Induction of regeneration in *Botrylloides diegensis*

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Master thesis in Biology

Colonial ascidians are marine invertebrates that belong to the Tunicata subphylum, which together with Vertebrata and Cephalochordata compose the Chordata phylum. Colonial ascidians form clonal colonies of adults, known as zooids, interconnected by a shared external vascular system embedded in a transparent and gelatinous matrix, termed tunic. Ascidiacea is the only know class of chordates with the ability to regenerate their whole body.

In *Botrylloides diegensis*, regeneration is triggered by isolating a small fragment of tunic containing part of the vascular system. One single functional individual is robustly restored after around 10 days by the proliferation of circulating stem cells within discrete loci of the vascular lumen called regeneration niches. The regulation of the regenerative capacity of these stem cells is mostly unknown. In particular, how regeneration is initiated and how spontaneous regeneration is prevented in uninjured colonies remain to be determined.

In this project, I investigate these two questions by combining surgical procedures, transfusions, chemical treatments and proteomics analysis. First, I tested the role of the various structures that compose the colony by comparing regeneration induction in the presence and absence of these factors. Second, I did transfusion between regenerating fragment and fragment not regenerating to determine whether inhibition is mediated by the haemolymph. Third, I chemically blocked regeneration to dissect how untreated haemocytes can rescue this process. Finally, I looked for candidate induction signals in the haemolymph using comparative proteomics.

This thesis starts with an introduction on the animals and their regenerative capacity. I then present the materials and methods that I developed, followed by a description of my experimental design. Finally, the results that were obtained are presented and interpreted. Overall, I have found that vascular connection to a developing zooid at any level (including the bud stage) is required for not inducing regeneration. Several candidate induction signals from haemolymph were determined and described, demonstrating the importance of the haemolymph in inducing regeneration. This thesis provides a first step towards the understanding of the molecular mechanisms underlying the induction of whole-body regeneration in *Botrylloides*.

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