Experimental Eye Research 144 (2016) 90–98

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

iFly: The eye of the fruit fly as a model to study autophagy and related trafficking pathways

Péter Lőrincz [a], Szabolcs Takáts [a], Manuéla Kárpáti [a], Gábor Juhász [a, b, *]

[a] Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Pázmány s. 1/C, Budapest H-1117, Hungary
[b] Momentum Drosophila Autophagy Research Group, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Temesvári krt. 62, Szeged H-6726, Hungary

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form
28 May 2015
Accepted in revised form 15 June 2015
Available online 16 June 2015

Keywords:
Autophagy
Drosophila
Eye
Neurodegeneration
Pigment granule
Retinula cell

ABSTRACT

Autophagy is a process by which eukaryotic cells degrade and recycle their intracellular components within lysosomes. Autophagy is induced by starvation to ensure survival of individual cells, and it has evolved to fulfill numerous additional roles in animals. Autophagy not only provides nutrient supply through breakdown products during starvation, but it is also required for the elimination of damaged or surplus organelles, toxic proteins, aggregates, and pathogens, and is essential for normal organelle turnover. Because of these roles, defects in autophagy have pathological consequences. Here we summarize the current knowledge of autophagy and related trafficking pathways in a convenient model: the compound eye of the fruit fly *Drosophila melanogaster*. In our review, we present a general introduction of the development and structure of the compound eye. This is followed by a discussion of various neurodegeneration models including retinopathies, with special emphasis on the protective role of autophagy against these diseases.

1. Overview of autophagy

Autophagy is a highly conserved catabolic process of eukaryotic cells, which is responsible for the turnover of cytoplasmic material via the lysosomal apparatus. Autophagy has three subtypes: macroautophagy, microautophagy, and chaperone mediated autophagy (Mizushima et al., 2008). Of these pathways, the first one is the best characterized, so we will focus on macroautophagy (hereafter simply referred to as autophagy) in this review.

The autophagic process begins with the emergence of a double membrane cistern called phagophore, which grows around selected portions of cytoplasm, often including organelles and protein aggregates as well. A double membrane-bound cargo transport vesicle called autophagosome is formed by the closure of the phagophore edges. The autophagosome can fuse with either an endosome or a lysosome, giving rise to an amphisome or autolysosome, respectively. Autophagic cargo and the inner membrane of the autophagosome are degraded by hydrolases inside the acidic lysosomal lumen. Finally, the resulting monomers are reused for biosynthesis of new proteins and energy production (Mizushima et al., 2008). Atg proteins that are necessary for autophagosome formation were first described in yeast (*Saccharomyces cerevisae*) (Tsukada and Ohsumi, 1993), and most of them have clear homologs among higher eukaryotes (Klionsky et al., 2012, 2003). These Atg proteins form functional units that are activated during autophagosome formation in a more-or-less stepwise manner. First, an Atg1/ULK1 serine–threonine protein kinase complex is activated, which is followed by the action of a Vps34-containing lipid kinase complex and its phospholipid effectors that include Atg18/WIPI family proteins and their binding partner Atg2. Atg9 is the only transmembrane protein among these gene products, and it is likely involved in a poorly characterized vesicle transport pathway that is important for phagophore formation. Lastly, two protein conjugation systems work together to mediate the covalent conjugation of Atg8/LC3 family members to phosphatidyl-ethanolamine, which is how these small ubiquitin-like proteins become anchored in the phagophore (Klionsky et al., 2012; Mizushima et al., 2011). Importantly, Atg8/LC3 remains bound to the inner membrane of autophagosomes. This property makes it a widely used marker for these vesicles: several antibodies against Atg8/LC3 and transgenic animals expressing tagged Atg8/LC3 reporters are readily available.

Some but not all of the factors mediating the fusion of the

* Corresponding author. Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Pázmány s. 1/C, Budapest H-1117, Hungary.
E-mail address: szmrt@elte.hu (G. Juhász).

http://dx.doi.org/10.1016/j.exer.2015.06.013
0014-4835/

autophagosome with the lysosome (called the vacuole in yeast) are also evolutionarily conserved. All six subunits of the heterohexameric vesicle tethering complex HOPS (homotypic fusion and protein secretion) have orthologs in higher eukaryotes, and they are also required for autophagosome clearance in Drosophila and mammalian cells (Jiang et al., 2014; Peng et al., 2012; Takats et al., 2014). However, the Vam3 and Vam7 SNARE proteins, which are important mediators of autophagosome-vacuole fusion in yeast (Hegedus et al., 2013), are not conserved. The SNARE proteins required for autophagosome-lysosome fusion in mammalian cells and in Drosophila have been recently identified. In Drosophila, the autophagosome-located Syntaxin17 (Syx17/mammalian STX17 homolog) forms a complex with a soluble SNARE protein called ubisnap (usnp/mammalian SNAP-29 homolog), and the late endosomal/lysosomal membrane protein VAMP7 (the closest Drosophila homolog of mammalian VAMP8) (Hegedus et al., 2013; Itakura et al., 2012; Takats et al., 2013).

A basal, low-level autophagic activity is observed in all eukaryotic cells, and this "housekeeping" function is important for the physiological turnover of damaged organelles or proteins (Birgisdottir et al., 2013). Autophagic adaptor proteins such as p62 (also known as Ref(2)P in Drosophila and SQSTM1 in mammals) bind to ubiquitinated proteins and organelles with their ubiquitin-binding UBA domain, and are able to form aggregates through homo-oligomerization mediated by a PB1 domain. The LIR (LC3 interacting region) motif of p62 directly binds to Atg8/LC3 and thus ensures the selective capturing of proper targets into autophagosomes (Johansen and Lamark, 2011; Pankiv et al., 2007). Since p62 itself is also degraded by autophagy, its levels are routinely used to indirectly follow the rate of autophagic degradation: autophagy defects lead to a massive accumulation of p62 aggregates (Bartlett et al., 2011; Pircs et al., 2012).

