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The aging lysosome: an essential catalyst for late-onset neurodegenerative diseases

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Abstract

Lysosomes figure prominently in theories of aging as the proteolytic system most responsible for eliminating growing burdens of damaged proteins and organelles in aging neurons and other long lived cells. Newer evidence shows that diverse experimental measures known to extend lifespan in invertebrate aging models share the property of boosting lysosomal clearance of substrates through the autophagy pathway. Maintaining an optimal level of lysosome acidification is particularly crucial for these anti-aging effects. The exceptional dependence of neurons on fully functional lysosomes is reflected by the phenotypes seen in congenital lysosomal storage disorders, which commonly present as severe neurodevelopmental or neurodegenerative conditions even though lysosomal deficits are systemic. Similar connections are now being appreciated between risk for late age-onset neurodegenerative disorders and primary lysosomal deficits. In diseases such as Alzheimer's and Parkinson's, as in aging alone, primary lysosome dysfunction due to acidification impairment is emerging as a frequent theme, supported by the growing list of familial neurodegenerative disorders that involve primary vATPase dysfunction. The additional cellular roles played by intraluminal pH in sensing nutrient and stress and modulating cellular signaling have further expanded the possible ways that lysosomal pH dysregulation in aging and disease can disrupt neuronal function. Here, we consider the impact of cellular aging on lysosomes and how these changes may create the tipping point for disease emergence in major late-age onset neurodegenerative disorders.

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Introduction

Aging lysosomes – a critical precondition for late-age onset neurodegenerative diseases

Lysosomes failure is implicated in the etiology of an increasing number of neurodegenerative diseases across the entire age spectrum(Nixon, 2013; Nixon et al., 2008). For decades, the unusually close relationship between lysosomal dysfunction and neurodegeneration has been appreciated for the family of more than 50 inherited Lysosomal Storage Disorders (LSD) arising prenatally or in childhood. Although the disease impact is systemic for most LSDs, the brain and especially neurons are disproportionately affected and most exhibit neurological disease as the most prominent or exclusive clinical feature(Wolfe et al., 2013) In addition, research has now uncovered pathogenic lysosomal dysfunction in major late age onset neurodegenerative disorders, such as AD, PD, FTD, and possibly ALS, which may be primary in some familial forms of the disease. Underscoring this concept is evidence for homozygous mutations of a single gene that cause a congenital LSD but may cause a late onset neurological disorder when in heterozygous form. For example, glucocerebrosidase (GBA) mutations cause the congenital disorder Gaucher's disease but, in the elderly, cause Parkinson's disease. Progranulin (PRGN) mutations, depending on gene dosage, cause either ceroid neuronal lipofuscinosis (NCL) in childhood or Frontotemporal Dementia in adults(Ward et al., 2017). Although early familial Alzheimer's disease (FAD) arises most frequently in the 4^{th} and 5^{th} decades of life, certain mutations of presenilin 1 (*PSEN1*), which cause primary defects in lysosomal acidification (Lee et.al. 2010), can induce very early onset AD in young adults. In one report, an early onset form of *PSEN*1 AD exhibited a neuropathological signature of Kuf's Disease, a form of NCL that usually arises in adolescence or early adulthood (Larner, 2013).

In most late onset neurodegenerative diseases, the causative misfolded mutant protein is produced throughout life yet only accumulates in the adult or aged brain as clinical disease emerges. This delayed appearance and the further evidence that most of these pathogenic proteins are substrates of lysosomes suggests that a significant, if not dominant, determinant of disease onset in some of these disorders is an aging-related decline in lysosomal system efficiency possibly compounding a disease-related compromise of lysosomes. Why the brain is preferentially vulnerable to systemic insults affecting the lysosomal system reflects in part that neurons are post-mitotic and have less active lysosomal exocytic activity than non-neural cells. Removal of waste by dilution during cell division or by exocytosis are therefore not major options for clearance and neurons must rely more on lysosomes to maintain cellular quality control.

Although research on lysosomes in disease states is now advancing at a rapid pace, efforts to understand the crucial declines in lysosomal efficiency due to brain aging have lagged. Here we will review what is known about the impact of aging on lysosomes in and outside of the brain. Although the evidence is still fragmentary, it establishes lysosomal functional declines as key determinants of senescence and of the vulnerability to neurodegenerative disease. A further intriguing theme emerging from research on lifespan and brain disorders, also addressed here, is the extent to which the various insults during aging converge to dysregulate the process of lysosome acidification. This deficit exerts a pervasive influence

on both hydrolytic and signaling functions of lysosomes, synergizing with additional disease-related lysosomal deficits.

