

# Studentship project descriptions Babraham website 2010

## PhD Student Opportunities 2010

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 teaching Department of the University of Cambridge. Starting October 2010 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, we will be continuing a new Babraham European Studentship scheme, funded jointly by the Babraham Institute and the Cambridge European Trusts, which will provide full funding for three further 3 year studentships.

Please see our website ([www.babraham.ac.uk](http://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.bio.cam.ac.uk/gradschool](http://www.bio.cam.ac.uk/gradschool)).

Details of our interactive scientific programmes can be found on [www.babraham.ac.uk](http://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 20<sup>th</sup> JANUARY 2010** 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 potential supervisors are given below the list of potential projects; our supervisors welcome informal enquiries.

Potential projects:

**Dr Geoff Butcher ([geoff.butcher@bbsrc.ac.uk](mailto:geoff.butcher@bbsrc.ac.uk))**

*Novel immune GTPases regulating lymphocyte apoptosis/survival*

**Dr Michael Coleman ([michael.coleman@bbsrc.ac.uk](mailto:michael.coleman@bbsrc.ac.uk))**

*Variations in axon survival within the human population*

**Dr Francesco Colucci ([francesco.colucci@bbsrc.ac.uk](mailto:francesco.colucci@bbsrc.ac.uk))**

*How do killer cells contribute to reproductive success?*

**Dr Simon Cook ([simon.cook@bbsrc.ac.uk](mailto:simon.cook@bbsrc.ac.uk))**

*Links between growth factor signalling pathways, apoptosis and autophagy.*

**Dr Anne Corcoran ([anne.corcoran@bbsrc.ac.uk](mailto:anne.corcoran@bbsrc.ac.uk))**

*The role of non-coding RNA transcription in immunoglobulin rearrangement and antibody diversity*

**Dr Sarah Elderkin ([sarah.elderkin@bbsrc.ac.uk](mailto:sarah.elderkin@bbsrc.ac.uk))**

*Understanding the regulation of Polycomb Repressor Complexes in embryonic and adult stem cell self renewal and cellular proliferation.*

**Dr Peter Fraser ([peter.fraser@bbsrc.ac.uk](mailto:peter.fraser@bbsrc.ac.uk))**

*Epigenetic control of gene expression by large non-coding RNAs.*

**Dr Myriam Hemberger ([myriam.hemberger@bbsrc.ac.uk](mailto:myriam.hemberger@bbsrc.ac.uk))**

*Transcription factor networks in trophoblast stem cell self-renewal and differentiation*

**Dr Jon Houseley ([jon.houseley@bbsrc.ac.uk](mailto:jon.houseley@bbsrc.ac.uk))**

*Investigating the roles of non-coding RNAs in genome variation*

**Dr Jenny Pell ([jenny.pell@bbsrc.ac.uk](mailto:jenny.pell@bbsrc.ac.uk))**

*The role of p38 MAPK in the epigenetic regulation of adult stem cell fate*

**Dr Wolf Reik ([wolf.reik@bbsrc.ac.uk](mailto:wolf.reik@bbsrc.ac.uk))**

**- CASE project with CellCentric**

*New regulators of epigenetic reprogramming and pluripotency in ES and iPS cells*

**Dr Wolf Reik ([wolf.reik@bbsrc.ac.uk](mailto:wolf.reik@bbsrc.ac.uk))**

*Potential for epigenetic inheritance across generations determined by genome scale analysis*

**Dr Martin Turner ([martin.turner@bbsrc.ac.uk](mailto:martin.turner@bbsrc.ac.uk))**

*Novel pathways for the regulation of lymphocyte development.*

**Dr Patrick Varga-Weisz ([patrick.varga-weisz@bbsrc.ac.uk](mailto:patrick.varga-weisz@bbsrc.ac.uk))**

*Links between Chromatin remodelling factors, noncoding RNAs, RNA interference and histone variant exchange*

**Professor Michael Wakelam ([michael.wakelam@bbsrc.ac.uk](mailto:michael.wakelam@bbsrc.ac.uk))**

*Regulation of mTOR signalling by phospholipase D during ageing*

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 ask two referees to write to the Institute on their behalf before the deadline; please include your contact details for 1- 20 January. Our website also provides a checklist of the information required to be provided in your application before it can be considered.

