fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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UNIVERSITY OF OXFORD

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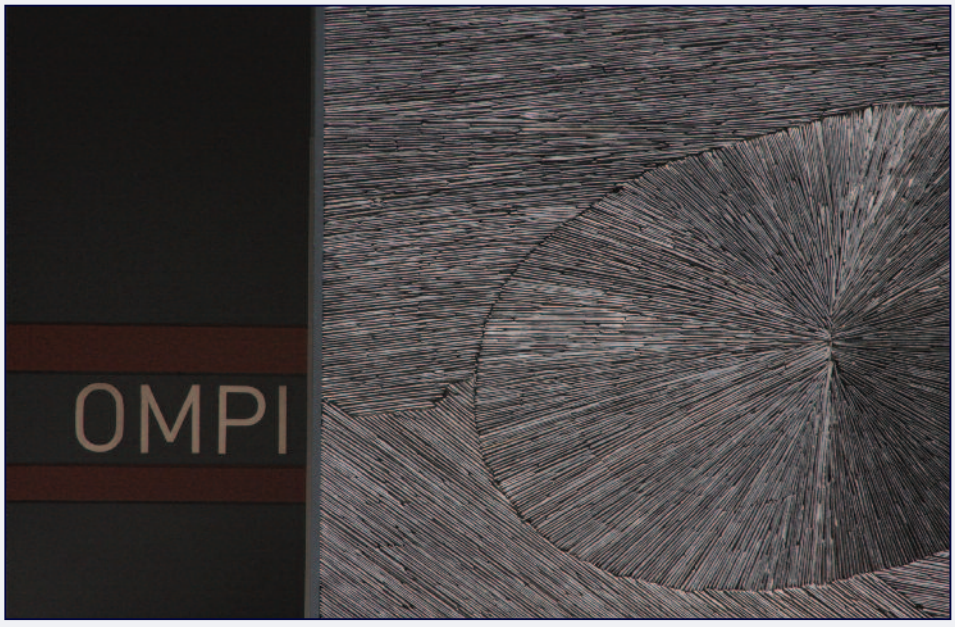
For a brief interlude (perhaps even longer!) we have had no building works going on at the Dunn School! Our extremely patient colleagues have emerged from their decant holes and, to our delight, are now well embedded in the new Oxford Molecular Pathology Institute (OMPI), albeit rehearsing their future responses to climate change, as we battle with the "modern" air conditioning systems that (thus far) seem to have minds of their own. With the welcome arrival of Prof Chris Tang (Microbiology) and his group, two floors of OMPI are now filled, leaving a further two floors as attractants to the next phase of the Department's evolution, once a new Professor of Pathology is appointed.

Dunn School science thrives on many fronts and this is reflected in the awards of the Professorial title of distinction to all of Stephen Cobbold, Ervin Fodor, David Greaves and David Vaux, the distinction of Fellowship of the Royal Society to Fiona Powrie, and independent research fellowships awarded to Eva Gluenz, Frank Vreede and Natalia Gromak.

Sadly, we have lost some valued colleagues this year. Mike Simpkins, who has been one of the long-standing Dunn School anchors, decided to retire. On a personal level Mike was always very adept and gentle at handling this particular Head of Department, if ever I got over-ambitious or unnecessarily involved in the minutiae of the service activities. Thomas Harder, a world authority of lymphocyte signalling, and his family have returned to careers in Germany. Nigel Saunders (Microbiology) has been appointed to a new Chair at Brunel University and will soon be building up his new Department there. Finally,

the sabbatical periods of Professor Tony Green (Cambridge) and Professor Ed Fisher (New York University) have proven too short for us — both have impeccably involved themselves in the life of our Department, acquired many friends, and left a long-lasting impression. Indeed, we are indebted to Tony Green for the photographs of the new OMPI building which appear throughout this edition of *Fusion* to mark its official opening. We hope that links with these fine friends of the Dunn School will continue once they return to their original habitats.

Herman Waldmann



Honours, Prizes, Awards

Fusion is delighted to publish this long list of distinctions awarded to Dunn School members in the last year. The first two appointments must be unique in Dunn School history. The editors are not aware that anyone from the Dunn School, who has moved into such high administrative office in the University. We wish Stephen Goss and William James well in their challenging and vitally important roles as Pro-Vice-Chancellors in these difficult times for universities.

Dr Stephen Goss was appointed Pro-Vice-Chancellor (Personnel and Equality) in July 2011. Dr Goss, a Fellow and Tutor in Medicine at Wadham College, a lecturer at the Dunn School of Pathology and Director of the Oxford Learning Institute, has held the position on an interim basis since January 2011.

As Pro-Vice-Chancellor for Personnel and Equality, he will provide leadership across the collegiate University and externally on a range of personnel and equality issues. The role includes overseeing the development and evolution of the University's Human Resources Strategy; and taking a lead on equality and diversity issues in relation to race, disability, gender and other equality strands.

Professor William James, Professor of Virology, Fellow of Brasenose College and James Martin Fellow, became Oxford University's Pro-Vice-Chancellor (Planning and Resources), in August 2011.

In the role, Professor James will be responsible for institutional and strategic planning and resource allocation. Among his duties as Pro-Vice-Chancellor, Professor James will chair the University's Planning and Resource Allocation Committee; take lead responsibility for ensuring the successful implementation and evolution of the objectives within the University's current Strategic Plan; and chair the Joint Teaching and Student Funding Review Group, developing policy following recent developments.

Professor Herman Waldmann has been elected a Fellow of the Royal College of Physicians (FRCP). The admission ceremony took place at the college on 16th June 2010.

Professor Fiona Powrie was elected a Fellow of the Royal Society in June 2011. Fiona, who is associated with the Dunn School, holds the Sidney Truelove Chair in Gastroenterology at the Nuffield Dept of Medicine.

Dr David R Greaves was awarded a 5-year Programme Grant from the British Heart Foundation in Nov 2010 to study: "Monocyte recruitment and macrophage retention in atherosclerosis".

Dr Ervin Fodor was appointed to the Readership in Experimental Pathology at the Dunn School. He delivered his inaugural lecture entitled: "Influenza virus RNA polymerase: from transcriptional control to innate immunity" on 12th May 2011.

Dr Anton van der Merwe's achievements in transforming our graduate training programme in the Dunn School have been recognised by his recent appointment as Divisional Director of Graduate Studies. Anton has also recently been awarded a project grant from CRUK.

Dr Eva Gluenz has been awarded a Royal Society University Research Fellowship starting in October 2011. She will work in the Dunn School on the project: "Host-parasite interactions: the role of the *Leishmania* flagellum in infection".

Dr Natalia Gromak has been awarded a Royal Society University Research Fellowship to study transcription and RNA processing in neurodegenerative diseases. She will start her project in October 2011.

Dr Chris Goulbourne was awarded an American Heart Foundation Fellowship in June 2011. Chris will move from David Vaux's lab to work in Steve Young's lab at UCLA. Chris was also awarded the Centenary Prize by the Biochemical Society for his presentation of his work at the **Nuclear envelope disease and chromatin organization** conference in Cambridge in July. This will involve him being flown back from UCLA by the Biochemical Society to attend an awards ceremony in London later in the year.

Dr Frank Vreede (Fodor lab) was awarded an MRC New Investigator Research Grant in June 2011 to study the regulation of influenza A virus replication and transcription. The grant will start in October 2011 at the Dunn School.

Dr Monika Gullerova was awarded one of four L'Oreal-UNESCO Woman in Science UK awards in June 2011. The Fellowships For Women In Science are awards offered by a partnership between L'Oréal UK & Ireland, the UK National Commission for UNESCO and the Irish National Commission for UNESCO, with the support of the Royal Society, to encourage and promote the role and achievements of women pursuing their research careers in the UK or Ireland in the fields of the life or physical sciences.

Finally congratulations to our colleagues, below, who have been awarded Professorial titles in the distinction awards announced in Aug 2011:

Stephen Cobbold: Professor of Cellular Immunology
Ervin Fodor: Professor of Virology
David Greaves: Professor of Inflammation Biology
Bass Hassan: Professor of Medical Oncology
David Vaux: Professor of Cell Biology


Stephen Goss


William James


Monika Gullerova

The Curious Tale of BRCA1

In this article, David Vaux discusses one of the topics of current research in his lab, without mentioning the rest of an eclectic mix of interests including neurodegeneration and gene expression regulation by the nuclear envelope.

