Transcriptional and epigenetic regulation of autophagy in aging

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Macroautophagy is a major intracellular degradation process recognized as playing a central role in cell survival and longevity. This multistep process is extensively regulated at several levels, including post-translationally through the action of conserved longevity factors such as the nutrient sensor TOR. More recently, transcriptional regulation of autophagy genes has emerged as an important mechanism for ensuring the somatic maintenance and homeostasis necessary for a long life span. Autophagy is increased in many long-lived model organisms and contributes significantly to their longevity. In turn, conserved transcription factors, particularly the helix-loop-helix transcription factor TFEB and the forkhead transcription factor FOXO, control the expression of many autophagy-related genes and are important for life-span extension. In this review, we discuss recent progress in understanding the contribution of these transcription factors to macroautophagy regulation in the context of aging. We also review current research on epigenetic changes, such as histone modification by the deacetylase SIRT1, that influence autophagy-related gene expression and additionally affect aging. Understanding the molecular regulation of macroautophagy in relation to aging may offer new avenues for the treatment of age-related diseases.

Introduction

The autophagy process

Autophagy is an evolutionarily conserved catabolic process through which damaged organelles and macromolecules are degraded and recycled within the cell. Three forms of autophagy can be distinguished based on the cellular components that are sequestered and degraded: microautophagy, the nonselective sequestration of cytoplasmic components directly into the lysosome; chaperone-mediated autophagy, the selective degradation of specific cargo proteins recognized and delivered to the lysosome by a chaperone complex; and macroautophagy, the degradation of cytoplasmic material via encapsulation in an autophagosome that subsequently fuses with the lysosome. This review focuses on macroautophagy (henceforth referred to as autophagy) since this is the best studied mechanism, including in the context of aging.

The process of autophagy proceeds through at least 5 mechanistically distinct steps: (1) induction, (2) double-membrane nucleation and autophagosome formation, (3) autophagosome elongation and sequestration of cellular debris, (4) autophagosome–lysosome fusion, and (5) degradation of sequestered components in the lysosome (**Fig. 1A–B**) (reviewed in ref. 1). Each of these sequential steps involves a number of conserved autophagy-related (ATG) proteins, as briefly described here. Activation of the ULK/Atg1 initiation complex is the first step in the autophagy process, permitting the creation of a phagophore membrane and formation of the autophagosome. The membrane used to form the phagophore can originate from different locations, such as the endoplasmic reticulum, mitochondria, Golgi, endosomes, and plasma membrane (reviewed in refs. 2, 3). Integration of membrane lipids into the phagophore requires the synthesis of phosphatidylinositol 3-phosphate (PtdIns3P) by the phosphatidylinositol 3-kinase (PtdIns3K) nucleation complex. PtdIns3P is then recognized by PtdIns3P-binding proteins,[4,5] which may possibly act as a shuttle by transferring membrane lipids between a membrane donor and the growing phagophore.[6] Phagophore elongation is further dependent on 2 ubiquitin-like conjugation reactions. First, the ubiquitin-like protein ATG12 is
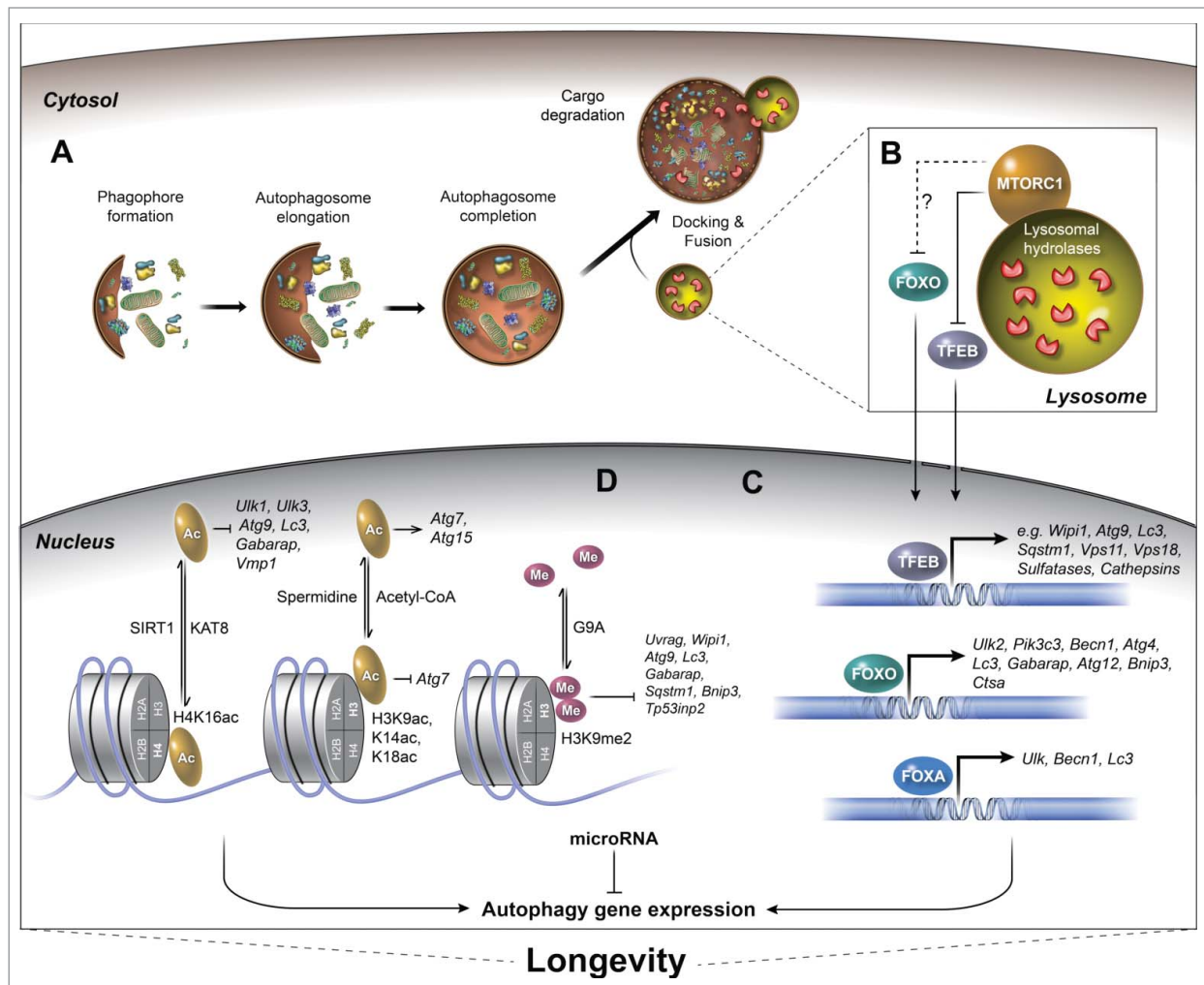
Figure 1. Regulation of autophagy-related gene expression associated with longevity. The process of autophagy and the transcriptional regulation of autophagy genes by the action of specific transcription factors, epigenetic modifications, and miRNAs have emerged as important conserved mechanisms to ensure longevity. (**A**) The autophagy process is initiated by cytoplasmic nucleation of the phagophore with double-membrane structures. The phagophore then elongates and sequesters cellular components until it matures into an autophagosome, which then docks and fuses with lysosomes to form an autolysosome. Upon fusion, the cargo and inner membrane of the autolysosome are degraded. (**B**) The nutrient sensor MTORC1 is a major regulator of the autophagy process. MTORC1 inhibits autophagy and positively regulates growth, mRNA translation, and ribosomal and lipid biogenesis. The nutritional status of the cell (e.g., amino acid levels) dictates the recruitment of MTORC1 to the lysosomal membrane and its subsequent activation. Active MTORC1 phosphorylates several targets, including the transcription factors TFEB and FOXO. Phosphorylation of TFEB, and possibly of FOXO, occurs at the lysosomal membrane and leads to their retention in the cytosol. (**C**) The nuclear translocation of transcription factors TFEB and FOXO, as well as activation of FOXA induces the expression of multiple autophagy-related and lysosomal genes (for FOXA, which is constitutively nuclear; only the *C. elegans* ortholog PHA-4 has so far been shown to regulate autophagy gene transcription and modulate longevity). TFEB and FOXO are regulators of longevity in several species. (**D**) Autophagy gene expression can also be regulated epigenetically by histone modifications such as acetylation (Ac) and methylation (Me), which affect the chromatin state thus altering expression of specific genes. For example, the deacetylation of histone mark H4K16 by SIRT1 leads to transcriptional repression of various autophagy-related genes (the acetylation reactions occurs by the acetyltransferase KAT8). High levels of acetyl-CoA can inhibit the expression of autophagy gene *ATG7*, and conversely, histone hypoacetylation via spermidine treatment can increase gene expression of *ATG11*, *ATG7*, and *ATG15*. The dimethylation of H3K9 through the methyltransferase EHMT2 leads to the repression of autophagy gene transcription. The deacetylase SIRT1, spermidine treatment and levels of nucleocytosolic acetyl-CoA are associated with the regulation of longevity in several species. Mammalian gene names are used exclusively in the figure. See text for reference to specific model systems and for further details.

