University of Oxford
DPhil in Biomedical and Clinical Sciences

Funded by The Wellcome Trust
Clinical Training Fellowship Scheme

Fellowship Scheme Details and
Project Proposals for 2008 – 2009

Address for enquiries and applications:

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I N D E X

Management Team

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Deputy Director:	Professor P Klenerman
Senior Mentor:	Professor M McMichael
Academic Medical Forum Leader:	Professor P Ratcliffe
Basic Science Leaders:	Professor KE Davies Professor K Nasmyth
Theme Leaders:	Professor H Watkins (Cardiovascular) Professor D Higgs (Haematology) Professor A McMichael (Infections / Immunity) Professor R V Thakker (Metabolic Medicine) Professor P Rothwell (Neurosciences)
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Administrative DTC Leader:	Mrs P O'Connor

All enquiries should be made via the DTC (email: dtc@medsci.ox.ac.uk).

INTRODUCTION

This handbook provides an overview to the DPhil programme in Biomedical and Clinical Sciences at the University of Oxford Medical Sciences Division (MSD) which is funded by the Wellcome Trust Clinician Training Fellowship Scheme to train academic clinicians in basic science. The handbook contains details of the projects in 5 theme areas – cardiovascular, haematology, infections and immunity, metabolic medicine and neuroscience – that are offered by the supervisors. In addition, details of the aims and content of the programme are provided, as well as the selection process and formal training activities.

The programme is administered by the Doctoral Training Centre (DTC) and all enquiries and applications should be made via the DTC (email dtc@medsci.ox.ac.uk).

AIMS OF THE PROGRAMME

- Provide high-quality research training in basic and applied molecular science for clinical fellows.

- Promote breadth of training opportunity through access to high quality basic science laboratories and by encouraging partnerships between basic and clinician scientist supervisors.

The principal aim of the programme is to provide high-quality research training in basic and applied molecular science that is tailored specifically to the needs of talented clinicians who aspire to a career in academic medicine.

Medical Sciences Division (MSD)

The MSD (University of Oxford), which integrates all Preclinical and Clinical Departments, is extremely well placed to achieve this. The University has maintained a strong tradition of rigorous scientific training in Medicine at all levels. The MSD is one of the largest and most productive biomedical research centres in Europe.

Fellowship programme and research in the MSD

The research activities of the MSD are exemplary in providing combined basic science and clinical environments that are strong, well supported, and facilitate the generation of integrated training pathways for young clinician scientists. A particular advantage of the proposed scheme is a greatly enhanced ability to connect trainees with innovative projects, and with the most appropriate supervisor, within this environment.

OVERVIEW OF CONTENT OF PROGRAMME

- Themed to match clinical training with opportunities across a broad range of research disciplines.
- Opening up training in basic sciences to clinicians.
- Enabling flexibility to accommodate trainee needs.

Fellowships

The fellowships are structured around existing research programmes that already provide first class research environments for clinician scientists, and which provide a framework for research and training in the combined fields of basic science and clinical medicine. The components of the programme have in common that they use molecular genetic, molecular biological and cellular approaches to important medical problems. The projects for the fellowships are grouped around five main themes – cardiovascular medicine; haematology and haematopoiesis; infection and immunity; metabolic medicine; and neurosciences.

Project Themes

The themes and titles have been specifically chosen, as they encompass important disorders and represent major strengths in the MSD, and also enable us to keep the programme "trainee – orientated", by choosing theme titles that are recognisable to clinical trainees, and thereby facilitating their recruitment and in developing their clinical training pathways. However, it is important to emphasise that these themes also encompass high quality basic science expertise in: biochemistry; structural biology; developmental biology; genetics; immunology; gene therapy; transcriptional regulation; cell biology; molecular physiology; and pharmacology. Moreover, each training fellowship has been designed to form a partnership between basic-scientists and clinician-scientists.

SELECTION PROCESS OF FELLOWS

- Trainees will have strong academic track record.
- Selection will be on basis of a written proposal and interview.
- Integration with ACF scheme.
- Rotation with commencement of fellowship, through basic science laboratories to enable trainees to make informed choices.

Trainees

Trainees should have: a strong academic track record, e.g. a BSc (or equivalent) with a 1st or 2:1; honours or distinction in the MB finals; academic prizes; and publications. Selection of trainees will be by interview and on the basis of academic ability, clinical competency, and a well-defined inter-disciplinary project.

Selection

Entry to the fellowship would be contingent on preparation by the trainee, in discussion with the supervisor, of a written proposal (2 pages maximum) describing the specific research goals, background, methodology and training opportunities. Selection will comprise assessment of the student and project by a panel consisting of internal members (e.g. the programme director, deputy director, medical forum leader, theme leaders, and two senior basic science leaders).

Integration with ACF scheme

Many trainees will have had opportunities to prepare a project during their 9 months of academic research time as part of the 3 year academic clinical fellowship (ACF) within Oxford, or other centres. However, some ACFs and other trainees of high academic potential may not have had such opportunities, and to enable them to make informed choices, the MSD will provide funding for up to 6 months salary, to facilitate 3 two-monthly rotations through basic science laboratories. At the end of this pre-fellowship programme, the trainee will have chosen a supervisor and written a well-defined inter-disciplinary project. Thus, the trainees will be empowered to make informed decisions regarding the selections of supervisors and projects, either during the ACF scheme or during the MSD funded pre-fellowship scheme.

Start of fellowships

Fellows in the programme should commence their DPhil studies at the beginning of the academic year, as this will enhance integration and interactions within their cohort and also with the other DPhil schemes.

FORMAL TRAINING ACTIVITIES

- Specific induction courses for generic skills and access to organised MSc course modules for research specific training.
- Programmes in basic science environments.
- Academic Medical Forum, a weekly event where trainees and mentors assemble for a mixed programme of presentations by trainees of their research plans and progress.
- Interface with ACF scheme and opportunities for selection of research projects.
- Annual one day symposium for all Wellcome Trust - Funded students / fellows to promote integration and interactions.

The objectives of the formal training are to ensure that all fellows receive tuition in generic skills as well as research-specific training. The MSD has excellent basic and clinical facilities to enhance this training. The MSD provides: 1) a high quality, structured, training environment for fellows; 2) a systematic training programme which can be used by all clinical research training fellows; and 3) clinical and basic science facilities that enhance the training and research of the fellows.

Courses

Induction Courses consist of a series of introductory lectures on basic science, research techniques, statistics, and ethics; graduate training courses; core training programmes e.g. at the WIMM, and WTCHG; and MSc courses e.g. in Neuroscience, Applied Immunology, and Genome Medicine. In addition, there are currently in excess of thirty seminars per week with open access and an electronic collation of these academic activities.

Academic Medical Forum

There is a weekly meeting of the Academic Medicine Forum, which specifically addresses the needs of the trainees, and promotes the aims of the clinical fellowship programme. Senior scientists and clinicians across the campus attend the Forum to provide an overview of on-going work, and fellows present their research project and progress. This is designed to create a trainee-centred collegiate environment that promotes cross-disciplinary interactions amongst trainees, mentors and potential trainers.

Annual one day symposium

In order to facilitate interaction between, and integration with, other WT-funded programs and students, an annual, one-day symposium will be held for all WT-funded D.Phil students in Oxford. All WT Fellows will present their work in poster or oral form with their contributions evaluated and prizes awarded for the best presentations.

TIPS ON CHOOSING A PROJECT

1. Read through as many projects as you can, with an open mind. Please don't decide on too rigid a field of interest without first seeing what else may be available.

2. Make appointments to see the supervisors to discuss the projects in which you are particularly interested.

3. Always make sure you contact a supervisor to say whether you're still interested in their project or not.

4. Read the references and also around the topic of the project.

5. Allow plenty of time to enable you to complete the application form and write the proposal.

6. We are happy to consider additional projects that you may have developed in discussions with other researchers in the University. In this case, please submit the project details under one of the themes listed (i.e. cardiovascular, haematology, infection and immunity, metabolic medicine, and neurosciences) to the DTC (email: dtc@medsci.ox.ac.uk) for approval and inclusion by the relevant theme leader. To expedite this it would be useful for the proposed supervisor to discuss matters initially with the relevant theme leader.

All enquiries should be made through the DTC (email: dtc@medsci.ox.ac.uk).

ROLE OF DOCTORAL TRAINING CENTRE (DTC)

The Doctoral Training Centre has been set up specifically to support the six Wellcome Trust funded doctoral programmes within the Medical Sciences Division. The purpose of the DTC is to enhance the research training environment provided by departments by ensuring that training provision and access to relevant academic forums is maximised for these programmes. The aim is to provide an enriching academic culture in which networking, career development, and scientific cross-fertilisation will be positively promoted.

All applications and enquiries should be submitted to the DTC.

In addition, the DTC will:

1. Inform applicants when they have been short listed for an interview and provide details of the venue and time.

2. Inform candidates regarding the outcome of their application.

3. Provide guidance and details for registering with the University of Oxford D.Phil graduate studies programme.

4. Oversee the arrangements for obtaining honorary clinical contracts.

5. Arrange for affiliation with an Oxford college.

6. Ensure that all departments involved with the Fellows training are notified of the arrangements.

7. Monitor the fellows' progress by collection of termly reports and by arranging an annual meeting with the members of the management team.

8. Organise seminars and an annual day for the fellows.

9. Provide a range of training programmes to equip fellows with complementary skills for career progression.

10. Generally facilitate the administrative support for the fellow.

PROJECT DETAILS

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SECTION 4	-	Metabolic Medicine
SECTION 5	-	Neurosciences

SECTION 1

CARDIOVASCULAR

Project title: A novel signalling pathway controlling vascular development and remodelling

Basic Science Supervisor: Dr. A. Russ (andreas.russ@bioch.ox.ac.uk), Department of Biochemistry

Clinical Supervisor / Sponsor: Prof. Keith Channon **(**keith.channon@cardiov.ox.ac.uk), Department of Cardiovascular Medicine

Brief description: The molecular mechanisms controlling the development, maturation, and remodelling of the vascular system are of key interest in cardiovascular medicine and oncology. A number of signalling pathways has been implicated in the regulation of vasculogenesis and angiogenesis, most prominently receptor tyrosine kinase signalling and the Notch pathway. Several successful therapeutic strategies have been recently introduced into clinical practice based on these insights.

While there is a large body of knowledge about the mechanisms controlling the development of vascular endothelium, very little is known about the essential cell types constituting the vascular wall, especially vascular smooth muscle cells (vSMCs). Our laboratory has identified a novel signalling pathway that is essential for the development of vSMCs. GPR126 is an orphan receptor of the Adhesion-GPCR class. Adhesion-GPCRs are hybrid molecules that consist of a long N-terminal domain with similarity to molecules implicated in cell-cell or cell-matrix interactions, and a C-terminal domain similar to Class B G-protein coupled receptors. We have generated mice deficient for GPR126 by gene targeting in mouse embryonic stem cells, and the mutant animals die at mid-gestation due to defects in vascular maturation and remodelling.

Spontaneous expression of GPR126 is only detected very transiently during development, but can be induced in mature vessels. The main objective of this project is to investigate the requirement for GPR126 signalling in vascular repair and neovascularization in the adult organism, using conditional mutagenesis and other transgenic approaches. As GPR126 is amenable to therapeutic modulation via its GPCR domain, this work might define the receptor as a desirable drug target for cardiovascular disease or tumour angiogenesis.

Training opportunities: Molecular, Cell, and Developmental Biology, in particular embryonic stem cell technology, targeted mutagenesis, the construction of reporter transgenes, in vivo imaging of cells labelled with fluorescent proteins.

References:

Adams et al. Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol (2007) vol. 8 (6) pp. 464-78

Bjarnadóttir et al. The Adhesion GPCRs: A unique family of G protein-coupled receptors with important roles in both central and peripheral tissues. Cell Mol Life Sci (2007) pp. 1-13

Project title: Investigation of the role of a novel intermediate-filament protein, syncoilin, in the heart

Basic Science Supervisor: Prof. Kay Davies, kay.davies@dpag.ox.ac.uk, Department of Physiology, Anatomy and Genetics).

Clinical Supervisor: Prof. Hugh Watkins (hugh.watkins@cardiov.ox.ac.uk), Department of Cardiovascular Medicine

Brief description: We have identified an intermediate-filament like protein called syncoilin via its interaction with the dystrophin associated protein, α-dystrobrevin (1). We demonstrated that syncoilin binds the intermediate filament desmin which is an early marker of muscle development. The interaction of syncoilin with dystrobrevin and desmin provides an alternative link between the cytoskeleton and the dystrophin associated protein complex (DAPC) at the sarcolemma. There are desmin related myopathies but to date no myopathy has been associated with syncoilin. However the majority of desmin related myopathies arise without mutations in the desmin gene, indicating binding partners may be a contributing factor. Syncoilin is predominantly expressed in skeletal and cardiac muscle. In skeletal muscle it is found throughout the sarcolemma and Z lines but concentrated at the neuromuscular junction (NMJ). Its function in cardiac muscle is unknown. In the dystrobrevin null mutant mice, syncoilin localisation is retained but it is lost in desmin null mutant mice. Like desmin, syncoilin appears to be upregulated in some dystrophic muscle models (mdx, dko) and the levels parallel the severity of the dystrophy. In situ hybridization analysis of syncoilin in mouse embryos reveals ubiquitous muscle expression and unique expression in the dorsal root ganglion. These data suggest that syncoilin may have an important function in skeletal and cardiac muscle. The aim of the project would be to investigate the role of synoilin in cardiac muscle through the detailed analysis of animals who are null for syncoilin which we have generated.

Training opportunities: Techniques would involve immunocytochemistry, tissue culture, mass spectrometry and standard molecular genetic techniques. Training would also be provided in *in vivo* cardiac phenotyping so that the fellow could evaluate the impact of loss of syncoilin on whole heart function. If a cardiac muscle phenotpype is found, the potential also exists for human genetic analyses of syncoilin as a candidate gene for cardiomyopathy in man.

References:

(1) Poon E, Howman EV, Newey SE, Davies KE (2002) Association of syncoilin and desmin: linking intermediate filament proteins to the dystrophin-associated protein complex. Journal of Biological Chemistry 277: 3433-3439.

(2) McCullagh KJ, Edwards B, Poon E, Lovering RM, Paulin D, Davies KE (2007) Intermediate filament-like protein syncoilin in normal and myopathic striated muscle. Neuromuscular Disorders, in press.

Project title: Galectin-3 as an amplifier of inflammation in atherosclerosis

Basic Science Supervisor: David R. Greaves (david.greaves@path.ox.ac.uk) Sir William Dunn School of Pathology, South Parks Road, University of Oxford.

Clinical Supervisor: Professor Keith Channon (keith.channon@cardiov.ox.ac.uk) Department of Cardiovascular Medicine, John Radcliffe Hospital, University of Oxford

Brief description: Galectin-3 (Gal-3) is a 26kDa lectin known to regulate many aspects of inflammatory cell behaviour. We identified Gal-3 as a gene that is expressed at a high level in atherosclerotic plaques in human carotid arteries and in aortic tissue of apoE-/- knockout mice (1,2). *In vitro,* recombinant Gal-3 and conditioned media from Gal-3 treated human macrophages induced an up to 6-fold and 11- fold increase respectively in human monocyte chemotaxis. Microarray analysis of Gal-3 treated human macrophages and subsequent qRT-PCR validation confirmed the up-regulation of known macrophage chemoattractant molecules such as CCL5 (7.6-fold), CCL2 (27-fold) and CCL8 (22.4-fold, $P<0.01$, ANOVA) in response to Gal-3 treatment (2).

Original hypothesis Our data suggest that Gal-3 could serve as both a novel biomarker of atherosclerotic plaque progression and a therapeutic target in anti-inflammatory strategies for the treatment of atherosclerosis.

Experimental aims 1. We will measure Gal-3 levels and bioactivity in patient plasma and endarterectomy samples to see if Gal-3 levels correlate with known risk factors for cardiovascular disease or other markers of systemic inflammation (e.g. CRP, ESR, IL-6).

2. We will screen for anti Gal-3 monoclonal antibodies and develop dominant negative forms of the Gal-3 protein that block monocyte chemotaxis in vitro.

3. We will test the ability of Gal-3 blocking antibodies and mutant Gal-3-Fc fusion proteins to reduce monocyte recruitment and macrophage activation in well established models of chronic inflammation including the apoE-/- model of atherosclerosis (3).

Training opportunities: The project offers training in a wide range of techniques in cell and molecular biology (RT-PCR, chemotaxis, FACS analysis, Luminex, ELISA, cloning and transfection), and the opportunity to combine the use of transgenic animal models of disease with patient based studies of novel biomarkers of inflammation (4).

References: - publications by the group

1. Papaspyridonos, M., Smith, A., Burnand, K. G., Taylor, P., Padayachee, S., Suckling, K. E., James, C. H., Greaves, D. R., and Patel, L. (2006) *Arterioscler Thromb Vasc Biol*
2. Papaspyridonos, M., McNeill, E., de Bono, J. P., Smith, A., Burnand, K. G., Channon, K. M., and Greaves, D. R. (2007) *Arterioscler Thromb Vasc Biol*
3. Bursill, C. A., Choudhury, R. P., Ali, Z., Greaves, D. R., and Channon, K. M. (2004) *Circulation* **110**(16), 2460-2466
4. Greaves, D. R., and Channon, K. M. (2002) *Trends Immunol* **23**(11), 535-541

Title: Isolation, expansion and characterisation of **human** cardiac stem cells for clinical application

Basic Science Supervisor: Kieran Clarke (kieran.clarke@dpag.ox.ac.uk) Professor of Physiological Biochemistry, Dept of Physiology, Anatomy and Genetics, University of Oxford

Clinical Supervisor: David Taggart (david.taggart@orh.nhs.uk), Professor of Cardiovascular Surgery, University of Oxford

Brief description: Heart failure, not amenable to conventional pharmacologic or invasive therapies, is a rapidly growing problem in Western society and makes heavy demands on hospital and community health care resources during progression to terminal cardiac dysfunction and death [1]. Autologous bone marrow and peripheral blood stem cells have been employed therapeutically in an effort to generate new cardiac myocytes and, although their use appears to be safe, their clinical benefits have at best been marginal and/or short-lived [2] and their ability to differentiate into cardiomyocytes questioned. It is now possible to isolate a number of different stem/progenitor cells from the **human** as well as other mammalian hearts [3,4]. We propose to determine the therapeutic potential of human cardiospheres on cardiac function and morphology in the immunotolerant infarcted female rat heart, using non-invasive MRI. Cardiospheres will be developed from male human myocardial biopsies using techniques that are in routine use in Prof Clarke's laboratory [5]. Specific questions to be answered in this project include:

- determination of optimal culture conditions for formation of human cardiospheres
- use of iron oxide labelling for cell tracking and retention
- use of paraCEST (chemical exchange saturation transfer) agents to provide positive contrast (vs negative contrast with iron oxide particles), increasing sensitivity and giving quantitative measurements of cell engraftment and viability
- use of tissue scaffolds (such as Matrigel) to maximize graft enhancement
- engraftment success, infarct size, global and regional cardiac function and morphology will be followed non-invasively using *in vivo* MRI for 16 weeks
- hearts will be explanted for confocal microscopy, histology and immunohistochemistry (using monoclonal antibodies directed against desmin, α-actinin, troponin-I, α myosin heavy chain and connexion) to determine cardiosphere differentiation into endothelial, smooth muscle and cardiac-specific proteins
- cardiospheres will be tracked using MRI to detect iron oxide, fluorescent *in situ* hybridization (FISH) or rtPCR for the Y chromosome, and immunohistochemistry for GFP expression contraction analysis on donor cardiomyocytes re-isolated using an electromagnet

Training opportunities: The Fellow will learn a variety of techniques, including cell culture, methods required to culture and characterise human cardiospheres, histology, immunohistochemistry, FACS analysis, western blotting, and magnetic resonance techniques.

References:

1. Cleland J, et al. ESC Guidelines for the diagnosis and treatment of chronic heart failure. Eur Heart J 2005; 26: 1115-40.
2. Cleland JG, et al. Clinical trials update from the AHA: REPAIR-AMI, ASTAMI, JELIS, MEGA, REVIVE-II, SURVIVE, and PROACTIVE. Eur J Heart Fail. 2006;8:105-110.
3. Smith RR, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation. 2007;115:896-908.
4. Barile L, et al. Cardiac stem cells: isolation, expansion and experimental use for myocardial regeneration. Nat Clin Pract Cardiovasc Med. 2007 Feb;4 Suppl 1:S9-S14.
5. Stuckey DJ, et al, Clarke K. Iron particles for non-invasive monitoring of bone marrow stromal cell engraftment into, and isolation of viable engrafted donor cells from, the heart. Stem Cells 2006; 24: 1968-1975.

Project title: Ion channels, intracellular calcium signalling and endothelial cell function.

Basic Science Supervisor: Prof Anant B. Parekh (anant.parekh@dpag.ox.ac.uk) Department of Physiology, Anatomy and Genetics.

Clinical Supervisor: Prof Keith Channon (keith.channon@cardiov.ox.ac.uk) Department of Cardiovascular Medicine

Brief description:
Vascular endothelial cell function is an essential part of the cardiovascular system. Endothelial cells express calcium channels in the surface membrane and calcium flux through these channels regulates a range of important processes including the secretion of key signalling molecules, such as nitric oxide and endothelin, that regulate blood pressure. Aberrant calcium influx is linked to endothelial dysfunction. In spite of their importance, little is known about how these calcium channels are controlled, both acutely (channel activity) and in the long term (numbers of channels expressed). Here, using a combination of powerful cellular and molecular approaches (single cell calcium imaging, electrophysiology, gene silencing as well as overexpression) this proposal will examine how intracellular signals (protein kinases, phosphates, reactive oxygen species and nitric oxide) affect calcium channel activity, cytoplasmic calcium levels, nitric oxide production as well as calcium channel gene expression. Resolution of these issues is of fundamental importance to understanding endothelial cell function.

Training opportunities: Training in methods of molecular biology (gene expression, transfection, western blotting, siRNA), cell physiology (measurement of intracellular calciu concentration in living cells), electrophysiology (patch clamp recordings of ion channels in the plasma membrane).

References:
Bakowski, D. and Parekh, A. B. (2007). Current Biology 17, 1076-1081.
Chang, W-C., Nelson, C. and Parekh, A. B. (2006). FASEB Journal, 20, 2381-2383.
Parekh, A. B. (2006). Nature 441, 163-164.
Bendall et al. (2007). Circulation Research 100, 1016-1025.
Antoniades et al. (2006). Circulation 114, 1193-1201.

Project title: Development of cardiomyocytes from induced pluripotent stem (iPS) cells

Basic Science Supervisor: Dr C. Porcher (catherine.porcher@imm.ox.ac.uk) MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine.

Clinical Supervisor: Prof S. Bhattacharya (sbhattac@well.ox.ac.uk) Department of Cardiovascular Medicine, Wellcome Trust Centre for Human Genetics.

Brief description: Repairing damaged myocardium is one of the challenges of regenerative medicine. Recent advances in cellular reprogramming have provided molecular tools to derive pluripotent stem (iPS) cells from genetically unmodified mouse and human primary cells[1]. The aim of this project is to induce the differentiation of iPS cells to cardiac fates.

iPS cells will be first derived from mouse somatic cells upon retrovirus-mediated expression of defined factors (Oct4, Sox2 and Klf4) or from non-integrating viruses or transient episomes (when such protocols become available) in order to minimise genetic alterations in donor cells. Based on embryonic stem (ES) cell differentiation protocols, culture conditions (ie growth factor cocktails) will be developed to favour iPS cell differentiation towards cardiogenic mesoderm. If enrichment in functional mature cardiomyocytes is low, two distinct approaches will then be considered to induce differentiation to cardiac fates.

(i) There is increasing evidence of interplay between lineage-specific transcription factors during early embryogenesis. Based on our knowledge of the transcriptional networks engaged in cardiac lineage commitment and a large-scale gene expression analysis we recently performed that revealed a molecular interface between blood and heart development pathways, we will define a set of key transcription factors that specifically and efficiently induce cardiomyocyte development from iPS-derived cells.

(ii) In parallel, we will test known chemical compounds and develop high-throughput assays to identify small molecules that stimulate pathways involved in differentiation of stem cells towards cardiomyocytes.

The cardiomyocytes thus produced will be extensively characterised morphologically, molecularly and functionally in mouse models. Protocols will then be adapted to human cells. Our combined expertise in mouse ES cell biology[2,3], and blood and heart development[3-5] will ensure that the work progresses in a timely fashion.

In summary, this project will open up new avenues of investigation in heart muscle regeneration and contribute to deciphering some of the regulatory networks involved in cardiac development.