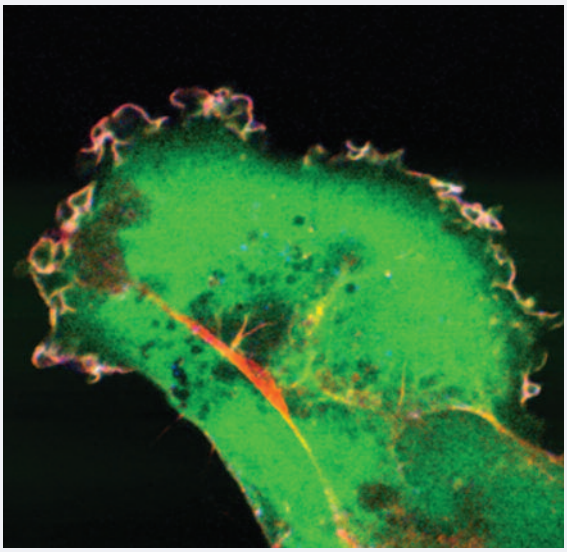


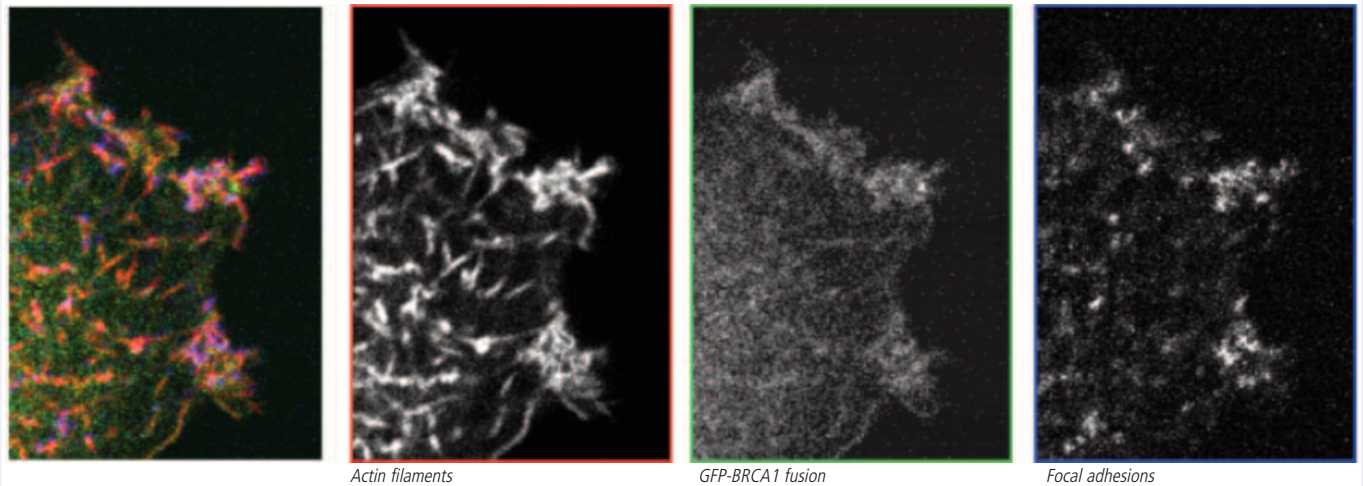
Figure 1

Human breast cancer cell. Red represents microfilaments, green BRCA1 and blue the ERM family proteins. White shows three-way co-localisation (at the light microscope level).

In the mid 90s I was interested in intriguing invaginations of the nuclear envelope that create a structure now called the nucleoplasmic reticulum. Shortly after our paper describing these structures appeared in 1997 in the *Journal of Cell Biology,* I was contacted by Professor Christian de Potter of the University of Gent who said that he found the breast cancer associated protein BRCA1 enriched in these nuclear membrane channels. And so began an ongoing fourteen year collaboration, firstly with Christian himself until his tragic early death at the age of forty two, and subsequently with his graduate student Elisabeth Coene, who then came to my lab for five years as a postdoctoral fellow.

To study the connection between a tumour suppressor gene product and an enigmatic nuclear structure we turned to antibody labelling at the electron microscope level. We quickly established that the story was not straightforward, and that enrichment of BRCA1 at nuclear channels was only rarely seen. On the other hand, we soon amassed a huge collection of images of BRCA1 staining in many different cell types and tissues, including human nasal epithelial cells generously donated by our electron microscopist Michael Hollinshead. Close examination of these images confirmed that much of the protein resided in the nucleus, as expected, but also revealed a substantial additional cytoplasmic pool in nearly all cells. When we turned our attention to this extranuclear BRCA1 we made two very unexpected observations; firstly, we found focal concentrations of the protein inside mitochondria, and secondly, we found BRCA1 associated with the plasma membrane and cytoskeleton at the cell periphery (Figure 1).

Puzzled at first, we soon realised that BRCA1 inside mitochondria made sense. The protein is intimately involved in sensing DNA damage in the nucleus and orchestrating the assembly of appropriate repair complexes, so it was not unreasonable to find the same protein pressed into service in the protection of mitochondrial DNA, the only other genetic material in the cell. Further microscopy supported this idea by revealing an association between the BRCA1 foci and the sites of clustered mitochondrial DNA. With helpful encouragement from demanding reviewers, we added detailed biochemical studies in which we systematically disassembled isolated mitochondria and confirmed that BRCA1 remains trapped inside until the very last moment. But this exposed a conundrum; the BRCA1 protein contains nuclear localisation sequences that explain its import into the nucleus, but none of the features of a conventional N-terminal mitochondrial import signal (MIS). We knew that the BRCA1 gene



Actin filaments *GFP-BRCA1 fusion* *Focal adhesions*

Figure 2.

HeLa cells expressing the GFP-BRCA1 C-terminus fusion protein showing association of the fusion protein with the microfilament cytoskeleton, especially at peripheral focal adhesion sites

generates a variety of transcripts, giving rise to alternative forms of the protein, so we set out to find an alternative form that might include an MIS. After a good deal of work, a number of new isoforms of BRCA1 were found, but not one gave a hint of an MIS. Fortunately, at this moment a paper appeared describing mitochondrial import driven by signals found at the C-terminus of several proteins. It was a quick experiment to hook up the C-terminus of BRCA1 to a green fluorescent protein reporter and look for mitochondrial import. But we couldn't see any. After a while the penny dropped: the GFP was folding in the cytoplasm before import and the subsequent import process permanently unfolded the reporter, destroying is fluorescence. Staining the cells with an anti-GFP antibody confirmed that the fusion protein had indeed been imported into the mitochondria. We now describe this embarrassing episode as a carefully designed experiment to confirm that the C-terminal import signal operates after the protein has folded in the cytoplasm. In support of this conclusion, recent proteomic experiments have implicated a chaperone, a heat shock protein homolog, in this novel import process.

Does it matter that BRCA1 can be imported into mitochondria? To answer this question we devised a quantitative PCR experiment to measure damage levels in mitochondrial DNA. Using this assay we were able to show that human breast cancer cells lacking BRCA1 seem to have higher levels of damage and poor repair. Expression of BRCA1 in these cells restored mitochondrial DNA repair levels. We are now planning further experiments to understand both the novel BRCA1 import pathway and the unexpected role of this protein within mitochondria.

The other concentration of extranuclear BRCA1, at the plasma membrane and in association with peripheral cytoskeleton, was less easy to rationalise. BRCA1 had been shown to have multiple functions, but all of these were nuclear, or at the spindle and centrosomes, especially during mitosis. None of these required protein at the plasma membrane. Mulling over this problem we returned to the images of cells expressing the GFP-BRCA1 C-terminus fusion protein and noticed that there was intense fluorescence at leading edges in motile cells. We wondered if this high level of a non-functional GFP fusion protein might interfere with the real function of BRCA1 at these sites. There followed a marathon of live cell imaging experiments which culminated in the demonstration that the GFP-BRCA1 C-terminus fusion protein expressed at high levels caused a motility phenotype, with increased spontaneous cell migration and severely distorted healing of an experimental wound in a cell monolayer (Figure 3). When we subsequently found that human breast cancer cells lacking BRCA1 are also hypermotile, and that this defect is reversed by engineering the expression of wild type BRCA1 into them, we knew we were on to something interesting. We got particularly excited in 2010 when transcriptome and proteome experiments from other groups on clinical samples led to the conclusion that reduced expression of BRCA1 correlated with metastatic disease and poor clinical outcome, both in breast cancer and other cancers, including prostatic, colorectal, and pancreatic carcinomas.

We are now beginning to believe that the unexpected localization results from our old EM data started an eight year convoluted trail that has led us to a potentially critical role for BRCA1 in regulating metastasis in multiple tumour types. The race is now on to try to understand how this regulatory machinery works at the molecular level, not least because it may reveal important new drug targets in the fight against cancer.