Please send your applications to: Ms Linda Notton, Graduate Studies Programme, The Babraham Institute, Babraham, Cambridge CB22 3AT, Tel: 01223 496338, Fax:

01223 496022 or email [babraham.graduate@bbsrc.ac.uk](mailto:babraham.graduate@bbsrc.ac.uk) by **FRIDAY 1st JANUARY 2010**.

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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 1<sup>st</sup> -20<sup>th</sup> January 2010**
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## Details of Projects for 2010

Dr Geoff Butcher ([geoff.butcher@bbsrc.ac.uk](mailto:geoff.butcher@bbsrc.ac.uk))

### **Novel immune GTPases regulating lymphocyte apoptosis/survival**

The GIMAP proteins are a small family of ~8/9 mammalian GTPases found predominantly in immune tissues. Roles for these proteins in the regulation of apoptosis have been proposed. Extensive studies of GIMAP5 have led to the conclusion that it is an anti-apoptotic protein protecting mature T lymphocytes from spontaneous cell death. Mutation of the *Gimap5* gene in rats confers susceptibility to autoimmune disease. In contrast to GIMAP5, studies of GIMAP4 have led to the proposal that this family member *promotes* apoptosis of T lymphocytes, a property that may be advantageous during the post-infection resolution of immune and inflammatory responses *in vivo*, in order to return lymphomyeloid cell populations to their pre-response sizes. Indeed, it has been suggested that GIMAP4 interacts with the pro-apoptotic protein Bax to achieve its effects, while GIMAP5 mediates its pro-survival effects via interaction with anti-apoptotic Bcl-2 family members.

Our laboratory has generated significant unique reagents to study these GTPases, including conditional knockout mice and a range of specific monoclonal antibodies. Through Amy Saunders' work on GIMAP1 we have established a powerful animal model for expanding our understanding of GIMAP protein function at the whole animal level. The purpose of this project is to explore the structural requirements for GIMAP protein function. The successful candidate will establish an *in vivo* reconstitution assays complementing *GIMAP* gene defects in haematopoietic stem cells, using mouse stem cell virus (MSCV) as the gene vector system and mature lymphocyte development as the read-out. Mutational analysis will address the requirements for enzymatic GTP cleavage by the GIMAP proteins and for the correct targeting of GIMAP proteins to intracellular organelles.

#### Relevant recent lab publications

Dion, C., Carter, C., Hepburn, L., Coadwell, W.J., Morgan, G., Graham, M., Pugh, N., Anderson, G., **Butcher, G.W.** & Miller, J.R. (2005) Expression of the *Ian* family of putative GTPases during T cell development and description of an *Ian* with three sets of GTP/GDP-binding motifs. *International Immunology* **17**:1257-1268.

Cousins, L., Graham, M., Tooze, R., Carter, C., Miller, J.R., Powrie, F.M., MacPherson, G.G. & **Butcher, G.W.** (2006) Eosinophilic Bowel Disease controlled by the BioBreeding rat-derived *lymphopenia/Gimap5* gene. *Gastroenterology* **131**:1475-1485.

Carter, C., Dion, C., Schnell, S., Coadwell, W.J., Graham, M., Hepburn, L., Morgan, G., Hutchings, A., Pascall, J.C., Jacobs, H., Miller, J.R. & **Butcher, G.W.** (2007) A natural hypomorphic variant of the apoptosis regulator *Gimap4/IAN1*. *Journal of Immunology* **179**:1784-1795.

Saunders, A., Lamb, T., Pascall J., Hutchings A., Dion C., Carter C., Hepburn L., Langhorne J. & **Butcher, G.W.** (2009) Expression of GIMAP1, a GTPase of the immunity-associated protein family, is not up-regulated in malaria. *Malaria Journal* **8**:53.

Saunders, A., Janas, M., Hutchings, A., Pascall, J., Webb, L., Carter, C., Pugh, N., Morgan, G., Turner, M. & **Butcher, G.W.** (2009) Putative GTPase GIMAP1 is critical for the development of mature B and T lymphocytes. *Submitted for publication*.

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**Dr Michael Coleman ([michael.coleman@bbsrc.ac.uk](mailto:michael.coleman@bbsrc.ac.uk))**

### **Variations in axon survival within the human population**

We have identified a protein in a healthy mutant mouse that is able to delay the degeneration of injured axons by tenfold, named the WldS, or slow Wallerian degeneration protein (Mack et al., 2001). WldS also protect axons in several neurodegenerative disorders, including models of motor neuron disease, multiple sclerosis and glaucoma (Coleman, 2005).

