

UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2015/16

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Name of student:

Miss Hannah Tjandra

Name of supervisor(s):

Dr Frank Schubert

Project Title: (no more than 220 characters)

Investigation of hypoxia in the embryonic chicken brain

Project aims: (no more than 700 words)

The brain is the most oxygen demanding organ of the human body (Lassen, 1959). Throughout neural tube development it utilises oxygen by way of diffusion until development of the complex vascular system by vascular ingression. From previous work in the lab (Amanda Corla, PhD thesis 2017), it has been established that in the chicken embryo the perineural vascular plexus, (PNVP) forms at 1.5 days post incubation; however, vascular ingression of endothelial sprouts does not occur until day 4 after incubation. This delay in vascular ingression is theorised to be due to the lack of oxygen in the developing tissues of the neural tube (Naňka et al., 2006). Hypoxic conditions would result in the activation of Hypoxia Inducible Factor-1 α (HIF-1 α), which in turn can be used to track the progression of hypoxia (Razorenova, 2005).

Under normoxic conditions, HIF-1 α is targeted for degradation by Proline Hydroxylase (PHD); this allows it to be recognised by Von Hippel Lindau ES-ubiquitin ligase, (VHL ES-ubiquitin ligase), which tags it for rapid degradation by the proteasome; the half-life for HIF-1 α is approximately 5 minutes (Semenza, 2000). Under hypoxic conditions PHD is inhibited, as it requires oxygen as a co-substrate for hydroxylation. This allows HIF-1 α to translocate to the nucleus and activate a range of more than 100 genes, including angiogenic genes such as VEGF-A (reviewed in Pugh & Ratcliffe, 2003). HIF-1 α , upon translocation to the nucleus, binds to HIF-1 β to form a stable complex, before binding to the HIF Responsive Element (HRE). As the HRE's are upstream of the angiogenic genes, binding of HIF allows for activation of these genes (Liu, Cox, Morita, & Kourembanas, 1995).

We aimed to utilise this mechanism to visualise hypoxia in the developing brain by developing an HRE-responsive reporter construct; a cassette of multiple copies of the HRE was coupled with reporter gene LacZ allowing track the activity of HIF in the embryonic chick brain using β -galactosidase staining. The construct was electroporated into the neural tube of chicken embryos. Subsequent staining showed LacZ in the developing brain by HH16.

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

Project Outcomes

1. Constructing the HIF1 reporter cassette

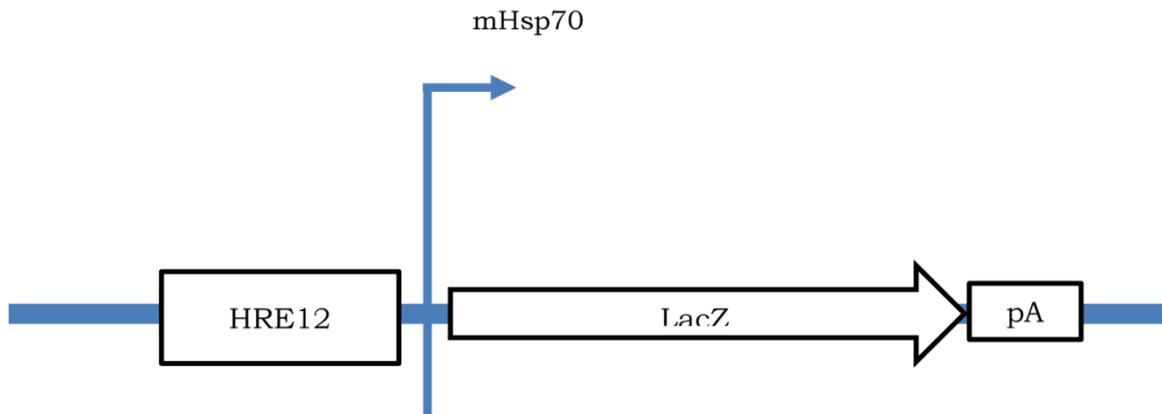


Figure 1 The functional coding regions of the pBluescript KS (+) HRE12-LacZ plasmid. Featured are the 12 repeats of the Hypoxia Responsive Element, the constitutive minimal Hsp70 Promoter, the LacZ reporter region and the polyadenylation signal.

The reporter construct for monitoring HIF activity was based on a prior retroviral construct in which 12 copies of the HRE in combination with the human Hsp70 minimal promoter controlled the expression of LacZ (Razorenova, 2005). We amplified the HRE12-Hsp70 cassette from pSIP-HRE12-Hsp70-LacZ (a kind gift by O. Razorenova) by PCR using primers for the flanking MoMLV gag region and LacZ gene of pSIP-HRE12-Hsp70-LacZ. This was combined with the LacZ gene and the rabbit beta-globin polyA site, cut from the pCAG-LacZ plasmid. The full expression cassette (Figure 1), was cloned into pBluescript KS (+) to give the pBluescript-HRE12-LacZ-pA construct (Figure 2).