covalently conjugated to ATG5 by ATG7 and ATG10 (E1- and E2-like enzymes, respectively). The ATG12–ATG5 conjugate, together with its interaction partner ATG16L1 is thought to act in part as a a E3-like ligase to promote conjugation of phosphatidylethanolamine (PE) to soluble LC3-I/Atg8, formed by cleavage of the ubiquitin-like protein LC3/Atg8 by the protease ATG4.[7] The resulting PE-conjugated, membrane-bound LC3-II/Atg8–PE is important for phagophore elongation as well as cargo recognition. LC3-II/Atg8–PE can be bound by various cargo receptors; for example, SQSTM1/p62, which recognizes ubiquitinated proteins or organelles targeted for degradation.[8]

The phagophore undergoes further maturation to fully encapsulate its cargo; the completed autophagosome releases outer membrane-associated autophagy-related proteins, after which it is ready to dock and fuse to the lysosome/vacuole to form an autolysosome. Upon fusion, the inner membrane of the autophagosome and the lumenal cargo are degraded, and the autolysosome reforms as a lysosome that is possibly again available for subsequent vesicular fusion events.[9] Incomplete processing of autolysosomes can instead produce a residual body containing indigestible material.[10] Notably, several age-related diseases, such as neurodegenerative disorders, are characterized by the accumulation of unprocessed autophagic vacuoles (reviewed in refs. 11, 12), suggesting that the ability of cells to efficiently coordinate and complete the autophagic process is gradually impaired with age.

Upstream regulators of macroautophagy

Autophagy is critical to cellular homeostasis under both normal and stressed conditions. Basal levels of autophagy ensure intracellular quality control, whereas autophagy induced by starvation and other stressors promotes cellular survival by maintaining adequate amino acid pools and cellular energy levels. Until recently, induction of autophagy was thought to be dependent primarily on post-translational regulation of nutrient-responsive pathways; however, it is now clear that alternative means of regulation exist. The emerging picture is that rapid induction of autophagy, e.g., in response to starvation, is mediated by post-translational protein modifications and protein-protein interactions, whereas transcriptional mechanisms are necessary for a sustained response (reviewed in ref. 47).

One key regulator of autophagy is the kinase MTOR/TOR (mammalian target of rapamycin), which is a component of 2 complexes, MTORC1 and MTORC2. As part of the MTORC1 complex, TOR regulates cell growth, proliferation, survival, protein synthesis, and autophagy, whereas the MTORC2 complex primarily regulates different downstream signaling cascades modulating cell shape and metabolism, but can also regulate autophagy indirectly (reviewed in ref. 13). The MTORC1 complex (hereafter referred to as MTOR) directly inhibits autophagy through phosphorylation and inactivation of ULK/Atg1 and ATG13/Atg13, which are essential for the induction of autophagy (reviewed in ref. 14). TOR activity can be modulated by changes in amino acid abundance via interaction with RAG small GTPases.[15] These enzymes are localized at the lysosomal surface through their interaction with the pentameric Ragulator complex.[16] Elevated lysosomal amino acid levels, which may reflect the overall cellular abundance of amino acids, promote the guanine-nucleotide exchange factor function of the Ragulator complex[17] and subsequent activation of RAG GTPases. These proteins recruit TOR to the lysosomal membrane where it is ultimately activated by the small GTPase RHEB.[18]

MTOR serves as a hub to integrate additional upstream signals from various sources, including INS/insulin, growth factors, and cellular energy levels (reviewed in refs. 13, 19). One notable pathway intersecting with MTOR is the INS-IGF1 pathway, which is involved in many functions necessary for metabolism,

growth, and longevity.[20] Formation of PtdIns(3,4,5)P$_3$ by activated PtdIns3K, a downstream effector of the INS-IGF1 pathway, recruits the protein kinase AKT to the plasma membrane, where it is phosphorylated and activated by the PDPK1/2 (3-phosphoinositide dependent protein kinase 1/2). AKT modulates the function of TSC2, a component of the heterodimeric tuberous sclerosis complex (TSC). The TSC1/2 complex can inhibit MTOR signaling by preventing activation of RHEB.[21] Upon growth factor or INS-IGF1 signaling, TSC2 is phosphorylated and inactivated by AKT, thereby allowing RHEB to activate MTOR.[22] The TSC-RHEB-MTOR cascade therefore represents a major signaling axis for the control of growth and autophagy.

