PhD Student Opportunities 2012

The Babraham Institute is an international focus for innovative research in post-genomics studying gene function in cells, organs and systems, supported principally by the Research Councils. It is a recognised postgraduate University Partner Institute of the University of Cambridge. Starting October 2012 a number of Research Council Quota studentships will be available at Babraham leading to a University of Cambridge PhD degree. These studentships can be awarded for up to 4 years. In addition, studentships funded by a range of University of Cambridge funding schemes can be held at the Babraham Institute (see the student pages of our website www.babraham.ac.uk and see http://webservices.admin.cam.ac.uk/gradfunds/gfinder.jsp?status=new&reset=reset for specific funding sources for EU and overseas graduate students at Cambridge).

Please see our website (www.babraham.ac.uk) and the BBSRC website (http://www.bbsrc.ac.uk/funding/training/eligibility.pdf) for details of eligibility and funding. Non-EU nationals must find funding for academic fees and personal support. In cases where applicants must find their own funding, we will require evidence that the level of funding is at least equal to the standard BBSRC/MRC PhD funding package. Students will join a thriving scientific community situated on an attractive parkland campus near Cambridge. Our 70 students are all members of Cambridge Colleges and participate fully in University social and academic life (www.biomed.cam.ac.uk/gradschool/).

Details of our interactive scientific programmes can be found on www.babraham.ac.uk. The Institute is fully equipped for state-of-the-art biological research including: innovative molecular biology, stem cell manipulation and transgenics, epigenetics, structural studies on chromatin, real-time laser scanning confocal microscopy, calcium imaging, fluorescence sorting of cells, gene targeting and knockouts, mouse models of disease, mouse behavioural testing, proteomics and lipidomics. The Institute PhD Recruitment Day will be held on **WEDNESDAY 18th JANUARY 2012** to which selected students will be invited to attend interviews, discuss their research interests and view the Institute's facilities.

Full details of potential projects and supervisors are given below; our supervisors welcome informal enquiries.

Potential projects (supervisor/title):

Michael Coleman (michael.coleman@babraham.ac.uk): *Healthy ageing for mice: refining the control of body weight*

Simon Cook (simon.cook@babraham.ac.uk): *The role of mRNA binding proteins in MAP Kinase signalling thresholds and cell fate decisions.*

Anne Corcoran (anne.corcoran@babraham.ac.uk): *Does non-coding RNA regulate development?*

Myriam Hemberger (myriam.hemberger@babraham.ac.uk): *Signalling-induced epigenetic reprogramming of stem cell fate*

Gavin Kelsey (gavin.kelsey@babraham.ac.uk): *Establishing a basis for DNA methylation in germ cells* (EU studentship project funded as part of the INGENIUM network).

Klaus Okkenhaug (klaus.okkenhaug@babraham.ac.uk): *Differential role of PI3K isoforms during tonic and agonist induced signalling by the B cell receptor.*

Jenny Pell (jenny.pell@babraham.ac.uk): *Epigenetic regulation of adult stem cell fate*

Wolf Reik (wolf.reik@babraham.ac.uk): *Genome wide reprogramming of DNA methylation in germ cells, stem cells, and early embryos*
(EU studentship project funded as part of the INGENIUM network).

Llew Roderick (llewelyn.roderick@babraham.ac.uk): *Signalling to and from the epigenome to control cardiomyocyte fate choices.*

Peter Rugg-Gunn (peter.rugg-gunn@babraham.ac.uk): *Epigenetic regulation of mammalian development and stem cell differentiation*

Partick Varga-Weisz (patrick.varga-weisz@babraham.ac.uk): *The role of a chromatin remodeling in stem cell biology, the immune system and development*

Sonja Vermeren (sonja.vermeren@babraham.ac.uk): *How is neutrophil activation regulated?*

Heidi Welch (heidi.welch@babraham.ac.uk): *A novel role for the guanine-nucleotide exchange factor P-Rex in vesicle trafficking*

Travel expenses will be paid to those invited to attend our Institute Recruitment Open Day. Applicants should submit a full *Curriculum Vitae* with a covering letter indicating the two projects in which they are most interested, in order of preference, and arrange for two referees to write to the Institute on their behalf before the deadline; please include your contact details for 3-18th January 2012. Our website also provides a checklist of the information required to be provided in your application before it can be considered. Incomplete applications will not be considered.