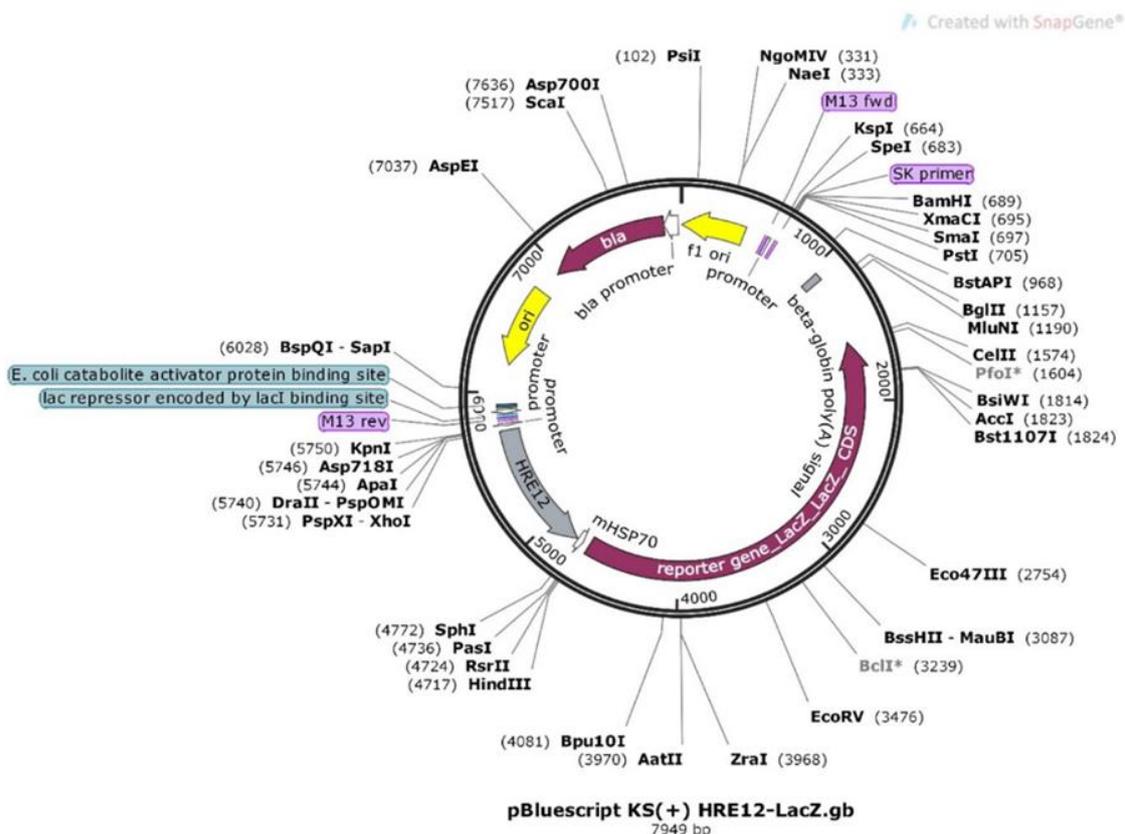


Figure 2 Plasmid map of pBluescript KS (+) HRE12-LacZ containing the HRE12 repeats, the LacZ reporter gene, the beta-globin polyadenylation signal and constitutive minimal Hsp70 promoter.

2. Monitoring HIF activity in the developing brain

The reporter construct was subsequently used to monitor HIF activity in the developing brain of chicken embryos. The construct was injected into the mesencephalon of HH11-12 (Hamburger & Hamilton, 1951) embryos. Electrodes were placed on either side of the neural tube, and a short electric pulse allowed the cells on the left side of the mesencephalon to take up the construct. A set of embryos were co-injected with the proline hydroxylase inhibitor IOX2 to trigger HIF activation. The eggs were incubated for 24 hours allowing the embryos to reach stage HH15-16, before the embryos were harvested and stained for β -galactosidase activity. Embryos injected with a control construct showed no staining, while a number of embryos showed distinct staining in cells in the mesencephalon and diencephalon (Figure 3A-D). Staining was further enhanced in IOX2-injected embryos (Figure 3 E-F).

