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Disorders of lysosomal acidification - the emerging role of v-ATPase in aging and neurodegenerative disease

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Abstract

Autophagy and endocytosis deliver unneeded cellular materials to lysosomes for degradation. Beyond processing cellular waste, lysosomes release metabolites and ions that serve signaling and nutrient sensing roles, linking the functions of the lysosome to various pathways for intracellular metabolism and nutrient homeostasis. Each of these lysosomal behaviors is influenced by the intraluminal pH of the lysosome, which is maintained in the low acidic range by a proton pump, the vacuolar ATPase (v-ATPase). New reports implicate altered v-ATPase activity and lysosomal pH dysregulation in cellular aging, longevity, and adult-onset neurodegenerative diseases, including forms of Parkinson Disease and Alzheimer Disease. Genetic defects of subunits composing the v-ATPase or v-ATPase-related proteins occur in an increasingly recognized group of familial neurodegenerative diseases. Here, we review the expanding roles of the v-ATPase complex as a platform regulating lysosomal proteolysis and cellular homeostasis. We discuss the unique vulnerability of neurons to persistent low level lysosomal dysfunction and review recent clinical and experimental studies that link dysfunction of the v-ATPase complex to neurodegenerative diseases across the age spectrum.

Keywords

Lysosome; acidification; pH; v-ATPase; endocytosis; autophagy; lysosomal storage disease; Alzheimer's Disease; Parkinson's Disease; mTORC; calcium; TFEB; cathepsin; caloric restriction

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INTRODUCTION

Defective lysosomal function is a primary factor in over 40 neurodevelopmental and neurodegenerative diseases of childhood and has been recently recognized to be a major factor in the pathogenesis of adult and late-age onset disorders (Menzies et al., 2015; Rubinsztein et al., 2005), including Alzheimer Disease (AD) (Nixon and Yang, 2011; Zare-Shahabadi et al., 2015), Parkinson Disease (PD) (Dehay et al., 2013), and frontotemporal lobar degeneration (FTLD) (Gotzl et al., 2014). Mutations in a single lysosomal gene can cause either a congenital disorder or a late-onset neurodegenerative disease, depending on the type of mutation and the subsequent severity of lysosomal disruption. Evidence from these genetic diseases reinforces the notion that additional pathogenic factors arising in adulthood and aging (Cuervo and Dice, 2000b; Rubinsztein et al., 2011; Yogendra et al., 2009), may tip a subclinical lysosomal impairment towards a more severe lysosomal dysfunction, and ultimately, progressive neurodegenerative disease. In genetic disorders that disrupt lysosome function in cells throughout the body, the brain is commonly the organ most prominently affected, which highlights the special vulnerability of the central nervous system (CNS) to autophagic-lysosomal compromise (Boland and Platt, 2015; Hara et al., 2006; Komatsu et al., 2006). Some possible reasons for this vulnerability will be discussed in this review.

Historically, research on lysosomal diseases has been mostly devoted to characterizing gene mutations that are causative for the family of congenital diseases known as lysosomal storage disorders (LSDs). In these devastating disorders, marked or complete loss of function of a single encoded mutant enzyme leads to defective processing of a substrate critical for neuron survival and often to the accumulation of digestion products that may exert further cytotoxicity. Recently, however, increasing attention is being paid to the disease relevance of lysosomal acidification, a process influencing activities of most of the lysosome's hydrolases as well as various additional signaling functions being ascribed to lysosomes, most notably nutrient sensing and regulation of nutrient homeostasis. The regulation of lysosomal acidification involves the proper coordinated function of multiple ion channels and most notably, the vacuolar ATPase, the macromolecular complex responsible for pumping protons (H^+) into lysosomes and lowering intraluminal pH to the acidic range needed to activate the dozens of hydrolases with acidic pH optima in lysosomes (Mindell, 2012). An abnormal rise in lysosomal pH, therefore, can have far-ranging effects on lysosomal digestion – strongly inhibiting hydrolases with the most acidic pH optima, but also potentially elevating activities of other hyrolases with pH optima closer to neutral. This shift would promote both substrate indigestion and atypical cleavages, possibly generating toxic digestion products and/or partially digested intermediates.

As we will discuss further in this review, altered substrate clearance is one of several key functions of lysosomes potentially affected by an acidification defect. Recognition of the broader range of regulatory influences of acidification on cell function has increased appreciation of how pH dysregulation may manifest in human disease and especially in ones involving neurodegeneration. Here, we review emerging literature linking genetic defects in v-ATPase components and lysosomal acidification to disorders varying in phenotype and age of onset, but almost invariably involving progressive neurodegenerative disease, which may

be the dominant feature of v-ATPase loss of function. The rapidly growing number of identified gene mutations underscores the pathogenic importance of lysosomal compromise in late age onset neurodegenerative diseases, such as AD and PD.

LYSOSOMAL ACIDIFICATION IN HEALTHY NEURONS

1.1: The lysosomal system

The major pathways of substrate delivery to lysosomes include multiple forms of autophagy (e.g. macroautophagy, chaperone-mediated autophagy (CMA), and microautophagy) (He and Klionsky, 2009) (Cuervo and Wong, 2014), and the endocytic pathway, which collectively comprise a dynamically interactive vesicular network referred to here as the lysosomal system (Figure 1). The properties of the different cellular delivery routes to lysosomes, their regulation, and their cross-talk have been well detailed in recent reviews (Behrends et al., 2010; Frake et al., 2015; Harris and Rubinsztein, 2012; Klionsky et al., 2010; Levine and Kroemer, 2008; Maxfield, 2014; Nixon, 2013a; Ravikumar et al., 2010). Macroautophagy is the cell's only option for degrading intracellular membranous organelles and large protein aggregates (Rubinsztein, 2006) while endocytosis is principally responsible for the turnover of internalized extracellular materials that are not sorted to other cellular destinations (He and Klionsky, 2009). Neurons constitutively degrade damaged and obsolete constituents (Boland et al., 2008) and, when healthy, are exceptionally efficient in clearing the diverse autophagic and endocytic substrates delivered by retrograde transport to the perikaryon. Because the final stages of digestion are mainly carried out in the perikaryon where lysosomes are concentrated, neurons are challenged, due to their extreme polar shapes, in delivering packaged waste materials from the periphery long distances to cell bodies. Given the uniquely large volumes of axonal and dendritic cytoplasm relative to the perikaryal volume of many neurons, lysosomal digestion of substrates is critically important and may be easily overwhelmed, as evidenced by the rapid build-up of waste within neurons when lysosomal proteolysis is impaired (Felbor et al., 2002; Ivy et al., 1989; Nixon, 2013b).

1.2: Lysosomal acidification and cellular regulation

The maintenance of a highly acidic pH (4.2-5.3) is essential for regulating many functions of lysosomes. With rare exceptions, lysosomal hydrolases of all classes operate optimally below neutral pH, although the pH optimum of individual acidic hydrolases varies considerably. The broadly specific protease cathepsin D, for example, is optimally active at the lowest end of the lysosomal pH range, while some other major cathepsins (such as cathepsin B) operate optimally in the range of pH 6.0. The wide range of pH optima implies that the rises in intraluminal pH that accompany introduction of substrates upon fusion with autophagosomes or endosomes and the gradual reacidification of the lysosomal lumen may coordinate the sequence of hydrolase activations that is most efficient for dismantling and digesting complex substrates, such as a mitochondrion, or for minimizing the generation of amyloidogenic or other potentially deleterious digestion products. Acidification also controls the maturation of lysosomal hydrolases, including the dissociation of newly-synthesized enzymes from mannose-6-phosphate receptors once they are delivered to late endosomes from the *trans*-Golgi network, and the processing of certain hydrolases via cleavage of immature pro-forms of the hydrolase within acidified lysosomes (Richo and Conner, 1994).

