SRF VACATION SCHOLARSHIP REPORT 2019

The form below should be completed by the student, then forwarded to the supervisor for approval and submission to srf@conferencecollective.co.uk within 8 weeks of completing the project. Please submit the form as a Word document.

A maximum of one figure (with legend of less than 100 words) may be appended if required.

Please note: excerpts from this form may be published on the SRF website, so please ensure content is suitable for website publication, and does not compromise future dissemination of data in peer-reviewed papers etc. The SRF reserves the right to edit responses to ensure suitability for publication on the website, newsletter or in promotional material.

Student's Name:	Katherine Morling	**Student's Institution/University:**	University of Bristol
Degree Title and year of study:	MSci Biochemistry, fourth year.		
Supervisor's Name:	Dr Binyam Mogessie	**Supervisor's Department and Institution:**	School of Biochemistry, University of Bristol
Project Title:	Actin-Based Mechanisms of Chromosome Segregation		

Briefly outline the background and aims of the project *(max 200 words)*

Meiosis is a specialised form of chromosome segregation whereby two rounds of divisions reduce genome size from a diploid to a haploid. This occurs during mammalian egg formation from oocytes. Inaccurate chromosome segregation leads to aneuploidy, a common cause of miscarriages and genetic disorders including Down's syndrome. Recently, spindle actin filaments have been shown to promote accurate chromosome segregation through interaction with spindle microtubules.

My project aimed to help identify spindle actin assembly proteins (SAAPs), using microscopy, to further understand the mechanism of spindle actin assembly and meiosis-specific actin-microtubule crosstalk. I aimed to build on previous work on potential SAAP Myosin-10, which has been shown to bind both actin and microtubules in frog oocytes and also to localize to the meiotic spindle in mouse oocytes. I had planned to use a protein degradation assay in mouse oocytes with both super-resolution live microscopy and immunofluorescence microscopy to determine the role of Myosin-10 in spindle actin formation and accurate chromosome segregation.

Did the project change from that proposed in the application? If so, what changes were made and why? *(max 100 words)*

After few initial experiments, we decided to instead focus on studying the function of actin filaments that reside inside the oocyte nucleus (nuclear actin), in order to make meaningful progress during my summer studentship. This study built on work that I undertook previously in my host lab during the summer of 2018, where actin was visualised inside the nucleus of mouse oocytes via phalloidin staining. A large part of my project involved cloning of fluorescently-labelled genes for live microscopy, and I was able to carry out experiments using super-resolution live microscopy and immunofluorescence microscopy, as described in the original proposal.

What were the main results/findings of the project? *(max 300 words)*

A large number of genes were successfully cloned and verified by sequencing. PCR and Gibson Assembly were used to tag proteins such as H2B with fluorophores such as GFP and mScarlet for live microscopy experiments. Some of these genes were then successfully transcribed into in mRNA

in vitro, for microinjection into mouse oocytes. The main aim of this cloning exercise was to adapt 'knock-sideways', a previously described method of protein inactivation, in mouse oocytes. By combining this tool with targeted degradation of nuclear actin in prophase-arrested oocytes, we sought to first degrade nuclear actin then stop this degradation when oocytes underwent nuclear envelope breakdown. This would be achieved by sequestering the proteins responsible for degrading nuclear actin to lysosomes after disassembly of the nucleus upon meiosis resumption.

I first cloned an actin disruption domain tagged with a nuclear localization signal and using confocal microscopy confirmed successful targeting of this domain to the nucleus. This construct in addition harboured an FRB domain that specifically dimerizes with FKBP domains in the presence of Rapamycin. I next cloned a construct encoding EGFP-tagged LAMP1, a lysosomal protein, that also contained a FKBP domain. In my first experiment, I tested whether the actin disruption domain could be targeted to lysosomes through the FRB-FKBP domain interaction. Here, I used confocal live imaging of oocytes undergoing nuclear envelope breakdown in the presence of Rapamycin. Excitingly, results from this experiment showed significant colocalization of the actin disruption domain to lysosomes only in the presence of Rapamycin, suggesting that the knocksideways approach indeed works in mouse oocytes. After live imaging, I fixed these oocytes and labeled actin with phalloidin. This experiment indicated specific reduction of actin only in oocytes where the actin disruption domain was freely present in the cytoplasm but not in oocytes where it was sequestered to lysosomes. While further optimization of mRNA expression levels is needed to perfect this method in oocytes, these results are already very encouraging.

What do you conclude from your findings? *(max 150 words)*

The findings from the FRBP-FKB experiment suggest that the actin disrupting domain was indeed sequestered to the lysosomes upon nuclear envelope breakdown, and therefore experiments following this design could be used to study the effects of nuclear actin loss specifically. However, further optimisation is needed to entirely sequester the chromosomes and actin. This could be achieved by injecting a higher concentration of LAMP1-FKBP mRNA.

How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice? *(max 150 words)*

My time in the Mogessie lab has been highly influential in my decision to pursue a career in science. As I thoroughly enjoyed the challenge of working in a research lab environment, it is likely I will apply for a PhD in a related area, or a graduate job working in a lab setting. As I was able to learn and practice techniques outside of the normal degree course, I also now feel much more confident in my lab skills and therefore more prepared for my Master's lab project starting in September. I feel I have a greater understanding of how to interpret unusual results and to design and improve experiments to address previous issues, which will be highly beneficial in my future work.

Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship *(max 100 words)*

Student: I am very grateful to the SRF for enabling me to undertake this project from which I have greatly improved my laboratory skills and gained an invaluable understanding of the workings of a research lab.

Supervisor: It was a great pleasure to have Kate in the lab and we are thankful to the SRF for making this possible. Kate showed strong interest in her project and made very good progress in her laboratory skills such as mammalian oocyte isolation and handling as well as a range of molecular biology techniques (GA cloning, *in vitro* mRNA synthesis, etc). The contribution of the SRF to training the next generation of scientists like Kate is immense and we look forward to taking advantage of this scheme again soon.