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Dynamin Regulates Autophagy by Modulating Lysosomal Function

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ABSTRACT

Autophagy is a central lysosomal degradation pathway required for maintaining cellular homeostasis and its dysfunction is associated with numerous human diseases. To identify players in autophagy, we tested ~1200 chemically induced mutations on the X chromosome in *Drosophila* fat body clones and discovered that *shibire* (*shi*) plays an essential role in starvation-induced autophagy. *shi* encodes a dynamin protein required for fission of clathrin-coated vesicles from the plasma membrane during endocytosis. We showed that Shi is dispensable for autophagy initiation and autophagosome−lysosome fusion, but required for lysosomal/autolysosomal acidification. We also showed that other endocytic core machinery components like clathrin and AP2 play similar but not identical roles in regulating autophagy and lysosomal function as dynamin. Previous studies suggested that dynamin directly regulates autophagosome formation and autophagic lysosome reformation (ALR) through its excision activity. Here, we provide evidence that dynamin also regulates autophagy indirectly by regulating lysosomal function.

KEYWORDS: Dynamin; Autophagy; Endocytosis; *Drosophila*

INTRODUCTION

Autophagy is a lysosomal degradation pathway important for removing damaged organelles, aggregated proteins, and invading microorganisms from cells (Mizushima and Komatsu, 2011; Rubinsztein et al., 2011; Boya et al., 2013). It occurs at low levels in virtually all cells but is up-regulated upon starvation or other stressful stimuli to produce nutrients for cells and maintain cellular homeostasis. Dysfunction of autophagy is associated with multiple developmental defects and diseases, such as cancer, neurodegeneration, and heart diseases (Levine and Kroemer, 2008; Rubinsztein et al., 2012).

There are three types of autophagy for delivering cytoplasmic contents to lysosomes and macroautophagy (hereafter referred to as autophagy) is the major autophagic mechanism in eukaryotic cells.

Autophagy is initiated by formation of isolation membranes that elongate and enclose cytoplasmic contents to form double membrane autophagosomes. Autophagosomes mature and fuse with lysosomes to form autolysosomes. The cytoplasmic contents are then digested for nutrient release. Subsequently, new lysosomes are regenerated from these autolysosomes (Mizushima et al., 2010; Feng et al., 2014). Autophagy is a highly regulated process that shares many common characteristics with the cellular membrane trafficking pathway of endocytosis (Lamb et al., 2013).

Endocytosis involves the uptake of soluble molecules or the delivery of membrane components or receptors to the

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endocytic pathway (McMahon and Boucrot, 2011). During clathrin-dependent endocytosis, receptors and their bound ligands are internalized by vesicles coated with clathrin and adaptor protein 2 (AP2). A number of adaptor proteins, including Eps15, are also localized in clathrin-coated pits to mediate the interactions between endocytic cargos and these vesicle coats. The fission of clathrin-coated vesicles (CCVs) from the plasma membrane is mediated by dynamin proteins. Upon internalization of CCVs, the clathrin coat is removed and some vesicles fuse with each other to form early endosomes (EEs). Some of the endocytosed material is then recycled back to the plasma membrane through recycling endosomes. The remaining contents are delivered to late endosomes (LEs) and digested in lysosomes (Scita and Di Fiore, 2010; Huotari and Helenius, 2011; McMahon and Boucrot, 2011).

Dynamin is a GTPase that can form rings or spirals that wrap around the necks of clathrin-coated pits (Hinshaw, 2000; Schmid and Frolov, 2011). Upon GTP hydrolysis, these necks become constricted and clathrin-coated pits are pinched off from the membrane. Dynamin is also thought to provide a source of membranes for autophagosomes by pinching off vesicles from the plasma membrane (Ravikumar et al., 2010). Moreover, dynamin is required for the regeneration of lysosomes by pinching off membrane from autolysosomes in hepatocytes (Schulze et al., 2013). Dynamin has also been proposed to complete the maturation of autophagosomes, leading to impairment in autophagy in *dynamin* mutant mice (Durieux et al., 2012). Finally, in yeast, the dynamin homolog Vps1 has also been shown to facilitate vacuolar fusion (Peters et al., 2004).

