Published in final edited form as:

Exp Eye Res. 2016 March ; 144: 64–72. doi:10.1016/j.exer.2015.08.021.

Autophagy in light-induced retinal damage

Yu Chen^{1,2}, Lindsay Perusek³, and Akiko Maeda^{3,4}

¹Yueyang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China.

²Clinical Research Institute of Integrative Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China.

³Department of Ophthalmology and Visual Sciences, Reserve University, Cleveland, OH44106

⁴Department of Pharmacology Case Western Reserve University, Cleveland, OH44106

Abstract

Vision is reliant upon converting photon signals to electrical information which is interpreted by the brain and therefore allowing us to receive information about our surroundings. However, when exposed to excessive light, photoreceptors and other types of cells in the retina can undergo light-induced cell death, termed light-induced retinal damage. In this review, we summarize our current knowledge regarding molecular events in the retina after excessive light exposure and mechanisms of light-induced retinal damage. We also introduce works which investigate potential roles of autophagy, an essential cellular mechanism required for maintaining homeostasis under stress conditions, in the illuminated retina and animal models of light-induced retinal damage.

Keywords

light-induced retinal damage; Beclin1; ATG7; Park2; all-*trans*-retinal; visual cycle

1. Introduction

Christian de Duve was the first to document lysosome-mediated intracellular degradation in 1963 and termed it autophagy which means “self-eating” in Greek (Klionsky, 2008). Autophagy is an evolutionarily conserved cellular process and in most cases characterized by the formation of specialized vacuoles termed autophagosomes. The initial stage of autophagy is the formation of the phagophore. The phagophore is a specialized membrane which sequesters intracellular cargo containing myriad cytoplasmic contents or organelles to form the autophagosome. Autophagosome formation in mammals requires microtubule-

To whom correspondence should be addressed: Akiko Maeda, M.D., Ph.D., Department of Ophthalmology and Visual Sciences, Case Western Reserve University, 10900 Euclid Ave, Cleveland, Ohio 44106-7286, USA; Phone: 216-368-0670, Fax: 216-368-1300, aam19@case.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

There is no conflict of interest.

associated protein 1 light chain 3 (LC3), a homologue of Apg8p essential for autophagy in yeast (Kabeya et al., 2000). LC3B has been associated with both canonical autophagy and LC3B-associated phagocytosis (LAP) which utilize double or single membrane phagophores respectively (Martinez et al., 2011). In order for double membrane autophagosome maturation to occur LC3B must undergo lipidation with phosphatidylethanolamine, therefore detection of modified LC3B (LC3B-II) is commonly used as an autophagosomal marker in molecular biology. Following fusion with a lysosome the contents of an autolysosome are degraded and recycled via lysosomal hydrolytic enzymes (Yang and Klionsky, 2010). Three major types of autophagy are unanimously recognized, namely macroautophagy, microautophagy, and chaperone-mediated autophagy (Choi et al., 2013). Macroautophagy, referred to as autophagy in this review, is the most extensively studied form and regarded as the major subtype of autophagy. Macroautophagy describes a sequence of cellular processes including the sequestration of cytoplasmic components into double-membrane autophagosomes, which then subsequently fuse with lysosomes. Mitophagy defines the selective targeting of damaged or dysfunctional mitochondria to autophagosomes for degradation through the canonical autophagy pathway. Basal level autophagy mainly preserves homeostatic functioning in the cell via protein and organelle turnover. Autophagy is highly inducible under starvation or various stress conditions and may assist in meeting the needs of increased intracellular nutrient and energy demand. In addition activated autophagy can result in elimination of misfolded or long-lived protein, superfluous or damaged organelles and invading microorganisms. Therefore, autophagy is an important cellular process in maintaining tissue homeostasis and disturbance of autophagy could be mechanistically implicated in the pathogenesis of various disease conditions (Levine and Kroemer, 2008). Moreover, increasing evidence has implied that multiple modes of crosstalk exist between autophagy and apoptosis. The balance between autophagy and apoptosis are dependent upon factors including cellular context, dose of stimuli and the activity of molecular switches contributing to both processes, etc. The net outcome of the complex crosstalk between autophagy and apoptosis may have significant impacts on determining the fate of cells, thus resulting in adaptive survival or death under stress conditions (Maiuri et al., 2007). One of the best characterized examples of the cross-regulation of autophagy and apoptosis is the molecular interaction of autophagic Beclin1 and anti-apoptotic Bcl-2 and Bcl-X_L (Liang et al., 1998). For instance, Beclin1-mediated autophagic activity is inhibited by Bcl-2 and Bcl-X_L under normal nutrient-rich conditions. However, when cells are undergoing starvation, the Beclin1 and Bcl-2 protein complex is disrupted leading to enhance autophagy, which thereby serves as a critical mechanism for coping with nutrient deprivation (He et al., 2012; Shimizu et al., 2004). Given that autophagy could function as a self-adaptive stress-response process, deficiency in autophagic genes can result in impaired autophagic activity and failed adaptation under stress conditions, which may increase the susceptibility to cell death.

The purpose of this review article is to highlight research pertaining to autophagy in the retina and the importance of functional autophagy in animal models of light-induced retinopathy. The works reviewed here support the notion that autophagy is an essential cellular mechanism required for maintaining retinal homeostasis under stress conditions.

2. Light-induced changes in the retina

2.1. Biochemical and morphological features of light-induced retinal degeneration

Excessive exposure to light can cause irreversible damage to the retina. Light-induced damage is mainly categorized into two types; thermal damage and photochemical damage. Thermal damage occurs when the retina is exposed to extremely bright light for a short duration of time. On the other hand, exposure to light for an extended period of time may result in photochemical damage. Photochemical damage is traditionally further divided into Noell damage (or Class I) (Noell et al., 1966) and Ham damage (or Class II) (Ham et al., 1978). Noell damage was initially examined in rats, and action spectrum analysis suggests involvement of the photo-pigment rhodopsin. Ham damage was initially reported to be associated with blue-light damage however now it is known that this type of damage is also caused by brief exposure to visible light with an action spectrum that matches that of rhodopsin absorption (van Norren and Gorgels, 2011). Recent accumulation of data suggests a complicated association between photochemical damage and light-induced retinal degeneration.

Roles of the visual/retinoid cycle are also known in pathogenesis of light-induced retinal degeneration. The visual cycle consists of a series of enzymatic reactions in the photoreceptors and the retinal pigment epithelial cells (RPE) to regenerate visual/rhodopsin chromophore, 11-*cis*-retinal (11-*cis*-RAL)(Fig. 1). Photoisomerization of the 11-*cis*-RAL chromophore of rhodopsin to all-*trans*-RAL triggers a complex set of metabolic transformations termed phototransduction which ultimately lead to light perception (Arshavsky et al., 2002; Palczewski, 2012). Photooxidation of all-*trans*-RAL and all-*trans*-retinol has been suggested as a possible sensitizer for light damage (Delmelle, 1977, 1979; Noell and Albrecht, 1971; Noell et al., 1966). An over-accumulation of all-*trans*-RAL may also lead to Noell type damage (Maeda et al., 2009)(please see more detail in the section of “All-trans-retinal and light-induced retinal degeneration”). Two molecules of all-*trans*-RAL and phosphatidylethanolamine produce A2E, and recent data has proposed that phototoxicity of A2E is generated by UV or blue light exposure (Delori et al., 1995; Sparrow et al., 2000). The speed at which the visual cycle regenerates 11-*cis*-RAL also plays an important role in light-induced retinal degeneration by modulating rhodopsin regeneration. Loss of RPE65 leads to protection from light-induced damage and pharmacologically inhibiting the speed of the visual cycle prevents light-induced retinal degeneration in mice (Grimm et al., 2000; Travis et al., 2007).

