オートファジーにおけ Myosin VI の役割

板倉千絵子、Cambridge Institute for Medical Research, University of Cambridge

I would like to report 2 projects.

1) Ultrastructural Insights into Pathogen Clearance by autophagy.

Autophagy serves as a defence mechanism to combat proliferation of bacteria in the cytosol *(*xenophagy). After invasion pathogens such as *Salmonella enterica* serotype typhimurium reside in a *Salmonella*-containing vacuole (SCV) for proliferation. A small percentage of the bacteria escape from damaged vacuoles into the cytosol, which exposes luminal glycans to cytosolic lectins such as Galectin-8. Recruitment of Galectin-8 to damaged SCVs provides the first signal to recruit selective autophagy receptors such as NDP52. Subsequently, the cytosolic bacteria are surrounded by a dense ubiquitin coat, which is recognised by further autophagy receptors including optineurin, p62 and TAX1BP1.

In this study we have analysed this sequence of events leading to selective autophagy induction and phagophore formation. We have used Transmission Electron Microscopy to examine antibacterial autophagy and performed a detailed localisation of Galectin-8, ubiquitin, p62, TAX1BP1 and LC3 surrounding cytosolic *Salmonella*. Our results show that ubiquitin is not only present on the *Salmonella* surface, but also enriched on the fragmented SCV. Similarly, we detected the autophagy receptors, TAX1BP1 and p62, on the *Salmonella* surface, at the fragmented SCV and in addition on the outside of the phagophore. Our preliminary observations indicate that “naked” bacteria without any visible SCVs are less likely to assemble phagophores. These results imply that ubiquitinated and ruptured SCV membranes may play a role in the recruitment of phagophore membranes to *Salmonella*, promoting degradation of the bacteria by autophagy. In summary our data suggests that the ubiquitinated, Galectin8-positive fragmented SCV is equivalent to the ubiquitinated outer mitochondrial membrane, thereby highlighting the similarity between evolutionary related xenophagy and mitophagy.

2) Myosin VI and Actin are required for phagophore formation after induction of nonselective autophagy.

Motor proteins are nanomolecular machines that generate the forces and movements critical for many cellular processes. Myosin VI is a unique actin based motor that moves cargo towards the minus ends of actin filaments. The diverse cellular functions of myosin VI are mediated by interactions with distinct cargo adaptor proteins. The ESCRT-0 protein Tom1 is a myosin VI binding partner on early endosomes. The loss of either myosin VI or Tom1 reduces delivery of endocytic cargo to autophagosomes, thereby preventing autophagosome maturation and autophagosome-lysosome fusion.

In this study we have used Transmission Electron Microscopy to perform the detailed localization of myosin VI and actin filaments on autophagosomes. Our results show that myosin VI and actin display a very distinct distribution on the outer membrane of the phagophore during non-selective autophagy. Depletion or over expression of mutant myosin VI cause defects in phagophore formation/autophagosome closure suggesting that forces generated by the actin-myosin VI complex are required for autophagosome formation.