UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2016/17

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Name of student:

Fiona Cronin

Name of supervisor(s):

Dr. Helen Dodson

Project Title: (no more than 220 characters)

Analysis of the expression, abundance and tissue-specific distribution of DNA damage response protein H2AX in the model organism *Hydractinia echinata.*

Project aims: (no more than 700 words)

DNA of all eukaryotic cells is packaged using histones. The histone variant H2AX has been established as having a crucial role in the DNA damage response. The aim of this project was to investigate the recently identified H2AX variants in the model organism *Hydractinina echinata*. Hydractinia has an exceptional capacity for regeneration and resistance to tumourogenesis and is therefore of interest to researchers in the fields of stem cell biology, regenerative medicine and cancer biology. Previously published work has shown that the histone complement of this model organism includes two H2AX variants among others that are *Hydractinina*-specific (Torok et al., 2016). Having analysed details of the histone complement of *Hydractinia*, we aimed to contribute towards the characterisation of H2AX.1 and H2AX.2 in *Hydractinia* by analysing the transcripts quantitatively and investigating the localisation of the proteins. Particular attention was to be given to the H2AX.2 variant localised to female germ cells to determine whether its presence potentially contributed to the repair capacity of these cells. The quantification of H2AX transcripts in this model organism could give us an insight into their contribution to the organism's specialised biology and consequently enhance our understanding of developmental biology and regeneration. Qualitative analysis could inform us of the contribution of H2AX to the DNA damage repair capacity of the cells in which it is found.

A variety of approaches to studying this organism will be applied, in particular qPCR to determine abundance of H2AX.1 and H2AX.2 and immunohistochemistry to investigate tissue specificity of these histone variants.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Three different polyp types were used in our experiments – feeding, female sexual polyps and male sexual polyps. The experiments carried out on these polyps broadly branched into three different paths; (1) quantitiative RT-PCR, (2) whole mount immuno-staining and (3) H&E staining and immunohistochemistry on paraffin embedded sections.

Quantitative reverse-transcription PCR for the H2AX.1 and H2AX.2 transcripts was optimised in the laboratory. An efficiency of over 90% was achieved for all primer pairs and GAPDH was found to be a suitable normaliser gene across all polyp types. Real time PCR data was collected in triplicate and mean CT values are shown in Table 1.

H2AX.1	mean CT
FSP	23.40
MSP	21.94

H2AX.2	mean CT
FSP	22.16
MSP	26.44

H4	mean CT
FSP	26.23
MSP	18.80

GAPDH	mean CT
FSP	19.55
MSP	18.03

Table 1*. q-PCR analysis of relative expression of H2AX.1, H2AX.2 and H4 in male and female polyps.*
The CT value data was analysed using the delta delta CT method to determine the relative expression of H2AX.1, H2AX.2 and H4 (Figure 1) in the male and female polyp types.

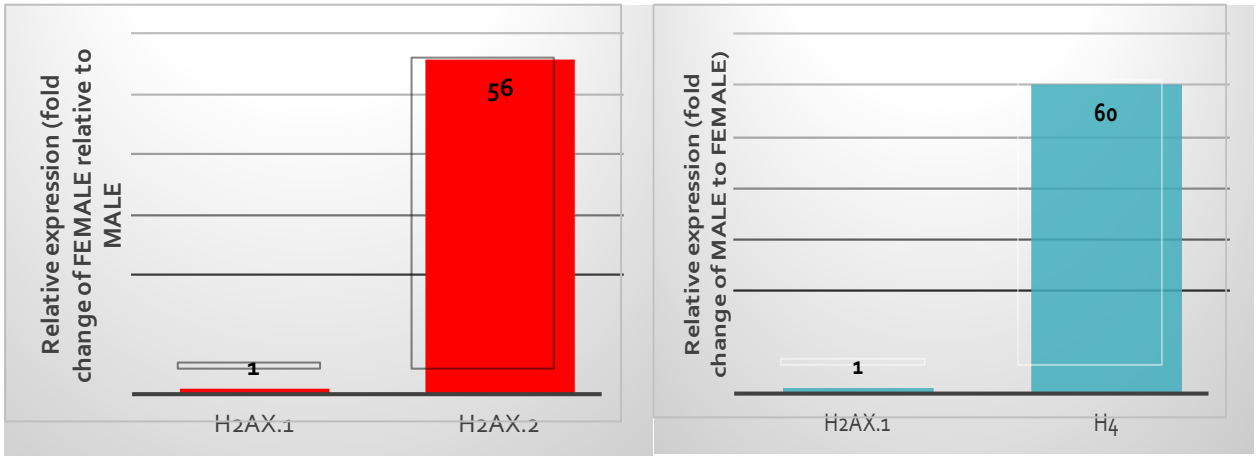

Figure 1. *(A) relative expression of H2AX.1 and H2AX.2 in female compared to male polyps. (B) relative expression of H2AX.1 and H4 in male compared to female polyps.*