Please send your applications to: Ms Caroline Coursol, Graduate Studies Programme, The Babraham Institute, Babraham, Cambridge CB22 3AT, Tel: 01223 496324, Fax: 01223 496046 or email babraham.graduate@babraham.ac.uk by **Friday 16th December 2011**

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Checklist of Information required for application

- **Full Curriculum Vitae including**
  - **Nationality and Residence in UK information**
  - **Details of Schooling including GCSE and A Level results (or other qualifications)**
  - **Details of University Education including courses taken and results of any examinations to date**
  - **Degree result (if already known)**
  - **Details of any lab based projects or laboratory placements**
  - **Details of any industrial placements**
  - **Details of any previous employment**

- **Covering Letter giving the details of the two projects in which you are most interested and your reasons for choosing them. These projects should be chosen from different Group Leaders.**

- **The names and contact information of the two referees you have asked to write to the Institute supporting your application for a PhD position**

- **Your contact details between 3rd and 18th January 2012**

****** See below for Full Descriptions of Projects for 2012******

PhD Studentship - Michael Coleman (michael.coleman@babraham.ac.uk)

Healthy ageing for mice: refining the control of body weight

Our studies have identified a novel mechanism of axon degeneration and genetic and pharmacological methods to preserve axons (Gilley & Coleman, 2010; Coleman & Freeman, 2010). Axon degeneration is a major factor in age-related disorders such as Alzheimer's disease, Parkinson's disease, glaucoma and diabetic neuropathy so we aim to place our findings in the context of the ageing nervous system. For example, we have found a substantial decline in the transport of mitochondria in axons during ageing (Gilley et al, 2011).

With a rapidly ageing population many scientists, like ourselves, are studying the physiological and pathological consequences of ageing. This brings new challenges for ensuring both the welfare of laboratory animals and high scientific standards. In this study, we aim to identify the best combination of dietary restriction and voluntary exercise for avoiding excessive weight gain in laboratory mice and study the consequences for nervous system health and function. This will allow studies across many scientific fields, including our work on axon degeneration, to proceed under the best practical animal welfare conditions while focussing specifically on the effects of ageing rather than changes that are secondary to obesity.

Using a new design of running wheel and collaborating with experts in animal diets, we will test the hypothesis that the health of ageing laboratory mice is best maintained using a combination of lower energy, *ad lib* food and provision for voluntary exercise. We will use recently-developed 'touch-screen' behavioural tests for mice, along with studies of axonal transport and synapse number to assess the optimum conditions and collaborate with other Babraham groups to study body systems such as skeletal and cardiac muscle and the immune system. Finally, we will relate the findings back to axon survival by testing whether Nampt, a key protein in NAD metabolism, links nutrient levels to age-related axon loss.

References

Gilley, J., and Coleman, M.P. (2010) Endogenous Nmnat2 is an essential survival factor for maintenance of healthy axons. *PLOS Biol* **8:** (1) e1000300. doi:10.1371/journal.pbio.10000300

Coleman, M.P. and Freeman, M.R. (2010) Wallerian degeneration, Wld^S and Nmnat. *Ann Rev Neurosci.* **33:** 245-67.

Gilley, J., Seereeram, A. Ando, K., Mosely, S., Andrews, S., Kerschensteiner, M., Misgeld, T., Brion, J-P., Anderton, B., Hanger, D.P. and Coleman, M.P. (2011) Age-dependent axonal transport and locomotor changes and tau hypophosphorylation in a 'P301L' tau knock-in mouse. *Neurobiol Aging* EPub ahead of print.

PhD Studentship – Dr Simon Cook (simon.cook@babraham.ac.uk)

The role of mRNA binding proteins in MAP Kinase signalling thresholds and cell fate decisions.

MAP Kinase pathways coordinate cell fate decisions (proliferation, senescence, death) in response to environmental stimuli. Even a single pathway (e.g., ERK1/2) can promote cell survival, proliferation or senescence depending on the duration and magnitude of pathway activation. This raises two key questions: (1) how is the magnitude and/or duration of pathway activation controlled and (2) how are such differences in pathway activation translated into changes in gene expression to direct specific cellular responses? We propose that the Tis11 family of mRNA binding proteins may be involved in both these processes.

Tis11 proteins bind AU-rich elements in mRNA to promote mRNA decay. They are expressed in response to growth factors and cellular stresses and are regulated by phosphorylation, thereby linking signalling to post-transcriptional gene regulation. DUSP6, a dual-specificity phosphatase that specifically inactivates ERK1/2, has recently been shown to be a target of Tis11 (J Cell Phys **226**:276) raising the possibility that MAPK signalling thresholds may be controlled by Tis11 proteins (1 above). In addition, Tis11 proteins control the abundance of mRNAs encoding proteins that control cell cycle progression and senescence (2 above).