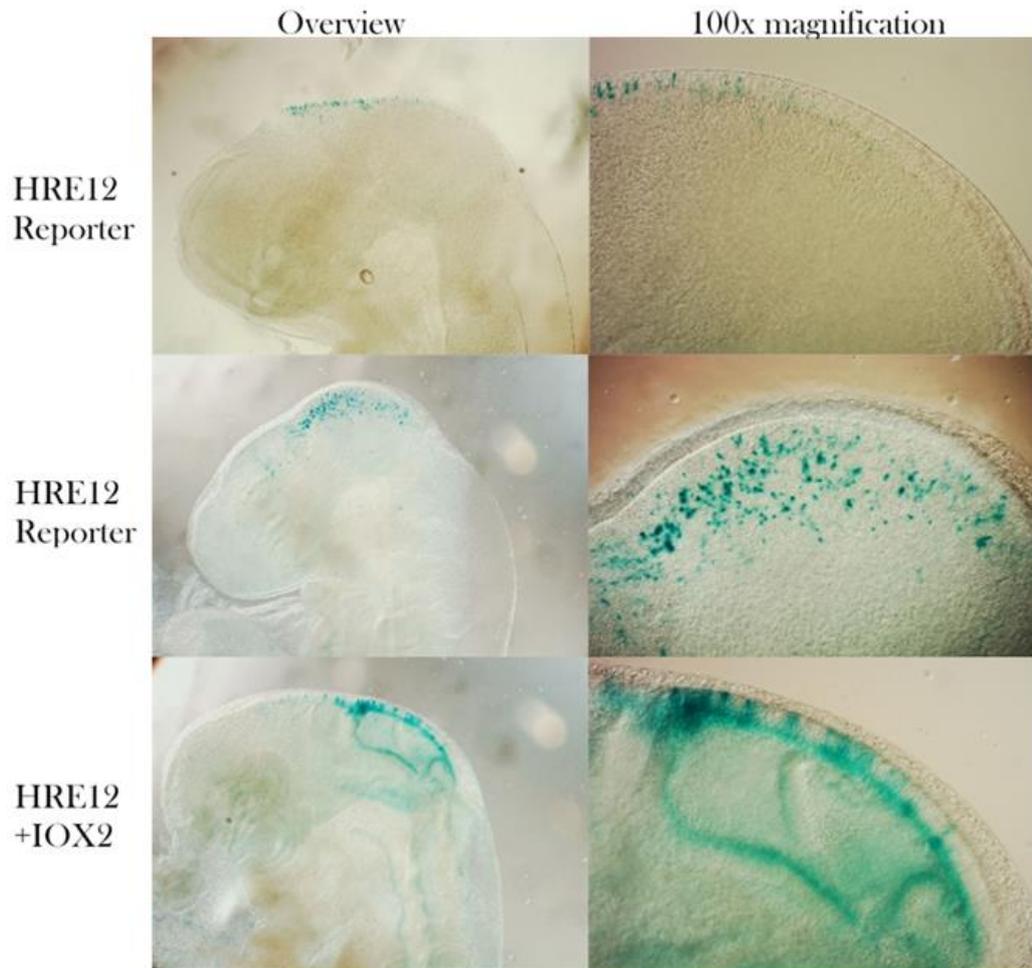


Figure 3 A panel depicting the results of the β -Galactosidase staining of the embryos at HH16. (a) The staining shown is focused along the dorsal midline (b) There is very little staining along the caudal midbrain. (c) The embryos injected with HRE12 show strong staining throughout the midbrain though slightly stronger staining in the rostral midbrain as opposed to the caudal. (d) The β -Galactosidase staining is very strong, in discrete cells and shows very little sign of staining by diffusion suggesting strong activity of HIF-1 α in these regions. (e) Staining in the HRE12 with IOX2 is strong, though the embryo forebrain is malformed possibly as an electroporation artefact. (f) The staining in the rostral midbrain is particularly strong, suggesting HIF-1 α activity.

Our results show that the HRE12-LacZ reporter construct is functional after electroporation in the embryonic chicken brain. It can be used in the future to monitor hypoxic conditions during brain development and to correlate hypoxia with the ingression of the brain vasculature.

Experiences Gained

The time spent in the Molecular Embryology laboratory at the University of Portsmouth has shown me how rewarding it can be to be a scientist, as well as gain insights into how a lab is run, what needs to be managed day to day, and how materials don't magically appear on a bench. The research conducted over the summer has been awesome in its complexity and the simplicity to which we can approach a complex problem. It has made working in a 'working lab' feel comfortable, knowing that the research conducted makes a difference and applying the knowledge gained from study.

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

Summer Meeting (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)

Title:

Poster Abstract

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.

Investigation of hypoxia in the embryonic chick brain

It is well known the brain undergoes rapid of growth during embryonic development. Theories have postulated previously that consequently the neural tissue becomes hypoxic, which in turn would promote the ingression of endothelial sprouts to initiate brain vascularisation. When tissue becomes hypoxic, Hypoxia Inducible Factor is stabilised; progression of hypoxia is then synonymous with HIF activity. To monitor HIF activity in vivo we developed a HRE12-Hsp70-LacZ reporter construct, wherein LacZ expression is controlled by 12 repeats of the hypoxia responsive element (HRE), and LacZ activity hence indicates activity of HIF.

The HRE reporter cassette was microinjected into embryonic chick midbrains at HH11-12, collected at HH15-16 and stained for β -galactosidase activity. We found LacZ staining in several embryos indicating that the neural tube already becomes hypoxic between HH11 and HH16.



Other comments: (no more than 300 words)

I would like to thank Frank Schubert for the continuous support, endless patience and use of the laboratory. I would also like to thank the Anatomical Society for the opportunity to work throughout the summer. Appreciation must also be given to Olga Razorenova for the use of the original HRE12 plasmid. Lastly, I would like to thank PhD student Amanda Corla, and MRes student Matthew Bryant for guiding me throughout the summer and providing foundation upon which my research could take place.

Signature of student.....



... Date 14/10/17

Signature of supervisor.....

..... Date 16/10/17

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Hamburger, V., & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *Journal of Morphology*, 88(1), 49-91.

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