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

*NB: This report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.*

**Name of student:**

Rosie Graham

**Name of supervisor(s):**

Susanne Dietrich

**Project Title: (no more than 220 characters)**

Tracing the boundary of the primary and secondary heart field (lateral and paraxial head mesoderm)

**Project aims: (no more than 700 words)**

The vertebrate paraxial head mesoderm (PHM) delivers parts of the skull, craniofacial skeletal muscle and the 2nd heart field (reviewed in [1]). The 2nd heart field encompasses the cells that are added on to the primitive heart to form the inflow and outflow tract of the heart and the entire pericardium. Importantly, the 2nd heart field produces cardiomyocytes for a prolonged time period, and these cells obey the beat set by the existing heart. Thus, 2nd heart field cells have properties that ideally, cells for the therapy of human heart defects should have.

My host laboratory wishes to molecularly characterise the cardiac competence of the 2nd heart field cells. The long-term aspiration is to apply this knowledge to potential therapeutic cells, rendering these cells more PHM/2nd heart field-like, subsequently transferring the cells to the site of cardiac lesion.

To characterise the properties of the PHM, the precise location of the 2nd heart field cells, in particular the boundary between the PHM and the lateral head mesoderm (LHM) that forms the primitive heart, needs to be established. Fate maps have been constructed, labelling 1st heart field and 2nd heart field cells in the primitive streak and analysing the position of the cells after they have contributed to the heart ([2]; reviewed in [1]). However, the position of the cells in the interim is unknown.

The aim of the work was to locate the PHM-LHM boundary from the time the tissues are first set up to the time PHM cells are being recruited into the heart. The objective was to generate a fate map for the PHM-LHM interface for this period. We used the chicken embryo as model since it is a recognised model for mammalian/ human heart development. The approach was to mount chicken embryos on filter rings as described by Chapman [3], apply DiI to the PHM-LHM border at the time this border is morphologically defined, followed by the recording of the position of the dye at HH10-11 and HH14 of development. To map the relative movement of PHM and LHM cells, DiI-DiO labelling of laterally adjacent areas at the PHM-LHM boundary was also carried out.

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

**Project Outcome**

In the first couple of weeks of the project, emphasis was on learning the embryological and photomicroscopical techniques. Moreover, the survival rate of embryos in culture was determined as 30-50 % per batch of embryos. Furthermore, HH7 embryos were labelled with DiI and subjected to in situ hybridisation for PHM and LHM markers, confirming that dye placed into the mesoderm underneath the neural plate border will label cells at the interface of PHM and LHM marker gene expression.

In the next experiments, DiI and DiO were placed into laterally adjacent areas mid-way along the rostrocaudal axis of the PHM-LHM boundary, and the embryos were allowed to develop to HH10. We observed that the two labelled cell populations remained distinct, suggesting that the cells move as a sheet of cells and do not overtake each other. Thus, label placed into the original PHM-LHM boundary will continue to demarcate the boundary between the two mesodermal cell populations.

Thereafter, HH7 embryos were labelled at three individual axial positions along the PHM-LHM boundary and cultured to stage HH10/11. An example is shown in Figure 1 below. In this specimen, the labelled cell population had moved into a sub-pharyngeal region, but did not yet contribute to the heart. Sectioning is under way to determine the precise location of the labelled cells. Moreover, experiments are under way to determine the position of the cells at stage HH14. Once this is accomplished, we will collate the data into a map representing the position of the labelled cells at HH10 and HH14.

**Experience Gained**

The skills and experience I have gained throughout this project have been:

- Understanding of developmental anatomy of the chicken heart,
- Culturing chicken embryos on filter rings (EC-culture),
- Harvesting embryos at defined developmental stages,
- Performing micro-injections of the fluorescent vital dyes DiI and DiO,
- Performing in-situ hybridisation,
- Specimen embedding and vibratome sectioning,
- Using Nomarski and fluorescence microscopy of whole and sectioned specimen,
- Photomicroscopy to document the findings.

References:

1. Diogo, R., et al., A new heart for a new head in vertebrate cardiopharyngeal evolution. Nature, 2015. 520(7548): p. 466-73.
2. Camp, E., S. Dietrich, and A. Munsterberg, Fate mapping identifies the origin of SHF/AHF progenitors in the chick primitive streak. PLoS One, 2012. 7(12): p. e51948.
3. Chapman, S.C., et al., Improved method for chick whole-embryo culture using a filter paper carrier. Dev Dyn, 2001. 220(3): p. 284-289.

