Abstract

Autophagy is a highly conserved cellular degradation pathway which is crucial for various cellular processes. The autophagic process is subdivided in the initiation, autophagosome maturation and lysosomal degradation phases and involves more than forty core and accessory autophagy-related (ATG) proteins. Autophagy 8 (ATG8, in mammals LC3) is a well-established marker of autophagy and is linked to the autophagic membrane from initiation until fusion with the lysosome.

We generated single and double knock-out mutants of the two *Dictyostelium* paralogues, ATG8a and ATG8b, as well as strains that re-expressed RFP-ATG8a and/or GFP-ATG8b, RFP-ATG8b, RFP-GFP-ATG8a or RFP-GFP-ATG8b in the knock-out background. The ATG8b$^-$ mutant displayed only subtle phenotypic changes in comparison to AX2 wild-type cells. In contrast, deletion of ATG8a resulted in a complex phenotype with delayed development, reduced growth, phagocytosis and cell viability, an increase in ubiquitinylated proteins and a concomitant decrease in proteasomal activity. The phenotype of the ATG8a/b double knock-out mutant was in all aforementioned aspects much more severe. This demonstrates a serious disturbance of cell homeostasis and shows that both protiens function in parallel during autophagy. Immunofluorescence analysis of KO strains re-expressing either RFP-GFP-ATG8a or RFP-GFP-ATG8b suggests a crucial function for ATG8b in autophagosome-lysosome fusion. Analysis of strains expressing RFP-ATG8b, RFP-ATG8a and/or GFP-ATG8b revealed that ATG8b generally localised to small and large vesicles, whereas ATG8a preferentially co-localised with ATG8b on large vesicles, indicating that ATG8b associated with nascent autophagosomes before ATG8a. High-resolution microscopy showed that ATG8b localised around ATG8a and was presumably present on the outer membrane of the autophagosome. In summary, ATG8a and ATG8b have distinct autophagic functions with ATG8b possibly predominantly acting as adapter for the autophagy machinery at the outer and ATG8a predominantly as cargo receptor at the inner membrane of the autophagosome. We infer that *Dictyostelium* ATG8b is likely the functional orthologue of the mammalian LC3 subfamily and ATG8a of the GABARAP subfamily.