Ciara Doody – Summer Studentship – Frank Lab, University of Galway

Sexual Development in the Cnidarian Model Organism *Hydractinia symbiolongicarpus*

As a BSc Biomedical Science student specialising in biochemistry, I have been fascinated by developmental biology since my first encounter with the subject. Seeking to gain hands-on experience, my attention was drawn to Professor Uri Frank, University of Galway, whose lab is one of few worldwide which study the cnidarian model organism *Hydractinia symbiolongicarpus*. In the wild, mature *Hydractinia* is sessile and resides on hermit crab shells. However, the animal is easily maintained in the lab, and grows on glass microscope slides in saltwater. *Hydractinia* appear as a colony of interconnected, genetically identical polyps, which are of the feeding or sexual type (Figure 1). This organism possesses a unique collection of attributes which make it a fascinating study model – it is clonal and colonial, does not age or develop cancer, and can regenerate whole body structures from few cells. The Frank lab aims to uncover the molecular mechanisms underlying these traits, and I was delighted to undertake my summer studentship here.

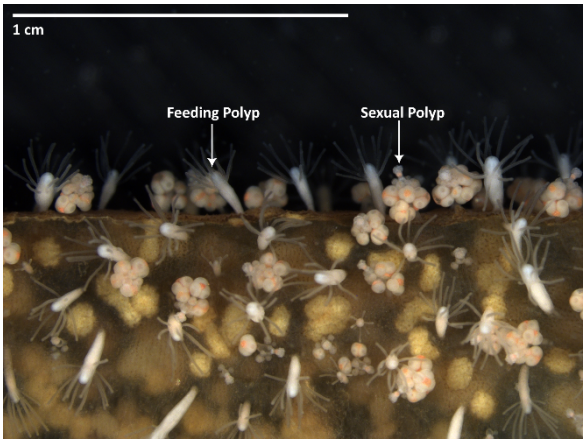

Figure 1: A mature colony of Hydractinia *on a microscope slide.*

A current project in the lab, headed by postdoc Dr. Camille Curantz, is focused on sexual development in *Hydractinia*. It is well characterised in Metazoa that the development of somatic gonad tissue dependents partly on the presence of germ cells, though exact nature of these interactions remain unclear [1]. Unlike mammals, *Hydractinia* does not sequester its germline during embryogenesis. After reaching sexual maturity at 3 months of age, the animal continuously produces germ cells, and in turn gonads, in the form of sexual polyps. This makes *Hydractinia* a convenient model in which to study sexual development. Previous research in the lab identified transcription factor AP2 (Tfap2) as a key player in germ cell fate, with *Tfap2* knock-out resulting in absence of germ cells and defective gonad development [2]. Differential expression analysis revealed the upregulation of *Dvr1*, a BMP-like gene, which Camille has found to be co-expressed with *Tfap2* in germ cells. Camille has engineered a number of *Dvr1*$^{-/-}$ mutants, which express a phenotype devoid of sexual polyps (Figure 2). The current model in the lab states that *Tfap2* specifies the germ cells, which then induce the patterning of the gonad by *Dvr1* expression. *Hydractinia* is a gonochoric organism, and the morphology of its sexual polyps is the only visual indicator of sex. This presents an obvious challenge in the case of the *Dvr1*$^{-/-}$ mutants, which do not possess any sexual polyps. In order to determine if *Dvr1* function is valid for both sexes, it is necessary to ascertain if the *Dvr1*$^{-/-}$ mutants are both male and female.

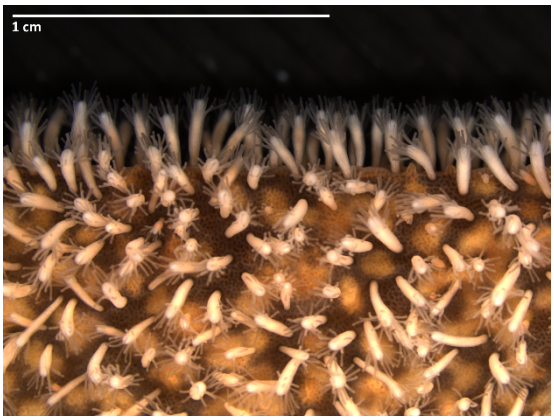

Figure 2: **Dvr1$^{-/-}$** ***mutant colony which lacks sexual polyps.***

I spent much of my time in the Frank lab attempting to design a PCR-based protocol for genotyping *Hydractinia.* PCR seemed an obvious choice, after a recent publication suggested that *Hydractinia* utilises an XY sex determination system [3]. I aimed to identify male-specific regions of the *Hydractinia* genome, design the appropriate primers, conduct PCR on male and female tissue samples, and discern the sex of the individuals based on observed gel products. In reality, the process was not so simple. The female genome assembly for *Hydractinia* (unpublished) is incomplete, which made primer design quite challenging. Genes I identified to be potentially male-specific bioinformatically, were shown to be also present in females by PCR. Cutting a long, agarose-filled story short, genotyping by PCR proved to be a herculean task. Indeed, whether an XY system is utilised by *Hydractinia* remains to be proven, and further still, the basis for sex determination in any cnidarian remains to be elucidated.