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

Summer 2018

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

**Tracing the boundary of the primary and secondary heart field (lateral and paraxial head mesoderm)**

The paraxial head mesoderm (PHM) delivers cells for the in- and outflow tract of the heart over a prolonged period of time. Moreover, cardiomyocytes from this source integrate into the primitive heart and follow the existing heartbeat. Thus, the PHM may teach us how to develop cells for cardiac therapy. Prerequisite for the molecular characterisation of the cardiac-competent cells in the paraxial head mesoderm (PHM) is an understanding of their precise location during development. Published work investigated the position of the PHM cells after they had contributed to the heart. Thus, their position at intermediate stages of development, and their spatial relationship with the lateral head mesoderm (LHM) that delivers the 1$^{st}$ heart field is not known. Performing labelling experiments in the chicken embryo using fluorescent dyes, we found that the PHM and LHM cells retain their relative mediolateral/dorsoventral position when they follow the endoderm ventrally. Moreover, the cells at the PHM-LHM boundary reach a sub-pharyngeal position during the development from HH7 to HH10/11. We will show the precise location of these cells, both for HH10/11 and for HH14 when the PHM cells are thought to contribute to the inflow and outflow tract of the heart.

**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.*

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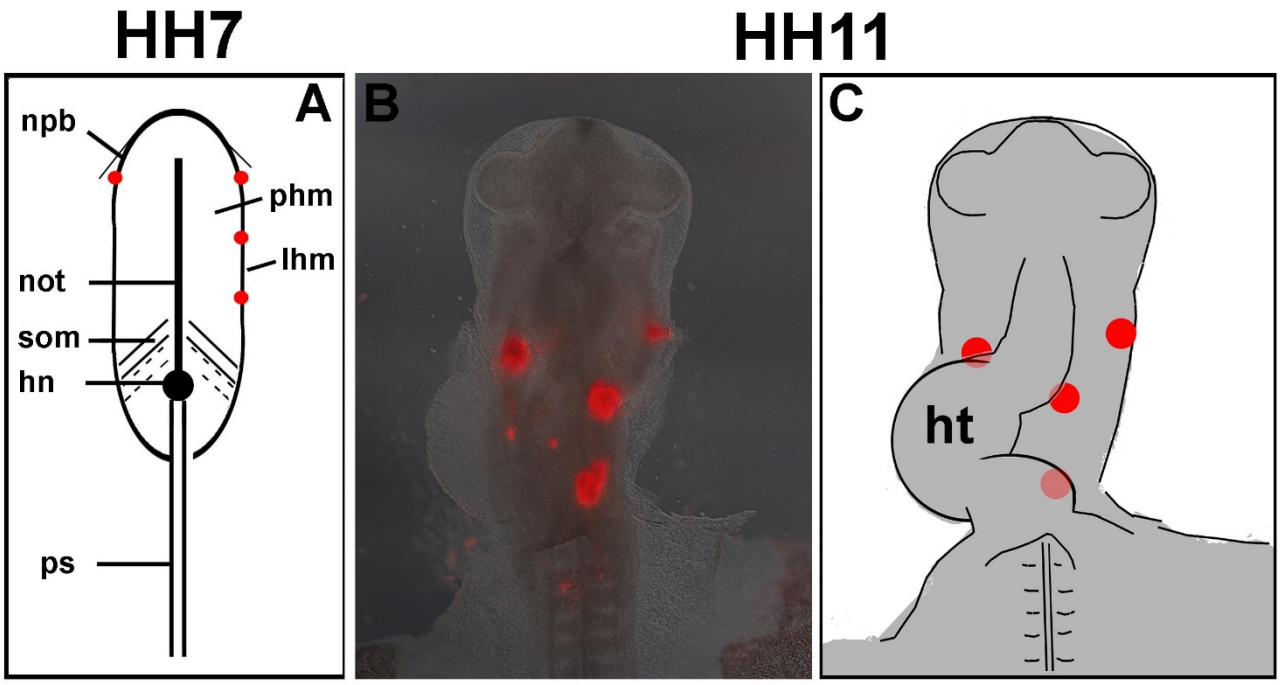


*Figure.1. (A) Schematic representation of a chicken embryo, ventral view, rostral to the top, labelled with DiI (red dots) at the quarter point, half-way point and three-quarter point along the rostrocaudal extent of the PHM-LHM boundary at stage HH7. This boundary is defined by the neural plate border dorsal to the mesoderm. (B) Combined Nomarski and fluorescence image for the labelled embryo at stage HH11, ventral view, rostral to the top. (C) Schematic representation of the embryo in (B). All labelled cell populations have moved into a ventral, sub-pharyngeal region, but have not yet contributed to the heart.*
*Abbreviations: hn, Hensen's node; ht, heart; lhm, lateral head mesoderm; npb, neural plate border, not, notochord; phm, paraxial head mesoderm; som, 1st somite.*

**Other comments: (no more than 300 words)**

*Signature of student.....Date…12/10/2017*

*Signature of supervisor..... Date…12/10/2017……*

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