fusion

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Editorial

Having completed my first full academic year at the Dunn School, it is perhaps a good time to take stock. It has been a steep learning curve and I am certainly not yet at the top; nevertheless, I know a lot more about how Oxford works, we have filled a floor of OMPI with young and exciting group leaders and we have negotiated the external processes of our REF submission and Athena SWAN accreditation. We have also had a couple of great parties.

The most interesting and important aspect of my role as Head of Department is recruitment of group leaders. I am, therefore, very pleased that, in addition to the four new recruits that I introduced in the last edition of *Fusion*, I can report the arrival of two further groups. Dr Conrad Nieduszynski joins us as an Associate Professor (the new term for University Lecturer) from the University of Nottingham, where his group has studied fundamental aspects of DNA replication and genome stability using a combination of yeast genetics and high throughout 'omics approaches. In addition, Dr Luis Alberto Baena has recently been awarded a very competitive Cancer Research UK Career Development Fellowship and will be establishing his group at the Dunn School. He works on the emerging field of non-apoptotic functions of caspases in growth, development, cancer and stem cells. He joins us from a postdoctoral fellowship at the MRC National Institute for Medical Research.

This total of six new recruits plus my own lab now completes the occupancy of the first floor of OMPI. Although there is more space to fill, we have decided to pause slightly, to allow everyone to settle in, and the rest of the Department to adjust, before energetically seeking more recruits (although we do have a couple of academic positions to fill in the next year).

This year we have also put effort into developing mentoring structures, supporting career progression, and ensuring that the Departmental policies are as family-friendly as possible. This was necessary for our Athena SWAN bronze award; more importantly, it was the right thing to do. Scientific careers, while potentially exciting, flexible and fulfilling, also come with high levels of uncertainty, insecurity at early stages and frequent long hours. It is also a fact of life that not everyone who embarks on the journey will reach their expected destination. It is, therefore, essential that we design processes to support people as they progress and reach important decision points. The Dunn School has a tradition of friendliness and support, and new developments build on those strong foundations. The government-mandated Athena SWAN process, for which we successfully applied, focuses specifically on women's academic careers, but actually most of the issues are relevant to all scientists.

I have been pleasantly surprised to find that, despite the rather arcane politics and bureaucracy of Oxford, it is not difficult to get things done. For example, I am very pleased to have been able to establish what may have been the first facility for CRISPR-mediated genome engineering in the UK. It was fast and simple to get the agreement of the heads of three other South Parks Road departments to contribute to the set-up costs and to recruit Dr Andrew Bassett, one of the people in the UK with the most experience of this revolutionary new technology, which he describes in some detail in this edition of *Fusion*. Despite only being in place since summer 2014, Genome Engineering Oxford (GEO), is already helping researchers at the Dunn School and the rest of Oxford to use CRISPR in many different applications.

Finally, we have started what is likely to become a nice new tradition: the Dunn School summer party. A barbecue in the University Parks and cricket pavilion, with music, games, families, and friends proved to be a roaring success and a lovely way of marking the end of the academic year. We had over 250 attendees this year, and my guess is that there will be even more next time, now the word has spread.

As always, keep an eye on the website for news throughout the year. In fact, by Christmas we should be ready to release our new website, which will be totally rebuilt in layout and content. You can also follow our departmental Twitter account @Dunn_School.

Matthew Freeman

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Photograph by Judie Waldmann.

News

The Dunn School featured in the Bodleian Library's exhibition of Great Medical Discoveries

This year marks the 800th anniversary of the birth of Roger Bacon, who became known as England's 'Doctor Mirabilis' for the work he performed in Oxford which led to the emergence of modern medicine as a science based on, and tested by, experiment. To celebrate the occasion, the Bodleian Library staged an exhibition, designed by Conrad Keating, of the Great Medical Discoveries that have emerged from Oxford over 800 years since Bacon's birth. Running from 22 November 2013 until 18 May 2014, the exhibition proved to be extremely successful, welcoming many thousands of visitors during this period and receiving exceptional reviews in journals such as the *Lancet*. The exhibition told of Robert Hooke's first description of the cell and the first appreciation of the circulatory system by William Harvey, through to more recent innovations such as Allen Hill's development of the glucose sensor, used by diabetics throughout the world to monitor their blood glucose levels. The Dunn School featured prominently in the exhibition, not only recognising the role played by Florey, Chain and Heatley in the development of penicillin as the very first antibiotic and the subsequent elucidation of its crystal structure by Dorothy Hodgkin, but also celebrating the pioneering work of James Gowans on lymphocyte life history and function and George Brownlee's on haemophilia.

Perspectives

Exhibition

An engaging tour through Oxford's medical history

To celebrate the 800th anniversary of the medieval scholar Roger Bacon's birth, and a similarly long history of medical research at the University of Oxford, the Bodleian Library's exhibition *Great Medical Discoveries* showcases some of the university's remarkable contributions to medicine. The Bodleian Library provides a fitting venue. It was there that Thomas Willis and Richard Lower made their dissections. With Christopher Wren, one of the most talented medical illustrators in the history of medicine and another Oxford familiar, these dissections led directly to Willis' account of the structure of the human brain in *Cerebri anatomie* (1664), a book that laid the foundations for modern neurology.