Training opportunities: The experimental work will provide training in basic and state-of-the-art methods in cellular and molecular biology as well as in bioinformatics; in addition, students will attend a mandatory course covering general methods and techniques. We hold extensive seminar series and weekly lab meetings/journal clubs. Opportunities to develop oral presentation skills will arise at our internal meetings. Participation at international meetings will be strongly encouraged.

References:

1- Park et al (2008) *Nature*, 451, 41-7 (and references therein).
2- Porcher et al (1996) *Cell*, 86, 47-57.
3- Porcher et al (1999) *Development*, 126, 4603-15.
4- Bamforth et al (2001) *Nat Genet*, 29, 469-74.
5- Bamforth et al (2004) *Nat Genet*, 36, 1189-96.

Project title: Iron and the integrative biology of the hypoxia-inducible factor pathway

Basic Science Supervisor: Prof. Peter A Robbins (peter.robbins@dpag.ox.ac.uk), Dept of Physiology, Anatomy & Genetics

Clinical Supervisor: Prof. Peter J Ratcliffe (pjr@well.ox.ac.uk), Nuffield Dept of Medicine

Brief description: Hypoxia-inducible factor (HIF) was first described as a transcription factor that increased the production of erythropoietin in response to hypoxia. Subsequently it was discovered that it was ubiquitously expressed in mammalian cells and that it was involved in the regulation of the expression of many other genes. As such, it has come to be seen as a master controller of the coordinated cellular response to hypoxia. Over the past decade the HIF regulatory pathway has been defined in much more detail. Of particular interest is that the abundance of HIF is regulated by hydroxylation of specific proline residues, which tag HIF for recognition by the von Hippel-Lindau tumour suppressor protein and then subsequent ubiquitination and proteosomal degradation. This hydroxylation step, catalysed by specific prolyl hydroxylase enzymes, confers the oxygen sensitivity on the pathway and, crucially for this project, is very sensitive to the level of free iron.

Investigation of the phenotype associated with systemic abnormalities of the HIF pathway has been undertaken in genetically-altered mice, and these studies have shown that HIF has a major regulatory role at the integrative level on both the cardiovascular and respiratory systems – the very systems on which cellular oxygen delivery ultimately depends. We have made similar observations in humans with Chuvash Polycythemia, a rare genetic disease producing systemic alterations in HIF under conditions of normal oxygen tension. Clonal abnormalities of the HIF pathway have also been implicated in a number of inherited cancer syndromes, and alterations in HIF abundance within tumours are strongly predictive of clinical outcome.

This particular project is based on some pilot observations that altering iron status in humans can substantially modify integrative human responses to hypoxia. The project will explore this observation much more fully, both by manipulating iron status through iron chelation and iron infusion in normal volunteers, and by studying patients with altered iron status or who may be otherwise informative. Depending on outcomes, there may also be an opportunity to explore interactions between iron status and the effects of exposure to high altitude through collaborations in Peru. Although the HIF-system is a potentially important therapeutic target, there are as yet no licensed therapies for its manipulation. In part, this project will help to assess whether careful manipulation of iron status could play any role in the clinical management of hypoxic patients.

Training opportunities: This project provides an opportunity for training in modern techniques for studying integrated responses in humans, both normal volunteers and patients. At Oxford, there is a large multidisciplinary grouping of scientists interested in hypoxia, and the fellow would become part of this grouping. The grouping has weekly scientific meetings at which the fellow would be expected to attend and to present their findings. These meetings aid the intellectual development of the fellow by providing awareness and understanding of a broader field of endeavour within the biology of hypoxia, together with an effective platform for more multidisciplinary collaboration.

References:

Schofield, CJ & Ratcliffe, PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5: 343-354, 2004.
Smith, TG et al. Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. PLoS Med 3: e290, 2006.

Project title: Antisense oligonucleotide exon skipping for the correction of cardiac dystrophin expression in Duchenne muscular dystrophy

Basic Science Supervisor: Dr Matthew Wood (matthew.wood@dpag.ox.ac.uk), Department of Physiology, Anatomy and Genetics).

Clinical Supervisor: Prof. Hugh Watkins (hugh.watkins@cardiov.ox.ac.uk), Department of Cardiovascular Medicine

Brief description: Duchene muscular dystrophy (DMD) is an X-linked muscle disorder mainly caused by nonsense or frame-shift mutations in the dystrophin gene. DMD patients suffer from severe, progressive muscle wasting which also affects cardiac muscle leading to cardiomyopathy. Heart disease in DMD can contribute to both morbidity and mortality. Becker muscular dystrophy (BMD), a milder allelic variant, is usually caused by in-frame deletions resulting in expression of a shortened, but partially functional dystrophin protein. The fact that some dystrophin domains are dispensable for its function has prompted attempts to alter pre-mRNA splicing as a means of genetic correction for DMD. Antisense oligonucleotide (AO)-mediated exon skipping has been shown to restore the open reading-frame of dystrophin, allowing the generation of partly functional protein in mdx mice (an animal model of DMD) and in myoblasts derived from DMD patients. Encouraging results have come from the first AO DMD clinical trial in the Netherlands. Currently the UK MDEX Consortium, of which my group is part, is conducting the first UK AO clinical trial for DMD.

Despite this progress, significant hurdles remain for the successful development of AO therapy in DMD. These include very poor AO delivery to cardiac muscle. This project will therefore investigate a range of novel approaches to enhance AO delivery to heart in animal models of DMD. Specifically the Clinical Fellow will;

- Investigate novel AO chemistries optimised for vascular stability and cardiac delivery
- Screen peptide and glycoconjugate ligand libraries, using phage display and other methods, to identify cardiac-specific ligands. These will be conjugated directly to AOs for further study
- Evaluate the utility of novel AO delivery formulations based on enhanced cardiac delivery through glucose uptake pathways. The mechanism of action will also be characterised further.

Training opportunities: Training will be provided in antisense oligonucleotide chemistry and RNA biology, cell culture, nucleic acid delivery systems, molecular analysis and pathological studies in mouse models.

References:

1. van Deutekom JC et al. Local dystrophin restoration with antisense oligonucleotide PRO051.N Engl J Med. 2007 Dec 27;357(26):2677-86.
2. van Deutekom JC, van Ommen GJ. Advances in Duchenne muscular dystrophy gene therapy. Nat Rev Genet. 2003 Oct;4(10):774-83.
3. Yin H, Lu Q, Wood M. Effective Exon Skipping and Restoration of Dystrophin Expression by Peptide Nucleic Acid Antisense Oligonucleotides in mdx Mice. Mol Ther. 2008 Jan;16(1):38-45.
4. Alter J et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat Med. 2006 Feb;12(2):175-7.
5. Lu QL et al. Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles. Proc Natl Acad Sci U S A. 2005 Jan 4;102(1):198-203.

SECTION 2

HAEMATOLOGY

Project title: Novel molecular regulators of stem cell numbers in the haematopoietic system

Basic Science Supervisors: Profs Tariq Enver, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine (tariq.enver@imm.ox.ac.uk) and Roger Patient, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine (roger.patient@imm.ox.ac.uk)

Clinical Mentor: Dr Paresh Vyas, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine and Department of Haematology John Radcliffe Hospital. (paresh.vyas@imm.ox.ac.uk)

Brief description: Haematopoietic stem cells (HSCs) are responsible for the continuous daily production of all mature blood cell lineages. HSCs are functionally defined at the single cell level by their dual capacity for self-renewal and multipotential differentiation. HSC self-renewal divisions may be symmetric or asymmetric. In symmetric self renewal both daughter cells retain stem cell properties. This type of cell division expands the stem cell pool, and is therefore thought to be important after transplantation or following haematopoietic injury. In asymmetric self-renewal, one daughter retains stem cell properties while the other differentiates. This allows for steady state maintenance of the HSC compartment. Regulating the balance of symmetric versus asymmetric divisions could afford amplification of stem cells and may be important in targeting cancer stem cells where this balance may be dysregulated. As part of a high throughput drug screen, initially in fruit flies, we have identified candidate small molecules that alter the balance of symmetric versus asymmetric divisions in both neural and gut stem cells. Testing in the vertebrate zebrafish model identified a subset with activity on HSCs. These will now be tested for ability to amplify mammalian (mouse and human) HSCs and their mechanism of action deconvoluted using the power of reverse genetics in zebrafish. We anticipate that these studies will illuminate how normal cell fate decisions are instigated, and inform approaches aimed at manipulating the expansion, directed-differentiation or reprogramming of stem cell fate for therapeutic or commercial advantage.

Training opportunities: Training in stem cell isolation culture and genetic manipulation, and in experimental transplantation. Training in developmental biology and genetic manipulation of zebrafish embryos. Training in bioinformatic and experimental approaches to molecular pathway deconvolution.

References:

1. Tsuzuki S, Hong D, Gupta R, Matsuo K, Seto M, Enver T (2007) Isoform-specific potentiation of stem and progenitor cell engraftment by AML1/RUNX1 *PLoS Med* 4(5), e172.
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Project title: Cancer stem cells in Acute Myeloid Leukaemia (AML) and Myelodysplasia (MDS)

Basic Science Supervisor: Professor Tariq Enver, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine (tariq.enver@imm.ox.ac.uk)

Clinical Supervisor: Dr Paresh Vyas, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine and Department of Haematology John Radcliffe Hospital. (paresh.vyas@imm.ox.ac.uk)

Brief description: Leukaemia, like other cancers, is sustained by a population of cancer stem cells (CSCs)[1,2]. CSCs arise from acquired genetic mutations and epigenetic changes that cause abnormal self-renewal and impaired differentiation. CSCs can arise from either normal haemopoietic stem cells or by reprogramming of more committed normal haemopoietic progenitors. CSC are important because (i) CSC are likely to be the key cellular source of relapse (ii) Failure to eradicate CSC is likely to provide an early cellular marker of treatment failure (iii) if we understand the molecular programs that control CSC growth, it may provide novel opportunities to target therapy.

Plan of investigation Building on our previous studies of molecular and cellular basis of abnormal haemopoiesis in both Acute Lymphoblastic Leukaemia[3,4]and MDS[5], we now focus on dissecting the abnormal molecular programmes critical for deregulated CSCs self-renewal/differentiation in AML, especially AML preceded by MDS, which is particularly hard to cure. We have established a programme of collecting bone marrow samples from a) A cross-section of patients with high risk MDS and AML. b) Samples from the same individuals with high risk MDS that progress to AML – this will allow us to study different stages of the disease. In this project the candidate will use FACS sorting techniques to prospectively isolate disease stem and progenitor cells and xenograft assays to characterise CSCs. The candidate will aim to identify the key molecular abnormalities in CSC self renewal/differentiation through gene expression profiling, analysis of gene mutations by high throughput sequencing and survey of epigenetic marks by chromatin immunoprecipitation and bisulphite sequencing. An important training component here is to work with the Computational Biology Research Group in the Weatherall Institute of Molecular Medicine to analyse the large data sets that will be generated by this work.

Training opportunities: Training in methods of molecular cellular biology of cancer and leukaemia in particular. This will include: a) Cell biology - cell culture, haemopoietic progenitor assays, FACS analysis, isolating stem cell enriched populations by FACS sorting and live cell imaging. b) Molecular biology - PCR, DNA/RNA extraction, gene expression microarray and high throughput DNA sequence analysis c) Protein analysis - Western blot analysis. d) Use of mouse models. e) Bioinformatics - computer programs for sequence analysis, protein and EST database searches.

References:

1. Clarke, M.F et al. *Cancer Res*. 66(19): p. 9339-44 (2006).
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3. Eguchi, M et al. *Proc Natl Acad Sci U S A*. 102(4): p. 1133-8 (2005).
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5. Sternberg, A et al. *Blood*. 106(9): p. 2982-91 (2005).

Project title: Epigenetic Programmes in Haematopoietic Differentiation

Basic Science Supervisors: Professor Douglas Higgs, MRC Molecular Haematology Unit Weatherall Institute of Molecular Medicine, University of Oxford (doug.higgs@imm.ox.ac.uk) and Professor Nick Proudfoot Dunn School of Pathology, University of Oxford (nicholas.proudfoot@path.ox.ac.uk).

Clinical Mentor: Dr Richard Gibbons Department of Clinical Genetics and the Nuffield Department of Clinical Sciences, University of Oxford (rgibbons@gwmail.jr2.ox.ac.uk)

Brief Description: The Polycomb group of proteins provide a mechanism for epigenetic memory during differentiation and development. First discovered in Drosophila, where they are required for silencing of homeotic gene expression, polycomb proteins have since been found to play a key role in mammalian development and the maintenance of stem cell identity. Their disregulation is associated with aberrant gene expression in a range of malignancies. The general aim of the project is to study the function of Polycomb group proteins at a well defined mammalian locus, the alpha globin gene cluster, in order to establish general principles of Polycomb function in mammals. In embryonic stem cells several hundred genes are bound by Polycomb. During development, removal of Polycomb is essential for activation of lineage-specific genes. The mechanisms governing this removal in mammals remain obscure. The specific goal of the project is to study this process at the alpha globin gene locus. This locus has been the subject of intense investigation by the host laboratory and is consequently amongst the best characterised mammalian gene clusters. The host laboratory has shown that Polycomb proteins bind to regions of the alpha globin cluster silencing expression in embryonic stem and non-erythroid cells but in differentiated erythroid cells binding is absent. The first aim of the project is to establish the timing of Polycomb removal during erythroid differentiation. We will isolate by FACS populations enriched in haematopoietic stem cells and various progenitor populations from primary bone marrow samples. The presence of Polycomb at the alpha globin locus will be determined by chromatin immunoprecipitation (ChIP) and real time PCR. Current ChIP protocols require a minimum of 10^6 cells, however only 10^3 to 10^5 cells of each progenitor population can be obtained from a single bone marrow sample. Therefore we will employ a novel ChIP method in which Drosophila cells are added as a 'Carrier'. This technique can reliably analyse as few as 100-1000 cells. The second aim of the project is to determine the sequences required for Polycomb recruitment and for removal during differentiation. We will employ mononucleosomal ChIP and a tiled array to determine Polycomb binding at single nucleosome resolution across the alpha globin locus. We will also characterise Polycomb binding in erythroid cell lines (interspecies hybrids) containing deletions of previously identified cis-regulatory regions and of the alpha globin promoters. We hope to discover general principles governing removal of Polycomb during mammalian development.

Training Opportunities: Training in basic molecular and cellular biology. Particular emphasis will be placed on the analysis of epigenetic analysis including DNA methylation and chromatin modification. In addition this work will involve the analysis of microarray platforms and high throughput sequence analysis. In addition the programme will involve training in the purification and evaluation of haematopoietic progenitors.

References:

1. Anguita E, Hughes J, Heyworth C, Blobel GA, Wood WG, Higgs DR. Embo J 2004;23(14):2841-52.
2. De Gobbi M, Anguita E, Hughes J, et al. Blood 2007;110:4503-10.
3. Vernimmen D, De Gobbi M, Sloane-Stanley JA, Wood WG, Higgs DR. Embo J 2007;26(8):2041-51.
4. Wallace HA, Marques-Kranc F, Richardson M, et al. Cell 2007;128(1):197-209.

Project title: Haemopoietic Defect in Myelodysplasia (MDS)

Basic Science Supervisor: Professor Sten Eirik Jacobsen, Haematopoietic Stem Cell Laboratory, Weatherall Institute of Molecular Medicine (sten.jacobsen@imm.ox.ac.uk)

Clinical Supervisor: Dr Paresh Vyas, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine and Department of Haematology John Radcliffe Hospital (paresh.vyas@imm.ox.ac.uk).

Brief description: *Background* There is a clinical need to identify the molecular and cellular basis of clonal MDS as a) there are few effective disease modifying therapies and b) ~30% cases progress to Acute Myeloid Leukaemia (AML). Even though MDS and AML are heterogeneous disorders we have hypothesised that there may be common pathogenetic haemopoietic abnormalities amongst apparently disparate patients that result in bone marrow proliferation and dysplasia. Previously, using a combination of cutting edge techniques, including isolation of stem-cells and gene expression profiling, we showed that MDS is a true stem cell disorder[1,2] and that there is a specific abnormality at the very earliest stages of myeloid-lymphoid differentiation[3].

Plan of investigation We will build on our previous work to define the molecular programs that regulate the earliest steps in normal human myeloid-versus-lymphoid differentiation, which are still unclear. We will then determine which of these programs is aberrant in MDS and how these programs are further corrupted in the transition to AML. To address these questions we have established the first national NCRN badged bone marrow sample collection programme from a) A cross-section of patients with high risk MDS and AML and b) The same individuals with high risk MDS that progress to AML – to allow us to study different stages of the disease. These samples are banked and associated with a comprehensive clinical database. In this project the candidate will use state-of-the-art FACS sorting techniques, previously used to identify novel normal and leukemic stem cells [4,5] to prospectively isolate normal and disease stem and early myeloid and lymphoid progenitor cells and use multiple assays to characterise their cell fate potential, including their ability to reconstitute normal haematopoiesis and MDS in vivo, using immune-deficient mouse models. The candidate will identify the key molecular abnormalities in self renewal/differentiation through gene expression profiling, analysis of gene mutations by high throughput sequencing and survey of epigenetic marks by chromatin immunoprecipitation and bisulphite sequencing. An important training component here is to work with the Computational Biology Research Group in the Weatherall Institute of Molecular Medicine to analyse the large data sets that will be generated by this work.

Training opportunities: Training in methods of molecular and cellular biology of cancer and leukaemia in particular. This will include: a) Cell biology - cell culture, haemopoietic progenitor assays, FACS analysis, isolating stem cell enriched populations by FACS sorting and live cell imaging. b) Molecular biology - PCR, DNA/RNA extraction, gene expression microarray and high throughput DNA sequence analysis c) Protein analysis - Western blot analysis. d) Use of mouse models. e) Bioinformatics - computer programs for sequence analysis, protein and EST database searches.

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2. Nilsson, L., et al. *Blood*. **110**(8): p. 3005-14 (**2007**).
3. Sternberg, A., et al. *Blood*. **106**(9): p. 2982-91 (**2005**).
4. Adolfsson, J., et al. *Cell*. **121**(2): p. 295-306 (**2005**).
5. Castor, A., et al. *Nat Med*. **11**(6): p. 630-7 (**2005**).

Project Title: Functions of cohesin during cell division and development

Basic Science supervisor: Kim Nasmyth (Kim.Nasmyth@bioch.ox.ac.uk) Department of Biochemistry.

Clinical Supervisor: Doug Higgs (doug.higgs@imm.ox.ac.uk).

Brief description: Sister chromatid cohesion is essential for chromosome segregation and is mediated by a multi-subunit complex called cohesin, which contains four core subunits: Smc1, Smc3, Scc1/Rad21, and Scc3/SA. Association of cohesin with chromatin and establishment of sister chromatid cohesion during S phase requires a second complex composed of Scc2 and Scc4 proteins. Sister chromatid disjunction at the metaphase to anaphase transition is triggered by cleavage of Scc1 by a thiol protease called separase. Cohesin's Smc1 and Smc3 both form rod shaped molecules. Each polypeptide folds back onto itself at a central "hinge" domain, forming 50 nm long intra-molecular antiparallel coiled-coils. This brings globular N- and C-terminal domains together to build an ABC-like ATPase "head" domain at one end of their coiled coils. At the other end, their hinge acts as a heterotypic dimerization domain used to form V-shaped Smc1/Smc3 heterodimers. The ATPases at the apices of cohesin's V are connected by Scc1, whose N- and C-terminal domains bind to Smc3 and Smc1 ATPase heads, respectively, thereby forming a huge tripartite ring, within which sister DNAs might be trapped. By severing Scc1's connection of cohesin's ATPase heads, separase is envisioned to open the ring and thereby release sister DNAs from their topological embrace. Remarkably, cohesin is associated with unreplicated chromatin in quiescent cells in metazoa (including all neurons) and has important non-mitotic functions, presumably in regulating gene expression. Patients with Cornelia de Lange syndrome, which is associated with pleiotropic developmental defects (including mental retardation, abnormal limb development, cardiac and gut defects), are known to possess either missense mutations in cohesin's Smc proteins or mutations of just one copy of the human ortholog of Scc2, known as Nipbl. Cells from these patients do not appear to have defective sister chromatid cohesion and it is thought that the syndrome is caused by misregulated gene expression rather than mitotic errors. The lab can offer projects in three areas.

1) The biochemical mechanism by which cohesin holds sister DNAs together. How, for example are sister DNAs trapped by the cohesin ring. This work is largely done using the budding yeast Saccharomyces cerevisiae as a model system but there are possibilities of working with mice.
2) How sister chromatid cohesion is regulated during meiosis. The cohesion that holds bivalent chromosomes together during meiosis I in mammalian oocytes is presumably generated at birth at the time of pre-meiotic DNA replication. Yet, in humans, oocytes do not undergo the first meiotic division until menstruation, which can be several decades later. Can cohesion be repaired during this period and might failures in maintaining sister chromatid cohesion contribute to age related infertility in females and to trisomy 21?
3) What genes are regulated by cohesin during development and how does cohesin perform this function? At the moment this work uses the fruit fly D.melanogaster as a model system, but we are starting to develop techniques to investigate non-mitotic cohesin functions in mice.

Training: How to think straight and pose important and answerable questions and how to use techniques from structural biology to mouse genetics to answer them.

References

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Project title: Defining the molecular basis of DNA Double strand break repair

Basic Science Supervisor: N. D. Lakin (nicholas.lakin@bioch.ox.ac.uk) and C. J. Pears (catherine.pears@bioch.ox.ac.uk), Department of Biochemistry

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: The genome is under continuous assault by agents that cause DNA damage. The cell has therefore evolved pathways that detect DNA damage for processing and repair. Defects in DNA repair pathways results in a variety of clinical symptoms including an increased predisposition to cancer. Understanding these pathways will provide insights into the molecular basis of cancer and uncover targets for diagnosis and treatment of this disease.

DNA double strand breaks (DSBs) can be repaired by two distinct but complementary pathways, homologous recombination and non-homologous end joining (NHEJ). The accuracy of these repair pathways is vital to maintain genome integrity and to protect against cancer. The analysis of DNA DSB repair in genetic model organisms has been vital in establishing how these pathways function in humans. However, in certain instances this approach has been hampered by the lack of human DNA repair proteins in genetically tractable organisms. For example, despite the sequencing of the entire *S. cerevisiae* and *C. elegans* genomes, no orthologues of the human NHEJ factors Artemis and DNA-PKcs have been identified in these organisms.

Recently we identified a functional orthologue of DNA-PKcs in the genetically tractable amoeba *Dictyostelium*. The aim of this research is to further exploit *Dictyostelium* to study NHEJ and therefore provide insights into this pathway in human cells. Analysis of the DNA end processing events that facilitate re-joining of non-compatible and/or blocked DNA termini *in vivo* has not been forthcoming. We have identified an orthologue of the human DNA end-processing factor Artemis in *Dictyostelium* and found that in addition to being involved in NHEJ, this protein is also required to repair more complex DNA damage architectures such as DNA inter-strand cross links (ICLs) which are generated by commonly used chemotherapeutic drugs such as cisplatin. The aim of this research is to assess how Artemis processes DNA termini and ICLs and identify novel components of the NHEJ pathway. Specifically it is proposed to: 1) Generate *Dictyostelium* cells defective in Artemis and other DNA processing factors and assess their requirement for processing a variety of different DNA termini to facilitate NHEJ. 2) Express and purify *Dictyostelium* and human Artemis and compare the processing of substrates *in vitro*. 3) Perform genetic screens that will identify novel factors required to process complex DNA termini to facilitate NHEJ. 4) Determine the role of these novel factors in NHEJ in human cells.

Training opportunities: Training in molecular and cell biology techniques will be provided including recombinant DNA technology, targeted gene disruption, PCR, Southern, northern and western blotting and analysis of signalling events using phospho-specific antibodies. Training will also be provided in cell culture, expression and purification of recombinant proteins, and cell based assays to assess the efficiency of DNA repair *in vivo*.

References:

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Project title: Selective killing of tumour cells

Basic Science Supervisor: Dr. L. S. Cox (lynne.cox@bioch.ox.ac.uk Department of Biochemistry).

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: Cancer is a multi-step disease resulting from loss of function of tumour suppressor genes and gain of function of oncogenes. The WRN helicase/exonuclease, which is mutated in the premature human ageing Werner's syndrome (WS) is an important tumour cell survival factor, and RNAi-mediated knockdown of WRN leads to apoptosis of a wide variety of neoplastic cells in culture, whilst WRN loss in normal cells leads to their senescence. As such, WRN inhibition or ablation may provide a route for selective killing of tumour cells.

