**How to build a spider: investigating the role of Delta in posterior segmentation**

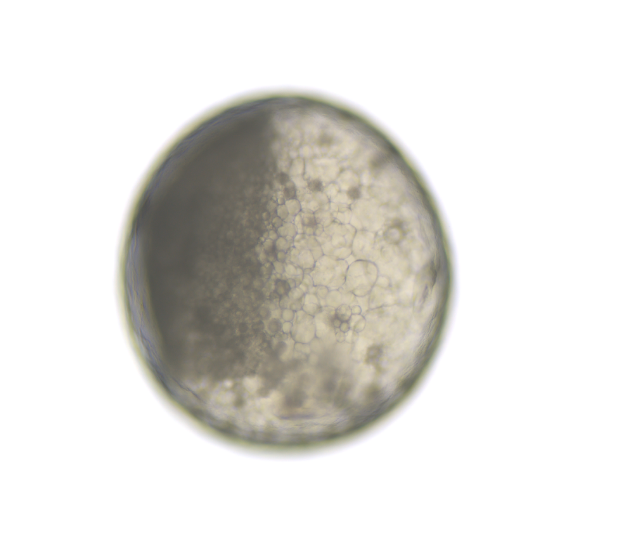
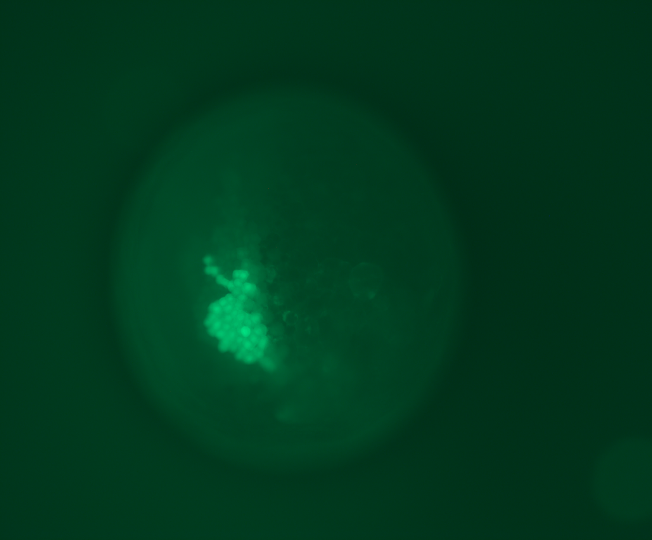
Three animal phyla have segmented bodies – the vertebrate chordates, the annelid worms and the arthropods. To understand the evolution and development of these segmented bodies it is necessary to identify what mechanisms regulate segmentation and the similarities and differences of these mechanisms among phyla. Amongst arthropods there are two main different mechanisms for segmentation: long-germ arthropods, such as *Drosophila melanogaster* which develop their segments simultaneously; and short-germ arthropods, constituting the majority of arthropods, which add their posterior segments sequentially from a segment addition zone (SAZ). Under supervision from Dr Anna Schonauer in Professor Alistair McGregor’s lab, I aimed to investigate the regulation of posterior segmentation in arthropods by further studying the Delta-Notch signalling pathway in the spider *Parasteatoda tepidariorum.*

Spiders are a useful model for answering questions about the regulation and evolution of segmentation as they develop their prosomal (anterior) segments simultaneous like *Drosophila* but their opisthosomal (posterior) segments sequentially in a manner analogous vertebrate segmentation. It is understood that the addition of posterior segments relies on the formation of the SAZ, which develops at around stage 6 in spider embryos through dynamic Wnt and Delta-Notch signalling. These genes are subsequently also required for segment addition. Specifically, Delta appears to differentially regulate the posterior and anterior regions of the SAZ to maintain *Wnt8* in the posterior SAZ but lower its expression in the anterior SAZ to facilitate the formation of nascent segments. However, little is known about the genes downstream of Delta that are involved in segment addition.

The gene *hairy* is thought to be involved in segment addition and it has been shown to have a similar oscillatory expression pattern to *Delta* leading to the hypothesis that it may be regulated by Delta as part of a gene regulatory network (GRN) that results in segment addition. Previous studies of the role of Delta in segment addition used parental RNA interference to knockdown this gene. However, the consequential loss of the SAZ resulting in a truncated germ band impedes investigation into any specific, localised downstream effects of loss of Delta. To be able to investigate the relationship between Delta and *hairy* I therefore aimed to use embryonic RNAi to knockdown Delta in subsets of posterior cells. This would allow embryonic development to progress as normal without truncating the germband, but create Delta knockdown clones allowing me to see within an embryo if *hairy* expression is different in cells with and without a knockdown of Delta. Thus I would be able to test whether *hairy* does act downstream of Delta and if so, might be able to infer what potential role it may have in segment addition.

Microinjections of Delta double stranded RNA and biotin into single cells at embryonic stage 1F (when there are only 32 cells) were carried out with the aim of knocking down Delta and at the same time staining the clone of cells derived from the injected cell. The microinjection technique itself was technically demanding, requiring a lot of patience and practice and it took me two weeks before I was able to successfully inject a cell without it bursting. Once I had injected successfully into a single cell (Figure 1) I gained experience in the correct technique needed and I was then able to successfully inject about 15% of embryos in a cocoon (a cocoon containing approximately 200 embryos). The other main technique I used was *in situ* hybridisations (ISH), which used labelled RNA probes to bind to the *hairy* mRNA in all the cells in the embryo to show where *hairy* is being expressed. I carried out the ISH two days after the microinjections as this is when embryos develop their SAZ and begin to add their first segments. As a control, I stained embryos solely for *Delta* or *hairy* as this allowed me to see the wildtype expression of each (Figure 2). I also performed a double ISH for Delta and *hairy* which confirmed their overlapping expression in the posterior of the embryo.

