SRF Vacation Scholarship Report 2014 – *CG9879* in the *Drosophila* Testis

Student: Rebecca Cole **Supervisor: Helen White-Cooper** **Faculty: Cardiff University**

Introduction and Project Aims

In all sexually reproducing animals, spermatogenesis is a highly complex process that requires the carefully timed interaction of thousands of genes. In *Drosophila,* the testis meiotic arrest complex (tMAC) plays a key role in spermatogenesis regulation (Beall *et al.* 2007), acting as a transcription factor that drives the expression of many testis-specific genes. Amongst the tMAC-dependent genes may be genes that encode further transcription regulators that go on to activate further testis-specific genes. *CG9879* has been proposed to be one such tMAC-dependent transcription regulator (Laktiniov *et al.* 2014).

The aim of this this eight-week summer project was to begin characterising *CG9879.* Two experimental techniques were carried out to this end. The first was *in situ* hybridisation (ISH) to determine the distribution of *CG9879* transcripts within the testis. Related genes were also examined using ISH to inform our understanding of the evolution of *CG9879*'s expression pattern. The second was the generation of GFP-CG9879 constructs, and their expression in fly testes. This would allow the behaviour of CG9879 within testis cells to be examined.

In situ Hybridisation

Five genes were under investigation in this part of the project; *TATA-binding protein* (*TBP*) and the related factors *TBP-related factor* (*TRF*), *TRF2*, *CG9879* and *CG15398*. *TBP* and *TRF* are ancient genes evolutionarily. *TRF2* is a dipteran specific paralogue of *TRF. CG9879* and *CG15398* are Drosophila specific paralogues of *TRF2*.

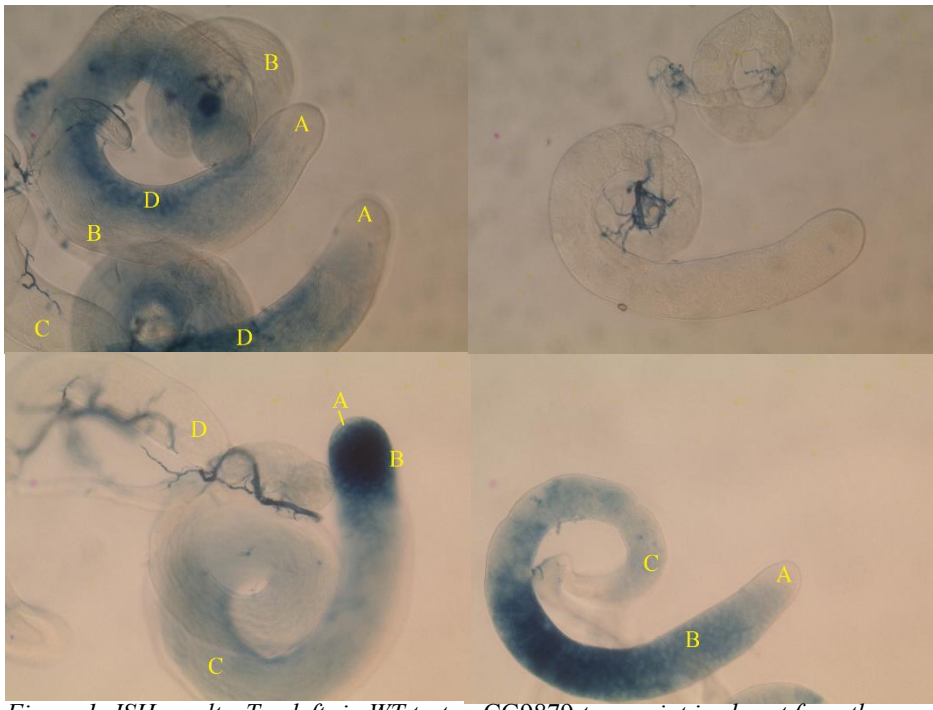

Figure 1: ISH results. Top left; in WT testes, CG9879 *transcript is absent from the apical tip (A) elongating spermatids (B) and associated somatic tissue (C), but present in late spermatocytes (D). Top right;* CG9879 *transcripts are totally absent from* aly *mutant testes. Bottom left; in WT testes,* TRF2 *transcript is absent from the hub and spermatogonia (A), present in early-mid spermatocytes (B),largely absent in elongating spermatids and completely absent in associated somatic tissue (D). Bottom right; in* aly *mutant testes,* TRF2 *transcript is absent from the apical tip (A), accumulates in the non-meiotic spermatocytes (B) before degrading (C). Strongly coloured thread-like structures are respiratory tracheae, which show non-specific staining.*

PCR was used to amplify 500-600bp of coding sequence from the genes of interest from fly gDNA. In each case the reverse primer had a T3 RNA polymerase site added at the 5' end. When products had been obtained and purified, they were used along with T3 RNA polymerase and Digoxygenin (Dig)-labelled NTPs to make Dig-labelled antisense mRNA probes according to Morris *et al*. (2009). Wild type (WT) and mutant testes were used in the ISH procedure. These were dissected from 0-1 day old male flies and fixed in paraformaldehyde. Mutant flies were *always early* (*aly*) and *tombola* (*tomb*) mutants, both mutants in tMAC components and both therefore having a meiotic arrest phenotype. In these mutants, meiosis fails so that the testis is filled with spermatocytes and contains no spermatids (reviewed in White-Cooper 2010).