We have recently generated several reagents suitable for reducing or inhibiting WRN activity in cells, based on WRN-directed microRNAi, and peptides that interact with either the exonuclease or helicase domain of WRN. The proposed project will investigate the impact of regulated loss of WRN function using these reagents. The microRNAi constructs will be cloned into inducible vectors for expression in mammalian cells, and used to knock down WRN protein levels in a regulated manner. Synthetic peptides that interact with WRN enzymatic domains will be optimised for *in vitro* inhibition of WRN, using highly sensitive time resolved fluorescence assays to monitor inhibition of recombinant WRN helicase or exonuclease, and informed by molecular modelling of interactions with WRN. Active peptides and inactive controls will then coupled with fluorescently-tagged penetratin to drive their uptake into cells. The possible outcomes of cell survival, proliferation, senescence or apoptosis will be measured upon WRN knockdown/ inhibition in a range of transformed and closely matched primary cells in culture. Molecular pathways known to be altered in Werner's syndrome will be studied in the experimentally WRN-depleted cells, including homologous DNA recombination, DNA replication, DNA damage (via markers such as γH2AX), and chromatin redistribution. It is anticipated that any miRNAi or peptides showing anti-tumour activity in this project will subsequently be pursued as models for rational design of small molecule inhibitors of WRN with value in cancer chemotherapy.

Training opportunities: Training in methods of molecular and cell biology. This will include generating novel RNAi expression vectors by restriction enzyme-based cloning and PCR techniques, *in vitro* enzyme analysis, cell culture, immunofluorescence microscopy, Western blotting, fluorescence activated cell sorting, and the use of bioinformatics to model molecular interactions.

References: (publications from the group)

1. Rodriguez-Lopez AM et al. (2007) Correction of Proliferation and Drug Sensitivity Defects in the Progeroid Werner's Syndrome by Holliday Junction Resolution. *Rejuvenation Research* 10 (1): 27-40

2. Cox, LS et al (2007) Modeling Werner's syndrome in *Drosophila melanogaster*: hyper-recombination in flies lacking WRN-like exonuclease. *Ann N Y Acad Sci.* 1119: 274-88

3. Cox LS, Faragher RG. (2007) From old organisms to new molecules: integrative biology and therapeutic targets in accelerated human ageing. *Cell Mol Life Sci.* 64(19-20): 2620-41.

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5. Rodríguez-López, AM et al. (2002) Asymmetry of DNA replication fork progression in Werner's syndrome. *Ageing Cell* 1: 30-39

Project title: Molecular mechanisms of DNA interstrand crosslink repair

Basic Science Supervisor: Prof. M. C. Whitby (matthew.whitby@bioch.ox.ac.uk) Department of Biochemistry

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: DNA interstrand crosslinks (ICLs) are highly cytotoxic and genotoxic lesions, which block both DNA replication and transcription. A variety of agents, including certain endogenously produced metabolites, induce ICLs, and some (e.g. psoralens, cis-platinum, and mitomycin C) are used in cancer chemotherapy. The repair of ICLs requires the interplay of several different DNA repair pathways (nucleotide excision repair, homologous recombination and translesion synthesis). However, the molecular details of how these consort to achieve ICL repair has not been worked out. Recently it was shown that the human Mus81-Eme1 endonuclease cleaves replication forks that are stalled at ICLs, and that this likely initiates repair. It was also suggested recently that the DNA helicase/translocase FANCM together with FAAP24 act as a sensor of stalled replication forks, and possibly remodels them to allow their processing. FANCM is encoded by one of 13 so-called *FANC* genes, any one of which, if mutated, can result in the rare autosomal recessive disorder Fanconi Anemia (FA) that is characterised by elevated rates of cancer and a cellular hypersensitivity to ICL-inducing agents.

The aim of this project is to investigate the interplay between Mus81-Eme1 and FANCM during ICL repair in the model eukaryote *Schizosaccharomyces pombe* (fission yeast), which has the benefit of relatively fast and facile genetic manipulation. Specifically, the involvement of the homologues of Mus81-Eme1 and FANCM in ICL repair in *S. pombe* will be confirmed by genotoxin sensitivity assays using mitomycin C and cis-platinum, and by monitoring DSB formation following ICL-induction using pulse field gel electrophoresis (PFGE). Indirect immunofluorescence and chromatin immunoprecipitation (ChIP) will then be used to investigate whether *S. pombe* Mus81-Eme1 and FANCM target stalled replication forks *in vivo*, and what the genetic requirements for this are. Finally, an *in vitro* assay will be established to investigate whether *S. pombe* FANCM can directly bind and remodel replication forks, and whether this results in a stimulation of Mus81-Eme1 cleavage activity.

Training opportunities: Training in methods of yeast genetics, standard molecular cloning techniques, microscopy and imaging, and protein purification and characterisation.

References:

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3. Whitby, M.C., *Junctions on the road to cancer.* Nat Struct Mol Biol, 2004. **11**: p. 693-695.
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5. Ciccia, A., et al., *Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM.* Mol Cell., 2007. **25**: p. 331-343.

Project title: Identification of novel epigenetic regulators in embryonic stem cells

Basic Science Supervisor: Prof. Neil Brockdorff (neil.brockdorff@bioch.ox.ac.uk). Department of Biochemistry, University of Oxford.

Clinical Supervisor: Prof D. R. Higgs (Doug.higgs@imm.ox.ac.uk), MRC Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital.
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description:

Mouse embryonic stem (ES) cells can give rise to all of the different cell lineages in an adult animal, a property termed pluripotency. As such the genome of ES cells exhibits a unique plasticity, allowing master regulatory genes required to establish different lineages to be expressed in response to specific cues. It has been shown that the promoters of many lineage determining genes have a unique form of chromatin packaging in ES cells. The core histones, around which DNA is wrapped, are marked by post-translational modifications normally associated with gene activity as well as those normally associated with gene silencing. This unique chromatin configuration has been termed bivalency. A recent study has demonstrated that in ES cells the transcription machinery is loaded onto the promoters of bivalent genes but that a specific repressive histone modification, ubiquitination of histone H2A, mediated by repressor complexes belonging to the Polycomb family, holds the RNA polymerase in a poised configuration.

The mechanism by which Polycomb repressors are recruited to the promoters of bivalent genes is unknown and this question forms the major focus of the project. The proposal is to identify novel factors involved in recruiting Polycomb repressors using RNA interference (RNAi) mediated genetic screening. Libraries of RNAi constructs directed towards all of the genes in the mouse genome are available and can be packaged in lentivirus and transduced into ES cells. The reporter for the screen will be produced by inserting a gene encoding a fluorescent protein marker, for example GFP and/or an antibiotic resistance gene under the control of the promoter of a bivalent gene(s). This will be achieved using homologous recombination in ES cells, the so called knock-in approach. Following transduction of the RNAi library, cells will be selected for based on GFP fluorescence and/or antibiotic resistance, thereby enriching for cells expressing RNAi constructs directed at factors required for repression of bivalent genes. The genes targeted by the RNAi constructs can be readily identified using conventional sequencing or microarray based approaches. Novel factors identified in this screen can be studied in detail with a view to further understand the mechanism of bivalency in ES cells.

Training opportunities:

The applicant will have the opportunity to learn a range of techniques and methods including gene-targeting in ES cells, use of RNAi libraries, and microarray approaches.

References:

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2. De Napoles, M., et al (2004). Polycomb-group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. *Dev. Cell* 7; 663-676.

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Project title: Maintenance of genome integrity during DNA replication

Basic Science Supervisor: N. D. Lakin (nicholas.lakin@bioch.ox.ac.uk), Department of Biochemistry

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: Fanconi anemia (FA) is a rare recessive disorder characterized by developmental abnormalities, progressive failure of the bone marrow and an increased risk for cancer. Many of the genes affected in FA patients have been identified and shown to be involved in repair of DNA damage as cells undergo DNA synthesis. However, the molecular basis of how FA proteins function during DNA replication remains unclear. In addition to providing insights into how genome stability is maintained during DNA synthesis, unravelling FA protein function will increase our understanding of the molecular basis of FA and provide possible targets of diagnostic and therapeutic value for cancer treatment.

Cells derived from FA patients display sensitivity to agents that interfere with DNA replication, including those that cause DNA interstrand cross-links (ICLs). FA proteins are recruited to sites of DNA damage in response to genotoxic stress and the FA pathway becomes activated specifically during S-phase in response to ICLs and other varieties of DNA damage. However, the specific DNA architectures and genomic loci that FA proteins recognize and the role of these proteins in regulating S-phase progression remain unclear. Recently our laboratory has developed chromatin immunoprecipitation (ChIP) technology to assess the recruitment of cell cycle checkpoint proteins to stalled replication forks. The aims of this research are to exploit this and other technologies to assess recruitment of FA proteins to sites of genotoxic stress encountered during S-phase and to establish how FA proteins contribute to processing and restart of stalled replication forks. This will be achieved by several approaches. 1) ChIP will be employed to quantitatively assess binding of FA proteins to stalled replication forks and DNA ICLs. Patient derived cell lines defective in components of the FA and other DNA damage signalling pathways will be used to assess the genetic requirements for these events. 2) These studies will be extended to establish whether FA proteins are recruited to sites of the human genome that are inherently difficult to replicate (fragile sites). 3) DNA fibre technology will be employed to assess the role of FA proteins in restart of DNA replication forks after they encounter and process sites of genotoxic stress.

Training opportunities: Training will be provided in a variety of biochemical, cellular and molecular biology techniques. This will include maintenance and manipulation of mammalian cell cultures, siRNA technology, chromatin immunoprecipitation, immunofluorescence microscopy and use of phospho-specific antibodies to assess cell-signalling events. Molecular biology training will include recombinant DNA technology, expression of recombinant proteins in bacterial and mammalian systems and real time PCR.

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Project title: Cellular and molecular pathways of haematopoietic stem cell self renewal and lineage commitment

Basic Science Supervisors: Professor Roger Patient, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine (roger.patient@imm.ox.ac.uk) and Professor Sten Eirik Jacobsen, Haematopoietic Stem Cell Laboratory, Weatherall Institute of Molecular Medicine (sten.jacobsen@imm.ox.ac.uk)

Clinical Mentor: Dr Paresh Vyas, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine and Department of Haematology John Radcliffe Hospital (paresh.vyas@imm.ox.ac.uk)

Brief description: Understanding how haematopoietic stem cells (HSC) choose between lineage commitment and self renewal is of key importance for promoting desirable cell fate options for clinical application (stem cell expansion and directed differentiation) and understanding leukaemia. Using non-mammalian model systems the Patient group has made key contributions to our understanding of the development of HSCs (1,2). The Jacobsen group, using mouse models and human progenitors, have identified alternative cellular pathways for the earliest lineage commitment steps of HSCs (3,4) and identified regulators of HSCs (5). This project will study lineage commitment and self renewal of HSCs at the single cell level. Using transgenic zebrafish lines expressing fluorescent reporters under the control of HSC genes, coupled with laser excitation in single cells, we will exploit the optical transparency of zebrafish embryos to follow the fates of individual HSCs as they emerge in the dorsal aorta. Existing evidence suggests that some of the population undergo differentiation while others become stabilised in the stem cell state and self renew. The fate of HSCs will also be traced in equivalent transgenic mouse lines as well as in cell culture. The fish and mouse lines will be crossed with lines carrying gain or loss of function mutations in key regulatory pathways to establish the molecular regulation of lineage commitment versus self renewal of HSCs. Through these approaches we hope to identify the signals that determine whether HSCs self renew or differentiate, which might be perturbed in leukaemia.

Training opportunities: Training with genetically modified zebrafish and mouse models, as well as using viral mediated vector overexpression and knock-down strategies in purified mouse HSCs. In vivo and in vitro imaging and advanced cell sorting, and cell tracing at the single cell level of the offspring of HSC divisions.

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Project title: Transcriptional regulation of GATA1 and its relevance to human MDS/AML.

Basic Science Supervisor: Professor Roger Patient and Dr Paresh Vyas, MRC Molecular Haematology Unit Weatherall Institute of Molecular Medicine (roger.patient@imm.ox.ac.uk)

Clinical Supervisor: Dr Paresh Vyas, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine and Department of Haematology John Radcliffe Hospital (paresh.vyas@imm.ox.ac.uk)

Brief description: *Background and Aims* Normal stem/progenitor cells can remain quiescent, self-renew, undergo apoptosis or proliferate and this ultimately leads to uni-lineage differentiation and cell cycle exit. When these processes go awry it can lead to diseases like leukaemia. An important class of regulators of blood cell fate are haemopoietic transcription factors (TFs)[1]. TFs are nuclear proteins that coordinate expression of large numbers of genes. The frequent finding of mutations in TFs in patients with cytopenias and leukaemia underlines their importance. An example of such a TF is GATA1, a myeloid TF critically required for normal erythroid and megakaryocyte (EM) differentiation (reviewed in[2]). Germ line GATA1 mutations in patients result in anaemia/thrombocytopenia; acquired GATA1 mutations are required event for myeloid leukaemia in children with Down Syndrome[3].

Sustained GATA1 expression promotes EM differentiation. Conversely, repression of GATA1 expression has to occur to allow granulocyte monocyte (GM) differentiation. Thus it is critical to regulate the precise level and spatio-temporal pattern of GATA1 expression; it determines myeloid lineage output. The aim of project is two-fold. First, to determine how GATA1 transcription is controlled? This will provide fundamental insight into control of normal myelopoiesis. Second, to determine if mutations in DNA sequences TFs that regulate GATA1 cause human myeloid disease, especially MDS and AML?

Plan of investigation Over the last 10 years, we have identified the DNA sequences, changes in chromatin structure at the GATA1 locus and some of the TF that regulate GATA1 expression[4,5]. However, we do not know the full complement of TFs that control GATA1 expression. The applicant will use gain-of-function (i.e. ectopically express proteins) and loss of function (i.e. knock down expression of proteins) screens in Xenopus and Zebrafish to identify transcriptional regulators of GATA1, in an unbiased manner. We use these model organisms as they allow high throughput analysis of regulators that may affect GATA1 expression. Experiments will then be performed to determine how proteins identified in these screens regulate GATA1 – e.g. are they TF that directly control GATA1 expression? In addition, the applicant will use high throughput sequencing to look for mutations in DNA sequences and in the genes of the proteins that control GATA1 expression in MDS and AML.

Training opportunities: Training in: a) Molecular biology - PCR, DNA/RNA/protein analysis, analysis of binding of transcription factors/cofactors and chromatin marks by chromatin immunoprecipitation, high throughput sequencing. b) Use of Xenopus/Zebrafish/tissue culture c) FACS analysis and sorting, haemopoietic progenitor assays and live cell imaging d) Bioinformatics - computer programs for genome analysis of chromatin immunoprecipitation and gene expression data.

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Project title: Histone deacetylase inhibitors in cancer therapy

Basic Science Supervisor: Catherine Pears (Catherine.pears@bioch.ox.ac.uk) and Louis Mahadevan (louis.mahadevan@bioch.ox.ac.uk), Department of Biochemistry
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.
Brief description: Background. DNA is packaged into chromatin which plays an important role in regulating the accessibility of the DNA to a variety of proteins such as those involved in gene expression. Specific modification of chromatin-associated histone proteins is associated with the transcriptional state of the associated genes. Alterations in the acetylation of histone proteins (especially acetylation of lysine9 on histone H3) during tumour formation have been linked mechanistically to the pathogenesis of cancer: the enzymes catalysing the removal of the acetyl groups (HDACs) have been found to be overexpressed and mutated in cancer cells and changes in histone acetylation patterns linked with disease progression. Small molecule inhibitors of HDACs (HDACis) have been shown to achieve significant biological effects in growth inhibition of preclinical models of cancer. Initial clinical trials using these inhibitors have shown promising effects. However results many of these drugs have toxic side effects and the development of resistance to HDACi growth inhibition is a problem. It is not clear that HDACs are the primary target of all of these drugs, and the pathway leading to tumour growth inhibition is not understood, precluding the development of more effective compounds.

A number of HDACis which are currently in clinical trials cause growth inhibition of a genetically tractable eukaryotic organism, *Dictyostelium discoideum*. This organism shows patterns of histone modification similar to those found in mammalian cells, but the ease of genetic manipulation allows rapid screening for resistant mutants and a pilot screen has successfully identified a mutant resistant to a number of HDACis, and in which histone modification is altered.

Aims and Plan of investigation. 1. To screen a library of randomly generated *Dictyostelium* mutants for resistance to the inhibition of growth by HDACis currently under consideration for clinical use. 2. To identify and characterise the genes mutated in these strains (including that already identified) by molecular genetic methods 3. To characterise the molecular mechanisms by which gene disruption leads to HDACi-resistance. This would involve characterising the consequences of gene disruption on a range of histone modifications in the presence or absence of HDACis, identifying consequent changes in gene expression profile and characterising the interaction partners and the molecular action of the proteins encoded by the disrupted genes in order to gain insight into mechanisms of HDACi action and resistance. 4. To identify homologous genes in mammalian cells and characterise their role in histone modification and in resistance to HDACis using over expression and siRNA technology.

Training opportunities:
Training will be provided in methods of molecular biology and genetics. This will include use of PCR, DNA sequence analysis, techniques to probe chromatin structure such as chromatin immunoprecipitation (ChIP) with modification-specific antisera, use of genetic model system, creation of null strains by homologous recombination, siRNA technology
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Project Title: A Study of Epigenetic Changes in FOXP3 in Regulatory T cells and Potential Clinical Application in Allogeneic Bone Marrow Transplantation

Basic Science Supervisor: Prof David Roberts, Nuffield Department of Clinical Laboratory Sciences, Level 2, John Radcliffe Hospital, Oxford (david.roberts@ndcls.ox.ac.uk)

Clinical Mentor: Dr Andy Peniket, Consultant Haematologist, Department of Haematology, John Radcliffe Hospital, Oxford (andy.peniket@orh.nhs.uk)

Brief Description: Allogeneic Bone Marrow Transplantation (BMT) is a well-established treatment for haematological malignancies. Its success depends on donor T-cells mediating a Graft-versus-Tumour (GvT) effect. Unfortunately, these cells can also cause significant morbidity and mortality via Graft-versus-Host Disease (GvHD). A deficiency in Regulatory T-cells (Tregs) appears to be involved in the pathogenesis of GvHD (1). Tregs are a subpopulation of $CD4^+$ T cells which maintain self-tolerance and prevent autoimmunity. They have potent suppressive activity, inhibiting proliferation and cytokine secretion of conventional $CD4^+$ and $CD8^+$ effector T-cells (2). Expression of forkhead box transcription factor FOXP3, is critically important for the development and function of Tregs (3). Intermediate levels of FOXP3 can also be transiently up-regulated in some activated conventional T cells (Tcon), conferring suppressive activity (4). Analysis of the FOXP3 promoter has identified several NF-AT and AP-1 binding sites which positively regulate FOXP3 expression after T cell stimulation (5). Epigenetic control may also be important with demethylation of two regions of the human FOXP3 locus, TSDR and CAMTA1, identified in natural Tregs compared to naïve T cells, activated Tcon or TGFβ induced Tregs (6). Acetylation of several regions of murine Foxp3 is required for optimal Treg function, binding to the IL2 promoter and suppressing Il2 production and histone deacetylase inhibitors (HDACi) enhance the number and function of Foxp3 Tregs (7). In this project, the candidate will study the epigenetic control of human FOXP3 both *in vitro* and *in vivo*. These changes will be studied both in healthy individuals and in patients undergoing Allogeneic BMT and correlated to the development of GvHD. The candidate will elucidate whether the epigenetic changes in FOXP3 can be modulated using demethylating agents (Decitabine and Azacitidine) or HDACi and if this can used to expand Tregs to control GvHD. Ethical approval to study Tregs and GvHD in Allogeneic BMT patients has already been obtained.

Training opportunities: Training in methods of a) Cell biology: Cell culture, FACS analysis, isolating Tregs by FACS sorting and magnetic beads, cell proliferation assays, cytokine assays (ELISA and FACS beads array); b) Molecular biology - DNA/RNA extraction, PCR, methylation and acetylation studies, gene expression microarray. Molecular studies of methylation status will be undertaken in collaboration with Prof Doug Higgs, WIMM, University of Oxford; c) Protein analysis - Western blot analysis; d) Bioinformatics.

Candidate: Dr Danby has been working as a Haematology Specialist Registrar in Oxford since February 2004 and has obtained Part1 MRCPath. He has a research based First Class Honours BSc degree and is widely considered as one of the outstanding registrars in Oxford. He was recently appointed as the Oxford Bone Marrow Transplant Research Fellow and has established an extensive clinical database and attained Oxford Research Ethics approval to study Allogeneic BMT patients.

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SECTION 3

INFECTION AND IMMUNITY

Project title: Immune selection of persistent viruses within populations; 1. HIV and HLA

Basic Science Supervisor: Angela McLean, Department of Zoology ,
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Clinical Supervisor: Philip Goulder, Peter Medawar Building for Pathogen Research
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Brief description: One of the major issues relating to HIV is the capacity to escape host adaptive immune responses through mutation. This is true for both antibody mediated responses and T cell mediated responses. The impact of such immunologic selection pressure can be seen at the level of the individual, studied over time, first described for HIV over 15 years ago. Recent studies using much larger cohorts of patients have shown that immunological "footprints" may also be seen at the population level. What is not yet known is how such mutations will persist or spread further within populations as a whole, and also what is the overall impact of such mutation on disease progression. This is because in making mutations which allow immune escape, HIV may suffer a fitness cost. The balance of fitness cost vs immune escape advantage will impact on disease progression within the individual and persistence of the mutations within specific populations.

The aim of the project in the Medawar Building/Zoology is to explore the host virus interaction in HIV to define the footprints of immune responses and relate this to disease outcome. The impact of key HLA molecules such as B57, B58 and B51 will be evaluated. This project will comprise 3 key components:
Immunological analyses. The key cohorts for HIV studies include a major effort in South Africa comprising a well-established study in Durban, KwaZulu Natal; these include a major paediatric component. Immunologic analyses include HLA Class I tetramer analyses, and large scale analyses of T cell responses using ELISpot and flow cytometry techniques.
Virological analyses. This is based around clonal sequence analysis and bulk sequence analyses of virus circulating in specific cohorts. Whole genome analyses are now possible. Specific mutations can be introduced into viral backbones to assess viral fitness costs directly in vitro.
Bioinformatics and modelling. We will use a range of statistical approaches to address the phylogenetic relationships of the viruses within hosts and populations. Prof. McLean's group have developed novel models for exploring the relationships between T cell responses and specific HLA types and viral variants within populations.

Training opportunities: The project will give the student the opportunity to explore cutting edge techniques in immunology, molecular biology, virology, epidemiology and bioinformatics/statistical modelling. The project can be weighted towards any aspect of these according to the talents/interests of the successful student.

References: - publications by the group

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Project title: Immune selection of persistent viruses within populations; 1. HCV and HLA

Basic Science Supervisor: Angela McLean, Department of Zoology ,
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Clinical Supervisor: Paul Klenerman, Peter Medawar Building for Pathogen Research
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Brief description: As in Medawar projects 1 and 3, for HIV, one of the major issues relating to hepatitis C virus (HCV) is the capacity to escape host adaptive immune responses through mutation. This is true for both antibody mediated responses and T cell mediated responses. The impact of such immunologic selection pressure can be seen at the level of the individual, studied over time. As for HIV, recent studies using much larger cohorts of patients have shown that immunological "footprints" may also be seen at the population level. What is not yet known is how such mutations will persist or spread further within populations as a whole, and also what is the overall impact of such mutation on disease progression. This is because in making mutations which allow immune escape, HCV may suffer a fitness cost. The balance of fitness cost vs immune escape advantage will impact on disease progression within the individual and persistence of the mutations within specific populations.

The aim of the project in the Medawar Building/Zoology is to explore the host virus interaction in HCV to define the footprints of immune responses and relate this to disease outcome. The impact of key HLA molecules such as A3 and B27 will be evaluated. This project will comprise 3 key components:
Immunological analyses. The key cohorts for HCV studies include an important single source outbreak in Ireland; cohorts of acutely infected donors, and a large cohort of chronically infected donors. An important set of donors with HCV/HIV co-infection are of major interest. We are using novel tetramer techniques ("magic tetramers") to explore the quality of T cell responses over time.
Virological analyses. This is based around clonal sequence analysis and bulk sequence analyses of virus circulating in specific cohorts. Whole genome analyses are now possible depending on the project. Specific mutations can now be introduced into viral backbones to assess viral fitness costs directly in vitro.
Bioinformatics and modelling. We will use a range of statistical approaches to address the phylogenetic relationships of the viruses within hosts and populations. Prof. McLean's group have developed novel models for exploring the relationships between T cell responses and specific HLA types and viral variants within populations.

Training opportunities: The project will give the student the opportunity to explore cutting edge techniques in immunology, molecular biology, virology, epidemiology and bioinformatics/statistical modelling. The project can be weighted towards any aspect of these according to the talents/interests of the successful student.