WldS is an unusual fusion protein where a short N-terminal sequence from a ubiquitin ligase is fused to the entire coding sequence of the NAD<sup>+</sup> synthesizing enzyme Nmnat1. Although both parts of WldS are required for it to function, the effects of the fusion can be mimicked by altering Nmnat1 in other ways. Recent data from our own and other groups also show that the related isoforms Nmnat2 and -3 also influence axon degeneration.

These new developments raise the exciting prospect of a variable axon phenotype within the human population. We hypothesize that polymorphisms in the Nmnat1, 2 and/or 3 proteins may help determine susceptibility to some neurodegenerative diseases, or influence the disease severity. Missense and other coding polymorphisms are already listed in the single nucleotide polymorphism (SNP) database. These include non-conservative changes to amino acids that are highly conserved throughout evolution and therefore likely to have important functions. The aim of this project is to test the hypothesis that some of these polymorphisms can alter protein function, resulting in phenotypic changes in axons.

First, mouse or human constructs will be engineered to introduce amino acids corresponding to human polymorphisms. These will be selected using predicted effects based on crystal structures, where available. Functional effects will then be tested in cell culture and recombinant proteins by assaying protein stability, intracellular location, interaction partners, enzyme activity and other parameters. Interesting changes can then be followed up in transgenic mice to determine their effects on axon degeneration phenotype.

An additional aim will be to further characterise SNPs within the human population. The population frequency of existing SNPs of interest will be determined and higher frequency SNPs may be investigated for any association to neurodegenerative disorders.

### **References**

Mack et al. (2001) Nature Neuroscience 4: 1199-1206

Coleman (2005) Nature Reviews Neuroscience 6: 889-898

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**Dr Francesco Colucci ([francesco.colucci@bbsrc.ac.uk](mailto:francesco.colucci@bbsrc.ac.uk))**

### **How do killer cells contribute to reproductive success?**

Natural Killer (NK) cells are bone marrow-derived cytotoxic lymphocytes of the innate immune system [1]. Unlike their counterparts in blood, uterine NK (uNK) cells found at the maternal-foetal interface are only weakly cytolytic but they do secrete a wide

range of angiogenic factors and cytokines that may influence placentation. Indeed, uNK cells are crucial in the development of a healthy materno-fetal nutritional supply line both in humans and mice [2, 3]. Research in our lab has recently shown that mouse uNK have a unique repertoire of cell surface receptors that bind possible target cells [4] and a unique repertoire is also observed in human uNK cells [5], suggesting that the mechanisms of cellular differentiation and selection of uNK cells are conserved in the two species. Foetal MHC molecules derived from paternal and maternal genes are the prime candidates the interactions between uNK cells and the foetal derived trophoblast. Genetic studies indicate that certain combinations of polymorphic NK receptors and foetal MHC molecules are associated with reproductive failure, including pre-eclampsia and recurrent miscarriage [6].

Our key question is: How do uNK cells recognise and respond to trophoblast and how does this affect trophoblast behavior resulting in reproductive success? The aim of this project is to define how uNK cells influence placental development. The project will be developed in collaboration with Ashley Moffett (University of Cambridge) and Myriam Hemberger (Babraham). You will be using advanced dissection techniques, transgenic mice, flow cytometry, histology, microscopy and gene expression analysis to test the hypothesis that there is a cooperative partnership between foetal antigens and the maternal immune system that leads to a successful pregnancy [7].

#### References:

1. Colucci F, Caligiuri MA, Di Santo JP (2003). What does it take to make a natural killer? *Nat Rev Immunol.* 3:413-25.
2. Moffett A, Loke C (2006). Immunology of placentation in eutherian mammals. *Nat Rev Immunol.* 6:584-94.
3. Croy BA, van den Heuvel MJ, Borzychowski AM, Tayade C (2006). Uterine natural killer cells: a specialized differentiation regulated by ovarian hormones. *Immunol Rev.* 214:161-85.
4. Yadi H, Burke S, Madeja Z, Hemberger M, Moffett A, Colucci F. (2008). Unique Receptor Repertoire in Mouse Uterine NK cells. *J Immunol.* 181:6140-7.
5. Sharkey AM, Gardner L, Hiby S, Farrell L, Apps R, Masters L, Goodridge J, Lathbury L, Stewart CA, Verma S, Moffett A (2008). Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. *J Immunol.* 181:39-46.
6. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CWG, Carrington M, Trowsdale J and Moffett A (2004). Combinations of maternal and paternal KIR and HLA-C genes influence the risk of pre-eclampsia. *Journal of Experimental Medicine* 200, 957-965.
7. Parham P (2004). NK cells and trophoblasts: partners in pregnancy. *J Exp Med.* 200:951-5.

