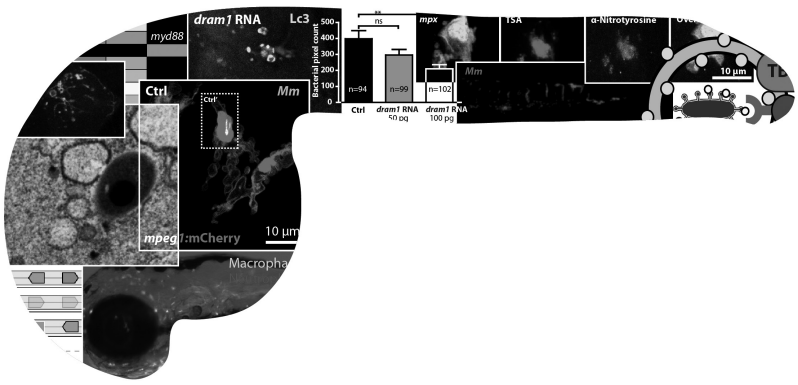
Chapter 1

Outline of the thesis



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Humans rely on their immune system for defense against invading microorganisms, such as pathogenic bacteria, fungi, parasites, and viruses. The defense against pathogens can be divided into two general types of host responses: (1) the innate immune response, and (2) the adaptive immune response. The innate immune response consists of cells and proteins that form the first line of defense against invading pathogens. Their function is to quickly arrive at the site of infection and fight off microbes that have breached physical barriers such as the skin or mucosal surfaces. The adaptive immune system is called into action when the innate immune response alone is not sufficient to kill the pathogen. Adaptive immunity involves the production of antibodies that bind and neutralize the pathogen, and the activation of T-cells that directly recognize and kill the pathogen. The innate immune system is the most ancient defense mechanism and many of its components are shared between invertebrate and vertebrate species. The adaptive immune system is unique to vertebrates and arose during the evolution of fishes. Nowadays the zebrafish is one of the most powerful model organisms for the study of the immune system.

This thesis focuses on the recognition of pathogenic bacteria and the defense mechanisms that are activated during the innate immune response. Innate immune detection of pathogens depends on a number of pattern recognition receptors (PRRs) capable of detecting the presence of evolutionary conserved structures on microbes, called pathogen-associated molecular patterns (PAMPs). In **chapter 2**, we introduce the different classes of PRRs available to the innate immune system and the defense mechanisms which they can activate upon detection of a pathogen. We also review the conservation of these receptors and mechanisms in zebrafish, and discuss recent studies using zebrafish infection models that have advanced our general understanding of the innate immune system.

The most extensively studied class of PRRs is the Toll-like receptor (TLR) family, which signals via adaptor molecules to initiate gene expression and activate the appropriate response upon recognition of a pathogen. In **chapter 3**, we present a zebrafish mutant line for the TLR signaling adaptor myeloid differentiation factor 88 (MyD88), which is central to the innate immune processes underlying infectious diseases, inflammatory disorders, and cancer. Zebrafish *myd88* mutant embryos were more susceptible to infection with the acute bacterial pathogens *Salmonella typhimurium* and *Edwardsiella tarda*, as well as the chronic bacterial pathogen *Mycobacterium marinum*. Further characterization revealed that gene expression of transcription factors and defense proteins important to innate immunity depends on Myd88 during these bacterial infections.

Mycobacterium marinum is a close relative of the human pathogen *Mycobacterium tuberculosis*, and causes tuberculosis disease in its natural zebrafish host. A potent innate defense mechanism against intracellular pathogens such as *Mycobacterium marinum* is the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). We studied the MyD88-dependent production of RNS in zebrafish innate

immune cells in **chapter 4**, and found that *Mycobacterium marinum* could attenuate this response and thereby avoid the cytostatic and cytotoxic effects of RNS.

Autophagy is a cellular homeostasis process that acts as a quality control system for eukaryotic cells. It has a central role in programmed cell death and survival by providing energy and nutrients and ridding the cytoplasm of unwanted components. Selective autophagy has recently emerged as defense mechanism activated by PRRs against tuberculosis disease, although the responsible molecular signaling pathway remained unclear. In **chapter 5,** we used zebrafish embryos and human macrophages to demonstrate that anti-mycobacterial autophagy is orchestrated by TLR-MYD88-NFκB signaling via DNA-damage regulated autophagy modulator 1 (DRAM1). Furthermore, *in vivo* overexpression of this autophagy regulator decreased mycobacterial infection by increased autophagosomal targeting of mycobacteria, presenting a potential novel therapeutic strategy for tuberculosis disease. Finally, the findings presented in this thesis are put into perspective of current knowledge of the innate immune system in **chapter 6**.