References: - publications by the group

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Project title: Immune selection of persistent viruses within populations; 2. Acute HIV infection

Basic Science Supervisor: Angela McLean, Department of Zoology ,
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Clinical Supervisor: Rodney Phillips, Peter Medawar Building for Pathogen Research
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Brief description: As described for project 1, one of the major issues relating to HIV is the capacity to escape host adaptive immune responses through mutation. This is true for both antibody mediated responses and T cell mediated responses. The impact of such immunologic selection pressure can be seen at the level of the individual, studied over time, first described for HIV over 15 years ago by this group (Phillips et al Nature, 1991, 354; 453-9). Recent studies using much larger cohorts of patients have shown that immunological "footprints" may also be seen at the population level. What is not yet known is how such mutations will persist or spread further within populations as a whole, and also what is the overall impact of such mutation on disease progression. This is because in making mutations which allow immune escape, HIV may suffer a fitness cost. The Phillips group is focusing on the evolution of such mutations in acute disease and the transmission patterns of mutations within large populations

The aims of the projects in the Medawar Building/Zoology are to explore the host virus interaction in HIV to define the footprints of immune responses and relate this to disease outcome. As for project 1 the impact of key HLA molecules (e.g. B51) will be evaluated, and the project will comprise 3 key components:

Immunological analyses. The key cohorts for HIV studies include a major international study of acute HIV infection, through which over 500 patients have been sampled; these include patients in the UK and South Africa. Immunologic analyses include HLA Class I tetramer analyses, including novel techniques ("magic tetramers') to evaluate T cell quality.

Virological analyses. This is based around clonal sequence analysis and bulk sequence analyses of virus circulating in specific cohorts. Whole genome analyses are now possible depending on the project.

Bioinformatics and modelling. We will use a range of statistical approaches to address the phylogenetic relationships of the viruses within hosts and populations. Prof. Mclean's group have developed novel models for exploring the relationships between T cell responses and specific HLA types and viral variants within populations.

Training opportunities: The project will give the student the opportunity to explore cutting edge techniques in immunology, molecular biology, virology and bioinformatics/statistical modelling. The project can be weighted towards any aspect of these according to the talents/interests of the successful student.

References: – publications by the group

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Project title: Chromosome Processing in Health and Disease.

Basic Science Supervisor: Professor DJ Sherratt FRS (david.sherratt@bioch.ox.ac.uk) Department of Biochemistry.

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: The faithful replication of the genetic material and its efficient transmission to daughter cells underlies the life process. Impairment of replication, recombination, repair or transmission to daughter cells, comprises life and leads to genetic instability and subsequent pathologies. The molecular mechanisms that act in these processes are conserved from bacteria to Man, and most of the mechanistic detail arises from studies of bacteria and yeast. In the Sherratt laboratory, studies of chromosome biology use the bacterium *Escherichia coli* not only because it is a superb model system, but because bacteria are hugely important not only in environmental bioconversions, but in their association with Man, in health and disease. Human bacterial disease is one of the huge challenges of the 21st century. Interdisciplinary work in the laboratory is uncovering how the processes of DNA replication, recombination and chromosome segregation shape bacterial chromosome organization, and how impairment of these processes will lead to genetic instability. There are research opportunities, in several areas of this research, all of which require the exploitation of a range of interdisciplinary techniques.

Training opportunities: Training in the methods and philosophy of molecular biology and genetics, biochemistry, cell biology and computational biology. These will include, in addition to standard techniques, the use of high resolution imaging in live cells to visualise genetic loci and molecular machines. Furthermore, techniques for the very rapid depletion of specific proteins in living cells and the consequent assessment of phenotype changes will be developed and used. There is also the opportunity to use single molecule methods to unravel the processes that assemble, drive and disassemble molecular machines.

References: (publications from the group)

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Project title: Molecular mechanisms of regulatory RNAs

Basic Science Supervisor: Dr James Parker (james.parker@bioch.ox.ac.uk), MRC Career Development Fellow, Department of Biochemistry

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: Short regulatory RNAs hold promise as tools to treat disease via selective knockdown of gene expression. Small interfering RNAs (siRNAs) are now widely used to selectively modulate gene expression in tissue culture in the laboratory. The power and versatility of this technique indicates a potential for effectiveness in humans. Proof-of-principle experiments demonstrate success in the treatment of some respiratory infections and sexually diseases, where the siRNAs can be applied directly to mucosal surfaces. The real excitement stems from the ability to selectively target any gene – if the sequence is known – thereby providing some lead against currently-untreatable disorders (such as genetic neurodegenerative diseases like Huntingdon's disease, amyotrophic lateral sclerosis and spinocerebellar ataxia type 1), in addition to providing new potential strategies against almost any viral infection.

To improve and optimise the effectiveness of the regulatory RNA strategy it is essential to understand the molecular bases for these mechanisms. My laboratory uses structural biology and biochemistry to analyse, understand and manipulate molecular machinery, focusing on components of the regulatory RNA pathways. We are interested in two principal systems: 1) the "Slicer" enzyme – otherwise known as "Argonaute" – that binds the "guide RNA" derived from the siRNA and targets the messenger RNA (mRNA) for degradation, and 2) the microRNA (miRNA) transporter complex (whose main component is the nuclear transporter Exportin-5) that is responsible for escorting miRNAs and small hairpin RNAs (shRNAs) from the nucleus where they are synthesized, to the cytoplasm where they function.

The project will involve a study of one of these two systems principally via structural biology. We will use molecular biology to generate expression clones for components of these systems, which will be expressed in heterologous hosts (either *E. coli* or insect cell culture). The proteins will be purified using standard techniques. Once isolated, the proteins will be mixed with siRNAs to form multi-component complexes that represent the functional molecular machines inside the cell. We will then analyse the molecular structures of these machines via either X-ray crystallography or single particle electron microscopy, techniques in which our laboratory specialises. The structures will reveal the molecular mechanisms of these systems and will be interpreted in the light of our current understanding of regulatory RNAs and siRNA function.

Training opportunities: The techniques of structural biology. This will involve molecular biology, protein purification and biochemistry, formation of molecular complexes, protein crystallisation, X-ray crystallography, electron microscopy and structure interpretation.

References:

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3) Raoul, C. *et al*. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat Med* **11**, 423-8 (2005).
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Project title: Structure-based drug design of potential autoimmune therapeutics

Basic Science Supervisor: Dr. Jim McDonnell (jim@biop.ox.ac.uk, Department of Biochemistry)

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description:

CD21 (or complement receptor 2) is a B-cell surface protein that acts as a receptor for cleaved fragments of the C3 component of complement. C3 is a principal component of the complement system and is central to all three complement pathways. Activation of C3 results in inflammation and elimination of self and non-self targets. The proteolytic fragment C3d acts in the inductive phase of the antibody response by covalently reacting with antigen via a thioester and then binding to CD21 on B cells. Mice deficient for the genes encoding CD21 or C3 exhibit significant impairment of antibody generation and a variety of T-cell dependent immune responses. The chronic inflammatory disease rheumatoid arthritis is correlated with local complement activation. C3 knockout mice display an almost complete absence of clinical symptoms in the collagen-induced arthritis (CIA) model system. Moreover, a critical role for the C3d/CD21 interaction has been demonstrated for the initiation of inflammatory arthritis in the CIA system, and has been indicated in several other autoimmune disorders.

We have been investigating CD21's role in B cell activation and its contribution to immunological responses via its interaction with a variety of ligands. Using NMR spectroscopy, we have recently elucidated the molecular interaction surfaces between C3d and CD21. These studies have identified a promising "druggable site" on C3d. Compounds that inhibit this interaction would be powerful and specific immunosuppressants. We are currently applying molecular and structural methods to develop compounds that bind to this site and disrupt the C3d/CD21 interaction, with the long term goal of developing novel immunotherapeutic compounds.

Training opportunities: Training in the methods of modern molecular and structural biology. These will include: cloning and expression of recombinant target proteins, *in silico* drug screening techniques, high throughput screening methods, and high resolution structural techniques (X-ray crystallography and NMR spectroscopy) for characterizing lead compounds.

References: (relevant publications from the group)

Hibbert *et al.* (2005) The structure of soluble CD23 and its interactions with IgE and CD21. **Journal of Experimental Medicine** 202:751-760.

Noble *et al* (2005) Exploiting structural principles to design cyclin-dependent kinase inhibitors. **Biochemica et Biophysica Acta** 1754:58-64.

Price *et al.* (2005) The role of protein flexibility in modulating IgE interactions. **Journal of Biological Chemistry** 280:2324-2330.

Eivazova *et al.* (2000) Cross-reactivity of anti-idiotypic antibodies with DNA in systemic lupus erythematosus. **Arthritis and Rheumatism** 43:429-439.

McDonnell *et al.* (1999) Solution structure of the pro-apoptotic molecule BID: a structural basis for apoptotic agonism and antagonism. **Cell** 96:625-634.

Project title: Innate immunity to bacterial infection in the nematode *C. elegans*

Basic Science Supervisor: Professor Jonathan Hodgkin (jonathan.hodgkin@bioch.ox.ac.uk)
Department of Biochemistry.

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description:

The nematode *Caenorhabditis elegans* is a major model organism, widely used for analysing biomedical problems, with many powerful experimental advantages. It provides a convenient system for examining processes of bacterial infection and resistance, in particular for studying innate immunity to pathogens. Infection by a variety of bacterial species, including a number of important human pathogens (e.g. *Salmonella enterica, Staphylococcus aureus, Enterococcus faecalis*) induces the production of antibacterial compounds such as lysozymes, antibacterial peptides and other candidate defense proteins. Processes of infection, pathogen detection, innate immune signalling, production and mechanism of defense proteins are under investigation, using a combination of forward and reverse genetic approaches, as well as genomic analysis.

The project will be directed at understanding the function and transcriptional induction of defense genes after infection. One set of such genes encode lysozymes, which are induced in pathogen-specific and tissue-specific patterns, as a result of a combination of signalling inputs. Another, much larger set of genes (>250 in the *C. elegans* genome) encode proteins with C-type lectin domains (*clec* genes). Each bacterial pathogen appears to induce a distinct set of multiple *clec* genes, and RNAi knockdown experiments show that at least some of these genes make significant contributions to defense. Their mechanism of action is currently unknown and may be novel. During the project, the function and expression of selected *clec* and lysozyme genes will analysed in detail. The complete genome sequence of *C. elegans* and related nematodes permits rapid investigation of transcriptional promoters, both by genomic comparison and by experimentation using transgenesis and genetic background manipulation. Expression of selected proteins in vitro will be used to test antibacterial function and other properties. Results will shed light on innate immunity pathways and novel antibacterial effectors.

Training opportunities: The project will provide experience in methods of molecular genetics (transcriptional promoter dissection, transgenesis), genetic analysis, genomics, in vitro protein expression, microbiology and nematology.

References:

1. Gravato-Nobre MJ, Hodgkin J (2005) *Caenorhabditis elegans* as a model for innate immunity to pathogens. Cell Microbiol. 7:741-751.
2. Mylonakis E, Aballay A (2005) Worms and flies as genetically tractable animal models to study host-pathogen interactions. Infect Immun. 73:3833-3841.
3. Nicholas HR, Hodgkin J (2004) The ERK MAP kinase cascade mediates tail swelling and a protective response to rectal infection in *C. elegans*. Current Biology **14:** 1256 –1261.
4. O'Rourke D, Baban D, Demidova M, Mott R, Hodgkin J (2006) Genomic clusters, putative pathogen recognition molecules and antimicrobial genes are induced by infection of *C. elegans* with *M. nematophilum*. Genome Research 16: 1005-1016.
5. Yook K, Hodgkin J (2007) Mos1 mutagenesis reveals a diversity of mechanisms affecting response of *C. elegans* to the bacterial pathogen *M. nematophilum*. Genetics 175: 681- 697.

Project title: Molecular Modelling and Simulation of Cell Membrane Systems

Basic Science Supervisor:
Prof. Mark Sansom, Structural Bioinformatics and Computational Biochemistry Unit, Department of Biochemistry, South Parks Road, OX1 3QU.
e-mail: mark.sansom@bioch.ox.ac.uk; phone: (2)75371; website: http://sbcb.bioch.ox.ac.uk
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.
Brief description:

Research in my group is concerned with multi-scale modelling of biomolecular systems, with a particular emphasis on membrane systems. This is relevant to signalling and transport in bacterial and mammalian systems, and has important biomedical implications, as 50% of drug targets are membrane proteins. The development of a computational systems approach is required as membrane proteins do not operate in isolation, but interact with their surrounding lipid bilayer environment and with other proteins in order to bring about their biological effects.

Dynamic interactions of proteins with membranes play a key role in many cellular processes. For example membrane fusion events are involved in release of neurotransmitters at synapses, and in the entry of enveloped viruses (e.g. influenza, HIV) into target cells. Structural biology continues to provide information on the proteins involved in such events. Cell biology and biophysical studies provide information on more dynamic aspects. However, in addition to these experimental approaches, there is a pressing need for a computational approach to membrane dynamics to enable integration of disparate data. Current work in MSPS's group has established a coarse-grained molecular dynamics approach which enables simulation of large scale membrane/protein events on a ~1 μsec timescale). A multi-scale approach enables accurate computational modelling of dynamic cell membrane processes and builds upon current developments in the physical sciences where multi-scale approaches are being used for soft matter and materials simulations.

An overview of research and people in my group can be found at: **http://sbcb.bioch.ox.ac.uk**
The following is a short list of possible projects. However, other projects may be possible.

1. Multi-scale modelling of PTEN, a membrane-associated tumour suppressor protein
2. Anti-cancer peptide interactions with cell membranes
3. Monotopic membrane proteins: multi-scale modelling of key drug targets

Training opportunities:

From a training perspective the project will provide experience in a wide range of computational and theoretical skills as applied to biological molecules and systems. These will include: biomolecular simulations, elastic network and/or finite element modelling, scientific programming and scripting (perl, python etc), use of parallel and distributed computing resources, and statistical analysis of data.

References:

1. Bond, P. J., and Sansom, M. S. P. (2007) Bilayer deformation by the Kv channel voltage sensor domain revealed by self-assembly simulations. *Proc. Natl. Acad. Sci. USA 104*, 2631-2636.
2. Bond, P. J., Derrick, J. P., and Sansom, M. S. P. (2007) Membrane simulations of OpcA: gating in the loops? *Biophys. J. 92*, L23-L25.
3. Deol, S. S., Domene, C., Bond, P. J., and Sansom, M. S. P. (2006) Anionic phospholipids interactions with the potassium channel KcsA: simulation studies. *Biophys. J. 90*, 822-830.
4. Fowler, P.W., Balali-Mood, K., Deol, S., Coveney, P.V. and Sansom, M.S.P. (2007) Monotopic enzymes and lipid bilayers: a comparative study. *Biochem.* 46: 3108-3115
5. Bond, P.J., Holyoake, J., Ivetac, A., Khalid, S., and Sansom, M.S.P. (2007) Coarse-grained molecular dynamics simulations of membrane proteins and peptides *J. Struct. Biol.* 157:593-605

Project title: HIV – breaking self tolerance
Basic Science Supervisor: Dr Nicole Zitzmann (nicole.zitzmann@bioch.ox.ac.uk) / Dr Chris Scanlan (chris.scanlan@bioch.ox.ac.uk), Department of Biochemistry, University of Oxford
Clinical Supervisor: Prof. Andrew McMichael (andrew.mcmichael@ndm.ox.ac.uk), Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital,

Brief description: The antigenic surfaces of viruses, prokaryotes, and eukaryotes are covered by 'shields' of specific polysaccharides or glycoconjugates whose diversity is driven by antigenic co-evolution between host and pathogen. For this reason, the humoral immune system is highly effective at discriminating self carbohydrates from non-self ones. However, in the case of viruses such as HIV, the relationship between host and pathogen is profoundly subverted: the carbohydrates on the pathogen are themselves synthesized by the host. Immune tolerance to these structures prevents an efficient anti-carbohydrate antibody response to the viral surface. However, in at least one known case, the adaptive immune system appears to have evolved a solution to this viral defence mechanism. A rare, protective, neutralizing anti-HIV antibody, IgG 2G12, has been isolated from HIV infected patient. Such antibodies protect against HIV infection by recognition of the "self" glycans of HIV envelope glycoprotein, gp120. We have previously characterised the 2G12 epitope on gp120, revealing that the carbohydrate neutralisation site on gp120 is formed from a set of mannose residues found within an unusually dense cluster of oligomannose glycans on the viral surface. To gain a better understanding of how immune tolerance to viral sugars is established and broken, we are designing and testing immunogens aimed at overcoming tolerance to the self glycans of HIV-1. There are three specific projects:

1. We have recently identified naturally occurring, immunogenic polysaccharides, from unrelated microbial sources (*Candida albicans, Klebsiella pneumoniae, Serratia marascens*), which share remarkable molecular mimicry with the HIV envelope. Antigenic and structural characterisation of these antigens, combined with immunisation trials, will reveal the extent of antigenic cross-reactivity between HIV-1 and these highly immunogenic microbial epitopes. The immune responses to these pathogens will be examined for protection against HIV infection.
2. We have established a chemo-enzymatic route to the creation of antigenic variants of gp120 (i.e. "host") sugars by introducing non-natural sugars (such as rhamnose or xylose) into the oligomannose structures found on gp120. Preliminary data indicate that this approach is a direct route to modifying the antigenic shield on HIV. These immunogenic motifs, within an immunologically self scaffold, will be tested to determine whether these modifications are, in themselves, sufficient to break tolerance.
3. Evidence from glycan microarray analysis suggests a structural/conformational basis for discrimination of non-self glycans. (Thus the Manα1-2Manα1-xMan neutralisation motif is highly antigenic when in the context of some pathogenic antigens such as mannan polysaccharides, but not within the context of self glycans such as the oligomannose structures found on HIV-1). NMR studies of self and non-self mannosides are exploring the molecular basis for this observation.

Training opportunities: Training in virology and glycobiology. Depending on the project, this may include cell culture, methods for antigenic characterisation, NMR spectroscopy The candidate will be linked to the translational HIV vaccine programme led by Professor McMichael in the Weaterhall Institue of Molecular Medicine.

References:
Scanlan, C.N. *et al.,* J Mol Biol. 2007 Sep 7;372(1):16-22.
Scanlan, C.N. *et al.,* Nature. 2007 Apr 26;446(7139):1038-45.
Lee, H.K., *et al.,* Angew Chem Int Ed Engl. 2004 Feb 13;43(8):1000-3.
Calarese, D.A., *et al.,* Science. 2003 Jun 27;300(5628):2065-71.
Scanlan, C.N., *et al.,* J Virol. 2002 Jul;76(14):7306-21.

SECTION 4

METABOLIC MEDICINE

Project title: New tumour suppressors in *C. elegans*

Basic Science Supervisor: Dr. Alison Woollard (alison.woollard@bioch.ox.ac.uk)
Biochemistry Department
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: In order to understand carcinogenesis, a thorough understanding of the molecular controls that balance cell proliferation and differentiation are necessary. Runx/CBFβ transcriptional complexes have been shown to have central roles in this process and have been postulated to act both as tumour suppressors and oncogenes. For example, both gain and loss of *Runx1* function has been associated with various leukaemias and *Runx3* is frequently deleted or silenced in gastric cancer. However, it is a tough challenge to rationalise the seemingly contradictory effects of Runx/CBFβ in carcinogenesis. Much of the current data relies on analyses of cell populations and tissues *in vitro*, making interpretation difficult. Model organisms such as Drosophila and *C. elegans* provide powerful systems for the *in vivo* analysis of gene function. In *C. elegans* in particular, the invariant lineage presents a unique platform for the study of cell proliferation at single cell resolution, and the presence of solo Runx and CBFβ homologues in this organism provides further opportunities to exploit this highly tractable system without complications caused by genetic redundancy.

This project will exploit the powerful *C. elegans* model system for studying how Runx/CBFβ transcriptional complexes help regulate the balance between cell proliferation and differentiation. Previous work from my group has highlighted the importance of two genes, *rnt-1* and *bro-1* (homologues of mammalian Runx and CBFβ, respectively) in controlling cell proliferation during *C. elegans* epithelial development. Mutations in either one of these genes causes failures of particular cell divisions, whereas over-expression of either gene causes epithelial hyperplasia, resulting in a tumour-like appearance. Excitingly, our preliminary data suggest that expressing mammalian Runx/CBFβ genes in *C. elegans* also causes hyperplasia, thus underpinning the likely conservation of Runx/CBFβ function.

RNT-1/BRO-1 induced tissue hyperplasia provides a unique system in which to dissect the molecular mechanisms by which these genes normally control the balance between cell proliferation and differentiation. Animals over-expressing RNT-1/BRO-1 have a distinctive appearance, caused by the hyperplasia, and do not move or thrive well. This makes them ideal candidates for suppressor screens, the aim of which is to identify genes that prevent tumour formation when inactivated. We propose a genome-wide RNAi screen for novel "tumour suppressors". The advantage of an RNAi based screen is speed: as soon as suppressors are isolated the identity of the gene is revealed, enabling rapid progress to a whole range of molecular genetic experiments using state-of-the-art approaches. It is hoped and expected that these studies will provide new insights into the oncogenic potential of Runx/CBFβ genes, and by determining the molecular pathways in which these factors operate, will highlight new avenues for potential drug discovery.

Training opportunities: Training in molecular biological, developmental biological, genetic and biochemical techniques, including RNA interference, microarray analysis, miRNA analysis, PCR, DNA sequence analysis, transgenic animals, bioinformatics

References: By the group: Nimmo, R. et al. (2005). *Development* 132, 5043-54.
Kagoshima, H. et al. (2007). *Development* 134, 3905-15.
Nimmo, R. and Woollard, A. (2008). *Dev. Biol.* 313, 492-500.
General: Antoshechkin, I. and Sternberg, P. W. (2007). *Nat Rev Genet* 8, 518-32.
Cameron, E. R. and Neil, J. C. (2004). *Oncogene* 23, 4308-14.

Project title: **Single molecule studies of the brain neurotensin receptor, NTS1**

Basic Science Supervisor: A. Watts (awatts@bioch.ox.ac.uk; www2.bioch.ox.ac.uk/~awatts)
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: The neurotensin receptor type 1 (NTS1) is a G-protein coupled receptor (GPCR) with seven transmembrane helice, and represents a putative target for the treatment of pain, obesity, schizophrenia, Parkinson's and (more recently) cancer – to date there are no drugs which directly address this receptor. The natural agonist is neurotensin (NT), an endogenous, tridecapeptide peptide hormone found in the central nervous system and gastrointestinal tract. We have been expressing the rat brain NTS1 receptor for some years in *E.coli* DH5α, as a fusion protein (termed NTS1A) from the pRG/III-hs-MBPP-T43NTR1-TrxA-H10 plasmid [1,2] for use in biophysical and expression studies, in particular solid state NMR [3].

There are indirect (mainly computationally driven) conflicting views about the consequences of ligand binding to GPCRs, from isolated conformational changes required to activate the relevant G-protein, to domain swapping in dimerization induced by ligand binding. Here, optical (FRET/TIRF) approaches will be used to identify hormone-receptor interactions directly, especially receptor oligo(di)merization which may be induced by hormone binding and a necessary step in activation.

We are fortunate that the activating peptide hormone (NT) only binds in the last six residues (8-13 of the 13-mer). Modification of the N-terminal (for example with a probe) does not affect binding. This has also permitted hormone binding to be monitored by novel surface plasmon resonance, which now opens up the method for other GPCRs, since this has been challenge for homologues to date [4].

Experimental approaches, possible outcomes:

Hormone-receptor interactions: As shown in Figure 1, fluorescence changes induced by a ligand binding can be monitored either from labelled agonist (NT) only, or in a study where both ligand and receptor are labelled (using FRET). This may reveal the ligand binding site, kinetics of binding and stoichiometry of binding.

Ligand-induced oligo(di)merization: With differently fluorescent-tagged NTS1 as fusions (NTS1B), any possible NT-induced associations with NTS1B will be monitored optically. By using different probes, some ideas of the stoichiometry of hormone-induced oligomerization and kinetics of binding will be deduced, and correlated with parallel SPR measurements. It may even be possible to estimate distances between ligand binding sites and contact faces, if oligomerization is detected. Putative competitors will be screened in an effort to discover possible drugs to address this important brain receptor.

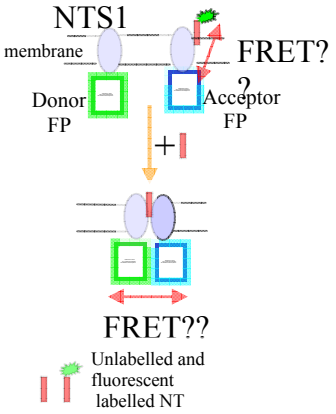

Figure 1: A range of single molecule approaches are envisaged to study the binding of the hormone (NT) to NTS1, and its consequences (oligomerization, domain swapping, etc) using labelled components.