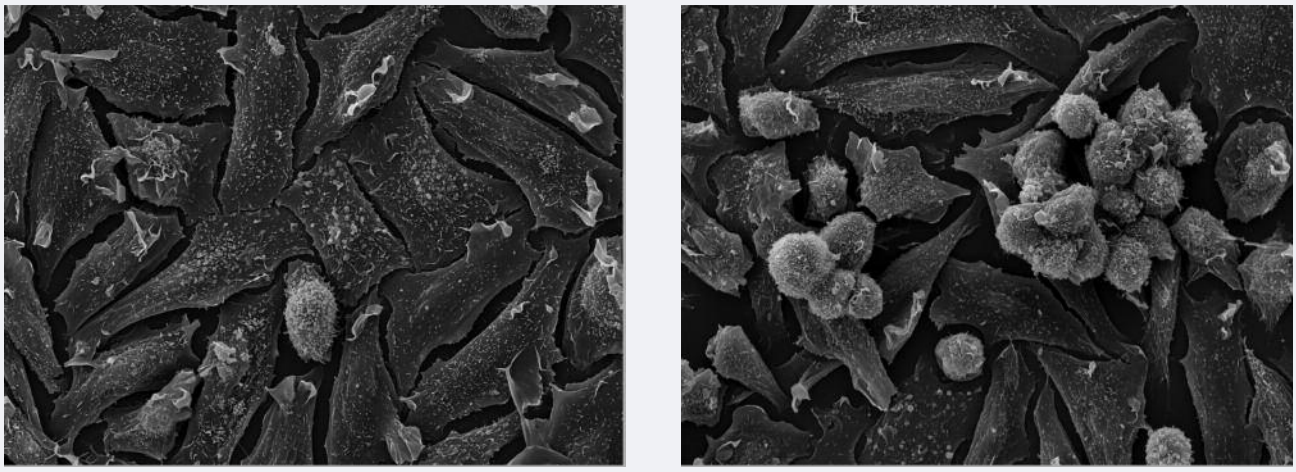


Figure 3. *SEM images from a wound healing experiment comparing wild-type CHO cells (left) with cells expressing the trans-dominant inhibitor of BRCA1 plasma membrane targeting (right). Cells lacking PM BRCA1 lose substrate contact, change morphology and heap into multiple cell layers*

A Niche of One's Own

Eva Gluenz

"...the Leishmania parasite has evolved mechanisms to subvert phagocyte defence mechanisms and proliferate inside the very cells that ought to kill it. If we understand how it does this we may, in time, be able to tip the balance of the immune response back in our favour." Here Eva Gluenz introduces the research project she intends to pursue as a newly appointed Royal Society University research fellow.

Figure 1. Leishmania mexicana *promastigotes.*

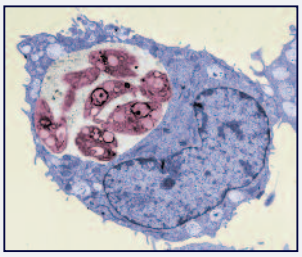


Figure 2. Leishmania *parasites (red) in the vacuole of an infected macrophage (blue).*

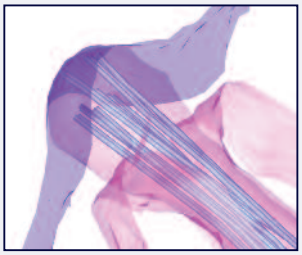


Figure 3. Electron tomography model showing the tip of the Leishmania *amastigote flagellum (pink) in intimate contact with the macrophage vacuole membrane (dark blue).*

Trypanosomatid parasites are remarkable in their ability to inhabit vastly diverse niches. Look closely and you will discover these single-celled flagellates attached to insect guts, swimming in plant sap and animal blood, crowded in cellular vacuoles or free floating in host cell cytoplasm. Even under crossfire from sophisticated immune defence mechanisms these parasites thrive, and by doing so cause much human suffering and death. Twelve million people worldwide are currently infected with parasites of the genus *Leishmania* (Figure 1). Blood-feeding phlebotomine sand flies deposit the parasites in the skin where they are taken up by neutrophils, macrophages and dendritic cells, whose role it is to destroy invading pathogens and alert the adaptive immune system to their presence. Rather than succumbing to these attacks however, *Leishmania* parasites set up residence in parasitophorous vacuoles inside these cells. Disease symptoms caused by a *Leishmania* infection range from localised self-healing skin lesions in cutaneous leishmaniasis to a systemic infection called visceral leishmaniasis, which is almost always fatal if untreated. Leishmaniasis disproportionately affects the poorest people in the world, as is the case in the recent upsurges of the disease in Afghanistan and Southern Sudan. The multitude of *Leishmania* species, diversity of vectors, zoonotic reservoirs and contributions of host genotype to disease outcome all complicate investigations of this disease. One central question remains unanswered however: how is the *Leishmania* parasite equipped to survive inside its very special niche in the phagocyte (Figure 2)?

The award of a Royal Society University Research Fellowship now allows me to investigate this question. This is a great chance to build on important recent insights into the cell biology of trypanosomatids, decoding of their genome sequence and investment in genetic tools. I joined Keith Gull's lab here in the Dunn School in 2004 because of its reputation for innovative research in all of these areas. Many of the key players orchestrating trypanosome cell division were discovered in the Gull lab. In my postdoctoral work on *Trypanosoma brucei* I studied the architecture of its nuclear and mitochondrial genome and showed how its elaborate network of mitochondrial DNA circles (a structure called the kinetoplast) is divided during cell division. This work also revealed a novel function of DNA topoisomerase II. These mechanisms are possibly widely conserved among all plant, insect and animal trypanosomes. Yet during their life cycle, as trypanosomes move from one environment to another, cell division must occasionally produce daughter cells equipped to sense and respond to changed nutrient availability, temperature shifts and potentially lethal host defence mechanisms.

How do *Leishmania* sense and respond to the environment they encounter inside a phagocyte? Exposure to acidic milieu and raised temperatures trigger a morphological change in the parasite: the slender promastigote form with a long motile flagellum turns into a smaller rounder amastigote form. These amastigotes have a short stubby flagellum whose function has received little attention; even its structure was uncertain until recently. With a graduate student, Amy Smith, I infected macrophages in the lab to study the intracellular *Leishmania* by 3D electron microscopy. Eukaryotic flagella and cilia are highly conserved microtubule-based structures and there are two major types. Motile flagella, including that of *Leishmania* promastigotes are built around a central pair of singlet microtubules surrounded by a ring of 9 microtubule doublets, with associated dynein motor proteins. Sensory cilia, by contrast, lack the central pair and associated structures; unexpectedly, it was this configuration we found in the amastigotes! This discovery, and the further observation that many of the amastigotes in the vacuole were positioned with their flagellum tip seemingly anchored to the vacuole membrane (Figure 3), led to my hypothesis that the amastigote flagellum acts as a cellular 'antenna' with a role in host-parasite interactions.

I will now test this idea using a combination of biochemical, microscopy-based and genomic approaches, and by assessing the phenotypes of mutant *Leishmania* in infection assays. Venturing into the domain of immune cells will, of course, provide exciting new opportunities to collaborate with macrophage groups in the Dunn School. Moreover, I have the privilege of working with exceptionally talented students, be they graduate students on the Wellcome Trust 4-year PhD programme in Infection, Immunity and Translational Medicine, or gap year students with a passion for science who come to the lab to gain their first research experience.

Being involved in a small amount of teaching for the Department is a great way of conveying to medical students here in Oxford the central themes of my research: the fascinating cell biology of parasites and the tough challenges faced when dealing with neglected tropical diseases. I will continue to run the Molecular Parasitology theme for FHS students here in Oxford together with Keith Gull. Moreover, the training workshops we run regularly for biomedical researchers in Africa provide important connections with people who have first-hand experience of the difficulties of diagnosing and treating these parasitic diseases.

Most importantly, this fellowship gives me time to work at the bench to tackle an important question: the *Leishmania* parasite has evolved mechanisms to subvert phagocyte defence mechanisms and proliferate inside the very cells that ought to kill it. If we understand how it does this we may, in time, be able to tip the balance of the immune response back in our favour.

Eva Gluenz

Interview with Natalia Gromak

Natalia Gromak was recently awarded a Royal Society University Research Fellowship and kindly agreed to be interviewed by Fusion about her career to date and her plans for the future...

Tell us a little about your background and what first brought you here to the Dunn School.

I was born in Minsk, the capital of Belarus, which, at that time, was a part of the USSR, but currently it is an independent country. In a sense, I was lucky to be brought up there, since the majority of former Soviet Union countries are still known for their high quality of education. I come from an academic family (both of my parents are mathematicians), therefore studying science at University was quite a natural path for me and I knew it from a very early age. In school I enjoyed all science subjects, in particular Maths and Biology. However, my family was a bit surprised when I chose to study Biochemistry at University, because even my sister followed in my family's footsteps and studied Maths.

In those days, Belarus was a closed country, internet did not exist and nobody was allowed to travel abroad, so it was very hard to get any information from outside the USSR. During the third year of my degree, I was fortunate enough to hear about the Darwin Trust scholarship, providing support for two final years of the Molecular Biology programme in the University of Edinburgh. The Darwin Trust was funded from the royalties to Professor Sir Kenneth Murray, whose team discovered the first genetically engineered vaccine against hepatitis B. Professor Sir Kenneth Murray became chairman of the Darwin Trust and co-founder of the first biotechnology company Biogen. To be honest, I had absolutely no idea what it would be like to live and study abroad. However, it was my Dad who strongly encouraged me to apply for this scholarship and supported me though all my subsequent years of living abroad. In the end I was very lucky to receive funding from the Darwin Trust and to be accepted in Edinburgh. However clichéd it may sound, this event really changed my life forever. I should add, that since that time, the Darwin Trust continued to support students from Belarus and increased its funding for graduate and Post-Doctoral levels. I feel honoured that I was one of the very first people funded by this generous Trust. The University of Edinburgh is famous for its strong standing in the RNA and gene expression fields, and since those early days it became a deciding factor in my future scientific career. Among my lecturers were Professors David Tollervey, Adrian Bird, Jean Beggs, Kenneth Murray, Noreen Murray, David Finnegan and Bill Earnshaw. I was extremely lucky to be in this environment, and I shall never forget those inspirational lectures and seminars given by such talented and very successful scientists. I found that two years in Edinburgh passed quite quickly, mainly due to the intensity of the Molecular Biology course. I graduated from Edinburgh University with high marks, allowing me to receive another scholarship from the Darwin Trust to do my PhD in the lab of Professor Chris Smith in the University of Cambridge.

