Visualization of Autophagy via Light and P Electron Microscopy in *D. rerio* Embryo Tailfins Infected by *M. marinum*

Zlatan Hodzic

The highly conserved cellular process of autophagy, from the Greek for self-eating, is a stress response system found in all multicellular organisms from yeast to humans. While it was first discovered over five decades ago, it is only in the last decade that extensive details about the mechanisms and pathways involved in the process have begun to be revealed. Autophagy is thought to have evolved as a nutrient recycling system, used by the cell in order to degrade non-essential cellular components for energy in the event of starvation. Not only that, it is also involved in degradation of foreign invaders such as infectious bacteria. Not surprisingly, autophagy has been found to be heavily implicated in countless human diseases, including cancer, neurodegeneration, and immune response to pathogenic infection.

Because of its highly conserved nature, autophagy can be readily studied and visualized in model organisms. The zebrafish (*Danio rerio*) in particular is an excellent model organism for autophagy studies due to the fact that zebrafish embryos are transparent, making visualization of transgenic cellular markers relatively easy. By utilizing transgenic zebrafish lines in which fluorescent proteins such as GFP are tagged to immune cells such as macrophages and leukocytes as well as autophagy related markers, one can visualize the microenvironment created by pathogenic infection via fluorescent light microscopy. In addition to this, a comprehensive study of autophagy related to infection requires a local infection site which can be sectioned for visualization via electron microscopy. Due to these stipulations, we have developed a method by which zebrafish embryos can be infected directly in the tailfin via microinjection of bacteria, giving both a local infection site and an extremely thin tissue which can be sectioned. Our experiments utilize the bacteria *Mycobacterium marinum* due to its ability to consistently grow in the tissue and form initial stage granulomas, similar to *M. tuberculosis* infection in humans.

In conclusion, we have shown that the injection of *M. marinum* into the tailfin tissue induces a local infection in which autophagy response as well as initial stage granuloma formation can be visually studied using both light and electron microscopy. The use of *M. marinum* has also been shown to be paramount in comparison to other bacterial strains in terms of growth and immune response stimulation. We are confident that with additional experimentation, other bacterial species can also be successfully implemented.

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Biology Education Center, Uppsala University, and Leiden University IBL, Gorlaeus Laboratory Cell Observatory, Einsteinweg 55, 2333 CC Leiden, The Netherlands
Supervisors: Rohola Hosseini, Dr. Marcel Schaaf