The acidic microenvironment of the lysosome is also critical for modifying certain cargoes delivered to lysosomes for further sorting and biological use, such as dissociation of cholesterol and other lipids from carrier proteins (Singh et al., 2009), dissociation of metal ions from degraded proteins (Asano et al., 2011),and receptor-ligand dissociation (Yamashiro and Maxfield, 1984). Recently, lysosomes have become recognized not only for their capabilities for digesting all manner of substrates and for recycling the subcomponents (Nixon and Yang, 2011), but also for serving as biosensors of nutritional status and general cellular “stress” (Wang et al., 2015). Release of amino acids from lysosomes, which reflects both substrate abundance and successful lysosomal digestion (and acidification), controls an amino acid-sensing apparatus on lysosomes that modulates autophagy induction (Zoncu et al., 2011). Additional pH-dependent signaling functions have been ascribed to lysosomes, which are mediated through a release of calcium from the highly abundant stores in lysosomes (Christensen et al., 2002), which may influence fusion of lysosomes with other membranous organelles (Nakamura et al., 1997) or with the cell surface (Kukic et al., 2014). The motor proteins modulating retrograde axonal transport of LAMP-positive compartments (Lee et al., 2011) are also influenced by pH-dependent calcium efflux (Lie and Nixon, unpublished data). Altered lysosomal pH in disease-related processes may have considerable effect on these lysosome functions (Lie and Nixon, unpublished data) while promoting lipid oxidation, ROS generation (Yokomakura et al., 2012), and impaired digestion of substrates (Lee et al., 2010a; Mangieri et al., 2014), which further compromise lysosomal function (Bergmann et al., 2004) and weaken integrity of lysosomal membranes, leading ultimately to cell death (Guicciardi et al., 2004).

1.3 The vacuolar (v-type) ATPase

The acidic pH of the lysosome is generated mainly by the vacuolar-type ATPase (v-ATPase), a multimeric protein complex which acts as an ATP-dependent proton pump, which is present and active in virtually all eukaryotic cells (Forgac, 2007; Saroussi and Nelson, 2009a). The v-ATPase is homologous to the F-type mitochondrial ATP synthase and these two enzyme complexes are thought to share genetic ancestors (Nelson, 1992). The v-ATPase uses energy from ATP hydrolysis to actively transport H^+ ions into the lysosome, thereby making the lumen more acidic. At least 13 different protein subunits form the complete v-ATPase complex, comprised of two “sub-complexes”; V0 (membrane-bound) and V1 (cytosolic). The V1 subcomplex includes 8 subunits, A-H, with three copies each of the catalytic A and B subunits, three copies of the “stator” (stabilizing) subunits E and G, and one copy each of the regulatory C and H subunits. The V1A and V1B subunits form a heterohexamer which mediates the ATP binding and hydrolysis that powers the v-ATPase. The V1 subcomplex also contains the subunits D and F, which form a central rotor axle. Rotation of this central rotor axle caused by the hydrolysis of ATP within the catalytic V1A/V1B subunits results in the movement of the barrel of six V0c subunits past the V0a subunit, which drives proton transport across the membrane. A stoichiometry of 2-4 protons translocated for each ATP hydrolyzed was validated in experimental models. Notably, this rate varies based on the pH of the compartment (Kettner et al., 2003). For more extensive information on the molecular biology of the v-ATPase, we refer readers to several comprehensive reviews (Cotter et al., 2015; Forgac, 2007; Marshansky et al., 2014; Masashi et al., 2010; Mindell, 2012; Muench et al., 2011; Rawson et al., 2015; Saroussi and Nelson,

2009a, b). Implicit in this complexity of the v-ATPase is the sizeable number of opportunities for v-ATPase function to be compromised in disease states.

The pH of the lysosome is also maintained through movement of anions and cations mediated by ion transporters in the lysosomal membrane. The v-ATPase is an electrogenic pump and so the electrogenic gradient generated by the v-ATPase must be dissipated by efflux of cations and/or import of anions to allow sustained proton import (Mindell, 2012; Steinberg et al., 2010). This is accomplished by counterion channels on the lysosome, such as ClC-7, a $2Cl^{-}/1H^{+}$ antiporter (Majumdar et al., 2011) and potentially TRPML1, a cation channel which mediates lysosomal calcium release and is linked to mucolipidosis type IV (Zhang et al., 2009). Although v-ATPase is most recognized as a component of lysosomes, varying forms of the complex differing in subunit composition are present in the membranes of many organelles, including endosomes, secretory vesicles and the plasma membrane. In neurons compared to glial cells, v-ATPase subunits are expressed at disproportionately higher levels relative to the lysosomal hydrolases (Nixon, unpublished data), possibly reflecting a uniquely high acidification need, higher turnover of v-ATPase components, or additional neuron-specific roles served by the complex. Acidification of synaptic vesicles involving v-ATPase (Wienisch and Klingauf, 2006), for example, is necessary for neurotransmitter packaging (Morel, 2003; Vavassori and Mayer, 2014).

1. 4: v-ATPase regulation and roles in nutrient sensing and calcium signaling

The v-ATPase regulates, and is itself regulated by, various signaling cascades controlling nutrient supply and metabolism. These inter-relationships reflect complexity in v-ATPase regulation and underscore the importance played by lysosomal acidification in cellular homeostasis beyond simply eliminating cellular waste, including nutrient sensing, intracellular calcium homeostasis, and lysosomal exocytosis. The activity of the v-ATPase is regulated by a process known as dissociation, whereby the cytosolic V1 subcomplex separates from the membrane-bound V0 subcomplex (Figure. 2), preventing the proton-pumping functions of the v-ATPase (Forgac, 2007). v-ATPase dissociation is regulated by a number of physiologic factors and signaling proteins, but these mechanisms are not fully understood. Many studies on v-ATPase dissociation are performed in non-mammalian systems (Breton and Brown, 2013), and confirmed regulatory factors differ between mammalian and yeast models (Cotter et al., 2015). Nonetheless, several factors are known to regulate v-ATPase dissociation in mammalian cells, including glucose (Kane, 1995; Nakamura, 2004), which promotes PI3K-dependent v-ATPase assembly (Sautin et al., 2005). Conversely, amino acid starvation promotes v-ATPase assembly and addition of amino acids causes dissociation (Stransky and Forgac, 2015). Rabconnectin3α (Einhorn et al., 2012; Sethi et al., 2010) , ERK (Marjuki et al., 2011), and EGF (Xu et al., 2012) all stimulate assembly of the v-ATPase complex. Increased assembly of the v-ATPase also occurs during maturation of dendritic cells (Trombetta et al., 2003), where it is mediated by mTORC1 and PI3K (Liberman et al., 2014). Notably, factors such as organelle localization of the v-ATPase (Johnson et al., 2016; Qi and Forgac, 2007), membrane composition (Crider and Xie, 2003; Finnigan et al., 2011; Ryu et al., 2010), PKA (Tiburcy et al., 2013), and serotonin (Zimmermann et al., 2003) all modulate v-ATPase assembly in non-mammalian systems. Future studies on novel factors (including, but not limited to, factors identified in

non-mammalian systems) which regulate v-ATPase assembly in neurons could allow for investigation of excessive v-ATPase dissociation as a potential mechanism underlying lysosomal dysfunction.

