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Introduction to autophagy in the eye (or “What’s eatin’ you?”)

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In this day and age, it’s possible to find most anything on the internet. Besides delivering an endless stream of information from all over the world (the validity of which is dubious, at best, in many instances), it provides forums to discuss critical issues related to politics, news, natural disasters, science and, of course, celebrities. In a recent search for information on “autophagy” (or self-eating), the topic of this Special Issue of *Experimental Eye Research*, there was a discussion thread debating the question: “If I eat myself, will I grow twice as big, or disappear?” Not surprisingly there were many serious attempts to answer this question, usually involving the consumption of specific body parts, emphatic declarations that you would most certainly disappear, and even something about anti-matter. Luckily for us, this Special Issue actually may address this philosophical dilemma as we explore autophagy and its importance to homeostatic and disease mechanisms of the eye.

Macroautophagy (hereafter simply called autophagy) is an intracellular pathway whereby cytoplasmic constituents are delivered to lysosomes for degradation; this represents a survival mechanism that supports cellular homeostasis. Under nutrient-rich conditions, autophagy is suppressed, but still occurs at low levels (*i.e.*, basal autophagy), where it supports tissue remodeling, mitochondrial quality control, and macromolecule turn over (Mizushima and Komatsu, 2011). When cells are subjected to starvation (or other stresses) autophagy is activated almost immediately (*i.e.*, induced autophagy) (Kuma and Mizushima, 2010). Digestion of cellular constituents releases metabolic by-products that are then routed as fuel to mitochondria– the energy factories of the cell– which in turn helps to promote survival (Mizushima and Komatsu, 2011). Autophagy is very important to post-mitotic cells, such as neurons: by removing damaged components that otherwise would accumulate and possibly lead to the *in situ* formation of cytotoxic molecules, it helps the cell maintain its functionality and viability (Degtarev et al., 2005; Hartleben et al., 2010; Komatsu et al., 2006; Nishiyama et al., 2007). Defects in autophagy have been associated with neurodegenerative diseases (Harris and Rubinsztein, 2012; Nixon, 2013; Rubinsztein et al., 2012; Wong and Cuervo, 2010), diabetes (Rivera et al., 2014; Shigihara et al., 2014), lysosomal storage diseases (Ordonez, 2012), and the loss of vision (Kim et al., 2014). In fact, many, if not most, age-related diseases may be the consequence of insufficient autophagy (Choi et al., 2013; Rubinsztein et al., 2012).

The eye is composed of many highly specialized cell types that function in concert to support the visual axis. Perhaps it is not surprising that each uses autophagy for its own unique purpose. Studies to date have shown that autophagy supports homeostasis of the cornea, trabecular meshwork, RPE, neural retina, and lens. Insufficiency or disruption of autophagy results in a host of pathological ocular diseases and conditions, including retinal degenerations (Murakami et al., 2012; Punzo et al., 2009; Rodriguez-Muela et al., 2015), retinal injury (Besirli et al., 2011), light-induced stress (Chen et al., 2013; Kunchithapautham et al., 2011), and hypoglycemia (Zhou et al. Autophagy 2015, In Press). Authors of this Special Issue explore ocular autophagy as part of a global tour (or, more correctly, tour of the globe) of various (self)-eating establishments, highlighting important new findings.

We start our tour of “self-eating bistros of the eye” at the ocular surface: two articles in this issue discuss the role of autophagy in corneal homeostasis and disease. Gordon Laurie and colleagues begin by cataloging all 460 known autophagy-associated genes of the normal human eye, including 15 NEI-designated ‘eye disease genes’. Particular attention is paid to the multifunctional tear protein lacritin, originally discovered in an unbiased biochemical screen for regulators of basal tearing. Surprisingly, the monomeric form of lacritin also has the ability to quickly and temporarily stimulate autophagic flux in dry eye-like stressed corneal epithelial cells and to thereby restore mitochondrial oxidative phosphorylation. The authors suggest that defective autophagy associated with lacritin monomer deficiency in different forms of dry eye perhaps may underlie the loss of ocular surface homeostasis associated with corneal staining. This is reversed by topical lacritin application in a dry eye mouse model. Eung Kweon Kim and associates discuss the involvement of autophagy in the pathogenesis of Granular Corneal Dystrophy Type 2 (GCD2), a consequence of a transforming growth factor β -induced gene (TGFB1) mutation whose protein product accumulates in autophagosomes. Interestingly, accumulation appears to be related to defective autophagy rather than merely the inability of mutant protein to undergo degradation. It is certainly possible that mutant TGFB1 protein may inhibit autophagy; however, damaged mitochondria are also observed in GCD2 fibroblasts, suggesting a general defect in autophagy in GCD2 disease. Data presented by both contributors suggest that a therapeutic approach to enhance autophagy might be beneficial in the treatment of ocular surface diseases. Thus, “on the surface”, self-eating seems very important.

We then move deeper into the eye to focus on the well-known lens “self-eatery” with a discussion by Hideaki Morishita and autophagy pioneer Noboru Mizushima. The lens is an avascular, transparent tissue that, together with the tear film and cornea, serves to focus light onto the retina. Differentiation of transparent lens fiber cells from the lens epithelium involves the degradation of all cytoplasmic organelles, a process once thought to be autophagy-dependent; however, gene knockout of autophagy mediators Atg5, Pik3c3 and FIP200 does not impede lens differentiation, although disrupting these genes postnatally is lethal. Currently, there is little evidence for a proposed alternative form of autophagy independent of these genes that would degrade the organelles. However, using lens-specific knockout mice, the authors establish that autophagy plays a critical role in suppressing cataract formation in the adult. Cataracts are a leading cause of age-dependent blindness, world-wide, particularly in under-developed countries and areas where healthcare disparities

are prevalent. These results provide significant insight into the process and biological importance of autophagy in the lens. Thus, while eating at the lens café is clarifying for adults, prenatal lens organelles are off the menu.