Once RNA probe and fixed testes had been acquired, ISH was carried out according to Morris *et al*. (2009). In brief, testes are incubated with probe, then phosphatase-conjugated anti-Dig antibody, then NBT and X-phosphate together. This results in a colour reaction at the site of the probe. The results of the ISH experiments for *CG9879* and *TRF2* is shown in figure 1. Results for *CG15398, TBP* and *TRF* (not shown) were similar to those for *TRF2.*

GFP-CG9879 Construct Generation

The vector used in the generation of a GFP-CG9879 was pP{UAS-EGFP} (Parker *et al.* 2001), which tags the inserted sequence with eGFP as an N-terminal fusion. PCR was used to acquire the full *CG9879* coding sequence from testis cDNA. An *Nde1* restriction site on the forward primer and a *Not1* restriction site on the reverse were included to facilitate ligation into the vector via complementary restriction sites on the vector's polylinker following the digestion of PCR product and vector with *Nde1* and *Not1*. After ligation, the recombinant plasmids were transformed into competent *Escherichia coli* cells for cloning. The cloned plasmids were then injected into 200 w^{1118} *Drosophila* embryos. Of the 19 surviving adults, only one produced transgenic progeny. The *BamGal4VP16* driver consists of the transcription factor Gal4 (recognises UAS) driven by the *bag-of-marbles* (*bam*) promoter, which drives transcription in late spermatogonia and early spermatocytes (Chen and McKearin 2003). Flies carrying *BamGalVP14* were crossed with the *GFP-CG9879* transgenics to bring these two components together. There was not time within the project to see the results of these crosses, however some predictions can be made. If CG9879 does indeed act as a transcription factor we would expect to see it enriched in the nucleus, where it is able to interact with chromatin. Other localisations would cast doubt on this proposed function.

Further Work

Flies expressing GFP-CG9879 in their testes must be examined to determine the stability and cellular localisation of the fusion protein. Furthermore, antibodies against CG9879 could be used to detect the localisation of CG9879 in non-transgenic testes, in case the over-expressed fusion protein does not accurately reflect the localisation of the natural protein driven by its own promoter. The mRNA distribution of *TRF2* transcripts in *aly* mutants compared with wild types is odd; expression in early spermatocytes is much weaker in the mutant testis, taking a while to accumulate to the levels seen in the wild type, but appears to reach a peak greater than that in the wild type. However, ISH is not a truly quantitative protocol. Quantitative RT-PCR on individual mutant and wild type spermatocytes of different ages could clarify whether these apparent patterns are real or simply a product of the abnormal morphology of mutant testes. Access to *CG9879* loss of function mutants, perhaps generated by CRISPR genome editing, would be extremely helpful.

References

Beall, E. L., Lewis, P. W., Bell, M., Rocha, M., Jones, D. L. and Botchan, M.R. (2007). Discovery of tMAC: a Drosophila testis-specific meiotic arrest complex paralogous to Myb-MuvB. *Genes and Development.* **21(8):**904-919.

Chen, D. and McKearin, D. M. (2003). A discrete transcriptional silencer in the *bam* gene determines asymmetric division of the *Drosophila* germline stem cell. *Development* **130:**1159–1170.

Laktiniov, P. P., White-Cooper, H., Maksimov, D. A. and Belyakin, S. N. (2014). Transcription factor Comr acts as a direct activator in the genetic program controlling spermatogenesis in *D. melanogaster*. *Molecular Biology.* **48(1):**130-140.

Morris, C. A., Benson, E. and White-Cooper, H. (2009). Determination of gene expression patterns using *in situ* hybridisation to *Drosophila* testes. *Nature Protocols*. **4(12):**1807-1819.

Parker, L., Gross, S. and Alphey, L. (2001). Vectors for the expression of tagged proteins in Drosophila. *BioTechniques.* **31(6):**1280-1286

White-Cooper, H. (2010) Molecular mechanisms of gene regulation during *Drosophila* spermatogenesis. *Reproduction. **139:** 11-21.*