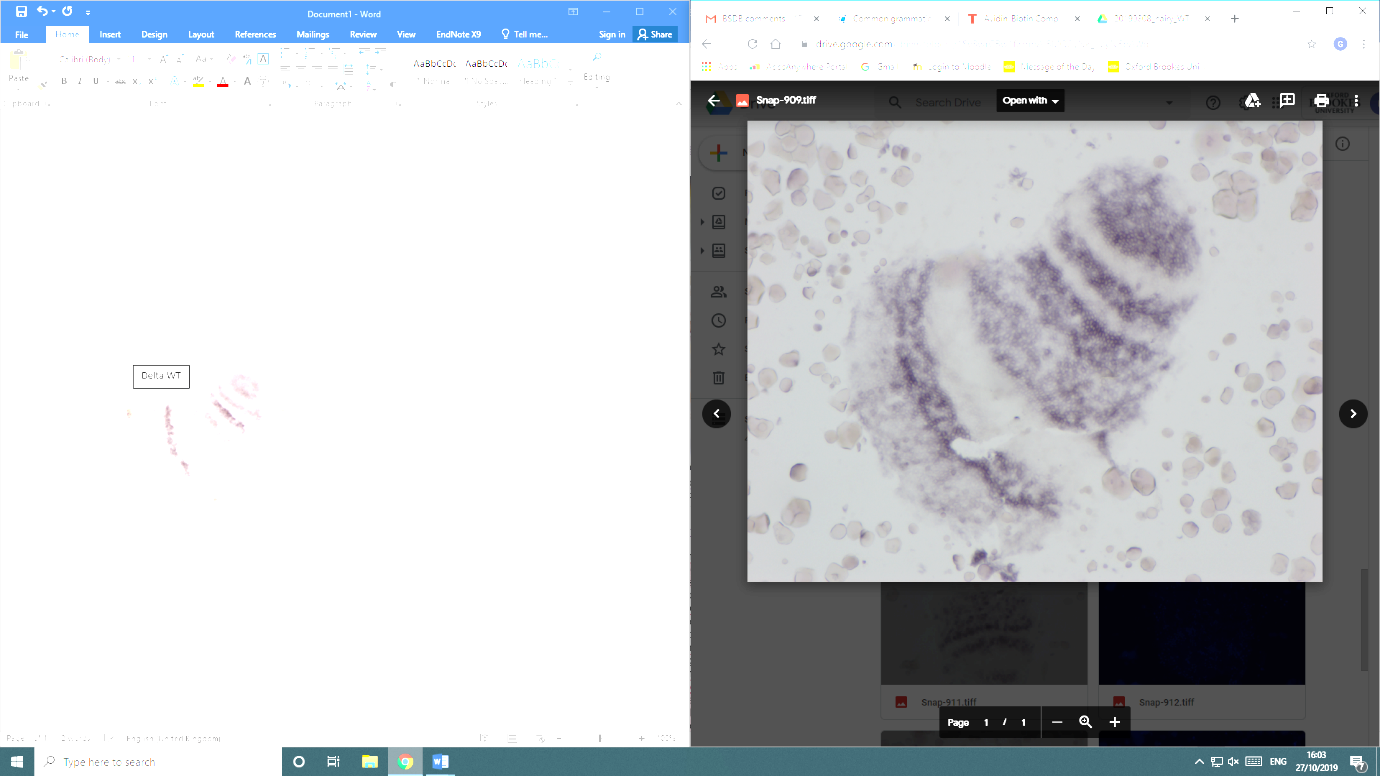
**Figure 1**: The first embryo I successfully injected with a practice injection mix containing Fluorescein isothiocyanate (FITC). (Left) A brightfield (BF) image of an injected embryo to show morphology and determine that the clone is located in the germ band (darker grey). (Right) The clone of cells derived from the cell indicated with a FITC dye which fluoresces green.



BF

GFP

Stage 5



*hairy*

*Delta*

**Figure 2**: Flatmounts of embryos at late stage 7 of development, the embryos are orientated so that the anterior of the germband is on the left. Wildtype expression of *Delta* (left image). Wildtype expression of *hairy* (right image)*.*

No blastodermal cell fate map exists for *P. tepidariorum* and as such it was never guaranteed that I would get clones in the posterior opisthosomal segments. Unfortunately, all my Delta knockdown clones occurred in the anterior prosomal segments so I was unable to draw conclusions about any *Delta-hairy* interaction in the SAZ. My anteriorly located clones however, do suggest an interaction between Delta and *hairy* in the prosomal segments. Here, I was able to detect downregulation of *hairy* expression in the subset of *Delta* knockdown cells. Most of our clones occur in a single prosomal segment, however in a stage 8 embryo, the clone spanned four developing prosomal segments, and showed downregulation of *hairy* across all of them. This suggests that *Delta* directs *hairy* in patterning of the anterior segments (data not shown).

Over the course of this project I learned many different things, most importantly that in science, patience and perseverance are key. I was surprised with how involved each technique was and quickly learned to take the advised protocol amendments suggested by my peers. Being part of a team all of whom supported me, helped me and questioned my work ultimately encouraged me to become a better researcher. The experience has confirmed my ambition to pursue a PhD and focused my interest on understanding the fundamental and complex molecular interactions that ultimately regulate and drive development.