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Dr Simon Cook ([simon.cook@bbsrc.ac.uk](mailto:simon.cook@bbsrc.ac.uk))

#### Links between growth factor signalling pathways, apoptosis and autophagy.

Correct nutrition has a profound influence on health and well being throughout life. For example, underfeeding the foetus during pregnancy can increase the lifetime risk for diabetes and heart disease. Conversely, caloric restriction after birth can

increase lifespan in flies, worms and mice. At the cellular level changes in nutrition (amino acid or glucose restriction) can influence cell cycle progression and cell viability by driving autophagy. This may be taken to extremes in tumour cells which undergo extensive re-modelling of their metabolism to adapt to the tumour environment. Amino acid withdrawal activates several discrete signalling pathways including the mTOR/autophagy pathway, the JNK and p38 stress kinases and the eIF2a kinase GCN2. This project will investigate how amino acid withdrawal stress response pathways are regulated by growth factor dependent signals such as the ERK1/2 and PKB pathways to control cell cycle progression and cell death/cell survival. This will be studied in primary and immortalised human fibroblast cells but also in human tumour cell lines including isogenic cell lines containing or lacking a single activated KRAS allele and cells which exhibit acquired resistance to ERK1/2 pathway inhibitors. Responses to amino acid withdrawal will be correlated with changes in tumour cell viability (apoptosis, autophagy) using defined biochemical inhibitors, conditional protein kinases and engineered tumour cell lines.

## References

- Weston et al (2003) *Oncogene* 22:1281-1293  
Ley et al (2003) *J Biol Chem* 278:18811-18816  
Ley et al (2005) *Cell Death Differ.* 12:1008-14  
Ewings et al (2007) *EMBO J.* 26:2856-67  
Wickenden et al (2008) *Oncogene* 27:7150-61  
Gilley et al (2009) *Cell Signal.* 21:969-77.  
Balmanno et al (2009) *Int J Cancer.* 125:2332-41  
Balmanno & Cook (2009) *Cell Death Differ* 16:368-77
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**Dr Anne Corcoran ([anne.corcoran@bbsrc.ac.uk](mailto:anne.corcoran@bbsrc.ac.uk))**

## **The role of non-coding RNA transcription in immunoglobulin rearrangement and antibody diversity**

Recombination of multiple genes in the immunoglobulin and T cell receptor DNA loci generates the vast repertoire of antibodies and T cell receptors required for a functional immune system. The focus of our research is to understand the chromatin remodelling mechanisms that open up these antigen receptor loci, to facilitate V(D)J recombination in lymphocytes. Since these DNA loci are the largest in the genome, containing hundreds of genes, they have evolved several dynamic processes to ensure appropriate chromatin opening, including nuclear relocalisation (movement within the nucleus), extensive histone modification, non-coding RNA transcription, 3D DNA looping (to bring distal genes together). Thus they also provide an excellent paradigm for chromatin regulation of all multigene loci. In particular, eukaryotic genomes have recently been shown to produce an enormous number of non-coding RNAs, which are thought to play a vital role in many nuclear processes, including developmental regulation of gene expression. However, the mechanisms are poorly understood. We have shown that large non-coding RNA transcripts are generated in the immunoglobulin heavy chain V (variable) region prior to V to DJ recombination (Bolland et al 2004, *Nature Immunology* 5; 630-637), and in the D (diversity) region prior to D to J recombination (Bolland et al 2007, *Mol Cell Biol* 27:5523-33). We are testing the hypothesis that this transcription opens up closed immunoglobulin

chromatin to make it accessible to the recombinase enzymes for V(D)J recombination. We have generated mouse models in which this transcription is interrupted. The aim of this project will be to determine the effect of transcription loss on D to J and V to DJ recombination, and to discover which of the processes above are inhibited. State-of-the-art techniques including high-throughput FISH (fluorescence in situ hybridization), chromatin immunoprecipitation and next generation sequencing will be used.