Autophagy is also regulated by intracellular energy levels via the energy sensor AMPK (AMP-activated protein kinase), which directly activates the ULK/Atg1 initiation complex (reviewed in ref. 23). AMPK activity is sensitive to AMP levels and is further regulated by phosphorylation by upstream kinases such as STK11/LKB1 and CAMKK2/CamKKβ (calcium/calmodulin-dependent protein kinase kinase 2, β).[24] Although STK11/LKB1 is constitutively active, CAMKK2 has been implicated in AMPK regulation and autophagy induction only in response to changes in intracellular Ca^{2+} levels.[25] AMPK can also influence autophagy by inhibiting MTOR, either by phosphorylating the MTORC1 subunit RPTOR/raptor or by inhibiting phosphorylation of TSC1/2.[26] Both AMPK and MTOR can themselves be phosphorylated by ULK/Atg1, providing an additional level of regulatory feedback to modulate and fine-tune autophagy (reviewed in ref. 27)

In addition to regulating autophagy, AMPK and MTOR also modulate organismal aging in a conserved fashion. Specifically, inhibition of MTOR extends the life span of organisms ranging from yeast to mice,[28] and overexpression of AMPK promotes longevity in worms and flies.[24] Collectively, these findings demonstrate that nutrient sensors are major regulators of life span in a variety of species and share autophagy as a downstream effector mechanism for the maintenance of health.

Direct links between autophagy and aging

Accumulating evidence over the past decade supports a direct role for autophagy in the aging process. Multiple genetic experiments have demonstrated a requirement for autophagy-related genes in many longevity paradigms, including inhibition of TOR activity in yeast (*ATG1, ATG11, ATG7*),[29] worms (*unc-51/Ulk/ATG1, bec-1/Becn1/VPS30/ATG6, vps-34, atg-18/Wipi1/2*)[30,31] and flies (*Atg5*).[32] Similarly, life-span extension by dietary restriction is abrogated in autophagy-deficient yeast (*ATG7, ATG5, ATG8, ATG15*, and v-SNARE genes *VAM3*, and *VAM7*)[33,34] and in worms (*unc-51/Ulk/ATG1, bec-1/Becn1/VPS30/ATG6, vps-34, atg-7*).[30,31,35] Similar links have been observed in other conserved longevity models (i.e., reduced INS-IGF1 signaling, germline removal; mitochondrial respiration, mRNA translation; as well as resveratrol and spermidine supplementation), which all cause induction of autophagy markers, and in each case, the resulting life-span extension is dependent on at least one autophagy gene (see **Table 1** for a summary of direct links between autophagy-related genes and longevity). In support of such a

Table 1. Effects of modulation of autophagy-related and lysosomal genes on life span including in conserved longevity paradigms.

ORGANISM	GENES	FUNCTION IN STEP	LIFE SPAN OF OE[1]	ROLES IN LONGEVITY PARADIGM[2]	TRANSCRIPTION FACTOR[3]
Yeast	ATG1	Autophagy initiation		Rapa ↓[§29]	
	ATG11	Phagophore formation		Rapa ↓[§29]	
	ATG7	AP^ elongation		Rapa ↓[§29], MetR ↓[§34], Sper ↓[§129]	
	ATG8	AP elongation		MetR ↓[§34]	
	ATG5	Conjugated protein of Atg12		MetR ↓[§34]	
	ATG8	AP elongation		MetR ↓[§34]	
	VAM3	SNARE protein, fusion		DR ↓[§33]	
	VAM7	SNARE protein, fusion		DR ↓[§33]	
	ATG15	Putative lipase required for intravacuolar disintegration of autophagic bodies		DR ↓[§33]	
C. elegans	unc-51/ ATG1/Ulk1	Autophagy initiation		LET-363/MTOR ↓[§§§30], DR ↓[§§30], GL ↓[§§§70], Mit ↓[§§30]	PHA-4/FOXA[70, 96]
	bec-1/ VPS30/ Becn1	Membrane nucleation		LET-363/MTOR ↓[§§§31], DR ↓[§§30, §§§31, §§§§35] IIS ↓[§§31, §§§§166, §§§§§167], GL ↓[§§§70] Mit ↓[§§30, §§§§64], Resv ↓[§§§115], Sper ↓[§§§129], miR-34 ↓[§§§146], CaN ↓[§§§§§§ 170]	PHA-4/FOXA[70, 96]
	vps-34/VPS34/Pik3c3	Membrane nucleation		LET-363/MTOR ↓[§§§31], DR ↓[§§§31] GL ↓[§§§70], Mit ↓[§§§§64]	
	atg-9	Phagophore formation		miR-34 ↓[§§§146]	
	atg-18/ Wipi4	Phagophore formation		GL ↓[§§§70], Mit ↓[§§30, §§§§64], RSKS-1/RPS6KB↓[§§§64]	HLH-30/TFEB[64, 168]
	atg-4.1	AP elongation		miR-34 ↓[§§§146]	
	atg-7	AP elongation		DR ↓[§§§§35], IIS ↓[§§§31, §§§§166], CaN ↓[§§§§§§ 17]	
	atg-12	Ubiquitin-like modifier		IIS ↓[§§§31, §§§§166]	
	lgg-1/ ATG8/Lc3	AP elongation	NE[31,Unp.]	GL ↓[§§§70], Mit ↓[§§§§64]	PHA-4/FOXA[70, 96]
	vha-16	Vacuolar pH		GL ↓[§§§64]	HLH-30/TFEB[64]
	lmp-1	Lysosomal membrane		GL ↓[§§§64]	HLH-30/TFEB[64]
	C08H9.1#	Lysosomal degradation		IIS ↓[§§§94]	DAF-16/FOXO[94]
	lipl-1	Lysosomal lipolysis	↑[67]		HLH-30/TFEB[67]
	lipl-3	Lysosomal lipolysis	↑[67]		HLH-30/TFEB[67]
	lipl-4	Lysosomal lipolysis	↑[69]	GL↓[§§§69], IIS ↓[§§§69]	DAF-16/FOXO[69]
Drosophila	Atg1/Ulk1	Autophagy initiation	↑[¶,¶¶39]	AMPK ↓[¶,§§§39]	dFOXO/Foxo?[39]
	Atg7*	AP elongation		Sper ↓[§§129]	
	Atg5	AP elongation		Rapa ↓[§§§§32]	
	Atg8/Lc3	AP elongation	↑[¶37]		
Mouse	Atg5	AP elongation	↑[36]		

(1) Life span of animals overexpressing (OE) autophagy gene: NE, no effect; ↓, decreased; ↑, increased. Note that most autophagy deletion mutants are short lived, likely due to developmental defects.