Autophagy and lysosome function are key determinants of senescence and longevity

Aging of cells and organisms involves a progressive loss of physiological integrity through multiple pathways(Cuervo and Dice, 2000d). Particularly prominent is the deterioration of proteostasis, a cellular network that governs protein life from synthesis to degradation (Douglas and Dillin, 2010). As the burden of misfolded and damaged constituents grows with age, cells face declining degradative capacity and other counterproductive alterations of intracellular proteolytic systems, Effects on the lysosomal system are arguably the most substantial, as further detailed below, while declines in ubiquitin-proteasome activity develop in aging but are generally less marked (DeMartino and Slaughter, 1999; Shibatani and Ward, 1996). Calpain proteinases are actually hyper-activated in many aging tissues and this change is considered an established marker of aging (Glaser et al., 1994; Saito et al., 1993). Because calpains only carry out limited proteolysis (Carafoli and Molinari, 1998; Glaser et al., 1994), increased generation of protein fragments in aged cells can further overburden lysosomes and the UPS. In addition, over-activated calpains may inhibit autophagy(Menzies et al., 2015; Yousefi et al., 2006). Among effects on the cell's clearance and quality control systems, the greatest impact of aging is on the lysosome's role in autophagy. Conversely, lysosomes impairment may well be the most influential determinant of senescence onset and lifespan.

Autophagy is a major lysosomal degradative process responsible for clearance and recycling of cellular constituents and is the only pathway available for intracellular turnover of organelles (Figure 1). While constitutively active in most cells, it can be strongly induced by various forms of stress and nutritional deficiency. In autophagy, cellular substrates may be sequestered and delivered to lysosomes via a variety of complementary mechanisms (Figure 1). The lysosome is the only organelle common to all of these autophagy routes, which include macroautophagy, chaperone-mediated autophagy, and microautophagy. Although it has become common to refer to an "autophagic-lysosomal pathway", it is important to note, especially for the discussion here, that autophagy requires substrate digestion by lysosomes and that autophagy rates depend as much on the efficiency of lysosomal as on autophagy induction and substrate sequestration rates. Importantly for considerations of lysosomes in aging, growing evidence points to declining lysosomal function as the most influential change in the autophagy pathway that lowers the organism's longevity (although mitophagy declines imply an importance of sequestration failure as well). Indeed, among the most established markers of neuronal aging are lipofuscin granules containing incompletely digested lysosomal substrates. The role of autophagy is especially critical for aging neurons and other post-mitotic cells, which are unable to use mitosis to dilute waste build-up and have low lysosomal exocytosis capability that allows non-neural cells to jettison waste in bulk. Neurons are further challenged by their asymmetry: distal axons and synapses that actively sequester substrates are often at long distances from lysosomes that are located in cell bodies.

The dysfunction of autophagy in aging-related neurodegenerative disorders has been extensively reviewed (Boland et al., 2018; Menzies et al., 2017; Nixon, 2016; Nixon, 2013; Scrivo et al., 2018). Although there are remaining questions about the extent to which macroautophagy is induced or impeded at the initial stages of induction, cargo recognition or sequestration, there is a general consensus that the clearance of autophagy substrates through the lysosome is progressively corrupted in Alzheimer's Disease, and likely in Frontotemporal Dementia and Parkinson's Disease (Boland et al., 2018; Bordi et al., 2016; Menzies et al., 2017; Nixon, 2013). The activity of CMA, in particular, has been extensively studied and shown to be markedly impaired in multiple neurodegenerative proteinopathies as well as in aging. The decline of CMA in aging involving mainly the declining levels of LAMP2a, the key chaperone for substrate delivery to lysosomes(Cuervo and Dice, 2000b, c), amply reflects the cumulative impact of aging-related processes on this vital function of lysosomes, including the involvement of increased oxidative stress, lipid alterations, and structural damage to the substrates. Experimental disruptions of CMA induce a phenotype of advanced aging in tissues while supra-physiological levels of LAMP2a that enhance CMA efficiency delay the onset of aging phenotypic changes(Kaushik and Cuervo, 2018).

In addition to inefficient proteostasis (Vilchez et al., 2014), contributions to cellular aging include shortening of telomeres (Wright et al., 1996), accumulation of extra-chromosomal DNA (Sinclair and Guarente, 1997) and oxygen free radicals (Harman, 1972), as well as dysregulation of the cell cycle (Afshari and Barret, 1996), signaling by insulin/mTOR, and secretion of proteins (Ivanov et al., 2013). The failure of these aging-associated pathways, in some cases, can be related back to antecedent declines in autophagy. For example, mitochondrial turnover is entirely dependent on autophagy (mitophagy). In aging cells, mitochondria become enlarged and are less efficiently sequestered by mitophagy (Terman et al., 2003) and more vulnerable to acute damage, which can then trigger mitochondrial membrane permeabilization and apoptosis or necrosis. Cell senescence, the state of irreversible cell cycle arrest, is accompanied by progressive nuclear changes including remodeling of chromatin and loss of total histones, which are, in part, lysosome-dependent processes. Autophagy of chromatin released from nuclei may drive senescence by making cell cycle re-entry less likely (Ivanov et al., 2013).

Lysosome acidification – the Achilles heel of aging cells?

The intimate relationship between lysosome activity and cellular aging is reinforced by the evidence that a dozen or so experimental manipulations known to extend lifespan in a wide range of invertebrate organisms share the property of increasing the efficiency of autophagy and, in particular, lysosomes (Chin et al., 2014; Vilchez et al., 2014) (Figure 2). While longevity pathways may involve different upstream triggers, all life-extension mechanisms converge on the autophagy pathway, including, for example, reduced insulin/IGF-1 signaling, mTOR signaling, and mitochondrial respiration, as well as dietary restriction and germline removal. The heightened autophagy activity seen in long-lived animals is required for their longevity(Nakamura and Yoshimori, 2018) while autophagy impairment or lysosomal deficits(Ivy et al., 1984) induce senescence-related changes (Kang et al., 2011; Markaki and Tavernarakis, 2013; Rubinsztein et al., 2011).