Training opportunities: Peptide synthesis, fluorescence labelling, bacterial expression, receptor purification, membrane reconstitution, SPR, drug screening, computational modelling and FRET are all planned activities.

References (from group)

1. Williamson, P.T.F., Hadingham, T., Roth, J.F. and Watts, A. (2000), *Expression and purification of recombinant neurotensin in E. coli.* **Prot. Expression & Purification**, 19, 271-275
2. Williamson, P.T.F., Bains, S., Chung, C., Cooke, R. & Watts, A. 2000, *Probing the environment of neurotensin whilst bound to the neurotensin receptor* **FEBS Letts**. 518, 111-115.
3. Watts, A. (2005) *Solid state NMR in drug design and discovery for membrane embedded targets*. **Nature Reviews Drug Discovery**, 4, 555-568
4. Harding, P.J., Hadingham, T.C., McDonnell, J.M. & A. Watts, (2007) *Direct analysis of a GPCR-agonist interaction by surface plasmon resonance.* **Eur. Biophys. J**. 35, 709-712 .

Project Title: Characterisation of *Rcalc1,* an ENU mouse model for kidney stone disease.

Basic science supervisor: Professor F Ashcroft (frances.ashcroft@physiol.ox.ac.uk Department of Physiology)

Clinical Supervisor: Professor R V Thakker (rajesh.thakker@ndm.ox.ac.uk Academic Endocrine Unit, Nuffield Department of Clinical Medicine).

Brief description: The overall aims are to further the understanding of molecular and physiological pathways of renal tubular transport, disorders of which lead to kidney stone disease. The specific aim is to identify the causative gene in a mouse model, RCalc1, with kidney stone disease and to characterize its function in renal tubular transport. Kidney stones (nephrolithiasis) affect ~5% of the population. Up to 45% of patients inherit the disease, either as a monogenic disorder or as a polygenic trait. The group has identified genes involved in monogenic forms of nephrolithiasis by positional cloning. They have shown that inactivating mutations of chloride channel 5 (CLC-5) and uromodulin, and activating mutations in calcium sensing receptor (CaSR) can cause nephrolithiasis in man. Studies of these proteins have given greater insights into the mechanisms that control the renal handling of calcium. The aim now is to identify other such genes. However, this approach is limited by the availability of large families with idiopathic nephrolithiasis. To overcome this and to facilitate further studies of renal calcium regulation, the group have established mouse models with autosomal dominant nephrolithiasis and in one of these, *Rcalc1*, have mapped the disease locus to a ~1.2Mb region on Chromosome 17 that contains 34 genes.

Thus, the aims of this project are to identify the disease gene and further characterize these mice to help elucidate the pathogenesis of the phenotype by the following approaches: 1) sequence analysis of candidate genes within this candidate region using a prioritized approach, and functional analysis of the mutant protein using standard *in vitro* and *in vivo* methods; 2) microarray analysis to identify pathways involved in the development of the disease in these mice; 3) detailed biochemical, radiological and histological studies of these mice to determine the underlying metabolic abnormality leading to the kidney stones. These studies will help further our understanding of the underlying molecular and physiological pathways involved in renal tubular transport mechanisms.

Training opportunities: Training in methods of molecular and cellular biology, and renal tubular physiology. Appropriate use of mouse models and their phenotypic characterisation.

References: (publications from the group)

1. Lloyd SE et al (1996). *Nature* 379: 445-449.
2. Lloyd SE et al (1997). *Journal of Clinical Investigation* 99: 967-974.
3. Pearce SH et al (1996). *N Engl J Med.* 335, 1115-22.
4. Turner JJO et al (2003). Journal of Clinical Endocrinology & Metabolism 88:1398-1401.
5. Hough TA et al (2004). *Proceedings of the National Academy of Sciences*, USA 101: 13566-13571.

Project title: Gene Therapy for Endocrine Tumours

Basic Science Supervisors: Dr M Wood (matthew.wood@dpag.ox.ac.uk Department of Physiology Anatomy and Genetics), and Dr L Seymour (Len.Seymour@clinpharm.ox.ac.uk Department of Clinical Pharmacology.

Clinical Supervisor: Professor R V Thakker (rajesh.thakker@ndm.ox.ac.uk Academic Endocrine Unit, Nuffield Department of Clinical Medicine).

Brief description: Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by the combined occurrence of parathyroid, pancreatic islets, adrenocortical tumours and anterior pituitary tumours, for which effective treatments are not available. The *MEN1* gene is located on chromosome 11q13 and encodes a 610 amino acid protein, MENIN. More than 450 germline mutations have been reported. Somatic mutations in *MEN1* and sporadic (non-*MEN1*) endocrine tumours are consistent with the Knudson hypothesis. MENIN has functions in transcriptional regulation and genome stability. The group has successfully generated a MEN1 knockout mouse model. The heterozygous (+/-) mice develop the expected tumours of the parathyroids, anterior pituitary, pancreatic islet cells and adrenocortical carcinomas. The MEN1 gene has many similarities to the retinoblastoma (Rb) gene. The tumour suppressor function of Rb was demonstrated via introduction of the WT Rb gene into neoplastic cells using virus-mediated gene transfer, and similar studies are proposed for MEN1.

Thus, the main purpose of the proposal is to suppress the tumourigenicity of MEN1 associated tumours by effective and sustained delivery of the wildtype (WT) MEN1 gene using a gene therapy vector. The *3 main approaches* are to: 1) develop a MEN1 gene therapy vector using either an adenovirus or retrovirus; 2) achieve an efficient delivery of this construct to MEN1 (-/-) tumour cells and assess its effects on cell proliferation and apoptosis; 3) determine its *in vivo* tumour suppressor potential and safety by injecting the vector into tumours that develop in immunocompetent MEN1 knockout (+/-) mice, and in tumours explanted onto the dorsal flanks of athymic nude mice. The results of these studies will establish the proof of principal and enable a clinical trial aimed at suppressing tumour growth in patients with MEN1 tumours.

Training opportunities: Training in methods of molecular and tumour biology. This will include generating a viral agent therapy construct; DNA sequence analysis and the use of PCR; cell culture and immunohistochemistry techniques; Southern, Northern and Western blot analyses; appropriate use of mouse models and the use of bioinformatic computer programs for sequence analysis, protein and EST database searches.

References: (publications from the group)

1. Pannett AAJ and Thakker RV (1999). *Endocrine-Related Cancer 6:449-473.*
2. Pannett AAJ and Thakker RV (2001). *Journal of Clinical Endocrinology and Metabolism* : 86:4371-4374.
3. Turner JJO et al (2002). *Journal of Clinical Endocrinology & Metabolism* 87:2688-2693.
4. Thakker RV (2006). *Endocrinology, 5th Edition*, Eds LJ De Groot, JL Jameson pp 3509-3531. (Publishers: Elsevier).
5. Lemos M, Thakker RV (2007). *Human Mutation* (in press).

Project title: The role of structural variants in monogenic and multifactorial diabetes

Basic Science Supervisor: Anna L Gloyn (anna.gloyn@drl.ox.ac.uk), Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM)

Clinical Supervisor: Mark I McCarthy (mark.mccarthy@drl.ox.ac.uk) OCDEM and Wellcome Trust Centre for Human Genetics (WTCHG)

Brief description: There has been substantial progress over the past 15 years in identifying rare causal mutations responsible for monogenic forms of diabetes (such as maturity onset diabetes of the young and neonatal diabetes). More recently, a growing number of common variants influencing typical multifactorial forms of type 2 diabetes have been uncovered through genome wide association studies. These efforts have focused almost exclusively on single nucleotide polymorphisms, neglecting other types of DNA sequence variants (particularly deletion, insertions, duplications and inversions – collectively "structural variants") that may also contribute to disease pathogenesis. However, recent studies have demonstrated that deletions are a common cause of HNF1B-MODY, and there is growing interest in the role that structural variants may play in common diseases such as T2D.

This project will seek to establish the contribution made by structural variants to the pathogenesis of non-autoimmune diabetes. One approach will involve the use of array-based approaches (genome-wide CGH arrays, and custom Nimblegen arrays targeting known MODY/T2D susceptibility genes) to detect and characterise structural variants causal for monogenic/syndromic forms of diabetes. A second approach will make use of data currently being generated by the Wellcome Trust Case Control Consortium (which is undertaking a genome-wide association study of ~10,000 copy number variants in 2000 T2D cases and 3000 controls over the next year), and will involve the large-scale replication and characterisation of the signals emerging in population-based samples and in case-series selectively ascertained for strong family history and early onset.

Finally, for structural variants shown to have a causal role in diabetes pathogenesis, the project will involve efforts to dissect the molecular mechanisms responsible. It is likely that most of these variants will exert their effects on diabetes risk through a primary effect on islet function, and relevant expertise and resources within OCDEM (including access to human islets) will be well-suited to these studies.

Training opportunities: Genetics: genotyping, sequencing, Q-RT-PCR, array-based approach to CNV discovery, statistical analysis, bioinformatics. **Functional studies:** cell culture, immunohistochemistry, *in vitro* manipulation of cellular function (eg siRNA knockdown, and overexpression)

References:

1. The Wellcome Trust Case Control Consortium. Genomewide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661-678

2. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JRB, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris, AD, Doney ASF, The Wellcome Trust Case Control Consortium, **McCarthy MI**, Hattersley AT. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 2007 316:1336-1341.

3. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JRB, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch A-M, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin M-R, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJF, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CNA, Doney ASF, Morris AD, Davey-Smith G, The Wellcome Trust Case Control Consortium, Hattersley AT, **McCarthy MI**. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;316:889-894

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5. Mefford HC, Clauin S, Sharp AJ, Moller RS, Ullman R< Kapur R, Inkel D, Copper GM, Netura M, Ropers HH, Tommerup N, Eichler EE, Bellanne-Chantelot C. Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. American Journal of Human Genetics, 2007 81(5):1057-69.

Project title: Defining the molecular basis of DNA Double strand break repair

Basic Science Supervisor: N. D. Lakin (nicholas.lakin@bioch.ox.ac.uk) and C. J. Pears (catherine.pears@bioch.ox.ac.uk), Department of Biochemistry

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: The genome is under continuous assault by agents that cause DNA damage. The cell has therefore evolved pathways that detect DNA damage for processing and repair. Defects in DNA repair pathways results in a variety of clinical symptoms including an increased predisposition to cancer. Understanding these pathways will provide insights into the molecular basis of cancer and uncover targets for diagnosis and treatment of this disease.

DNA double strand breaks (DSBs) can be repaired by two distinct but complementary pathways, homologous recombination and non-homologous end joining (NHEJ). The accuracy of these repair pathways is vital to maintain genome integrity and to protect against cancer. The analysis of DNA DSB repair in genetic model organisms has been vital in establishing how these pathways function in humans. However, in certain instances this approach has been hampered by the lack of human DNA repair proteins in genetically tractable organisms. For example, despite the sequencing of the entire *S. cerevisiae* and *C. elegans* genomes, no orthologues of the human NHEJ factors Artemis and DNA-PKcs have been identified in these organisms.

Recently we identified a functional orthologue of DNA-PKcs in the genetically tractable amoeba *Dictyostelium*. The aim of this research is to further exploit *Dictyostelium* to study NHEJ and therefore provide insights into this pathway in human cells. Analysis of the DNA end processing events that facilitate re-joining of non-compatible and/or blocked DNA termini *in vivo* has not been forthcoming. We have identified an orthologue of the human DNA end-processing factor Artemis in *Dictyostelium* and found that in addition to being involved in NHEJ, this protein is also required to repair more complex DNA damage architectures such as DNA inter-strand cross links (ICLs) which are generated by commonly used chemotherapeutic drugs such as cisplatin. The aim of this research is to assess how Artemis processes DNA termini and ICLs and identify novel components of the NHEJ pathway. Specifically it is proposed to: 1) Generate *Dictyostelium* cells defective in Artemis and other DNA processing factors and assess their requirement for processing a variety of different DNA termini to facilitate NHEJ. 2) Express and purify *Dictyostelium* and human Artemis and compare the processing of substrates *in vitro*. 3) Perform genetic screens that will identify novel factors required to process complex DNA termini to facilitate NHEJ. 4) Determine the role of these novel factors in NHEJ in human cells.

Training opportunities: Training in molecular and cell biology techniques will be provided including recombinant DNA technology, targeted gene disruption, PCR, Southern, northern and western blotting and analysis of signalling events using phospho-specific antibodies. Training will also be provided in cell culture, expression and purification of recombinant proteins, and cell based assays to assess the efficiency of DNA repair *in vivo*.

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Strmecki, L., D.M. Greene, and C.J. Pears (2005). Dev Biol **284:**25-36
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Leiber, M. R. (2008). *J. Biol. Chem*., 283:1-5

Project title: Selective killing of tumour cells

Basic Science Supervisor: Dr. L. S. Cox (lynne.cox@bioch.ox.ac.uk Department of Biochemistry).

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: Cancer is a multi-step disease resulting from loss of function of tumour suppressor genes and gain of function of oncogenes. The WRN helicase/exonuclease, which is mutated in the premature human ageing Werner's syndrome (WS) is an important tumour cell survival factor, and RNAi-mediated knockdown of WRN leads to apoptosis of a wide variety of neoplastic cells in culture, whilst WRN loss in normal cells leads to their senescence. As such, WRN inhibition or ablation may provide a route for selective killing of tumour cells.

We have recently generated several reagents suitable for reducing or inhibiting WRN activity in cells, based on WRN-directed microRNAi, and peptides that interact with either the exonuclease or helicase domain of WRN. The proposed project will investigate the impact of regulated loss of WRN function using these reagents. The microRNAi constructs will be cloned into inducible vectors for expression in mammalian cells, and used to knock down WRN protein levels in a regulated manner. Synthetic peptides that interact with WRN enzymatic domains will be optimised for *in vitro* inhibition of WRN, using highly sensitive time resolved fluorescence assays to monitor inhibition of recombinant WRN helicase or exonuclease, and informed by molecular modelling of interactions with WRN. Active peptides and inactive controls will then coupled with fluorescently-tagged penetratin to drive their uptake into cells. The possible outcomes of cell survival, proliferation, senescence or apoptosis will be measured upon WRN knockdown/ inhibition in a range of transformed and closely matched primary cells in culture. Molecular pathways known to be altered in Werner's syndrome will be studied in the experimentally WRN-depleted cells, including homologous DNA recombination, DNA replication, DNA damage (via markers such as γH2AX), and chromatin redistribution. It is anticipated that any miRNAi or peptides showing anti-tumour activity in this project will subsequently be pursued as models for rational design of small molecule inhibitors of WRN with value in cancer chemotherapy.

Training opportunities: Training in methods of molecular and cell biology. This will include generating novel RNAi expression vectors by restriction enzyme-based cloning and PCR techniques, *in vitro* enzyme analysis, cell culture, immunofluorescence microscopy, Western blotting, fluorescence activated cell sorting, and the use of bioinformatics to model molecular interactions.

References: (publications from the group)

1. Rodriguez-Lopez AM et al. (2007) Correction of Proliferation and Drug Sensitivity Defects in the Progeroid Werner's Syndrome by Holliday Junction Resolution. *Rejuvenation Research* 10 (1): 27-40
2. Cox, LS et al (2007) Modeling Werner's syndrome in *Drosophila melanogaster*: hyper-recombination in flies lacking WRN-like exonuclease. *Ann N Y Acad Sci.* 1119: 274-88
3. Cox LS, Faragher RG. (2007) From old organisms to new molecules: integrative biology and therapeutic targets in accelerated human ageing. *Cell Mol Life Sci.* 64(19-20): 2620-41.
4. Rodríguez-López AM et al.(2003) Characterisation of the interaction between WRN, the helicase/exonuclease defective in progeroid Werner's syndrome, and an essential replication factor, PCNA. *Mech. Ag. Dev.* 124: 167-174
5. Rodríguez-López, AM et al. (2002) Asymmetry of DNA replication fork progression in Werner's syndrome. *Ageing Cell* 1: 30-39

Project title: Engineering antibodies for more sensitive diagnosis of cancer

Basic Science Supervisor: Dr Mark Howarth (mark.howarth@bioch.ox.ac.uk , Department of Biochemistry)

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description:

Background:

Antibodies are a central tool in biological research and disease diagnosis and they are starting to fulfil their potential in therapy. Antibodies can be raised to almost any molecular structure, often with high specificity and typically nanomolar affinity. Antibody-based assays have advanced enormously over the last fifty years, opening up whole new fields of research from the ability to detect lower and lower abundance species. Assay methods, such as isotope- or enzyme-coupled assays, are now so advanced that the assay is not the limitation on sensitivity any more- it is the antibody itself that is the limiting factor. Whatever the amplification method in an assay, they all depend on the initial antigen binding event. If the antigen dissociates from the antibody, there is no signal to amplify. One area where sensitivity of detection may be most important is for cancer, where early diagnosis leads to high survival rates.

Overall aim:

Previous approaches to improve antibody affinity have depended upon finding optimal combinations of the natural 20 amino acids. The approach of our laboratory is to extend the range of chemical groups present in the antigen binding site, to achieve specific and irreversible antigen recognition. We will apply this to lower the detection limit in immunoassays, with the goal of more sensitive detection of tumour markers, including for prostate cancer and breast cancer.

Plan of investigation:

(i) express and modify an antibody against a model protein, for which we have an antibody-antigen crystal structure.

(ii) test the sensitivity and specificity of the modified antibody for cellular imaging and for detection *in vitro* and in blood by ELISA and immunoPCR.

(iii) use the information from these studies to improve the sensitivity of detection of human Hepsin, for application to diagnosis and monitoring therapy of prostate cancer.

Training opportunities: Training in methods of molecular and cellular biology. This will include use of PCR, DNA sequence analysis, mutagenesis, recombinant protein expression in bacteria, ELISA, Western blotting, mammalian cell culture, and fluorescence microscopy.

References:

Previous work of ours on protein engineering for high affinity binding and ultrasensitive imaging:
A monovalent streptavidin with a single femtomolar biotin binding site.
Howarth M et al. Nature Methods. 2006 Apr;3(4):267-73.
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Site-Specific Labeling of Cell Surface Proteins with Biophysical Probes using Biotin Ligase.
Chen I, Howarth M, Lin W, Ting AY. Nature Methods 2005 Feb;2(2):99-104.
Antibody engineering and immunoassay background:
Wu,A.M. and Senter,P.D. (2005). Arming antibodies: prospects and challenges for immunoconjugates. Nat.Biotechnol. *23*, 1137-1146.
Nam, J.M., Thaxton, C.S., Mirkin, C.A. (2003) Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. Science 301, 1884-6.

Project title: Molecular Modelling and Simulation of Cell Membrane Systems

Basic Science Supervisor:
Prof. Mark Sansom, Structural Bioinformatics and Computational Biochemistry Unit, Department of Biochemistry, South Parks Road, OX1 3QU.
e-mail: mark.sansom@bioch.ox.ac.uk; phone: (2)75371; website: http://sbcb.bioch.ox.ac.uk
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.
Brief description:

Research in my group is concerned with multi-scale modelling of biomolecular systems, with a particular emphasis on membrane systems. This is relevant to signalling and transport in bacterial and mammalian systems, and has important biomedical implications, as 50% of drug targets are membrane proteins. The development of a computational systems approach is required as membrane proteins do not operate in isolation, but interact with their surrounding lipid bilayer environment and with other proteins in order to bring about their biological effects.

Dynamic interactions of proteins with membranes play a key role in many cellular processes. For example membrane fusion events are involved in release of neurotransmitters at synapses, and in the entry of enveloped viruses (e.g. influenza, HIV) into target cells. Structural biology continues to provide information on the proteins involved in such events. Cell biology and biophysical studies provide information on more dynamic aspects. However, in addition to these experimental approaches, there is a pressing need for a computational approach to membrane dynamics to enable integration of disparate data. Current work in MSPS's group has established a coarse-grained molecular dynamics approach which enables simulation of large scale membrane/protein events on a ~1 μsec timescale). A multi-scale approach enables accurate computational modelling of dynamic cell membrane processes and builds upon current developments in the physical sciences where multi-scale approaches are being used for soft matter and materials simulations.

An overview of research and people in my group can be found at: **http://sbcb.bioch.ox.ac.uk**
The following is a short list of possible projects. However, other projects may be possible.

4. Multi-scale modelling of PTEN, a membrane-associated tumour suppressor protein
5. Anti-cancer peptide interactions with cell membranes
6. Monotopic membrane proteins: multi-scale modelling of key drug targets

Training opportunities:

From a training perspective the project will provide experience in a wide range of computational and theoretical skills as applied to biological molecules and systems. These will include: biomolecular simulations, elastic network and/or finite element modelling, scientific programming and scripting (perl, python etc), use of parallel and distributed computing resources, and statistical analysis of data.

References:

6. Bond, P. J., and Sansom, M. S. P. (2007) Bilayer deformation by the Kv channel voltage sensor domain revealed by self-assembly simulations. *Proc. Natl. Acad. Sci. USA 104*, 2631-2636.
7. Bond, P. J., Derrick, J. P., and Sansom, M. S. P. (2007) Membrane simulations of OpcA: gating in the loops? *Biophys. J. 92*, L23-L25.
8. Deol, S. S., Domene, C., Bond, P. J., and Sansom, M. S. P. (2006) Anionic phospholipids interactions with the potassium channel KcsA: simulation studies. *Biophys. J. 90*, 822-830.
9. Fowler, P.W., Balali-Mood, K., Deol, S., Coveney, P.V. and Sansom, M.S.P. (2007) Monotopic enzymes and lipid bilayers: a comparative study. *Biochem.* 46: 3108-3115
10. Bond, P.J., Holyoake, J., Ivetac, A., Khalid, S., and Sansom, M.S.P. (2007) Coarse-grained molecular dynamics simulations of membrane proteins and peptides *J. Struct. Biol.* 157:593-605

Project title: Molecular Mechanisms of Parathyroid Gland Development

Basic Science Supervisor: Prof K E Davies (kay.davies@anat.ox.ac.uk) Department of Physiology, Anatomy and Genetics.

Clinical Supervisor: Professor R V Thakker (rajesh.thakker@ndm.ox.ac.uk Academic Endocrine Unit, Nuffield Department of Clinical Medicine).

Brief description: Parathyroid developmental anomalies result in hypoparathyroidism and may occur as part of a complex congenital defect as in the DiGeorge syndrome, which occurs in 1:4,000 live births, or the hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome, or as an isolated endocrinopathy. The overall aim of this project is to characterise the molecular and cellular mechanisms regulating parathyroid development from the pharyngeal pouches. The specific aims are to determine the roles of the transcription factors GATA3 and SOX3 in these developmental pathways.

The involvement of SOX3 in parathyroid development was identified by studies of families with X-linked hypoparathyroidism (XLHPT), in a deletion-insertion involving chromosomes 2p25.3 and Xq27.1 was identified. The deletion-insertion was located 67 kilobases (kb) downstream of SOX3, which was demonstrated to be expressed in the developing parathyroids of mouse embryos, and hence is likely to result in a position effect on SOX3 expression. This demonstrated an important role for genetic abnormalities that involve non-coding regions in causing disease, a feature that is likely to be of significance in the search for the molecular basis of other inherited diseases. Investigation of such positional effects, which represents a major challenge in human developmental biology, and the team is pursuing studies in cellular and mouse models. The group have identified 3 non-coding evolutionarily conserved regions (NCCRs), that may represent regulatory sequences e.g. enhancers or repressors. These NCCRs will be assessed in an artificial gene expression system, using the luciferase reporter vector pGL3, deletional analysis, DNase I footprinting and one-hybrid studies. The temporo-spatial expression of *Sox3* in relation to the other parathyroid developmental genes, e.g. *Hoxa3*, *Pax1*, *Gcm2* and *Gata3* will also be established. In addition, the transcriptional mechanisms that regulate levels of *SOX3* in a tissue and developmental stage-specific manner will be investigated by using transgenic reporter approaches.

The group identified that GATA3 mutations leading to haploinsufficiency result in the HDR syndrome. The aim of the project is to: 1) functionally characterize GATA3 mutations in patients with HDR Syndrome by the use of a luciferase reporter assay; 2) establishing the role that GATA3 plays in the parathyroid gland developmental pathway by determining the genes that this transcription factor regulates, by for instance, using the chromatin immunoprecipitation (ChIP) assay; 3) investigating the expression of other parathyroid developmental genes in haploinsufficient $Gata3^{+/-}$ mice.

Training opportunities: Training in methods of molecular and developmental biology. This will include use of PCR, DNA sequence analysis, transcription reporters, and appropriate use of mouse models.