(2) Effect of autophagy gene inactivation on conserved longevity models: AMPK, overexpression of PRKAA/α-subunit of AMPK; CaN, reduced calcineurin signaling; DR, dietary/caloric restriction; GL, germline removal; IIS, reduced INS/insulin-IGF1 signaling; MetR, methionine restriction; *miR-34, miR-34* loss of function; Mit, reduced mitochondrial respiration; Rapa, rapamycin treatment; Resv, resveratrol treatment; Sper, spermidine treatment; LET-363/MTOR, reduced TOR signaling.

(3) Transcriptional regulation of autophagy gene by noted transcription factor in at least 1 longevity model (not specified).

^AP, autophagosome.

*, known epigenetic regulation.

§, chronological life span was assessed.

§§, genetic mutant used. Genetic mutation of autophagy genes shortens somewhat the life span of wild-type *C. elegans*.

§§§, adult-only RNAi treatment. This treatment generally does not shorten the life span of wild-type *C. elegans*.

§§§§, RNAi treatment from L4 stage. This treatment generally does not shorten the life span of wild-type *C. elegans*.

§§§§§, whole-life RNAi treatment. This treatment generally shortens somewhat the life span of wild-type *C. elegans*.

§§§§§§, RNAi treatment for 2-4 generations

#, *C08H9.1* is a putative lysosomal serine carboxypeptidase/CTSA homolog, i.e., C08H9.1.

¶, overexpression from neuron-specific promoter.

¶¶, overexpression from intestinal promoter.

Unp., unpublished by Hansen lab.

link, overexpression of specific autophagy genes has been found to promote longevity in several different species. In mice, heterologous overexpression of ATG5 is sufficient to stimulate autophagy, promote a youthful appearance, and extend life span.[36] In *Drosophila*, overexpression of Atg8a in the neurons and muscle of adult flies extends their life span.[37,38] Similarly, neuron-specific overexpression of Atg1 in adult *Drosophila* induces autophagy both cell autonomously and non-cell autonomously, and also results in life-span extension.[39] Consistent with the physiological relevance of these observations, many autophagy genes (i.e., *Atg1, Atg6, Atg7, Atg5, Atg8*) show reduced expression with age in flies,[37,40] and LC3 and ATG7 protein levels in muscle also decline with age in mice and humans.[41] This culminates in the loss of autophagic capacity generally observed with normal aging (reviewed in ref. 42).

Notably, genetic and age-related loss of adequate autophagic and lysosomal function has been linked to the development of several metabolic and neurodegenerative diseases (reviewed in ref. 11). For example, loss-of-function mutations in several genes with autophagy-related functions (e.g., *Becn1/VPS30/ATG6*,[43] *Atg7*[44], *Atg5*[45]) result in decreased autophagy and increased accumulation of disordered and aggregated proteins in neurodegenerative disorders such as Huntington disease (HTT/huntingtin), Alzheimer disease (Aβ and MAPT/tau), and Parkinson disease (SNCA/α-synuclein) (reviewed in ref. 46).

Accumulating evidence thus supports a beneficial role for autophagy in aging, although the underlying mechanisms of autophagy regulation in long-lived organisms are not fully understood. In this review, we focus on the role of transcriptional and epigenetic regulation of autophagy in the context of aging by highlighting studies in longevity models, and noting relevance to age-related diseases where applicable.

Transcriptional Regulation of Autophagy Relevant to Aging

Transcriptional mechanisms are emerging to play an important role in the regulation of autophagy. Specifically, several transcription factors are now known to regulate the sustained expression of specific autophagy-related or lysosomal genes (reviewed in refs. 47–48, see also refs. 49-50); however, only a few have been carefully examined to determine their conserved roles in inducing autophagy to promote longevity. Nevertheless, the helix-loop-helix transcription factor TFEB and the forkhead transcription factors FOXO and FOXA have been shown to be key transcriptional regulators of autophagy and lysosomal biogenesis. These factors have also been associated with the transcriptional induction of beneficial autophagy found in long-lived organisms. Below, we review recent research on the role of these transcription factors in autophagy regulation and aging.

TFEB and MITF/microphthalmia-associated transcription factors

Beyond its role in phosphorylating autophagy-related proteins, MTOR kinase phosphorylates several transcription factors

with roles in autophagy, thereby preventing their translocation to the nucleus.[51] The most prominent example of an autophagy-related MTOR-regulated transcription factor is TFEB, a member of the MITF (microphthalmia-associated transcription factor) family.[52-57] TFEB was first described as a key transcription factor in lysosomal biogenesis,[58] and subsequent studies revealed additional roles in the regulation of genes involved in autophagosome formation (e.g., *Atg9, Wipi1/2/Atg18, Lc3/Atg8*), cargo recognition (e.g., *Sqstm1*), vacuolar fusion (e.g., *Vps11, Vps18*), vacuolar proton pumping (e.g., V-ATPase subunits), and lysosomal degradation (e.g., sulfatases and cathepsins).[52] Thus, TFEB regulates autophagic flux by coordinating the expression of genes with functions at all stages of the autophagy process, from vesicle initiation to cargo degradation.

Under nutrient-rich conditions, MTOR phosphorylates TFEB at the lysosomal surface, which results in the binding of YWHA/14–3–3 proteins and the retention of TFEB in the cytosol.[56,57] The TFEB sequence contains several predicted MTOR and MAPK1/ERK2 phosphorylation sites, and mutation of serine 142 or serine 211 to nonphosphorylatable residues has been reported to induce nuclear localization of TFEB.[55–57] Additionally, TFEB nuclear localization is induced by the TOR inhibitors rapamycin and Torin.[55,56] Similarly, TFEB translocates to the nucleus in response to nutrient deprivation, causing increased lysosomal calcium release and activation of the phosphatase calcineurin (CaN) to directly dephosphorylate TFEB.[169] Interestingly, another member of the MITF family, TFE3, can bind to similar promoter sequences found on autophagy-related target genes of TFEB (so-called CLEAR sites), displays conserved regulation by TOR, and has overlapping functions in lysosomal biogenesis and autophagy gene regulation under nutrient-scarce conditions. Moreover, overexpression of TFE3 promotes lysosomal biogenesis and autophagy. These findings suggest the potential nuclear coordination of MITF transcription factors in controlling autophagy.[59] Nevertheless, TFEB and TFE3 must have distinct functions, as deletion of *Tfeb* in mice results in embryonic lethality, whereas deletion of *Tfe3* has no apparent phenotype.[60,61] Recent work has shown that TFEB is recruited to the lysosome for TOR phosphorylation by interaction with FLCN/folliculin,[62,63] suggesting a complex regulatory network governs the intracellular localization of TFEB, and thus its activity (**Fig. 1C**). Taken together, recent studies demonstrate an important function for lysosomes, beyond their role in degradation, as sites for the integration of signaling by the nutrient sensor TOR.