This project will test two hypotheses: (1) Tis11 proteins control the abundance of DUSP proteins and thereby the magnitude and/or duration of MAPK activation and (2) by re-modelling patterns of MAPK-dependent mRNA expression Tis11 proteins control cell fate decisions. These will best be tested in cell culture models including fibroblasts derived from Tis11 KO mice provided by our colleague Dr Martin Turner. These studies will focus on (i) activation of ERK1/2 by growth factors and (ii) ERK1/2, JNK and p38 signalling in the context of ER stress, cell cycle arrest and senescence. The student will use established cell biochemistry and molecular biology techniques together with analysis of cell cycle & cell death, mRNA profiling, RNAi and single cell imaging.

Recent relevant references from the Cook lab (* denotes a PhD student paper)

* Little AS, Balmanno K, Sale M, Newman S, Dry J, Hampson M, Edwards PAW, Smith PD & **Cook SJ.** (2011) Up-regulation of the driving oncogene, KRAS or BRAF, drives acquired resistance to MEK inhibitors in colorectal cancer cells. *Science Signalling*. **4** (166) ra17

Wickenden JA, Jin H, Johnson M, Gillings AS, Newson C, Austin M, Chell SD, Balmanno K, Pritchard CA & **Cook SJ** (2008) Colorectal cancer cells with the BRAFV600E mutation are addicted to the ERK1/2 pathway for growth factor-independent survival and repression of BIM. *Oncogene* **27**:7150- 7161

* Densham RM, Todd DE, Balmanno K & **Cook SJ** (2008) ERK1/2 and p38 cooperate to delay progression through G1 by promoting cyclin D1 protein turnover. *Cell Signal.* **20**:1986-1994

*Arkell RS, Dickinson RJ, Squires M, Hayat S, Keyse SM, **Cook SJ**. (2008) DUSP6/MKP-3 inactivates ERK1/2 but fails to bind and inactivate ERK5. *Cell Signal*. **20**:836-43

* Chalmers CJ, Gilley R, March HN, Balmanno K, **Cook SJ**. (2007) The duration of ERK1/2 activity determines the activation of c-Fos and Fra-1 and the composition and quantitative transcriptional output of AP-1. *Cell Signal*. **19**:695-704.

PhD Studentship - Anne Corcoran (anne.corcoran@babraham.ac.uk)

Does non-coding RNA regulate development?

The long-held central dogma that 'DNA makes RNA makes protein' has been overturned by recent discoveries that non-coding RNAs have several important functions as chaperones of histone modifying enzymes, as initiators of nuclear substructures, and as enhancers. Furthermore, overexpression of individual ncRNAs can alter expression of hundreds of target genes, leading to diseases including cancer. Thousands of conserved non-coding RNAs have now been discovered and understanding their function is the current major challenge in gene regulation and epigenetics.

We have previously discovered extensive non-coding RNA transcription preceding rearrangement of the immunoglobulin DNA loci in B lymphocytes. This V(D)J recombination process ensures that the 100s of genes in the immunoglobulin loci are cut and pasted together in multiple combinations to generate the millions of antibodies required by the immune system to fight infection. We have used in vitro and in vivo models to uncover a crucial role for non-coding RNA transcription in making genes available for recombination.

It is unknown whether non-coding RNAs play a role in development. We are currently testing the hypothesis that non-coding RNAs have a pivotal function in B lymphocyte development. We propose that key differentially expressed ncRNAs may play a 'master regulator' role in a similar way to tissue-specific transcription factors. We have used next generation sequencing to generate a genome-wide profile of non-coding RNAs and associated histone modifications, and their dynamic alterations during B lymphocyte development. We have found numerous RNAs that are up and down regulated at key stages of B cell development. The aim of this project is to investigate the function of key candidates currently undergoing preliminary characterisation, that we predict will control lymphocyte development. Objectives will include identification of protein-binding partners, visualization of nuclear organization, analysis of enhancer function, analysis of gene expression and developmental alterations following shRNA knockdown. State-of-the-art techniques including genome-wide next generation sequencing (RNA-seq ChIP-seq, RIP-seq), shRNA knockdown, high-throughput fluorescence in situ hybridization (FISH), and bioinformatics will be used.

