Abstract

Macroautophagy is an intracellular degradative pathway that is highly conserved in all eukaryotic cells. The process is crucial for cellular homeostasis and serves as a response to different stresses such as e.g. starvation or the appearance of protein aggregates. During macroautophagy, cytosolic material becomes enclosed in newly generated double-membrane vesicles, the so-called autophagosomes. Upon maturation, the autophagosome fuses with the lysosome for degradation of the cargo. *Atg12* is one of the core autophagy genes (ATG) and has been shown to be involved in the expansion of the double-membrane of the growing autophagosome. For autophagosome formation, two ubiquitin-like conjugation reactions are indispensable. The ubiquitin-like protein ATG12 plays an essential role in the first conjugation reaction which results in the formation of a multimeric complex composed of an ATG16 homodimer and two ATG12~ATG5 heterodimers. This complex then acts as an E3-like enzyme in the covalent linkage of the ubiquitin-like ATG8(LC3) to the phospholipid phosphatidylethanolamine.

To decipher the cellular function of ATG12, gene replacement mutants of *atg12* in *Dictyostelium discoideum* AX2 wild-type and ATG16$^-$ cells were generated. The generated ATG12$^-$, ATG16$^-$ and ATG12$^-$/16$^-$ cells had complex phenotypes and showed significant similar defects in fruiting body formation, autolysosome maturation and cellular viability, which implies that both proteins together with ATG5 act, as expected, as a functional unit in autophagy. In contrast, ablation of ATG16 or ATG12 and ATG16 resulted in slightly more severe defects in axenic growth, macropinocytosis and protein homeostasis than ablation of ATG12. This suggests that ATG16 fulfils an additional function in these processes which is independent of its role in the ATG12~ATG5/ATG16 complex. Furthermore, phagocytosis of yeast, spore viability and the maximal cell titre in liquid culture were much more affected in ATG12$^-$/16$^-$ cells than in the single knock-out cells, suggesting that both proteins have also independent functions in these cellular processes or that the ATG12~ATG5/ATG16 complex without either ATG12 or ATG16 has still some residual activity in these processes. RNA_{Seq} and qRT-PCR analyses of AX2 and mutant strains revealed major transcriptional changes in a large number of genes. Among other changes we observed an up-regulation of several core autophagy genes, as e.g. *atg5*, *atg9* and the two *atg8* paralogues, indicating a positive feedback loop for the expression of autophagy genes in the mutant strains. Future studies will focus on: i) the identification of novel ATG12 interacting proteins, ii) a possible role of ATG12 in the crosstalk between autophagy and proteasomal degradation and iii) the role of ATG12 in autophagy-independent cellular processes.