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Divergent Roles of Atg8 Orthologues in Autophagy

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Editorial

Autophagy is a fundamental pro-survival cellular process in eukaryotic cells that recycles nutrients under starvation conditions. Autophagy also plays a role in maintaining cellular homeostasis by degrading damaged organelles or aberrantly folded proteins [1-3]. During autophagy, a double membrane vesicle, termed an autophagosome, takes up bulk cytoplasm and fuses with the lysosome where its contents are degraded [4,5]. A key step in autophagosome biogenesis is the lipidation of the Atg8 proteins by conjugation to phosphatidylethanolamine (PE) on the autophagic membrane [6-8]. Although it has been found that genetic abolition of Atg8 lipidation prevents completion of the autophagosome, the exact function of the lipidation process remains largely unknown [9-13].

In mammalian cells, there are at least six Atg8 orthologues that can be subdivided into the LC3 (light-chain 3) and the GABARAP (γ-amino butyric acid receptor-associated protein) families. LC3s, comprised of LC3A, LC3B, and LC3C, were first identified as microtubule-associated proteins. GABARAPs, including GABARAP, GABARAPL1, and GABARAPL2 (also known as GATE-16, Golgi-associated ATPase enhancer of 16 kDa) were initially identified as intracellular trafficking factors [14,15].

The Atg8 proteins are expressed universally although tissue-specific gene expression varies for the different homologues [15]. LC3C is expressed primarily in the lung. GABARAP is expressed markedly in the endocrine gland. GABARAPL1 and GABARAPL2 are expressed primarily in the central nervous system [16]. The reason for this divergence in tissue distribution is unknown. The Atg8 homologues could potentially have a cell or tissue specific role beyond their involvement in autophagy.

Extensive studies of LC3 lipidation in cell models have given insight into how the process of lipidation is regulated. Stresses, such as starvation, induce autophagosome biogenesis and LC3 lipidation. Signaling factors, such as the mammalian target of rapamycin complex 1 (mTORC1) and the AMP-activated protein kinase (AMPK), have been implicated as upstream regulators of autophagy [17]. Starvation leads to the inhibition of mTORC1 and the activation of AMPK, which in turn activates the ULK1 kinase complex consisting of FIP200, ULK1/2, ATG13, and ATG101 [18-21], and then the autophagic phosphatidylinositol 3-kinase (PI3K) complex including ATG14, Beclin-1, P150, and VPS34 [19-22]. The former induces the assembly of pre-autophagosomal structures by scaffolding and phosphorylation of downstream factors and the latter catalyzes the formation of phosphatidylinositol 3-phosphate on the target membrane, which is essential for the recruitment of downstream effectors. The two kinase complexes direct the site of LC3 and regulate the magnitude of lipidation [19-21].

LC3 lipidation relies on the ubiquitin-like conjugation systems for the ATG12 protein and the LC3 protein [4]. These partially overlapping systems serve to conjugate ATG12 to ATG5 in a reaction that requires ATG7 and ATG10 and to conjugate LC3 to PE in a reaction that requires ATG7, ATG3, and a protein complex consisting of the ATG12-ATG5 conjugate and ATG16 [4]. Studies have shown that LC3 lipidation is dependent on the activity of the conjugation systems because lipidation is completely eliminated in the absence of ATG5, ATG16, ATG3, or ATG7 [9,11-13].

The lipidated form of the Atg8 protein is attached to both faces (convex and concave) of the phagophore membrane, the cup-shape precursor of the autophagosome [23,24]. Lipidation is a reversible process since the cysteine protease Atg4, which is specific to the Atg8 proteins, de-conjugates Atg8 from the autophagic membrane by hydrolyzing the bond between the C-terminal glycine of Atg8 and PE [25,26]. Like the protein it interacts with, Atg4 has homologs and while ATG4A is specific to the GABARAP family, ATG4B acts on both the LC3 and the GABARAP families [16,27,28].

