Summary

D. discoideum is a well suitable model organism to investigate basic cellular processes as well as host-pathogen interactions. Infection studies of *D. discoideum* with the bacterial pathogen *S. typhimurium* revealed autophagy as a possible host defence mechanism. The core autophagy gene *atg8* and a closely related gene, which we called *atg8-like,* were upregulated upon infection with *S. typhimurium* and *M. marinum*. ATG8 is a known autophagosomal marker, that undergoes C-terminal modification and becomes linked to PE in the autophagosomal membrane. The protein sequences of ATG8 and ATG8-like are 54 % identical. Phylogenetic analysis revealed that they are closely related to each other and to ATG8 from *Acanthamoeba* as well as fungi and plants, but more distant related to LC3/GABARAP from animals. We decided to further investigate ATG8 and ATG8-like. To learn more about their cellular function we generated polyclonal antibodies and different mutants that expressed either mRFP-ATG8 or mRFP-ATG8-like or co-expressed mRFP-ATG8 and GFP-ATG8 or GFP-ATG8-like in AX2 wild-type, ATG9$^-$ and AAG cells. Using these tools we performed RFP- and GFP-trap co-immunoprecipitation experiments followed by mass spectrometric analysis. We found a large number of common potential interaction partners for ATG8 and ATG8-like. As for example the E1- and E2-like proteins ATG7 and ATG3, which are involved in ATG8 modification. This result, the conserved C-terminal glycine where proteolytical cleavage occurs, as well as mass spectrometric analysis suggest that ATG8 and ATG8-like are modified in the same way as yeast ATG8 and mammalian LC3. Statistical analysis of the mRFP-ATG8 and GFP-ATG8/-ATG8-like co-expressing cells revealed that ATG8-like is present on newly synthesized small autophagosomes, whereas ATG8 is associated mainly with larger autophagosomes. Furthermore we found that ATG8 and ATG8-like partially co-localise with ubiquitin and ATG9. The latter co-localisation was observed in live-cell imaging studies using the AAG/mRFP:ATG8 and AAG/mRFP:ATG8-like strains. These studies showed a highly dynamic and brief co-localisation between ATG9 and ATG8/ATG8-like. ATG9 appeared first on vesicles, followed by ATG8-like and ATG8. From our data we infer that ATG8-like has a function in the early phase of autophagosome formation and ATG8 in the late phase. ATG8 could, similar to GABARAP, have a function in the sealing of autophagosomes. Therefore we propose that ATG8-like and not ATG8 is the true LC3 orthologue in *D. discoideum*.