Abstract

Macroautophagy is used as a highly conserved process by eukaryotic cells to degrade cytoplasmic components and thereby maintain a balance of synthesis and degradation. In addition to proteins, whole organelles can be enclosed by a double membrane forming the autophagosome. By merging the autophagosome with the lysosome, its contents can be degraded. More than 40 autophagy (ATG) proteins are involved in this process, including the ATG12~5-16 complex, which connects ATG8 to phosphatidylethanolamine as an E3-like enzyme in a ubiquitin-like conjugation reaction.

In this work the ATG5$^-$, ATG5$^-$/12$^-$, ATG5$^-$/16$^-$ and ATG5$^-$/12$^-$/16$^-$ knock-out strains were generated in the *Dictyostelium discoideum* wildtype background AX2. The mutants showed similar defects in the development and the cell viability during amino acid deficiency. Liquid culture growth, macropinocytosis and phagocytosis of yeast were more affected as more components of the ATG12~5-16 complex were knocked out at the same time. This indicates minor activity of the complex in the single or double mutants and/or further autophagy-independent functions of the proteins of the complex. The growth on *Klebsiella aerogenes* and the phagocytosis of *Escherichia coli* shows a more complex phenotype, suggesting that ATG5 and ATG16 have an inhibitory, ATG12 a slightly stimulating effect on growth on *K. aerogenes* which are independent of the canonical autophagy.

Protein homeostasis was severely disturbed in the mutants, as evidenced by increased ubiquitination of proteins and formation of ubiquitin-positive aggregates. These could not be effectively degraded anymore by the ubiquitin-proteasome-system, as shown by the reduced proteasomal activity of the mutants. In the absence of ATG5 and presence of ATG16, a greater impairment was observed than in the absence of both proteins. RNA_{seq} showed massive transcriptional changes compared to the wild-type, including increased expression of autophagy genes *atg8a*, *atg8b*, *atg11* and *atg18*. This is consistent with a possible autophagosome sensing system. By bioinformatic analysis, all known domains in *D. discoideum* ATG5 could be detected and a 3D homology model of the ATG12~5-16 complex could be generated.

A polyclonal ATG5 antibody was generated that can be used in the future to identify potential posttranslational modification of the ATG12~5 conjugate. In addition, autophagy-independent functions and the interaction of the ATG12~5-16 complex with the UPS should be further investigated in future work.