Furthermore, excessive phototransduction signaling can cause light-induced retinal degeneration. In 2002, a study conducted by Hao et al demonstrated that transducin/G-protein, essential for phototransduction signaling, contributes to light damage and that two different pathways, a bright-light pathway and a low-light dependent pathway, mediate light-induced retinal degeneration (Hao et al., 2002). Bright light induces apoptosis that is independent of transducin and accompanied by induction of the transcription factor AP-1. By contrast, low light induces an apoptotic pathway that requires transducin. They also confirmed the previous observation by Noell and colleagues that both pathways are initiated by excessive activation of the photo-pigment rhodopsin (Noell et al., 1966). In addition,

other genes involved in phototransduction signaling, for example rhodopsin kinase and arrestin, were found to regulate sensitivity to light-induced damage.

Other factors also affect sensitivity of light-induced retinal degeneration. RPE cells are sensitive to light-induced damage because they contain melanin, lipofuscin and retinoids which absorb light (Hunter et al., 2012; Wenzel et al., 2005). Conversely melanin has been observed to contribute a protective effect against light-induced damage by scavenging radicals (Rozanowski et al., 2008). Anatomical factors also affect the susceptibility to light induced damage. In wild type rodents, more prominent retinal degeneration is documented in the superior retina by light exposure (LaVail et al., 1987; Tanito et al., 2008). In contrast, some rodent models with genetic modifications displayed sensitivity of the inferior hemisphere to light-induced damage rather than the superior (Chen et al., 1999). The complexity underlying molecular mechanisms related to light induced retinal damage are still unclear and require further investigation. Light-induced damage may be modified by multifactorial effects, including different light sources, environmental insults, and genetic backgrounds as well as the nature of aging (Hunter et al., 2012; Wenzel et al., 2005).

2.2. All-trans-retinal and light-induced retinal degeneration

All-*trans*-retinal (all-*trans*-RAL) is a major intermediate of the visual cycle (Travis et al., 2007). Continuous regeneration of the chromophore, 11-*cis*-RAL, from all-*trans*-RAL, is essential for renewal of light-sensitive visual pigments, rhodopsin and cone opsin, in the vertebrate retina. Although all-*trans*-RAL is essential to maintain our eyesight, impairment of its metabolism is also detrimental. This reactive aldehyde forms condensation products including A2E, one of the main components of lipofuscin, when clearance from the retina is delayed (Parish et al., 1998). Age-dependent A2E accumulation in the RPE is recognized as a marker for ineffective all-*trans*-RAL clearance, even in healthy individuals (Delori et al., 2001). All-*trans*-RAL is also known to be a potent photosensitizer. All-*trans*-RAL can mediate the generation of superoxide radical anion ($O_2^{\cdot -}$), singlet oxygen (1O_2), and peroxides ($ROO^{\cdot -}$) when irradiated with UVA or blue light (Rozanowska and Sarna, 2005). Unless effective antioxidants and repair enzymes offer protection, reactive oxygen species (ROS) produced by all-*trans*-RAL can cause oxidative damage to lipids, proteins and DNAs that compromise their structures and functions.

Clearance of all-*trans*-RAL consists involves two processes; 1) Translocation of all-*trans*-RAL across photoreceptor disc membranes by ATP-binding cassette transporter 4 (ABCA4) (Tsybovsky et al.), and 2) Reduction of all-*trans*-RAL to all-*trans*-retinol by retinol dehydrogenases (RDHs), including RDH8, expressed in the outer segments of photoreceptors (Parker and Crouch) (Fig. 1). Deletion of these two critical enzymes in mice can cause retinal degeneration which is accelerated by light exposure. Furthermore, retinal degeneration in *Abca4*^{-/-}*Rdh8*^{-/-} mice can be prevented by all-*trans*-RAL buffering by amines (Maeda et al., 2012a). These critical findings indicate roles of all-*trans*-RAL in light-induced retinal damage.

2.3. Human disease relevance of light-induced retinal damage

Light is a double-edged sword in the visual system. Light is essential for visual perception but conversely light has detrimental effects on the retina under specific conditions. Light-induced retinal damage was initially noted in humans who presented with solar retinopathy after solar observations. This condition was recognized early in human history and acknowledged by the famous Greek philosopher Plato's *Phaedo* in the 4th century BC: "people may injure their bodily eye by observing and gazing on the sun during an eclipse." (Plato 360 BC, II 99d-e). The severity and permanent consequence of solar retinopathy was scientifically documented in 1988 (Hope-Ross et al., 1988). In this article, four cases of solar retinopathy were reported and all the patients suffered irreversible vision loss. Solar retinopathy in humans is typically observed in the cone-rich macular region since, with a functional pupil/lens, light is focused to this region. Therefore damage to this area predominately affects cones and night vision is not affected. Solar retinopathy is an example which demonstrates that excessive light is harmful to the retina. Sensitivity to light-induced damage is higher in rods than in cones and is demonstrated in many species (Organisciak and Vaughan, 2010). Retinal light damage has been investigated under experimental conditions to model human retinal degenerations arising from environmental insult, aging and genetic disease, based primarily on similar end point morphologies. For instance the dominant pathology in light-induced retinal damage, degeneration and death of photoreceptor cells, is the cause of vision loss in an array of retinal disorders, including retinitis pigmentosa (RP), geographic atrophy in dry age-related macular degeneration (AMD) and neovascular AMD. Intense light exposure was originally shown to cause irreversible photoreceptor cell damage in rats (Noell et al., 1966), which has since been extensively reproduced in various other species and adopted as an experimental paradigm in evaluating the mechanisms of photoreceptor death and protective strategies. Many model animals of human retinal degenerative diseases, including mice with genetic mutations of *Rhodopsin*, *Rdh12*, *Abca4*, *Cralbp*, *Mertk*, *Crb1*, and *peripherin/Rds*, show light-dependent acceleration of retinal damage or modified severity of diseases by illumination (Cideciyan et al., 2005; Maeda et al., 2009; Maeda et al., 2008; Maeda et al., 2006; Paskowitz et al., 2006; Saari et al., 2001; Tam and Moritz, 2007). Therefore, understanding the events associated with light damage is important to develop therapeutics for retinal degeneration in humans.