The TFEB homolog in *C. elegans*, HLH-30, plays a role similar to that of TFEB in the induction of orthologous target genes and activation of autophagy.[64] Indeed, HLH-30 localization to the nucleus is induced by RNA interference (RNAi)-mediated inhibition of *let-363/Tor*,[64] nutrient deprivation,[65] and by 4 additional conserved longevity paradigms (reduced insulin signaling, mRNA translation, and mitochondrial respiration, and removal of the germline) that induce autophagy markers and require autophagy genes for life-span extension.[64] The induction of autophagy gene expression by either *let-363/Tor* inhibition or germline removal requires *hlh-30*, and *hlh-30* is indispensable for

the life-span extension observed in all of these 6 autophagy-dependent *C. elegans* longevity models.[64,66] These observations are consistent with HLH-30 transactivation causing an increase in autophagic flux, which is necessary for life-span extension in these longevity paradigms. In support of this, overexpression of HLH-30/TFEB is sufficient to activate autophagy and to moderately extend *C. elegans* life span.[64] HLH-30/TFEB is negatively regulated by another helix-loop-helix transcription factor, MXL-3, which provides precise temporal control of HLH-30/TFEB activity to maintain homeostasis.[67] In mammals, the transcription factor ZNF24/ZSCAN3 works in opposition to TFEB by acting as a repressor of TFEB target genes.[68] Nuclear TFEB levels are elevated in liver cells of long-lived, dietary-restricted mice,[64] indicating that TFEB is a component of a conserved longevity mechanism.

Other targets of HLH-30/TFEB suggested to play a role in life-span extension (**Table 1**) include the lysosomal acid lipases *lipl-1* and *lipl-3*,[67] supporting previous observations suggesting that increased lipolysis[69] via lipophagy by the homologous lysosomal acid lipase *lipl-4*,[70] may represent a central life-span-extending mechanism. Of note, TFEB has been linked to changes in lipid metabolism induced by starvation.[65,67] Lipophagy-mediated remodeling and utilization of lipids for energy generation and signaling via lipophagic products may represent possible mechanisms by which cellular homeostasis, organelle biogenesis, and survival are ensured during nutrient deprivation. Indeed, elevation of TFEB activity by overexpression or by starvation leads to an increase in PPARA/PPARα and PPARGC1A/PGC1α expression,[65,71] suggesting an enhanced cellular ability to respond to lipid signals. Thus, TFEB-mediated transcriptional induction of autophagy may be central for increasing autophagic flux in order to provide a dynamic pool of metabolites, particularly lipids, for synthetic and signaling pathways conducive to longevity. The conserved regulation of TFEB activity by MTOR supports the notion that nutrient signaling is pivotal for the control of autophagic flux. Thus, TFEB stimulation of autophagy-related and lysosomal gene expression coordinates each step of the process to provide a vital source of metabolites during periods of nutrient deprivation.

Recent work suggests an emerging role for TFEB, beyond aging and metabolism, in the pathology of diseases. For example, overexpression of TFEB has beneficial effects on cellular clearance in models of lysosomal storage diseases, Parkinson disease, and α1-antitrypsin deficiency.[52,72,73] In addition, heterologous overexpression of TFEB in mouse brain increases the clearance of aberrant MAPT/tau protein, a key player in Alzheimer disease, in part by improving lysosomal function.[74,74] Benefits of TFEB induction in the turnover of disease-related proteins such as HTT/huntingtin also involves interactions with PPARGC1A,[75] a transcriptional cofactor involved in mitochondrial biogenesis and recently linked to longevity in *Drosophila*.[76] Conversely, in X-linked spinal and bulbar muscle atrophy, TFEB transactivation is inhibited by interaction with polyglutamine-expanded ARs/androgen receptors, with a subsequent impairment of autophagy.[77] This recent finding raises the possibility that TFEB activity may be impaired by the age-related accumulation and

aggregation of disordered proteins in a variety of neurodegenerative diseases. Interestingly, TFEB is also required for the response to infection in both worms and mice, suggesting a broad and conserved role for this transcription factor in survival associated with a variety of stressors.[78] Taken together, these studies highlight a critical role of TFEB in promoting autophagy under physiological and pathological conditions, including the increasingly prevalent age-related diseases.

Forkhead transcription factors

Another major family of transcriptional regulators of autophagy with a conserved role in aging is the forkhead transcription factors (FOXO), which play well-recognized and central roles in cellular homeostasis through the regulation of genes involved in lipid and glucose metabolism and in mitochondrial function.[79] A role for the FOXO family in autophagy was first described in murine models of muscle atrophy, an age-related condition[80,81] to which several degradative pathways contribute, especially autophagy.[82] In the muscle, FOXO1 and FOXO3 elevate the autophagic flux by increasing the expression of autophagy genes mainly working as part of the core machinery (*Ulk2/ATG1, Pik3c3/VPS34, Becn1/VPS30/ATG6, Atg4B/ATG4, Lc3b/ATG8, GabarapL1/ATG8, Atg12/ATG12, Bnip3*)[80,81,83,84] and additionally increase protein degradation via the proteasomal pathway.[80,85,171] In particular, FOXO3 increases the capacity of the lysosome to degrade incoming cargo, indicating a role for lysosomal function in muscle atrophy. Other FOXOs (FOXO1, FOXO4, and FOXO6) also play roles in proteostasis and autophagy (reviewed in refs. 40, 86, 87). Interestingly, AMPK directly activates FOXO family members in mammalian cells to promote protein breakdown and the production of alternative energy sources, even in the presence of INS/insulin and normal AKT activation.[88-90] AMPK also activates FOXO in worms[88] and possibly in flies,[39] and overexpression of AMPK in flies is sufficient to induce autophagy gene expression (*Ulk/ATG1, Lc3a/ATG8, Lc3b/ATG8*).[39] Moreover, human FOXO3 and AMPK coordinately enhance autophagy during ultra-endurance running, suggesting that transcriptional upregulation of autophagy genes may also contribute to induced autophagic flux in exercised skeletal muscles.[91] Thus, several nutrient-signaling pathways converge to modulate FOXO for activation of autophagy and metabolism.

