

How animals sense (and are related to) the microbial world: the impact of microorganisms on gut environment maturation of the protochordate *Ciona robusta*

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Abstract

This PhD project focuses on understanding the role of host-microbial interactions on the physiology of the marine invertebrate *Ciona robusta*. Host-microbial symbiosis is essential for the physiology of multicellular organisms, influencing diverse processes, i.e., immune system and digestive tract, as well as other organs, development, and metabolic and neurobehavioral functions. Most of these interactions take place in the digestive tract, where, often, microbial dysbiosis is linked to gastro-intestinal pathologies, as well as neurological diseases. Hence, a stable intestinal microenvironment is vital for host development and physiology/health, and it is maintained by cooperation between intestinal microbes, intestinal immune system, enteric nervous system, and endocrine system. Both the immune and nervous systems can be considered the systems that interface animals and the external world, and thus they are particularly susceptible, but also resilient, to the environmental changes.

Studies in this research field have been primarily focused on humans, however, fundamental questions remain, and the use of diverse model systems, including those that are “simpler” or possessing reduced complexity in genetic pathways (e.g. invertebrates) may be advantageous to answer diverse biological questions. The marine environment offers a huge catalog of invertebrate organisms that can help inform us of the central principles orchestrating homeostasis in the gut and, specifically, will help refine our recognition of the role(s) of innate immunity (the only immune system presents in invertebrates) in governing the complex gut ecosystem, and the effect of these interactions on the development and formation of the enteric nervous system.

Protochordates (e.g. tunicates and cephalochordates), or basal invertebrate chordates, are distant relatives of vertebrates, representing an attractive link in the study of animal physiology and evolution. In recent years, the tunicate *Ciona robusta* has been considered and studied as a holobiontic entity, identifying and characterizing diverse components of the gut environment, such as: *i)* a chitin-rich mucus that layers the epithelium; *ii)* a subset of secreted immune effectors, able to bind and influence the behaviors of diverse components of the gut microbiota (e.g., bacteria and fungi) and *iii)* the microbiota and virome communities, as well as the culture of diverse bacterial and fungal strains. As a valuable tool in this research field, a protocol has been developed for generating *C. robusta* germ-free juveniles. This PhD project leverages this established protocol and aims to investigate the role of the microbial community in shaping the digestive tract environment, comparing germ-free animals, from early stages of metamorphosis until stage 4, with animals reared under standard conditions (namely “conventional animals”). This aim will be achieved by investigating three main aspects: *i)* morphology of the digestive tract (from the esophagus to the intestine), through optical and electron microscope observations and histological approach; *ii)* expression of immune molecules and their localization in the digestive tract, through molecular biology techniques, such as RT-qPCR and whole mount *in situ* hybridization and *iii)* enteric nervous system characterization (i.e. cell type identification, localization, and development/maturation), with a molecular approach as well.

These findings will allow to answer the basic question on the role of microbes on the development of the innate immune and enteric nervous system in a marine invertebrate organism closely related to vertebrates, with thus an evolutionary relevance. These finding will pave the way to future, comparative, ecological and translational studies, for investigating the effect of both *i)* environmental changes on the microbial community and the consequent effect on host physiology (comparing to germ-free phenotype), and *ii)* manipulations of the microbial community for proper development of the gut environment or for the onset (and study) of pathologies linked to microbial dysbiosis and affecting the innate immune and enteric nervous systems.