References:

Corcoran AE. (2010) The epigenetic role of non-coding RNA transcription and nuclear organization in immunoglobulin repertoire generation. (Semin Immunol 22(6):353-61)

Featherstone, K., Wood, A., Bowen, A.J. and Corcoran, A.E. (2010) The mouse immunoglobulin heavy chain V-D intergenic sequence contains insulators that may regulate ordered V(D)J recombination (*J. Biol Chem. 285(13):9327-3*

Bolland, D., Wood, A., Afshar, R., Featherstone, K., Oltz, E.M. and Corcoran A.E. (2007) Antisense intergenic transcription precedes IgH D to J recombination, and is activated by the intronic enhancer, Eu (Mol. Cell. Biol., 27(15):5523-33)

PhD Studentship - Myriam Hemberger (myriam.hemberger@babraham.ac.uk)

Signalling-induced epigenetic reprogramming of stem cell fate

We are interested in the epigenetic regulation of cell fate decisions in mammalian development and their impact on the potency of stem cells, notably embryonic (ES) and trophoblast (TS) stem cells. Specifically, this project is aimed at identifying the signalling pathways through which exogenous factors, such as growth factors (e.g., FGF, TGFb, BMPs etc) and hormones (e.g., estrogens, prolactins), can modify the epigenome to induce profound changes in cell fate. Mechanistic studies will then determine whether manipulation of the signalling cascade, and specific epigenetic modifiers, can reprogram (stem) cell fate. Insights into this crosstalk are important to understand the impact of the extracellular environment on the epigenome which can have dramatic consequences for early development, later life and, if passed through the germ line, even for the next generation. These mechanisms are also central to the sequence of events involved in malignant transformation of cells in tumorigenesis.

The student will learn a range of cutting-edge technologies including stem cell culture, Next Generation Sequencing and bioinformatic analysis to integrate epigenomic and transcriptomic datasets, embryology, state-of-the-art fluorescence imaging techniques, in addition to a wide range of molecular techniques (stem cell transfection/transduction, conditional knockdown/overexpression strategies, ChIP-qPCR, ChIP-Seq, DNA methylation analyses including meDIP-Seq, Western blotting, IP, mass spec,RT, qPCR, ISH, immunostaining, etc). The student will also interact with computational biologists in order to unravel key events in environmentally induced epigenetic (re)programming. These approaches have great relevance to stem cell and developmental biology, regenerative medicine, healthy aging, and cancer epigenetics. The student will greatly benefit from the expert environment in the fields of Epigenetics & Chromatin Organization, Cell Signalling and Mathematical Modelling, as well as from the Epigenomics and Bioinformatics Facilities at the Babraham Institute.

Recent, relevant publications:

Hemberger M. and Pedersen R.A. (2010). Epigenome Disruptors (Perspective) ***Science***,330:598-599.

Senner C.E. and Hemberger M. Regulation of Early Trophoblast Differentiation - Lessons from the Mouse. ***Placenta***, 2010 Aug 24.

Hemberger M., Udayashankar R. Tesar P., Moore H. and Burton G. (2010). ELF5-enforced transcriptional networks define an epigenetically regulated trophoblast stem cell compartment in the human placenta. ***Hum. Mol. Gen.***, 19: 2456-2467.

Hemberger M., Dean W. and Reik W. (2009). Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. ***Nature Reviews Mol. Cell Biology***, 10: 526-537.

Ng R.K., Dean W., Dawson C., Lucifero D., Madeja Z., Reik W. and Hemberger, M. (2008). Epigenetic restriction of embryonic cell lineage fate by methylation of *Elf5*. ***Nature Cell Biology***, 10: 1280-1290.

Ph.D. Studentship -Gavin Kelsey (gavin.kelsey@babraham.ac.uk)

Establishing a basis for DNA methylation in germ cells
(EU studentship project funded as part of the INGENIUM network).