Natalia Gromak

At that time Professor Smith was a young PI, who just recently started his independent lab, working on the regulation of alternative RNA splicing. I am very grateful to Chris for accepting me to do a PhD in his lab and for teaching me all the basics of scientific research, including critical thinking, problem solving and never forgetting about all-important controls! In those days, alternative splicing was something quite novel and because the human genome was not yet sequenced, people did not really realise the extent of this process in human cells. We were one of the first labs in the UK to work on molecular mechanisms of alternative splicing. We were interested in understanding how different alternative splicing pathways are regulated in different cell types. During my PhD studies I discovered a novel splicing factor 'Raver', involved in regulation of muscle-specific splicing decisions. I also characterised the mechanism by which antagonising splicing regulators can interact and eventually lead to different splicing outcomes.

Following my PhD studies, I wanted to expand my knowledge of RNA regulation, and decided to join the lab of Professor Nick Proudfoot, here in the Dunn School. Professor Proudfoot's lab attracted me by the breadth of the research topics related to RNA metabolism and the lab's expertise in different eukaryotic experimental systems (human and yeast).

What has been the focus of your research so far during the years you have spent here?

During my scientific career to date, I have always been interested in RNA metabolism at different stages of the gene expression process. During my PhD years it was RNA splicing. During my Post-Doctoral career in Professor Proudfoot's lab I have been fortunate to be involved in a number of different exciting projects. Among the main topics, which I have been investigating, are transcriptional termination, RNA processing and co-ordination between these two processes in humans. The most recent avenues of my research include the study of RNA degradation enzymes, such as exosome and 5′-3′ exonuclease Xrn2, miRNA biogenesis and post-translational modifications in splicing. I am very happy that Nick has given me the freedom to explore various avenues within the gene expression field. It extended my experimental expertise and knowledge, but also allowed me to closely collaborate with many top labs in the field and develop my own research programme, which I aim to pursue as an independent PI.

What has been the most exciting discovery you have made?

During my Post-Doctoral career in Oxford, one of the major discoveries was made with a graduate student at that time, Steven West. Together, we discovered the mechanism of transcriptional termination in humans. This process was over-looked for many years, as the majority of studies in the field were carried out on initiation and elongation of transcription. Prior to our work, a number of theoretical models of transcriptional termination existed, however, they were not tested experimentally in human cells. We were lucky that RNA interference technology appeared which allowed us to knock down proteins in human cells to test these models. We discovered that the 5′-3′ exonuclease Xrn2 in humans plays a key role in the process of transcriptional termination of protein-coding genes. Xrn2 is essential for nuclear degradation of the nascent RNA downstream of the polyadenylation (pA) signal, prior to transcriptional termination of RNA Polymerase II. The Xrn2-mediated transcriptional termination process is conserved among other eukaryotic organisms, including yeast. Our discovery initiated many studies to uncover further molecular details of the termination process. Our work has also lead to subsequent studies on the Xrn2 protein and identifying its key nuclear RNA degradation function in many other regulatory steps of gene expression.

What will be the focus of your research as you embark on your Royal Society Fellowship?

During my Royal Society Fellowship, I would like to apply my knowledge of fundamental RNA biology in transcription and RNA processing to the study of human disease. It is known that quite a large proportion of human diseases arise due to mutations in proteins involved in RNA metabolism. I am particularly interested in looking at neurodegenerative diseases. It is quite fascinating how mutations in the proteins governing fundamental processes of gene expression in each human cell, can cause such devastating consequences as neurodegeneration.

In particular, I am going to study the function of human senataxin protein, mutations of which cause neurodegenerative ataxia AOA2, which is considered to be the second most frequent type of recessive ataxia after Freidrich ataxia. My work demonstrates that mutations in the senataxin protein affect alternative RNA splicing, potentially providing an explanation for disease symptoms. So far, the molecular function of senataxin in splicing has not been investigated. It is not known, for instance, why the mutations in senataxin cause neurodegeneration, or what the genome-wide effects of senataxin-deficiency might be. In the long term, I am very interested in applying the findings from this research to development of molecular therapies, which can improve the health and quality of life of AOA2 patients. After many years working on fundamental questions of gene expression, I am very excited about the opportunity of doing research which can have direct medical applications.

How has the environment of the Dunn School helped you in your research so far?

Throughout my research career this Department was excellent in terms of its scientific reputation, research facilities, extremely collaborative environment and supportive infrastructure. However, for me the main highlight of the Dunn School has always been the people who work here. During my application process to the Royal Society, I received a lot of support and inspiration from the senior members of this Department. In particular, I am very grateful to Professors George Brownlee, Peter Cook, Herman Waldmann and Iain Campbell (Biochemistry Department), who always found time to talk to me and give me their invaluable advice. I think without the existence of such people, it would be very hard for any young PI to start their independent career. Dr Shona Murphy, Professor Elizabeth Bikoff, Professor Liz Robertson, Dr Chris Norbury and Professor Stephen Bell were particularly helpful at the time of my interview preparation. Last but not least, I would like to thank Professor Nick Proudfoot, for his positive approach to life and continuing inspiration during my stay in his lab.

Now when I am embarking on my independent research programme, I feel that the Dunn School is a very welcoming and inclusive place to be and it has a lot to offer to a young group leader, like myself. From a scientific point of view, the Dunn School has well-renowned expertise in the field of gene regulation, and I am extremely fortunate to be able to build upon my existing connections with these labs. In addition, I am interested in establishing collaborations with the Oxford Stem Cell Institute, mainly because of my interest in induced pluripotent stem cells and neuronal differentiation from fibroblasts. This will be particular valuable in studying senataxin mutations and their function in neurodegeneration. Further afield, I am looking forward to establishing new collaborations with labs in the Department of Anatomy, Physiology and Genetics, working on ataxias, and learning from their experience in neuronal cultures and mouse models of neurodegeneration.

What advice would you give to those who are considering embarking on a career in research?

Embarking on a career in academic research is certainly not an easy path. On occasions, it can be tough and quite frustrating, but overall I think it is a rewarding and very exciting job. If you have chosen a career in research, believe in yourself and your ideas, pursue your dreams and don't be put off by small bumps along the way!

The James Martin Stem Cell Facility

Sally Cowley

Sally Cowley is a Wellcome Trust Career Re-entry Fellow, and Head of the James Martin Stem Cell Facility. Here, she describes the establishment of the Facility and the numerous interdisciplinary collaborations it has fostered.

Since human embryonic stem cells (hESCs) were first derived from the inner cell mass of blastocysts by James Thomson in 1998, there has been enormous interest in their use both therapeutically and as human cellular models. This interest has been based on the fact that they can be expanded indefinitely as karyotypically normal lines, and that they are pluripotent, with the potential to differentiate into all types of cell of the human body.

As if this wasn't exciting enough, the stem cell field has recently been completely revolutionized, from a somewhat unexpected angle, by the discovery that differentiated somatic cells can be reprogrammed to a pluripotent state very similar to that of hESCs. This was achieved by Yamanaka's group in 2007 by retroviral transduction of four key pluripotency-associated transcription factors into human fibroblasts (using Oct4, Sox2, Klf4, and c-Myc), a feat achieved concurrently by Thomson's group (using Oct4, Sox2, Lin28, and Nanog). Cells reprogrammed in this way are called induced pluripotent stem cells (iPSCs).

Human iPSCs (hiPSCs) by-pass the ethical issues surrounding hESCs (which require the destruction of a human blastocyst in order to generate a self-renewing line) and somatic cell nuclear transfer, which has the potential to create viable cloned organisms. The use of hiPSCs therapeutically is greatly anticipated, as it would be expected to reduce the problem of histoincompatibility encountered by hESCs; however, there are significant safety issues to address before this is achievable. Of more immediate benefit to medical science is the use of hiPSCs to study the etiology of disease. Because hiPSCs can be derived with relative ease from patients' own skin biopsy fibroblasts and can then be differentiated to potentially any cell type, they enable the study of human tissues that are not normally available in the context of a patient's genetic background.