References: - publications by the group

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2. Nesbit, M.A et al (2004). *J Biol Chem* 279:22624-22634.
3. Thakker, R.V et al (1990). *J Clin Invest* 86:40-45.
4. Bowl, M.R (2005). *J Clin Invest* 115:2822-2831.
5. Ali A et al (2007). *Human Molecular Genetics*, 16: 265-275.

Project title: Identification of novel candidate biomarkers for diabetic nephropathy

Basic Science Supervisor: Dr Nicole Zitzmann (nicole.zitzmann@bioch.ox.ac.uk), Oxford Glycobiology Institute, Department of Biochemistry.
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: More than 180 million people worldwide have diabetes mellitus, this number is predicted to more than double by 2030 (WHO, 2006). Poor glucose control in diabetes can lead to several complications including diabetic nephropathy (DN; for a review, see [1]). During DN, persistently high glucose levels, hypertension, and related problems result in kidney damage. High glucose levels increase the expression of cytokines resulting in kidney fibrosis that alters the architecture of the basement membrane leading to elevated glomerular pressure and microalbuminuria (the presence of small amounts of albumin in the urine). 50% of all diabetic patients develop microalbuminuria, of these 20–30% will develop proteinuria and eventually end-stage renal disease (ESRD). DN is the most common cause of ESRD worldwide and is associated with elevated cardiovascular morbidity and mortality.

Detection of microalbuminuria is currently used to diagnose DN. However, by this point the kidney has suffered some pathology and the diagnosis may be unreliable as microalbuminuria does not necessarily imply DN and other factors for kidney disease need to be ruled out. Therefore there is a need for reliable diagnostic biomarkers for DN at an earlier stage than the current benchmark urine albumin test. Novel urinary biomarkers are most favourable since plasma/serum predominately consists of liver derived proteins and these samples may not reflect renal disease specifically.

We propose to conduct a gel-based proteomics study to identify novel DN biomarkers. My group has extensive experience in using gel-based proteomics to discover novel biomarkers for different disease states (e.g. [2-5]). Previously we have successfully used proteomics to identify novel candidate biomarkers for hepatic fibrosis [2]. As the same profibrogenic cytokines and growth factors cause both hepatic fibrosis and DN-related kidney fibrosis we propose to apply a similar strategy to identify early biomarker candidates for DN. Candidate biomarkers identified from the proteomics study would initially be validated by Western blotting and further characterised.
N.B. The proposed project is dependent on the acquisition of appropriate samples by the Clinical Fellow.

Training opportunities: Training in gel-based proteomics, including sample preparation and optimisation, gel-running, image analysis, and Western blotting to validate proposed candidate biomarkers.

References:
1. Marshall, S.M., *Recent advances in diabetic nephropathy*, **Postgrad. Med. J.**, 2004. 80(949): p. 624-33.
2. Gangadharan, B., R. Antrobus, R.A. Dwek, and N. Zitzmann, *Novel serum biomarker candidates for liver fibrosis in hepatitis C patients*, **Clin. Chem.**, 2007. 53(10): p. 1792-9.
3. Pardo, M., R.A. Dwek, and N. Zitzmann, *Proteomics in uveal melanoma research: opportunities and challenges in biomarker discovery*, **Expert Rev. Proteomics**, 2007. 4(2): p. 273-86.
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5. Pardo, M., A. Garcia, B. Thomas, A. Pineiro, A. Akoulitchev, R.A. Dwek, and N. Zitzmann, *The characterization of the invasion phenotype of uveal melanoma tumour cells shows the presence of MUC18 and HMG-1 metastasis markers and leads to the identification of DJ-1 as a potential serum biomarker*, **Int. J. Cancer**, 2006.

Project title: "Kiss-and-run" insulin secretion as a cause of type-2 diabetes?

Basic Science Supervisor: Professor Patrik Rorsman, OCDEM, Nuffield Department of Clinical Medicine, University of Oxford, Churchill Hospital, Oxford OX3 7LJ. Email: patrik.rorsman@ocdem.ox.ac.uk

Clinical Supervisor: To be decided depending on the candidate's background

Brief description: Insufficient insulin secretion is a key component of type-2 diabetes (1). Insulin is secreted from the pancreatic β-cells in response to hyperglycaemia. The β-cell is an electrically excitable cell and uses electrical signals to couple changes in plasma glucose to increases or decreases in insulin release. During electrical activity, calcium enters the β-cell from the extracellular space and the resultant increase in cytoplasmic calcium concentration triggers the fusion of insulin-containing vesicles with the plasma membrane (exocytosis) (2). Exocytosis in β-cells exhibits many similarities with release of neurotransmitters in the neuronal synapses. During exocytosis, a narrow pore ("fusion pore") is established between the lumen of the secretory granule and the exterior. This pore has initially a diameter of only ~1.5 nm (3). Whilst this is sufficient to allow the exit of low molecular weight neurotransmitters like GABA, ACh and glutamate, it is too narrow to allow the exit of larger and bulkier molecules like insulin (diameter: ~4 nm). In order to ensure efficient release of insulin, it is therefore critically important that the fusion pore expands. Interestingly, up to 60% of the exocytotic events in rodent β-cells are aborted prior to expansion of the fusion pore and insulin remains trapped in the granule lumen (4). In this form of exocytosis, the granule only makes brief contact with the plasma membrane and it is often referred to as "kiss-and-run". It is not known whether the same applies to human β-cells but this can be tested as we now have regular access to human islets. In rodent cells there is some evidence that long-term exposure to lipids and glucose has results in a switch from full fusion (associated with insulin secretion) to "kiss-and-run" exocytosis (5 and references therein). Type-2 diabetes (even when treated) is associated with moderately elevated plasma and lipid levels for very long periods (years). However, if human β-cells respond the same way as their rodent counterparts to long-term hyperglycaemia and hyperlipidaemia then an increase in "kiss-and-run" exocytosis might contribute to the insulin secretion defect of type-2 diabetes. We will study this by a combination of several techniques. We will *i*) use standard biochemical techniques such as RIA to measure insulin secretion from human islets under a variety of experimental conditions; *ii*) correlate the hormone release measurements to changes in the cell surface area that can be measured by electrophysiological techniques and that include graules which have not undergone full fusion; and *iii*) monitor insulin granule release by optical methods following transfection of the cells with fluorescent granule markers that label either insulin itself or the granule membrane. These techniques will allow us to determine the relative number of full fusion and kiss-and-run exocytotic events in human β-cells maintained in tissue culture under a variety of experimental conditions that simulate pathophysiological states (hyperglycaemia, hyperlipidaemia etc).

Training opportunities: The project will provide extensive training in advanced electrophysiological and optical techniques. The project will also involve molecular biology, PCR analyses and cell culture. All techniques are already established within the host laboratory and staff is available to provide intense training as required.

References:

(1) Ashcroft FM, Rorsman P. *Hum Mol Genet*. **13**: R21-31, 2004
(2) Rorsman P, Renstrom E. *Diabetologia* **46**:1029-45, 2003
(3) MacDonald PE, Braun M, Galvanovskis J, Rorsman P *Cell Metab*. **4**:283-90, 2006.
(4) Barg S, Olofsson CS, Schriever-Abeln J, Wendt A, Gebre-Medhin S, Renström E, Rorsman P. *Neuron* **33**:287-99, 2002.
(5) Olofsson CS, Collins S, Bengtsson M, Eliasson L, Salehi A, Shimomura K, Tarasov A, Holm C, Ashcroft F, Rorsman P. *Diabetes* **56**:1888-97, 2007.

Project title: Histone deacetylase inhibitors in cancer therapy

Basic Science Supervisor: Catherine Pears (Catherine.pears@bioch.ox.ac.uk) and Louis Mahadevan (louis.mahadevan@bioch.ox.ac.uk), Department of Biochemistry
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.
Brief description: Background. DNA is packaged into chromatin which plays an important role in regulating the accessibility of the DNA to a variety of proteins such as those involved in gene expression. Specific modification of chromatin-associated histone proteins is associated with the transcriptional state of the associated genes. Alterations in the acetylation of histone proteins (especially acetylation of lysine9 on histone H3) during tumour formation have been linked mechanistically to the pathogenesis of cancer: the enzymes catalysing the removal of the acetyl groups (HDACs) have been found to be overexpressed and mutated in cancer cells and changes in histone acetylation patterns linked with disease progression. Small molecule inhibitors of HDACs (HDACis) have been shown to achieve significant biological effects in growth inhibition of preclinical models of cancer. Initial clinical trials using these inhibitors have shown promising effects. However results many of these drugs have toxic side effects and the development of resistance to HDACi growth inhibition is a problem. It is not clear that HDACs are the primary target of all of these drugs, and the pathway leading to tumour growth inhibition is not understood, precluding the development of more effective compounds.

A number of HDACis which are currently in clinical trials cause growth inhibition of a genetically tractable eukaryotic organism, *Dictyostelium discoideum*. This organism shows patterns of histone modification similar to those found in mammalian cells, but the ease of genetic manipulation allows rapid screening for resistant mutants and a pilot screen has successfully identified a mutant resistant to a number of HDACis, and in which histone modification is altered.

Aims and Plan of investigation. 1. To screen a library of randomly generated *Dictyostelium* mutants for resistance to the inhibition of growth by HDACis currently under consideration for clinical use. 2. To identify and characterise the genes mutated in these strains (including that already identified) by molecular genetic methods 3. To characterise the molecular mechanisms by which gene disruption leads to HDACi-resistance. This would involve characterising the consequences of gene disruption on a range of histone modifications in the presence or absence of HDACis, identifying consequent changes in gene expression profile and characterising the interaction partners and the molecular action of the proteins encoded by the disrupted genes in order to gain insight into mechanisms of HDACi action and resistance. 4. To identify homologous genes in mammalian cells and characterise their role in histone modification and in resistance to HDACis using overexpression and siRNA technology.

Training opportunities:
Training will be provided in methods of molecular biology and genetics. This will include use of PCR, DNA sequence analysis, techniques to probe chromatin structure such as chromatin immunoprecipitation (ChIP) with modification-specific antisera, use of genetic model system, creation of null strains by homologous recombination, siRNA technology
References:
Glozak and Seto, E. 2007. Histone deacetylases and cancer. Oncogene **26,** 5420-5432
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Clayton, A.L., C.A. Hazzalin, and L.C. Mahadevan 2006. Enhanced histone acetylation and transcription: a dynamic perspective. Mol. Cell **13**, 289-296
Strmecki, L., D.M. Greene, and C.J. Pears 2005. Developmental decisions *in Dictyostelium discoideum.* Dev Biol **284:**25-36.
Williams, R., Cheng, L., Mudge, A. and Harwood, A. 2003. A common mechanism of action for three mood stabilising drugs. Nature **417**, 292-295

Project Title: Genetics and epigenetics of hypoxia pathways
Basic Science Supervisor: Professor C J Schofield (christopher.schofield@chem.ox.ac.uk) Department of Chemistry
Clinical Supervisor: Professor P J Ratcliffe (pjr@well.ox.ac.uk) Nuffield Department of Clinical Medicine
Brief description: Recent insights into the cellular response to hypoxia have revealed major alterations in patterns of gene expression that are directed by a transcription factor termed hypoxia inducible factor (HIF). HIF itself is regulated by novel oxygen sensitive signal pathways involving the hydroxylation of specific amino acids by a set of non-haem Fe (II) and 2-oxoglutarate (2-OG) dependent dioxygenases. In any given cell type hundreds or thousands of genes respond directly or indirectly to the HIF pathway regulating a broad range of responses including angiogenesis, inflammation, differentiation, stem cell capacity, energy metabolism, vasomotor function, motility and apoptosis. These responses are however cell-specific with many responses that occur in cancer or inflammatory cells not being observed in quiescent cells. The aim of this project is to analyse the hierarchical controls that constrain patterns of hypoxia-inducible gene expression in different types of neoplastic and non-neoplastic cells.
Chromatin immunoprecipitation (ChIP) methods will be combined with gene expression array analysis to define relationships between promoter occupancy by components of the HIF hydroxylase pathway and the regulation of gene expression. Histone modifications at target gene loci will also be analysed. Of particular interest is the recent finding that several other Fe(II) and 2-OG dependent dioxygenases, closely related to the HIF hydroxylases function as histone demethylases (HDMs). This has raised the possibility of different levels of control of gene expression by hypoxia; i.e. through histone modifications that are permissive for HIF promoter occupancy, as well as HIF itself.
The Schofield laboratory has expertise in structural, kinetic and bioinformatic analysis of the Fe(II) and 2-OG dependent dioxygenases and in the design of small molecule inhibitors. Coupled with increased understanding of how particular patterns of hypoxia-inducible gene expression are regulated in different cells it is envisaged that this may enable the design of small molecules with appropriate activity profiles for specific therapeutic manipulation of hypoxia pathways in cancer or ischaemic vascular disease.
Training opportunities: Multi-disciplinary training in cell biology, particularly gene expression analysis (including state-of-the-art whole genome platforms), transcriptional control, epigenetics, introduction to medicinal chemistry. Scientific training would complement clinical training in a broad range of specialities encompassing oncology, cardiac, pulmonary or metabolic medicine.
References:
(from group)
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(general)
Schofield and Ratcliffe Nature Reviews Molecular Cell Biology 5 343-354 (2004); Shi, Y. Nature Reviews Genetics 8 829-833 (2007); Barski et al. Cell 129 823-837 (2007).

Project title: Iron and the integrative biology of the hypoxia-inducible factor pathway

Basic Science Supervisor: Prof. Peter A Robbins (peter.robbins@dpag.ox.ac.uk), Dept of Physiology, Anatomy & Genetics

Clinical Supervisor: Prof. Peter J Ratcliffe (pjr@well.ox.ac.uk), Nuffield Dept of Medicine

Brief description: Hypoxia-inducible factor (HIF) was first described as a transcription factor that increased the production of erythropoietin in response to hypoxia. Subsequently it was discovered that it was ubiquitously expressed in mammalian cells and that it was involved in the regulation of the expression of many other genes. As such, it has come to be seen as a master controller of the coordinated cellular response to hypoxia. Over the past decade the HIF regulatory pathway has been defined in much more detail. Of particular interest is that the abundance of HIF is regulated by hydroxylation of specific proline residues, which tag HIF for recognition by the von Hippel-Lindau tumour suppressor protein and then subsequent ubiquitination and proteosomal degradation. This hydroxylation step, catalysed by specific prolyl hydroxylase enzymes, confers the oxygen sensitivity on the pathway and, crucially for this project, is very sensitive to the level of free iron.

Investigation of the phenotype associated with systemic abnormalities of the HIF pathway has been undertaken in genetically-altered mice, and these studies have shown that HIF has a major regulatory role at the integrative level on both the cardiovascular and respiratory systems – the very systems on which cellular oxygen delivery ultimately depends. We have made similar observations in humans with Chuvash Polycythemia, a rare genetic disease producing systemic alterations in HIF under conditions of normal oxygen tension. Clonal abnormalities of the HIF pathway have also been implicated in a number of inherited cancer syndromes, and alterations in HIF abundance within tumours are strongly predictive of clinical outcome.

This particular project is based on some pilot observations that altering iron status in humans can substantially modify integrative human responses to hypoxia. The project will explore this observation much more fully, both by manipulating iron status through iron chelation and iron infusion in normal volunteers, and by studying patients with altered iron status or who may be otherwise informative. Depending on outcomes, there may also be an opportunity to explore interactions between iron status and the effects of exposure to high altitude through collaborations in Peru. Although the HIF-system is a potentially important therapeutic target, there are as yet no licensed therapies for its manipulation. In part, this project will help to assess whether careful manipulation of iron status could play any role in the clinical management of hypoxic patients.

Training opportunities: This project provides an opportunity for training in modern techniques for studying integrated responses in humans, both normal volunteers and patients. At Oxford, there is a large multidisciplinary grouping of scientists interested in hypoxia, and the fellow would become part of this grouping. The grouping has weekly scientific meetings at which the fellow would be expected to attend and to present their findings. These meetings aid the intellectual development of the fellow by providing awareness and understanding of a broader field of endeavour within the biology of hypoxia, together with an effective platform for more multidisciplinary collaboration.

References:

Schofield, CJ & Ratcliffe, PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5: 343-354, 2004.
Smith, TG et al. Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. PLoS Med 3: e290, 2006.

Project title: Functional genetic studies of the hypoxia-sensing pathway and metabolism

Basic Science Supervisor: Prof Peter Robbins (peter.robbins@dpag.ox.ac.uk), Dept of Physiology, Anatomy and Genetics; Prof Keith Frayn (keith.frayn@oxlip.ox.ac.uk), OCDEM

Clinical Supervisor: Dr Fredrik Karpe (fredrik.karpe@ocdem.ox.ac.uk), OCDEM

Brief description:

Background: Cells sense hypoxia through a pathway involving the hypoxia-inducible factor (HIF) family of transcription factors. Clonal abnormalities of components within the HIF pathway have been implicated in a number of inherited cancer syndromes, and alterations in HIF abundance within tumours are strongly predictive of clinical outcome. A systemic abnormality of the HIF system is found in the rare disease of Chuvash Polycythemia, and we have shown that these patients have gross alterations in cardiorespiratory control (1). At the molecular level, it is known that the HIF pathway regulates the expression of a number of key metabolic enzymes, and in a pilot study investigating the metabolic consequences of this mutation, we have found that the normal relationship between lactate production during exercise and exercise intensity is perturbed. The aim of this project is to search for other, more common, sources of genetic variation within in the HIF pathway that have metabolic sequelae.

Plan of investigation: The project is comprised of three main stages: 1) identification of candidate genetic variation that may give rise to metabolic variation; 2) identification of genetically informative normal individuals for the study of this variation and 3) detailed investigation of the metabolism of individuals identified in the preceding step.

A number of relatively low power genetic association studies suggest that certain allelic variants within the HIF pathway may be associated with cardiovascular or metabolic disease. Further candidates will be sought by screening the Wellcome Trust Case Control consortium SNP database for genes involved in the HIF pathway for significant association with either type 2 diabetes or cardiovascular disease. Once these have been identified, the student will then screen DNA samples from the Oxford BioBank (OBB), a population-based biobank with ~ 1200 members to date (2), to identify genetically informative individuals with respect to these variants. The biochemical and anthropometric data of the OBB would then be analysed for associations with the polymorphisms. Selected individuals within the OBB would then be invited to attend for detailed metabolic study using both stable-isotope tracer methodology together with arterio-venous difference techniques. Biopsies of skeletal muscle and, if appropriate, adipose tissue, will be studied for evidence of altered gene expression linked to the metabolic characteristics observed (3).

Training opportunities: The student will be a member of the active graduate students' programmes within both DPAG and OCDEM. Training will include mutation analysis, screening for polymorphisms, and techniques for detailed human physiology. Mass spectrometric analysis of stable isotopes tracers will be included. The nature of the project lends itself to a genuine training in 'functional genetics'.

References:

1. Smith TG, Brooks JT, Balanos GM, et al. Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. *PLoS Med* 2006;3:e290.

2. Tan GD, Neville MJ, Liverani E, Humphreys SM, Currie JM, Dennis AL, Fielding BA, Karpe F. The in vivo effects of the Pro12Ala PPARγ2 polymorphism on adipose tissue NEFA metabolism: the first use of the Oxford Biobank. *Diabetologia* 2006;49:158-168.

3. Risérus U, Sprecher D, Johnson T, et al. Activation of PPARδ promotes reversal of multiple metabolic abnormalities, reduces oxidative stress and increases fatty acid oxidation in moderately obese men. *Diabetes* 2007;10.2337/db07-1318

SECTION 5

NEUROSCIENCES

Project title: **Single molecule studies of the brain neurotensin receptor, NTS1**

Basic Science Supervisor: A. Watts (awatts@bioch.ox.ac.uk; www2.bioch.ox.ac.uk/~awatts)
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: The neurotensin receptor type 1 (NTS1) is a G-protein coupled receptor (GPCR) with seven transmembrane helice, and represents a putative target for the treatment of pain, obesity, schizophrenia, Parkinson's and (more recently) cancer – to date there are no drugs which directly address this receptor. The natural agonist is neurotensin (NT), an endogenous, tridecapeptide peptide hormone found in the central nervous system and gastrointestinal tract. We have been expressing the rat brain NTS1 receptor for some years in *E.coli* DH5α, as a fusion protein (termed NTS1A) from the pRG/III-hs-MBPP-T43NTR1-TrxA-H10 plasmid [1,2] for use in biophysical and expression studies, in particular solid state NMR [3].

There are indirect (mainly computationally driven) conflicting views about the consequences of ligand binding to GPCRs, from isolated conformational changes required to activate the relevant G-protein, to domain swapping in dimerization induced by ligand binding. Here, optical (FRET/TIRF) approaches will be used to identify hormone-receptor interactions directly, especially receptor oligo(di)merization which may be induced by hormone binding and a necessary step in activation.

We are fortunate that the activating peptide hormone (NT) only binds in the last six residues (8-13 of the 13-mer). Modification of the N-terminal (for example with a probe) does not affect binding. This has also permitted hormone binding to be monitored by novel surface plasmon resonance, which now opens up the method for other GPCRs, since this has been challenge for homologues to date [4].

Experimental approaches, possible outcomes:

Hormone-receptor interactions: As shown in Figure 1, fluorescence changes induced by a ligand binding can be monitored either from labelled agonist (NT) only, or in a study where both ligand and receptor are labelled (using FRET). This may reveal the ligand binding site, kinetics of binding and stoichiometry of binding.

Ligand-induced oligo(di)merization: With differently fluorescent-tagged NTS1 as fusions (NTS1B), any possible NT-induced associations with NTS1B will be monitored optically. By using different probes, some ideas of the stoichiometry of hormone-induced oligomerization and kinetics of binding will be deduced, and correlated with parallel SPR measurements. It may even be possible to estimate distances between ligand binding sites and contact faces, if oligomerization is detected. Putative competitors will be screened in an effort to discover possible drugs to address this important brain receptor.

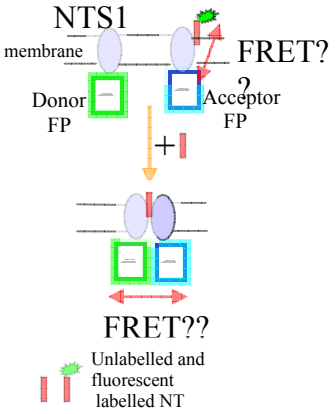

Figure 1: A range of single molecule approaches are envisaged to study the binding of the hormone (NT) to NTS1, and its consequences (oligomerization, domain swapping, etc) using labelled components.

Training opportunities: Peptide synthesis, fluorescence labelling, bacterial expression, receptor purification, membrane reconstitution, SPR, drug screening, computational modelling and FRET are all planned activities.

References (from group)

1. Williamson, P.T.F., Hadingham, T., Roth, J.F. and Watts, A. (2000), *Expression and purification of recombinant neurotensin in E. coli.* **Prot. Expression & Purification**, 19, 271-275
2. Williamson, P.T.F., Bains, S., Chung, C., Cooke, R. & Watts, A. 2000, *Probing the environment of neurotensin whilst bound to the neurotensin receptor* **FEBS Letts**. 518, 111-115.
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4. Harding, P.J., Hadingham, T.C., McDonnell, J.M. & A. Watts, (2007) *Direct analysis of a GPCR-agonist interaction by surface plasmon resonance.* **Eur. Biophys. J**. 35, 709-712 .

Project title: New treatments in inherited disorders of the neuromuscular synapse

Basic Science Supervisor: Prof D M W Beeson (dbeeson@hammer.imm.ox.ac.uk)
Neurosciences Group, Weatherall Institute of Molecular Medicine

Clinical Supervisor: Dr Jacqueline Palace (Jacqueline.palace@clneuro.ox.ac.uk)
Department of Clinical Neurology

Brief description: Congenital myasthenic syndromes result from mutations in proteins located at the neuromuscular junction which affect synaptic transmission.
Mutations in at least 11 different genes may underlie these syndromes. Dok7 is a recently identified protein that binds to muscle-specific kinase (MuSK) and is essential for clustering AChR at the motor endplate and for the maturation of the neuromuscular junction structure. We have recently demonstrated that mutations of the DOK7 gene underlie a form of congenital myasthenia with a limb girdle pattern of muscle weakness that shows recessive inheritance. These patients do not respond to conventional therapies used in congenital myasthenia but there is increasing anecdotal evidence that treatment with ephedrine is beneficial. We propose to study the underlying pathogenic mechanism of disease in these patients and to establish a rational explanation for the reported response to ephedrine.

Thus the objectives of this project are: i) to understand at the molecular level the mechanisms through which the DOK7 mutations cause disease; ii) to confirm the effectiveness of ephedrine using a mouse model of this disorder; and subsequently iii) to determine the mechanism through which ephedrine enhances neuromuscular transmission in this and other disorders.