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**Dr Sarah Elderkin ([sarah.elderkin@bbsrc.ac.uk](mailto:sarah.elderkin@bbsrc.ac.uk))**

### **Understanding the regulation of Polycomb Repressor Complexes in embryonic and adult stem cell self renewal and cellular proliferation.**

Polycomb-group (PcG) repressor proteins are key epigenetic regulators involved in both establishing gene expression patterns and maintaining long-term cellular memory. Maintenance of cellular gene expression memory is an important process in regulation of embryonic stem cell self renewal, cell identity, cell proliferation and tumor development (Sparmann and van Lohuzin 2006).

PcG proteins form large multi-protein complexes, which can modify histone tails within chromatin. Polycomb complexes are highly regulated, both at the transcriptional and post-translational levels. One method of regulating polycomb repressive complexes is through the post-translational modification of proteins within the complex (Elderkin et al. 2007). In mammalian cells there are multiple polycomb repressive complexes and we have recently shown that their enzymatic activity can be regulated by phosphorylation.

We are particularly interested in understanding how epigenetic modifying polycomb repressive complexes are potentially regulated by different signalling pathways. The PhD project in our laboratory will involve identifying the specific signalling pathways that regulate polycomb repressive complexes and determine the role these pathways play in polycomb mediated gene repression in embryonic/adult stem cells. This project will provide strong training in embryonic/ adult stem cell, signalling and epigenetics research, using protein and chromatin biochemistry, molecular biology, and bioinformatics.

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**Dr Peter Fraser ([peter.fraser@bbsrc.ac.uk](mailto:peter.fraser@bbsrc.ac.uk))**

### **Epigenetic control of gene expression by large non-coding RNAs.**

Recent transcriptome studies have revealed that the mammalian genome produces an enormous number of non-coding RNAs that far outweigh the number of protein-coding or gene mRNAs. Though this vast RNA world is only beginning to be explored the early indications are that these non-coding RNAs play highly diverse roles in control of multiple genome functions. Large non-coding RNAs such as Xist and Air are involved in silencing cis-linked genes over wide genomic regions. The molecular mechanisms of this epigenetic control are unknown though recent studies in the host lab indicate that these RNAs interact directly with chromatin and influence

histone modifications and nuclear positioning relative to transcription factories. This project will use novel technologies developed by the host lab to investigate the molecular mechanisms of newly discovered large non-coding RNAs in control of gene expression. The applicant will become proficient in state-of-the-art molecular and cell biology techniques in combination with bioinformatic analyses of data from massively parallel sequencing technologies.

Chakalova L, Debrand E, Mitchell JA, Osborne CS, Fraser P. (2005) Replication and transcription: shaping the landscape of the genome. *Nat Rev Genet.* 6, 669-677.

Fraser, P. and Bickmore W. (2007) Nuclear organisation of the genome and the potential for gene regulation. *Nature*, 447, 413-417.

Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R and Fraser P (2008) The *Air* non-coding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* 322,1717-1720.

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Dr Myriam Hemberger ([myriam.hemberger@bbsrc.ac.uk](mailto:myriam.hemberger@bbsrc.ac.uk))

### Transcription factor networks in trophoblast stem cell self-renewal and differentiation

We are interested in the genetic and epigenetic regulation of cell fate decisions and stem cell potency in early mammalian development. The first cells to adopt a clear fate after fertilization are those that will form the placenta, the so-called trophoblast cells. We have recently identified a key transcription factor that is required in these cells to reinforce trophoblast cell fate and to maintain the self-renewing capacity of trophoblast stem cells. This student project will build on these findings and investigate the molecular targets of transcription factor networks in trophoblast stem cells, how they maintain self-renewal and how differentiation into functional trophoblast cell types is achieved. You will use a combination of epigenomics, embryology, microscopy, and computational biology in order to unravel the key events in early development. These approaches have great relevance to stem cell biology, reprogramming, regenerative medicine, mammalian reproduction and developmental biology.

Recent publications:

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.

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

Roper S. and Hemberger M. (2009). Defining pathways that enforce cell lineage specification in early development and stem cells. *Cell Cycle*, 15: 1515-1525.