Using hESCs and hiPSCs, and their differentiated progeny as models for studying human disease, is the central aim of the James Martin Stem Cell Facility. The Facility was established in 2008 by William James, together with Sally Cowley, to address a growing need within Oxford for human pluripotent stem cell expertise. It is part of the Oxford Stem Cell Institute (directed by Paul Fairchild) and has received significant investment from the Oxford Martin School. It has recently relocated to the glorious new environment of the 2nd floor of the OMPI building (Figure 1), and has two dedicated research assistants. Cathy Browne (James Martin Facility Research Assistant) maintains the high level of quality control and information integrity required by the demanding and complex projects hosted by the Facility. Jane Vowles (Parkinson's Disease UK Research Assistant) specializes in the reprogramming of human skin cells to iPSCs. Through collaborative arrangements with many laboratories in Oxford and beyond, we are able to share these technologies with those working in diverse fields. These can currently be grouped into the four following interdisciplinary research themes:

Sally Cowley

Macrophages in Human Health and Disease

The James lab has established a method for differentiating monocytes and macrophages from hESCs. These macrophages are useful disease models - we have shown that they are susceptible to HIV (see theme 2 below) and Dengue virus and have an immunomodulatory phenotype. Genetic manipulation at the stem cell level can be used to achieve genetic modification at the macrophage level (Figure 2), although careful vector design, selection and experimental planning is required to counteract potential silencing of transgenes during differentiation.

Figure 1. The new premises of the James Martin Stem Cell Facility, OMPI level 2.

Chronic Granulomatous Disease is a serious monogenetic disease in which mutations in components of the phagocyte oxidase lead to deficient oxidative burst capacity in neutrophils and macrophages, leading to recurrent infections which severely reduce the quality and length of life. In a project to evaluate gene therapy approaches to CGD, the Stem Cell Facility is successfully producing macrophages from iPSCs from CGD patients, and these cells demonstrably reproduce the disease phenotype (collaboration with Reinhart Seger and Janine Reichenbach, University Children's Hospital, Zurich, and Majlinda Lako and Lyle Armstrong, University of Newcastle).

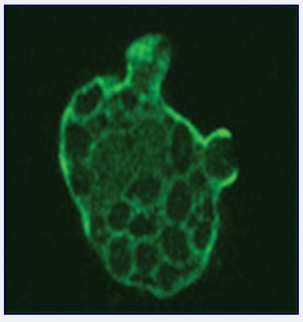


Figure 2. hESC-derived macrophage expressing GFP-actin, phagocytosing yeast particles (unstained).

HIV and AIDS

Macrophages are a key target for HIV during infection, creating a viral reservoir in these long-lived cells. The development of hESC-derived macrophages means we are in a unique position to undertake precise genetic experiments to investigate the molecular interactions between HIV and the macrophage in order to understand disease pathogenesis and identify potential therapeutic pathways (Figure 3).

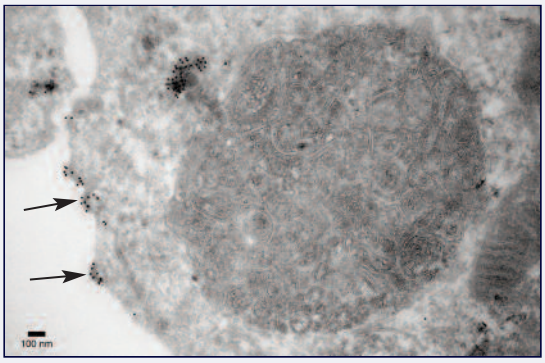


Figure 3. *HIV budding from an hESC-derived macrophage (arrows, p24 immuno-gold staining).*

James Martin Stem Cell Research Fellow Kenny Moore is developing a new set of powerful genetic vectors both to engineer precise genetic corrections into stem cells and to express potentially therapeutic genes in a controlled manner. He is using these methods, amongst other things, to investigate molecular pathways during macrophage infection by HIV. Quentin Sattentau's group, on the other hand, is interested in how phagocytes recognise and ingest HIV-1-infected T cells and, in the case of macrophages, become infected by the virus. We are collaborating with them on ways of visualising this phenomenon using ES-derived macrophages and neutrophils.

The Stem Cell Facility has been collaborating on two separate projects investigating the molecular pathways by which the HIV core reaches the nucleus of infected macrophages (Ariberto Fassati , Wohl Viron Centre UCL, and Greg Towers, UCL). By working with genetically modified hESC-derived macrophages generated by the Stem Cell Facility, they have been able to demonstrate the role of nuclear import proteins in HIV infection of authentic macrophages.

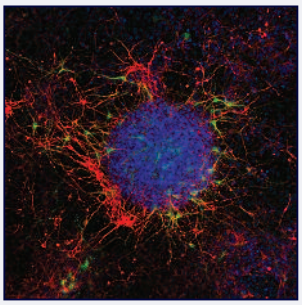


Figure 4. *Dopaminergic neurons differentiated from in-house-generated iPSCs. βIII tubulin (red) is a neuronal marker, while tyrosine hydroxylase (green) is the rate-limiting enzyme for dopamine synthesis.*

Neurodegenerative Diseases

Richard Wade-Martins (Department of Physiology, Anatomy and Genetics) leads the Oxford Parkinson's Disease Centre (OPDC), the aim of which is to better understand the causes of Parkinson's Disease and to develop therapies to halt the disease progression at the earliest stages. Human iPSCs from PD patients play a central role in the OPDC program. A small sample of skin is taken from a patient and reprogrammed into iPSCs by Jane Vowles in the Stem Cell Facility. The iPSCs are then directed to generate midbrain dopaminergic neurons (Figure 4) by OPDC Fellow Elizabeth Hartfield and Hugo Ribiero Fernandes, in order to study the cellular pathology of Parkinson's. James Martin Stem Cell Research Fellow Michiko Yamasaki-Mann (DPAG) is also involved in the project, studying calcium flux in the PD neurons produced in this project. Anybody who happened to watch the BBC news on June 17 will have seen the PD iPSCs we have generated and their dopaminergic neuron progeny, being described by Pallab Ghosh.

Matthew Wood (DPAG) is developing gene silencing therapies for human neurodegenerative diseases including Spinocerebellar ataxia type 7. A crucial requirement is demonstrating activity and benefit in relevant human cell models, prior to proceeding to clinical trials in patients. Using skin biopsies from SCA7 patients, iPSCs have been derived and are being characterised with help from the Stem Cell Facility. Matthew and Janine Scholefield can now produce the relevant neuronal types *in vitro* with which to monitor the effects of potential new treatments.

Developmental Molecular Biology

Shona Murphy's group in the Dunn School is investigating the function of a novel group of small RNAs in alternative splicing. Dawn O'Reilly has shown that several of these novel small RNAs are differentially expressed in hESCs, suggesting they play a role in stem cell maintenance and/or early development. Using the protocols established in the Stem Cell Facility to differentiate hESCs, Dawn and graduate student Pilar Vasquez Arango are able to study the function of these RNAs during differentiation.

Zoia Monaco's group (Wellcome Trust Centre for Human Genetics) is interested in understanding the mechanisms of genome stability in stem cells as a prerequisite to developing strategies for gene therapy. The focus is on investigating human chromosome organization and function using artificial chromosomes (HAC) as a research model. HAC are extrachromosomal elements, which behave as normal chromosomes within cells, and are successful as gene vectors, since they have the capacity to accommodate larger genes and regulatory regions for long term expression studies. In collaboration with the Stem Cell Facility, they have shown that stable artificial chromosomes are successfully generated in hESCs and express genes.

We also have many smaller-scale collaborations and are always happy to discuss potential projects of any scale with groups within Oxford and beyond. Pilot and short-term projects can be accommodated with reciprocal requisition arrangements to offset the high costs of stem cell maintenance. Larger projects would be expected to be funded through collaborative grant proposals.

Acknowledgements

Work described here has been funded by Parkinson's UK, the Oxford Martin School, the CGD Trust, the John Fell Fund, the MRC and the Wellcome Trust.

Exploiting Dendritic Cells to Overcome the Rejection of Stem Cell Derived Tissues

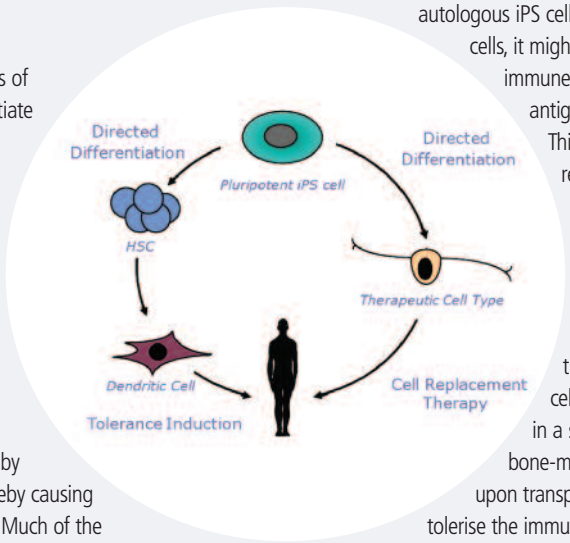
Simon Hackett

Simon Hackett holds an MRC Capacity Building Studentship awarded to the Oxford Stem Cell Institute and describes here one of the aspects of the project he has been undertaking in Paul Fairchild's laboratory.

Barely a week passes without the mention of the words 'stem cell' in the media. Indeed, these potentially revolutionary cells have become favourite 'buzz-words' for journalists, scientists, politicians and even religious figures. So what exactly is all the hype about?