This association-dissociation mechanism of regulation allows the v-ATPase to respond to nutrient availability, as dissociation is in turn regulated by glucose (Sautin et al., 2005) as well as by amino acids (Stransky and Forgac, 2015). High levels of amino acids promote v-ATPase dissociation which suppresses the activity of the v-ATPase, which likely conserves energy by down-regulating ATP hydrolysis as fully acidified lysosomes become relatively dormant. When amino acids are scarce, v-ATPase is expected to be more active to support an influx of substrates from up-regulated autophagy requiring increased capacity for rapid re-acidification following autophagosome-lysosome fusion, which will accelerate digestion rate and protein recycling by the lysosome. This process occurs via amino acid-regulated associations between the v-ATPase, Rag-GTPases, and Ragulator, ultimately allowing the v-ATPase to promote lysosomal function while functioning as a signaling molecule for nutrient sensing (Efeyan et al., 2012; Sancak et al., 2010; Sancak et al., 2008). One of the key elements in the cycle of reciprocal regulation of lysosomal activity and nutrient homeostasis is an amino acid-sensing protein complex on lysosomes composed of v-ATPase, Rag-GTPases, and Ragulator, which allows the v-ATPase to function as a signaling molecule for nutrient sensing. Amino acids, in large part stemming from lysosomal proteolysis, modulate this sensing mechanism, which in turn controls the association of mTORC1 with lysosomes. In high nutrient conditions, mTORC1 is stably associated with the Ragulator complex and autophagy induction and lysosomal biogenesis are down-regulated, while the reverse is true under nutrient poor conditions (Zoncu et al., 2011).

v-ATPase-mediated lysosomal acidification is also linked to the control of Ca2+ efflux from lysosomes (Christensen et al., 2002), at least when low v-ATPase activity elevates lysosomal pH. Reducing the acidity of lysosomes by inhibiting v-ATPase activity activates the lysosomal TRPML1 channel causing a calcium efflux from lysosomes and a rise in the cytosolic level of calcium (Lee et al., 2015). Loss of function of Presenilin-1, the most common cause of early-onset AD, induces an identical sequence of events (Lee et al., 2015). Lysosomal calcium efflux in these conditions is associated with hyper-activation of calpains and CDK5, both of which are implicated in the neurodegenerative cascade in AD (McBrayer and Nixon, 2013).

1.5: v-ATPase, neurodegeneration, and aging

The importance of v-ATPase and lysosomal acidification in mechanisms of cellular aging, the *sine qua non* for late-onset neurodegenerative diseases, is exemplified by studies showing that increased autophagy flux is a mechanism shared by experimental manipulations that extend life-span in yeast, drosophila, and mouse models. Recently, v-ATPase-mediated acidification of the vacuole (the metazoan lysosome equivalent) has been identified as a positive regulator of long-term mitochondrial stability and lifespan in yeast (Hughes and Gottschling, 2012). Increased vacuolar pH early in life contributed to age-related mitochondrial dysfunction and a shortened lifespan in this study and vacuolar acidity further declined with aging. Vacuolar acidification seems also 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). Additionally, overexpression of v-ATPase components promotes increased lifespan in yeast models (Hughes and Gottschling, 2012; Ruckenstuhl et al., 2014).

The role of the v-ATPase in nutrient sensing is an intriguing area of study with regard to the importance of lysosomal acidification in mammalian models of caloric restriction (Schleit et al., 2013), considering the aforementioned interactions between v-ATPase and mTORC1 signaling and nutrient sensing. mTORC1 regulates lysosomal biogenesis via signaling interactions with the transcription factor TFEB (Pena-Llopis and Brugarolas, 2011; Pena-Llopis et al., 2011; Sardiello et al., 2009). TFEB, a transcription factor which regulates expression of many lysosomal genes (Settembre et al., 2011), has been shown to promote lysosomal clearance of waste in animal models of AD (Polito et al., 2014; Xiao et al., 2015) and PD (Decressac et al., 2013). Expression of v-ATPase subunits is also regulated by TFEB (Sardiello et al., 2009), and so it is reasonable to expect that upregulated v-ATPase function promoting lysosomal acidification contributes to the positive effects of TFEB induction in these models, although this remains to be tested.

Given the vital functions served by acidification, it is not surprising that a loss-of-function mutation of a v-ATPase subunit in *Drosophila* induces a phenotype exhibiting failed protein degradation and aging-dependent neurodegeneration (Williamson et al., 2010). Loss of the V0a1 subunit, in particular, increases the susceptibility of neurons to Aβ- and tau-induced toxicity (Williamson and Hiesinger, 2010), but only in the context of aging or toxic stress, reminiscent of the delayed synergy among AD-related pathogenic proteins and the striking reduction in v-ATPase function caused by Presenilin-1 mutations. Similar effects of v-ATPase impairment follow the conditional deletion of the *ATP6AP2* gene (which encodes for a critical v-ATPase-regulating protein). Loss of *ATP6AP2*, which reduces v-ATPase activity (Korvatska et al., 2013), led to neurodegeneration and cognitive impairment in both fly and mouse models, along with the appearance of autophagic vacuoles, suggesting a failure of lysosomal proteolysis (Dubos et al., 2015). These animal models highlight the importance of the v-ATPase and lysosomal acidification in the aging brain, as v-ATPase defects are sufficient to induce neuropathological phenotypes similar to those observed in AD and PD. These results also suggest that delayed effects of a partial loss-of-acidification function do not necessarily halt the autophagic-lysosomal system immediately but instead render the system more vulnerable to failure over time, which is analogous to the long-term gradual onset of aging-related neurodegenerative diseases such as AD.

v-ATPase –RELATED LYSOSOMAL ACIDIFICATION FAILURE IN DISEASE

More than 50 genetic diseases have been traced to mutations in genes encoding for lysosomal proteins (Ballabio and Gieselmann, 2009; Coutinho and Alves, 2015; Futerman and van Meer, 2004). Lysosomal storage diseases (LSDs), which are present in 1:4000 to 1:9000 live births (Meikle et al., 1999), are marked by the lysosomal accumulation of undigested cellular waste products, which contributes to disease development. As genetic diseases that most often affect infants and children, LSDs are the leading cause of pediatric neurodegeneration (Coutinho and Alves, 2015). The pathological presentation and clinical

phenotype of these diseases varies depending on the gene defect and the nature of the substrate deposited within cells. Many of the genes mutated in LSDs encode for acidic hydrolases, explaining the buildup of specific macromolecular products in the lysosomal lumen, while other LSDs involve mutation in structural proteins (e.g. Danon disease (D'Souza R et al., 2014)) or ion channels (e.g. Mucolipidosis type IV (Wakabayashi et al., 2011)) of the lysosome, causing impairments of CMA, reduced vesicle maturation, and impaired acidification, which collectively may contribute to secondary failure of lysosomal hydrolysis. In less common cases, lysosomal dysfunction arises through mutations in genes encoding proteins that reside in another organelle but impact the function of a lysosomal constituent as their primary disease effect. Examples include osteopetrosis (Bhargava et al., 2012), Wolfram Syndrome (Gharanei et al., 2013), and PS1-FAD (Lee et al., 2010a; Lee et al., 2015), all of which are disorders where the mutated protein residing in the ER impairs the stability, delivery, or function of a lysosomal constituent. While most LSDs involve dysfunction of proteins that are ubiquitous in lysosomes throughout the body, it is remarkable that many LSDs have particularly devastating effects on the CNS. Well over 50% of LSDs have neuropsychiatric symptoms (Futerman and van Meer, 2004), which are frequently the dominant feature of the phenotype and may be the sole presenting clinical sign in some LSDs with adult onset (Rucker et al., 2004). The observation that later-onset lysosomal disorders usually have more prominent CNS involvement could reflect the neuron's vulnerability to cumulative lysosomal compromise exerted over decades and aging-related factors that further tip the balance toward overt disease (Boland and Platt, 2015). Although in this review we are focusing specifically on CNS disorders of lysosomal acidification, we have previously reviewed evidence that lysosomal impairments involving varying underlying mechanisms drive pathogenesis and age of onset of neurodegenerative diseases across the entire lifespan (Nixon et al., 2008).