3. Autophagic events in the retina

3.1. Autophagic events detected in the retina under steady-state conditions

Autophagy is increasingly recognized as a protective mechanism employed by cells to maintain homeostasis in healthy tissues and to protect tissues from damage in various degenerative conditions (Choi et al., 2013; Levine and Kroemer, 2008). The morphological documentation of autophagic events in RPE cells and photoreceptors was first derived from examining the retinas of ground squirrels under active and hibernating conditions (Reme and Young, 1977). In this seminal article, the authors described mitochondria at various stages of degradation within autophagic structures. Mitochondrial degeneration, via autophagy in the ellipsoid portion of the cone cell inner segment, was documented under active conditions. Autophagic vacuoles were also noted in the myoid of cone cell inner segments. The outer part of the fiber extending from the outer limiting membrane to the nucleus was

found to be densely packed with a heterogeneous population of vesicles, most of which appeared to be autophagic vacuoles. The central regions of microtubules were prominently dominated by autophagic vacuoles of various sizes. In RPE cells, autophagic vacuoles engulfing partially degraded smooth endoplasmic reticulum were also noted. Under hibernating conditions, increased numbers of mitochondria in the ellipsoid portion of the cone outer segment were observed to be undergoing autophagy-mediated degradation. Autophagic vacuoles were found to progressively enclose cellular contents. However, in RPE cells, autophagic events were rarely identified under hibernation. Additional studies further confirmed that autophagy is ubiquitous in RPE and photoreceptor cells under normal conditions in various species (Remé, 1977), which therefore generated a general profile of autophagy in these cell types. Autophagy is widely distributed in RPE cytoplasm except for the basal infoldings and apical cell processes. In photoreceptor cells, it is frequently encountered in the myoid, ellipsoid and perinuclear region of inner segment, where the major synthetic pathways are localized. Autophagy is observed less frequently in connecting fibers between nuclei and synaptic bodies, and has not been documented in outer segment. In addition, autophagosomes in RPE and photoreceptor cells contain all types of cytoplasmic constituents and invariably display the same range of morphological features regardless of cell type or species. These pioneering observations suggest that autophagy is part of a basal cellular process common to RPE and photoreceptor cells (Fig. 2). Note that all of the major content of photoreceptor cells undergoes continuous renewal and the majority of molecules in RPE cells should be perpetually replaced. In addition to daily phagosomal handling of shed outer segment tips from rod photoreceptor cells, autophagy is speculated as a plausible mechanism implicated in the renewal of cellular constituents in photoreceptor and RPE cells. Additionally, autophagy related proteins, including ATG5/ATG12 and ATG7, have recently been shown to display cyclic expression patterns indicating that autophagy may be regulated by circadian rhythm and daily phagocytosis of shed outer segments in RPE cells (Kim et al., 2013; Yao et al., 2014).

Recently the expression of genes that are important regulators of autophagy was shown to be readily detected in the mouse retina (Chen et al., 2013). The mRNA expression of autophagy-related genes (Atg) including *Atg5*, *Atg7*, and *Beclin1* is abundantly present in the mouse retina. Autophagy in the mouse retina was also examined biochemically by analyzing the level of LC3B. During autophagy nascent LC3B (proLC3B) is processed by ATG4 into LC3B-I upon synthesis and thus is undetectable under normal conditions. LC3B-I is localized in the cytosol, and conjugation with phosphatidylethanolamine enables conversion of LC3B-I to LC3B-II, which is mostly present on isolated membranes and autophagosomes serving as a widely adopted marker for autophagosome formation. Therefore, the amount of LC3B-II is commonly used to monitor autophagic activity (Mizushima and Yoshimori, 2007). LC3B was shown to be ubiquitously present in the mouse retina with prominent cellular and subcellular localization in RPE, photoreceptor inner segments (IS), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), and ganglion cells (GC). Both LC3B-I and LC3B-II forms of LC3B could be detected in the mouse retina under steady state (Chen et al., 2013).

3.2. Changes in autophagic events in the illuminated retina

Enhanced autophagic events have been documented under light exposure conditions which cause damage in RPE and photoreceptor cells (Remé et al., 1999). In this early study, the number of autophagic vacuoles increased significantly after switching from 3 Lux to 200 Lux at days 1 and 2 and declined at day 3, whereas the reverse intensity change did not cause any increase. This observation strongly suggests roles of autophagy to cope with photo-induced stress responses. Recent studies utilizing both *in vitro* and *in vivo* techniques have suggested a role for autophagy in both RPE and neural retina homeostasis during aging and light insult. Work in 661W cells, a cone derived cell line, demonstrated that the autophagy activator rapamycin significantly decreases light induced endoplasmic reticulum (ER) stress and cell death (Li et al., 2014). Autophagy is highly reliant upon the lysosomal pathway for degradation of autophagosome contents and defects in RPE lysosomal clearance has been linked to increased inflammation and retinal degeneration (Valapala et al., 2014). Similarly RPE conditional knockout of the essential macroautophagy gene RB1-inducible coiled-coil 1 (RB1CC1) in mouse led to age-dependent RPE death and accumulation of drusen-like deposits ultimately leading to the loss of photoreceptors thus suggesting a role for autophagy in retinal homeostasis (Yao et al., 2015). *In vitro* experimentations with cultured ARPE19 cells, a human-derived RPE cell line, demonstrated a dynamic change of autophagic events that is inversely correlated with the dose-dependent cytotoxicity of all-*trans*-RAL, a photo-activated visual chromophore associated with light-induced retinal toxicity (Maeda et al., 2012b). Accumulation of bisretinoids, found commonly in patients with macular degeneration, has been shown to negatively affect autophagy in RPE cells, cause cholesterol buildup and ultimately lead to cell dysfunction (Toops et al., 2015). In the *Abca4*^{-/-}*Rdh8*^{-/-} mouse model, which exhibits increased susceptibility to light-induced retinopathy (Maeda et al., 2009), an increase in LC3B-II conversion is observed prior to the extensive loss of the photoreceptor cells after light exposure, suggesting that increased autophagosome formation is an early event during light-induced photoreceptor degeneration (Chen et al., 2013). Though examining LC3-II and LC3-I by Western blot has been a golden standard for measuring autophagy, additional measurements including the autophagy receptor protein p62/SQSTM1 have been suggested as an indicator of autophagic activity (Klionsky et al., 2012). Furthermore, measurements of LC3 and p62/SQSTM1 in the presence or absence of pharmacological lysosomal blockade (chloroquine or bafilomycin A1) provides critical information about the rate of transit of autophagosome cargo through lysosomal degradation (Gottlieb et al., 2015). Autophagy in retinal cells was clearly observed in ARPE19 cells examined in the presence or absence of bafilomycin A1 (Chen et al., 2013).

Mouse models lacking autophagy modulators, such as Beclin1 and parkin RBR E3 ubiquitin protein ligase (Park2), are highly susceptible to light induced damage therefore suggesting that autophagy is a protective mechanism against cell death (Chen et al., 2013a). Other work suggests that loss of Atg5 in photoreceptor cells leads to retinal degeneration and dysregulation of phototransduction protein turnover independent of light exposure (Rodriguez-Muela et al., 2013; Zhou et al., 2015). Excessive light exposure in the retina could potentially alter the metabolic needs of retinal tissue thereby facilitating increased autophagy to meet these demands; on the other hand perhaps increased autophagy is a result

of autophagic programmed cell death and merely a secondary event in light damage (Shimizu et al., 2004). Based on the current literature light insult in the retina and RPE activates specific autophagy pathways but it is still poorly understood whether these pathways are involved in cell protection or the induction of autophagic cell death. (Rodriguez-Muela et al., 2013; Shimizu et al., 2004; Zhou et al., 2015)

4. Autophagy regulates light-induced photoreceptor cell death in *Drosophila*

The *Drosophila* visual system has proven to be a powerful genetic model for dissecting the molecular mechanisms underlying neurodegenerative diseases including light-induced retinal degeneration (Alloway et al., 2000; Bilen and Bonini, 2005; Kiselev et al., 2000; Wang et al., 2010). Using the *Drosophila* visual system, Wong et al discovered that activation of TOR signaling leads to photoreceptor cell death in an age- and light-dependent manner, and this is mediated via the suppression of autophagy (Wang et al., 2009). Their study also revealed that autophagy induction greatly suppresses light-induced retinal degeneration by mediating the turnover of toxic rhodopsin–arrestin complexes generated during phototransduction.