Autophagy is one of the most important stress responses of eukaryotic cells including Drosophila, as it is induced by various stimuli including nutrient limitation, oxidative stress, or serum deprivation (Mizushima et al., 2008; Scott et al., 2004). Moreover, several signal transduction pathways are known to regulate autophagy during animal development (Rusten et al., 2004; Zhang et al., 2009). Autophagy is important for maintaining the homeodynamic balance of the intracellular milieu during development and adult life, and its perturbation is known to contribute to various diseases. Autophagy is involved in tumorigenesis (White, 2012), innate immunity (Deretic, 2012) and the elimination of intracellular pathogens (Randow and Youle, 2014). Autophagy is also essential for the survival of terminally differentiated neurons: numerous studies showed that the loss of autophagy results in accumulation of protein aggregates, leading to neurodegeneration and decreased lifespan (Bartlett et al., 2011; Hara et al., 2006; Juhasz et al., 2007; Komatsu et al., 2006; Simonsen et al., 2008; Takats et al., 2013).

2. The structure and development of the Drosophila compound eye

The compound eye of the fly develops from the larval eye primordium, the so-called eye-antennal disc (Fig. 1A). This small, paired organ belongs to the group of imaginal discs that all consist of two epithelial monolayers opposing each other. One cell layer of a disc is a squamous epithelium and it is called the peripodial membrane, and the other layer is a columnar epithelium, the so-called disc proper. Besides its structural role, the function of the peripodial membrane is to secrete signaling molecules that govern the fate of columnar cells, which give rise to the imaginal appendices and the adult fly exterior (Held Jr, 2005). The larval eye-antennal disc is the progenitor of the compound eyes, ocelli, antennae, palpi and the head capsule (Fig. 1A). During the last - L3 - larval stage, a morphogenetic wave starts at the apex of the eye disc (at the base of the optic stalk that connects the disc to the brain) and moves towards the antennal part. Ahead of the wave, the cells are undifferentiated, unordered and scattered mitoses occur. Behind the wave, the cells are organized into nascent ommatidia. Because of its "cell-transforming" feature this wave is called the morphogenetic furrow (Fig. 1A). After the furrow has passed by, further divisions take place, and via subsequent differentiation steps, the final structure of the ommatidia is formed. The furrow reaches the end of the eye disc during the first 10 h of metamorphosis, and in most cases the ommatidia become organized into 33 columns (the number of ommatidia and columns may change in different genetic backgrounds). The eye discs evaginate during the pupal period, and finally ommatidia mature into their functional state (Held Jr, 2005).

The adult eye is no longer a monolayer but a stratified complex organ, which is composed of various differentiated cells with different functions (Fig. 1B–F, Fig. 2). Each compound eye contains approximately 750 ommatidia and each ommatidium contains 8 photoreceptor (Retinula - R) cells. The six large photoreceptor cells (R1-R6) are called the outer receptors, and ensure high light sensitivity. The smaller inner receptors (R7-R8) are more important for sharp vision and color vision. As R7 is distal to R8, only 7 receptor cells can be seen in cross sections (Fig. 1B–F, Fig. 2). Each photoreceptor cell has an apical microvillar extension facing the center of the ommatidium. This extension is called the rhabdomere, and the rhabdomeres of the eight retinula cells form the rhabdom. The rhabdomeres in the Drosophila retina are not fused and are separated by the inter-rhabdomeral space, therefore fruit flies have an "open" type of rhabdom. Microvillar membranes in the rhabdomeres contain the rhodopsins that mediate phototransduction (Fig. 1B–F, Fig. 2). Inner and outer photoreceptors express different rhodopsin subtypes, so they have different spectral sensitivity (Held Jr, 2005; Katz and Minke, 2009).

Ommatidia are isolated by lateral pigment cells, and neighboring ommatidial units share these pigment cells among each other. Two different types of pigment granules are found in pigment cells: drosopterine (red) and ommochrome (brown) pigments, and the former one gives the fly eye its bright red color (Shoup, 1966). Four cone cells (also known as Semper cells) are found above the photoreceptor cells, and their function is to secrete the dioptric elements of the eye: the crystalline cone and the lens. This latter structure is seen when one looks at the fly head through a dissecting microscope (Fig. 1E and Fig. 3A). Lenses are arranged in a hexagonal symmetry and are surrounded by three alternating bristles. Lenses are translucent, so eye color is determined by the pigment cells that are found beneath them. During the pupal period, the eye disc has approximately 2000 excess cells, which are later eliminated by cell death (Rusconi et al., 2000). The balance between cell proliferation and death, together with the sophisticated signaling events mediating differentiation, ultimately give rise to a very regularly patterned organ (Held Jr, 2005; Rusconi et al., 2000). Even a slight disturbance in eye development disrupts this symmetry, producing for example a rough eye phenotype that can be easily detected by simply looking at the adult eye. A rough eye phenotype occurs for example when eye disc cells undergo excessive apoptosis during development, giving rise to a disorganized ommatidial pattern. As a result, the surface of the compound eye looks rugged and irregular. For example, the expression of pathogenic tau in the developing eye produces a rough eye phenotype and disorganization of ommatidial units (Fig. 3A–C). Multiple genetic strategies can be used to alter gene expression in the whole eye or in different regions of the eye disc in mosaic animals, and eye discs or retinas can be easily dissected with some experience. These make the Drosophila eye a versatile