SRF VACATION SCHOLARSHIP REPORT 2016

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Degree Title and year of study:	BSc Biomedical Sciences, third year		
Supervisor's Name:	Daniel Cooney	**Supervisor's Department and Institution:**	Institute of Genetic Medicine
Project Title:	Regulation of cohesin in non-growing oocytes		

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

The growing trend for women to delay pregnancy into later in life has resulted in an increased risk of infertility, miscarriage and developmental defects. This is due to an age-related increase in chromosomal segregation errors (aneuploidy) in oocytes that are thought to arise due to a loss of chromosomal cohesion (1). In humans, Oocytes can remain at the primordial stage of development in prophase arrest for up to 40 years before undergoing the meiotic divisions (2). Work in mice indicates that chromosomal cohesin is depleted in oocytes ovulated late in life. (1-3). Research in our lab carried out by a PhD student revealed that cohesin is lost in the primordial stage before the oocyte resumes meiosis.
Accurate chromosome segregation is dependent upon the regulation of a cysteine protease called Seperase that cleaves the Rec8 subunit of cohesion (2). In meiosis I, centromeric cohesion between sister chromatids is protected from Seperase cleavage by shugoshin (Sgol2 in mammals) and has to remain intact until the second meiotic division. Therefore, disruption of the correct regulation of Seperase could result in "leaky" separase which could be cleaving cohesin prematurely and thus causing chromosome missegregation. (4)
It is therefore essential to first determine if Separase is present in the primordial oocyte before investigating if it could be responsible for loss of cohesin.

References:
1. Age-related decrease of meiotic cohesins in human oocytes.
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0096710
2. Un Ménage à Quatre: The Molecular Biology of Chromosome Segregation in Meiosis
http://www.sciencedirect.com/science/article/pii/S0092867403000837
3. Chromosome Cohesion Established by Rec8-Cohesin in Fetal Oocytes Is Maintained without Detectable Turnover in Oocytes Arrested for Months in Mice
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4791431/
4. Shugoshin protects cohesin complexes at centromeres
http://europepmc.org/articles/PMC1569468

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

The project investigated the regulation of cohesin as proposed, however the focus was entirely on separase. There was no time to progress to investigating expression of Esco1 due to the time taken to optimise the Separase antibody.

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

In order to determine the location of the primordial oocyte and its cytoplasm I used immunofluorescence staining of PFA fixed cryosections from Rec8-Myc mice. These were stained for DDX4 and REC8 before being mounted using Vectashield DAPI mounting media.

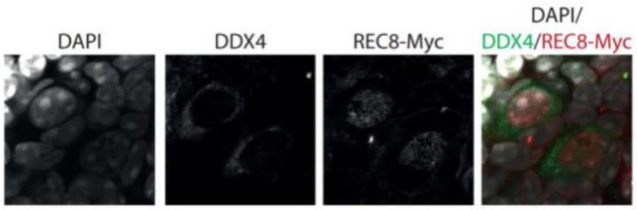

Figure1. Cryosections of PFA-fixed ovarian sections from Rec-Myc transgenic mice showing nuclear localisation of Rec8-Myc and cytoplasmic staining of DDX4 in a representative primordial-stage oocyte.

The images in Figure 1 show that in primordial stage oocytes (which could be distinguished by the surrounding flattened granulosa cells and REC8-Myc staining) the REC8-Myc signal is located in the nucleus while the DDX4 staining is strictly in the cytoplasm. As the DDX4 staining is only found in the cytoplasm of primordial-stage oocytes, this could be used to distinguish primordials from other cells and to distinguish the primordial cytoplasm from the cytoplasm of other cells.

I next investigated whether Separase is expressed in primordial-stage oocytes. Figure 2A shows ovarian cryosections stained with DDX4, Separase (with and without blocking peptide) and mounted using Vectashield DAPI mounting media. Primordial oocytes were selected based on the presence of DDX4 staining using immunofluorescence imaging. Figure 2B shows the location of Seperase detected, in relation to DDX4 and DAPI, with or without the blocking peptide. DDX4 staining and Separase staining co-localise together in the cytoplasm of the primordials while separase staining is markedly weaker in the nucleus. This would suggest that Separase is present in primordial stage oocytes but predominantly in the cytoplasm. No signal was detected in the presence of a blocking peptide. Thus, these findings indicate Seperase is expressed in primordial-stage oocytes and co-localises with DDX4 in the cytoplasm, but appears to be markedly reduced in nucleus of primordial-stage oocytes.