Roles of autophagy in rhodopsin-mediated light induced photoreceptor cell death was further investigated and reported by Midorikawa et al (Midorikawa et al., 2010). They demonstrated that light-dependent retinal degeneration in the *Drosophila* visual system is caused by the knockdown or mutation of autophagy-essential components, such as *Atg7* and *Atg8*, or genes essential for PE (phosphatidylethanolamine) biogenesis and autophagosome formation, including phosphatidylserine decarboxylase (*Psd*) and CDP-ethanolamine:diacylglycerol ethanolaminephosphotransferase (*Ept*). Additionally they provided evidence that accumulation of rhodopsin is localized in Rab7-positive late endosomes, and overexpression of Rab7 reduces rhodopsin accumulation in the ER and therefore protects photoreceptors from degeneration. This study provided the first evidence that degradation of rhodopsin occurs via cross talk between the autophagy and endosomal degradation pathways, and that autophagy acts as a compensatory pathway when the endosomal/lysosomal pathway is impaired. Accumulating evidence collected by studies with *Drosophila* implies that autophagy is an important regulator for light-induced photoreceptor degeneration.

5. Autophagy gene deficiency and light-induced retinal degeneration in mice

Table 1 shows the summary of mouse studies for autophagy.

5.1. Beclin1 deficiency increases the susceptibility of light-induced retinal degeneration

Autophagy is tightly regulated and mediated by an array of autophagy-related proteins over 30 of which have been identified to date. *Beclin1* is the mammalian ortholog of the yeast *Atg6/Vps30* gene. It is one of the essential regulators of autophagy functioning at the initial stage of autophagosome formation (Aita et al., 1999; Liang et al., 1999). Beclin1 interacts

with anti-apoptotic protein Bcl-2 and this interaction inhibits Beclin1-dependent autophagic activity in yeast and mammalian cells (Pattingre et al., 2005). *Beclin1* homozygosity is embryonically lethal in mice (Yue et al., 2003). *Beclin1* heterozygosity is characterized by decreased autophagosome formation in various cell types (Qu et al., 2003; Yue et al., 2003). *Beclin1* gene deficiency in mice results in a predisposition to tumorigenesis and thus initiated investigation to the biological significance of autophagy (Qu et al., 2003). *Beclin1* deficiency has since been shown to be involved in the pathogenesis of Alzheimer disease in mice, demonstrating the significance of autophagy in neurodegeneration (Pickford et al., 2008). Beclin1 expression could be readily detected in the mouse retina at both the mRNA and protein level (Chen et al., 2013). Immunohistochemistry further localized expression of Beclin1 to RPE, ONL, OPL, INL and GC layers. *Beclin1*^{+/-} mice displayed normal retinal morphology compared to that from *Beclin1*^{+/+} mice (Fig. 3). When bright light is applied at the intensity of 5,000 Lux for 2 hours, *Beclin1*^{+/-} mice develop retinal degeneration prominently in the superior retina, which is characterized by severely disrupted OS, IS and ONL structure (Fig. 4). Light-induced damage of photoreceptors was also marked by diminished expression of rhodopsin and PNA immunohistochemical staining in *Beclin1*^{+/-} mice, validating the loss of rod and cone photoreceptor cells as a result of *Beclin1* heterozygosity. In addition, high magnification electron microscopy demonstrated that in photoreceptors and RPE cells, swollen mitochondria with disintegration of cristae are frequently encountered as a result of *Beclin1* heterozygosity. Moreover, endoplasmic reticulum (ER) was observed to be extremely dilated causing vacuolation of the RPE in light-exposed *Beclin1*^{+/-} mice. *Beclin1*^{+/-} RPE nucleus displayed heterochromatin and vacuolation 24 hours after light exposure and RPE hyperplasia marked by multiple nuclei and RPE apoptosis indicated by condensation of the chromatin in large masses at the periphery of the nucleus was noted in *Beclin1*^{+/-} mice 10 days after light exposure. *Beclin1*^{+/-} RPE cells were highly vacuolated as a result of increased ER luminal space (Chen et al., 2013), indicating ER stress. These results indicate that both photoreceptor and RPE cells are impacted by *Beclin1* gene deficiency and hence loss of Beclin1 may alter the retinal response to photo-oxidative stress. These observations therefore imply the essentiality of intact autophagy machinery for preserving photoreceptor and RPE integrity under photo-oxidative stress conditions.

5.2. ATG7 in light-induced retinal degeneration

Of the many ATG genes, seven are known to constitute two ubiquitin-like conjugation systems, ATG12/ATG8 conjugation systems, which are essential for autophagy (Mizushima et al., 1998). The conjugation of ubiquitin-like protein ATG12p to ATG5p occurs in a stepwise manner. ATG12p is first activated by an E1-like enzyme, ATG7p (Tanida et al., 1999), transferred to an E2-like enzyme, ATG10p, and then covalently attached to ATG5p. ATG8p, another ubiquitin-like protein, however, conjugates to phosphatidylethanolamine. ATG8p is activated by ATG7p and is transferred to ATG3p, an E2-like enzyme (Ichimura et al., 2000). In mammalian cells three known ATG8 homologues, GATE-16, GABARAP, and LC3B, can be activated by ATG7 and localized to the autophagosome. Therefore, by activating both of the above mentioned ubiquitin-like proteins, ATG7 plays an important role in the process of autophagosome formation. In mice the global knockout of *Atg7* results in early neonatal death (Komatsu et al., 2005). Mice lacking *Atg7* specifically in the central

nervous system display behavioral defects due to massive neuronal loss in the cerebral and cerebellar cortices (Komatsu et al., 2006). To investigate the function of Atg7 in photoreceptors in the murine system, rod photoreceptor specific knockout of *Atg7* gene (*Atg7^{flox/flox};LMOP-Cre⁺*) was created (Chen et al., 2013). Rod specific deletion of *Atg7* did not cause overt retinal abnormality under normal laboratory lighting conditions at 3 and 6 months of age. However, when challenged by bright light exposure at 5,000 Lux for 2 hours, *Atg7^{flox/flox};LMOP-Cre⁺* mice exhibited features of photoreceptor degeneration with decreased numbers of ONL along with disrupted photoreceptor structure. Moreover, in retinal explant cultures, co-incubation with all-*trans*-RAL, photo-activated visual chromophore, caused a higher rate of retinal cell death in the retinas collected from *Atg7^{flox/flox};LMOP-Cre⁺* mice compared with that from *Atg7^{flox/flox};LMOP-Cre⁻* mice, indicating that ATG7 plays a protective role in rod photoreceptors under stresses caused by light and all-*trans*-RAL, respectively.

