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:	Neil Taylor	**Student's Institution/University:**	University of Edinburgh
Degree Title and year of study:	BSc (Hons) Reproductive Biology 3rd Year		
Supervisor's Name:	Roseanne Rosario	**Supervisor's Department and Institution:**	MRC Centre for Reproductive Health, University of Edinburgh
Project Title:	Validation of protein sequestration by CGG RNA aggregates in Fragile X-associated premature ovarian insufficiency		

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

Fragile X-associated primary ovarian insufficiency is an X-linked genetic condition which causes early-onset menopause in women carrying the premutation allele. This premutation consists of 55-200 CGG repeats in the 5' untranslated region of the FMR1 gene, however it is not known whether these repeats cause disease by an RNA- or protein-mediated mechanism. Previous research in the host lab has shown that CGG-repeat RNA forms aggregates that increase cell death in granulosa cells, and that these aggregates bind and deregulate specific proteins *in vitro*, suggesting an RNA-mediated mechanism of disease.

The aim of the project was to validate whether a selection of these proteins, chosen due to their key roles in the cell, are sequestered by these CGG aggregates *in vivo*. HGrC1 granulosa cells were transfected with plasmids causing expression of CGG-repeat RNA and plasmids to overexpress proteins of interest, most of which were GFP-tagged for immunocytochemistry detection. Immunocytochemistry and RNA *in situ* hybridization were used in combination to visualise the proteins of interest and the CGG aggregates under a confocal microscope.

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

Initially, the project's aim was to validate whether proteins RBM14, DDX5 and TAF15 colocalised with CCG aggregates *in vivo* and quantify any such colocalisation. However, due to issues with cloning the overexpression plasmids, candidate proteins Tra2β, DHX9, PA2G4 were investigated instead, as well as TAF15. In addition, a low transfection efficiency and suspected toxicity of some of the overexpressed proteins meant few double-transfected cells survived and few colocalisation events could be observed, limiting quantification. As such, this project operated as a preliminary

investigation into which proteins show some colocalisation and would be suitable for further study by the supervising laboratory.

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

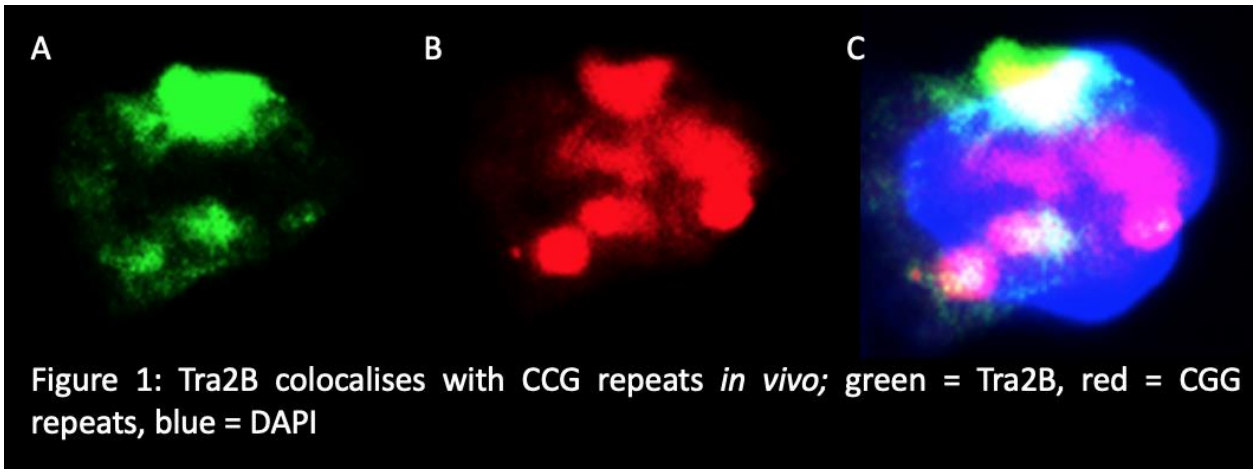

Figure 1: Tra2B colocalises with CCG repeats *in vivo;* green = Tra2B, red = CGG repeats, blue = DAPI

Of Tra2β, DHX9, PA2G4 and TAF15, Tra2β had evidence for colocalisation and DHX9 had evidence against colocalisation; not enough evidence was gathered either way for PA2G4 or TAF15.

Figure 1 shows a representative image of Tra2β colocalisation with CCG repeats *in vivo*; nearly all immunocytochemistry staining of Tra2β directly overlaps with *in situ* hybridisation staining of CCG repeats in this granulosa cell nucleus. In contrast, DHX9 was found to be distributed in the cytoplasm of the cells and therefore showed no co-localisation with CGG repeats in the nucleus.

However, it should be noted that fewer than 10 cells with good double staining were identified and imaged in this project across all 4 proteins due to the issues outlined above; as such, thorough further investigation is needed to validate and quantify this early evidence.

This project also helped to identify and troubleshoot problems with the protocol, including in plasmid cloning and the transfection process, to aid the host lab with future experiments.

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

This project demonstrated how it is much more difficult to detect sequestration *in vivo* than *in vitro*, as examinations can easily cause cell death and invalidate experiments *in vivo*, whereas this is not a problem *in vitro*. In addition, acquiring quantitative as opposed to qualitative results is much more difficult.

Despite this, it can be concluded that some sequestration of proteins *in vivo* by CCG-repeat RNA aggregates occurs. As such, it is feasible that an RNA-mediated mechanism causes pathology in FXPOI and that key RNA-binding proteins colocalise with these repeats *in vivo* in granulosa cells; there is evidence that these proteins include Tra2β and evidence against DHX9.

The sequestration of bound proteins may cause pathology by disrupting their key functions; however, further investigation of any proteins found to colocalise with CCG repeats by the protocol used in this project would be needed to confirm whether they were involved in FXPOI. Knockdown of Tra2β, an mRNA splicing regulator, has previously been shown to cause reduced proliferation and promote apoptosis, so this could be a key candidate protein for FXPOI research.

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

The experiences of carrying out this project, being able to work in the environment of the MRC Centre for Reproductive Health, and spending time with various people involved in research,

including researchers, postgraduate students and technicians, have only fuelled my interest and ambition to go on to work in the biomedical sciences. It has given me invaluable experience that will undoubtedly aid in my future study and, hopefully, career. I have garnered further insight into the research process while also gaining experience in a variety of research techniques and protocols pertinent to both my current and future study.

I enjoyed the whole project, despite the frustration of setbacks and problems, again giving myself renewed confidence in my chosen career path. In addition, I hope to carry out my honours-level research project in the host lab and continue contributing to this project, something I am looking forward to.

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

Student: *The SRF Vacation Scholarship has been invaluable in helping me complete this project and gain valuable insight into my potential future career; thank you to the SRF for this opportunity!*

Supervisor: *SRF Vacation Scholarships not only provide the opportunity for students to gain insight into research, but also young scientists to gain mentoring experience. We are very grateful to the SRF for funding Neil, which enabled him to undertake a summer lab project with us. With the funds contributed by the SRF, Neil has been able to further this project, and identify key proteins which will be the focus of future work.*