The activity of mTOR, a molecular rheostat that balances autophagy and protein synthetic activities, is particularly critical in mediating lifespan extension and is influenced by many different signaling pathways in response to environmental and disease factors (Kenyon, 2010; Zoncu et al., 2011b) . Arguably of greatest importance is the reciprocal signaling between lysosomes and mTOR that binds to the vATPase complex of lysosomes in its inhibited state. mTOR is regulated in part by lysosomal signals that release mTOR enabling it to phosphorylate transcription factors. Among the most important of these factors are TFEB and TFE3, which modulate the expression of genes encoding most lysosomal components and additional components of the autophagosome machinery (Lapierre et al., 2013; Vilchez et al., 2014). TFEB is negatively regulated by mTOR under conditions of changing nutrient supply. mTOR on the lysosome surface senses amino acid supply through several mechanisms, including inside-out signaling through the v-ATPase(Zoncu et al., 2011a) and the lysosomal arginine transporter SLC38A9(Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015). Starvation or other stress states inhibit TOR allowing TFEB to be dephosphorylated and translocated into the nucleus where it initiates the transcription of target genes encoding most lysosomal components and additional components of the autophagosome machinery(Lapierre et al., 2013; Vilchez et al., 2014) The activity of v-ATPase via its association with other regulator molecules is vital to the sensing functions of lysosomes: inhibiting-ATPase prevents mTORC1 activation by amino acids(Zoncu et al., 2011a)

Although longevity research has mainly focused on autophagy “flux” (i.e. the complete process of substrate sequestration and digestion), recent studies in yeast have identified lysosomes and specifically the lysosomal proton pump, vATPase as the critical determinants of longevity (Hughes and Gottschling, 2012; Ruckenstuhl et al., 2014; Stephan et al., 2013). When aging yeast cells lose lysosomal acidity, limited lifespan results partly from the inhibition of mitochondrial function(Carmona-Gutierrez et al., 2016). The transport of lipids and possibly nutrients between these organelles involves a protein-tethering complex (vCLAMP) that forms a physical connection between lysosomes and mitochondria(Elbaz-Alon et al., 2014; Honscher et al., 2014). Cells lacking subunits of the v-ATPase or treated with v-ATPase inhibitors have a very short lifespan and exhibit a range of mitochondrial impairments(Dimmer et al., 2002; Hughes and Gottschling, 2012; Merz and Westermann, 2009; Schleit et al., 2013). In the *C. elegans* model, overexpression of HLH-30, a homolog of TFEB, is sufficient to extend lifespan similarly to pro-longevity signaling by inhibiting insulin/IGF-1 signaling, mitochondrial respiration, TOR signaling, protein translation or germline removal, and is required for longevity(Hughes and Gottschling, 2012; Ruckenstuhl et al., 2014). Acidification of the vacuole, the metazoan equivalent of the lysosome, also seems to be critical in mediating lifespan-extending effects of caloric restriction(Hughes and Gottschling, 2012; Molin and Demir, 2014) and methionine restriction(Ruckenstuhl et al., 2014). Exercise-induced autophagy requires lysosomal Ca2+ release through the lysosomal calcium channel mucolipin 1 (TRPML1) and the subsequent activation of calcineurin, which in turn promotes nuclear translocation of TFEB(Medina et al., 2015). Interestingly, lysosomal exocytosis is modulated by Ca2+ and TFEB(Medina et al., 2011), both of which have regulatory functions during aging. The transcription of most subunits of the v-ATPase complex is also regulated by TFEB(Sardiello et al., 2009) making it likely that the positive

effects of TFEB induction in these models involves upregulated v-ATPase function and greater lysosomal acidification. Lysosomal function is also intricately connected to regulation of the PKA pathway(Bond and Forgac, 2008; Dechant et al., 2010; Hlavata et al., 2008) which influences lifespan. PKA pathway stimulation facilitates the ER to lysosome delivery of the ClC7 chloride channel (Lee et al 2020, in press) and its effector. OSTM1(Majumdar et al., 2011). The import of chloride ions promotes lysosomal acidification by reducing the electrogenic gradient caused by vATPase-mediated proton import.

Animal models in which the vATPase complex has been modulated highlight the potential impact of chronic aging related impairments of lysosomal acidification on the aging brain, revealing that v-ATPase defects are sufficient to induce neuropathological phenotypes similar to those observed in AD and PD. A loss-of-function mutation of a v-ATPase subunit in Drosophila induces a phenotype exhibiting failed protein degradation and aging-dependent neurodegeneration(Williamson and Hiesinger, 2010). Loss of the V0a1 subunit, in particular, increases the susceptibility of neurons to abeta- and tau-induced toxicity(Williamson and Hiesinger, 2010), but only in the context of aging or toxic stress. These observations are reminiscent of the delayed synergy among AD-related pathogenic proteins and the striking reduction in v-ATPase function caused by Presenilin-1 mutations (Lee et al., 2010; Nixon, 2017). Conditional deletion of the ATP6AP2 gene, which encodes for a critical v-ATPase-regulating protein, reduces v-ATPase activity(Korvatska et al., 2013) leading to autophagic vacuole accumulation, neurodegeneration and cognitive impairment in both fly and mouse models(Dubos et al., 2015). These results suggest that delayed effects of a partial loss-of-acidification function do not necessarily impede the autophagic-lysosomal system immediately but instead render the system more vulnerable to failure over time, similar to the pattern in aging-related neurodegenerative diseases, such as AD.