This project investigates mechanisms required to establish DNA methylation at CpG islands (CGIs) in germ cells. It builds on work that examines how methylation is directed to the control regions of imprinted genes (Chotalia et al., 2009) and genome-wide profiling of CGIs methylation in sperm and oocytes (Smallwood et al., 2011). CGIs methylation in gametes has a special significance since it defines imprinted genes (genes with monoallelic expression) and may provide the basis for transgenerational inheritance. Moreover, germ cells are an important model system, because methylation occurs in arrested cells without the complication of maintenance methylation or histone remodelling at DNA replication. The project's premise is that CGIs are protected against DNA methylation by default by binding of factors that ensure that the modification of histones occupying CGIs is hostile to DNA methylation. The DNA methylation complex is blocked by methylation of histone H3 at lysine 4 (H3K4) but promoted by trimethylated H3K36, and factors such as Cfp1 and Kdm2a, which are bound at many CGIs in somatic cells, promote H3K4 methylation and H3K36 demethylation. Transcription across CGIs may help remodel chromatin to a state permissive for methylation by destabilising binding of protective factors. Here, we shall use genome-wide techniques to comprehensively map the binding of histone modifying factors at CGIs in germ cells. This will involve purification of fetal germ cells and optimisation of chromatin immunoprecipitation methods for low cell numbers. We shall classify CGI binding patterns and correlate them with CGIs destined for methylation in oocytes and with changes in transcription as germ cells enter the *de novo* methylation phase. This will lead to identification of some critical factors that protect CGIs. One or more candidate factors will be tested functionally using conditional knock-out alleles obtained from within the EU Initial Training Network 'Ingenium' which funds this project.

Key recent references:

Chotalia M, Smallwood SA, Ruf N, Dawson C, Lucifero D, Frontera M, James K, Dean W, Kelsey G. (2009) Transcription is required for establishment of germline methylation marks at imprinted genes. *Genes & Dev*. 23, 105-117.

Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Sato S, Hata K, Andrews S, Kelsey G. (2011) Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat. Genetics* 43, 811–814.

Smallwood SA, Kelsey G. De novo DNA methylation: a germ cell perspective. *Trends in Genetics* (in press).

Tomizawa S, Kobayashi H, Watanabe T, Hata K, Kelsey G, Sasaki H. (2011) Dynamic stage-specific changes of imprinted differentially methylated regions during early mammalian development and prevalence of non-CpG methylation in oocytes. *Development* 138, 811-820.

PhD studentship - Klaus Okkenhaug (klaus.okkenhaug@babraham.ac.uk)

Differential role of PI3K isoforms during tonic and agonist induced signalling by the B cell receptor.

B cells express modified antibody protein on their cell surface where they act as receptors called the B cell receptor (BCR). In recent years, PI3Ks have emerged as key signalling proteins engaged by the BCR. PI3Ks phosphorylate membrane inositol lipids on the D3 position and thus trigger various signal transduction cascades within cells. We have shown that the pre-BCR (the Ig heavy chain in complex with an invariant surrogate light chain) is capable of activating either the p110α or p110δ isoform of PI3K and that a signal generated by either of these PI3K isoforms is essential for pre-B cells to differentiation to become mature B cells. By contrast, the BCR expressed by mature B cells rely almost exclusively on the p110δ isoform. We would like to know why this is. One hypothesis is that when a pre-BCR or mature BCR generates low-level signals in without having been engaged by an antigen, either p110α or p110δ can transmit these so-called tonic signals. However, when the BCR is clustered by antigens, p110δ is preferentially engaged. Using genetically engineered mice that have lost p110α, p110δ or both in B cells, the student will use biochemical techniques to determine whether p110α and p110δ can both generate the same phosphorylated inositol species with the same efficiency. The student will also use mass spectroscopy-based techniques to determine whether p110α and p110δ are found within comparable complexes in activated pre-B cells and mature B cells.

Reference:

Ramadani, F., D. J. Bolland, F. Garcon, J. L. Emery, B. Vanhaesebroeck, A. E. Corcoran, and K. Okkenhaug. 2010. The PI3K isoforms p110alpha and p110delta are essential for pre-B cell receptor signaling and B cell development. *Sci Signal* 3:ra60.

PhD Studentship - Jenny Pell (jenny.pell@babraham.ac.uk)

Epigenetic regulation of adult stem cell fate

Adult stem cells generate daughters to fulfil two essential divergent roles: differentiation to repair damaged tissue, and self-renewal to maintain the stem cell population for future use. The balance of these decisions is crucial; excessive self-renewal may result in cancer but insufficient stem cell expansion will compromise tissue maintenance.

We use the adult muscle stem cell (satellite cell) as a paradigm for the investigation of signalling pathways and epigenetic mechanisms that control adult stem cell fate. Satellite cells occupy a specific niche, and are usually quiescent in adult animals; however they possess an extraordinary capacity for regeneration, one cell being able to provide 1000s of progenitor myoblasts. Clearly, it is important to understand the mechanisms by which the lineage choice of these cells is controlled.