Screen M., Dean W., Cross J.C. and Hemberger M. (2008). Cathepsin proteases have distinct roles in trophoblast proliferation and vascular remodelling. *Development*, 135: 3311-3320.

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Dr Jon Houseley ([jon.houseley@bbsrc.ac.uk](mailto:jon.houseley@bbsrc.ac.uk))

## Investigating the roles of non-coding RNAs in genome variation

Only a tiny fraction of the human genome encodes proteins, but recent studies show that almost the entire genome is transcribed into RNA. This means that many more genes produce RNA than produce proteins, and the key aim of my research is to find functions for these non-protein coding RNAs.

Cells have multiple mechanisms by which the number of active copies of a gene can be changed, such as X-inactivation and genomic imprinting. Less well appreciated is that many genes lie in repeated regions of the genome, so there are multiple copies of these genes on single chromosomes. The number of copies can be highly variable between individuals, and this variation is therapeutically important as changes in gene copy number have been linked to multiple diseases.

We and others have found evidence that non-protein coding RNAs and their associated enzymes can affect copy number in repeated regions. This raises the fascinating possibility that non-coding RNAs can influence genome changes, and therefore cells could use transcription to alter their genomes in response to changing circumstances. Understanding these processes is likely to be important in understanding the occurrence of many genetic disorders and cancers.

The aim of this PhD research is to elucidate mechanisms by which non-protein coding RNAs and their interacting enzymes can influence changes in copy number. Budding yeast will be employed as a model system, and the work will involve a wide variety of molecular biology techniques.

The realisation that cells produce so much non-coding RNA provides new areas of fundamental biology to explore, and non-coding RNAs are of considerable pharmaceutical interest as they greatly enhance the target range of RNA interference methodologies. This project will provide much sought-after experience in this fast-moving area of biology, which should be very beneficial to the successful candidate's future scientific career.

Further reading:

Hastings, P. J., Lupski, J. R., Rosenberg, S. M., and Ira, G. (2009). Mechanisms of change in gene copy number. *Nat Rev Genet* 10, 551-564.

Houseley, J., Kotovic, K., El Hage, A., and Tollervey, D. (2007). Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. *Embo J* 26, 4996-5006.

Houseley, J., and Tollervey, D. (2009). The many pathways of RNA degradation. *Cell* 136, 763-776.

Kobayashi, T., and Ganley, A. R. (2005). Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. *Science* 309, 1581-1584.

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Dr Jenny Pell ([jenny.pell@bbsrc.ac.uk](mailto:jenny.pell@bbsrc.ac.uk))

## The role of p38 MAPK in the epigenetic regulation of adult stem cell fate

Postnatal health depends upon adult stem cells; they must have the ability to differentiate into specialized cells as well as to self-renew and replace the original

stem cell population. Regulation by complex transcriptional and epigenetic networks is fundamental to these decisions. Understanding these is important because uncontrolled stem cell proliferation can induce tumour formation and cancer but insufficient stem cell activation will lead to impaired tissue repair.

p38 MAPK is a key signaling molecule that is required for muscle stem cell differentiation (1). It exists as four isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ), of which the  $\alpha$  isoform is most important (2). p38 MAPK has multiple intriguing functions in muscle stem cells, via its kinase activity: it regulates chromatin remodeling complexes, has a role in regulating mRNA stability, increases the activity of key myogenic transcription factors and can facilitate efficient RNA polymerase activity. It is thus likely that p38 has multiple and different targets at different stages of myogenic stem cell activation. The hypothesis to be examined in this study is that p38 has the ability to modulate epigenetic targets, in particular differential histone methylation, that will have a key role in dictating stem cell fate.

The project will use a variety of *in vivo* (genetically modified mice: p38 flox, muscle-specific Cre recombinase, and ER-inducible Cre recombinase), *ex vivo* (isolated primary muscle stem cells and myofibres) and *in vitro* (myoblast cell lines). Epigenetic modification will be determined using the powerful genome wide Illumina 'next generation' sequencing and bioinformatic analysis.

This project will suit a candidate who is interested in the way in which cell signalling can communicate with and modify chromatin structure to modify cell behavior. It will provide training in key cell and molecular biology techniques, as well as providing scope for intellectual development.

#### References:

1. Gonzalez I, Tripathi G, Carter EJ, Cobb LJ, Salih DA, Lovett FA, Holding C, **Pell JM**. Akt2, a novel functional link between p38 mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways in myogenesis. 2004 *Mol Cell Biol* **24**:3607-22.
2. 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.
3. 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.
4. 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.