The exact role of Atg8 in autophagy, especially in the formation of the autophagosome, has yet to be determined. Nonetheless, recent studies have established that the Atg8 proteins have a dual role in autophagy in regulating the growth of the autophagosome and in acting as cargo adaptors in selective autophagy [16,29]. A study using an in vitro system composed of purified proteins and liposomes indicated that Atg8 lipidation causes membrane tethering and hemifusion activity essential for the expansion of the autophagosome membrane, which was later supported by a genetic study demonstrating the importance of Atg8 for autophagosomal size [30]. A role for the Atg8 proteins in cargo recruitment has also been proposed due to their binding to the autophagic cargo adaptors P62, NBR1, NDP52, NCOA4, Optineurin, and Alfy via the LC3-interacting motif (LIR) [16,31-37].

Although the Atg8 family proteins are homologous to each other, they are not redundant. A study in which each of the Atg8 subfamilies was selectively deleted has shown that the LC3 and GABARAP families are both essential and act at different stages of autophagy. The removal of one subfamily does not result in compensation by the other since autophagy as a whole is inhibited [14]. In addition, overexpression of LC3B in the absence of GABARAPL2 leads to accumulation of the ATG12-ATG5/ATG16 complex whereas overexpression of GABARAPL2 in the absence of LC3B leads to dissociation of the ATG12-ATG5/ATG16 complex from the autophagic membrane [14]. These results were consistent with the proposition that LC3s are involved in the elongation of the autophagosome membrane and that GABARAPs are involved later, possibly in regulating the sealing process for the maturation of the autophagosome or in the fusion between the autophagosome and lysosome.

A recent study confirms the role of GABARAPs in a late step of autophagosome formation. GABARAP was identified in

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binding phosphatidylinositol 4-kinase IIα (PI4KIIα) to generate phosphatidylinositol 4-phosphate (PI4P) from phosphatidylinositol [38]. This step is crucial for the fusion of autophagosomes with lysosomes since live cell imaging studies have shown that PI4KIIα depletion inhibits fusion [38]. PI4KIIα does not bind LC3s indicating that the Atg8 subfamilies have distinct roles. Further studies are needed to elucidate the regulation of GABARAP, PI4KIIα, and PI4P in autophagosome and lysosome fusion. It has been proposed that PI4P may provide a platform for assembling the GABARAP scaffolding network, which has been implicated in tethering, cargo recruitment, and now fusion [38].

Although the LC3s have been proposed to act upstream of GABARAPs in mammalian autophagy, a study of Atg8 proteins conducted in *C. elegans* which undergoes allophagy, an autophagic process, suggests a reversed role of the two Atg8 subfamilies [39]. The two Atg8 orthologues in *C. elegans*, LGG-1 and LGG-2, correspond to the GABARAP and LC3 families respectively. The study found that LGG-1 acts upstream of LGG-2 and is essential for autophagosome biogenesis while LGG-2 mediates the tethering between autophagosomes and lysosomes [39]. The question remains whether the functions of the Atg8 proteins in nematodes are conserved in the human orthologs since these results oppose the scheme that the LC3 family acts at an earlier step than the GABARAP family in the autophagic pathway.

In addition to the association with downstream effectors, the GABARAPs are also able to cooperate with early autophagic factors. A study that investigated the interaction of the ULK1 complex with the six ATG8 homologues found that ULK1 interacted most strongly with GABARAP and GABARAPL1 although it interacted with GABARAPL2, LC3A, LC3C, and weakly with LC3B [40]. Thus, the ULK1 complex has a strong preference for the GABARAP family and this interaction is mediated by LIR motifs on ULK1, ATG13, and FIP200 [40]. The interaction of the ULK1 complex with GABARAPs presumably facilitates or stabilizes association of the ULK1 complex with the phagophore.

In summary, accumulating evidence indicates functional divergence between members of the Atg8 orthologues in the aspects of: 1) stages of autophagosome development, 2) autophagic factor interaction, and 3) autophagic cargo selection. Still, the roles of each Atg8 orthologue in autophagosome biogenesis as well as the roles of each beyond autophagy have not yet been resolved. The emergence of powerful genetic approaches such as CRISPR/Cas9 have made it possible to dissect the individual role of each Atg8 orthologue [41,42]. In addition, super-resolution microscopy will allow us to determine the details of Atg8 protein localization on a growing phagophore. Finally, in vitro reconstitution approaches will enable us to resolve the detailed molecular actions on the process of lipidation, cargo recognition, and phagophore elongation.

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