Reversible histone N-terminal modifications provide sophisticated epigenetic regulation of genomic reprogramming, ultimately via changes in chromatin structure. We have recently obtained exciting novel data to suggest that H3K27me3 (catalysed by Ezh2 within the PRC2 complex) is essential for satellite cell proliferation and function. Key candidate differentiation and cell cycle genes are mis-regulated, and we aim to characterise their function further in stem cells. Muscle stem cell fate is dictated extrinsically, and an emerging new area is the identification of signalling pathways that are translated into epigenetic regulation e.g. Ezh2 phosphorylation.

This project will thus characterise the role of epigenetic modifications in adult stem cell fate choices, and the key signalling pathways that control them. It will use a combination of techniques (*in vivo* conditional knockouts, primary stem cell culture, next generation techniques e.g. ChIP-seq, signalling) and will provide the student with an excellent range of competitive skills.

Lovett FA, Cosgrove RA, Gonzalez I, **Pell JM**. Essential role for p38α MAPK but not p38γ MAPK in *Igf2* expression and myoblast differentiation. 2010 ***Endocrinology*** 151:4368-4380.

Carter EJ, Cosgrove RA, Gonzalez I, Eisemann JH, Lovett FA, Cobb LJ, **Pell JM**. MEK5 and ERK5 are mediators of the pro-myogenic actions of IGF-2. 2009 ***J Cell Sci*** 122:3104-3012.

Tripathi G, Salih DA, Drozd AC, Cosgrove RA, Cobb LJ, **Pell JM**. IGF-independent effects of insulin-like growth factor binding protein-5 (Igfbp5) in vivo. 2009 ***FASEB J*** 23:2616-26.

Lovett FA, Gonzalez I, Salih DA, Cobb LJ, Tripathi G, Cosgrove RA, Murrell A, Kilshaw PJ, **Pell JM**. Convergence of Igf2 expression and adhesion signalling via RhoA and p38 MAPK enhances myogenic differentiation. 2006 ***J Cell Sci.*** 119:4828-40.

PhD Studenship - Professor Wolf Reik (wolf.reik@babraham.ac.uk)

Genome wide reprogramming of DNA methylation in germ cells, stem cells, and early embryos (EU studentship project funded as part of the INGENIUM network).

We are interested in the biology and mechanisms of genome-wide erasure of DNA methylation in mammalian germ cells, stem cells such as ES cells, and the early embryo after fertilization. Reprogramming is important for the return of the genome to pluripotency and for the erasure of acquired epimutations. A number of possible mechanisms for demethylation have recently been discovered, including the modification of methylcytosine by deaminases or by hydroxylases. This may be followed by engagement of specific DNA repair pathways such as the base excision repair pathway. This studentship project will explore pathways of demethylation by employing specific mouse mutants in deaminases, TET hydroxylases, and BER components. The student will analyse the phenotypes of mutant mice and of stem cells and carry out genome-wide profiling studies of methylation, hydroxymethylation, formylcytosine, and carboxylcytosine by high throughput sequencing techniques and associated computational methods. You will join an interactive and collaborative team of about 15 scientists who are engaged in related projects on epigenetic reprogramming.

Recent references: Ficz et al 2011 *Nature* **473**, 398-402; Wossidlo et al 2011 *Nature Comm* **2**, 241; Feng et al 2010 *Science* **330**, 622-627; Popp et al 2010 *Nature* **463**, 1101-1105.

PhD Studentship - Llew Roderick (llewelyn.roderick@babraham.ac.uk)

Signalling to and from the epigenome to control cardiomyocyte fate choices.

Epigenetic mechanisms are central to regulation of gene expression during development and differentiation and are increasingly invoked in diseases including cancer. Although, wholesale transcriptional remodelling is observed in cardiac myocytes during development, differentiation and hypertrophic growth associated with ageing and disease, aside from histone acetylation, the role of epigenetics in the heart is not established. Significantly, progression through the myocyte lifetime is also associated with decreased plasticity, such as loss of proliferative capacity and inability to adapt to stress. These properties of cardiomyocytes endow the heart with poor capacity for repair/regeneration and make hypertrophy an indicator of future heart failure, which is the leading cause of mortality in the ageing population.

Given their key role in development and differentiation, we hypothesise that the prevalence of epigenetic marks associated with transcriptional silencing such as methylation of lysines 27 and 9 of histone H3 (H3K9 and H3K27) and methylation of DNA contribute to loss of myocyte plasticity. Through understanding how these modifications are regulated, we aim to develop strategies that can be employed to induce cell cycle re-entry of adult myocytes. Development of these strategies will inform research into cardiac regeneration and into methods to replace myocytes lost through hypertrophy and ageing.