2.1: v-ATPase-Related Mutations Linked to Congenital and Early-Onset CNS Disease

Renal tubular acidosis with deafness was the first human disease to be linked to causal mutations in a v-ATPase subunit (Blake-Palmer and Karet, 2009). Notably, in addition to impaired acid secretion by renal intercalated cells, metabolic acidosis, and bone disorders, renal tubular acidosis in its recessive, more severe form, causes neurological defects; sensorineural deafness, and mental retardation (Fry and Karet, 2007). This form of the disease is arises from loss-of-function mutations in the v-ATPase subunits V0a4 and V1B1 (Karet et al., 1999a; Karet et al., 1999b; Stover et al., 2002) and is reproduced in $V1B1^{-/-}$ and $V0a4^{-/-}$ mice (Hennings et al., 2012; Lorente-Canovas et al., 2013; Norgett et al., 2012; Paunescu et al., 2012; Vedovelli et al., 2013), which exhibit defective endocytic trafficking and build-up of lysosomal storage material in proximal tubule cells, suggesting lysosomal hydrolase impairment (Hennings et al., 2012). Mice lacking V1B1 display upregulation of the homologous V1B2 isoform (Vedovelli et al., 2013), suggesting a compensatory mechanism to promote v-ATPase assembly when certain v-ATPase components are lost. Mutations in the v-ATPase subunit V1B2, the brain-specific isoform of the V1B subunit, are found in *Dominant deafness-onychodystrophy syndrome* and *Zimmermann-Laband syndrome,* two very rare genetic disorders (Kortum et al., 2015). Besides sharing features of deafness (reversible via cochlear implant) and digital abnormalities, the latter syndrome

causes intellectual disability. These familial mutations in V1B2 reduce v-ATPase function and impair lysosomal acidification (Yuan et al., 2014).

Mutations in subunits of the V0 complex are associated with several severe disorders that reduce longevity. Autosomal recessive osteopetrosis (ARO), is a severe life-threatening disorder associated with osteosclerosis (hardening of bones), auditory and visual impairments and neurodegeneration (Keith, 1968) among other systemic deficits (Tolar et al., 2004). About half of ARO patients studied carry diverse mutations in the *OC116* gene, which encodes for the V0a3 subunit of the v-ATPase (Kornak et al., 2000) (Sobacchi et al., 2001). One of these mutations was shown to impair maturation and processing of V0a3 in osteoclasts. The mutated V0a3 is retained in the ER instead of being localized to lysosomes where it would normally be integrated as part of the v-ATPase (Bhargava et al., 2012). In addition to V0a3 being a component of the lysosomal v-ATPase, osteoclasts express the V0a3 isoform of the V0a subunit and use the v-ATPase to acidify extracellular compartments for bone reabsorption (Toyomura et al., 2003). Retinal degeneration was observed in mice lacking V0a3 which could explain the visual impairments observed in ARO (Kawamura et al., 2010). Mutations in the V0a2 gene cause *autosomal recessive cutis laxa type II (ARCLII)* and *Wrinkly Skin Syndrome (WSS*), two related developmental disorders characterized by decreased skin elasticity connective tissue weakness, osteoporosis, (Allanson et al., 1986; Kornak et al., 2008; Morava et al., 2008; Patton et al., 1987). Notably, these individuals are susceptible to mental retardation, CNS abnormalities, visual impairment, and risk for aging-related mental deterioration and seizures (Guillard et al., 2009; Kornak et al., 2008; Morava et al., 2008).

Mutations of accessory proteins required for v-ATPase function cause severe congenital disorders associated with neurodegeneration. *X-linked Parkinson Disease with Spasticity (XPDS)* is an extremely rare progressive form of Parkinsonism (Poorkaj et al., 2010) with a disease onset varying between 14 and 58 years of age. Interestingly, in the only case so far available for neuropathological evaluation, features common to AD and PD were reported, including neurofibrillary tangle pathology (Braak stage III) and amyloid-β deposits below levels necessary for an AD diagnosis, enlarged ventricles, and “mild to moderate neuronal loss” in the substantia nigra, but no Lewy Body pathology (Poorkaj et al., 2010). Genetic analysis of individuals with XPDS yielded a novel candidate gene locus on the X chromosome (Poorkaj et al., 2010) and it was later shown that a point mutation (c.345C>T) in exon 4 of the *ATP6AP2* gene causes altered splicing of ATP6AP2 in XPDS (Korvatska et al., 2013). ATP6AP2 (prorenin receptor) is a v-ATPase-interacting protein essential for coordinating proper v-ATPase assembly, specifically mediating the assembly of the membrane-bound V0 sub-complex (Kinouchi et al., 2010; Malkus et al., 2004). Ablation of ATP6AP2 in cells reduces expression of several V0 subunits, impairs v-ATPase function, de-acidifies intracellular compartments, and elevates numbers of autophagic vacuoles (Kinouchi et al., 2011; Kinouchi et al., 2013), Neuropathological analysis in patient brain supports these findings by showing “massive” accumulation of p62/SQSTM1 in the striatum, the brain region with the most severe ATP6AP2 protein loss (Korvatska et al., 2013). Mutations in exon 4 of the *ATP6AP2* gene (c.321C>T), leading to altered ATP6AP2 splicing, are also linked to another neurological condition, *X-linked Mental Retardation Hedera type (MRXSH)* (Hedera et al., 2002; Ramser et al., 2005), a congenital form of mental retardation

with epilepsy and sometimes ataxia (Hedera et al., 2002). Recently, conditional CNS-specific knockdown of ATP6AP2 was shown to cause cognitive impairment, neurodegeneration, and autophagy failure in both mouse and fly models (Dubos et al., 2015).

A final example of v-ATPase involvement in early onset neurodegenerative disease is the childhood disorder Wolfram syndrome (Venzano et al., 1980), an autosomal-recessive neurodegenerative disease associated with broad sensory, autonomic nervous system deficits and childhood-onset diabetes mellitus often leading to premature death (Rigoli and Di Bella, 2012). It also causes optic atrophy, brain stem atrophy, peripheral neuropathy, and seizures (Genis et al., 1997; Urano, 2016). Wolfram Syndrome is caused by mutations in the gene WFS1, which encodes for a nine-pass transmembrane protein of the endoplasmic reticulum (ER) (Inoue et al., 1998; Strom et al., 1998). While WFS1 itself is not involved directly in lysosomal acidification, it is required to stabilize the V1A subunit of the v-ATPase. This process occurs via interaction between ER-bound WFS1 and cytosolic V1A subunits via the cytosolic N-terminus of WFS1. The interaction of WFS1 with the V1A subunit prevents the degradation of V1A subunits through an unknown proteasome-independent mechanism, and WFS1 loss leads to reduced protein levels of V1A (Gharanei et al., 2013). WFS1 loss in pancreatic β-cells impairs the acidification of compartments (Hatanaka et al., 2011) which is thought to underlie diabetes mellitus development in Wolfram syndrome. It remains to be determined if loss or mutation of WFS1 impairs v-ATPase function in neurons.