Most recently, rod photoreceptor specific *Atg5* knockout mice (*Atg5^{flox/flox};iCre75-Cre⁺*) were established, and the role of *Atg5* in light-induced retinal degeneration was examined (Zhou et al., 2015). *Atg5^{flox/flox};iCre75-Cre⁺* mice exhibited age-related retinal degeneration, however, retinal degeneration in this mouse was not altered by dark rearing or accelerated by intense light exposure at 13,000 Lux for 7 hours. ATG5, like ATG7, is an essential molecule for autophagy, and mice deficient in *Atg5* or *Atg7* die soon after birth (Kuma et al., 2004). It is worth noting that *Atg5^{flox/flox};iCre75* mice carry a natural p.L450M mutation of Rpe65. RPE65 is one of the key enzymes of the visual cycle and is responsible for the conversion of all-*trans*-retinyl ester to 11-*cis*-retinal in RPE cells. Mice with the p.L450M mutation have a 5-10 fold slower activity of this enzyme resulting in slow regeneration of visual chromophore 11-*cis*-retinal (Danciger et al., 2004; Lyubarsky et al., 2005). *Atg5^{flox/flox};iCre75* mice are more resistant to light-induced damage, and therefore different results could be observed between mice with *Atg5* deletion and with *Atg7* deficiency. Additionally deletion of *Atg5* in rod cells leads to decreased phototransduction via depletion of transducin- α suggesting a role for ATG5 in transducin- α recycling (Zhou et al., 2015). Decreased phototransduction also protects the retina from light-induced retinal degeneration (Hao et al., 2002).

Considering the complex and multifactorial nature of mechanisms involved in light-induced retinal degeneration, phenotypic differences of retinal degeneration in rod photoreceptor specific *Atg5* knockout and *Atg7* knockout mice raise additional questions as to how autophagy modulates stress responses associated with different retinal degenerative pathologies.

6. Mitophagy gene Park2 is required for the retina to cope with the stress upon bright light exposure

Mitochondria are the master regulators of cells due to their essential functions in energy production and critical role in promoting apoptosis. Autophagy has been demonstrated as one of the regulatory mechanisms for mitochondrial quality control, specifically involved in the removal and subsequent recycling of damaged or excess mitochondria in the cell. This autophagy-mediated elimination of mitochondria is defined as mitophagy (Ashrafi and

Schwarz, 2013). Mitophagy is specifically regulated at the molecular level by several molecules including Park2. The function of Park2 in maintaining mitochondrial integrity has been supported by Park2 acting in parallel with the PTEN-induced putative kinase1 (PINK1) to facilitate autophagy-mediated mitochondrial clearance of damaged and defective mitochondria. Park2 selectively accumulates in the depolarized and fragmented mitochondria and ubiquitinates mitochondrial proteins, which is followed by induction of autophagosome formation. Park2 is recruited preferentially to electrochemically compromised mitochondria compared to electrochemically active mitochondria, supporting the selective targeting of Park2 to impaired mitochondria. Park2 is recruited to damaged mitochondria not only in cultured cell lines (Narendra et al., 2008) but also in primary neurons (Wang et al., 2011). Healthy mitochondria produce ATP by the respiratory chain complex and thus, generate a membrane potential. The mitochondrial membrane potential is essential for membrane structure and indispensable for functional maintenance of mitochondria. Disruption of mitochondrial potential leads to mitochondrial fragmentation. Depolarized mitochondria are captured by autophagosomes and subsequently removed via autophagy-mediated degradation. Park2 has been shown to be a critical mediator in this form of selective clearance of defective mitochondria. In cells without Park2 expression, little colocalization of mitochondria and autophagosomes were observed, while in cells expressing Park2, this colocalization occurs under mitochondrial depolarization conditions. Furthermore, Park2-mediated mitochondria elimination is autophagy-dependent and requires intact function of autophagy since Park2 failed to clear depolarized mitochondria in the absence of autophagy machinery (Narendra et al., 2008). The functional significance of Park2-mediated elimination of damaged mitochondria is to protect cells from the adverse effects of damaged mitochondria by removing these severely damaged organelles. Increased expression of Park2 suppresses cell death and Park2 deficiency is associated with mitochondrial dysfunction and increase in intracellular oxidative stress in Parkinson's patients and related animal models (Fishman and Oyler, 2002; Kitada et al., 1998; Lucking et al., 2000; Periquet et al., 2003).

In the mouse retina, Park2 is localized in various cell types, including RPE cells, photoreceptor ONL and IS (Chen et al., 2013; Esteve-Rudd et al., 2010). The expression of Park2 protein is significantly increased by introducing damaging light in *Abca4*^{-/-}*Rdh8*^{-/-} mice suggesting that Park2 is involved in photoreceptor degeneration (Chen et al., 2013). Under regular laboratory lighting conditions, *Park2*^{-/-} mice displayed normal retinal morphologies comparable to that seen in *Park2*^{+/+} counterparts. However, severe photoreceptor damage is induced when *Park2*^{-/-} mice are exposed to the intermittent light delivered at 10,000 Lux for 20 min with an interval of 2-hour dark adaptation, manifesting massive disorganization of photoreceptor OS and IS, reduction in the thickness of ONL, the presence of pyknotic photoreceptor nuclei, and nucleic acid deposition. Ultrastructural examination revealed that numerous phagocytized photoreceptor OS at different stages of phagocytic clearance in *Park2*^{-/-} RPE cells 24 hours after light exposure and the presence of juxtanuclear mitochondria in photoreceptor cells 7 days after light exposure. These findings indicate that defective mitophagy may result from *Park2* gene deficiency. More importantly, these observations supported the notion that Park2-mediated mitochondrial quality control is required for protection against photo-oxidative stress in the retina.

7. Conclusion

Autophagy is basally present in the retina. Deficiencies in genes related to autophagy or mitophagy have been shown to be causatively implicated in the pathogenesis of light-induced retinopathy. Independently or through cross-regulation with apoptosis, autophagy may exert important functions in maintaining retinal homeostasis under photo-oxidative stress conditions. Strategies promoting autophagy and mitophagy could have therapeutic significance in treating related degenerative retinal disorders.

Acknowledgements

We thank Dr. K. Palczewski and members of Maeda laboratory for their comments. This work was supported by funding from the National Eye Institute of the National Institutes of Health (EY022658, EY021126 and EY11373), Research to Prevent Blindness Foundation, and Ohio Lions Eye Research Foundation.

