

## 7. Summary

Macroautophagy contributes to many physiological processes including starvation, cell differentiation, neurodegenerative diseases and the response to pathogens. More than 30 autophagy-related (*atg*) genes have been identified so far, mainly in yeast, of which 18 constitute the core machinery for starvation induced autophagy. To investigate the function of autophagy in *D. discoideum*, we selected the highly conserved *atg9* gene. ATG9 is thus far the only transmembrane protein of the core autophagy machinery and is predicted to provide membrane material to the pre-autophagosomal structure (PAS) in yeast or the phagophore in mammals, the site of autophagosome biogenesis. In yeast, part of ATG9 is localized in close proximity to mitochondria while in mammalian cells ATG9 localizes to the *trans*-Golgi network and to late endosomes. In *D. discoideum* we found ATG9 in small ATG9-containing vesicles (ACVs). ACVs were distributed throughout the cytoplasm, often localized along microtubules and were enriched at the microtubule organizing center (MTOC). Live cell imaging showed that ACVs were often “born” in the cell periphery and then swiftly travelled to the MTOC. Upon treatment of cells with Nocodazole, the enrichment of ACVs at the MTOC was lost but many of them remained associated with the disrupted microtubules that were evenly distributed in the cytosol. These results suggest that ACVs utilized the microtubular network for its transport towards to the MTOC. Disruption of the *atg9* gene in *D. discoideum* cells resulted in a pleiotropic phenotype. Autophagy was blocked as deduced from an autophagic flux assay and the ubiquitin proteasome system (UPS) was also severely affected. Proteasomal activity was strongly inhibited (as found in a parallel study), the level of ubiquitinated proteins was increased and frequently ubiquitin-positive aggregates were observed in the ATG9<sup>-</sup> strain. The ATG9<sup>-</sup> mutant had also severe growth defects which were probably caused by decreased in pinocytosis and phagocytosis activity. Consistent with a role of autophagy in development, the ATG9<sup>-</sup> mutant also displayed severe developmental defects, which could be partially rescued by the Addition of Differentiation inducing factor-1 (DIF-1).