Autophagy, or not to be:
The delicate balance of cellular self-digestion
In neurons and neurodegeneration

Amy Lassen

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Masters of Science

University of Washington

2012

Committee:

Susan Brockerhoff, Ph.D.

Jim Hurley, Ph.D.

Maureen Neitz, Ph.D.

Michael Ailion, Ph.D.

Rachael Wong, Ph.D.

Program Authorized to Offer Degree:

Biochemistry

ABSTRACT

Neurons rely on autophagy for some critical functions, perhaps even more so than non-neuronal cells [4], but the process does not have such a clear-cut role in these cells. In some cases, autophagy can assist the cell by clearing out toxic aggregates. In other cases, however, autophagy has the opposite effect and can instead spell death for the cell [5]. The control of autophagy in neurons is delicately balanced and a tip of the scale to one or the other side can have disastrous consequences for these post-mitotic cells. The role of autophagy in neurodegenerative diseases is an especially controversial topic, with studies on the one hand arguing that autophagy is protecting neurons from death. On the other hand, there have been studies that show autophagy hastens cell death, and blocking autophagy can actually partially rescue neurons. Depending on which direction a cell takes, induction of autophagy could answer a neuron's question, "To be, or not to be?"

DEDICATION

To my parents: who invested a lot of time and love into my education and formation as a woman and scientist.

Thank you.

I love you both.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the help of my Principal Investigator, Professor Susan Brockerhoff, and the members of her lab during my time there: Alaron Lewis, Eva Ma, Ashley George and Sara Hayden.

My committee members: Jim Hurley, Maureen Neitz, Michael Ailion, and Rachael Wong. Their suggestions and questions were stimulating, thoughtful, and helpful.

In addition, the Department of Biochemistry graciously paid me and provided tuition to the University of Washington. I also briefly received funding from the Cell and Molecular Biology Training Grant.

TABLE OF CONTENTS

LIST OF FIGURES

1. Introduction

When cells undergo differentiation, need to dispose of unneeded or damaged cellular components, or are exposed to stress, they can use a variety of methods to traffic the proteins or organelles to the vacuole, in yeast, or the lysosome, in mammalian cells [1]. One of these methods, autophagy, has three major types: microautophagy, chaperone-mediated autophagy and macroautophagy (fig 1). Microautophagy happens directly at the lysosomal or vacuolar membrane. The membrane can either invaginate or protrude to engulf the adjacent cytoplasm and then fuses to form a vesicle already contained within the lytic compartment. The resulting vesicular structure and its contents are subsequently degraded [2]. For chaperone-mediated autophagy to occur, proteins targeted for this pathway contain a specific peptide motif, KFERQ , and are recognized and transported from the cytosol across the lysosomal membrane for degradation [3]. Macroautophagy begins with the nucleation of an engulfing membrane and is followed by the sequestration of cytoplasm or organelles, autophagic vesicle formation, fusion and docking with the lytic compartment and, finally, degradation of the contents of the vesicle [1] (fig 1). Macroautophagy will be the focus of this review and will be referred to as “autophagy” for simplicity.

Autophagy is evolutionarily conserved and occurs in all eukaryotic cells. It is induced in yeast by starvation, but is also active at a basal level. In mammalian cells it appears to be constitutively activated and can be further regulated, up or down, depending on environmental conditions; e.g. oxidative stress or nutrient deprivation. In addition, autophagy has different functions and levels of activity in different cell types. During normal cellular function, autophagy can perform the role of housekeeper, recycling proteins regularly before they denature. In the event proteins do break, or are mutated, and form aggregates, autophagy can help the cell avoid the toxic effects of this accumulation. In addition, autophagy has been implicated in a secondary death pathway cells can use instead of, or in addition to, apoptosis. Autophagy is so important to some cells, specifically those requiring a high metabolism, that

knockdown or complete disruption of autophagy causes dysfunctions, such as myopathy, or cell death outright [15, 39, 40].

Neurons in particular rely on autophagy for some critical functions, perhaps even more so than non-neuronal cells [4], but the process does not have such a clear-cut role in these cells. In some cases, autophagy can assist the cell by clearing out toxic aggregates. In other cases, however, autophagy has the opposite effect and can instead spell death for the cell [5]. The control of autophagy in neurons is delicately balanced and a tip of the scale to one or the other side can have disastrous consequences for these post-mitotic cells. The role of autophagy in neurodegenerative diseases is an especially controversial topic, with studies on the one hand arguing that autophagy is protecting neurons from death. On the other hand, there have been studies that show autophagy hastens cell death, and blocking autophagy can actually partially rescue neurons. Depending on which direction a cell takes, induction of autophagy could answer a neuron's question, "To be, or not to be?"

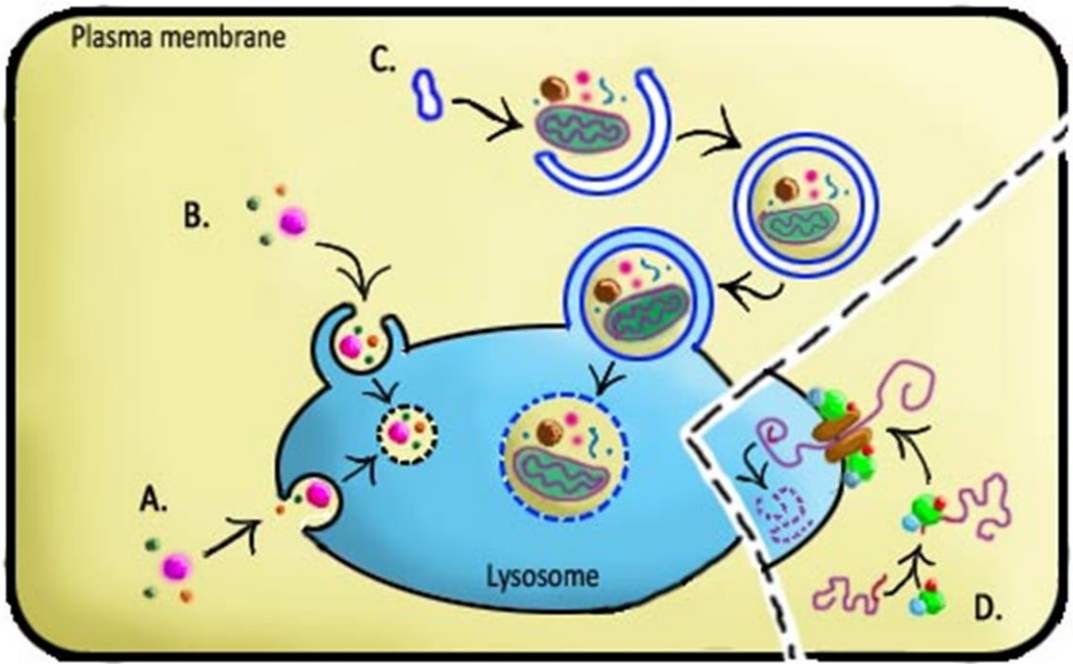

Figure 1. Schematic of the different subtypes of autophagy. Microautophagy occurs via direct engulfing of substrates by the lysosome. It can either invaginate (A) or protrude (B) to surround cargo. (C) Macroautophagy forms an isolating membrane to engulf cytoplasm and organelles (e.g. mitochondria). Membrane fuses to form an autophagosome, then fuses with the lysosome where the inner membrane and its cargo are degraded. (D) Substrates for chaperone mediated autophagy are recognized by chaperones and then transported across the lysosomal membrane to be degraded. Lassen, A., 2012

2. Autophagy: A brief history

A general form of autophagy can be traced back as far as 1905, perhaps even farther. In his book, *The Yeasts*, Alexandre Guilliermonde briefly explained "the curious phenomenon known as autophagy." Yeast cells in a culture with "a quantity of yeast greater than 40 per cent of sugar by weight" continued to undergo fermentation, using the glycogen they stored prior to starvation, and performed "a sort of autodigestion." The yeast cells used proteases, digesting their own proteins, for amino and nucleic acid supply [6]. In addition, Jean Effront asserted that when placed under starvation conditions, 7% alcohol and "a little hydrofluoric acid", enzymes the cells made upon inanition were able to keep the cells going up until 6 days after the start of nutrient deprivation, at which point they died quickly [7].

Despite their knowledge of autophagy, it is unclear whether they understood anything about the process and its relationship to the yeast vacuole. The identification of the vacuole predates that of the lysosome by at least fifty years, but it was the lysosome that was first connected with cellular autophagy. The term 'lysosome' was coined by Christian de Duve and his colleagues in 1955. Using albino rat liver cells, they performed nineteen 6-hour fractionations, separating the cell lysate into five parts through centrifugation, and then assayed the specific activity of enzymes contained within the fractions. Their fractionation technique varied from previously conducted studies in that they had two mitochondrial fractions, heavy and light, rather than one. The purpose of this extra fraction was to try to separate out a "special group of granules, comparable in size to small mitochondria and possessing [a] sac-like structure" they had noticed in earlier experiments. The light mitochondrial fraction showed very high activities of several lytic enzymes when compared with the other fractions; specifically acid phosphatase, ribo- and deoxyribonuclease, cathepsin and the majority of β-glucuronidase [8]. Based on these findings and the "richness in hydrolytic enzymes", de Duve and his colleagues resolved to call the organelle in this fraction a 'lysosome'.

A little over ten years later, de Duve and his colleague Robert Wattiaux published an extensive review of the accumulated knowledge on the lysosome. By this time others had noticed that cells seemed to be performing autophagy. Electron microscopy showed autophagic vesicles containing cytoplasm and pieces of, or whole, mitochondria. In the review, de Duve recounts the various cell types and conditions under which autophagy was observed. It was seen in normal cellular environments as well as in cells that were undergoing differentiation, starvation, and stress. The cell types included, but were not limited to: liver, kidney, brain, heart, lung, skin, as well as many others [9].