Reduced INS-IGF1 signaling, which increases the activity of FOXO, extends longevity in *C. elegans, Drosophila*, and mice, and is observed in human centenarians.[20] In *C. elegans*, life-span extension through reduced INS signaling is mediated by DAF-16/FOXO, which regulates the transcription of numerous genes, including those with relevance to proteostasis.[92,93] Similar to HLH-30/TFEB, FOXOs can enhance autophagic flux in *C. elegans*, at least in part by increasing the expression of autophagy-related genes. For instance, C08H9.1, a lysosomal serine carboxypeptidase similar to CTSA/cathepsin A, is induced by DAF-16/FOXO in long-lived *daf-2/Igf1r* (insulin-like growth factor 1 receptor) mutants and is linked to thermal stress resistance in these animals.[94] Consistent with these observations, animals overexpressing DAF-16/FOXO have an increased abundance of autophagosomes and enhanced resistance to bacterial

infections.[95] Nevertheless, autophagosome numbers are not affected by the loss of *daf-16/FoxO* in *daf-2/Igf1r* mutants,[70] suggesting a more complex and/or late-acting role for DAF-16/FOXO in autophagy regulation.[70] Another Forkhead transcription factor, PHA-4/FOXA, stimulates autophagic flux in *C. elegans* by binding to the promoter region of several early-acting genes involved in autophagosome formation, including *unc-51/Ulk/ATG1, bec-1/Becn1/VPS30/ATG6*, and *lgg-1/Lc3/ATG8*.[96,97] PHA-4/FOXA-mediated induction of these genes may stimulate the longevity-inducing autophagy observed in germline-less, dietary-restricted, and *let-363/Tor*-inhibited animals, which all require *pha-4/FoxA* for their survival.[31,70,98,99] *let-363/Tor* is known to regulate DAF-16/FOXO in *C. elegans*[70,100] and may also function as an upstream regulator of PHA-4/FOXA in longevity models.[70,99,100] Current studies are aimed at understanding the complexity of signaling between FOXO and MTOR[101] and its impact on autophagy, metabolism, and aging.

The role of FOXO in intracellular proteostasis is well established in *C. elegans*[20] and is conserved in *Drosophila* muscle.[40] Studies in these organisms have also highlighted the importance of global, tissue-specific, and cell-specific functions of FOXO in modulating aging and age-related diseases.[20,102] Long-lived *C. elegans* display increased proteasomal degradation[103] that likely acts in concert with elevated autophagy to facilitate the maintenance of proteostasis.[104,105] Interestingly, autophagy and proteasomal activity increase concomitantly in embryonic and hematopoietic stem cells.[106,107] This is in line with a report that somatic cells acquire stem cell-like properties in long-lived *daf-2/Igf1r* mutants.[108] These findings suggest that FOXO plays a central role in aging by activating longevity-related gene expression and by maintaining a proteostatic status conducive to cell survival and longevity.

Epigenetic Regulation of Autophagy Relevant to Aging

In addition to transcriptional regulation, sustained expression of autophagy genes can be regulated by several epigenetic mechanisms, such as chromatin modulation, histone modification, and microRNAs (miRNAs). The histone deacetylase SIRT1 influences aging and age-related disorders, at least in part, via effects on autophagy; however, the extent to which other epigenetic mechanisms of autophagy regulation contribute to aging and age-related diseases remains unclear. Here, we review some of the epigenetic factors that have been implicated in the regulation of autophagy gene expression in the context of aging.

Chromatin-modifying enzymes

Chromatin is defined as the complex of condensed DNA molecules wound around histones and associated proteins. Histones are alkaline proteins that act as spools to support the compact packaging of DNA into nucleosomes. Posttranslational modification of histones, including acetylation, methylation, phosphorylation, SUMOylation, ubiquitination, and ADP-ribosylation, occur mainly in the N-terminal tails and have profound effects on chromatin structure and gene expression. Of these modifications, several histone acetylation and methylation marks have been linked to autophagy regulation (reviewed in ref. 109) (**Fig. 1D**). Histone-modifying enzymes and ATP-dependent chromatin-remodeling complexes can reorganize chromatin structure, and both types of modifiers can affect longevity. For instance, ATP-dependent remodeling complexes have been implicated in longevity in yeast (Isw2)[110] and in worms (SWI/SNF Switch/Sucrose Non-fermentable), which colocalizes with DAF-16/FOXO at the promoters of longevity-promoting genes).[111]

Among the histone-modifying enzymes, the NAD-dependent deacetylase SIRT1 (sirtuin 1) is a particularly well-known modulator of aging. Specifically, the life spans of yeast, worms, and flies can be extended by overexpression and/or pharmacological activation of SIRT1 (by small molecule activators and resveratrol) (reviewed in refs. 112,113) and the life span of mice is extended by ubiquitous overexpression of another sirtuin, SIRT6, or brain-specific overexpression of SIRT1 (reviewed in ref. 114). Notably, in *C. elegans*, the life span-extending effect of the SIRT1 activator resveratrol requires the expression of *bec-1/Becn1/VPS30/ATG6*,[31,115,116] suggesting that autophagy is necessary for this longevity paradigm. Several lines of evidence support this notion. SIRT1 regulates autophagy gene expression through histone deacetylation, with lysine 16 on histone H4 (H4K16) as the primary deacetylation target (reviewed in ref. 117). H4K16 deacetylation inhibits the transcription of genes involved in the early and late steps of autophagy in multiple cell types, including yeast and mouse embryonic fibroblasts (*Ulk1/ATG1, Ulk3/ATG1, Atg9A/ATG9, Lc3/ATG8, GabarapL2/ATG8*, and vacuolar membrane protein *Vmp1*), resulting in decreased autophagic flux measured by LC3/Atg8 conversion and turnover.[48] An antagonist to the histone deacetylation activity of SIRT1 is the histone acetyltransferase (HAT) KAT8/MOF/MYST1/YBF2. Induction of autophagy by starvation or rapamycin treatment is accompanied by deacetylation of H4K16 and downregulation of KAT8. Therefore, the acetylation status of H4K16 represents a molecular switch that can regulate autophagy.[118]

While the activity of SIRT1 in the nucleus limits autophagy, SIRT1-mediated deacetylation of cytoplasmic proteins is required for autophagy induction, as shown in enucleated cells subjected to starvation or rapamycin treatment.[119] This may be due to the direct interaction of SIRT1 with autophagy proteins, since SIRT1 co-immunoprecipitates with ATG5, ATG7, and LC3/Atg8, and directly binds and deacetylates these proteins in vitro.[120] Moreover, SIRT1 indirectly regulates autophagy by deacetylation of FOXO3, leading to increased expression of autophagy-related genes, including *Bnip3*, which are critical for autophagy induction.[121,122] Thus, although it is clear that SIRT1 regulates autophagy by both epigenetic and posttranslational mechanisms, the relative contribution of each mechanism to SIRT1-mediated longevity remains to be fully established.

SIRT1 has protective effects in several neurodegenerative disease models, which may in part be due to its role in autophagy induction. For example, in cellular models of Parkinson disease,

resveratrol increases the degradation of SNCA/α-synuclein via autophagy induction,[123] and SIRT1 overexpression can protect against prion-peptide toxicity in cultured neurons.[124] Similarly, SIR-2.1/SIRT1 suppresses the formation of SNCA inclusions in worms.[125] In yeast, expression of human SNCA is involved in determining chronological life span, which is accompanied by an increase in autophagy via Sir2/SIRT1.[126] Collectively, the evidence to date suggest that SIRT1 is a regulator of life span and autophagy, and plays a role in age-related diseases.