In this study, we showed that loss of dynamin results in lysosomal acidification defects and autophagy dysfunction in flies due to failed lysosomal degradation. We also showed that these defects are not due to defects in autophagosome formation or their fusion with lysosomes. Moreover, the lysosomal defects in *shi* mutants are independent of starvation-induced autophagy and loss of clathrin and AP2 also results in similar autophagy defects as dynamin mutants.

RESULTS

Identification of a complementation group with autophagy defects

To isolate genes involved in autophagy, we conducted a forward genetic screen in the *Drosophila* fat bodies. We used a collection of lethal mutations on the X chromosome (Yamamoto et al., 2014) and the FLP/FRT system to create mosaic clones in the fat bodies. We isolated third *instar* larvae 90 h after egg laying (AEL) and induced autophagy by amino acid starvation for 4 h. The fat bodies were subsequently stained with LysoTracker Green. In the absence of starvation, there is minimal LysoTracker staining in the wild-type cells. However, upon induction of autophagy, LysoTracker staining appears as very bright puncta inside fat body cells (Mauvezin et al., 2014; Nagy et al., 2015).

Two alleles of one complementation group, *XG12A* and *XG12O*, displayed dramatically reduced LysoTracker staining, suggesting possible defects in autophagy (Fig. 1A and B). Using 20 kb and 80 kb P[acman] duplications (Venken et al., 2009, 2010), we mapped *XG12* to four putative genes (Fig. 1C). Sanger sequencing revealed point mutations in *shi* in both alleles. We renamed them shi^{XFRT1} and shi^{XFRT2} as the chromosome carries an FRT site. shi^{XFRT1} has a missense mutation at p.Gly55Asp, whereas shi^{XFRT2} has a nonsense mutation at p.Arg10X. *shi* encodes the dynamin protein with an N-terminal GTPase domain, a central domain, a pleckstrin homology (PH) domain, and a GTPase effector domain (GED) (Fig. 1C). The mutated Gly in shi^{XFRT1} is in the GTPase domain and is conserved across species (Fig. 1D).

shi mutants display lysosomal and autophagy defects

Reduced LysoTracker staining in *shi* mutant clones upon starvation can either be due to defects in autophagy initiation or autolysosomal acidification. Autophagy initiation requires type III PI3K activity, which can be monitored by the intracellular distribution of GFP-FYVE (Juhasz et al., 2008). Without PI3K activity, GFP-FYVE signals distribute diffusely inside cells. Upon PI3K activation, GFP-FYVE exhibits punctate staining that is enriched in early endosomes. In *shi* mutant cells, GFP-FYVE reveals enlarged puncta, indicating that PI3K activity is present in mutant cells (Fig. 2A−C, E). Because GFP-FYVE primarily accumulates on endosomes, the increased GFP-FYVE punctate staining suggests an accumulation and enlargement of early endosomes in mutant fat body cells. GFP-Rab7, a protein that labels late endosomes and lysosomes, also exhibits enlarged punctate staining in the mutant clones (Fig. 2F−H, J), suggesting enlargement of late endosomes and lysosomes. Consistent with this observation, lysosomal marker LAMP-GFP also forms larger punctate structures in the mutant cells compared to the controls (CTL) (Fig. 2K−M, O), indicating there are lysosomal defects in the mutant cells. Interestingly, there is also an accumulation of LAMP-GFP on the plasma membrane, indicating that the trafficking of LAMP is affected in *shi* mutants.

To analyze autophagosome formation, we examined the patterns of Atg8, a ubiquitin-like protein that marks autophagosomes, in *shi* mutant cells. Without starvation, Atg8 signals are diffusely distributed intracellularly. Upon starvation, Atg8 is modified and specifically labels autophagosomes. Finally, it is degraded inside the autolysosomes (Scott et al., 2004). *shi* mutant clones exhibit a dramatic accumulation of GFP-Atg8 punctate structures upon starvation. These data indicate that either there is an increase in the cellular autophagy activity or a decrease in the autophagic flux downstream of autophagosome formation (Fig. 2P−R, T).

To distinguish these possibilities, we monitored the levels of p62, an autophagy adapter protein digested through an autophagic process (Komatsu et al., 2006). p62 is elevated in the mutant cells, supporting a reduction in autophagic flux in *shi* mutant cells (Fig. 2U−W, Y).