Although it was widely accepted that autophagy occurred in all of these cells, the exact mechanism remained under debate. Some thought the membrane responsible for autophagy was generated *de novo* in the cytosol [10], others thought that it originated from the golgi or smooth endoplasmic reticulum. It was also unclear how autophagosomes became acidified. If they originated from the golgi, it was possible they came pre-packaged with acid hydrolases. On the other hand, the autophagosome could fuse with pre-lysosomes containing these enzymes and gradually become acidified on its way to degradation. These questions and related others were investigated over the next few decades and, in conjunction with recently renewed interest in autophagy, have contributed to the current understanding of its function in cells.

As mentioned earlier, electron microscopy was the go-to method used to study autophagy in mammalian cells. The morphology and contents of autophagosomes could be seen and described. Inhibiting or enhancing molecules were identified, based on counting the number of autophagic vesicles present. But electron micrographs were not useful to study the formation of the membrane and the complexity of mammalian cells made it difficult to break down the process enough to say which proteins were responsible for different stages in autophagy. Little progress was made understanding the mechanism until the late 1990's, and it came not from mammalian cells, but from yeast. Homologous

autophagy proteins, the close similarity between the vacuole and the lysosome, and yeast's comparative simplicity made studying autophagy much easier in this model organism.

3. Autophagy: The Mechanism

The yeast cytoplasm to vacuole trafficking (Cvt) pathway overlaps with autophagy through the precursor to aminopeptidase I (ApeI). Yeast use the Cvt pathway during nutrient rich conditions to transport the immature hydrolase (prApeI) from the cytosol where it is synthesized to the vacuole lumen where it matures (mApeI) [1]. prApeI can also be trafficked to the vacuole through autophagy and continues to reach the vacuole under starvation conditions. By studying this overlap, numerous proteins required for autophagy were discovered and have been better categorized [2] and the progression of autophagy from nucleation of the membrane to fusion with the lysosome has become more clear.

Autophagosomes have double membranes and, unlike vesicles involved in other transport pathways within the cell (e.g. endocytosis), the vesicles used for autophagy do not bud off from an existing organelle membrane. Instead, the membrane is formed by what is known as the pre-autophagosomal structure, also known as the phagophore assembly site (PAS) [1]. The phagophore, or isolation membrane, is a flat membrane cisterna that will eventually fuse, engulfing cytoplasm and organelles, to form the autophagosome. There has been debate as to where the PAS gets the lipids necessary to form a phagophore, but one very recent opinion posits that several of the organelles in the cell – the ER, golgi and, in starvation conditions, the mitochondria— as well as the plasma membrane can contribute lipids for phagophore formation [11]. In particular, the only transmembrane protein associated with autophagy, Atg9, is supplied to the PAS via the golgi in a vesicle and it seems reasonable that the lipids making up that vesicle can be used for the process.

After initial formation of the phagophore, the membrane continues to elongate with the help of the protein Atg8, in yeast, or LC3, GABARAP and GATE-16 in mammalian cells [12]. proAtg8/LC3 is converted into Atg8/LC3-I when Atg4, as cysteine protease, cleaves off its carboxyl terminus, exposing a glycine. The protein is lipidated in an ubiquitin-like mechanism. It is activated by E1-like protein Atg7, transferred to E2-like enzyme Atg3 and conjugated to phosphatidylethanolamine (PE) at the C-terminal glycine [13]. This lipidation allows Atg8-PE/LC3-II to be inserted into the membrane of the forming phagophore. The other proteins that are involved in the formation of the phagophore (Atg12-Atg5/Atg6) dissociate from the membrane and are recycled before it fuses to become a vesicular autophagosome, but Atg8-PE/LC3-II does not. Instead, it dots the outer and inner membranes. The outer membrane-attached protein is delipidated, removed, and recycled, but the inner membrane

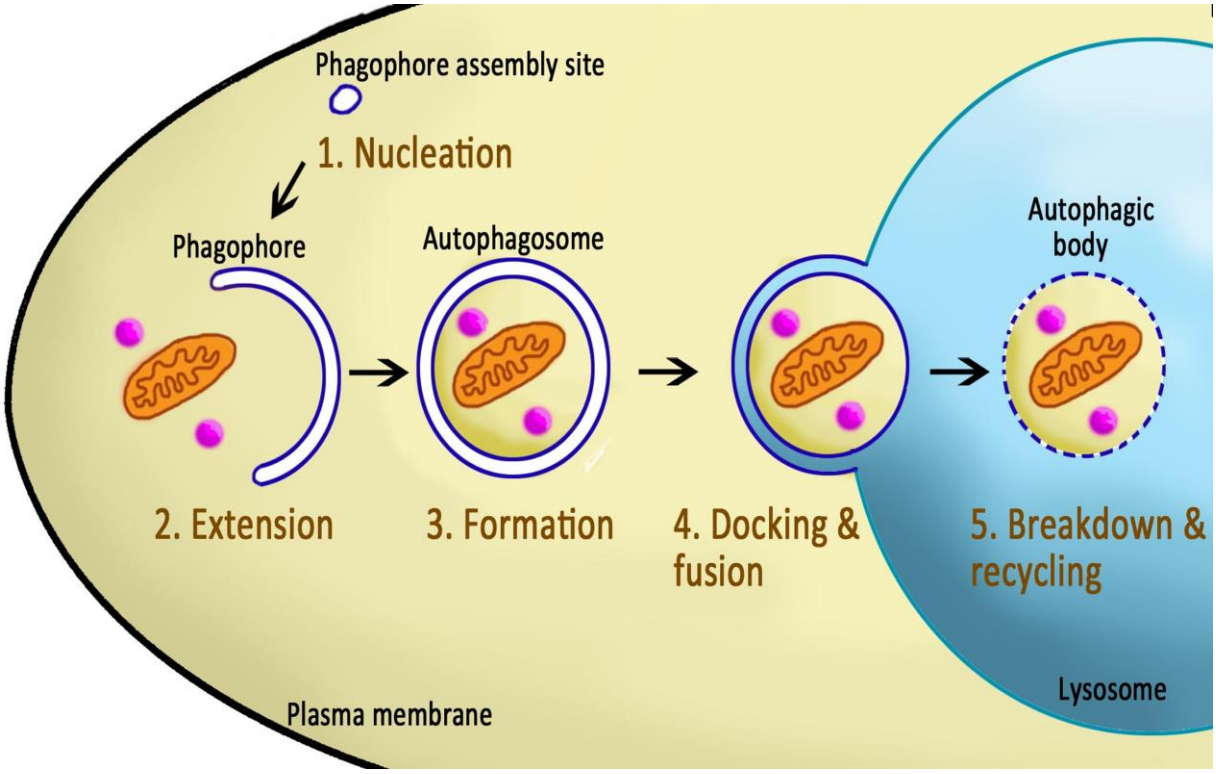

Figure 2. Schematic model of (macro)autophagy in a mammalian cell. (1) Nucleation of the membrane begins at the PAS. (2) Membrane extends. (3) Phagophore fuses, engulfing cytoplasm and organelles (eg. Mitochondria). (4) Autophagosome matures and fuses with the lysosomal membrane. (5) Inner membrane and contents are degraded by lytic enzymes within the lysosome and are recycled. Adapted from [1]

protein cannot be removed until the autophagosome fuses with the lysosome. The remaining Atg8-PE/LC3-II is degraded.

Autophagosomes ultimately fuse with lysosomes to become autolysosomes, but they may also fuse with early and late endosomes prior to making that final transition [14]. Autophagosomes originally have roughly the same pH as the cytosol [15], but become acidified during maturation. By fusing with late endosomes before the lysosome, autophagosomes could acquire proton pumps such as ATPases that lower the pH [16]. Once they fuse with the lysosome, lytic enzymes degrade the inner membrane compartment and its contents, allowing the constituent parts to be recycled and reused by the cell (fig 3).

4. Popular Techniques to Study Autophagy

4.1 Fluorescent LC3 labeling and immunoblotting

LC3-I/II has become incredibly important in the study of mammalian autophagy. It can be labeled with a fluorophore, typically GFP, on its N-terminus and the presence and progress of autophagosomes throughout the cell are monitored with fluorescence microscopy [17] (fig 3 A-B). A potential pitfall for this method is that LC3 has a tendency to form aggregates when it is overexpressed through transfection. It can also be included in aggregates like inclusion bodies in cells that express polyglutamine (polyQ) or neurons that cannot perform autophagy [18]. GFP-LC3 that is stably expressed, however, does not form such aggregates, so this method is used in preference to transient transfections. Alternatively, controls are done to ensure results are interpreted properly, such as immunoEM to examine the structures to which GFP-LC3 is localized.

Polyclonal antibodies for LC3 are commercially available for SDS-PAGE and subsequent Western blot analysis. Because LC3-II is lipidated, it runs at a slightly faster rate than LC3-I (14kDa for LC3-II vs. 16kDa for LC3-I), even though it has a higher molecular weight [19] (fig 3, C-D). Western blotting does

not detect proLC3, the LC3 precursor form, because of its immediate processing by Atg4, so the immunoblots represent the conversion of LC3-I to LC3-II rather than the processing. Despite that, immunoblotting cannot by itself indicate an increase in autophagosome formation. As described above, LC3-II on the outer membrane is delipidated and recycled, which skews results. In addition, the LC3-II that reaches the lysosome gets degraded and also will not be present on a blot. To control for these problems, lysosomal inhibitors are used and then the levels of LC3-II should be compared across different samples [19].