Training opportunities: The trainee will part of the neurology/neuroscience myasthenia team and will attend National Specialist Clinics and ward rounds for adults and children with congenital myasthenic syndromes. They will learn molecular biology techniques such as PCR, cloning, gene expression in mammalian cells and analysis of fluorescence-tagged synaptic proteins. Training will involve fluorescence microscopy and the appropriate use of mouse models. Finally, they will have the opportunity to learn techniques in neurophysiology and electrophysiology.

References: - publications by the group

1. Hamuro J et al (2007) Mutations causing DOK7 congenital myasthenia ablate functional motifs in DOK-7. *J Biol Chem.* [Epub ahead of print]
2. **Palace J, et al (2007)** Clinical features of the *DOK7* neuromuscular junction synaptopathy. *Brain*, 130:1507-1515.
3. Beeson D, et al (2006) Dok-7 mutations underlie a neuromuscular junction synaptopathy. *Science*, 313:1975-1978.
4. Beeson D, et al (2005). 126th International Workshop: congenital myasthenic syndromes, 24-26 September 2004, Naarden, the Netherlands. *Neuromuscul Disord*., 15:498-512.
5. Cossins J, et al (2004) A mouse model of AChR deficiency syndrome with a phenotype reflecting the human condition. *Hum Mol Genet,* 13:2947-2957.

Project title: Adult olfactory-derived stem cell transplants in Parkinson's disease

Basic Science Supervisor: Professor J Paul Bolam (paul.bolam@pharm.ox.ac.uk) MRC Anatomical Neuropharmacology Unit & Dr Deborah J Clarke (deborah.clarke@psy.ox.ac.uk) Department of Experimental Psychology

Clinical Supervisor: Professor Tipu Z. Aziz (tipu.aziz@dpag.ox.ac.uk) Nuffield Department of Surgery & Dr Kevin Talbot (kevin.talbot@dpag.ox.ac.uk) Department of Clinical Neurology / Department of Physiology, Anatomy & Genetics

Brief description:
Parkinson's disease (PD) is a degenerative neurological disease that has a population prevalence of 0.3%, increasing to 1% in those aged over 60 years. Despite current best management (using drugs or surgery) the disease inevitably progresses.
Several neurorestorative therapies have previously been attempted to treat PD. Early work successfully transplanted human foetal mesencephalic tissue. Unfortunately the randomised trial evidence is equivocal (Freed, Greene et al. 2001; Olanow, Goetz et al. 2003) and there are significant practical, legal and ethical complications in obtaining sufficient quantities of this tissue. Embryonic stem cell transplants have been shown to be effective in rat (Bjorklund, Sanchez-Pernaute et al. 2002) and non-human primate (Takagi, Takahashi et al. 2005) models of PD. There remain concerns about using embryonic derived tissue for ethical reasons and due to the long-term risk of uncontrolled differentiation following transplantation. In addition these cells are still allografts and therefore patients receiving them require prolonged immunosuppression.
Multipotent progenitor cells (adult stem cells) are found in a number of adult tissues. These offer the potential of autologous transplantation. Our international collaborators in Brisbane have shown the olfactory mucosa (OM) is an alternative source of multipotent neural progenitor cells in adult humans and rats (Murrell, Feron et al. 2005).
This project will investigate the potential of neural stem cells derived from the OM to treat the motor symptoms of PD in a rat 6-OHDA model of the disease. The aims of the project are: 1) To harvest and culture neural stem cells. 2) To demonstrate multipotency of these cells in vitro. 3) To transplant cultured cells into Parkinsonian animals and to assess their behavioural response. 4) To perform post-mortem analysis of cell differentiation. In addition it is envisaged that the investigator will utilise recently established collaborations to investigate the in vivo migration of transplanted cells.
Facilities also exist to allow more detailed in vitro characterisation of the cultured cells using electrophysiological and genetic techniques.
This work will provide the basis for extending the experiments to truly autologous transplants in non-human primates and if indicated a clinical trial.

Training opportunities:
The project offers training in systems neuroscience, cell biology and molecular biology. This will include cell culture, histology, immunohistochemistry and the use of animal models including neurological surgery. The training may be extended at the discretion of the investigator to include a combination of in-vivo imaging (MRI), genetic analysis and cell electrophysiology.

References:
Bjorklund, L. M., R. Sanchez-Pernaute, et al. (2002). Proc Natl Acad Sci U S A 99(4): 2344-9.
Freed, C. R., P. E. Greene, et al. (2001). N Engl J Med 344(10): 710-9.
Murrell, W., F. Feron, et al. (2005). Dev Dyn 233(2): 496-515.
Olanow, C. W., C. G. Goetz, et al. (2003). Ann Neurol 54(3): 403-14.
Takagi, Y., J. Takahashi, et al. (2005). J Clin Invest 115(1): 102-9.

Project title: Mouse models of motor neuron degeneration

Basic Science Supervisor: Prof K E Davies (kay.davies@dpag.ox.ac.uk) Department of Physiology, Anatomy and Genetics.

Clinical Supervisor: Dr Kevin Talbot (kevin.talbot@clneuro.ox.ac.uk), Department of Clinical Neurology

Brief description: a number of diseases are characterised by specific degeneration of motor neurons innervating voluntary muscle. Amyotrophic lateral sclerosis is a rapidly fatal adult onset neurodegenerative disorder which is untreatable. With the exception of 2% of patients who carry gain of function mutations in the SOD1 gene the cause is unknown. Spinal muscular atrophy is a childhood onset motor neuron disorder due to inactivating mutations in the SMN gene. Preliminary work in our group has shown that crossing the G93A SOD1mutant mouse with a mouse deficient in SMN leads to a more severe phenotype, suggesting that SMN may be a general motor neuron survival factor and that the pathways leading to cell death in these diseases may have overlaps. The aim of this project is to explore whether increasing SMN levels will protect motor neurons from SOD1 mediated damage by crossing the G93A mouse with a mouse which expresses SMN at high levels under the neuron specific prion (PrP) promoter. Mice will be phenotyped using behavioural and motor testing such as the rotarod. The number of motor neurons dying in the spinal cord will be assessed using standard histochemical methods. In parallel experiments a panel of neuronal (NSC-34) cell lines which stably express various SOD1 mutants will be transfected with SMN and assayed for effects on survival under conditions of oxidative stress. Cells will further be analyzed to compare the proteomic profile of cells overexpressing SMN to identify potential pathways which are modified. Any proteins which are differentially expressed will then be verified in the mouse model. The overall aim of this work is to determine if SMN is a plausible therapeutic target for therapy in patients with amyotrophic lateral sclerosis. A number of drugs are being developed as a treatment for SMA which act by increasing SMN levels.

Training opportunities: phenotypic analysis of neurological mouse mutants, primary neuronal and cell line culture, molecular biology and cloning, transfection of constructs in cell culture, transcriptional and proteomic analysis, rt-PCR and antibody staining.

References: recent publications from the group:

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Oliver PL, Bitoun E, Davies KE. Mamm Genome. 2007 Jul;18(6-7):412-24

Oliver PL, Keays DA, Davies KE. Behavioural characterisation of the robotic mouse mutant. Behav Brain Res. 2007 Aug 6;181(2):239-47.

McCullagh KJ, *etal* Neuromuscul Disord. 2007 Dec;17(11-12):970-9.

Jeans AF *et al*Proc Natl Acad Sci U S A. 2007 Feb 13;104(7):2431-6.

Ackerley S, *et al*. Hum Mol Gen. 2006 15(2):347-54

Project title: Ca2+ mediated axonal degeneration in multiple sclerosis

Basic Science Supervisors: Prof Lars Fugger (lars.fugger@imm.ox.ac.uk) Department of Clinical Neurology

Clinical Supervisor: Jackie Palace, Department of Clinical Neurology

Brief description:
Multiple Sclerosis (MS) is the commonest autoimmune disorder of the central nervous system (CNS), afflicting about 1 million people worldwide. The cause of MS is still unknown and disease pathways are poorly understood. MS is clinically reflected by neurological symptoms including motor dysfunction, hyperreflexia, spasticity, ataxia, visual and sensory impairment, bladder and bowel disturbances and fatigue. Although inflammation and primary demyelination are the most characteristic features of CNS lesions in MS, axonal degeneration correlates best with clinical deficits in the patients. It has been suggested that the inflammatory insult in MS leads to axonal degeneration by causing neuronal mitochondrial dysfunction with reduced ATP production and energy failure and alteration of ion exchange mechanisms. The final pathway of axonal degeneration would then be mediated by Na^+ and Ca^{2+} influxes which activate proteases and disrupt the cytoskeleton, similar to the pathophysiology seen in ischemia. We have recently show that acid-sensing ion channel 1 contributes to Na^+ and Ca^{2+} influx into neurons during inflammation with a deleterious outcome for axonal integrity. However, it is clear that these channels are only partly contributing to the progressive axonal degeneration in MS and that other channels are also involved in fluxing cations into neurons. In addition, it is likely that inflammation disturbs Ca^{2+} metabolism and storage.

Thus, the main purpose of the proposed research is to investigate several cation channel families for their expression in inflammatory CNS lesions in multiple sclerosis and its animal model (experimental autoimmune encephalomyelitis). In addition we will look at several newly discovered proteins, which regulate Ca^{2+} metabolism and storage. The aim of this research will be to identify channels or regulator molecules which are changed in their activation state by inflammation in the CNS and contribute to cation influx during a chronic inflammatory setting in the CNS. These proteins will then be tested for their relevance *in vivo* by using animal models for EAE and the respective knockout or genetically modified animals. Functionally these proteins will be analysed by electrophysiology and *in vitro* models for axonal degeneration. Final aim of this study is to identity new target molecules for a neuroprotective treatment strategy in MS patients.

Training opportunities: Training in methods of molecular and cellular biology and in electrophysiology. This will include immunohistochemistry, Western blot analyses, DNA sequence analysis and the use of PCR; cell culture and electrophysiology; appropriate use of genetically modified mouse models with the use of immunological techniques; the use of bioinformatic computer programs for sequence analysis.

References: (most recent publication from the group)

Friese MA, Craner MJ, Etzensperger R, Vergo S, Wemmie JA, Welsh, MJ, Vincent A and Fugger L. Acid-sensing ion channel-1 contributes to axonal degeneration in autoimmune inflammation of the central nervous system. **Nature Med**. 2007;13:1483-9.

Project title: The role of mRNA localization and translation in memory and learning in neurons

Basic Science Supervisor: Prof. Ilan Davis, (ilan.davis@bioch.ox.ac.uk) Dept. of Biochemistry.
Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description:

Background: mRNA localization is a common and important post-transcriptional method of regulation of the spatial distribution of proteins within cells. Examples are known for most types of proteins in all major model systems. In *Drosophila*, mRNA localization is essential for the establishment of the major body axes in the oocyte and embryo. Our lab has been using *Drosophila* to elucidating the molecular mechanisms of mRNA localization. We have previously shown that the main microtubule associated minus end directed molecular motor, Cytoplasmic Dynein, is responsible for transporting a number of key axis specification mRNAs.

mRNA localization is also thought to play a key role in memory and learning within neurons of all animals. mRNA localization and its translational repression at the tips of axons and dendrites allows neurons to respond very rapidly to specific signals by de-repressing translation and synthesizing particular proteins. This bypasses the lengthy process of sending a signal back to the nucleus to activate the expression of specific genes and transporting proteins to the cell periphery.

Aims: The goal of the project is to extend our studies to the *Drosophila* nervous system. The fellow will use reagents and methods that have been in routine use in our lab and will work together with two experienced senior postdocs, Dr Ana Maria Valles and Dr Richard Parton, who are experts in *Drosophila* nervous system physiology and cutting edge live-cell imaging techniques, respectively. The project will involve imaging GFP tagged molecular motors, trans-acting factors and fluorescent RNAs, as they move along axons and dendrites in wild type and mutant neurons. This work will build on our preliminary evidence using a GFP tagged subunit of the Dynein motor and trans-acting factors in larval neurons. Initial observations will be made using a widefield deconvolution microscope optimised for live cell imaging. The fellow will then progress to using a new microscope in our lab, known as OMX, which is particularly well suited for simultaneous multi-colour live cell imaging and has a revolutionary high level of sensitivity. It is the second replica, world wide, of a prototype of the microscope created in UCSF, San Francisco, by our collaborator Prof. John Sedat, a pioneer of widefield digital cinematography and image de-blurring.

Plan of investigation: During the project, the student will characterize the movement of Dynein containing particles to establish whether they are transported bi-directionally or uni-directionally in axons and dendrites. They will then determine whether the particle movements are also governed by the plus end directed motor Kinesin 1. Finally, they will attempt to image the movement of fluorescently tagged mRNAs and determine which of a number of known trans-acting factors are required for their motility, using *Drosophila* genetics. The longer term goal of the project is to study the influence of neuronal activity on the distribution and translational regulation of mRNA in neuronal termini, processes that are believed to be the basis of memory and learning.

Training opportunities: The fellow will be trained in the practice of basic biomedical research using molecular, cell biological and diploid genetic methods. They will have a unique opportunity to be trained in advanced live cell imaging including the use of revolutionary new microscope systems.

References:

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MacDougall, N., Clark, A., MacDougall, E. and **Davis, I.** (**2003**). **Developmental Cell** 4: 307-319.
Delanoue, R. and **Davis, I.** (**2005). Cell,** 122: 97-106.
Delanoue, R., Herpers, B., Soetaert, J., **Davis, I.** and Rabouille, C. (**2007**). **Developmental Cell**, *13*, 523-538.
Meignin, Alvarez-Garcia, **Davis** and Palacios (**2007**). **Current Biology 17, 1871-1878.**

Project title: Molecular mechanisms of regulatory RNAs

Basic Science Supervisor: Dr James Parker (james.parker@bioch.ox.ac.uk), MRC Career Development Fellow, Department of Biochemistry

Clinical Supervisor / Sponsor: To be decided, depending upon applicant's requirements.

Brief description: Short regulatory RNAs hold promise as tools to treat disease via selective knockdown of gene expression. Small interfering RNAs (siRNAs) are now widely used to selectively modulate gene expression in tissue culture in the laboratory. The power and versatility of this technique indicates a potential for effectiveness in humans. Proof-of-principle experiments demonstrate success in the treatment of some respiratory infections and sexually diseases, where the siRNAs can be applied directly to mucosal surfaces. The real excitement stems from the ability to selectively target any gene – if the sequence is known – thereby providing some lead against currently-untreatable disorders (such as genetic neurodegenerative diseases like Huntingdon's disease, amyotrophic lateral sclerosis and spinocerebellar ataxia type 1), in addition to providing new potential strategies against almost any viral infection.

To improve and optimise the effectiveness of the regulatory RNA strategy it is essential to understand the molecular bases for these mechanisms. My laboratory uses structural biology and biochemistry to analyse, understand and manipulate molecular machinery, focusing on components of the regulatory RNA pathways. We are interested in two principal systems: 1) the "Slicer" enzyme – otherwise known as "Argonaute" – that binds the "guide RNA" derived from the siRNA and targets the messenger RNA (mRNA) for degradation, and 2) the microRNA (miRNA) transporter complex (whose main component is the nuclear transporter Exportin-5) that is responsible for escorting miRNAs and small hairpin RNAs (shRNAs) from the nucleus where they are synthesized, to the cytoplasm where they function.

The project will involve a study of one of these two systems principally via structural biology. We will use molecular biology to generate expression clones for components of these systems, which will be expressed in heterologous hosts (either *E. coli* or insect cell culture). The proteins will be purified using standard techniques. Once isolated, the proteins will be mixed with siRNAs to form multi-component complexes that represent the functional molecular machines inside the cell. We will then analyse the molecular structures of these machines via either X-ray crystallography or single particle electron microscopy, techniques in which our laboratory specialises. The structures will reveal the molecular mechanisms of these systems and will be interpreted in the light of our current understanding of regulatory RNAs and siRNA function.

Training opportunities: The techniques of structural biology. This will involve molecular biology, protein purification and biochemistry, formation of molecular complexes, protein crystallisation, X-ray crystallography, electron microscopy and structure interpretation.

References:

1) Parker, J. S. *et al*. *EMBO J* **23**, 4727-37 (2004).
2) Bitko, V. et al. Inhibition of respiratory viruses by nasally administered siRNA. *Nat Med* **11**, 50-5 (2005).
3) Raoul, C. *et al*. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat Med* **11**, 423-8 (2005).
4) Parker, J. S. *et al*. Structural insights into mRNA recognition from a PIWI domain-siRNA guide complex. *Nature* **434**, 663-6 (2005).
5) Palliser, D. *et al.* An siRNA-based microbicide protects mice from lethal herpes simplex virus infection. *Nature* **439**, 89-94 (2006).

Project title: ***Drosophila*** **as a model system to study small RNA regulation in the nervous system**

Basic Science Supervisor: **Dr J-L Liu (jilong.liu@dpag.ox.ac.uk)** MRC Functional Genetics Unit, Department of Physiology, Anatomy, and Genetics
Clinical Supervisor:

Brief description: Small RNAs play roles at various levels of gene expression in development, such as chromatin architecture, transcription, and RNA processing, turnover and translation. We are interested in addressing how various small RNAs (mi/si/pi/snRNAs) may be related in complicated situation such as the nervous system. We are using *Drosophila melanogaster* as a model system because of the potential for combining genetic, molecular and developmental approaches.

We have recently demonstrated that uridine-rich small nuclear RNAs (U snRNAs) are concentrated in discrete cytoplasmic foci, which we have called U bodies (Liu and Gall, PNAS 2007). The enrichment of snRNA, snRNA-associated proteins and Survival Motor Neuron protein (SMN, critical for snRNP assembly) in the U body suggest that it might be the site for snRNP assembly. Intriguingly, U bodies always associate with cytoplasmic processing bodies (P bodies), which are specialized foci containing siRNAs, miRNAs and factors for mRNA degradation and translational repression. While the U body exhibits a different pattern and composition from the P body, there is mounting evidence to suggest a functional relationship between the two organelles.

Projects for a DPhil student will address any of the following questions: What is the significance of the U body-P body interaction in the nervous systems? What events are occurring underneath the physical contact of these two bodies? Do small RNAs have a common maturation site or share a common way station? Do various small RNAs have any cross-talk at all? What processes are reflected by the U body-P body interaction?

By taking advantage of the genetic tractability and the wealth of information available for *Drosophila*, we are now able to precisely dissect these cellular problems in a developmental manner. Our long-term goal is to understand small RNA regulatory networks, through which we hope to gain new insights into the molecular mechanisms of human diseases such as spinal muscular atrophy, a devastating neurodegenerative disorder.

Training opportunities: Training in methods of genetics, molecular and developmental biology. This will include Drosophila genetics, fluorescence in situ hybridization, laser-scanning confocal microscopy, immunochemistry, single-neuron analysis, and advanced molecular and biochemistry techniques.

References:
Liu JL and Gall JG (2007). Proc Natl Acad Sci U S A, 104(28):11655-9.

Project title: Neural Signals underlying Reward Processing and Pain

Basic Science Supervisor: Professor Morten L Kringelbach (morten.kringelbach@queens.ox.ac.uk Department of Psychiatry, University of Oxford and CFIN, Aarhus University, Denmark)

Clinical Supervisor: Professor Tipu Z Aziz and Mr Alexander L Green (tipu.aziz@dpag.ox.ac.uk and alex.green@nds.ox.ac.uk Department of Neurosurgery, John Radcliffe Hospital, Oxford)

Brief description: From both functional imaging in humans and animal studies, we know that the cingulate cortex and the medial prefrontal cortex are areas that is important in both pain processing (Petrovic and Ingvar, 2002) and the processing of reward-related behaviour (Amodio and Frith, 2006). Similarly, the periventricular grey area in the midbrain is important for pain control and blood pressure (Green et al., 2005). We routinely stimulate these areas (using 'deep brain stimulation') in patients with intractable neuropathic pain as well as depression (Owen et al., 2006; Kringelbach et al., 2007). However, we do not fully understand the underlying neural mechanisms that encode these behaviours. Patients with Deep Brain Stimulators provide a unique opportunity to study these areas as we can record from the electrodes as well as stimulate through them. We have also shown that it is possible to use magnetoencephalography (MEG) to map the effects of DBS. Our preliminary studies have shown that that there are frequency changes in the electrical activity in the PVG during pain, as well as a 'spindle' shaped signal that appears to be related to pain intensity. In the cingulate cortex, our preliminary studies indicate that dissociable areas of the cingulate cortex encode positive and negative reward behaviours

Thus, the main aim of this project is to study the local field potentials in various brain areas during different aspects of reward tasks as well as different pain states. Analysis of these potentials will enable us to determine how these behaviours are electrically encoded in the human brain. The project will also involve functional imaging with MEG during the same tasks to see if these signals can be detected externally (thus leading to similar studies on a large number of patients as well as 'normals'). In addition, Diffusion Tensor Imaging (DTI) will be used to look at the functional connectivity of the areas in question to build up a map of the functional circuits within the brain.

Training opportunities: Training will be provided in mathematical techniques of signals analysis including looking at local field potentials and recording from patients. Also, it will be necessary to learn the techniques of MEG and DTI.

References:

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Project title: Functional characterization of the KIAA0319 encoded protein and its role in neuronal migration and developmental dyslexia

Basic Science Supervisor: Prof Anthony Monaco (Anthony.monaco@well.ox.ac.uk), Dr. Silvia Paracchini (silviap@well.ox.ac.uk), and Dr. Antonio Velayos (avelayos@well.ox.ac.uk), Wellcome Trust Centre for Human Genetics.

Clinical Supervisor: Professor John Stein (john.stein@dpag.ox.ac.uk), Dept. of Physiology, Anatomy and Genetics.

Brief description: Dyslexia is specific difficulty in learning to read caused in large part by genetic factors[1]. We have recently identified the *KIAA0319* gene as a candidate for dyslexia susceptibility, by conducting genetic association analysis in large independent samples of families[2] and by gene functional characterisation. We showed that *KIAA0319* is specifically expressed during brain development and that it might be involved in neuronal migration, which is one of the key steps for the formation of the cortex[2,3]. Using cell line models over-expressing *KIAA0319* we have shown that this gene encodes a highly glycosylated protein which locates to the plasma membrane and is mainly exposed outside the cell[4,5]. KIAA0319 contains several PKD domains that have been implicated previously in cell adhesion functions. We are hypothesising that the *KIAA0319* gene might contribute to neuronal migration by mediating proper adhesion between the migrating neurons and the scaffold provided by the glia fibers. Interestingly, other candidate genes for dyslexia have also been implicated in neuronal migration[1].
We have also found that KIAA0319 undergoes ectodain shedding, a process by which plasma membrane proteins might be involved in signalling. We are currently characterising this process and performing studies to detect proteins interacting with either the C-terminal cytoplasmic domain or the N-terminal extracellular domain.

The main aim of this project is to investigate the role that the KIAA0319 protein may play in neuronal migration. Three different approaches will be used: 1) cell aggregation, cell adhesion and cell migration assays in cell culture models, using stable cell lines expressing KIAA0319 full-length and a number of deletion constructs; 2) use of human neuronal cell lines and mouse and/or rat primary neuron systems to detect and characterise the endogenous KIAA0319 protein; and 3) development of a functional assay to study KIAA0319. This assay will be based on the effect that KIAA0319 has in neuronal migration, and would involve the use of siRNA and different KIAA0319 constructs. It will also be used to test candidate KIAA0319-interacting partners and to analyse the effect that ectodomain shedding of KIAA0319 may have on neuronal migration.

Training opportunities: Training in cell and molecular biology techniques. These will include standard tissue culture and immunochemistry techniques; generation and characterisation of stable cell lines; generation of mammalian expression plasmids; siRNA knowdown; DNA sequence analysis, PCR and use of bioinformatics computer programs; Northern and Western blot analysis.

References: publication from the group

1. Paracchini, S., Scerri, T. & Monaco, A.P. *Annu Rev Genomics Hum Genet* **8**, 57-79 (2007).
2. Francks, C. et al. *Am J Hum Genet* **75**, 1046-1058 (2004).
3. Paracchini, S. et al. *Hum Mol Genet* **15**, 1659-1666 (2006).
4. Velayos-Baeza, A., Toma, C., da Roza, S., Paracchini, S. & Monaco, A.P. *Mamm Genome* **18**, 627-634 (2007).
5. Velayos-Baeza, A., Toma, C., Paracchini, S. & Monaco, A.P. *Hum Mol Genet* (2008), *in press*.

Project title: Whole genome association study of depression

Basic Science Supervisor: Professor Andrew Morris (amorris@well.ox.ac.uk , Wellcome Trust Centre for Human Genetics)

Clinical Supervisor: Professor Jonathan Flint Mott (jf@well.ox.ac.uk , Wellcome Trust Centre for Human Genetics)

Brief description: Major depression (MD) is among the commonest causes of disability worldwide but its biological basis remains poorly understood and treatments relatively ineffective: current regimes result in remission for about 55% of cases at 38 months. Following family, adoption and twin studies that reported a significant heritable component to MD, there has been considerable hope that a genetic approach would identify the condition's molecular basis, thereby opening the path to better diagnosis, prognosis and treatment.