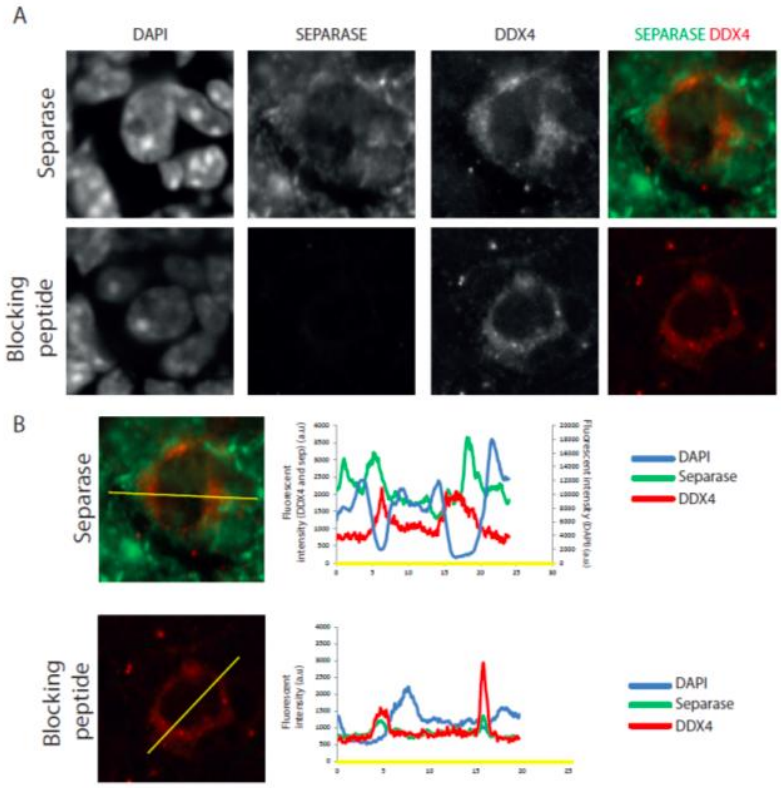

Figure 2. (A) Images showing representative primordial-stage oocyte in a PFA-fixed ovarian cryosection. The top panel shows sections were separase blocking peptide was not included in the staining procedure with the bottom panel showing when it was (B) Shows linescan analysis carried out to investigate localization of separase with regards to DAPI and DDX4 on images with and without separase blocking peptide.

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

The results suggest that the cysteine protease Separase is located in the primordial stage oocyte. Seperase appears to be present predominantly in the cytoplasm. By comparison, localisation to the nucleus appears to be very much reduced. This may limit access for separase to chromosomal cohesin. However, this experiment included only 2 month old mice and it would be interesting to determine

whether the mechanisms responsible for keeping separase levels low in the nucleus become defective with advancing female age, which could contribute to age-related depletion of cohesion. This work also shows that DDX4 can be used as a useful tool for detecting primordial stage oocytes in ovarian sections. Future work will focus on (1) Separase expression and localization in oocytes from aged mice and (2) on expression and localisation of the Seperase inhibitor securin.

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

This project has been an invaluable experience, that helped me improve my practical and technical skills and thus, has prepared me for my third year project. The numerous challenges faced in the lab have helped me enhance my critical thinking and allowed me to put my scientific knowledge into practice. I also had the chance to learn new interesting techniques. Working independently in the lab enhanced my confidence, since I had to deal with new challenges, take decisions, evaluate results and manage time efficiently. This project has been really helpful for me; I seriously consider research as a future choice for my masters.

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

Student: It is the perfect opportunity for students to gain practical experience, preparing them efficiently for their third year project. It improved my understanding of how it is working in the lab, and thus helped me become more independent, while learning from experts in the research area.

Supervisor: