PBMC, whereas young men did not exhibit these changes. These results from King et al. suggest an age-related impairment in cold-induced autophagy, which may limit the health benefits of cold exposure in older adults. Determining whether this decline affects the health benefits of cold exposure will require longitudinal studies in humans, ideally combined with mechanistic work in model systems or cell cultures to establish causality. If validated, such findings could inform personalized lifestyle strategies: younger individuals might achieve optimal benefits from cold therapy alone, whereas older adults may require combined approaches, such as cold exposure paired with pharmacological or dietary interventions that enhance autophagy. Ultimately, understanding these age-dependent responses could inform safe, tailored use of sauna, thermal therapy, or cold bathing as accessible interventions to promote healthy aging.

Conclusions and future prospects

Autophagy is a central mediator of healthspan across species [16], yet its precise role in humans remains incompletely defined. As reviewed here, across interventions ranging from diet and exercise to sleep and temperature, autophagy emerges as a conserved mechanism linking lifestyle factors to cellular and organismal homeostasis (Figure 3). Despite these many exciting links, significant gaps remain, including in understanding how lifestyle factors-induced autophagy differs across age and sex groups and how these differences influence overall outcomes, a key question when considering potential translational value to humans.

Several limitations must be addressed to fully realize the therapeutic potential of autophagy modulation by lifestyle factors. A particularly important unmet need is the development of robust, non-invasive assays to measure autophagic flux *in vivo* in humans [15,130]. Most human studies still rely on indirect or static readouts, which cannot capture dynamic flux and may confound interpretations. Some progress has been made toward direct flux measurements in human samples. One approach measures autophagy flux in peripheral blood mononuclear cells (PBMCs) by treating freshly drawn blood with lysosomal inhibitors such as CQ, isolating PBMC populations, and quantifying autophagy-related markers like LC3B-II [131]. This method, analyzing overall PBMC populations, has been applied to studies of aging [132] and DR (specifically, iTRF) in overweight individuals (middle-aged to old, both males and females) [133]. These studies reported notable findings, including increased autophagy flux with iTRF [133] and a positive correlation between autophagy flux and age, indicating higher autophagy in older individuals [132]. However, several limitations remain. Considerable inter-individual variability in flux measurements suggests that large sample sizes are needed for robust conclusions. One contributor to this variability is the heterogenous composition of PBMC populations between human individuals [134]. Indeed, isolating specific PBMC subsets, such as $CD4^+$ T-cells, has revealed cell-type specific differences in autophagy [135] and yielded more consistent results, including increased flux upon activation in offspring of centenarians [136] and a positive correlation between age and autophagy flux [135,137], consistent with the findings from overall PBMC populations by Bensalem et al [132]. The increase in autophagy with age in certain PBMC populations is notable considering the general trend of a decline in autophagy with age, and may reflect cell-type specific autophagy changes during aging or a reactive autophagy response to age-related cellular damage [138]. As autophagy flux in PBMCs may not accurately reflect autophagy status in other tissues, future studies examining autophagy activity across different human tissues will be important for a more complete understanding of age-related changes in autophagy. To this end, tissue biopsies represent one possible approach to directly assess autophagy in different organs, although they have important limitations: while tis-

sue biopsies could provide complementary information, *ex vivo* culture conditions may alter physiological responses. Finally, longitudinal human studies linking autophagy activity to health outcomes remain scarce, limiting our ability to draw correlational inferences. Ultimately, the discovery of reliable circulating biomarkers of autophagy flux could enable routine, minimally invasive monitoring in clinical and research settings, allowing for longitudinal tracking, individualized assessment, and more precise evaluation of lifestyle factors.

While this review primarily focuses on macroautophagy, it is important to recognize that health benefits from lifestyle factors may also be mediated by other types of autophagy, such as CMA and microautophagy. While we highlighted relevant roles for CMA in DR [139] and CR/CRMs [53], the link between CMA and other lifestyle factors requires further investigation. Given that different autophagy pathways vary in cargo selectivity and operate on distinct temporal scales, it will be important to determine how lifestyle factors influence each pathway in spatial and temporal contexts. Such insights could enable the tailoring of therapeutic strategies for healthy aging to individual needs. In addition, alternative, ATG5/ATG7-independent macroautophagy, in which autophagosomes are formed in the absence of ATG8 lipidation [140], remains poorly understood. This alternative autophagy pathway, which depends on ULK1, Beclin1 and RAB9 may play unique roles in aging, but its relevance *in vivo* is still unclear. Furthermore, recent studies have revealed that autophagy-related proteins can have additional functions beyond autophagy. In particular, ATG8 can be attached to different non-autophagosomal membrane structures via conjugation of ATG8s to single membranes (CASM or membrane atg8ylation) [141,142] or to proteins (protein atg8ylation) as post-translational modifications [143], which have both been connected to inflammation and neurodegeneration. A recent study suggests a potential role of these non-canonical pathways in aging [144], but how non-canonical pathways intersect with aging biology remains an exciting question for future studies.