Pluripotent stem cells are cells that are able to give rise to cell types from each of the three germ layers and, therefore, potentially any cell type of the body. As such, they are quite distinct from adult stem cells which exist in many organ systems, such as in the sub-ventricular zone of the brain and the crypts of Lieberkühn in the gastric mucosa. These populations of cells permit the continuous regeneration of the surrounding tissues and are, therefore, already lineage-restricted. Much research has focused on the application of pluripotency to cell replacement therapy: the replacement of diseased tissue by the differentiated progeny of stem cells. Two major types of pluripotent stem cell exist: embryonic stem (ES) cells and induced pluripotent stem (iPS) cells.

ES cells are derived from the inner cell mass of blastocysts. These cells are able to differentiate into any cell type within the body, allowing for the possible regeneration of cell types and tissues damaged by disease or a genetic pre-disposition. However, as these cells would more than likely be derived from a genetically mismatched donor, they would be rapidly rejected by the immune system upon transplantation.



iPS cells were first developed by Shinya Yamanka and colleagues in Japan in 2006 by genetically manipulating somatic cells, thereby causing them to revert back to a pluripotent state. Much of the therapeutic focus has been on these cells as they can be derived directly from the donor and, since they could be developed in an autologous fashion, should theoretically escape rejection by the immune system.

So do iPS cells have the ability to revolutionise our approach to regenerative medicine? Recent evidence suggests not. The belief that iPS cells would not be immunogenic has recently been called into question by the publication of work by Zhao and colleages who showed that, upon transplanting iPS cell-derived tissue into the same strain of mice from which they were first derived, the immune system recognised and rejected the transplanted tissue. Why were these cells rejected despite, being autologous to the recipient's immune system? It is thought that the genetic manipulation involved in generating iPS cells from somatic cells may have resulted in the unexpected up-regulation of genes which subsequently acted as minor tissue antigens, sufficiently immunogenic to provoke an immune response. Publication of this work dealt a significant blow to the field: will this discovery prevent iPS cells from ever being used in the clinic in the future?

Not necessarily. In our lab, we are currently developing methods which may one day overcome this immunological barrier. One aspect of our lab's work focuses on differentiating dendritic cells from both mouse and human pluripotent stem cells. Dendritic cells are immune cells whose primary function is to process antigenic material and present it on their cell surface, resulting in a response by the immune system. They were discovered in the 1970s by Ralph Steinman and our understanding of the way in which this cell type functions physiologically has rapidly expanded, with the identification of different sub-types and populations. Dendritic cells have the ability to balance the immune system carefully between tolerance towards antigens or by inducing immunogenic responses against a particular antigen. We have shown in the past, in collaboration with Herman Waldmann and Stephen Cobbold, the potential of dendritic cells to induce tolerance to minor tissue antigens, precisely the situation likely to be encountered following transplantation of tissues derived from autologous iPS cells. By differentiating dendritic cells from iPS cells, it might, therefore, be possible to 'trick' the immune system into inducing tolerance to the very antigens up-regulated during reprogramming. This might be sufficient to prevent the rejection of tissues differentiated from iPS cells without the need for powerful, often damaging, immunosuppressive drugs (Figure 1).

Previous work in the lab has demonstrated that it is possible to differentiate mouse ES cells into dendritic cells which function *in vitro* in a similar manner to conventional bone-marrow derived dendritic cells. However, upon transplantation into mice, they were unable to tolerise the immune system sufficiently to prevent the rejection of skin grafts across a minor tissue-antigen barrier. This was most likely due to low expression of major histocompatibility complex (MHC) class II molecules on their surface. I have expanded upon the foundations laid by this work to derive dendritic cells from iPS cells which might allow me to investigate whether these cells display a tolerogenic capacity. Even though ES and iPS cells show similar characteristics, the epigenetics of iPS cells suggest that the same cell type differentiated from the two populations may have significant phenotypic differences. Recent results have shown that these so called iPS-derived dendritic cells (ipDC) function in a similar way to bone-marrow derived dendritic cells: they are able to process and present antigen and, promisingly, appear to display MHC class II, suggesting the potential to function in a tolerogenic manner in vivo. The next step in my work will look at modulating these dendritic cells pharmacologically, so as to render them stably tolerogenic, which might allow their administration *in vivo* in advance of cell replacement therapy. We are hopeful that dendritic cells, derived in this way, may one day enable the therapeutic application of iPS cells in humans also.

Reflections on a Long-Overdue Sabbatical

Tony Green

Professor Tony Green, currently Head of the Department of Haematology at the University of Cambridge, spent the first half of 2011 at the Dunn School on a six month sabbatical as Newton-Abraham Visiting Professor. He finally succumbed to gentle pressure to reflect on his brief stay in Oxford.

'What *did* you do on your sabbatical?' The frequency with which this question is asked, the wistful expressions on the faces of the inquisitors and the guilt associated with Paul Fairchild's gentle but persistent reminder of my promise to produce an article for *Fusion*, all coalesced to prompt this attempt to answer the question.

I think it was Aaron Klug who pointed out that 'a full diary is the enemy of good research' and it was with this aphorism in mind that I made plans to move away from Cambridge for six months, to escape the tyranny of the email and the tribulations of University and NHS bureaucracy. The timing was good. I had been Head of the Department of Haematology for 10 years and was, therefore, in need of an opportunity to think ahead to the next decade. In addition, my research group had enjoyed a particularly exciting time for the previous seven years as we sought to understand the cellular and molecular consequences of gain-of-function JAK2 mutations in human myeloproliferative neoplasms (MPNs). These pre-leukaemic conditions are experimentally tractable, permit powerful clonal analysis and provide a window into the very earliest stages of malignancy that are inaccessible in other forms of cancer.

Following the discovery of the JAK2 mutation in 2005, we were well placed to begin dissecting the pathogenesis of these disorders, and our studies have led us to explore diverse areas of biology including stem cells, chromatin biology, cytokine signaling, DNA replication, DNA damage response pathways and transcriptional networks. Our results, together with those of others, have already had a major impact on the clinical management of MPN patients, with assays for JAK2 mutations now a front-line diagnostic tool embedded in international guidelines and with multiple JAK2 inhibitors in clinical trials. Some of our findings have been particularly surprising. For example the MPNs can evolve from an eminently treatable chronic phase into a lethal acute leukaemia and yet the JAK2 mutation present in the chronic phase is frequently absent from the subsequent acute leukaemia, an observation that appears to reflect unexpected clonal complexity within an individual MPN patient. Most recently, in collaboration with the Kouzarides lab, we have described a novel nuclear function for JAK2 in the phosphorylation of histone H3, thus providing a mechanism for "non-canonical" JAK signaling directly to chromatin and challenging current concepts of cytokine signaling. For each biological process that we have explored, reductionist approaches have been needed to dissect the biochemical or cellular consequences of the JAK2 mutations. Grappling with fields outside our normal comfort zone has been enormously enjoyable, not least because we have been fortunate in our collaborators from whom we have learnt so much. However, integrating these diverse perturbations to create an understanding of pathology at the level of the whole organism remains a considerable challenge, and the concept of a sabbatical tempted me with the lure of protected thinking time.

But where should I go? This required some delicate negotiations on the home front. Boston and Singapore were impractical given a new job for my wife and the recent arrival of our first grandchild in Cambridge. Staying in Cambridge was a possibility but felt unwise in view of the inverse square law of sabbaticals, namely that the danger of being sucked back into administrative tasks is inversely proportional to physical distance. The opportunity to come to Oxford was, therefore, perfect – far enough but not too far, and with an extraordinary scientific environment into the bargain. And being based at the Dunn School was the icing on the cake.

So what have I been doing? Having persuaded several colleagues in Cambridge to kindly cover various aspects of my normal day job, the first step was to cover my tracks carefully and put as many people as possible off my scent. To this end, I obtained an Oxford email and resolved not to look at my Cambridge inbox, which was left in the custody of my long-suffering secretary. Step two was to get to grips with Skype. As a consequence, I've been able to spend more time with members of my lab than I managed when in Cambridge, much to my enjoyment, although I am not convinced the feeling is fully reciprocated! The rest of my time has been filled with a productive mélange of writing, reading, joining in the weekly lab meetings of several groups, going to seminars in the Dunn School, the WIMM and on the Churchill site, and dealing with the steadily increasing number of emails that, despite my best efforts, have succeeded in tracking me down. I have especially enjoyed meeting individual scientists in the Dunn School and elsewhere to learn about their work, interactions which have already lead to several collaborative links. So my working week has been pretty much as full as in Cambridge but the fulfillment to frustration ratio has been markedly higher.

What about extramural activities? The Fellows at Lincoln College have been exceptionally welcoming and wonderfully tolerant of my ignorance of their customs, not to mention my idiosyncratic Latin. It has been a real privilege to live so centrally in Norham Gardens, to explore Oxford, to cycle everywhere and to be able to walk to work through the University Parks. And then there are my two extra curricular projects. The first has been to improve my photography and, with the help of Judie Waldmann together with Keith Barnes at the Oxford School of Photography, I have had enormous fun trying to get in touch with my well-hidden creative side whilst pestering friends and family to pose in unlikely settings. My second goal was to move my internet skills into the 21st century – Facebook seemed a step too far and so the goal was to master iTunes, thus following Herman's example, although not perhaps his level of addiction. This has been a striking success and I am now the proud owner of five Apps, one of which has actually been useful. In addition, the set of computer speakers that were a thoughtful gift from my wife nine months ago, but which still lie in their unopened box, have been transported to

Oxford. If I'm not downloading music and playing it through these by the end of my time here, spousal support for future sabbaticals is highly unlikely.