Oxidative stress – an independent aging factor corrupting lysosome acidification and hydrolase function

The free radical theory of aging(Hekimi et al., 2011) proposes that reactive oxygen species (ROS), when excessively produced in cells under stress conditions, are detrimental to cell components and homeostasis(Scherz-Shouval and Elazar, 2007). Many of the covalent modifications of proteins common in aging such as oxidation, glycation, phosphorylation, deamidation, carbonyl modification, and misfolding (Gafni, 1997), reduce the proteolytic susceptibility of the proteins delivered to lysosomes (Sukharev et al., 1997) . Besides effects on the proteolytic substrates, oxidative stress associated with cell aging may also adversely impact the lysosome directly. Age-dependent declines in CMA(Cuervo and Dice, 2000a) may be promoted by increased oxidative stress and attempts to eliminate oxidatively damaged proteins (Massey et al., 2006). Cellular iron deficits arise due to lysosomal dysfunction(Diab and Kane, 2013; Kurz et al., 2011) and can significantly impact the functions of mitochondria(Tai et al., 2017), which are enriched in iron requiring enzymes(Stehling and Lill, 2013). Another manifestation of this impact, seen especially in postmitotic cells, is accumulation of the age pigment(Jolly et al., 1993) lipofuscin within lysosomal-related compartments (Terman and Brunk, 2004).

Lipofuscin is an autofluorescent polymeric pigment composed of aldehyde cross linked protein fragments, oxidized lipids, carbohydrates and trace amount of metals, especially iron (Brunk and Terman, 2002; Seehafer and Pearce, 2006), which is stored at high levels in lysosomes. Lipofuscin is formed by the iron-catalyzed peroxidation of decomposed lipids principally from vesicular organelles such as autophagocytosed mitochondria and its accumulation, which is linearly correlated with age in various organisms (Strehler et al., 1959), reflects the impaired intralysosomal degradation of autophagic substrates under conditions of growing oxidative stress within aging cells. Loss of lysosomal enzyme activity, as seen experimentally using inhibitors of protease or acidification, increases the opportunity for lipid peroxidation and accelerates lipofuscin generation as well as other cardinal manifestations of brain aging (Bednarski et al., 1997) (Terman and Brunk, 1998; Terman et al., 2008). (Ivy et al., 1984). Although initially thought to have negligible influence on lysosomal function, further studies demonstrated lipofuscin-dependent decreases in activities of lysosomal cysteine proteases (Amano et al., 1995; Seehafer and Pearce, 2006). Components of lipofuscin may also inhibit v-ATPase, promoting its own accumulation and lysosomal failure(Bergmann et al., 2004). In a model of chronic oxidative stress, lysosomal acidification and autophagic flux were decreased in trabecular meshwork cells(Porter et al., 2013). Similarly, hydrogen peroxide inhibits synaptic vesicle v-ATPase activity and causes impaired uptake of glutamate into synaptic vesicles in isolated bovine brain synaptosomes (Wang and Floor, 1998). The v-ATPase itself is a target of oxidative stress in aging(Barone, 2016; Butterfield et al., 2014b), including carbonylation of the V1B2 subunit in aged rat brain tissue (Di Domenico et al., 2010), increased nitration of the V1E1 subunit in early AD (Butterfield and Sultana, 2007), and increased oxidative modification of the v-ATPase in AD and DS brain(Butterfield et al., 2014a). Nitrative stress in neuronal cells has been shown to reduce the activity of the lysosomal v-ATPase (Colacurcio, unpublished data). Oxidative/ nitrative modifications impair the F-type mitochondrial ATPase, a homologue of the v-ATPase(Fujisawa et al., 2009; Haynes et al., 2010).

Lysosomal hydrolase changes in aging and the influence of pH regulation

Scattered reports of aging-related changes in the activities of cathepsins are generally consistent with an overall decline in lysosomal hydrolysis in aging in various tissues(Stoka et al., 2016). This conclusion accords with the accumulation of lipofuscin, most evident in neurons, and the enlargement of the lysosomal compartment (Kurz et al., 2000) which is also possibly indicative of slowed substrate clearance. Published data on individual enzyme activities in lysosomes mainly reflect measurements in tissues rather than lysosomes and are difficult to interpret given the brain's cellular heterogeneity, changes in neural cell composition in brain during aging and disease, and varied properties of lysosomes even those within a single cell that are due to dynamic changes in intralumenal pH during the lysosome's "life cycle". Compounding these measurement challenges are the limited methods available to assess lysosomal activity *in vivo.* Hydrolase activities measured *in vitro* are a poor reflection of the total activities within lysosomes in living cells. Age-related changes of lysosomal pH, levels of endogenous lysosomal cysteine protease inhibitors (e.g., cystatins B, C, etc) (Kay et al., 2014), membrane stability (Nakamura et al., 1989), or changes in myriad other possible conditions within the intralumenal environment of the lysosome, may well account for the overall decreased lysosomal activity suspected to

develop in aging cells; however, the inter-relationships among these factors and individual hydrolases that establish net *in situ* activity are difficult to discern when tissues or cells are lysed for protease assays.