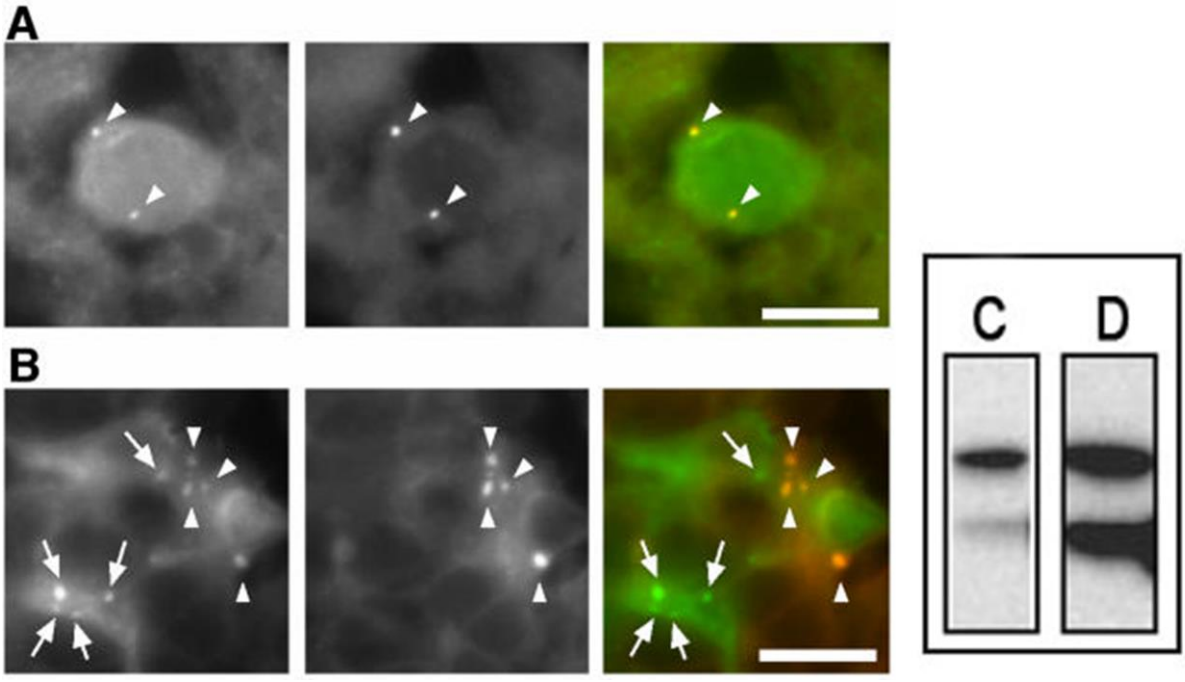

Figure 3. Distinction between GFP-LC3 and autofluorescence signals. Samples from the frontal cortex of the brain (A) and the medulla of the thymus (B) were analyzed for green (left panels) and red (middle panels) fluorescence. Merged images are shown in the right panels. GFP-specific signals (arrows) and autofluorescent signals (arrowheads) are indicated. A neuron-like cell (A) and stromal cells show autofluorescence. Similar autofluorescent signals were also observed in samples from nontransgenic mice. Bar, 10 µm. [Reprinted from Mol. Cell. Biol., 15:3, Mizushima, N. et al, In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosomemarker, 1101-1111, Copyright (2004), The American Society for Cell Biology.] (C, D) Example of LC3 immunoblot. PC12 cells were cultured in the absence of serum and amino acids for 2 hours. Total cell lysates were then prepared and subjected to immunoblot analysis using monoclonal anti‐LC3 antibody [Molecular & Biological Laboratories (MBL)]; Code #M115‐3, Clone #51‐11) (C) and polyclonal antibody raised against the N-terminal peptide of LC3 (D). [Reprinted from Autophagy, 3:6, Mizushima, N. and Yoshimori, T., How to interpret LC3 immunoblotting, 542-545, Copyright (2007), Landes Bioscience.]

4.2 Autophagy Inhibitors and Enhancers

There are many drugs available that make it easier to study autophagy and that, potentially, could be used as therapeutic treatments for disease. Experimentally, these drugs can be used in conjunction with immunoblotting to study the progression of autophagy within cells. Some drugs work by inhibiting autophagy, whereas others induce autophagy. Inhibitors include 3-methyladenine (3-MA), LY294002, and wortmannin. These act by suppressing class III phosphatidyl inositol-3 kinases, which are required for autophagy [20, 21]. Other drugs, such as Bafilomycin A and chloroquine, inhibit the fusion of autophagosomes with the lysosome, preventing the final step of autophagy and, thus, degradation of the inner membrane and its contents [22-24]. Inducers are ABT737, CCI-779, RAD001, rapamycin, resveratrol, spermidine, and xestosongin B [25-28]. Rapamycin is the most well-known inducer of autophagy. It and its derivatives, CCI-779 and RAD001, inhibit TOR complex 1, a major inhibitor of autophagy [2]. ABT737 and xestospongin B act on other proteins that negatively regulate autophagy; eg. Bcl-2 and IP_3R. Many of these drugs, most commonly 3-MA and rapamycin, are used currently to investigate the effects of an increase or decrease in autophagy on many different types of cells.

4.3 Acidic compartment labeling

Another technique that is used in conjunction with fluorescent microscopy and EM is fluorescent labeling of acidic compartments. Lysosome-associated membrane protein 2 (LAMP2) can be labeled with RFP to show the colocalization with GFP-LC3 once autophagosomes fuse with the lysosome [29] (fig 4A). This is useful for tracking the movement and location of autophagosomes as they mature and fuse with the lysosome. It is especially useful because GFP is pH sensitive (no fluorescence at low pH, pKa = 5.8 [30]) and its fluorescence is quenched once it reaches the lysosome. Using this method, a forming autophagosome would appear green and, as the vesicle matures and fuses with late endosomes and lysosomes, should appear yellow. Once it is fully mature, the green is quenched and the

autolysosome is red. Similar experiments are done using acidic dyes such as LysoTracker [31] (fig 4B) and autophagic vacuole specific marker monodansylcadaverine (MDC) [32].

5. Functions of Autophagy

5.1 Immunity, growth, starvation, and death

Autophagy can protect cells from invasion by pathogens. This specific process is called xenophagy. *Micobacterium tuberculosis* (MTB) inhibits acidification of phagosomes as well as their fusion with the lysosome, preventing the bacterium from being digested and neutralized by the macrophage that originally phagocytosed it. If autophagy is induced in infected macrophages, it can bypass the inhibition and the bacteria are killed [33]. MTB and other pathogens (e.g. *Listeria, Salmonella, Shigella,* and viral capsids*)* can be cleared via autophagy through recognition by sequestrsome-1/p62-like receptors (SLRs). Recent evidence has shown that SLRs such as p62, bind mono- and polyUbiquitin and have LC3-interacting regions (LIRs). SLRs can also recognize pathogens,

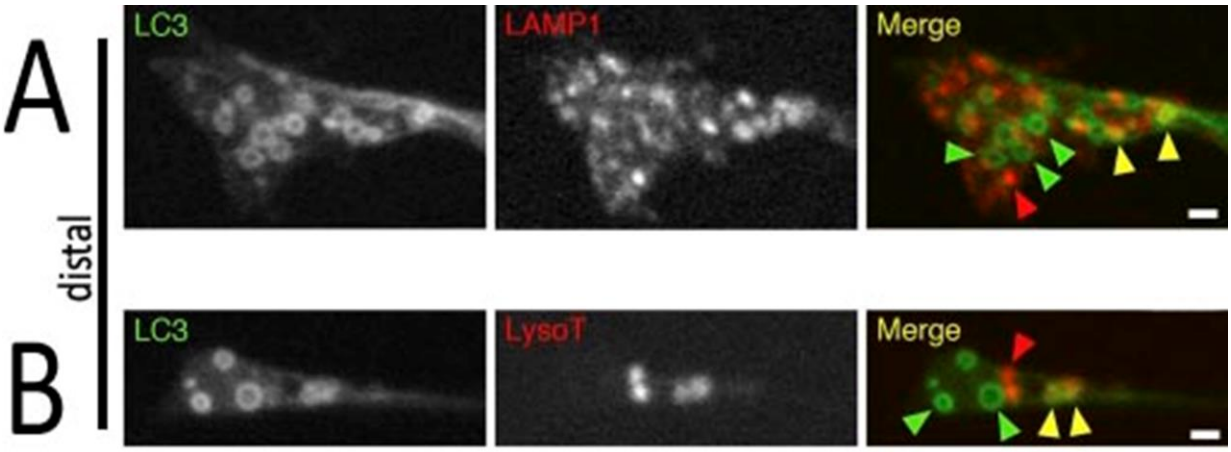

Figure 4. (A) GFP-LC3 and LAMP1-RFP distribution at the distal end of the neurite. Yellow, green, and red arrowheads designate vesicles positive for both markers, GFP-LC3 only, or LAMP1-RFP only, respectively. GFP-LC3 and LysoTracker (LysoT) red localization at the neurite tip. (B) Yellow, green, and red arrowheads designate vesicles positive for both markers, GFP-LC3 only, or LysoTracker red only, respectively. [Reprinted from J. Cell. Biol., 196:4, Maday, S., Wallace, K.E., and Holzbaur, E.L.F., Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons, 407-417, Copyright (2012), Maday et al]

allowing autophagosomes to form around bacteria through LIRs [34] (fig 5).

Programmed cell death was previously thought to be limited to apoptosis, but autophagy also participates in cellular suicide. This is a common occurrence in growing tissues and organ formation, which requires some cells to die so that the organ can develop normally. Apoptosis is signaled and autophagy is then induced to help clear the cell of its components. Interestingly, the mitochondria residing in the cell remain until the nucleus collapses and the dying cell is phagocytosed by another cell. This is, presumably, so that the cell will have enough energy to carry out programmed cell death.

Alternatively, autophagy can be a cell's protective response to stress or a toxic environment. In an attempt to prevent apoptotic cell death, the cell may perform autophagy to get rid of any offending molecules [35]. Cells can also stave off death through autophagy's most recognizable role during starvation. If the cell cannot get enough nutrients, it will begin to break down its own proteins in order to stay alive. Autophagy can prevent a cell from dying if it is starving by providing it with a limited supply of nutrients [36]; otherwise cells would just die when faced with starvation.