In both yeast and mammals, autophagy can be regulated by acetyl coenzyme A (acetyl-CoA), which serves as the donor for acetylation reactions. In yeast, nucleocytosolic acetyl-CoA is produced through the action of the synthetase Acs2, and inhibition of *ACS2* enhances autophagy during chronological aging.[127] On the one hand, reduced acetyl-CoA levels have conserved beneficial effects, as illustrated by the extended life span of *Drosophila* in which AcCoAS (acetyl-CoA synthetase) is specifically reduced in the brain.[127] On the other hand, accumulation of nucleocytosolic acetyl-CoA represses autophagy in yeast via hyperacetylation of histone 3 (on K9, K14, and K18) and repression of *ATG7* transcription.[127] In mice and cultured human cells, inhibition of the enzymes required to maintain cytosolic acetyl-CoA levels leads to induction of autophagy, and conversely, high cytosolic acetyl-CoA levels inhibit the ability of starvation to induce autophagy.[128] In human cell lines, the HAT EP300 is required for the repression of autophagy under these conditions. In contrast to yeast, in human cell lines acetyl-CoA influences autophagy through cytosolic effects that are independent of transcriptional changes of autophagy genes.[128] Taken together, these findings point to the emergence of nucleocytoplasmic acetyl-CoA as a novel modulator of autophagy and life span; future experiments should clarify its impact on age-related diseases.

Histone acetylation and the activity of HATs and HDACs are also affected by the action of cationic polyamines, such as spermine and spermidine. The levels of specific polyamines decrease with age in several tissues of many organisms and the external addition of spermidine has beneficial affects on life span and aging in model organisms including yeast, worms, flies, mice, and human cells.[129,130] These effects are likely to involve, at least in part, changes in autophagy since spermidine extends life span in a manner dependent on autophagy genes in both *C. elegans* and *Drosophila* (*bec-1/Becn1/VPS30/ATG6* and *Atg7*, respectively). Consistent with this notion, spermidine treatment stimulates autophagy in *S. cerevisiae, C. elegans, Drosophila*, and cultured mammalian cells, as detected by LC3 lipidation and increased formation of LC3-positive puncta.[129,131] Spermidine treatment leads to hypoacetylation of histone 3 (K9, K14, K18) due to the inhibition of histone acetyltransferase activity. Interestingly, the *ATG7* promoter remains hyperacetylated upon spermidine treatment and the expression of *ATG11, ATG7*, and *ATG15* is increased.[129] These data support a model in which spermidine treatment leads to the favored transcription of autophagy-related genes, whereas the majority of genes are downregulated by global hypoacetylation.[129] Despite the beneficial effects of spermidine treatment on life span, high levels of polyamines are associated with

diseases, especially various cancers. In vitro studies as well as studies in yeast suggest that polyamines, including spermidine, increase aggregation of SNCA implicated in Parkinson disease.[132–134] However, it remains unclear whether the increase in polyamines is directly implicated in causing disease and whether changes in autophagy-gene expression occur under these circumstances.

In addition to histone acetylation, several histone methylation marks have been implicated in autophagy regulation. The H3K9 methyltransferase EHMT2/G9A is thought to act as a repressor of autophagy under basal conditions. Upon autophagy induction EHMT2 dissociates from histone H3K9, and H3K9 dimethylation decreases. In human cell lines, this is accompanied by an increase in H3K9 acetylation and increased transcription of multiple autophagy genes such as *UVRAG, WIPI1/ATG18, ATG9B, ATG9 LC3B/ATG8, GABARAPL1/ATG8, GABARAPL2/ATG8, SQSTM1, BNIP3*, and *TP53INP2/DOR*.[135] Interestingly, H3K9me3 repressive marks accumulate in aging flies, although it is unclear whether this age-dependent increase is causally linked to changes in autophagy gene expression.[136]

Another histone mark, H3K4me3, has also been associated with longevity in *C. elegans* and *Drosophila*, as well as with autophagy regulation in yeast and mammalian cells.[118,137] In *C. elegans*, reducing the expression of the H3K4 demethylase RBR-2 leads to a concomitant increase in H3K4me3 and a decrease in life span, and conversely, reducing components of the H3K4-methylation complex (ASH-2/Set1/Ash2, WDR-5.1/Tag-125, and SET-2) decreases H3K4 methylation levels and extends longevity.[138] Likewise, downregulation of the *Drosophila* ortholog of RBR-2, Lid, shortens the life span while increasing H3K4me3 levels.[139,140] Although these observations are consistent with the notion that longevity is associated with increased autophagy, it remains to be determined whether long-lived animals with reduced H3K4 methylation levels display increased autophagy flux and require autophagy genes for their longevity. Overall, the emerging concept from these studies is that age-related alterations in histone marks can affect autophagy gene expression with relevance to longevity.[141] Future investigations of the relationship between histone marks in autophagy regulation and in life-span determination will be important to assess the impact of epigenetically regulated autophagy in age-related diseases.

MicroRNAs

RNA interference is an epigenetic mechanism of gene regulation that, in contrast to chromatin and histone alterations, occurs post-transcriptionally. miRNAs are small (21–25 nucleotides) single-stranded RNAs that bind to complementary nascent mRNAs and render them susceptible to degradation prior to translation, thereby inhibiting the expression of specific target genes. miRNAs are highly conserved, and to date, thousands have been identified in plants and animals.[142] Several miRNAs (e.g., *lin-4, lin-14, miR-34, miR-71, miR-238, miR-239*, and *miR-246*) regulate aging in *C. elegans*, and many miRNAs that affect tissue- and cell-specific aging phenotypes have been identified in mammals (reviewed in ref.

142). Similarly to other epigenetic regulators, numerous miRNAs exhibit age-related changes in expression.[143-145]

One particular example is *miR-34*, which is linked to autophagy and longevity in several species. *C. elegans* with *miR-34* loss-of-function mutations show an extended life span that is eliminated by RNAi of autophagy genes *bec-1/Becn1/VPS30/ATG6, atg-9,* and *atg-4.1.*[146] Accordingly *miR-34* levels are decreased in long-lived dietary-restricted mice.[147] *miR-34* is upregulated in *C. elegans* during aging and inhibits the expression of the autophagy gene *Atg9a* in vitro.[146] In contrast, increased levels of *Mir34* extend life span and reduce neurodegeneration induced by polyglutamine expansion proteins in *Drosophila*;[148] however, the contribution of autophagy genes to these *miR-34*-mediated beneficial effects has yet to be addressed. Several additional studies have linked *MIR34* to autophagy regulation in mammalian cells, but the contribution to aging in mammals remains unclear. For example, in human cells, *MIR34* targets the cell death-regulating protein BCL2, which directly binds to and inhibits the autophagy protein BECN1/VPS30.[149] *MIR34* also inhibits the expression of the HDAC SIRT1.[150] Thus, the age-related upregulation of *MIR34* could contribute to the aging process by directly modulating the expression of autophagy-related proteins.