The foregoing caveats notwithstanding, the *in vitro* activities of a few lysosomal hydrolases (eg.cathepsin D and certain lysosomal cysteine proteases) have been reported to be higher in aging rat liver (Keppler et al., 2000) and brain (Nakanishi et al., 1994b; Porta et al., 1995) and *in vitro* β-galactosidase activity may be elevated in aging human fibroblasts. In brain, certain cell types may possibly account for observed higher activities is whole brain tissue. For example, microglia, comprising up to 20% of the brain's glial cell population and representing a prominent source of cathepsins, are activated during aging (von Bernhardi et al., 2015). Upregulated expression of cathepsins during brain aging, including cathepsins B, X, and S, has been suggested as a possible mechanism underlying the priming of microglia (Nakanishi, 2003; Nakanishi and Wu, 2009);(Wendt et al., 2007). By contrast, the activity, but not the protein levels, of cathepsin L was reported to be strikingly decreased in all brain regions of aged rats(Nakanishi et al., 1994a) suggesting generation of a catalytically inactive form of cathepsin L due to a higher lysosomal pH(Stoka et al., 2016). Inactivation of cathepsin L in this study was followed by induction of cathepsin D activity and aberrant tau proteolysis (Bednarski and Lynch, 1996). Age-related translocation of neuronal cathepsin D from lysosomes to cytosolic granules seen in about a third of rat cerebral cortex neurons was considered important in leading to age- related in tau proteolysis and cell death (Jung et al., 1999).

The regulation of pH within the lysosomal system plays an outsized role in influencing lysosomal acid hydrolase functions. Newly synthesized lysosomal cathepsins reaching endolysosomal compartments are activated by cleavage of their inhibitory propeptide domain under mild acidic conditions by autocatalysis or by another protease (Fox et al., 1992; Guay et al., 2000). Most cathepsins are inactivated and relatively unstable structurally in the neutral pH of the cytosol and extracellular milieu(Almeida et al., 2001; Turk et al., 1995; Turk et al., 1993; Turk et al., 1994); however, cathepsin S is stable even at neutral pH (Kirschke et al., 1989) and some cathepsins briefly remain somewhat active, which could account for their extra-lysosomal activity in metastasis and other disease states (Jordans et al., 2009; Turk and Turk, 2009). The pH optima of different cathepsins range from 3.5 to 6,0 and prolonged exposure to pH ranges outside these ranges can denature the enzyme and promote its degradation by a second cathepsin (Turk et al., 1999). What seems clear, therefore, is that aging-related changes in pH are likely to dramatically change the balance of proteolytic activities within aging lysosomes and have disruptive effects on the proteolysis of certain substrates.

Aging effects on lysosomal lipids and membrane stability

Lysosomal membrane permeabilization (LMP) is a possible outcome of cumulative insults of lysosomal aging that may herald the degeneration or death of a compromised neuron or other long-lived cell(Gomez-Sintes et al., 2016). LMP is defined as the selective destabilization of the lysosomal membrane allowing translocation of lysosomal contents to the cytoplasm. Some of the lysosomal constituents released, most notably cathepsins, act as

the main initiators and executors of lysosome-dependent cell death. LMP is a well-reviewed topic (Boya and Kroemer, 2008; Groth-Pedersen and Jaattela, 2013; Johansson et al., 2010; Repnik et al., 2014; Stoka et al., 2007) and the detailed mechanisms of LMP interface with mechanisms of cell death beyond the scope of this review. The major relevant concept here is that aging of cells provides the key pre-conditions for LMP through routes described earlier, such as damage from free radicals, membrane incorporation of damaged proteins and oxidized lipids, and ionic shifts that alter osmotic equilibrium, which contribute to the increased volume of the lysosomal compartment seen in aging and senescence(Kurz et al., 2000) and is associated with enhanced fragility and LMP (Ono et al., 2003). The demonstration of lipofuscin in cytoplasm suggests the possibility of LMP(Reeg and Grune, 2015). Increasing lysosomal pH using lysosomotropic agents (e.g., certain antibiotics) induces LMP and cell death in several cell types(Boya et al., 2003a; Boya et al., 2003b)

Beyond the foregoing factors, lipids are key to protecting against membrane permeabilization and changes in lipid composition of membranes during aging likely reduce this protection. Liver lysosomes from 22 month old mice, compared to 3 month mice, contain increased levels of cholesterol, ceramide, glucosylceramide, and, notably, lysophosphatidylcholine that shows a comparatively greater increase than the other lipids(Rodriguez-Navarro et al., 2012). The latter change may be relevant to a reported ability of LysoPC to increase lysosomal permeability to potassium ions and protons, an effect that enhances the osmotic sensitivity of these organelles(Hu et al., 2007) and could destabilize older lysosomes and disrupt pH control. Higher levels of ceramide in lysosomes are also associated with membrane destabilization(Blom et al., 2015).The chaperone Hsp70 contributes to lysosomal stabilization by binding to the endolysosomal anionic phospholipid BMP (Kirkegaard et al., 2010), a co-factor that enhances the activity of acid sphingomyelinase, which mediates sphingomyelin catabolism. Consistent with this observation, acid sphingomyelinase deficiency leads to LMP and cytosolic release of cathepsins(Gabande-Rodriguez et al., 2014).