Moving laterally, we next partake of some elegant waterside dining in two articles that consider the role of autophagy in the regulation of aqueous outflow pathways in the trabecular meshwork (TM), which is constantly exposed to mechanical, oxidative, and phagocytic stress. Paloma Liton discusses how stress-dependent activation of autophagy can in turn diminish TM stress, yet when chronic can lead to alkalization of lysosomes and reduction of autophagic flux. Outflow defects are associated with elevated intraocular pressure that can lead to glaucoma, one of the top three leading causes of visual disability and blindness world-wide. They posit that glaucoma may be due to a dysregulation of autophagy in the TM and that this may be a new target for glaucoma therapy. Chad Dickey and associates also examine the role of autophagy in aqueous humor outflow pathways, but focus on the link between myocilin and autophagy. Myocilin is a widely expressed protein thought to be involved in cytoskeletal functions in many cell types. In glaucoma 70–80% of cases show mutations in myocilin gene (MYOC) and myocilin accumulation possibly leads to TM toxicity. Misfolded myocilin can associate with the stress protein GRP94 in the endoplasmic reticulum (ER), thereby preventing autophagic degradation. Data such as these suggest that targeting autophagic degradation in glaucoma with a myocilin bill of fare might prove efficacious. Hence, at this waterside tavern, self-eating certainly keeps the water flowing.

Cells in the retina must also self-eat to survive. If autophagosomes are akin to cellular ‘stomachs’, then lysosomes comprise the ‘digestive tract’ as the final resting place of cargos being degraded. Debasish Sinha et al. discuss the importance of mTOR (‘mammalian target of rapamycin’) as a critical nutrient sensor regulating autophagy and lysosomal function, and how mTOR signaling is proving to be an important target for therapeutic interventions. β A3/A1 crystallin regulates PI3K/AKT/mTOR signaling and, when subjected to conditional knockout (*Cryba1* gene) in RPE, disrupts V-ATPase leading to progressive degeneration and an Age-Related Macular Degeneration (AMD)-like phenotype. This highlights the importance of the RPE autophagy brasserie to normal homeostasis and that age-related self-eating disorders may be involved in blinding eye diseases, such as AMD. Akiko Maeda et al. explore the idea that proper self-eating can overcome stress in the retina. Vision relies on the retina to convert light as photons into electrical energy for perception by the brain. When light is excessive, photoreceptors and other cells of the retina can undergo damage and death. Here the authors discuss recent data on the role of autophagy in resistance to retinal light damage, and how defects in essential molecules (e.g., retinoids) can modulate the severity of light-induced damage. Hence, strategies that promote autophagy in the setting of light stress might prove efficacious in treating retinal degenerative disorders. Although stressful eating is considered a bad thing, stressful self-eating might be a good thing for vision.

Our next stop visits the “restaurant at the end of the retina” (not to be confused with *The Restaurant at the End of the Universe*, by Douglas Adams). Here we find retinal ganglion cells (RGCs) self-consuming to maintain their function. Ganglion cells are the last stop in

the retina (hence, the location of the restaurant) for visual information before it is transmitted to the brain via the optic nerve. Ghanshyam Swarup and Beatrice Yue discuss how the loss of ganglion cells as a consequence of glaucoma contributes to ensuing blindness. The authors discuss the potential role of optineurin, an autophagy adaptor protein, in the development of glaucoma as mutations of the optineurin gene (OPTN) are associated with primary open angle glaucoma. Data presented therein suggest that the mutations impair autophagy, leading to ganglion cell death and disease progression. [Note of caution: Please be advised that when visiting the ganglion cell diner, do not order the RGC-5 plate; no one really knows what's in it.]

As we exit the “restaurant at the end of the retina”, we enter the optic nerve, the trail for visual information on its way from the retina to the brain. Jan Koch and Paul Lingor explore trail-side eateries as they review the role of autophagy in optic nerve trauma, hereditary diseases, and glaucoma. They point out that autophagy is critically involved in these processes, but caution that there are opposing data along the path(way). While many studies suggest that autophagy serves a neuroprotective role in RGCs and that autophagy enhancement may be a promising therapeutic strategy, others suggest a detrimental role of enhanced autophagy, particularly in glaucoma. Some differences can be accounted for by differences in experimental models and that there might be a narrow therapeutic window for optimum effects. However, they also point out that some differences may be due to the methods employed for measurement of autophagic flux, where increases in metrics, such as the number of autophagosomes and the levels of autophagy-associated molecular markers (*e.g.*, LC3-II, beclin, etc.), can be interpreted as either increases or decreases in autophagy (this is an appropriate caution for all of us). They suggest more rigorous tests for autophagic flux in many systems, but conclude that understanding autophagy in these disease models will be critical to therapy development. Thus, in some establishments, one can “over self-eat” or “under self-eat”, depending on the bill of fare.

We certainly don't want flies in our restaurant, but we must consider the “fly” as an excellent model to study autophagy. In the final article in this issue, Gabor Juhasz and collaborators highlight the power of *Drosophila* as a model to study ocular autophagy. The short lifecycle, reproductive prowess, and powerful genetics of *Drosophila*, coupled with the easy scoring of eye phenotypes, have made this organism a useful model to study many pathways, including autophagy in retinopathies and neurodegeneration. Data are presented that demonstrate that autophagy is dispensable for eye development, yet plays a protective role in neurodegeneration induced by light and genetic mutations. We must conclude, then, that any respectable self-eating venue would not be complete without flies.

So, if someone asks, “What eating you?,” the answer is “Me”! We hope you enjoy the feast.

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