Several other miRNAs (e.g., *MIR376A* and *MIR376B*) prevent starvation-induced autophagy in human cell lines by blocking the expression of *BECN1* and *ATG4C*.[151,152] Similarly, overexpression of *MIR181A* in starved human cells inhibits the induction of *ATG5* expression and reduces autophagy, as measured by LC3 lipidation.[153] Autophagy can also be regulated by *MIR30D*, which inhibits *BECN1* expression,[154,155] and by *MIR101*, which blocks *ATG4D, RAB5A,* and *STMN1* expression.[156,157] In addition to targeting autophagy-related gene expression, miRNAs also target transcription factors and MTOR pathway components with roles in aging. For instance, hypoxia-induced *Mir155* blocks *Rheb1* expression and stimulates autophagy.[158] Interestingly, TFEB is a target of the miRNA *Mir128*, highlighting a mechanism for fine-tuning of the transcriptional regulation of autophagy.[52]

The expression of several autophagy-regulating miRNAs is altered during physiological aging as well as in neurodegenerative diseases such as Alzheimer, Huntington, and Parkinson diseases (reviewed in ref. 159). However, it is not yet known whether the disease pathology is directly affected by such deregulated miRNAs. Future studies should continue to identify small RNAs with effects on autophagy regulation and determine their corresponding impact on aging.

Conclusions and Future Perspectives

Common denominators in the development of age-related diseases are the inability of cells to maintain adequate metabolism, proteostasis, and organelle function. Emerging evidence strongly supports autophagy as a major mechanism to maintain cellular homeostasis and prolong organismal life span. The capacity of cells to induce and sustain autophagic flux is predominantly dependent on the function of nutrient sensors such as MTOR and AMPK, and on the INS-IGF1-signaling pathway. While MTOR and AMPK regulate metabolism and autophagy via direct phosphorylation of proteins, likely to ensure rapid induction of autophagy, studies in various organisms suggest that these master regulators of homeostasis also govern the transcriptional regulation of autophagy necessary to sustain long-term responses. In turn, several studies have linked the dysfunction of autophagy-relevant transcription factors to diseases (reviewed in ref. 160), and it is becoming evident that alterations in the function of such transcription factors can modulate the aging process itself (reviewed in ref. 66).

Although numerous transcription factors influence autophagy (reviewed in refs. 47, 48, see also refs. 49-50), TFEB and FOXO are the most prominent factors currently known to enhance autophagic flux by direct stimulation of autophagy-related and lysosomal gene expression. While FOXO so far has been reported to regulate genes especially in the core machinery, TFEB controls genes with functions in autophagy initiation, phagophore formation and elongation, and lysosomal degradation and lipolysis, suggesting that initiating autophagy or enhancing lysosomal lipid remodeling may suffice to stimulate autophagic flux. Importantly, many of these autophagy-related and lysosomal genes modulate life span and aging in various genetic contexts (summarized in **Table 1**). An increasing number of transcriptionally regulated autophagy genes are being identified,[161] and determining their specific contributions to autophagy flux and aging may help to define novel targets for the pharmacological manipulation of autophagy for the treatment of age-related diseases.

Cellular regulation of FOXO and TFEB relies predominantly on phosphorylation and nuclear exclusion to prevent the activation of autophagy gene expression. MTOR phosphorylation of TFEB occurs at the lysosomal membrane surface, suggesting that this is an important site for autophagic flux regulation. Indeed, lysosomal–nuclear signaling is emerging as a regulatory platform not only for aging but also for metabolism.[46] From this standpoint, it is possible that hyperactivation of MTOR makes a major contribution to aging by actively preventing autophagy activation in various metabolic and pathological contexts. As an example, sustained activation of skeletal muscle MTORC1 causes myopathy by inhibiting autophagy.[162] Taken together, current evidence suggests that nutrient signaling via MTOR provides a central mechanism by which cells regulate the induction of autophagy-related and lysosomal gene expression, which in turn, modulates stress resistance and life span.

Expression of autophagy-related genes can also be regulated epigenetically. Epigenetic changes are thought to be responsible for acute fine-tuning and long-term transcriptional regulation of the autophagy response. Histone modifications also play a role in preventing prolonged autophagy through negative regulatory effects.[109] Epigenetic mechanisms have been implicated in the regulation of life span, but it is not yet clear whether epigenetic regulation of autophagy genes is linked to their involvement in longevity. Spermidine treatment is a prominent and conserved example of a life-span-extending regimen that epigenetically regulates autophagy gene expression. In addition, several histone modifications and chromatin-modifying enzymes have been identified that can alter

the expression pattern of multiple early-acting autophagy-related genes, such as *Ulk/ATG1, ATG11, Atg9B/ATG9, Atg7/ATG7, Lc3/ATG8 family*, and *Bnip3*. Among these, *Atg9B* and *Lc3* are upregulated in mice that overexpress TFEB,[52] but they have yet to be directly implicated in mammalian longevity. It will be interesting to further explore autophagy gene regulation in model organisms in which the homologous genes have known effects on life span; for example, *Atg7*, which is required for normal life span in mice and for spermidine-induced life span extension in *Drosophila*.

Aging is accompanied by several prominent changes that influence chromatin structure, including a decline in histone protein levels, an increase in histone acetylation, and alterations in the histone methylation signature.[163,164] In age-related pathologies, however, the nature of these chromatin changes, the identity of the enzymes associated with these changes, and the contribution of transcriptional changes of specific autophagy-related genes have yet to be defined. Additionally, it is conceivable that stable changes in the chromatin state could reprogram the profile of transcription factors occupying the promoters of autophagy-related genes, leading to a sustained change in the autophagy response throughout the organism's life span. Future studies should determine whether such chromatin changes can be engineered to sustain and increase autophagic flux in aging cells to improve proteostasis, thereby delaying aging and the onset of age-related diseases.

To summarize, recent evidence has emphasized a major role for autophagy in life-span extension in multiple organisms. The pathways underlying this role, including the transcriptional and epigenetic regulatory mechanisms described here, may provide attractive entry points to explore pharmacological avenues for the therapeutic induction of autophagy; for example, by enhancing the function of longevity-enhancing transcription factors. Alternatively, appropriately increased levels of autophagy induced by exercise[91,165] might be sufficient to promote organismal health, in particular by combating autophagy-related diseases. It seems clear that the successful development of therapeutic autophagy enhancers will require an increased understanding of the regulatory networks surrounding autophagy-promoting transcription factors, their potential interactions with other factors in the nucleus, and the epigenetic modifications necessary to enhance autophagy.

Note

Nomenclature: mammalian genes/proteins are stated first, followed by the yeast name, if different. The nomenclature for other model organisms is used where applicable.

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