Mucolipin 1 (TRPML1), a major lysosomal calcium channel, is inhibited by high sphingomyelin levels, but is potentiated by sphingomyelinases(Shen et al., 2012). Moreover, abnormally elevated lysosomal pH, which is a prominent feature in Alzheimer's Disease and other vATPase deficient conditions(Colacurcio and Nixon, 2016) and is suspected to develop during aging, induces release of calcium into the cytosol via TRPML1 channels which, in turn, activates calpains. Calpain cleavage products increase during aging, reflecting the greater activation of calpains, due in part to aging-related declines in its endogenous inhibitor, calpastatin(Nixon et al., 1994). In many circumstances, calpain activation lies upstream of cathepsin release during lysosomal associated cell death. In light induced retinal degeneration photoreceptor cell death, calpain inhibition rescued lysosomal permeabilization and calpain-mediated cleavage of the lysosomal membrane protein LAMP 2A, which was essential for this permeabilization(Villalpando Rodriguez and Torriglia, 2013). Finally, in addition to the role of Hsp70.1 in regulating BMP as described above, oxidative stress-induced carbonylation of Hsp70.1 together with calpain-mediated cleavage, leads to lysosomal destabilization and rupture resulting in neuronal death due to release of cathepsins(Yamashima, 2016).

Conclusion:

Lysosomes in the aging brain – gateway to late-onset neurodegeneration

A remarkable convergence of genetic and molecular- biological data underscores the central importance of the lysosome and its interacting compartments in the pathogenesis of late age-onset neurodegenerative diseases. Given the unique properties of neurons, the heavy reliance on efficient lysosomal clearance mechanisms is not surprising nor is the vulnerability to lysosomal impairments of a postmitotic neuron expected to survive over the lifetime of the organism. Lysosomal vulnerability is amplified by aging, which further impedes efficient substrate delivery and clearance during late life when increasing damage to proteins and membranes requires even greater capacity for protein quality control. In increasing numbers of late age onset neurodegenerative diseases (eg. AD, PD, FTD), milder degrees of primary lysosomal dysfunction related to the disease may remain subclinical until it is compounded by effects of cellular aging. Understanding the critical role evidently played by the lysosomal system in cellular aging and organismal lifespan is, therefore, an important frontier of biology holding many clues to neurodegenerative disease development and therapeutic strategies for new therapies.

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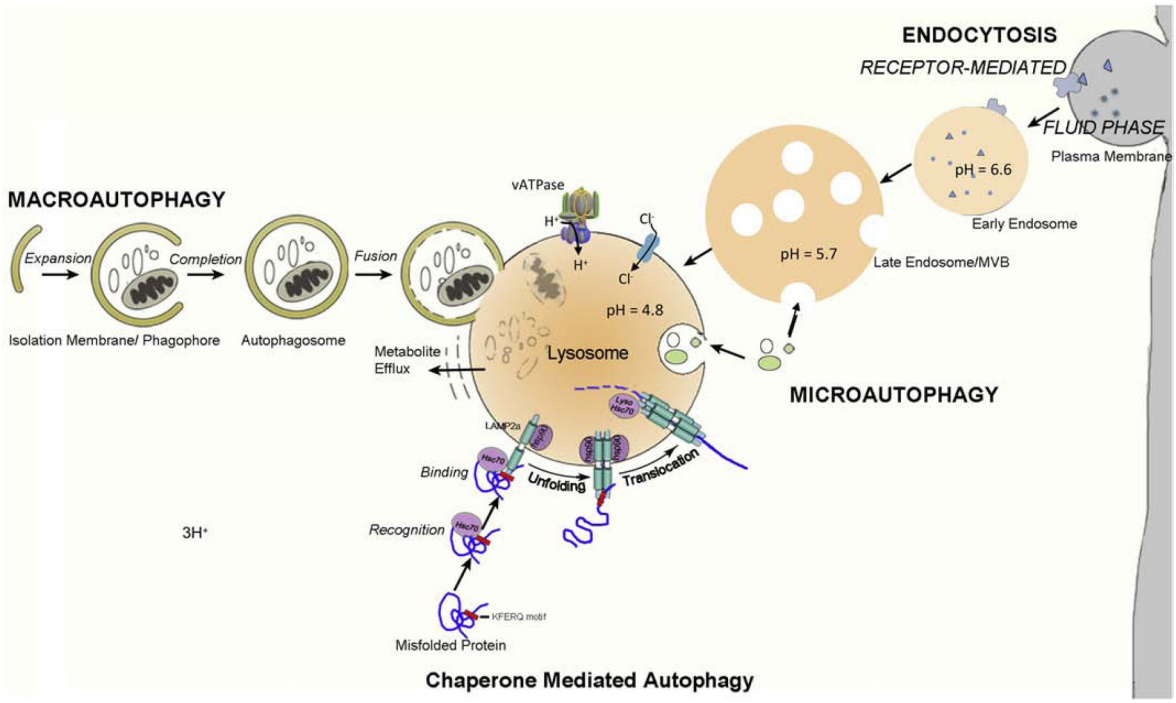

Figure 1. Major routes of substrate delivery to lysosomes .