And finally a word of thanks – to Herman for inviting me, to the Newton Abraham fund for supporting my visit, and to all who have made my time here so memorable. I am sure you don't need me to tell you that the Dunn School is a very special place, with its outstanding science and strong sense of community; I feel immensely fortunate and privileged to have been able to spend time here.

"...the Dunn School is a very special place, with its outstanding science and strong sense of community..."

Edwin Wheal — The First Pathology Technician

Eric Sidebottom

I recently had the pleasure of meeting the descendents of John Edward (Edwin) Wheal who was undoubtedly the first laboratory attendant, or 'technician', to work in Oxford. His family were keen to learn more about their illustrious forbear who was rewarded by the University in 1926 with an Honorary MA degree. With their help, I have pieced together this short story of his career.

We think Edwin was born in 1877 and started work about 1891 when he was only 14. He worked in the various University and hospital pathology departments for 45 years until his untimely death in 1934 at the age of only 57. He worked under five Regius Professors, Sir William Osler, perhaps the most famous physician of his time who probably appointed him, Sir John Burdon Sanderson, who introduced pathology teaching into the University, Sir Archibald Garrod, who introduced the concept of genetic diseases and Sir Farquhar Buzzard, friend of Lord Nuffield, who gave so much money to the Oxford hospitals and medical school. The first formal University Pathology Department was not set up until 1897 when James Ritchie was appointed as the first University lecturer in pathology. The Department did not have its own home; it was accommodated in the Regius Professor of Medicine's rooms in the University Museum. A purpose-built laboratory was opened in 1901 and Edwin would have been much involved in helping to design and set this up.

Ritchie, a Scotsman from Edinburgh, was eventually enticed to return to Edinburgh in 1906 and Georges Dreyer, a Dane, was appointed as his successor. Edwin features in Dreyer's biography, being variously described as "the faithful laboratory attendant with willing and capable hands" and "the indispensable Edwin [who was] needed when anything was amiss". It was clearly a happy partnership for 27 years and Edwin's obituary even suggested that Dreyer's sudden death in 1934 contributed to his own early demise.

It is interesting to note from the Dunn School archives there is just one scientific paper written by Edwin and a younger technician colleague, Arnold Chown. It was published in 1911 in the *Journal of Pathology and Bacteriology* and describes a new method for staining the bacterial species *Actinomyces*. This must have been Edwin's initiative since no 'research scientist' contributed to the paper.

By the end of World War I, the 1901 laboratory was bulging at the seams and Dreyer's reputation and influence persuaded the Trustees of Sir William Dunn's estate to give £100,000 to enable the University to build a new, much larger and grander laboratory. Once again Edwin would have been much involved in the planning and commissioning (in 1927) of the current Sir William Dunn School of Pathology. It is perhaps no coincidence that Edwin had been awarded an honorary MA by the University the previous year, clear recognition of his importance to Oxford Pathology.

His obituary states that "his life and work were an example to all who worked under or over him. Willing and able to tackle all kinds and quantities of work, from the most delicate manipulations to the dullest and most laborious routine, he was sometimes too generous in giving his time to spare the less skilled labour of others". He set the high standard for laboratory technicians which has been maintained through numerous generations and which continues to benefit so many at the Dunn School today.

My Time at the Dunn School

Mike Simpkins

After 34 years at the Dunn School, Mike Simpkins retired earlier this year, leaving a void that will be difficult to fill. He will be greatly missed by all who knew him but offers here his parting recollections of the changes he has witnessed over the years.

Mike Simpkins

I remember arriving at the Dunn School in December 1974, approaching those foreboding front steps up to what looked, for all the world, like a school. On entry, that feeling was reinforced, with the marbled entrance hall and oak staircase, lined with portraits of former professors including Howard Walter Florey, who was unknown to me at that time.

I was greeted by Mrs Turner, the administrator, and taken up to the office of the Professor's secretary, a Mrs Finch-Mason, known locally as Mrs. FM, although I didn't have the courage to call her that for some years! She was, indeed, a formidable woman who protected and filtered out 'unnecessary' visitors to the professor. She introduced me to Professor Henry Harris, a small man with a huge intellect, who carried an air of intense thought. He invited me to sit in a comfy chair by the window. I was completely terrified! The interview must have gone well because I started working in January. New technicians were trained in the central Cell Bank run by Mary Williams, a softly spoken lady with very definite views about how things were to be done. This gave new arrivals an introduction to other staff and techniques used throughout the building and, more importantly, an idea of the pecking order of senior staff, which was much more apparent then, than it is now.

Most senior academic staff wore shirt and tie and usually a tweed jacket with Oxford brogues. In fact the afternoon tea club, which was exclusive to senior academic staff, required a jacket to be worn on all except the hottest days of the year. Tea was served with biscuits and cake. Technical staff, on the other hand, quite literally took their tea at the bench.

My first placement was with two young chaps, Roy Bullingham, a trauma surgeon on secondment from the John Radcliffe Hospital and a very bright D.Phil student, Alan Jones, who was also a chain smoker! I well remember him balancing his cigarettes on the edge of the bench while he prepared buffers, stopping only to take a deep draught before continuing with the preparations. These were early days with DNA technology and I can remember helping Alan to make a restriction enzyme, EcoR1 from bacteria produced at Porton Down, what is now the ECACC. I later worked with Mike Bramwell's group, before spending a short time with Edward Abraham and finally with Peter Cook until 1990, when I took over the Cell Bank and services, including oversight of the cleaning of the Departmental, which was externally-contracted. This was a time before computers were widely used in the lab and all writing was, therefore, done with pen and paper and typed up later. Now it is rare to see a PI away from one.

The person I most admired and respected was Norman Heatley. Here was a hero of the penicillin era, a great innovator, able to fashion almost anything from old biscuit tins and bits of string while remaining totally unassuming. Norman was called in from retirement to get down the rope from the flagpole: a knot had developed and nobody could think of a way of extracting it, other than shimmying up the pole! He fashioned a device, literally out of an old biscuit tin on the end of a pole, which poked the rope out. Norman was quietly spoken and had respect for everyone, sadly a trait in short supply nowadays. I had the singular honour of presenting him for his Honorary Doctor of Medicine at a degree ceremony at the Sheldonian Theatre. This was the first time the degree had been presented to a non-medic.

As the Dunn School was relatively small in the early days it had a rich lab-wide social life with lunches in the park, Christmas parties (the food was served in the teaching lab where the bacteriology practicals were held!). Mike Bramwell, ably assisted by Sue Humm, were the main driving force behind most events. I think there were more characters around in those days: perhaps the pressure was not so great. There were annual mixed fancy dress football matches between the former Lesley Martin Building and the main Dunn School building and, as now, a thriving cricket team.

As Henry Harris neared the end of his reign, he was to be succeeded by Alan Williams, another energetic Australian, but sadly his life was cut tragically short by cancer and the search began for another replacement. Enter Herman Waldmann in 1994. He immediately began to transform the Department, ably assisted by William James steering the planning and production of new buildings, first completing the PSB and then adding, in short succession, the EPA, The Medical Sciences Teaching Centre and, lastly, the OMPI, making it the hugely-successful and highly-rated department it is today. In contrast to previous times, Herman has been about as hands-on as a person can be, taking personal interest in individuals and regularly pointing out to me the dust on the main stairs to help inform my ongoing negotiations with cleaning staff! I thoroughly enjoyed my time at the Dunn School especially the many friends I have made and have now left behind.

Homemade Penicillin

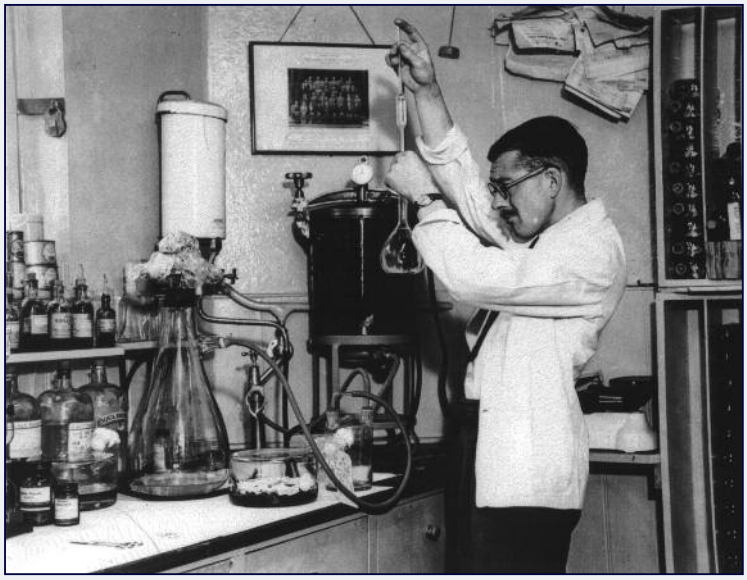
Gilbert Shama

The first newspaper account of penicillin was published by *The Times* on 27th August, 1942. Its appearance was to precipitate the now celebrated exchange via its letters columns between those two influential scientists, Sir Almroth Wright of St. Mary's Hospital, Paddington and Robert Robinson of Oxford University, each anxious to ensure that credit for penicillin went to the right man — Alexander Fleming and Howard Florey respectively. These were to be the first of many articles that were to follow. In 1942 there were just 7 articles in *The Times*, but by 1944 this number had increased to 93. Naturally all other print media, both newspapers and periodicals, followed suite, and a more or less common theme began to emerge. This was one of extolling the curative powers of penicillin, whilst at the same time explaining that all supplies of the antibiotic were reserved exclusively for military use.