Abbreviations

A2E	<i>N</i> -retinylidene- <i>N</i> -retinylethanolamine
ATG	autophagy-related gene
RAL	retinal
RPE	retinal pigment epithelium
ROL	retinol

References

- Aita VM, Liang XH, Murty VVVS, Pincus DL, Yu W, Cayanis E, Kalachikov S, Gilliam TC, Levine B. Cloning and Genomic Organization of Beclin 1, a Candidate Tumor Suppressor Gene on Chromosome 17q21. *Genomics*. 1999; 59:59–65. [PubMed: 10395800]
- Alloway PG, Howard L, Dolph PJ. The formation of stable rhodopsin-arrestin complexes induces apoptosis and photoreceptor cell degeneration. *Neuron*. 2000; 28:129–138. [PubMed: 11086989]
- Arshavsky VY, Lamb TD, Pugh EN Jr. G proteins and phototransduction. *Annual review of physiology*. 2002; 64:153–187.
- Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. *Cell death and differentiation*. 2013; 20:31–42. [PubMed: 22743996]
- Bilen J, Bonini NM. *Drosophila* as a model for human neurodegenerative disease. *Annual review of genetics*. 2005; 39:153–171.
- Chen J, Simon MI, Matthes MT, Yasumura D, LaVail MM. Increased susceptibility to light damage in an arrestin knockout mouse model of Oguchi disease (stationary night blindness). *Investigative ophthalmology & visual science*. 1999; 40:2978–2982. [PubMed: 10549660]
- Chen Y, Sawada O, Kohno H, Le YZ, Subauste C, Maeda T, Maeda A. Autophagy protects the retina from light-induced degeneration. *The Journal of biological chemistry*. 2013; 288:7506–7518. [PubMed: 23341467]
- Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. *The New England journal of medicine*. 2013; 368:651–662. [PubMed: 23406030]
- Cideciyan AV, Jacobson SG, Aleman TS, Gu D, Pearce-Kelling SE, Sumaroka A, Acland GM, Aguirre GD. In vivo dynamics of retinal injury and repair in the rhodopsin mutant dog model of human retinitis pigmentosa. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:5233–5238. [PubMed: 15784735]

- Danciger M, Lyon J, Worrill D, Hoffman S, Lem J, Reme CE, Wenzel A, Grimm C. New retinal light damage QTL in mice with the light-sensitive RPE65 LEU variant. *Mammalian genome : official journal of the International Mammalian Genome Society*. 2004; 15:277–283. [PubMed: 15112105]
- Delmelle M. Retinal damage by light: possible implication of singlet oxygen. *Biophysics of structure and mechanism*. 1977; 3:195–198. [PubMed: 890057]
- Delmelle M. Possible implication of photooxidation reactions in retinal photo-damage. *Photochemistry and photobiology*. 1979; 29:713–716. [PubMed: 221949]
- Delori FC, Dorey CK, Staurengi G, Arend O, Goger DG, Weiter JJ. In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. *Investigative ophthalmology & visual science*. 1995; 36:718–729. [PubMed: 7890502]
- Delori FC, Goger DG, Dorey CK. Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. *Investigative ophthalmology & visual science*. 2001; 42:1855–1866. [PubMed: 11431454]
- Esteve-Rudd J, Campello L, Herrero MT, Cuenca N, Martin-Nieto J. Expression in the mammalian retina of parkin and UCH-L1, two components of the ubiquitin proteasome system. *Brain research*. 2010; 1352:70–82. [PubMed: 20638372]
- Fishman PS, Oyler GA. Significance of the parkin gene and protein in understanding Parkinson's disease. *Current neurology and neuroscience reports*. 2002; 2:296–302. [PubMed: 12044248]
- Gottlieb RA, Andres AM, Sin J, Taylor DP. Untangling autophagy measurements: all fluxed up. *Circulation research*. 2015; 116:504–514. [PubMed: 25634973]
- Grimm C, Wenzel A, Hafezi F, Yu S, Redmond TM, Reme CE. Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. *Nature genetics*. 2000; 25:63–66. [PubMed: 10802658]
- Ham WT Jr, Ruffolo JJ Jr, Mueller HA, Clarke AM, Moon ME. Histologic analysis of photochemical lesions produced in rhesus retina by short-wave-length light. *Investigative ophthalmology & visual science*. 1978; 17:1029–1035. [PubMed: 100464]
- Hao W, Wenzel A, Obin MS, Chen CK, Brill E, Krasnoperova NV, Eversole-Cire P, Kleyner Y, Taylor A, Simon MI, et al. Evidence for two apoptotic pathways in light-induced retinal degeneration. *Nature genetics*. 2002; 32:254–260. [PubMed: 12219089]
- He C, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, An Z, Loh J, Fisher J, Sun Q, et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature*. 2012; 481:511–515. [PubMed: 22258505]
- Hope-Ross M, Travers S, Mooney D. Solar retinopathy following religious rituals. *The British journal of ophthalmology*. 1988; 72:931–934. [PubMed: 3228548]
- Hunter JJ, Morgan JI, Merigan WH, Sliney DH, Sparrow JR, Williams DR. The susceptibility of the retina to photochemical damage from visible light. *Progress in retinal and eye research*. 2012; 31:28–42. [PubMed: 22085795]
- 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. [PubMed: 11100732]
- Kim JY, Zhao H, Martinez J, Doggett TA, Kolesnikov AV, Tang PH, Ablonczy Z, Chan CC, Zhou Z, Green DR, Ferguson TA. Noncanonical autophagy promotes the visual cycle. *Cell*. 2013; 154:365–376. [PubMed: 23870125]
- Kiselev A, Socolich M, Vinos J, Hardy RW, Zuker CS, Ranganathan R. A molecular pathway for light-dependent photoreceptor apoptosis in *Drosophila*. *Neuron*. 2000; 28:139–152. [PubMed: 11086990]
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*. 1998; 392:605–608. [PubMed: 9560156]
- Klionsky DJ. Autophagy revisited: a conversation with Christian de Duve. *Autophagy*. 2008; 4:740–743. [PubMed: 18567941]
- Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy*. 2012; 8:445–544. [PubMed: 22966490]

- Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*. 2006; 441:880–884. [PubMed: 16625205]
- 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. *The Journal of cell biology*. 2005; 169:425–434. [PubMed: 15866887]
- 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. [PubMed: 15525940]
- LaVail MM, Gorin GM, Repaci MA, Yasumura D. Light-induced retinal degeneration in albino mice and rats: strain and species differences. *Progress in clinical and biological research*. 1987; 247:439–454. [PubMed: 3685038]
- Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008; 132:27–42. [PubMed: 18191218]
- Li GY, Fan B, Jiao YY. Rapamycin attenuates visible light-induced injury in retinal photoreceptor cells via inhibiting endoplasmic reticulum stress. *Brain research*. 2014; 1563:1–12. [PubMed: 24607296]
- Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*. 1999; 402:672–676. [PubMed: 10604474]
- Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, Herman B, Levine B. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *Journal of virology*. 1998; 72:8586–8596. [PubMed: 9765397]
- Lucking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Deneffe P, Wood NW, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. *The New England journal of medicine*. 2000; 342:1560–1567. [PubMed: 10824074]
- Lyubarsky AL, Savchenko AB, Morocco SB, Daniele LL, Redmond TM, Pugh EN Jr. Mole quantity of RPE65 and its productivity in the generation of 11-cis-retinal from retinyl esters in the living mouse eye. *Biochemistry*. 2005; 44:9880–9888. [PubMed: 16026160]
- Maeda A, Golczak M, Chen Y, Okano K, Kohno H, Shiose S, Ishikawa K, Harte W, Palczewska G, Maeda T, Palczewski K. Primary amines protect against retinal degeneration in mouse models of retinopathies. *Nature chemical biology*. 2012a; 8:170–178. [PubMed: 22198730]
- Maeda A, Maeda T, Golczak M, Chou S, Desai A, Hoppel CL, Matsuyama S, Palczewski K. Involvement of all-trans-retinal in acute light-induced retinopathy of mice. *The Journal of biological chemistry*. 2009; 284:15173–15183. [PubMed: 19304658]
- Maeda A, Maeda T, Golczak M, Palczewski K. Retinopathy in mice induced by disrupted all-trans-retinal clearance. *The Journal of biological chemistry*. 2008; 283:26684–26693. [PubMed: 18658157]
- Maeda A, Maeda T, Imanishi Y, Sun W, Jastrzebska B, Hatala DA, Winkens HJ, Hofmann KP, Janssen JJ, Baehr W, et al. Retinol dehydrogenase (RDH12) protects photoreceptors from light-induced degeneration in mice. *The Journal of biological chemistry*. 2006; 281:37697–37704. [PubMed: 17032653]
- Maeda T, Golczak M, Maeda A. Retinal photodamage mediated by all-trans-retinal. *Photochemistry and photobiology*. 2012b; 88:1309–1319. [PubMed: 22428905]
- Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nature reviews. Molecular cell biology*. 2007; 8:741–752. [PubMed: 17717517]
- Midorikawa R, Yamamoto-Hino M, Awano W, Hinohara Y, Suzuki E, Ueda R, Goto S. Autophagy-dependent rhodopsin degradation prevents retinal degeneration in *Drosophila*. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2010; 30:10703–10719. [PubMed: 20702701]
- Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, Ohsumi Y. A protein conjugation system essential for autophagy. *Nature*. 1998; 395:395–398. [PubMed: 9759731]

- Mizushima N, Yoshimori T. How to interpret LC3 immunoblotting. *Autophagy*. 2007; 3:542–545. [PubMed: 17611390]
- Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *The Journal of cell biology*. 2008; 183:795–803. [PubMed: 19029340]
- Noell WK, Albrecht R. Irreversible effects on visible light on the retina: role of vitamin A. *Science (New York, N.Y.)*. 1971; 172:76–79.
- Noell WK, Walker VS, Kang BS, Berman S. Retinal damage by light in rats. *Investigative ophthalmology*. 1966; 5:450–473. [PubMed: 5929286]
- Organisciak DT, Vaughan DK. Retinal light damage: mechanisms and protection. *Progress in retinal and eye research*. 2010; 29:113–134. [PubMed: 19951742]
- Palczewski K. Chemistry and biology of vision. *The Journal of biological chemistry*. 2012; 287:1612–1619. [PubMed: 22074921]
- Parish CA, Hashimoto M, Nakanishi K, Dillon J, Sparrow J. Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:14609–14613. [PubMed: 9843937]
- Parker RO, Crouch RK. Retinol dehydrogenases (RDHs) in the visual cycle. *Experimental eye research*. 2010; 91:788–792. [PubMed: 20801113]
- Paskowitz DM, LaVail MM, Duncan JL. Light and inherited retinal degeneration. *The British journal of ophthalmology*. 2006; 90:1060–1066. [PubMed: 16707518]
- Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B. Bcl-2 Antiapoptotic Proteins Inhibit Beclin 1-Dependent Autophagy. *Cell*. 2005; 122:927–939. [PubMed: 16179260]
- Periquet M, Latouche M, Lohmann E, Rawal N, De Michele G, Ricard S, Teive H, Fraix V, Vidailhet M, Nicholl D, et al. Parkin mutations are frequent in patients with isolated early-onset parkinsonism. *Brain : a journal of neurology*. 2003; 126:1271–1278. [PubMed: 12764050]
- 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 β accumulation in mice. *The Journal of Clinical Investigation*. 2008; 118:2190–2199. [PubMed: 18497889]
- Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *The Journal of clinical investigation*. 2003; 112:1809–1820. [PubMed: 14638851]
- Remé CE. Autophagy in visual cells and pigment epithelium. *Investigative Ophthalmology & Visual Science*. 1977; 16:807–814. [PubMed: 302253]
- Remé CE, Wolfrum U, Imsand C, Hafezi F, Williams TP. Photoreceptor Autophagy: Effects of Light History on Number and Opsin Content of Degradative Vacuoles. *Investigative Ophthalmology & Visual Science*. 1999; 40:2398–2404. [PubMed: 10476808]
- Reme CE, Young RW. The effects of hibernation on cone visual cells in the ground squirrel. *Investigative ophthalmology & visual science*. 1977; 16:815–840. [PubMed: 893032]
- Rozanowska M, Sarna T. Light-induced damage to the retina: role of rhodopsin chromophore revisited. *Photochem Photobiol*. 2005; 81:1305–1330. [PubMed: 16120006]
- Rozanowski B, Burke JM, Boulton ME, Sarna T, Rozanowska M. Human RPE melanosomes protect from photosensitized and iron-mediated oxidation but become pro oxidant in the presence of iron upon photodegradation. *Investigative ophthalmology & visual science*. 2008; 49:2838–2847. [PubMed: 18326697]
- Saari JC, Nawrot M, Kennedy BN, Garwin GG, Hurley JB, Huang J, Possin DE, Crabb JW. Visual cycle impairment in cellular retinaldehyde binding protein (CRALBP) knockout mice results in delayed dark adaptation. *Neuron*. 2001; 29:739–748. [PubMed: 11301032]
- Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, Tsujimoto Y. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nature cell biology*. 2004; 6:1221–1228. [PubMed: 15558033]