2.2: Adult-Onset CNS Diseases Caused by v-ATPase Defects

Alzheimer Disease (AD): AD is the most prevalent neurodegenerative disease in old age although, in less than 5% of all cases, an “early onset” form of familial AD (FAD) caused by autosomal dominant mutations of Presenilin-1 (PS1), Presenilin-2 (PS2), or amyloid precursor protein (APP) genes arises mainly in the fourth to fifth decades of life (Querfurth and LaFerla, 2010). Common late-onset AD has a multifactorial origin involving brain aging as well as environmental and genetic risk factors, including most importantly the *APOE4* allele (Verghese et al., 2011) but also polymorphisms in genes involved in endocytosis, lipid trafficking, or inflammatory responses. In all forms of AD, progressive abnormalities of the endosomal lysosomal system are a prominent neuropathological feature (Cataldo et al., 2000; Maxfield, 2014; Nixon and Cataldo, 2006). The diagnostic hallmarks of AD are intraneuronal aggregates of tau protein („neurofibrillary tangles”) and “neuritic plaques”, which are patches of extracellular ß-amyloid associated with dystrophic neurites (Serrano-Pozo et al., 2011). Importantly, the abundant grossly swollen neurites are filled almost exclusively with autolysosomes containing incompletely digested waste, including Aß peptide immunoreactivity, (Nixon et al., 2005). The huge burden of stored waste in AD brain, reminiscent of LSDs, reflects both an impaired lysosomal substrate hydrolysis and a slowed retrograde transport of these organelles, which in turn is linked to compromised acidification and proteolysis (Lee et al., 2011; Nixon and Yang, 2011). Interestingly, certain primary lysosomal storage disorders (LSDs) are among the very few diseases where neuropathology resembling AD, including tauopathy, robust neuritic dystrophy, and autophagy deficits are seen (Nixon, 2004). Contributing to this pathology in AD is an increased delivery of substrates to compromised lysosomes, which is due to the upregulation

of both autophagy (Bordi and Nixon, unpublished data) and endocytosis – the latter driven by the ß-secretase-cleaved C-terminal fragment of APP (C99 or ßCTF) (Kim et al., 2015). Impairing lysosomal acidification or proteolysis experimentally induces a similar pathological phenotype (Lee et al., 2011; Nixon and Yang, 2011). Remediating lysosomal dysfunction ameliorates AD-related phenotypes in mouse models of AD (Butler et al., 2011; Vinicia et al., 2014; Yang et al., 2011a; Yang et al., 2014), underscoring the pathogenic significance of these lysosomal abnormalities.

Considerable evidence links v-ATPase deficiency directly to the pathogenesis of early onset AD. In the most common form of early-onset AD, mutations of Presenilin-1 confer loss of function for PS1 roles in proteolysis and lysosomal acidification (Coffey et al., 2014; Lee et al., 2010a; Lee et al., 2015; Wolfe et al., 2013a). PS1 holoprotein, a specific ligand of the v-ATPase V0a1 subunit (Lee et al., 2010a; Lee et al., 2015), is required for proper N-glycosylation, stability, and targeting to lysosomes (Lee et al., 2015; Lee et al., 2010b; Wolfe et al., 2013b). Cells from AD patients with PS1 mutations exhibit defective V0a1 maturation and increased lysosomal pH (Coffey et al., 2014; Lee et al., 2010a; Wolfe et al., 2013b). Cells lacking PS1 or both PS1 and PS2 display even greater elevations of lysosomal pH, lysosomal proteolysis impairment, and AD-like autophagic vacuole pathology (Lee et al., 2010a; Lee et al., 2015). Lysosomes isolated from PS1-KO cells have 70% lowered levels of V0a1 subunit, and commensurately reduced v-ATPase proton-pumping capacity and ATPase activity (Lee et al., 2015). Correcting the pH deficit in these cells with lysosomally-targeted acidic nanoparticles reverses all aspects of lysosome and autophagy dysfunction (Lee et al., 2015). A number of AD mouse models demonstrate lysosomal dysfunction. PS1M146L/APP751SL mice display impaired maturation and activity of cathepsins, suggesting a de-acidification of lysosomes in this model (Torres et al., 2012). In 5XFAD mice, levels of mature V0a1 subunit are reduced and can be restored by inhibiting GSK3, leading to improved lysosomal acidification and cathepsin maturation in this AD model (Avrahami et al., 2013). Notably, GSK3 inhibition also promoted mTOR activation, reduced Aβ burden, and improved cognitive function, suggesting a number of positive effects following lysosomal re-acidification by modulating this novel signaling pathway regulating v-ATPase function. Lysosome-localized V0a1 is reduced in PS/APP mutant mouse brain without changes in total cellular levels of V0a1 or V1B2, suggesting a failure of subunit localization onto lysosomes (Wolfe et al., 2013a). Reduced PS1 levels (78% of control) induced by antisense oligonucleotides in the senescence-accelerated SAMP8 mouse model are associated with increased expression of v-ATPase subunit V1B2, suggesting a possible compensatory response to impaired V0a1 maturation (Fiorini et al., 2013). Acidification deficits in late endosomes in PS1-KO cells are also responsible for abnormalities in Wnt processing (Dobrowolski et al., 2012). Although changes in v-ATPase were not detected in brains of mice after *neuron-specific conditional* knockdown of PS1 (Zhang et al., 2012), the knockdown of PS1 was only partial and in a subpopulation of neurons: the large pool of PS1 and V0a1 in glial cells may have masked alterations in these neurons. In a second study, primary neurons reported to lack PS1 and have normal V0a1 levels (Coen et al., 2012) displayed none of the expected phenotypic features of PS1-null cells, such as altered nicastrin modification and elevated APP-ßCTF (Lee et al., 2015).

Elevated levels of wild-type APP or FAD-mutant APP cause lysosomal dysfunction that implicates a deficit in acidification. In multiple AD mouse models in which FAD-mutant APP alone is expressed, lysosomal abnormalities develop and, where studied, these lysosomal deficits contribute substantially to the progression of AD-related neuropathological, synaptic and cognitive deficits (Manuel et al., 2012; Yang et al., 2011b; Yang et al., 2014). In cultures of primary glial cells from mice over-expressing FAD-mutant APP via a prion promoter, elevation of lysosomal pH was suggested by a markedly impaired cathepsin activation and substrate proteolysis and reduced Lysotracker signal despite expansion of the lysosomal compartment. Down syndrome (DS) causes early-onset AD mainly due to the extra copy of APP on the trisomic segment of chromosome 21. Our studies of primary DS fibroblasts and cell models of APP overexpression (Colacurcio and Jiang, unpublished data) have also revealed an APP-dependent compromise of lysosomal acidification.

The common late-onset forms of AD are strongly aging-dependent, rising exponentially in incidence after age 65. Aging-related compromise of lysosomal function is evidenced by the progressive drop in chaperone-mediated autophagy rates (Cuervo and Dice, 2000a) and oxidative modification of partially degraded proteins and lipids in autolysosomes, which gives rise to reactive oxygen species through interaction with lysosomal iron (Kurz et al., 2010), (Kiffin et al., 2006; Kurz et al., 2008). Components of lipofuscin may act as v-ATPase inhibitors and, in a positive feedback loop, promote lipofuscin accumulation and lysosomal failure (Bergmann et al., 2004). Oxidative stress is a well-recognized factor in cellular aging and AD (Barone, 2016) and, 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). Hydrogen peroxide also impairs v-ATPase-mediated vacuolar acidification in *plasmodium falciparum* (van Schalkwyk et al., 2013). The v-ATPase is a target of oxidative stress in AD, DS and aging (Barone, 2016; Butterfield et al., 2014b). Proteomic screens have revealed increased nitration of the V1E1 subunit of v-ATPase in brains from patients with early AD (Butterfield and Sultana, 2007) and increased oxidative modification (carbonylation) of the V1B2 subunit in aged rat brain tissue (Di Domenico et al., 2010). Moreover, proteomic screens have found that oxidative modification of the v-ATPase is increased in both AD and DS brain tissue, compared to controls (Butterfield et al., 2014a). Such oxidative/nitrative modifications are known to impair the enzymatic function of the F-type mitochondrial ATPase (which is highly homologous to the v-ATPase) (Fujisawa et al., 2009; Haynes et al., 2010). Nitrative stress also reduces the activity of the lysosomal v-ATPase (Colacurcio, unpublished data).