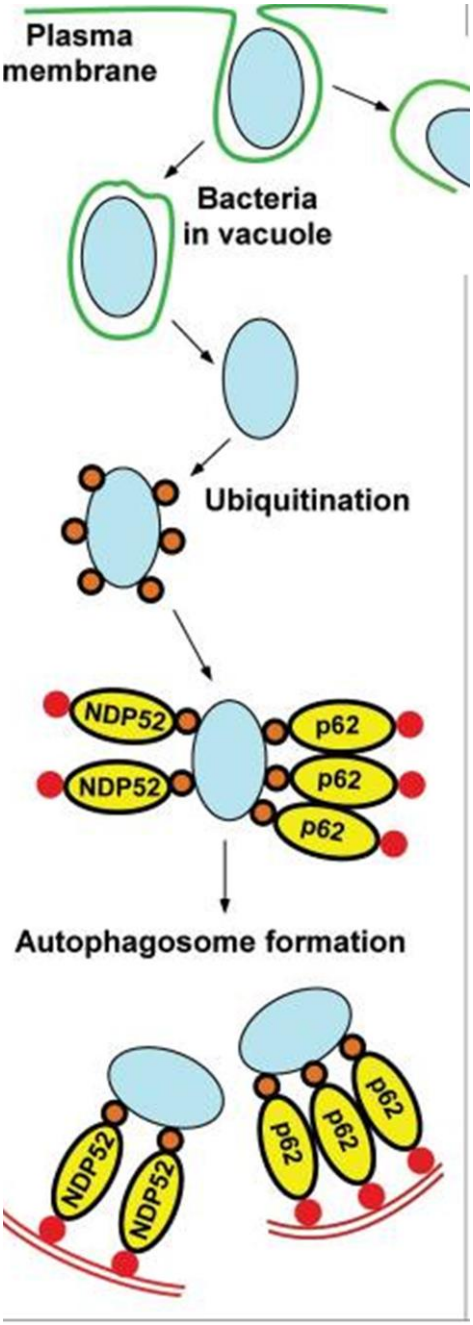

Figure 5. "Removal of intracellular bacteria by selective autophagy…p62 acts as a cargo receptor for the delivery of bactericidal precursors to the autolysosomes via selective autophagy... p62 also acts as a cargo receptor for autophagy of vacuolar membrane remnants after a bacterium escapes from the phagosome after cell entry." [Adapted from Autophagy, 7:3, Johansen, T. and Lamark, T., Selective autophagy mediated by autophagic adapter proteins, 279-296, Copyright (2011), Landes Bioscience]

5.2 Housekeeping and aggregate removal

Autophagy is also instrumental for housekeeping within the cell. The cell needs to have functional proteins and regularly recycles them as a preemptive measure [37]. In addition, organelles such as mitochondria need to be maintained as a part of this housekeeping. In cells dependent on aerobic metabolism, being able to recycle spent mitochondria through a specific form of autophagy, known as mitophagy, is critical. Developing mice do not survive past one day after birth if they lack Atg5, a protein critical to formation of the autophagophore. Instead they die of starvation because they cannot oxidize nutrients [38]. Human patients and mice develop myopathy and cardiomyopathy when they have a deficiency in LAMP-2, a lysosomal membrane protein required for autophagosome fusion with the lysosome. The muscles involved cannot perform optimally likely due to excess non-functional proteins and organelles [15, 39, 40].

Another form of housekeeping through autophagy is the removal of protein aggregates. When some proteins malfunction or are misfolded they become prone to aggregation. Amyloid beta plaques, α-synuclein, and proteins with polyQ repeats, such as huntingtin, are some obvious examples of protein build-up that forms within cells. In some of these cases, enhancement of autophagy can help clear out these toxic aggregates [41]. Autophagy performs this clearing to some extent on a regular basis in cells, as in the example of SLRs and autophagy. An SLR will recognize ubiquitinated cytosolic proteins and induce an autophagosome to form around the protein by interacting with LC3, leading to degradation of the protein in the lysosome. If autophagy fails, these proteins cannot be dealt with appropriately. For example, when Atg7, a protein required for autophagosome formation, is knocked out conditionally in mouse tissues, aggregates of ubiquitinated proteins begin to form [42].

6. Autophagy in Neurons

Autophagy was noticed in neurons in cases of neurodegenerative diseases and was thought originally to be the cause of cell death. More recent research into neuronal autophagy has led to the proposition that autophagy has a more complex role. In some cases it can be detrimental, but it can also act as part of a protective mechanism [43]. Although a lot of focus on autophagy in neurons has been directed towards diseased cells, very recent research has shown that the mechanism of autophagy in neurons is essentially the same as in other cells. The autophagosomes form and engulf cytoplasm and organelles, fuse to form vesicles, acidify, and finally fuse with the lysosome. In cultured dorsal root ganglion neurons, autophagosomes are seen forming in the distal tip of the axon. They are trafficked via retrograde motion to the cell body by dynein along microtubules [29] (fig 6). One difference between neurons and non-neurons is that neurons seem to use autophagy more on a regular basis, whereas non-

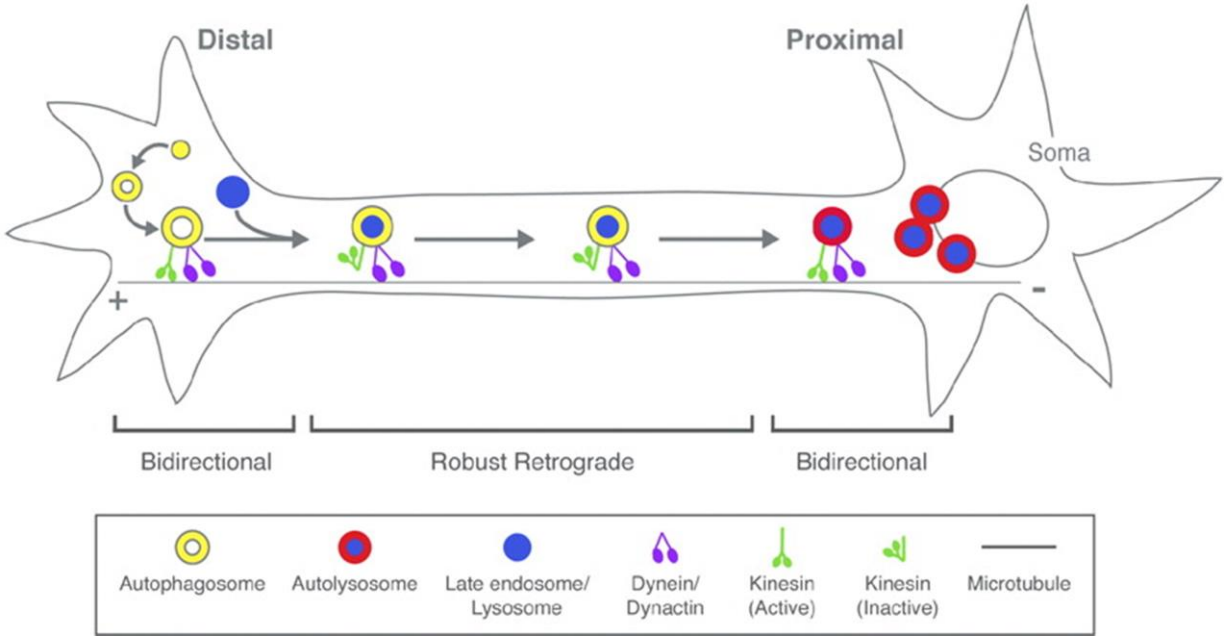

Figure 6. Model for autophagosome biogenesis and maturation along the axon in primary neurons [Reprinted from J. Cell. Biol., 196:4, Maday, S., Wallace, K.E., and Holzbaur, E.L.F., Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons, 407-417, Copyright (2012), Maday et al]

neurons tend to reserve it for starvation situations. Starvation, also, may be handled differently by neurons. In a study using striatal and cortical neurons in comparison with HeLa cells, the HeLa cells showed an increase in LC3-II when they were starved, but the neurons, even after being starved for extended periods of times, showed no signs of increased autophagy levels [4].

The role of autophagy in the clearance of aggregated proteins is especially important for neurons. These highly specialized cells are different from other cells within the body because they are post mitotic. Once a neuron is differentiated, it will never divide. It will survive as long as it can and then die without producing any daughter cells. Other cells in the body that are continuously dividing have the advantage of being able to dilute potentially toxic aggregates. In a neuron, however, if aggregates begin to form, it cannot simply divide and leave some of the aggregate in a daughter cell. It must either deal with the aggregates or suffer the consequences as the aggregates continue to form: decreased function and eventual death (fig. 7).