A similar message was transmitted in BBC radio broadcasts. One of the problems programme-makers faced was how to convey the potency of penicillin to a largely lay audience. The script of a transmission (Ariel in Wartime) made on 4th September 1942 survives, and provides a fine example. Quoting directly from the script: 'Laboratory tests show that [penicillin] completely prevents the growth of staphylococcus in a dilution of between 1 in 24 million and 1 in 30 million …Let me try and give you a picture of what this means…Now supposing we were able to have a very large container of about 300 gallons of culture medium, and we added staphylococci in suitable amount, followed by only about 1 grain of the purest penicillin and then kept the container at blood heat for 24 hours, this minute amount of penicillin would prevent the multiplication of germs and the liquid would remain quite clear.'

Florey had never courted publicity for his work, and he found the attention of the media intrusive. Following a further broadcast on penicillin (Marching On) made in October 1942, he wrote angrily to the BBC asking if the Corporation had considered whether it was in the public interest to draw attention to a substance which was 'unprocurable, except in minute amounts.' He also complained that the publicity had resulted in his receiving a 'flood of pathetic letters from as far away as Western Australia and Saskatchewan'.

This situation was mirrored to a very great extent in the United States. Letters addressed to the President pleading for penicillin have been preserved at the National Archives in Maryland. They too make for pathetic reading. Some penicillin was in fact released for civilian use, often with much publicity. The ensuing press accounts were often accompanied by photographs or drawings. Accompanying one such article in



Kenneth White in full swing…

The American Weekly in October 1943 are two drawings. The first depicts a desperate mother with a baby swaddled in her arms at the entrance of a clinic, and the second, the same woman laying flowers at her child's grave. 'Would you', asks the article 'be willing to swap jobs with the allocator of life-saving drugs who decides whether a soldier, sailor, civilian, child or adult must face death so that others can live?' The 'allocator' was Chester S. Keefer of Harvard Medical School, described in the article as the 'final authority' in America on the distribution of 'the small civilian allotment' of penicillin.

Ordinary GPs, like some of their patients, must have experienced deep frustration at hearing about the efficacy of penicillin whilst knowing that their chances of actually getting hold of any were virtually nil. However, there was one man who decided to do something about this. Dr Julius A. Vogel, from Aliquippa, Pennsylvania, and a physician for a steel corporation addressed a meeting of the Industrial Hygiene Association in November 1943 to this effect: 'Dare any man say that penicillin is scarce? Certainly money can't buy it, but each and everyone here present can easily produce his own penicillin in his own family kitchen at an initial outlay of less than $5, and at a production cost of less than 5 cents per Petrie dishful.'

The Times Herald newspaper decided to take him at his word. Its Science Editor, Wilson L. Scott, who, as chance would have it, had received 'sound laboratory training' as a postgraduate student in chemistry at Yale University from a certain Robert D. Coghill, decided to try it for himself. Coghill had by then become the leader of a team investigating the scale-up of penicillin production at the US Department of Agriculture's (USDA) laboratories at Peoria, Illinois.

UNIVERSITY OF OXFORD

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On 28th November, 1943 the newspaper published a step-by-step guide, with photographs. Splashing out $10 on the necessary glassware and constituents of the growth medium, Scott demonstrated how a piece of gauze should be cut to size and placed in the bottom of a petri dish, whereupon it was moistened with some starch-based growth medium before being inoculated with spores of *Penicillium*. He could not resist passing on his 'sound training' in asepsis: 'Note cotton plug [of a test tube] in right hand' appears in the caption to one of the photographs. The petri dish was incubated for 4 days after which the gauze could be applied directly as a poultice onto external 'cuts, wounds and abrasions'.

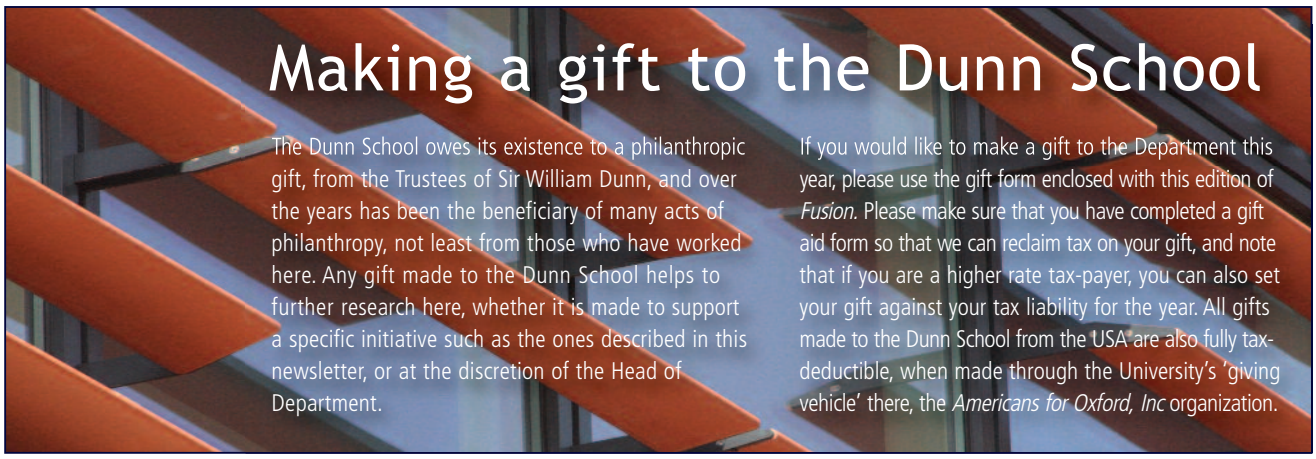
The most crucial component in all this – the *sine qua non* — was the right mould culture, and here Scott simply states that he received a culture 'free of charge from a co-operative bacteriological laboratory'. Scott also drew attention to some of the risks. One of his moistened gauzes became contaminated with a 'soil bacillus' and he points out the dangers of applying a potential pathogen to a wound or cut.

The cosy domesticity of homemade penicillin production was emphasised in other newspaper articles. Familiar everyday items could be pressed into service, and even one's wife could lend a hand! Mrs Vogel assisted Julius with the sterilization of the growth medium on the stove. 'Mrs Vogel's glass coffee maker also came in handy' – whether for a well-earned cuppa after all that hard work, or to throw at Julius for patronising her, the article does not specify.

Whether or not it was the intention of such articles to induce ordinary people to undertake their own penicillin production is hard to ascertain. It seems certain that a few at least must have made some attempt. Following the publication of the 'how to' articles a number of warnings about experimenting with unidentified moulds hastily appeared in the press. One published in the American periodical Science News Letter in January 1944 is a good case in point. This article pointed out that the greatest obstacle any would-be-producer of penicillin would have had to face was that of getting hold of a suitable penicillin-producing strain of *P. notatum*. USDA scientists, the article revealed, had not found a single one belonging to the *P. notatum* group in 50 blue-green moulds they had examined.

Getting hold of a suitable culture of *P. notatum* was not something that Mrs Nancy Good was going to allow to deter her. She had read an article on penicillin in the British periodical News Review in April, 1944. The article made reference to 'home-made penicillin', which was carefully placed in quotation marks. Ignoring the nuance, Mrs Good wrote directly to the Curator of National Collection of Type Cultures. She stated that where she lived she enjoyed a warm climate and often found mould growing on cheese and curds; would the *Penicillium* mould grow on cheese she wondered? She would never be given the chance to find out; scrawled at the bottom left of her letter are the words 'ans'd, refused' and the signature of R. St. John Brooks, the Curator.

However, some do-it-yourself enthusiasts were to fare better. Mr Kenneth E. White was a pharmacist, who the Daily Mail told its readers on 3rd June, 1944, led a 'double existence'. By day he supplied the villagers of Ripley with medicines, but by night manufactured penicillin for distribution free of charge to local doctors and hospitals. White paid £50 for an autoclave but he needed a refrigerator for storing the penicillin and here he had a stroke of luck. He wrote to a 'well-known firm of teashops' suggesting that as they were unable to sell ice cream because of wartime restrictions, they might like to consider lending him one of their refrigerators. They readily obliged, and this enabled White to undertake small scale penicillin manufacture. The picture shows him surrounded by his equipment, in the bottom centre is a flask manufactured by Chance Glass of Smethwick for growing the mould. Despite the familiarity of the procedures for many current scientists, in the interests of Health and Safety, the author feels under an obligation to advise readers not to attempt manufacture of penicillin for themselves at home…



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