q-PCR analysis revealed that H2AX.1 is expressed at equivalent levels in both male and female polyps (we were unable to analyse the feeding polyps due to low quality RNA extraction). H2AX.2 is much more highly expressed in female polyps as compared to male (56 fold more). This finding is expected as previous reports had identified H2AX.2 as being localised specifically to the female germ cells. However, the level H2AX.2 transcript compared to H2AX.1 is quite striking. A less expected result was that H4 was found to be very highly expressed in male polyps as compared to female (60 fold). The reasons for this is likely that a very high abundance of the canonical histones are required for sperm packaging in this organism.

To gain some insight into the localisation of H2AX and the potential of available H2AX antibodies we performed an immunostaining experiment on whole mount feeding polyps. The polyps were fixed in PFA and permeabilised prior to blocking and incubation with an antibody raised against human H2AX (Abcam ab11175). Following incubation with an Alexa-488 conjugated secondary antibody the animals were counterstained with TRITC labelled phalloidin and hoechst (Figure 2).

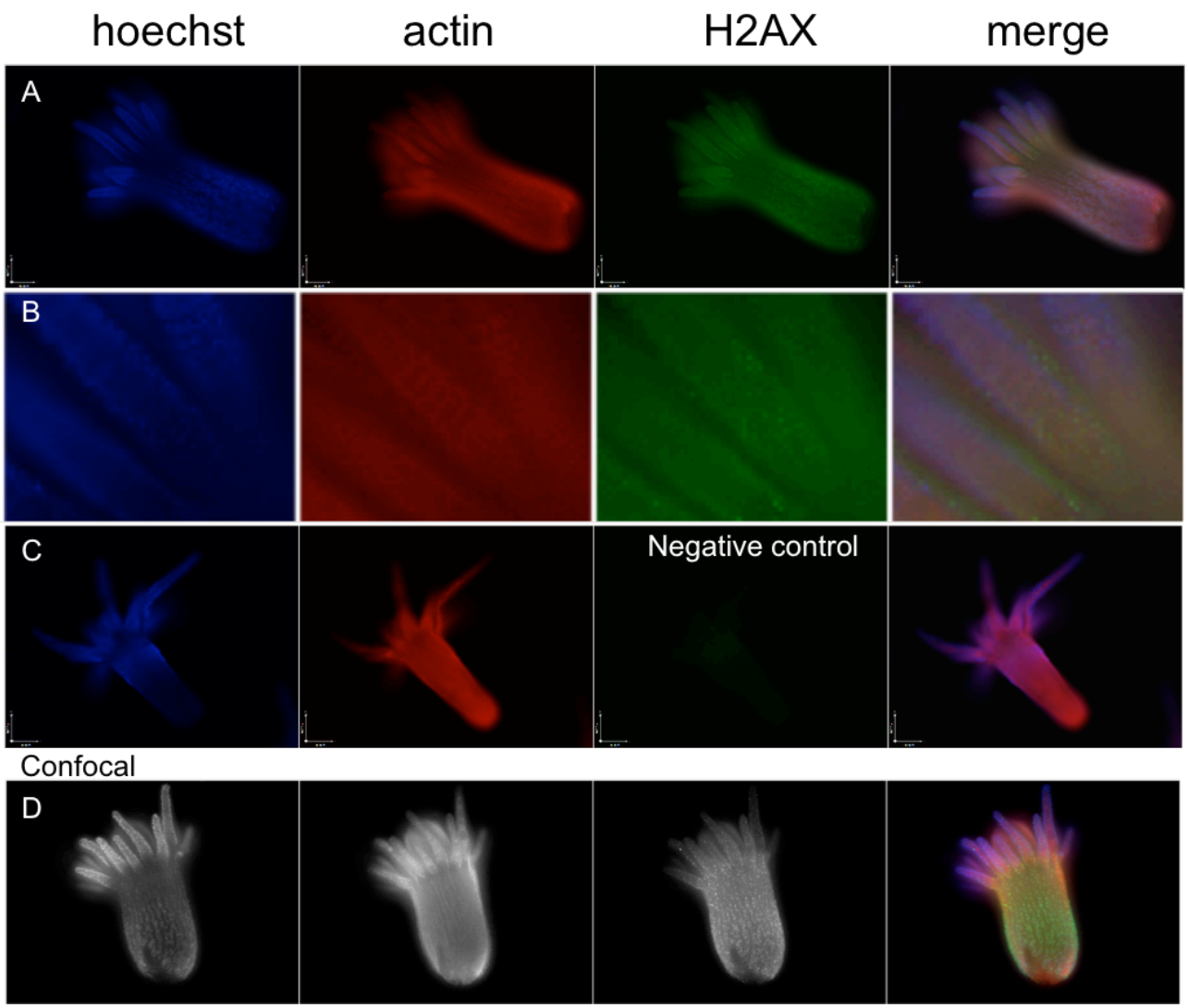

Figure 2. Whole mount feeding polyps imaged by fluorescent microscopy. *(A and B) cells stained with anti-H2AX (green), Hoechst (blue) and phalloidin (red) were imaged by widefield microscopy. (C) Negative control where no primary antibody was used. (D) Samples imaged using spinning disk confocal microscopy.*

While the nuclei of cells is clearly stained with the hoechst and the actin cytoskeleton visible with the phalloidin stain the H2AX antibody did not stain specifically. Hydractinia are notorious for non-specific binding of antibodies to stinging cells on the surface of the animal and this is what we believe to be responsible for the green signal, as it does not co-localise with