Whether related to aging or other factors, V0a1 mRNA is reduced in hippocampal neurons in sporadic AD, suggesting a potential transcriptional change which could underlie reduced v-ATPase function in this common form of the disease (Ginsberg et al., 2010). V1E1 subunit expression is altered over the course of neurofibrillary pathogenesis in a mouse tauopathy model with increased expression early in the disease process, but decreased expression later in the development of the disease (Chang et al., 2013). V1A subunit protein expression is increased relative to controls in the AβPPswe/PS1dE9 mouse line (Fu et al., 2015). These

studies suggest dynamic regulation of v-ATPase subunits at a transcriptional level in response to stress within the lysosomal system may alter acidification.

The APOE ε4 allele, the strongest genetic risk factor for late-onset AD, accelerates rab5 mediated upregulation of endocytosis (Cataldo et al., 2000; Ji et al., 2002; Ji et al., 2006; Mahley and Huang, 2006; Troncoso et al., 1998), which increases delivery of endocytosed cargoes to lysosomes and challenges acidification and proteolytic capabilities. Consistent with this idea, APOE ε4, but not APOE ε3, in a thiorphan-treated APP mouse model of AD, expands lysosomal compartments, increases Aβ co-localization with lysosomes, and causes learning and memory impairment (Belinson et al., 2008). Cholesterol, the principal lipid carried into neurons by ApoE, and a suspected AD risk factor (Chen et al., 2014a; Chen et al., 2014b), also induces neuronal rab5 activation and endocytic upregulation when administered to animals through a high-fat diet (Braccini et al., 2015). Evidence suggests that membrane cholesterol may also influence v-ATPase function more directly. A rise in cholesterol levels in isolated lysosomes reduces lysosomal proton import (Cox et al., 2007), while addition of exogenous ApoB-containing LDL cholesterol increases endolysosomal pH in cultured neurons (Hui et al., 2012). Factors affecting membrane composition, such as lipid rafts (Finnigan et al., 2011; Ryu et al., 2010) and sphingolipids (Chung et al., 2003; Finnigan et al., 2011) may also influence v-ATPase function and lysosomal acidification. Because the V0 component of the v-ATPase resides within the lysosomal membrane, and must rotate to transport protons into the lysosomal lumen, it is conceivable that changes in lysosomal membrane lipid composition may affect the ability of V0 to rotate.

Parkinson Disease (PD): PD, the second most common neurodegenerative disease (Poewe and Wenning, 1998) is characterized by dopaminergic neuron loss in the substantia nigra *pars compacta*, impaired dopaminergic signaling, and aggregation of alpha-synuclein (α-syn) within inclusions known as Lewy Bodies (Dauer and Przedborski, 2003). PD usually presents as a movement disorder, causing resting tremor, bradykinesia, stiffness, and poor balance but less commonly can also cause progressive dementia (Emre et al., 2007). Most PD cases are sporadic, but a number of genes are implicated in familial forms which point to failure of the autophagic-lysosomal system as a unifying concept in PD causation (Bras et al., 2014; Dehay et al., 2013; Gan-Or et al., 2015; Kalinderi et al., 2016).

Two early-onset, genetic forms of PD, XPDS (described above) and Kufor-Rakeb syndrome (KRS) (Di Fonzo et al., 2007; Ramirez et al., 2006) are both caused by mutations affecting lysosomal acidification, and these rare diseases give insight into how lysosomal de-acidification accelerates the development of PD-like neuropathology. In XPDS, *ATP6AP2* mutations cause v-ATPase failure and lysosomal de-acidification, leading to lysosomal system failure in neurons and to juvenile-onset Parkinsonism (Kinouchi et al., 2011). KRS, a very rare autosomal-recessive hereditary form of juvenile-onset Parkinsonism, is caused by homozygous or compound heterozygous mutations in the lysosomal P-type ATPase *ATP13A2/PARK9* (Park et al., 2015; Ramirez et al., 2006). Interestingly, *ATP13A2* mutations also cause a lysosomal storage disease, neuronal ceroid lipofuscinosis (NCL) (Bras et al., 2012), and NCL shares overlapping pathological features with both PD and KRS (Deng et al., 2015; van Veen et al., 2014). Murine and canine models of *ATP13A2* mutation or deficiency exhibit aging-related motor deficits, cognitive decline, brain atrophy,

impaired axonal trafficking, gliosis, endolysosomal abnormalities, accumulation of ubiquitinated proteins, and lipofuscinosis, strongly suggesting aging-related lysosomal system failure (Farias et al., 2011; Kett et al., 2015; Schultheis et al., 2013; Wohlke et al., 2011). Some animal models of *ATP13A2* loss accumulate α-syn in the brain (Schultheis et al., 2013), but pathology can also develop independently of synucleinopathy (Kett et al., 2015). ATP13A2 may protect against toxicity of α-syn by promoting its lysosomal clearance (Gitler et al., 2009; Usenovic et al., 2012). Loss of *ATP13A2* causes impaired lysosomal acidification and reduced lysosomal proteolysis (Dehay et al., 2012b), while increased lysosomal pH is observed in fibroblasts from PD patients with ATP13A2 mutations (Bourdenx et al., 2016).

Several *ATP13A2* mutations were found to be risk factors for early-onset PD in separate cohorts (Chen et al., 2011; Di Fonzo et al., 2007; Djarmati et al., 2009; Lin et al., 2008). The early-onset form of PD in a subject with *ATP13A2* missense mutations was found to be a milder form than that observed in KRS (Di Fonzo et al., 2007). It is speculated that ATP13A2 mutations may cause partial loss of ATP13A2 function in early PD cases, while mutations causing complete loss of ATP13A2 function lead to KRS. Single heterozygous mutations in *ATP13A2* are suspected risk factors for sporadic PD, but confirmation in larger cohorts is needed (Park et al., 2015). In sporadic PD brain, ATP13A2 protein levels are reduced in dopaminergic nigral neurons, and ATP13A2 is present in the cores of Lewy body inclusions (Dehay et al., 2012b), suggesting that Lewy body formation might originate at lysosomes or undigested autophagosomes during PD (Benjamin et al., 2012; Dehay et al., 2012a). Significantly upregulated mRNA for ATP13A2 is observed in surviving dopaminergic neurons in the brains of sporadic PD patients (Ramirez et al., 2006), suggesting that ATP13A2 expression may increase occurs as a protective response to lysosomal dysfunction.

A number of mutations that cause familial PD are found on the *SNCA* gene, which encodes for α-synuclein (α-syn) (Polymeropoulos et al., 1997; Singleton et al., 2003). It is not known if α-syn affects the v-ATPase, but α-syn over-expression induces lysosomal de-acidification *in vitro* (Stefanis et al., 2001) and, in several mouse models, disrupts lysosomal function (Cuervo et al., 2004; Martinez-Vicente et al., 2008; Xilouri et al., 2009). Mutations of other genes which are causative for PD,, such as LRRK2, impair the autophagic-lysosomal system, including mitophagy (Gan-Or et al., 2015; Kalinderi et al., 2016; Su and Qi, 2013; Wang et al., 2012). The most common pathological mutation of LRRK2 (G2019S) causes a phenotype exhibiting lysosomal expansion and diminished lysosomal degradation of substrate. This phenotype is dependent on the catalytic activity of LRRK2 (Henry et al., 2015). The G2019S mutation also leads to decreased lysosomal pH and impaired cathepsin function, as well as increased expression of the lysosomal ATPase ATP13A2 in brains from mouse and human LRRK2 G2019S carriers (Henry et al., 2015), possibly as a compensatory response. Notably, in a *c. elegans* tauopathy model, LRRK2 mutations lead to HNE modification of the subunits V1A and V1B of v-ATPase (Di Domenico et al., 2012), reminiscent of the oxidative modifications of v-ATPase in AD and DS. Mutations in glucocerebrosidase (GBA), which can be associated with either PD or Gaucher Disease (an LSD), also impair lysosomal function, as GBA is a lysosomal enzyme essential for processing of substrates (Mazzulli et al., 2011). Additionally lysosomal de-acidification has

been observed in human fibroblasts from PD patients with GBA mutations (Bourdenx et al., 2016).