Recent advances have made it possible a new to test the involvement of every gene in the genome, a methodology that in principle makes it possible to proceed directly from phenotype to molecular variant. However, this technology requires the analysis of large, well characterized patient samples. This project deals with the assessment and genetic analysis of 1,000 cases of MD and 1,000 controls from Shanghai, China. Collecting such a cohort is feasible in China where we have access to a hospital network that has already been developed for large scale studies of physical disease.

The aims of the project are as follows:

1. Recruitment of an ethnically homogeneous sample of 1,000 Chinese women with recurrent MD from Shanghai and 1,000 controls.
2. Extraction of DNA from 2,000 saliva samples and the completion of a whole genome association analysis.
3. Single-locus and haplotype association with MD will be modelled in a logistic regression framework, taking account of the effects of environmental and other non-genetic risk factors, and indicators of ancestry association analyses.

The student will work with our team in the UK and in Shanghai to collect data using a computer based assessment protocol. We have designed a comprehensive interview to collect a wide range of phenotypes related to depression; this will allow the student to explore, both at a phenotypic and genetic level, possible predisposing and precipitating factors to MD. Data will be uploaded directly to a server in Oxford for analysis. Saliva samples will be sent by post for DNA extraction and genotypes collected by one of the high throughput genotyping systems available in the Wellcome Trust Centre for Human Genetics. Single-locus association analyses will be performed within a generalised linear regression modelling framework. Within this framework, genetic effects can be tested in the presence of environmental and other non-genetic risk factors by covariate adjustment. Interactions with covariates can be incorporated to allow for gene-by-environment interaction effects. The project will be based at the Wellcome Trust Centre for Human Genetics where there a number of whole-genome association studies are already in progress.

References:

1. Wellcome-Trust-Case-Control-Consortium (2007): Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447:661-678.

2. Shifman S, Bhomra A, Smiley S, Wray NR, James MR, Martin NG, et al (2007): A whole genome association study of neuroticism using DNA pooling. Mol Psychiatry (epub ahead of print).

Training opportunities: Training in methods of genetic analysis of complex traits, including opportunities to develop either laboratory skills in genomic applications (DNA preparation, PCR, chip based genotyping) and/or analytical skills in dealing with whole genome data.

Project title: Translational investigations of the schizophrenia susceptibility gene NRG1

Basic Science Supervisor: Dr Ole Paulsen, Department of Physiology, Anatomy and Genetics.
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Clinical Supervisor: Professor Paul Harrison, Department of Psychiatry.
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Brief description: Neuregulin 1 (NRG1) is a leading schizophrenia susceptibility gene, implicated by a number of genetic association studies (1). NRG1 is a growth factor with many roles in neurodevelopment and plasticity, consistent with the prevailing view as schizophrenia as being a neurodevelopmental disorder (2). Recent studies have identified a putative functional polymorphism which impacts on the expression and function of one variant (isoform) of NRG1 (3,4). As part of our program of NRG1 research, we are studying a transgenic mouse that overexpresses the type I isoform of NRG1 under the Thy-1 promoter (5). We already have data showing that there is a behavioural phenotype to the mouse and initial electrophysiological studies are underway. The project will extend this work, using both electrophysiological and molecular techniques.

The main aim of the project is to elucidate the neurobiological effects of overexpressing NRG1 in the cortex of the brain, using the mouse hippocampus as a primary model. To this end, a multilevel approach of in vivo and in vitro techniques will be used. At a molecular level, you will characterise changes in expression of other NRG1 isoforms as well as downstream genes in the NRG1 signalling pathway (e.g. ErbB4 receptors, PI3K isoforms). At a microcircuit level, spontaneous network activity and synaptic plasticity (long-term potentiation) will be compared between NRG1 overexpressing mice and wild-type littermate controls. At a behavioural level, there would be an opportunity to study alterations in hippocampus-dependent behaviour. As well as studying the mice, the project will include the opportunity to further characterise NRG1 expression in schizophrenia, using a large collection of post mortem brains, as well as functional studies in cell lines. The work will be carried out in laboratories in the Department of Physiology, Anatomy and Genetics (DPAG; Dr Paulsen) and Psychiatry (Prof Harrison).

The project has potential to identify novel neurobiological mechanisms involved in schizophrenia.

Training opportunities: In addition to the standard Divisional training programme, this project would give you additional project-specific as well as generic skills: Electrophysiological (field recordings and patch-clamp) and molecular (qPCR, in situ hybridization, immnuohistochemistry, morphometry) methods. Use of both animal models and human brain tissue. Understanding of molecular and genetic underpinnings of schizophrenia, and opportunity to work with patients as part of clinical training. Attendance at seminars and lectures in both DPAG and the Dept of Psychiatry. Weekly lab meetings and journal clubs.

References:

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2. Harrison PJ, Weinberger DR (2005) *Mol Psychiatry* 10: 40-68.
3. Law AJ et al (2006) *Proc Natl Acad Sci USA* 103: 6747-6752.
4. Tan W et al (2007) *J Biol Chem* 282: 24343-24351.
5. Michailov et al (2004) *Science* 304: 700-703.

Project title: Combining Essential Dynamics with Docking for Ionotropic Glutamate Receptors.

Basic Science Supervisor:
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Clinical supervisor/sponsor: To be decided, depending upon applicant's requirements.
Brief description:
We are particularly interested in developing and applying computational methods including docking and molecular dynamics simulations to receptor proteins such as the ligand-gated ion channels and in particular the ionotropic glutamate receptors. These are receptors that upon binding of a ligand change their conformation such that ions can pass through a central pore and down their electrochemical gradient. They are essential for fast synaptic neurotransmission and as such play a central role in learning and memory. They have also been implicated in a variety of neurological disease states including Alzheimer's, Parkinson's, epilepsy and the effects of stroke. Upon binding of agonist (glutamate) the receptor undergoes a conformational change such that the transmembrane channel domain opens to allow the passage of ions to partially depolarize the surrounding membrane on the post-synaptic cleft. The receptor is a tetramer that can be comprised of identical or different subunits. Each subunit has several domains, and although there is no structural information for the whole receptor, there are currently over 60 X-ray structures for the ligand-binding domain of the receptor (Mayer 2006). The ligand-binding domain is comprised of two discreet lobes (D1 and D2) that bind glutamate at their interface in the manner of a Venus fly-trap (Figure 1). This information has provided static information about the apo and ligand-bound states of the domain from various different subtypes.

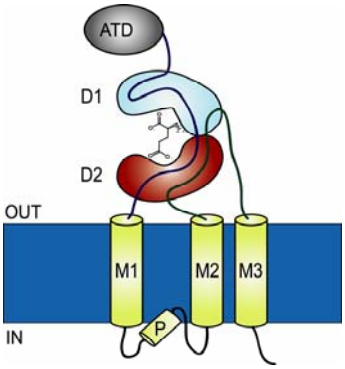

Figure 1. Subunit architecture of ionotropic glutamate receptors.

Essential dynamics is a method that can be used to extract out the important motions from the simulations trajectory. We can use this information to accelerate certain transitions (for example the cleft-opening/closing) in the protein. This is particularly useful as normal docking procedures are unable to take into account large structural deformations. My laboratory has a substantial amount of simulation trajectories for various ligand-binding cores from ionotropic glutamate receptors where the bound-state is already known and thus this represents a good test set that can be expanded to compounds for which there is currently no crystal structure. The project will extract out the "essential dynamics" and combine them with docking procedures to investigate whether docking can be improved for cases such as these where large conformational changes are expected. More information can be found at: **http://sbcb.bioch.ox.ac.uk**. The above project is only an example and alternative projects can be discussed.

Training opportunities:
From a training perspective the project will provide experience in a wide range of computational and theoretical skills as applied to biological molecules and systems. These will include: biomolecular simulations, drug-docking calculations, elastic network and/or finite element modelling, scientific programming and scripting (perl, python etc), use of parallel and distributed computing resources, and statistical analysis of data.

References:
Arinaminpathy Y, Sansom MSP, Biggin PC. 2006. Binding site flexibility: Molecular simulation of partial and full agonists within a glutamate receptor. Mol. Pharm. 69:11-18.
Mayer ML. 2006. Glutamate receptors at atomic resolution. Nature 440:456-462.

Project title: Reinforcement and mood elevation in bipolar disorder

Basic Science Supervisor: Dr RD Rogers (robert.rogers@psych.ox.ac.uk) Department of Psychiatry, Warneford Hospital, Oxford.

Clinical Supervisor: Professor Guy Goodwin (guy.goodwin@psych.ox.ac.uk) Department of Psychiatry, Warneford Hospital, Oxford.

Brief description: Bipolar disorder involves the experience of manic states characterised by elevated mood and a preoccupation with pleasurable activities to the detriment of affected individuals' health and well-being. Despite the centrality of mood elevation to the clinical presentation of bipolar disorder, there has been very little research into its underlying psychological and neural causes. Although disturbances in dopamine activity are almost certainly a factor, we know almost nothing about factors that precipitate the elevation of mood or the processes that convert elevated mood in vulnerable individuals into clinically significant illness. Improving our understanding of mood elevation will help us understand both the psychological and pathophysiological processes associated with the development, and relapsing character, of bipolar illness. Recently, we have found experimentally that deliberating about the good and bad possible outcomes of risky actions involves activity within the same neural circuits associated with emotional regulation, and that such deliberation about possible outcomes is particularly prolonged in individuals at risk for bipolar, but not unipolar, mood disorder. This suggests that altered reinforcement processing during value-based decision-making — known to involve mid-brain dopamine activity —is an important mechanism that destabilises mood in vulnerable individuals and might presage illness onset.

The project will investigate the relationships between reinforcement processing and mood elevation in both healthy, non-clinical control volunteers and in individuals at risk for bipolar disorder. We will use cognitive psychology and brain-imaging to investigate whether altered attention towards reinforcement cues (good versus bad outcomes) in value-based decision-making in individuals with a history of bipolar disorder reflects disturbed functioning and, possibly, connectivity within defined cortico-striatal-thalamic pathways known to be implicated in the pathophysiology of major mood disorders. The project will also investigate whether learning about the reward value of risky actions predicts elevated mood in individuals with a history of bipolar illness compared to healthy non-clinical controls. The research might also be extended to investigate whether individuals with a history of bipolar disorder are differentially susceptible to the emotional (and mood-destabilising) impact of choices promoting risk-aversion or risk-seeking behaviour, and whether altered reward processing is not a consequence of previous bipolar illness but can be observed in individuals at risk for bipolar illness but who have not been ill already (e.g. unaffected relatives of bipolar relatives).

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Training opportunities: The project involves a combination of cognitive psychology, the modelling of reinforcement learning and neuroeconomics, functional brain-imaging (fMRI), structural brain imaging (in the form of diffusion-tensor-imaging) as well as the opportunity to link basic science to clinical research in an important but relatively neglected area.

References: -

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Ochsner KN et al (2005). Trends in Cognitive Neurosciences 9(5): 242-249.
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Lawrence NS et al (2004). Biological Psychiatry 55(6): 578-587.
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Rogers RD et al (2004). Biological Psychiatry 55(6): 594-602.
Scarna A et al (2005). Psychopharmacology 179(4): 761-768.

Project title: Serotonin and inflammation in the pathophysiology of major depression

Basic Science Supervisor: Dr Trevor Sharp (trevor.sharp@pharm.ox.ac.uk) Department of Pharmacology

Clinical Supervisor: Professor P J Cowen (phil.cowen@psych.ox.ac.uk) Department of Psychiatry.

Brief description: Recent studies have indicated that major depression is associated with infection and acute inflammatory processes. In particular, specific proinflammatory cytokines including interferon-α, which are known to induce mood-lowering effects in both animals and humans, have been shown to have important effects on serotonin (5-HT), a neurotransmitter that plays a key role in the pathophysiology and treatment of depression. The aim of the present studies is to use translational methodology to link these two lines of investigation and thereby provide novel approaches to the understanding and treatment of depressive disorders.

Proinflammatory cytokines can alter 5-HT neurotransmission through two main routes: (a) reduced availability of the 5-HT precursor, tryptophan, through induction of the tryptophan metabolising enzyme, indoleamine dioxygenase (IDO); (b) altering the genetic expression of the 5-HT transporter and 5-HT-related downstream effector molecules involved in synaptic plasticity such as brain-derived neurotrophic factor (BDNF). We will investigate the effect of proinflammatory cytokines on 5-HT function using an integrated molecular through to whole systems approach.

We have recently established an animal pharmacofMRI model of 5-HT function, and found a striking decrease in response to the immune system stimulant lipopolysaccharide (LPS) (Raley et al, Proc of Brit. Pharm Soc, Dec. 2007). We will investigate the effect of LPS and proinflammatory cytokines (including interferon) using this model, and specifically examine the site of action (pre- versus postsynaptic), and the role of tryptophan and specific 5-HT receptor subtypes. In separate studies, we find that LPS and proinflammatory cytokines increase mRNA expression of the 5-HT transporter, and decrease that of BDNF. We will investigate these effects and their underlying mechanisms. Given recent evidence that early life changes in 5-HT transporter expression alter emotional behaviour in the adult (see Jennings et al, 2006), we will investigate the molecular and behavioural sequelae to immune system activation in young animals.

The treatment of patients with hepatitis C by Peginterferon will be used as a translational clinical model. Studies will be informed and guided by findings from the animal experimental work. Patients receiving interferon treatment experience high rates of depression and anxiety which can be studied in a prospective manner. 5-HT function in interferon-treated patients will be assessed using both peripheral markers (plasma IDO activity, tryptophan, BDNF) as well as two brain imaging techniques, (a) fMRI in a pharmacological paradigm with 5-HT challenge (pharmacofMRI) and ligand PET to measure the binding potential of the 5-HT transporter and specific 5-HT receptor subtypes. The latter studies will be carried out in collaboration with Professor Paul Grasby (MRC Cyclotron Unit, Hammersmith Hospital).

Training opportunities: gene expression, receptor autoradiography, animal imaging, animal models of depression and anxiety, clinical fMRI/PET

References: - publications by the group

Jennings KA, et al (2006) *J Neurosci* **26,** 8955-8964.
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Project title: Neuroimaging of central sensitization in chronic pain.

Basic Science Supervisor: Professor Irene Tracey (irene@fmrib.ox.ac.uk) Nuffield Department of Anaesthetics and Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB)

Clinical Supervisor: Professor Henry McQuay (henry.mcquay@pru.ox.ac.uk) Nuffield Department of Anaesthetics.

Brief description: Chronic pain is one of the largest medical health problems in the developed world affecting approximately 20% of the population and costing Europe €200 billion per annum. Until recently it has been difficult to obtain reliable objective information from normal subjects and patients regarding their subjective pain experience. Relating specific neurophysiologic markers to perceptual changes induced by central sensitisation, psychological or pharmacological mechanisms and identifying their site of action within the CNS has been a major goal for scientists, clinicians and the pharmaceutical industry. This information provides a powerful means of understanding not only the central mechanisms contributing to the chronicity of pain states but also potential diagnostic information (1). Identifying non-invasively where sensitisation and other amplification processes (psychological) might occur along the pain neuraxis for an *individual* and relating this to their specific pain experience or measure of pain relief has been the focus of our research (2-4). Using functional neuroimaging methods, such as functional magnetic resonance imaging (FMRI), positron emission tomography (PET) and electroencephalography (EEG) we have revolutionised our understanding of central pain mechanisms. The central relevance of descending brainstem modulatory pathways in the generation and maintenance of central sensitisation in chronic pain states is becoming increasingly apparent (5). In this project high-resolution functional magnetic resonance imaging in humans will be employed to characterise: (a) the brainstem neural correlates of descending facilitation, (b) their involvement in neuropathic pain states and (c) whether pharmaceutical agents commonly used to treat chronic pain can pre-emptively prevent the establishment of descending facilitation prior to injury and therefore subsequent chronic pain symptoms. A range of studies exploiting the capscaicin model of central sensitisation in normal subjects as well as neuropathic pain patients will be used. Drugs to be investigated will include duloxetine and pregabalin.

Training opportunities: Neuroimaging methodologies (experimental design, basic physics of MR and image analysis), CNS pain processing mechanisms and neuroanatomy, pharmacology of analgesia. The FMRIB Centre (where Professor Tracey is also Director) provides a recognized world-class extensive graduate and postdoctoral training program with lectures, seminars and tutorials occurring throughout the week during term-time. Professor Tracey's research team comprises over twenty postdoctoral and clinical researchers and who provide a rich environment for students to train within and be expertly mentored in the fields of neuroimaging and pain mechanisms. Professor McQuay will provide clinical expertise for the patient related work.

References: (from Tracey imaging group)

1. Schweinhardt et al., Imaging pain in patients: is it meaningful? Curr Opin Neurol. 2006;19(4):392-400. Review.
2. Ploghaus et al., Exacerbation of pain by anxiety is associated with activity in a hippocampal network. Journal of Neuroscience. 2001;21(24):9896-903.
3. Dunckley et al., A comparison of visceral and somatic pain processing in the human brainstem using FMRI. Journal of Neuroscience 2005;25(32):7333-41.
4. Iannetti et al, Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. Proc Natl Acad Sci U S A. 2005;102(50):18195-200.
5. Tracey I & Mantyh P. The cerebral signature for pain perception and its modulation. Neuron 2007;55(3):377-91. Review.

Project title: Translational investigations of COMT in cognitive function.

Basic Science Supervisor: Dr Elizabeth Tunbridge, Department of Psychiatry.
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Clinical Supervisor: Professor Paul Harrison, Department of Psychiatry.
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Brief description:
The human catechol-O-methyltranferase (COMT) gene contains a functional polymorphism ($Val^{158}Met$) that affects enzyme activity and is associated with cognition and prefrontal cortex (PFC) function (1, 2). Consistent with this, we have demonstrated that pharmacological manipulation of COMT in rats modulates PFC dopamine levels and alters PFC-dependent behavioural performance (3). We are currently investigating the role of COMT in hippocampal function, which may underlie genetic associations between COMT and obsessive compulsive disorder (4) and anxiety (5). The project will extend these findings by comparing the effect of brain penetrant (tolcapone) vs. non-brain penetrant (entacapone) COMT inhibitors on cognitive function in humans and animals. The project will test the effect of tolcapone and entacapone on cognitive function and brain activity, using functional imaging (fMRI and MEG). The mechanisms underlying the effects of these drugs on human brain function will then be investigated in animals. The work will be carried out in the Department of Psychiatry and will build on our strong collaborative links with other University departments.

Training opportunities:
The applicant will have the opportunity to learn a wide range of methods including functional imaging and cognitive testing in humans, and molecular, neurochemical and behavioural analysis in animals, including most of the methods used in references 2 and 3. The applicant will also gain understanding of molecular and genetic underpinnings of brain function, and the opportunity to work with patients as part of clinical training. The applicant will be joining an active and well-funded group with existing translational programs in COMT and other genes involved in psychiatric disorders. They will have access to appropriate research training, including attendance at seminars and lectures in the Dept of Psychiatry.

References:
6. Tunbridge et al (2006). *Biol Psychiatry*. 60:141-51
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10. Enoch et al (2003) *Psychiatr Genet* 13: 33-41.

Project title: Dissecting the role of insulin signalling in age-related degenerative processes

Basic Science Supervisor: Dr. Clive Wilson (clive.wilson@dpag.ox.ac.uk), Department of Physiology, Anatomy and Genetics.

Clinical Supervisor: To be decided, depending upon applicant's requirements.

Brief description: Specific diseases of ageing, in particular neurodegenerative disorders such as Alzheimer's Disease (AD) and Parkinson's disease (PD) are becoming increasingly prevalent in the ageing population. AD and PD typically arise sporadically in the population, but rare familial forms have been identified and several of the affected genes cloned. For example, mutations in *parkin* and *PTEN-induced putative kinase 1* (*pink1*) are associated with familial PD[1]. Recent studies of *Drosophila parkin* and *pink1* have revealed a linked role for these molecules in maintaining normal mitochondrial structure and resistance to oxidative stress, and controlling protein ubiquitination[2-5]. Flies mutant for either gene survive to adulthood, but they display a flightless phenotype at eclosion due to degeneration of indirect flight muscle, which like mammalian dopaminergic neurons, appears to be highly susceptible to *pink1* loss[2-5]. AD and Huntington's models in flies have also revealed that mutations leading to reduced signalling by the nutrient-sensing TOR kinase suppress the degeneration phenotype[6-7]. The TOR signalling cascade is positively regulated by insulin/insulin-like growth factor signalling (IIS), an evolutionarily conserved link that was again first demonstrated in flies[8]. Elevated IIS not only enhances neurodegenerative processes, but also promotes ageing[9], suggesting that some common cellular events underlie these two processes.

When the flightless phenotype of *pink1* flies was published last year, it immediately drew our attention to a recessive viable mutation in *PTEN*, a major antagonist of IIS, which we generated some years ago. When this allele is combined with several different strong loss-of-function *PTEN* alleles, about 60% of all mutant flies exhibit a progressive flightless phenotype over a four week period. Remarkably, the proportion of flies with this phenotype can be reduced by dietary restriction and mutations in other IIS components. Surprisingly, we have also found that *pink1*, which at least in mammals is upregulated by PTEN, must be maintained at low levels in *PTEN* mutant animals to prevent catastrophic degeneration of flight muscle in flies. This has led us to propose a model in which IIS modulates the effects of several degenerative and protective pathways, including PINK1/Parkin, and where imbalance of these pathways leads to degenerative events, ie., we postulate that ubiquitinated proteins produced by elevated PINK1/Parkin accumulate in our *PTEN* mutant because IIS suppresses mechanisms of protein clearance[7].

The aims of this project are: 1) to determine the cellular events that occur in flight muscle when IIS is elevated; 2) to establish the genetic and molecular links between PTEN and PINK1 in this system; 3) to identify other IIS-dependent pathways that affect indirect flight muscle survival and test our hypothesis that imbalance of these pathways is a critical factor in degeneration. Our findings should allow us to design approaches to begin to test for similar mechanisms in mammals.

Training opportunities: Training in methods of molecular, cellular and developmental biology, as well as genetics. This will include use of PCR, molecular cloning, immunohistochemistry, light, EM and confocal microscopy, and model systems for degeneration, particularly in *Drosophila*.

References:

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8. Goberdhan, D. & Wilson, C. (2003) Differentiation **71**, 375-97
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Project title: RNA interference based gene therapy for Spinocerebellar ataxia type 7

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Brief description: Spinocerebellar ataxia type 7 (SCA7) is a chronic neurodegenerative disease presenting with a classic autosomal dominant ataxia but which uniquely is associated with macular degeneration. It is one of the polyglutamine (polyQ) group of disorders, arising due to an expanded CAG repeat mutation in the ataxin 7 gene. Removal of the accumulated toxic polyQ protein has been shown to ameliorate the disease phenotype in mouse models. My laboratory is currently working to develop a novel RNA interference (RNAi) based therapy for SCA7. RNAi has been shown to have significant therapeutic potential for dominant genetic disease, where selective knockdown of a mutant allele would leave the wild-type allele largely intact for normal cellular function. However, mutation-specific silencing cannot be used to distinguish between the mutant and wild-type transcripts in polyQ disorders, given that the mutations are CAG repeat expansions. We have therefore taken a novel approach targeting a disease-linked single nucleotide polymorphism (SNP) linked to the mutation. We have generated promising preliminary data demonstrating the potential for allele-specific RNAi silencing, and have shown that silencing eliminates mutant protein aggregates and reverses the SCA7 disease phenotype. This approach has significant therapeutic potential not just for SCA7 but as proof-of-concept for RNAi therapies for neurodegenerative disease general. We now wish to carry out extensive pre-clinical studies to evaluate this therapeutic approach in animal models of the disease. The project will therefore comprise:

- the development of microRNA based vectors to optimise allele-specific silencing of the mutant gene
- the investigation of siRNAs as an alternative mode of silencing, given that synthetic siRNAs are in currently use in RNAi clinical trials targeting the eye
- the generation of lentiviral and adeno-associated viral vectors for RNAi delivery
- evaluation of mutant ataxin 7 silencing in patient-derived lymphoblasts
- extensive studies to evaluate this approach in transgenic mouse models of the SCA7, giving priority to targeting the retinal phenotype in the first instance

Training opportunities: Training will be provided in RNA and RNAi biology, cell culture, the generation of viral vectors, molecular analysis and behavioural phenotyping.

References:

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4. Xia H, Mao Q, Eliason SL, Harper SQ, Martins IH, Orr HT, Paulson HL, Yang L, Kotin RM, Davidson BL. RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia.Nat Med. 2004 Aug;10(8):816-20.