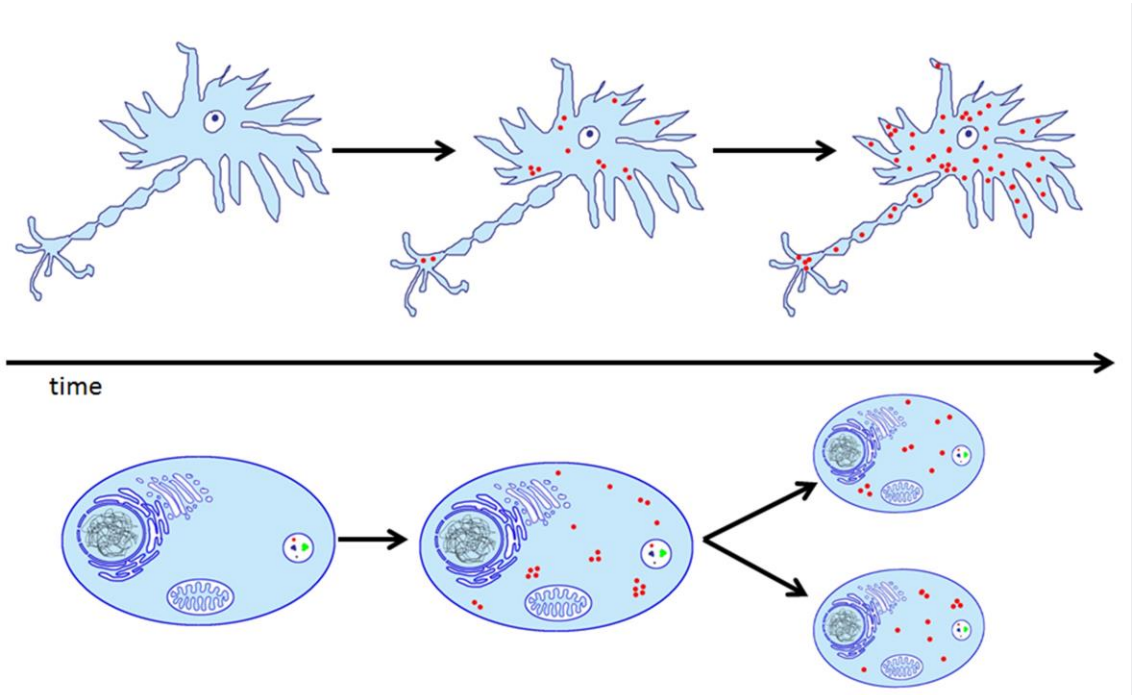

Figure 7. Representation of aggregation in neurons (above) versus mitotically diving cells (below). Lassen, A., 2012

Huntington's, Alzheimer's and Parkinson's diseases are all characterized at the cellular level by accumulation of proteins in the neurons: huntingtin, amyloid beta or tau and alpha-synuclein, respectively. Autophagy is, naturally, of great interest to those studying these diseases. Its normal function is to clear out aggregated proteins, so there must be something malfunctioning in the process to allow these diseases to progress. One thing that has been noticed is autophagy declines as normal aging occurs [44]. This, in correlation with the late onset of these neurodegenerative diseases, implies an important role for autophagy in removal of the aggregates that, when left within the cells, cause neurons to die and patients to suffer.

6.1 Autophagy and Alzheimer's disease

With around 35 million people affected worldwide as of 2010, Alzheimer's disease (AD) is the most common neurodegenerative illness and cause of dementia, and that number is projected to reach 115 million by 2050 [45]. AD is an age-associated disease, except in cases of autosomal dominant-AD, with signs in 1% of patients aged 65 affected and increasing to 30% of individuals aged 80 [46]. Early symptoms of AD include mild mental difficulty: trouble remembering, learning, and speaking. It progresses to increased loss of vocabulary, reading and writing, and personality changes such as increased irritability. Patients with advanced stages of the disease become completely reliant on care due to loss in muscle mass and extreme memory loss. AD does not cause death directly. Rather, it increases risk of external infections, like pneumonia, which ultimately result in a patient's demise [47].

AD was first noted by Alois Alzheimer over 100 years ago, but what is thought to be the cause of the disease was discovered in the 1980's. AD is characterized by the accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles, intra- and extracellularly [48]. The Amyloid Cascade Hypothesis proposes that these plaques and tangles are the cause of neurodegeneration. Aβ is a short peptide, ranging from 38 – 43 amino acids (aa's), whose length variants are designated by the exact number of

aa's they contain. The peptide is created through a pair of cleavages of the type-1 transmembrane amyloid precursor protein (APP) (fig 8). APP is first cleaved by a beta-site APP-cleaving enzyme (BACE), releasing the C-terminal fragment (APP-CTF) [49]. The second cleavage, which determines the Aβ peptide length and amount, is performed by a γ-secretase, a protein complex consisting of presenilin (PS1/2), nicastrin, PEN2, and Aph-1 [50, 51]. The processing of APP occurs in various intracellular compartments, but most of it is cleaved through the endolysosomal system in acidic compartments [52]. Aβ then tends to aggregate, with the most commonly seen peptides being Aβ40 and Aβ42.

In some forms of AD, a positive regulator of autophagy, Beclin-1, is down-regulated and, in turn, the level of autophagy within neurons decreases. In the $APP^{+}Beclin1^{+/-}$ mouse model, expressing human APP and a deficiency in Beclin-1, Aβ deposits accumulate within neurons, more so than in the simpler APP^{+} mouse, and are reduced upon overexpression of Beclin-1. Affected neurons were examined with EM and contained a lot of abnormally large lysosomal structures filled with electron dense material [53].

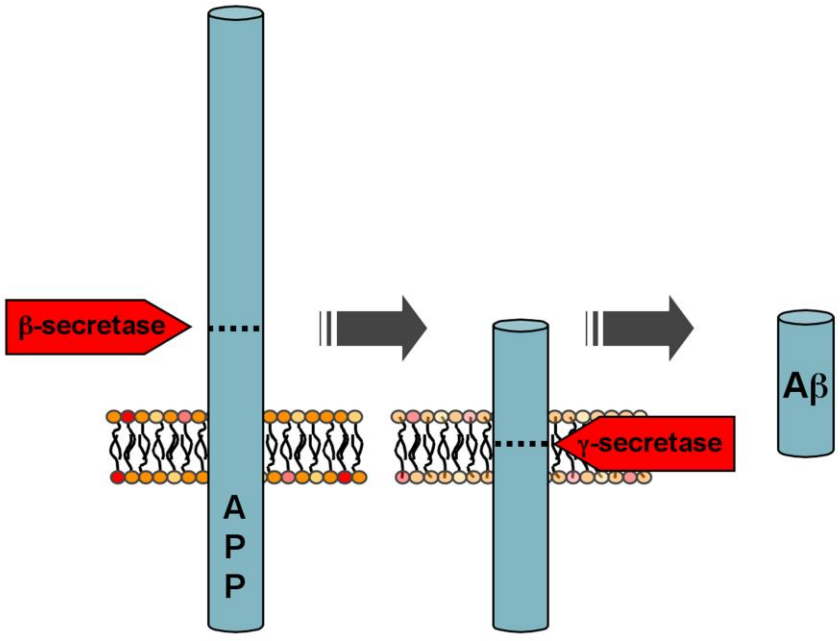

Figure 8. Model of APP processing. [Reprinted from Wikipedia (http://en.wikipedia.org/wiki/File:APP _processing.png), public domain]

At first glance, this seems to imply Beclin-1 can negatively regulate APP processing, but the Beclin-$1^{+/-}$ mice (not expressing APP) show no signs of increased Aβ. The mechanism of Beclin-1 in relationship to APP is unknown, but it could be that the lack of autophagy in Beclin-1-deficient AD causes misregulation of intracellular flux, lysosomal disruption, and, consequently, an increase of APP processing.

Autophagy has a role in both the clearance of Aβ plaques and the generation thereof. Autophagosomes purified from fractionated liver cells of APP^{+} mice have been shown to contain APP, APP-CTF, and the majority of the cell's γ-secretases [54]. In the $PS1^{+}/APP^{+}$ mouse model Aβ colocalizes with LC3, suggesting it is cleared via autophagy. Given these findings, the autophagosome is a sort of Aβ production factory. This is not detrimental in and of itself because the product is degraded if the autophagosome fuses with the lysosome and the resulting autolysosome acidifies enough that the lytic enzymes can degrade everything. However, it has been shown in some AD mice mutants that proper fusion with the lysosome, as well as acidification of the autolysosome, is impaired [55, 56]. In the case of impaired autophagosome/lysosome fusion, Aβ plaque-rich autophagosomes build up within the cell, as demonstrated in the brains of patients [57]. In the other case, in which acidification does not occur, the autolysosome cannot effectively degrade its contents, leading to the same consequence—buildup of Aβ plaques and neurodegeneration.

6.2 Autophagy and Parkinson's disease

After Alzheimer's, Parkinson's (PD) is the second most common neurodegenerative disease. Early sufferers of the disease show four characteristic muscular symptoms: rigidity, slouching (postural instability), bradykinesia (sluggish movement), and tremor. Some people will also show signs of neuropsychiatric symptoms impairing cognition, speech, behavior, mood and thought [58]. As the disease progresses, the symptoms worsen. Individuals have trouble walking and older people begin to

show signs of dementia if they did not already. Reliance on care comes with the middle to later stages, at which point PD has caused severe muscular and mental disabilities.

Unfortunately, PD cannot be as easily diagnosed as AD because it does not have secreted protein present in the blood. Diagnoses must be made based on symptoms and by ruling out other diseases that cause similar symptoms. A final diagnosis is usually done upon the patient's death, when the brain can be autopsied and presence of the protein aggregates is found. PD-affected neurons can be subject to a variety of proteins accumulating in clumps called Lewy Bodies, but the major occupant of those clumps is α-synuclein [59] (fig 9). These aggregates form in dopaminergic neurons in the substantia nigra and, ultimately, cause the neurons to degenerate and die. The vast majority of PD cases are idiopathic, but some of the remaining cases are caused by genetically inherited α-synuclein mutations, leading to the current hypothesis that α-synuclein is at the root of the problem. These