the hoechst as would be expected for a nuclear protein. Upon further analysis of the protein sequence of hydractinia H2AX.1 and H2AX.2 isoforms and the probable epitope (proprietary information not available from Abcam) used to produce this antibody it was concluded that this antibody is unlikely to bind the hydractinia proteins and to move the project further specific hydractinia antibodies would have to be developed.

To gain insight into the anatomy of this organism female and male sexual polyps were fixed, paraffin embedded and processed. Sections were stained with H&E (Figure 3, female polyps) and also immunohistochemistry was attempted (data not shown).

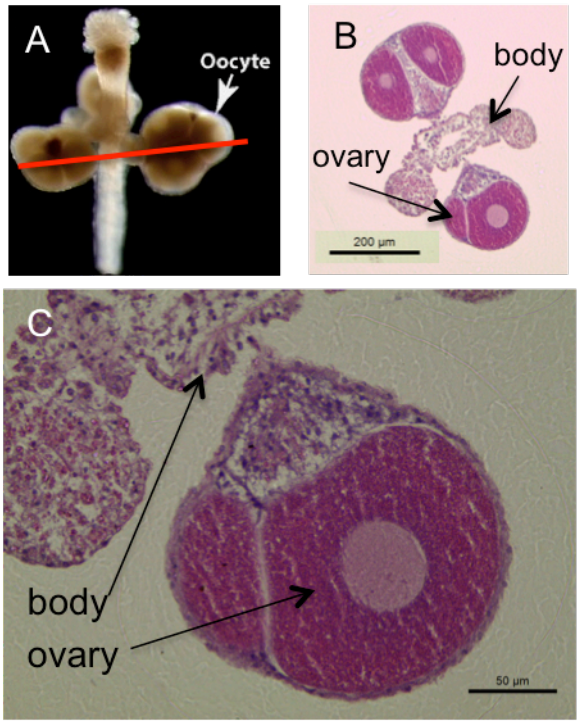

Figure 3. Analysis of female polyps. *(A) Photograph of female polyp taken from Bradshaw et al., 2015. Red line indicates the approximate plane of sectioning of the sample show in B and C. (B) H & E stained female polyps, the body and ovary are indicate, scale bar 200 µm. (C) high magnification image of H & E stained female polyps, the body and ovary are indicate, scale bar 50 µm.*

Experience Gained:

This research project allowed me to experience first-hand how biomedical research is conducted and allowed me to take responsibility for my own experiments. It encouraged me to think about the scientific method and to question results in a way I feel I haven't done before. Getting the opportunity to discuss results with academics that have extensive experience with this model organism and who are experts in the field of histone biology was both helpful and inspiring. It provided me with a number of role models to aspire to as I think about my future career in scientific research. Attending journal clubs, seminars and in particular presentations given by current PhD students exposed me to novel research and provided an experience of both formal and relaxed discussions that were enjoyable and enlightening.