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Dr Wolf Reik ([wolf.reik@bbsrc.ac.uk](mailto:wolf.reik@bbsrc.ac.uk)) - CASE project with CellCentric

#### New regulators of epigenetic reprogramming and pluripotency in ES and iPS cells

We are undertaking genome-wide epigenomics screens in pluripotent stem cells and differentiated cells in order to understand epigenetic reprogramming and the epigenetic regulation of pluripotency. We have identified a number of new candidate

factors and candidate mechanisms that might play a role in epigenetic reprogramming and in regulating pluripotent states. In this student project you will manipulate these new gene candidates by stable knockdown strategies in ES cells in order to determine their role in epigenetic reprogramming and pluripotency. You will examine the potential of manipulated ES cells to self-renew and differentiate, as well as their epigenetic properties and reprogramming capability. You will learn techniques for culture and manipulation of ES cells and for genome scale epigenetic analysis and computational evaluation. Depending on what you find in the ES cell system, you may also test the new candidates by introducing them into differentiated cells in order to derive iPS (induced pluripotent stem cells). This is an exciting opportunity for research at the forefront of epigenetics, stem cell biology and regenerative medicine. The work is in close collaboration with an industrial partner (CellCentric, Cambridge) which will give you the opportunity to learn how collaborations with the biomedical industry can help to accelerate translating and applying your research findings.

Recent references: Nature 447, 425-432 (2007), Nature Genet. 40, 971-976 (2008), Nature Cell Biology 10, 1280-1290 (2008), PLoS Genet. 4, e1000116 (2008), Development 136, 525-530 (2009), Cell 136, 629-641 (2009), Nature Rev. Mol. Cell Biol. 10, 526-537 (2009).

**Dr Wolf Reik ([wolf.reik@bbsrc.ac.uk](mailto:wolf.reik@bbsrc.ac.uk))**

### **Potential for epigenetic inheritance across generations determined by genome scale analysis**

Reprogramming of epigenetic information (DNA methylation as well as histone marks) occurs in mammalian germ cells and early embryos. Reprogramming erases existing epigenetic information and is probably important for the return to pluripotency of embryonic cells, and the erasure of acquired epigenetic information. The balance between erasure and maintenance of epigenetic information in the germline will determine the potential for acquired epigenetic marks to be inherited by future generations. The mechanisms of erasure are also of great importance; as far as DNA methylation is concerned, we are examining some of the key candidate pathways for demethylation in knockout mice. In this exciting project you will examine epigenetic marks (DNA methylation and histone marks) by genome-scale analysis using High Throughput Sequencing in germ cells and early embryos. You will also carry out epigenomic analyses in selected mouse mutants of reprogramming factors. You will learn to use advanced computational methods for epigenomic analyses. In this project, you will gain insights into how and to what extent the epigenome becomes reprogrammed, and what the potential is for epigenetic inheritance across generations. This is important not least for human disease susceptibilities for which transgenerational influences have been suggested from epidemiological studies.

Recent references: Nature 447, 425-432 (2007), Nature Genet. 40, 971-976 (2008), Nature Cell Biology 10, 1280-1290 (2008), PLoS Genet. 4, e1000116 (2008), Development 136, 525-530 (2009), Cell 136, 629-641 (2009), Nature Rev. Mol. Cell Biol. 10, 526-537 (2009).

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**Dr Martin Turner ([martin.turner@bbsrc.ac.uk](mailto:martin.turner@bbsrc.ac.uk))**

## **Novel pathways for the regulation of lymphocyte development.**

B lymphocytes develop in the mammalian bone marrow and T lymphocytes develop in the thymus after passing through a well-characterised series of intermediate stages. The progression and phenotype of cells can be assessed by their cell surface phenotype and their transcriptional profile. It is now routine to use microarray technology to assess the whole transcriptome of cells at a given developmental stage. This type of approach has led to the emergence of a concept of transcriptional networks controlling lymphocyte development, with one or a few transcription factors acting as “master regulators”. Recent work from our group and others has placed emphasis on post-transcriptional processes and their impact on the development and function of lymphocytes.

Post-transcriptional processes affect the handling of RNA after transcription and can influence the expression of genes by altering the stability of the mRNA or by changing the rate at which the mRNA is translated into protein. Small RNAs called microRNAs are one aspect of this level of control. RNA binding proteins are also key regulators of mRNA stability and translation. In this project the student will examine the role of RNA binding proteins using conditional gene expression mouse models of lymphocyte development. In vivo approaches will be combined with cutting edge molecular biology and whole genome approaches to identify post-transcriptional networks which determine cell fate.

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**Dr Patrick Varga-Weisz** ([patrick.varga-weisz@bbsrc.ac.uk](mailto:patrick.varga-weisz@bbsrc.ac.uk))

## **Links between Chromatin remodelling factors, noncoding RNAs, RNA interference and histone variant exchange**

Chromatin remodelling factors play key roles in stem cell biology and development. Mutations in genes coding for these enzymes are linked to cancer. We wish to illuminate existing, novel links between chromatin remodeling factors, the RNAi silencing pathway, heterochromatin assembly, histone variants and gene regulation. The Varga-Weisz laboratory uses studies in yeast to obtain fundamental insights into the mechanisms of chromatin remodelling factors. These insights are then extended to test the roles of these conserved factors in mammalian cell function and development. We combine biochemical characterization with genetic experiments and genome wide localization analysis (chromatin immunoprecipitation coupled to high throughput sequencing, CHIP-seq) to obtain comprehensive pictures about the roles and regulation of these proteins.

We identified a remodelling factor that is enriched in heterochromatin, especially upon cell stress. We want to elucidate what is the role of this factor in heterochromatin. Our working hypothesis is that this factor is involved in facilitating the expression of noncoding RNA molecules to drive the generation of small interfering RNA for heterochromatin formation. Future studies will test and elaborate this model. Histone variants define structurally and functionally distinct chromatin domains. We will examine if the mechanism of this chromatin remodelling factor involves the regulation of histone variant deposition within transcriptionally repressed chromatin.

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## **Regulation of mTOR signalling by phospholipase D during ageing – (last year's project)**

Rapamycin is a potent antifungal metabolite that inhibits proliferation of mammalian cells, and possess immunosuppressive properties. The mammalian target of Rapamycin, mTOR is a serine/threonine kinase that is present in all eukaryote organisms, and has been implicated in regulating life span. During development mTOR primarily regulates growth, but in the adult, where there is relatively little growth, mTOR controls ageing.

TOR integrates signals received from growth factors, nutrients, stress and the energy status of cells, to regulate cellular growth, proliferation and shape. Aberrant mTOR activity results in the initiation and progression of several age-related diseases. These include human cancers, the development of autoimmune disorders such as Rheumatoid arthritis and Parkinson's disease, cardiac hypertrophy and type II diabetes. Consequently, mTOR inhibitors such as Rapamycin may be useful therapeutic agents for several age-related human diseases.

The Ras-related small G protein, Rheb activates mTOR, and has been shown to induce oncogenic transformation *in vitro*, and to produce the rapid development of lymphomas and prostrate tumours *in vivo*. The tumourigenic activity of Rheb is dependent on mTOR activity.

Rheb activates mTOR in response to growth factors and nutrients by two distinct mechanisms; (1) Rheb binds to an endogenous inhibitor of mTOR, FKBP38, thereby antagonising the inhibition of mTOR by FKBP38, and (2) Rheb binds to and activates phospholipase D1 (PLD1), thereby elevating the levels of the lipid second messenger phosphatidic acid (PA), a direct activator of mTOR.

Mammalian cells express a second isoform of phospholipase D, PLD2, which has also been reported to also regulate mTOR. However in contrast to PLD1, PLD2 does not appear to be regulated by Rheb, but instead binds to Raptor, an mTOR-interacting protein that is required for mTOR signalling.

The aim of this studentship is to (1) investigate the physiological significance of the Rheb/PLD1/mTOR and PLD2/Raptor/mTOR pathways in regulating mTOR signalling in response to growth factors, nutrients, stress and the energy status of the cell, (2) determine if the tumourigenic activity of Rheb is also dependent on PLD activity, and (3) elucidate the mechanism(s) of PLD/PA regulation of mTOR activity.

These aims will be achieved through the use of shRNA knockdown of PLD1 and PLD2 levels in established model cell lines, and the use of primary cells isolated from mice that no longer express PLD1 and/or PLD2.