- Sparrow JR, Nakanishi K, Parish CA. The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. *Investigative ophthalmology & visual science*. 2000; 41:1981–1989. [PubMed: 10845625]
- Tam BM, Moritz OL. Dark rearing rescues P23H rhodopsin-induced retinal degeneration in a transgenic *Xenopus laevis* model of retinitis pigmentosa: a chromophore-dependent mechanism characterized by production of N-terminally truncated mutant rhodopsin. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27:9043–9053. [PubMed: 17715341]
- Tanida I, Mizushima N, Kiyooka M, Ohsumi M, Ueno T, Ohsumi Y, Kominami E. Apg7p/Cvt2p: A novel protein-activating enzyme essential for autophagy. *Molecular biology of the cell*. 1999; 10:1367–1379. [PubMed: 10233150]
- Tanito M, Kaidzu S, Ohira A, Anderson RE. Topography of retinal damage in light-exposed albino rats. *Experimental eye research*. 2008; 87:292–295. [PubMed: 18586030]
- Toops KA, Tan LX, Jiang ZC, Radu RA, Lakkaraju A. Cholesterol-mediated activation of acid sphingomyelinase disrupts autophagy in the retinal pigment epithelium. *Molecular biology of the cell*. 2015; 26:1–14. [PubMed: 25378587]
- Travis GH, Golczak M, Moise AR, Palczewski K. Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. *Annual review of pharmacology and toxicology*. 2007; 47:469–512.
- Tsybovsky Y, Molday RS, Palczewski K. The ATP-binding cassette transporter ABCA4: structural and functional properties and role in retinal disease. *Adv Exp Med Biol*. 2010; 703:105–125. [PubMed: 20711710]
- Valapala M, Edwards M, Hose S, Grebe R, Bhutto IA, Cano M, Berger T, Mak TW, Wawrousek E, Handa JT, et al. Increased Lipocalin-2 in the retinal pigment epithelium of Cryba1 cKO mice is associated with a chronic inflammatory response. *Aging cell*. 2014; 13:1091–1094. [PubMed: 25257511]
- van Norren D, Gorgels TG. The action spectrum of photochemical damage to the retina: a review of monochromatic threshold data. *Photochemistry and photobiology*. 2011; 87:747–753. [PubMed: 21410704]
- Wang T, Lao U, Edgar BA. TOR-mediated autophagy regulates cell death in *Drosophila* neurodegenerative disease. *The Journal of cell biology*. 2009; 186:703–711. [PubMed: 19720874]
- Wang X, Wang T, Jiao Y, von Lintig J, Montell C. Requirement for an enzymatic visual cycle in *Drosophila*. *Current biology : CB*. 2010; 20:93–102. [PubMed: 20045325]
- Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell*. 2011; 147:893–906. [PubMed: 22078885]
- Wenzel A, Grimm C, Samardzija M, Reme CE. Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. *Progress in retinal and eye research*. 2005; 24:275–306. [PubMed: 15610977]
- Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. *Nat Cell Biol*. 2010; 12:814–822. [PubMed: 20811353]
- Yao J, Jia L, Khan N, Lin C, Mitter SK, Boulton ME, Dunaief JL, Klionsky DJ, Guan JL, Thompson DA, Zacks DN. Deletion of autophagy inducer RB1CC1 results in degeneration of the retinal pigment epithelium. *Autophagy*. 2015; 11:939–953. [PubMed: 26075877]
- Yao J, Jia L, Shelby SJ, Ganios AM, Feathers K, Thompson DA, Zacks DN. Circadian and noncircadian modulation of autophagy in photoreceptors and retinal pigment epithelium. *Investigative ophthalmology & visual science*. 2014; 55:3237–3246. [PubMed: 24781939]
- Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:15077–15082. [PubMed: 14657337]
- Zhou Z, Doggett TA, Sene A, Apte RS, Ferguson TA. Autophagy supports survival and phototransduction protein levels in rod photoreceptors. *Cell death and differentiation*. 2015; 22:488–498. [PubMed: 25571975]

Highlights

1. Excessive exposure to light can cause light-induced retinal damage.
2. Complex mechanisms involving autophagy have been reported in light-induced retinal damage.
3. Defects in essential molecules for autophagy can modulate the severity of light-induced damage.
4. Autophagy could play an important role in coping with stress during the perception of light in the retina.

Figure 1. Vitamin A metabolism in the eye

Vitamin A (all-*trans*-ROL, retinol) is supplied from blood circulation by the concerted action of serum retinol-binding protein (RBP), stimulated by retinoic acid 6 (STRA6), cellular retinol-binding protein type 1 (CRBP1) and lecithin:retinol acyl transferase (LRAT). Vitamin A is stored as all-*trans*-retinyl ester (all-*trans*-RE) in the retinosomes of RPE cells. RPE65 isomerizes all-*trans*-RE to 11-*cis*-ROL and then 11-*cis*-retinol dehydrogenases (RDHs), such as RDH5 and RDH11, oxidize 11-*cis*-ROL to 11-*cis*-RAL. 11-*cis*-RAL binds to retinoid binding proteins, including cellular retinaldehyde-binding protein (CRALBP) and inter-photoreceptor retinoid-binding protein (IRBP), and is then transported to photoreceptors to regenerate light-sensitive rhodopsin. When light hits the light-sensitive form of rhodopsin, 11-*cis*-RAL is photo-isomerized to all-*trans*-RAL. This conformational change initiates rhodopsin activation. All-*trans*-RAL is largely released from rhodopsin into the cytosolic lumen of rod photoreceptor cell outer segments, but a fraction remains in the intra-discal lumen. ATP-binding cassette transporter (ABCA4) removes the remaining aldehyde from the intra-discal lumen. Collectively all-*trans* RAL is reduced by all-*trans*-retinol dehydrogenases (RDHs, including RDH8 and RDH12) to all-*trans*-ROL. All-*trans*-ROL is transported back to RPE cells by binding with IRBP and CRBP1.

Figure 2. Autophagic vesicles in the rod photoreceptor cell and the RPE cell

The distribution of dots demonstrates the localization of autophagic vesicles in the rod photoreceptor cell and the RPE cell (Remé, 1977). Reme CE et. al. examined localization of autophagic vacuoles in photoreceptors of normal animals from five different species (cat, rat, frog, goldfish, ground squirrel) by electron microscopic analysis. In the rod photoreceptor cells, autophagic vesicles are found more often in the myoid (M), the ellipsoid (E) and the perinuclear area (PN). Autophagic vesicles are less frequently observed in the connecting fiber between the nucleus and synaptic body. Autophagic events have not been documented in the outer segment. Autophagic vesicles are found widely dispersed in the cytoplasm of RPE cells.

Figure 3. Normal retinal morphology in *Beclin1*^{+/-} mice raised under room lighting conditions *Beclin1*^{+/-} and littermate wild-type control *Beclin1*^{+/+} mice were raised under room lighting conditions (12 h light at 10 Lux /12 h dark), and retinal histology was evaluated at the age of 2 months. *Beclin1*^{+/-} retinas show normal morphology similar to the retinas of *Beclin1*^{+/+} mice. Rod photoreceptors, cone photoreceptors and nuclei were stained by anti-rhodopsin antibody (red), peanut agglutinin (PNA, green) and DAPI (blue), respectively. RPE, retinal pigmented epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars indicate 30 μ m.

Figure 4. Increased susceptibility to light-induced retinal damage as a result of *Beclin1* deficiency

Beclin1^{+/-} mice and *Beclin1*^{+/+} littermate controls at 2 months of age were exposed to white light at 5,000 Lux for 2 hours. Plastic sections were prepared from light-exposed *Beclin1*^{+/-} mice and *Beclin1*^{+/+} littermate controls 7 days after the illumination. Severely disrupted photoreceptor structure was marked by degenerated photoreceptor outer segment and inner segment and significantly reduced thickness of ONL in *Beclin1*^{+/-} eyes as compared to the intact photoreceptor structure displayed by *Beclin1*^{+/+} eyes. RPE, retinal pigmented epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars indicate 30 μm.

Effect of Knockout in mouse	Retinal Pigmented Epithelium	
	Retina	
Atg5	Atg5 deficiency in rod cells leads to retinal degeneration independent of light (Zhou et al 2015)	Loss of Atg5 in RPE leads to reduction of 11-cis-retinal and diminished photoreceptor response to light stimulus (Zhou et al 2013)
Atg7	Loss of Atg7 in rods leads to increased susceptibility to light induced retinal damage (Chen et al 2013)	N/A
Beclin1	<i>Beclin1</i> ^{+/-} genotype results in increased susceptibility to light induced retinal damage (Chen et al 2013)	N/A
Park2	Loss of Park2 leads to increased susceptibility to light induced retinal damage (Chen et al 2013)	N/A