This project will involve genome-wide analysis of the epigenomes and transcriptomes of myocytes at different stages of differentiation (isolated by FACS) as well as how these change in response to stimuli known to induce cell cycle re-entry and terminal differentiation. Gain and loss of function studies of enzymes responsible for mediating the identified changes in the epigenome will be performed to test causality of these epigenetic marks in myocyte fate choices.

Higazi, D.R., Fearnley, C.J., Drawnel, F.M., Talasila, A., Corps, E.M., Ritter, O., McDonald, F., Mikoshiba, K., Bootman, M.D., and Roderick, H.L. (2009). Endothelin-1-stimulated InsP3-induced Ca2+ release is a nexus for hypertrophic signaling in cardiac myocytes. Mol Cell *33*, 472-482.

PhD Studentship – Peter Rugg-Gunn (peter.rugg-gunn@babraham.ac.uk)

Epigenetic regulation of mammalian development and stem cell differentiation

Polycomb-group (PcG) proteins mediate transcriptionally repressive epigenetic information and are critical regulators of cell fate decisions. Our group is interested in understanding how PcG proteins modulate the epigenome during early mammalian development and stem cell differentiation. The specific objective for this PhD Studentship is to determine the role of the PcG-protein Ezh2 during the transition from pluripotent epiblast cells to mesoderm and endoderm lineages. Investigating these processes will provide crucial insight into how distinct cell types are formed in the embryo and will uncover new ways to use stem cells for cell-replacement therapies.

You will use mouse embryonic stem cells and early embryos, in combination with state-of-the-art genomic and epigenomic approaches, to uncover the dynamics of genome-wide epigenetic changes that occur during cell differentiation. These data will provide the first analysis of epigenetic states that are reprogrammed during mesoderm and endoderm formation and in doing so will identify key target genes of PcG proteins. Transgenic stem cell lines and embryos will be used to test the functional role of Ezh2 in modulating epigenetic information and gene transcription during these processes. The results will define the key role of Ezh2 during early development and stem cell differentiation and lead to better understanding of why PcG proteins are required for cell fate decisions. Identifying the detailed mechanisms of how this process occurs has major relevance for stem cell and developmental biology, and other broad areas of biomedical research, including cancer epigenetics, tissue homeostasis and healthy aging.

You will join a thriving Epigenetics Program at the Babraham Institute, with training in stem cell biology, embryology, high-throughput sequencing, bioinformatics, advanced imaging techniques, flow cytometry and a range of current molecular approaches.

Recent, relevant publications:

1. Rugg-Gunn et al. Distinct histone modifications in stem cell lines and tissue lineages from the early mouse embryo. *Proc. Natl. Acad. Sci. USA*. 2010; 107(24):10783.
2. Ahmed et al. Global chromatin architecture reflects pluripotency and lineage commitment in the early mouse embryo. *PLoS One*. 2010; 5(5):e10531.
3. Vallier et al. Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways. *PLoS One*. 2009; 4(6):e6082.
4. Brons et al. Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature*. 2007; 448(7150):191.
5. Rugg-Gunn et al. Epigenetic status of human embryonic stem cells. *Nature Genetics*. 2005; 37(6): 585.

PhD Studentship - Partick Varga-Weisz (patrick.varga-weisz@babraham.ac.uk)

The role of a chromatin remodeling in stem cell biology, the immune system and development

Embryonic stem cells are characterized by a special chromatin status: their chromatin is often referred to as 'hyper-dynamic'. What underlies this dynamics and what is its importance is poorly understood. Our hypothesis is that the special nature of embryonic stem cell chromatin is linked to chromatin remodeling factors.

We are studying a chromatin remodeling factor that is highly expressed in pluripotent stem cells, is regulated by and interacts with key stem cell transcription factors and has been suggested to be required for the embryonic stem cell phenotype in cells in culture (Wang et al., Nature, 2006; Boyer et al., Cell, 2005; Hong et al., PLOS Computational Biology, 2009). Our work suggests a role of this factor in maintaining heterochromatin during cell proliferation and facilitating correct chromosome segregation (Rowbotham et al., Mol Cell, 2011). We have created a conditional knockout system for this factor in mouse and will study what is its role in early development, how it affects chromatin in the early embryo and how it maintains epigenomic and genomic stability. In a parallel effort, we will explore the role of this factor in the development and function of the immune system in collaboration with Dr Anne Corcoran, Babraham Institute. We will use massive parallel sequencing to map the sites of action of this factor in the genome and to explore how it impacts genic and intergenic transcription. We are developing new techniques, involving *in vivo* protein tagging, to address how this factor remodels chromatin in the living cell. These techniques should be widely applicable, for example to unravel the mechanisms of chromatin enzymes in a systematic approach.