(a) Macroautophagy is characterized by the sequestration of organelles or cytoplasmic constituents targeted for degradation into double-membrane vesicles called autophagosomes. Fully formed autophagosomes fuse with hydrolase-filled lysosomes releasing hydrolases into the lumen of the created autolysosome. Introduction of a proton pump (v-ATPase) induces full acidification of the autolysosomal lumen necessary to activate acid hydrolases. The metabolites resulting from digestion are transported into the cytoplasm and used for synthesis of new macromolecules or for energy. (b) During chaperone-mediated autophagy, proteins carrying the pentapeptide KFERQ-like sequence are recognized by the Hsc70 chaper-one, which then associates with the integral lysosome membrane protein LAMP-2A, triggering its oligomerization. This event leads to the translocation of the bound protein into the lysosome interior through a process that requires Hsc70. (c) Microautophagy involves “bulk” or chaperone-mediated internalization and degradation of cytoplasmic substrates within late endo-some/MVB or lysosomal compartments by a process of membrane invagination followed by membrane scission to release the cargo into the lysosomal lumen for degradation. (d) Heterophagy involves the lysosomal degradation of plasma membrane components and exogenous substrates after they are internalized by receptor-mediated or bulk endocytosis. Selected proteins are sorted to different cellular destinations or recycled to the plasma membrane. Proteins targeted for degradation are trafficked to late endosomes/ MVB, which mature to lysosomes to effect complete degradation.

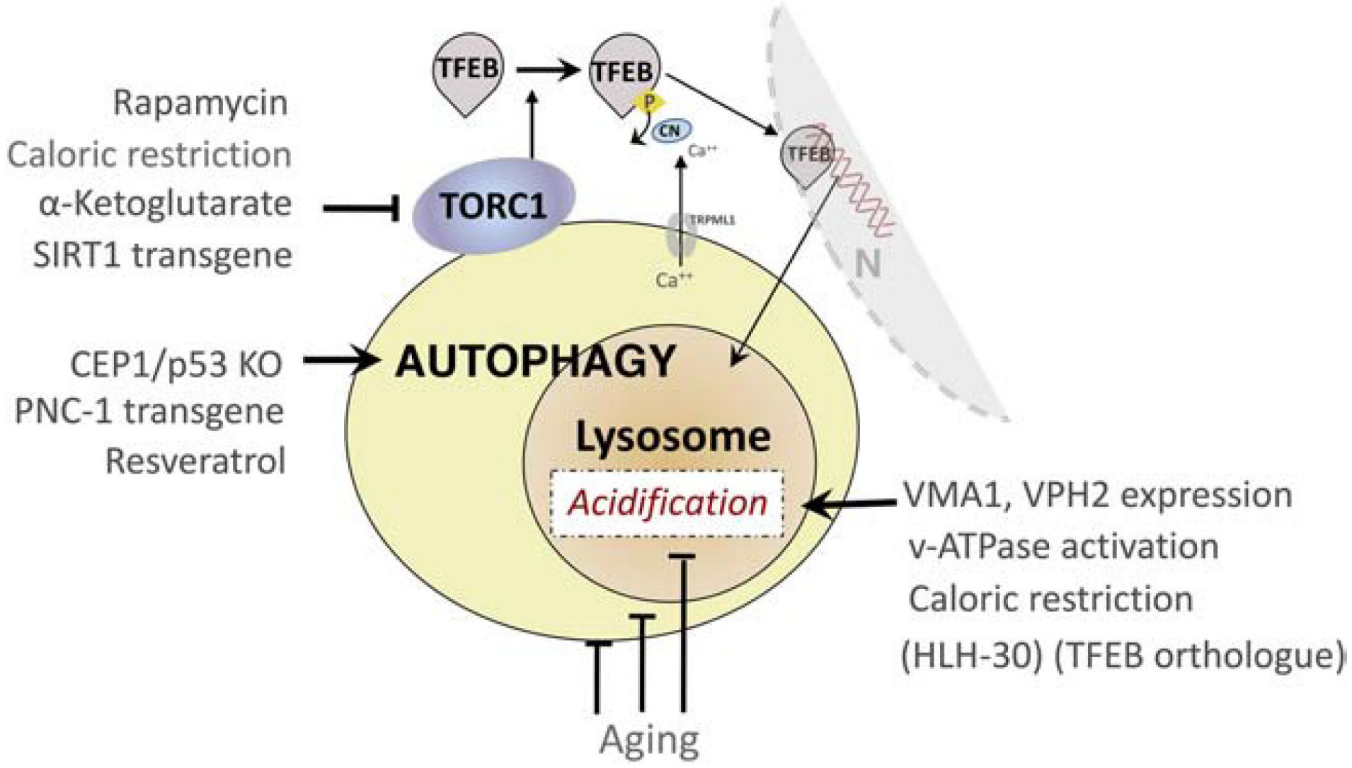

Figure 2. Disparate strategies that enhance autophagy or preserve full acidification of lysosomes are able to extend lifespan in lower species.