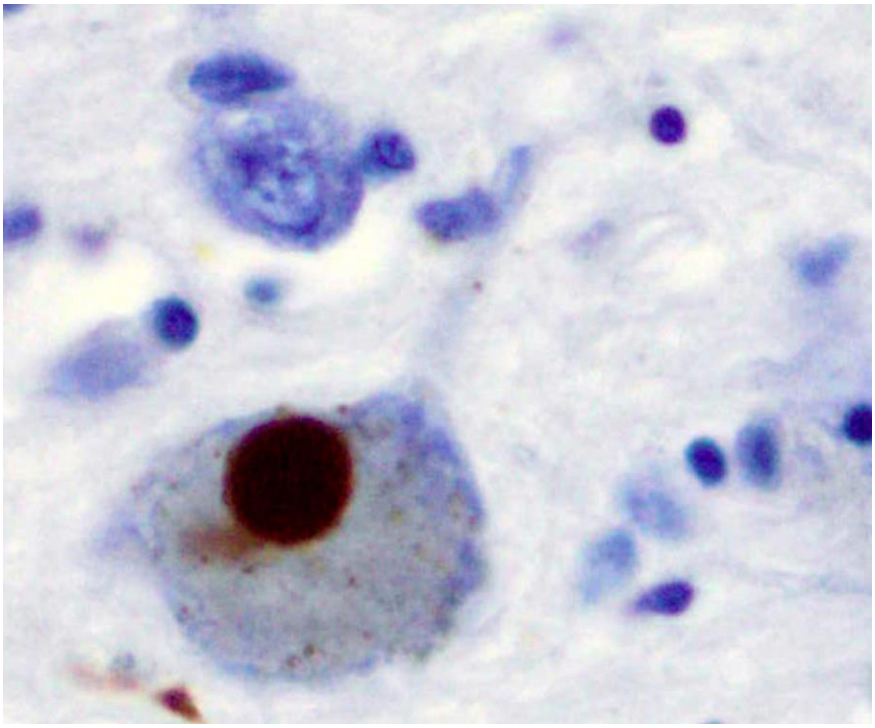

Figure 9. Immunohistochemistry for α-synuclein (brown) of an intraneural Lewy-body in the substantia nigra in Parkinson's disease. [Reprinted from Wikipedia (http://en.wikipedia.org/wiki/File:Lewy_Body_alphaSynuclein.jpg), Creative Commons Attribution-Share Alike 3.0 Unported: Marvin 101]

missense mutations, A53T and A30P, result in autosomal-dominant early-onset PD [60, 61]. The A53T mutation is very good at forming aggregates, whereas the second, A30T, is capable only of forming oligomers [62].

Autophagy again can play both the role of a savior or that of a killer in α-synuclein metabolism in neurons. As per its function in aggregate removal, autophagy decreases greatly the accumulation and toxicity of α-synuclein (WT, A30T and A53T) when autophagy is induced in pheochromocytoma 12 (PC12) cells that were differentiated into neuron-like cells and express α-synuclein [62]. The protein can have an effect on autophagy, too: it can mislocalize the important Atg9 when α-synuclein is overexpressed, preventing autophagosomes from forming [5, 63]. This, in light of the findings that α-synuclein is degraded by autophagy, suggests that α-synuclein can exacerbate its own accumulation by disrupting proper metabolism.

In addition to this direct interaction between autophagy and α-synuclein, mutations in key autophagy proteins such as PINK-1, a serine-threonine kinase that can interact with Beclin-1 to induce autophagy, cause autosomal-recessive PD [64]. Another protein that is mutated commonly and causes early, familial PD is parkin, an E3 ubiquitin ligase. It localizes to damaged mitochondria and then assists in mitophagy. Mitophagy is disrupted in cells that have mutated parkin and PINK-1[65], which localizes to the outer membrane of damaged mitochondria and serves as a recognition point for parkin [66]. If the cells are unable to get rid of malfunctioning or dysfunctional mitochondria, they are more fragile; they cannot withstand oxidative or heat stress [67].

On the other hand, autophagy can aggravate neuronal cell degradation. When the dopamine neuron-specific protein Oxi-α is down-regulated, the neurons experience an accumulation of autophagic vesicles and autophagic cell-death [68]. Oxi-α activates mTORC1, which usually prevents autophagy through a cascade of signaling. In the normal neuron exposed to oxidative stress, mTORC1 activation would prevent the cell from dying. Up-regulation of autophagy by addition of rapamycin mimics the

effect seen by Oxi-α down-regulation. Likewise, if A53T α-synuclein is overexpressed, autophagic vacuoles accumulate and the neurons undergo autophagic cell-death [69]. Although these results suggest that there is a role for autophagy in the pathology of PD and cell death, it needs to be studied in more depth to show that higher than basal levels of autophagy can be dangerous for PD-affected neurons.

6.3 Autophagy and Huntington's disease

Huntington's disease (HD), formerly known as Hungtinton's chorea because of the characteristic symptom of involuntarily, writhing muscle movements, causes neurodegeneration that leads to problems with muscle coordination, cognitive impairment and personality changes. As with the other age-associated diseases, the symptoms worsen over time, but the onset can happen anywhere from infancy to the mid-thirties. Those affected will suffer from inability to control their muscle movements and sudden, unbidden jerking of those muscles, as well as dementia as they age. As with AD and PD, death is not directly caused by HD itself, but is brought about by pneumonia in 1/3 of cases, heart attack is second most common, and suicide the third [70].

The cause of HD is a genetically inherited (autosomal-dominant) mutation in the Huntingtin gene that codes for a protein of the same name. Consistent with other genes in trinucleotide repeat disorders, the Huntingtin (htt) gene naturally has a string of CAG repeats that are amplified by mutation and HD is seen in patients with repeats of 35 or longer [70]. The length of the repeats also tends to predict how early the disease will appear, with the longer repeats predicting earlier onset [71, 72]. When the protein (Htt) is transcribed, the repeats form a long, uninterrupted string of glutamines (Q or PolyQ) at the N-terminus. Although it is usually assumed that PolyQ proteins will form aggregates that become toxic to the cell, there have also been proteins found that recognize CAG repeats on RNA which could also be detrimental for cells [73].

Autophagy has more of a protective role in HD than it appears to in AD or PD, and no evidence of autophagy causing further problems in HD, as yet, has been found. Autophagy clears PolyQ and PolyA aggregates from cells, and in cells that usually die from exposure to these aggregates, rapamycin induction of autophagy prevents them from undergoing cell death [74]. While autophagy can degrade Htt and protect the cell, it seems Htt can have its own, negative effect on autophagy. Htt forms aggregates around some key autophagy regulation proteins, causing dysregulation of autophagy. As explained earlier, mTORC1 is critical in autophagy regulation. Specifically, mTORC1 down-regulates autophagy. Htt forms aggregates that sequester mTORC1, preventing it from suppressing autophagy [41]. At first glance, this seems like a good thing because autophagy is turned on and can clear out the aggregates. But Htt also sequesters Beclin-1 in these aggregates [75], which reduces autophagy levels, as in the example of the $APP^{+}Beclin\text{-}1^{+/-}$ mice[53]. With reduced autophagy levels, neurons can no longer clear out Htt aggregates and they build up, poisoning the cells. Htt also appears to have an effect on phagophores and their ability to recognize and engulf cargo before fusing to become autophagosomes. The autophagosomes in HD cells from humans and mouse models form, fuse with the lysosome and are degraded as usual, but the cytosolic cargo is never integrated, exacerbating the accumulation of Htt within the cells [76].

6.4 Autophagy and photoreceptors

The retina contains many different, highly specialized neurons, several of which have been shown to express Atg9 and LC3 [77] (for a review on retinal structure, see[78]). Most of these cells perform a lot of metabolic capacities and must deal frequently with damaged mitochondria [79], providing a role for autophagy within the cells. Photoreceptors, both rods and cones, perform autophagy within their inner segments, digesting many cellular components, organelles (mitochondria) and synaptic vesicles [80] (fig 10).

There is far less information about autophagy in photoreceptors and the problems that affect them. Photoreceptors also experience degeneration and the cause can be of environmental or genetic origin. An example of environmental stress would be exposure to a sudden and drastic change in the illumination. Rod photoreceptors in rats up-regulate autophagy when faced with a change from 2 to 200 lux. Autophagy does not persist after the cells have adapted, and the reverse change has no effect on autophagy. The autophagic vesicles in the rod photoreceptors largely contained opsin, suggesting that the sudden change damaged the proteins and they needed to be recycled [81].

Retinal detachment can also be a cause of photoreceptor degeneration. If the retina becomes detached from the retinal pigment epithelium (RPE), which can happen as a result of an injury or in conjunction with macular degeneration or retinopathy, patients usually have about a week to get treated before their photoreceptors begin to die off and chance of recovery of vision decreases with the length of time. In a model of this, rat retinae were detached from the RPE and levels of autophagy-specific proteins were monitored. The photoreceptors up-regulated autophagy, prolonging their survival and delaying apoptotic cell death [82].

Genetic mutations affect photoreceptors and cause them to degenerate and die. Retinitis pigmentosa (RP) is one of the most common of

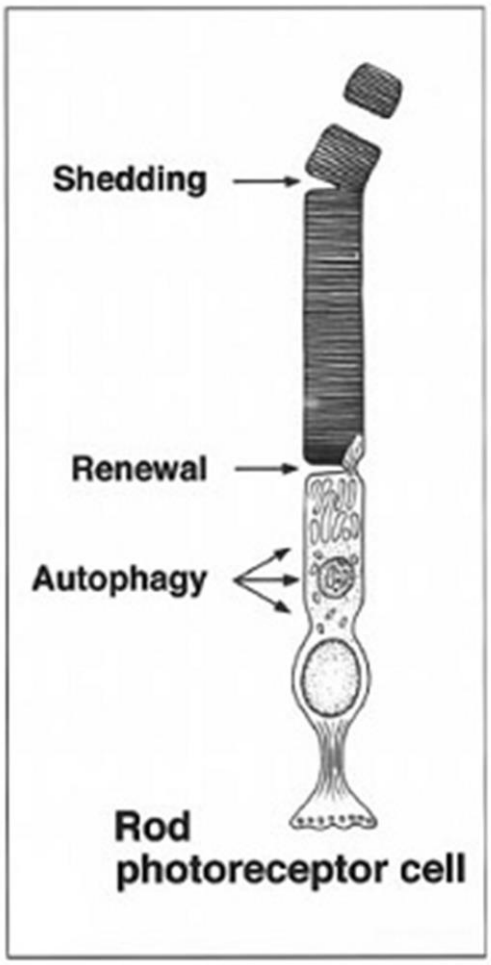

Figure 10. Autophagic vesicle position in rod photoreceptor. [Reprinted from IOVS, 40:10, Reme, C.E. et al, Photoreceptor autophagy: effects of light history on number and opsin content of degredative vacuoles, 2398-2404, Copyright (1999) Association for Research in Vision and Ophthalmology]

these retinal dystrophies and results in thus far incurable blindness. RP is caused by mutations in rod photoreceptor-specific genes, with over 30 different genes causing the disease when mutated. 25% of mutations in RP occur in rhodopsin and cause autosomal dominantly inherited RP [83]. Rod photoreceptors in RP cases die off, causing patients to become night blind. An unexpected side-effect of rod death is subsequent death of the cones in the retina. This can happen at a variety of rates after rod death, resulting in either a slow, gradual loss of day vision from the periphery inward, or rapid cone death and complete blindness.