Aside from this I feel I gained invaluable experience in numerous techniques that I will use in my final year project next semester. Various lab techniques such as fixing and staining tissues, carrying out end point and real-time qPCR, running gels, extracting RNA and preparing cDNA as well as learning about and using various types of microscopy will no doubt be skills I will apply to my forthcoming project. Perhaps the most valuable experience I have gained from this project is the appreciation for the need to question results and methods. Having responsibility for my own project encouraged me to think about my results and why they occurred, an invaluable lesson that will be highly beneficial in all my research endeavours.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Summer meeting

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

All eukaryotic organisms use histones to package their DNA into chromatin. A variety of histones contribute numerous functions to the cell, one such histone is H2AX whose role in DNA damage repair is essential in maintaining genetic stability. The model organism *Hydractinia echinata* has specialised cells that provide unique characteristics to the organism including an ability to regenerate, to tolerate ionizing radiation and to resist tumourogensis. Although advances have been made that characterise the histone complement of *Hydractinia*, little is known of the role of specific histone variants in the specialised functions of this model organism. This project aims to characterise the role of histone variants H2AX.1 and H2AX.2 in *Hydractinia* through qualitative analysis and quantitative measurements. Quantitative PCR was used to determine relative abundance of both histone variants in male and female polyps. Immunohistochemistry techniques utilising an H2AX antibody in combination with tissue specific markers were employed to begin investigation of tissue specificity of H2AX variants in *Hydractinia*. H2AX.2 transcript was found to be 56 times more abundant in female polyps compared to males, while histone H4 transcript was found to be 60 times more abundant in males compared to females. H2AX.1 was found at equivalent levels in both male and female animals. Further analysis of the role of H2AX variants in this organism, with a unique regenerative capacity, will facilitate further understanding of the mechanisms of regeneration and stem cell biology. Applying this knowledge to human health and disease could lead to exciting advances in regenerative medicine.

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Title: Analysis of the expression, abundance and tissue-specific distribution of DNA damage response protein H2AX in the model organism *Hydractinia echinata*

All eukaryotic organisms package their DNA using histones, of which there are several types. The H2AX histone has been well established as having a crucial role in DNA damage repair. The model organism *Hydractinia echinata* has specialised cells and tissues that confer unique characteristics including the ability to regenerate, immunity to tumourogenesis and ability to tolerate ionizing radiation. The aim of this project was to begin to characterise the two recently identified variants of H2AX in *Hydractinia*. One of these H2AX.1 has been found in all polyp types feeding, male and female, whereas H2AX.2 is oocyte (female) specific. Utilising quantitative measurements and qualitative analysis we aimed to determine their abundance and localisation. The H2AX.2 variant is specific to female germ cells and was found to be 56 times more abundant in female polyps relative to male polyps using comparative qPCR analysis. The H4 histone was also investigated and found to be 60 times more abundant in male relative to female polyps which is proposed to have an important role in sperm packing. Immnohistochemistry approaches did not yield definitive results either on whole mount samples or paraffin embedded sections most likely due to the divergence of hydractinia and human proteins. The reagents available in the lab are human specific and not fully optimised for this organism yet. Once appropriate protocols have been established, this organism will be an invaluable tool in relating advances in stem cell and developmental biology and regeneration to human health and disease.

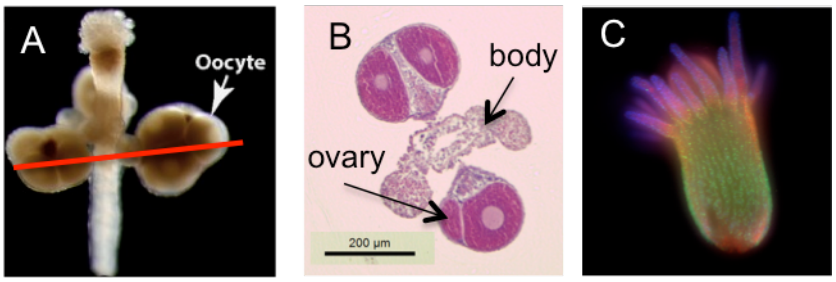

Figure 1. **Imaging Hydractinia.** (A) Photograph of female polyp taken from Bradshaw et al., 2015. Red line indicates the approximate plane of sectioning of the sample show in B. (B) H & E stained female polyp, the body and ovary are indicate, scale bar 200 µm. (C) Confocal whole-mount image of feeding polyp, the external stinging cells are highlight in green, the actin cytoskeleton is stained red and the nuclei of cells are blue.

Other comments: (no more than 300 words)

References

Torok *et al.* (2016). The cnidarian Hydractinia echinata employs canonical and highly adapted histones to pack its DNA. Epigenetics Chromatin. Sep 6;9(1):36

Bradshaw *et al*. (2015). Distinct mechanisms underlie oral vs aboral regeneration in the cnidarian Hydractinia echinata. Elife Apr 17;4

Signature of student.. *.....Date……18/09/2017……..*

Signature of supervisor…… *…….. Date……18/09/2017……*

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