Rowbotham, S.P., Barki L.,Neves-Costa, A., Santos F., Dean. W., Hawkes, N. Choudhary, P., Will, W. R., Webster, J., Oxley, D., Green, C. M., Varga-Weisz, P.* and MermoudJ.E.* (2011) Maintenance of silent chromatin through replication requires SWI/SNF-like chromatin remodeller SMARCAD1. ***Mol Cell*** *in press (*joint corresponding authors, Dr J. Mermoud is a senior postdoctoral fellow in the Varga-Weisz laboratory)*

PhD Studentship - Sonja Vermeren (sonja.vermeren@babraham.ac.uk)

How is neutrophil activation regulated?

Neutrophils form the first line of defense against invading pathogens. They reside in the blood stream until activated, when they become increasingly adhesive. They roll along and then adhere to the vessel wall, finally migrate through it and travel to the site of insult, guided by a gradient of chemoattractants. Once arrived, neutrophils produce reactive oxygen species and cytotoxic enzymes to kill and phagocytose invaders, generating inflammation in the process.

It is crucial that neutrophils become activated only in the right circumstances. Insufficient activation leaves the organism prone to recurrent infections, as exemplified in leukocyte adhesion deficiency patients. Too much neutrophil activity is also detrimental, causing excessive inflammation, as in autoimmune diseases.

We identified that a signalling protein called ARAP3 is important for the regulation of the activation status of neutrophils. It does this by controlling the neutrophils' integrins, which determine the ability to adhere to the substratum. ARAP3 is regulated by PI 3-kinase and Rap, a known regulator of integrin activity. ARAP3 in turn regulates Arf and Rho proteins, which can also influence integrins.

Aim of the present project is to find out how ARAP3 regulates integrins. Many questions remain to be solved. Is this function of ARAP3 downstream of Rap, PI 3-kinase or both? Integrins are complex receptors that can change their ligand binding affinity and avidity; they signal once ligand has bound. Are both or just one of these processes affected? Finally, to what extent does integrin signalling cross-talk with immunoreceptor (Fc receptor) signalling?

To address these questions, you will mostly work with primary neutrophils and carry out many tests in the laboratory; some questions may be addressed using cell lines. You will perform functional assays (ROS, degranulation and chemotaxis assays etc) and analyse cellular signalling pathways using biochemical assays (e.g. enzyme activity assays, Western blots).

References.

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PhD Studentship - Heidi Welch (heidi.welch@babraham.ac.uk)

A novel role for the guanine-nucleotide exchange factor P-Rex in vesicle trafficking

P-Rex guanine-nucleotide exchange factors (GEFs) activate the small G protein Rac in response to stimulation of GPCRs and PI3K [1]. P-Rex1 is important in leukocytes that confer immunity against bacterial and fungal infections [2] and P-Rex2 in neurons that control motor coordination [3]. P-Rex1 and P-Rex2 are also currently emerging as important in the progression and metastasis of a range of cancers [4,5].

Surprisingly few proteins are currently known to interact with P-Rex GEFs, so we have recently conducted a search for more (unpublished). This identified several new P-Rex1 binding partners, two of which are known regulators of vesicle trafficking. We hypothesize that these interactions control P-Rex1 function as a Rac-GEF and/or the process of vesicle trafficking. The latter possibility is particularly intriguing, as control of trafficking would be a novel function of P-Rex.

This project analyses the novel interaction between P-Rex1 and the two vesicle trafficking proteins. We will characterise the interaction in vivo and in vitro to establish whether binding is direct or indirect, constitutive or subject to regulation, and which protein domains confer it. We will determine whether P-Rex1 GEF activity is required for the interaction, whether the interaction affects P-Rex1 localisation or GEF activity, and whether it affects the localisation of the vesicle trafficking proteins. Impact on cell morphology and vesicle trafficking will be assessed. Technically, the project will involve exogenous protein expression and RNAi-mediated down-regulation in cell lines, protein purification, protein binding assays, in vitro and in vivo GEF activity assays, cell fractionation, fluorescence microscopy, PCR-based mutagenesis and, depending on results, potentially a murine genetics approach.

References:

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[4] Fine B et al, 2009, Science 325, 1261-1265.
[5] Sosa MS et al, Mol Cell 40, 877-892.