Overcoming these obstacles required some deeper thinking. We hypothesised that, since the *Tfap2* gene was intact in the *Dvr1*$^{-/-}$ mutants, some of their 'feeding' polyps may actually contain germ cells, but due to the absence of *Dvr1*, lack the somatic sexual structure. Experimentally, a germ cell in Hydractinia can be defined as a Piwi1-positive cell, present in the gastrodermis. With that, I conducted an immunofluorescence (IF) staining experiment on the *Dvr1*$^{-/-}$ mutants probing Piwi1. In all of the 8 mutant animals examined, germ cells were observed in ~50% of polyps, which is the proportion of sexual-to-feeding polyps in a wild-type colony. It appears as though 7 individuals are male, with 1 individual possessing large germ cells resembling immature oocytes – a likely female (Figure 3). While further experimentation probing spermatogenesis and oogenesis markers is required to confirm the identity of these germ cells, these results provide an alternative avenue to answer the question at hand.

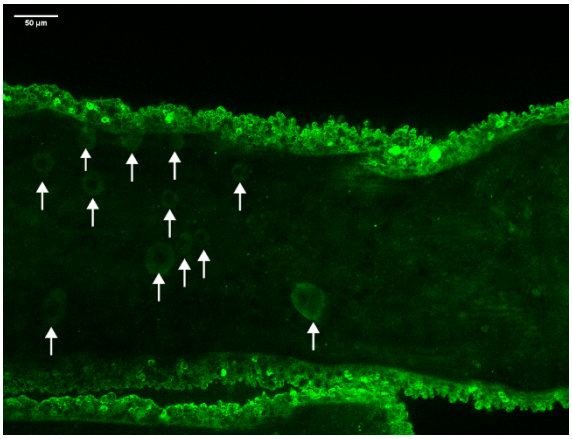

Figure 3: Cross-section of IF stained* Dvr1$^{-/-}$ *mutant polyp using Piwi1 antibodies. *Large Piwi1 positive cells – likely immature oocytes - are observed in the gastrodermis (arrows).*

A goal I had for my stay in the Frank lab was to expand my lab skills, which I certainly achieved. Aside from becoming well acquainted with PCR and gel techniques, I also gained the skills of microinjection, plasmid preparation, IF staining, experimental design and was able to contribute to the progression of the project. However, I never anticipated the level of confidence the studentship would provide me with, which is equally as valuable as these manual skills. Engaging in weekly lab meetings, scientific discussions with lab members, and being in an environment of active research has been of enormous value. Indeed, exposure to the realities of the field - not every experiment works out - has shown me the resilience it requires to be an academic researcher. Undoubtedly, this experience has consolidated my desire to pursue a PhD, and I would implore anyone considering the same to apply for a BSDB summer studentship.

I would like to extend an enormous thank you to everyone in the lab for being so welcoming and supportive during my stay. I had a fantastic time and was genuinely excited to come into the lab each day. Most particularly, thank you to Camille for being an excellent teacher, and to Uri for his exceptional guidance and support throughout.

Figure 4: Me in the lab, and my amassed collection of gel images!

References:

1. Rios-Rojas C, Bowles J, Koopman P: **On the role of germ cells in mammalian gonad development: quiet passengers or back-seat drivers?** *Reproduction* 2015, 149:R181-191.

2. DuBuc TQ, Schnitzler CE, Chrysostomou E, McMahon ET, Febrimarsa, Gahan JM, Buggie T, Gornik SG, Hanley S, Barreira SN, et al.: **Transcription factor AP2 controls cnidarian germ cell induction.** *Science* 2020, 367:757-762.

3. Chen R, Sanders SM, Ma Z, Paschall J, Chang ES, Riscoe BM, Schnitzler CE, Baxevanis AD, Nicotra ML: **XY sex determination in a cnidarian**. *BMC Biol* 2023, 21:32.