Extensive studies in yeast, C. elegans, flies have identified distinct metabolic pathways modulated by drugs or genetic manipulations that extend lifespan and also share the property of facilitating substrate clearance through autophagy. Many of these pathways are linked to those independently implicated in the evolution of cellular aging, as discussed in this review. Notably, recent evidence from investigations on the vATPase complex establish the special importance of maintaining optimal lysosomal acidification as a key factor mediating the extension of lifespan through autophagy modulation.

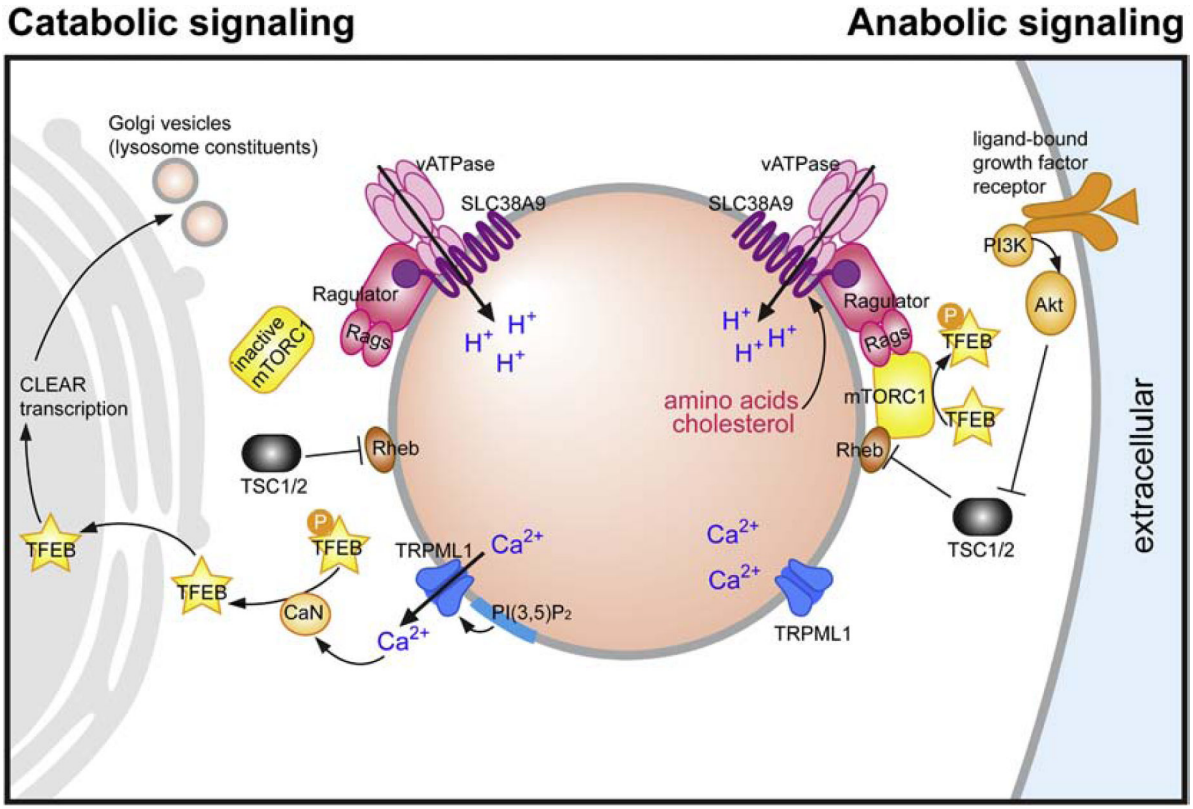

Figure 3. Components and activities of the lysosome

Lysosomes are composed of over two hundred identified proteins and likely hundreds associated with lysosomes but also localized to additional organelles. The diagram here illustrates only a subset relevant to the discussion in this review. In the presence of abundant cellular nutrients, mTORC1 is recruited to the lysosomal surface by the vATPase-SLC38A9-Ragulator-Rag GTPase complex, which senses amino acids and cholesterol levels within lysosomes. These levels are responsive to rates of substrate hydrolysis, which in turn is influenced by intraluminal pH. Both TRPML1-mediated Ca2+ release and the ATP-sensitive TPC-mediated Na+-release are inhibited under nutrient-replete conditions. Conversely, catabolic signaling is favored by nutrient depletion or certain forms of cell stress. Under these conditions, mTORC1 is released from the vATPase SLC38A9-Ragulator-Rag GTPase complex and the inactivated mTORC1 is no longer able to phosphorylate TFEB. PI (3,5)P2-mediated activation of TRPML1 channel triggers lysosomal Ca2+ efflux, activating CaN, which in turn dephosphorylates TFEB and stimulates its nuclear translocation. Nuclear TFEB activates CLEAR gene transcription for lysosome biogenesis. Abnormally elevated lysosomal pH similarly effects Ca2+ release and, when persistent, can over-activate calpains leading to cytotoxicity. Perinuclear redistribution of lysosomes, regulated in part by pH, facilitates the delivery of nascent lysosomal components from the Golgi, thereby promoting lysosomal degradative function. Under low level of ATP, TPC-mediated Na+ release affects the lysosomal membrane potential in a manner that helps maintaining vATPase proton pumping activity and acidification during starvation. (Figure adapted from Lie et al, 2019 with permission)