Class-II mutations in rhodopsin lead to misfolding of the protein. The rhodopsin then accumulates in the ER and cytoplasm and if the aggregates are not degraded, they become detrimental to the cell [84]. Rods affected by RP usually die through apoptosis, but one very brief study suggested that autophagy also had a role in killing the cells. This study only investigated levels of genes indicative of autophagy, however, and did not delve farther into its exact role in RP, only stating that it must be causing death alongside apoptosis [85, 86]. This assumption ignores one of the main functions of autophagy. In light of the earlier mentioned study of retinal detachment and the mechanisms of autophagy in clearance of protein aggregates, it seems likely that instead of causing cell death in RP with rhodopsin accumulation, autophagy functions as the cell's effort to get rid of aggregated rhodopsin and stave off death.

In support of this, rhodopsin-1 (Rh1) was shown to accumulate in late endosomes and autophagosomes within phosphatidylserine decarboxylase mutant *Drosophila* photoreceptors exposed to prolonged intense light. These photoreceptors survive in dark situations, but cannot survive under light. Similarly, knockdown of Atg8 (the Drosophila homologue of LC3) or Atg7 had the same effect after light exposure: accumulation of Rh1 and subsequent photoreceptor degeneration [87]. Both of these results show that rhodopsin, although degraded by endocytosis, is also degraded by autophagy and lack of autophagy causes the cells to degenerate under light conditions. This suggests that autophagy could

have the same role in RP, although it may not keep the cell alive for long because of the extent of damage caused by the genetically mutated rhodopsin.

7. Conclusions

Autophagy plays a critical role in neurons and neurodegeneration, but whether that role benefits or further harms the cells is an ongoing debate. Evidence has been shown from both sides that autophagy can help cells, as in the case of clearing protein aggregates in Alzheimer's, Parkinson's, and Huntinton's diseases, or shorten their lifespan, as with autophagic cell death in these same diseases and in photoreceptors affected by retinitis pigmentosa. Rather than one or the other side of the dispute being ultimately correct, it seems more likely that autophagy is controlled in a delicate balance in these cells and may play both roles, dependent on the conditions the cell finds itself in.

A lot of interest has been placed on autophagy, specifically in the three neurodegenerative diseases discussed, as well as RP-associated cone death. Because these diseases are characterized by a slow die-off of cells, the hope is that a therapy could be found that might prevent or slow down the neurodegeneration. Autophagy is the ideal candidate for targeted therapy for two reasons: its role in clearance of aggregated proteins, and the ability to use rapamycin to induce autophagy. The drug rapamycin is already being used on human patients to prevent rejection of organ transplants, so if it could be shown that autophagy up-regulation would attenuate the effects of Aβ, α-synuclein or huntingtin, rapamycin might be a good choice for clinical studies. The opposite is also true of autophagy: if it were found to be the cause of neurodegeneration, there are inhibitors available that could be administered as a preventative measure.

In the case of the dementia-causing diseases, however, treatments would be long in coming due to the requisite length of drug trials. Once neurons die, they cannot be resurrected, so the most useful time of treatment would be before the cells begin to die in the first place. In the cases of AD and HD,

which can be detected through Aβ deposition in the blood or genetic testing for the Htt CAG repeat, early treatment is plausible, but the studies needed to prove a treatment useful would need to be followed for more than twenty years. Treatment of PD, on the other hand, poses more of a problem. Treatment of patients should begin before onset of the disease, but unless it was only administered to the small number of people who inherit a genetic mutation that will result in PD, the entire at-risk population would have to be treated prophylactically. Because it cannot as yet be conclusively diagnosed until an autopsy at death, treatment would only be speculative and such a trial would be expensive, considering some of the participants may not have even developed the disease, regardless of treatment.

The exact function of autophagy in neurons and neurodegeneration remains elusive and poorly defined. It is even possible that autophagy only plays a small part and some other process is more responsible for the devastating effect on neurons. The CAG-recognition proteins in HD [73] could be a candidate. Also possible is the exposure of neurons to oxidative stress (OS) in neurodegenerative diseases. Evidence exists to implicate OS in AD and PD, but little more is known about how neurons react to it [88]. Either way, it is clear the role of autophagy in these diseases needs to be studied in greater depth before a conclusion can be reached.

References

1. Wang CW, Klionsky DJ: **The molecular mechanism of autophagy.** *Mol Med* 2003, **9:**65-76.
2. Klionsky DJ, Baehrecke EH, Brumell JH, Chu CT, Codogno P, Cuervo AM, Debnath J, Deretic V, Elazar Z, Eskelinen EL, et al: **A comprehensive glossary of autophagy-related molecules and processes (2nd edition).** *Autophagy* 2011, **7:**1273-1294.
3. Cuervo AM, Dice JF: **Lysosomes, a meeting point of proteins, chaperones, and proteases.** *J Mol Med (Berl)* 1998, **76:**6-12.
4. Tsvetkov AS, Mitra S, Finkbeiner S: **Protein turnover differences between neurons and other cells.** vol. 5. pp. 1037-1038. Autophagy; 2009:1037-1038.
5. Son JH, Shim JH, Kim KH, Ha JY, Han JY: **Neuronal autophagy and neurodegenerative diseases.** *Exp Mol Med* 2012, **44:**89-98.
6. Guilliermond AaT, F. W: *The Yeasts.* Norwood, Massachusets: The Plimpton Press; 1920.
7. Effront J: **Sur l'autophagy de la levure.** *Moniteur scientific* 1905, **16**.
8. de Duve C, Pressman, B.C., Gianetto, R., Wattiaux, R., & Appelmans, F.: **Intracellular distribution patternsof enzymes in rat-liver tissue.** *Biochem J* 1955, **60:**604-617.
9. de Duve CW, R.: **Functions of lysosomes.** 1966, **28:**435-492.
10. Ashford TPP, K. P.: **Cytoplasmic components in hepatic cell lysosomes.** *J Cell Biol* 1962**:**198 - 202.
11. Rubinsztein DC, Shpilka T, Elazar Z: **Mechanisms of autophagosome biogenesis.** *Curr Biol* 2012, **22:**R29-34.
12. Shpilka T, Weidberg H, Pietrokovski S, Elazar Z: **Atg8: an autophagy-related ubiquitin-like protein family.** *Genome Biol* 2011, **12:**226.
13. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M, et al: **A ubiquitin-like system mediates protein lipidation.** *Nature* 2000, **408:**488-492.
14. Eskelinen EL: **Maturation of autophagic vacuoles in Mammalian cells.** *Autophagy* 2005, **1:**1-10.
15. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lüllmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P: **Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice.** *Nature* 2000, **406:**902-906.
16. Dunn WA: **Studies on the mechanisms of autophagy: maturation of the autophagic vacuole.** *J Cell Biol* 1990, **110:**1935-1945.
17. Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y: **In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker.** *Mol Biol Cell* 2004, **15:**1101-1111.
18. Kuma A, Matsui M, Mizushima N: **LC3, an autophagosome marker, can be incorporated into protein aggregates independent of autophagy: caution in the interpretation of LC3 localization.** *Autophagy* 2007, **3:**323-328.
19. Mizushima N, Yoshimori T: **How to interpret LC3 immunoblotting.** *Autophagy* 2007, **3:**542-545.
20. Seglen PO, Gordon PB: **3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes.** *Proc Natl Acad Sci U S A* 1982, **79:**1889-1892.
21. Blommaart EF, Krause U, Schellens JP, Vreeling-Sindelárová H, Meijer AJ: **The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes.** *Eur J Biochem* 1997, **243:**240-246.
22. Klionsky DJ, Elazar Z, Seglen PO, Rubinsztein DC: **Does bafilomycin A1 block the fusion of autophagosomes with lysosomes?** *Autophagy* 2008, **4:**849-950.
23. Yamamoto A, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y: **Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells.** *Cell Struct Funct* 1998, **23:**33-42.
24. Fedorko M: **Effect of chloroquine on morphology of cytoplasmic granules in maturing human leukocytes--an ultrastructural study.** *J Clin Invest* 1967, **46:**1932-1942.
25. Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P, Tasdemir E, Pierron G, Troulinaki K, Tavernarakis N, et al: **Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1.** *EMBO J* 2007, **26:**2527-2539.
26. Opipari AW, Tan L, Boitano AE, Sorenson DR, Aurora A, Liu JR: **Resveratrol-induced autophagocytosis in ovarian cancer cells.** *Cancer Res* 2004, **64:**696-703.
27. Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, et al: **Induction of autophagy by spermidine promotes longevity.** *Nat Cell Biol* 2009, **11:**1305-1314.
28. Criollo A, Maiuri MC, Tasdemir E, Vitale I, Fiebig AA, Andrews D, Molgó J, Díaz J, Lavandero S, Harper F, et al: **Regulation of autophagy by the inositol trisphosphate receptor.** *Cell Death Differ* 2007, **14:**1029-1039.
29. Maday S, Wallace KE, Holzbaur EL: **Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons.** *J Cell Biol* 2012, **196:**407-417.
30. Haupts U, Maiti S, Schwille P, Webb WW: **Dynamics of fluorescence fluctuations in green fluorescent protein observed by fluorescence correlation spectroscopy.** *Proc Natl Acad Sci U S A* 1998, **95:**13573-13578.
31. Chazotte B: **Labeling lysosomes in live cells with LysoTracker.** *Cold Spring Harb Protoc* 2011, **2011:**pdb.prot5571.
32. Biederbick A, Kern HF, Elsässer HP: **Monodansylcadaverine (MDC) is a specific in vivo marker for autophagic vacuoles.** *Eur J Cell Biol* 1995, **66:**3-14.
33. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V: **Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages.** *Cell* 2004, **119:**753-766.
34. Johansen T, Lamark T: **Selective autophagy mediated by autophagic adapter proteins.** *Autophagy* 2011, **7:**279-296.
35. Bursch W: **The autophagosomal-lysosomal compartment in programmed cell death.** *Cell Death Differ* 2001, **8:**569-581.
36. Bauvy C, Gane P, Arico S, Codogno P, Ogier-Denis E: **Autophagy delays sulindac sulfide-induced apoptosis in the human intestinal colon cancer cell line HT-29.** *Exp Cell Res* 2001, **268:**139-149.
37. Cuervo AM: **Autophagy: many paths to the same end.** *Mol Cell Biochem* 2004, **263:**55-72.
38. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N: **The role of autophagy during the early neonatal starvation period.** *Nature* 2004, **432:**1032-1036.
39. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y, et al: **Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease).** *Nature* 2000, **406:**906-910.
40. Nishino I: **Autophagic vacuolar myopathies.** *Curr Neurol Neurosci Rep* 2003, **3:**64-69.

41. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC: **Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease.** *Nat Genet* 2004, **36:**585-595.
42. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, et al: **Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice.** *J Cell Biol* 2005, **169:**425-434.
43. Yue Z, Friedman L, Komatsu M, Tanaka K: **The cellular pathways of neuronal autophagy and their implication in neurodegenerative diseases.** *Biochim Biophys Acta* 2009, **1793:**1496-1507.
44. Lipinski MM, Zheng B, Lu T, Yan Z, Py BF, Ng A, Xavier RJ, Li C, Yankner BA, Scherzer CR, Yuan J: **Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease.** *Proc Natl Acad Sci U S A* 2010, **107:**14164-14169.
45. International A: **World Alzheimer Report 2010.** 2010.
46. Tam JH, Pasternak SH: **Amyloid and Alzheimer's disease: inside and out.** *Can J Neurol Sci* 2012, **39:**286-298.
47. Förstl H, Kurz A: **Clinical features of Alzheimer's disease.** *Eur Arch Psychiatry Clin Neurosci* 1999, **249:**288-290.
48. Tung YT, Wang BJ, Hu MK, Hsu WM, Lee H, Huang WP, Liao YF: **Autophagy: a double-edged sword in Alzheimer's disease.** *J Biosci* 2012, **37:**157-165.
49. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, et al: **Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE.** *Science* 1999, **286:**735-741.
50. Sinha S, Lieberburg I: **Cellular mechanisms of beta-amyloid production and secretion.** *Proc Natl Acad Sci U S A* 1999, **96:**11049-11053.
51. Edbauer D, Winkler E, Regula JT, Pesold B, Steiner H, Haass C: **Reconstitution of gamma-secretase activity.** *Nat Cell Biol* 2003, **5:**486-488.
52. Olanow CW: **Rationale for considering that propargylamines might be neuroprotective in Parkinson's disease.** *Neurology* 2006, **66:**S69-79.
53. Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, Levine B, Wyss-Coray T: **The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice.** *J Clin Invest* 2008, **118:**2190-2199.
54. Yu WH, Kumar A, Peterhoff C, Shapiro Kulnane L, Uchiyama Y, Lamb BT, Cuervo AM, Nixon RA: **Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease.** *Int J Biochem Cell Biol* 2004, **36:**2531-2540.
55. Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, Nixon RA: **Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease.** *J Neurosci* 2008, **28:**6926-6937.
56. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM: **Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study.** *J Neuropathol Exp Neurol* 2005, **64:**113-122.
57. Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, Mohan PS, Mercken M, Farmery MR, Tjernberg LO, et al: **Macroautophagy--a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease.** *J Cell Biol* 2005, **171:**87-98.
58. Jankovic J: **Parkinson's disease: clinical features and diagnosis.** *J Neurol Neurosurg Psychiatry* 2008, **79:**368-376.
59. Kahle PJ, Haass C, Kretzschmar HA, Neumann M: **Structure/function of alpha-synuclein in health and disease: rational development of animal models for Parkinson's and related diseases.** *J Neurochem* 2002, **82:**449-457.
60. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, et al: **Mutation in the alpha-synuclein gene identified in families with Parkinson's disease.** *Science* 1997, **276:**2045-2047.
61. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schöls L, Riess O: **Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease.** *Nat Genet* 1998, **18:**106-108.
62. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC: **Alpha-Synuclein is degraded by both autophagy and the proteasome.** *J Biol Chem* 2003, **278:**25009-25013.
63. Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, Lichtenberg M, Menzies FM, Ravikumar B, Imarisio S, et al: **α-Synuclein impairs macroautophagy: implications for Parkinson's disease.** *J Cell Biol* 2010, **190:**1023-1037.
64. Michiorri S, Gelmetti V, Giarda E, Lombardi F, Romano F, Marongiu R, Nerini-Molteni S, Sale P, Vago R, Arena G, et al: **The Parkinson-associated protein PINK1 interacts with Beclin1 and promotes autophagy.** *Cell Death Differ* 2010, **17:**962-974.
65. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ: **Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants.** *Proc Natl Acad Sci U S A* 2003, **100:**4078-4083.
66. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, Youle RJ: **PINK1 is selectively stabilized on impaired mitochondria to activate Parkin.** *PLoS Biol* 2010, **8:**e1000298.
67. Gautier CA, Kitada T, Shen J: **Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress.** *Proc Natl Acad Sci U S A* 2008, **105:**11364-11369.
68. Choi KC, Kim SH, Ha JY, Kim ST, Son JH: **A novel mTOR activating protein protects dopamine neurons against oxidative stress by repressing autophagy related cell death.** *J Neurochem* 2010, **112:**366-376.
69. Stefanis L, Larsen KE, Rideout HJ, Sulzer D, Greene LA: **Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death.** *J Neurosci* 2001, **21:**9549-9560.
70. Walker FO: **Huntington's disease.** *Lancet* 2007, **369:**218-228.
71. Rubinsztein DC, Carmichael J: **Huntington's disease: molecular basis of neurodegeneration.** *Expert Rev Mol Med* 2003, **5:**1-21.
72. Djoussé L, Knowlton B, Hayden MR, Almqvist EW, Brinkman RR, Ross CA, Margolis RL, Rosenblatt A, Durr A, Dode C, et al: **Evidence for a modifier of onset age in Huntington disease linked to the HD gene in 4p16.** *Neurogenetics* 2004, **5:**109-114.
73. Li LB, Yu Z, Teng X, Bonini NM: **RNA toxicity is a component of ataxin-3 degeneration in Drosophila.** *Nature* 2008, **453:**1107-1111.
74. Ravikumar B, Duden R, Rubinsztein DC: **Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy.** *Hum Mol Genet* 2002, **11:**1107-1117.

75. Shibata M, Lu T, Furuya T, Degterev A, Mizushima N, Yoshimori T, MacDonald M, Yankner B, Yuan J: **Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1.** *J Biol Chem* 2006, **281:**14474-14485.
76. Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, de Vries R, Arias E, Harris S, Sulzer D, Cuervo AM: **Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease.** *Nat Neurosci* 2010, **13:**567-576.
77. Mitter SK, Rao HV, Qi X, Cai J, Sugrue A, Dunn WA, Grant MB, Boulton ME: **Autophagy in the retina: a potential role in age-related macular degeneration.** *Adv Exp Med Biol* 2012, **723:**83-90.
78. **Webvision: The Organization of the Retina and Visual System** [http://webvision.med.utah.edu/book/]
79. Jarrett SG, Lewin AS, Boulton ME: **The importance of mitochondria in age-related and inherited eye disorders.** *Ophthalmic Res* 2010, **44:**179-190.
80. Remé C: **Autophagy in rods and cones of the vertebrate retina.** *Dev Ophthalmol* 1981, **4:**101-148.
81. Remé CE, Wolfrum U, Imsand C, Hafezi F, Williams TP: **Photoreceptor autophagy: effects of light history on number and opsin content of degradative vacuoles.** *Invest Ophthalmol Vis Sci* 1999, **40:**2398-2404.
82. Besirli CG, Chinskey ND, Zheng QD, Zacks DN: **Autophagy activation in the injured photoreceptor inhibits fas-mediated apoptosis.** *Invest Ophthalmol Vis Sci* 2011, **52:**4193-4199.
83. Hartong DT, Berson EL, Dryja TP: **Retinitis pigmentosa.** *Lancet* 2006, **368:**1795-1809.
84. Mendes HF, Zaccarini R, Cheetham ME: **Pharmacological manipulation of rhodopsin retinitis pigmentosa.** *Adv Exp Med Biol* 2010, **664:**317-323.
85. Kunchithapautham K, Rohrer B: **Autophagy is one of the multiple mechanisms active in photoreceptor degeneration.** *Autophagy* 2007, **3:**65-66.
86. Lohr HR, Kuntchithapautham K, Sharma AK, Rohrer B: **Multiple, parallel cellular suicide mechanisms participate in photoreceptor cell death.** *Exp Eye Res* 2006, **83:**380-389.
87. Midorikawa R, Yamamoto-Hino M, Awano W, Hinohara Y, Suzuki E, Ueda R, Goto S: **Autophagy-dependent rhodopsin degradation prevents retinal degeneration in Drosophila.** *J Neurosci* 2010, **30:**10703-10719.
88. Jimenez-Del-Rio M, Velez-Pardo C: **The bad, the good, and the ugly about oxidative stress.** *Oxid Med Cell Longev* 2012, **2012:**163913.