Looking ahead, advancing our understanding of autophagy in aging will require both deeper mechanistic insight and translational innovation. Several autophagy-inducing compounds, including spermidine and urolithin A, have shown promise in preclinical models. However, many currently known autophagy inducers lack specificity, impacting multiple cellular pathways beyond autophagy and potentially causing off-target effects or toxicity. Moreover, optimal dosing, tissue specificity, and long-term outcomes remain poorly understood. Thus, a major priority is the development of pharmacological strategies that can safely and selectively enhance autophagy in humans. Beyond pharmacology, the gut microbiome is emerging as a critical yet underexplored regulator of autophagy [145]. Microbial composition and metabolism influence nutrient signaling, inflammation, and epithelial barrier function [146], all of which intersect with autophagic pathways. Because the microbiome changes markedly across the lifespan [145–148], targeted modulation of microbial communities, e.g., through diet, probiotics, or microbial metabolites, could offer a route to fine-tuning autophagy activity and, ultimately, aging trajectories. In parallel, environmental exposures, collectively termed the exposome, are increasingly recognized as modulators of aging [149,150]. Defining how specific exposures influence autophagy, and whether mitigating harmful exposures or enhancing beneficial ones can shift autophagic capacity, will be critical for developing comprehensive strategies to promote healthy aging. Together, these emerging avenues, from pharmacological agents to microbiome-targeted approaches and environmental modulation, underscore the potential of diverse strategies. Integrating these interventions could allow sustained and synergistic enhancement of autophagy and pave the way for translational applications in human aging.

In summary, in this review we have highlighted how diverse lifestyle factors can modulate autophagy and improve healthspan across model systems. By influencing autophagy and possibly other processes, these interventions help maintain cellular homeostasis and promote healthy aging. Understanding the context-specific regulation of autophagy by different lifestyle factors lays the groundwork for developing targeted strategies in humans, such as optimizing timing, intensity, and duration of interventions; tailoring approaches to individual biological profiles; and combining different autophagy activating factors. Such combinations might include lifestyle-based interventions paired with pharmacological inducers, or multi-lifestyle approaches (e.g., intermittent fasting with structured exercise), potentially yielding additive or synergistic benefits. As the field moves forward, integrating mechanistic insights with longitudinal human studies and translational approaches will be essential to harness autophagy as a modifiable and actionable pathway of healthy aging.

Declaration of AI and AI-assisted technologies

ChatGPT.com was used in the writing process for minor grammatical refinement during the drafting of this article.

CRediT authorship contribution statement

Hiroshi Ebata: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding

acquisition, Conceptualization. **Malene Hansen:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

DATA AVAILABILITY

No data was used for the research described in the article.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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proteostasis

Abbreviations:
AMPK, AMP-activated protein kinase; BafA1, bafilomycin A1; BAG3, BCL2-associated athanogene 3; BAT, brown adipose tissue; BECN1, Beclin1; CASA, chaperone-assisted selective autophagy; CASM, conjugation of ATG8s to single membranes; CMA, chaperone-mediated autophagy; CQ, chloroquine; CRM, caloric restriction mimetics; DR, dietary restriction; eIF, eukaryotic translational initiation factor; EM, electron microscopy; FIP200, FAK family-interacting protein of 200 kDa; FN1, fibronectin 1; GABARAP, gamma-aminobutyric acid type A receptor-associated protein; HSC70, heat-shock cognate protein of 71 kDa; HSP, heat shock protein family; IF, intermittent fasting; IGF-1, insulin-like growth factor 1; LAMP2A, lysosome-associated membrane protein type 2A; LC3, microtubule-associated proteins 1A/1B light chain 3; LD, lipid droplet; LIR, LC3-interacting region; mTOR, mechanistic target of rapamycin; NAD, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; PBMC, peripheral blood mononuclear cell; PE, phosphatidylethanolamine; PI3K, phosphoinositide 3-kinase; PI3P, phosphoinositide 3-phosphate; SIRT1, sirtuin 1; SQSTM1/p62, sequestosome 1; STX7, syntaxin 7; TFEB, transcription factor EB; TRF, time-restricted feeding; ULK1, UNC-51-like autophagy-activating kinase 1; VPS, vacuolar protein sorting; WIPI, WD-repeat protein interacting with phophoinositide

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