Notably, a number of neurotoxic agents which promote PD and PD-like symptoms can impair lysosomal function, including pH regulation. Methamphetamine, a significant environmental risk factor for PD (Curtin et al., 2015), promotes oxidative stress and vacuolation of endocytic compartments in a dopamine-dependent manner (Callaghan et al., 2012; Cubells et al., 1994). As a weak base, methamphetamine collapses the pH gradient across acidic organelles, including lysosomes (Funakoshi-Hirose et al., 2013). Similarly, rotenone, another environmental risk factor for PD (Betarbet et al., 2000), impairs lysosomal acidification and lysosomal activity in an NADPH oxidase-dependent manner (Pal et al., 2016). MPP^+, a neurotoxin which promotes Parkinsonian symptoms, also causes de-acidification of lysosomes in cultured cells (Bourdenx et al., 2016). Together, these studies suggest that lysosomal de-acidification and dysfunction are common factors in genetic, sporadic, and toxicity-induced forms of PD/Parkinsonism. Considering previous studies on how catecholamines (Martinez-Vicente et al., 2008) and oxidative stress (Kiffin et al., 2006; Porter et al., 2013) in conjunction with α-syn (Cuervo et al., 2004) may negatively affect lysosomal function, further study on the susceptibilities of dopaminergic neurons to endosomal-lysosomal dysfunction may shed light on the pathogenesis of PD.

3.1: Conclusions and prospects

Mutations of subunits composing the v-ATPase or the proteins regulating the maturation and assembly of this complex have been identified at an accelerating pace in the past 6 years. Although the v-ATPase complex is the universal proton pump acidifying lysosomes of all cells, most v-ATPase disorders preferentially involve CNS neurodegeneration, especially prominent in diseases with adult onset. The association of lysosomal dysfunction with neurological dysfunction is well appreciated from earlier research on congenital lysosomal storage disorders, but we advance the concept in this review that milder degrees of defective lysosomal acidification may remain subclinical until they are compounded by effects of cellular aging and additional disease factors. Cellular aging in experimental systems critically involves declining v-ATPase-mediated lysosomal acidification, among other possible challenges to the lysosomal network. The unique properties of neurons, including their exceptionally long life spans, large cytoplasmic volumes, and specialized functions such as synaptic transmission involving acidified vesicles, make vesicular acidification in general, and lysosomal acidification in particular, especially critical for function and survival of neurons. The growing list of cellular roles played by intraluminal pH in nutrient and stress sensing and homeostasis and in modulating cellular signaling and trafficking have also expanded the possibly ways that v-ATPase can disrupt neuronal function, subtly or catastrophically.

A further understanding the molecular mechanisms underlying lysosomal acidification and v-ATPase regulation, which are still largely uncharted research areas, holds considerable promise for pharmacological development to reverse lysosomal dysfunction in neurodegenerative conditions. As an example, stimulation of the transcription factor TFEB improves lysosomal functions in models of LSDs (Spampanato et al., 2013) and improved

behavioral and synaptic functions in murine models of tauopathy, (Vinicia et al., 2014) by upregulating the expression of most lysosomal genes, including the v-ATPase components. The improved clearance of amyloid-β in PS1/APP mice by TFEB expression (Xiao et al., 2015) is particularly notable because the positive effects of TFEB occur in a PS1 mutant background that causes deficiency of the V0a1 subunit of the v-ATPase (Lee et al., 2010a). TFEB induction has also been shown to promote improved behavioral and synaptic functions in murine models of tauopathy, (Vinicia et al., 2014).

Several lines of evidence also show that lysosomal pH can also be corrected in experimental models in a v-ATPase-independent manner. While the v-ATPase is the primary driver of lysosomal acidification, the pH of the lysosome is a product of multiple factors, which can also be targeted in efforts to re-acidify lysosomes (Mindell, 2012). Application of nanomaterials is an intriguing approach towards stimulating lysosomal function, and multiple studies show that these agents can re-acidify lysosomal pH in cultured cells. Acidic nanoparticles, which can be taken up by lysosomes and promote the acidification of the lysosome, induce increased cathepsin activity and lysosomal proteolysis (Baltazar et al., 2012). Additional studies demonstrate that acidic nanoparticles restore lysosomal acidification, and subsequently lysosomal function, in cells lacking PS1 (Lee et al., 2015) and promote lysosomal proteolysis and cathepsin activity in primary glial cells from CRND8 mice, a model of AD which exhibits APP-associated lysosomal system defects (Xue et al., 2014; Yang et al., 2011a; Yang et al., 2014). Finally, β-adrenergic/cAMP/PKA pathway stimulation by the β-adrenergic receptor agonists, isoproterenol or cAMP, in cultured retinal pigment epithelial cells lowered lysosomal pH (Liu et al., 2008) and cAMP restored lysosomal acidification in primary fibroblasts from patients with FAD (Coffey et al., 2014). These recent attempts to modulate lysosomal pH are clearly just the earliest stage in development of innovative approaches to neurodegenerative disease treatment by targeting lysosomal dysfunction.

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Highlights

- pH regulates hydrolytic, signaling, and homeostatic functions of lysosomes
- The v-ATPase complex is mainly responsible for lysosomal acidification
- Mutations of v-ATPase components underlie numerous neurodegenerative disorders
- Deregulation of lysosomal acidification is implicated in cell aging and longevity
- Modulation of lysosomal pH in disease states is a promising therapeutic avenue

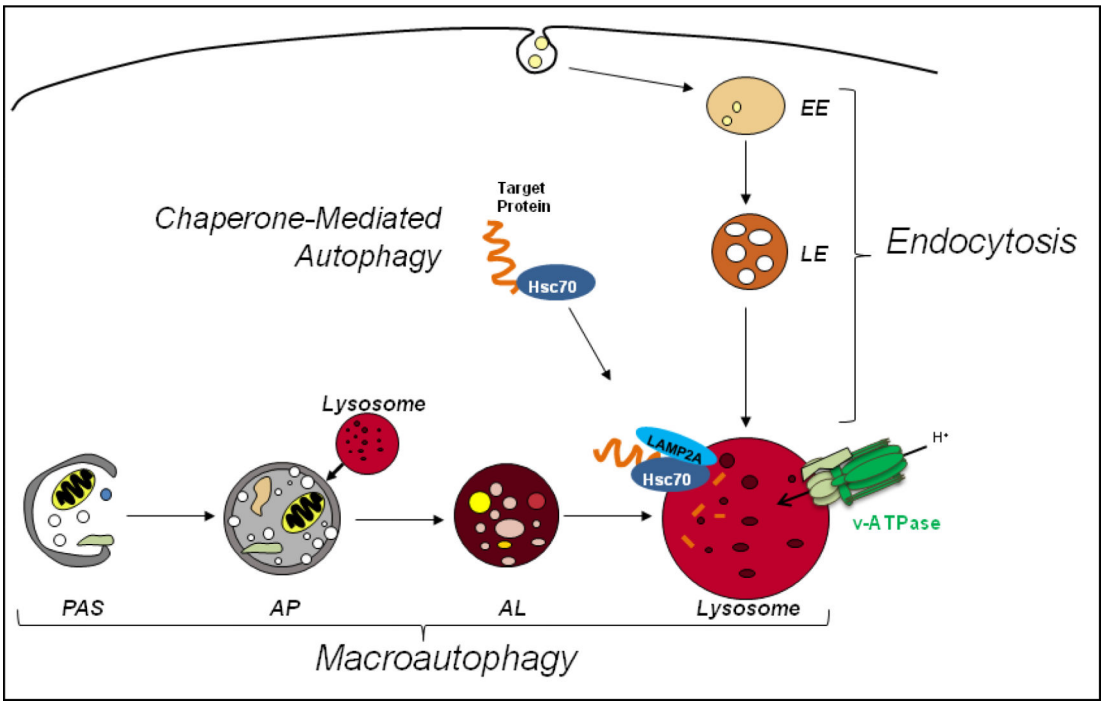

Figure 1. The Lysosomal System

The lysosomal system refers to the autophagic pathway and the endocytic pathway, and mediates the transport and proteolytic degradation of cellular waste. In the endocytic pathway, early endosomes (EE) mature into late endosomes (LE) prior to full acidification (Lysosome). In the autophagic (macroautophagy) pathway, a preautophagosomal structure (PAS) is formed, enveloping an area of cytoplasm or a selected substrate, and developing into a double-membrane autophagosome (AP). Lysosomes fuse with autophagosomes, generating single-membrane autolysosomes (AL), and ultimately lysosomes. Upon fusion with autophagosomes, lysosomes introduce proteolytic enzymes which carry out the degradation of substrates as the compartment becomes more acidic. The acidification of these compartments is mediated by the v-ATPase. Chaperone-mediated autophagy (CMA) is another type of autophagy, during which a chaperone protein complex (Hsc70 complex) recognizes a cytoplasmic target protein via a KFERQ motif, and shuttles the target protein to the lysosomal lumen for digestion via interaction with the LAMP2 protein complex, which serves as the lysosomal CMA receptor. (Figure modified from Nixon, 2013, Nat Med).

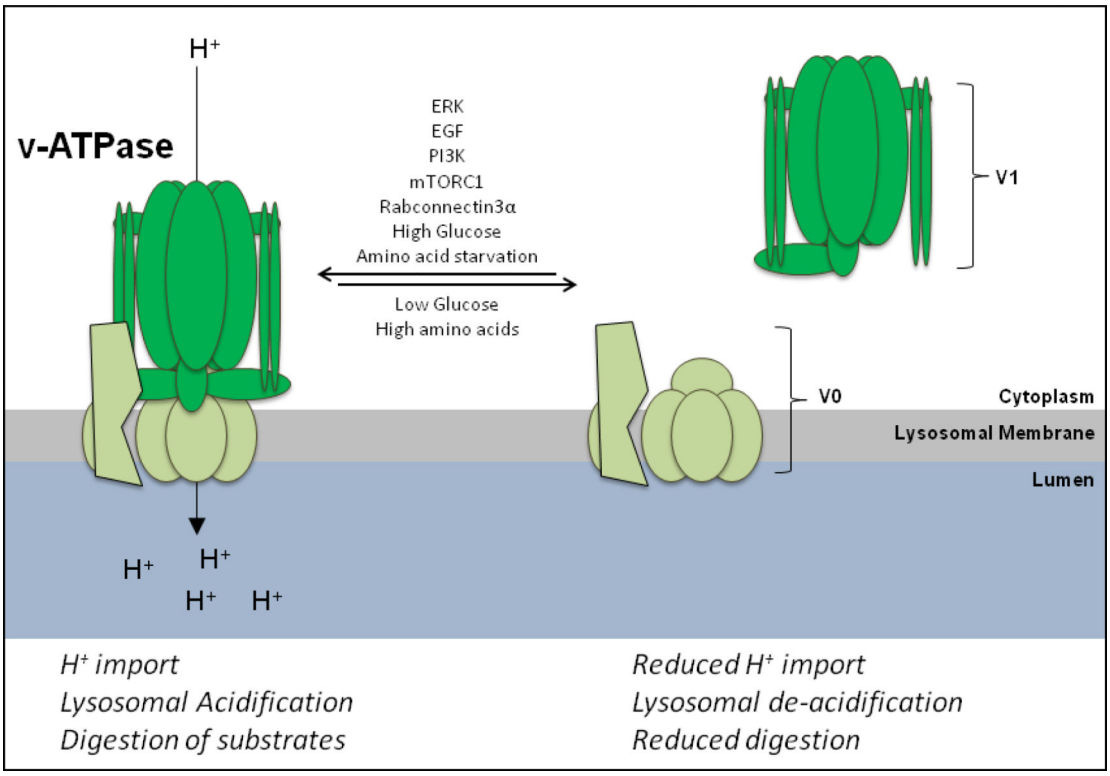

Figure 2. v-ATPase structure and regulation by dissociation

The v-ATPase promotes the acidification of lysosomes via ATP-dependent transport of protons across the lysosomal membrane. The v-ATPase is a multimeric complex composed of two sub-complexes, the cytosolic V1, and the membrane-bound V0. These two sub-complexes can reversibly dissociate, which diminishes v-ATPase activity. In mammalian cells, v-ATPase assembly is induced by glucose, leading to a feedback mechanism by which lysosomal acidification is modulated by intracellular nutrient availability. v-ATPase assembly is also regulated by several other factors, allowing v-ATPase function and lysosomal acidification to be regulated by various intracellular signaling pathways.

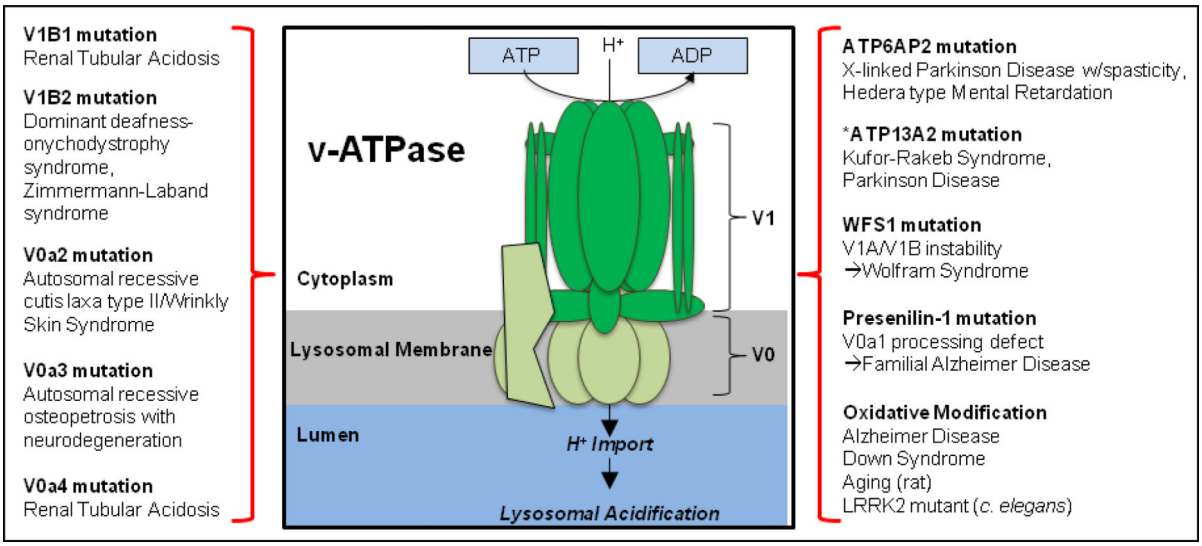

Figure 3. v-ATPase defects and associated neurodegenerative diseases

Shown in the left column are disease-associated mutations in the genes encoding for individual v-ATPase subunits (bold). Shown in the right column are changes in v-ATPase-related proteins (bold) and corresponding neurodegenerative diseases. *ATP13A2, while not directly linked to v-ATPase function, is shown due to its suspected role in lysosomal acidification and its implication in both Kufor-Rakeb Syndrome and Parkinson Disease.