While the organiser of the exhibition, Conrad Keating, Writer-In-Residence at Oxford's Wellcome Unit for the History of Medicine, acknowledges that the topics addressed are somewhat idiosyncratically selected, the exhibition explores themes of medical discovery that have much broader resonance. Beautiful manuscripts, tales of bold experimentation (such as with Lower's first blood transfusion from sheep to man), carefully annotated laboratory notebooks, and ingenious devices all tell of much more than Oxford's science.

"There are genuine delights, particularly in the details of letters or notebooks on display, which bring the ideas and lives of Oxford's physicians vividly to life."

The exhibits are organised in a series of display cases arranged roughly in chronological order, each of which highlights a different clinically related discovery or development. The examples chosen bring out two major traditions in medical discovery. Most prominent is the Baconian conception of an experimental medical science, abundantly illustrated in the exhibition by examples from Robert Hooke, William Harvey, Thomas Willis, and a string of modern scientists. Bacon studied, lectured, and died in Oxford. His encyclopedia of all human knowledge, *Majus Opus* (1267), included both empirical observations and speculation. He enunciated a rigorous approach to new knowledge based on observation and induction. However, set against this is an older tradition derived from Hippocrates that relies on clinical observation agnostic of theory. This began in Oxford with Thomas Sydenham. His scepticism for all but bedside observation is illustrated by his response when asked to name the best textbook of medicine. He demurred, better to "read *Don Quixote*" he is said to have replied. Famously, Sir William Osler's contribution as the father of the modern Oxford medical school, the discovery of penicillin, and Oxford's strong tradition of epidemiology all partly arise from roots in Sydenham's focus on observation of the patient.

Developing the theme of experimental science in Oxford medicine, the other-worldly landscape of the very small is displayed in a copy of Hooke's best-selling *Micrographia* (1665), one of the first publications of the Royal Society and one which Samuel Pepys described as "the most ingenious book that ever I have read". Hooke championed the use of technology to study nature and the concept of a cell as the fundamental building block for an organism. Groundbreaking studies of the cell continuing into the present day in Oxford are illustrated by descriptions of James Gowans' characterisation of the lymphocyte and the Nobel Prize winning work of John Gurdon, who discovered that terminally differentiated cells could be re-programmed as stem cells. A related story of how "artificial organs", to use Hooke's term, can extend human senses for medical discovery moves from Hooke's microscope through x-ray crystallography to Oxford's contributions to the development of brain imaging.

Among the most influential of all of the Oxford pioneers of early experimental medicine was William Harvey. His great work, *De motu cordis* (1628), describes an experimental approach to the first elucidation of mechanisms for circulation of the blood. Its impact extended far beyond an understanding of the heart by introducing the radical idea that organs can be described in mechanical terms and follow the laws of physics.

Dorothy Hodgkin (1910–94)
Photograph from University of Oxford MS Eng.C.5715 folder 36.

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A review of the exhibition by Paul Matthews was published in the* Lancet *383:1200–1201

CAMPATH approved by NICE for use in the treatment of multiple sclerosis

The UK's National Institute for Health and Care Excellence (NICE) has recently approved a drug therapy developed by Herman Waldmann and his group for the treatment of multiple sclerosis. The monoclonal antibody, originally called Campath but now formally known as Lemtrada, was made available in NHS hospitals from September. Approval of the drug by NICE follows a similar endorsement by the European Medicines Agency in September 2013 and acknowledges Lemtrada as a cost-effective and clinically-efficacious treatment for a disease that affects 2.5 million people worldwide and approximately 100,000 people between 20 and 40 years of age in the UK. Lemtrada, developed in collaboration with Cambridge University, ''is a major step forward in the treatment of people with multiple sclerosis'' said Nick Eijke, director for policy and research at the MS Society.

Lemtrada's original name, Campath, reflects the long and sometimes complex history of a drug that has taken decades to reach the market. Having been developed by Herman Waldmann and his colleagues Stephen Cobbold and Geoff Hale while in the Department of Pathology at Cambridge University, work on this therapeutic antibody continued in Oxford when Professor Waldmann became Head of Department at the Dunn School in 1994. Nevertheless, links with Cambridge have continued over the years, leading ultimately to the clinical trials that have proven its utility in the treatment of MS. "We are very pleased and proud of this outcome," said Professor Waldmann. "In particular, we have great admiration for the neurology team in Cambridge, with whom we have worked on this project for so many years. Their commitment and focus has been exemplary, and this has been a good example of basic and clinical science collaboration at its best".

Research Focus:

Zsofia Novak, a DPhil student in Jordan Raff's laboratory, won the prestigious Young Cell Biologist of the Year award from the British Society for Cell Biology (BSCB) during its Spring meeting in March. Here, Zsofia describes the research which has earned her this coveted title.

Keeping the centrosome under control

Zsofia Novak

The centrosome is important in animal cells for organizing the microtubule cytoskeleton, particularly the mitotic spindle that segregates DNA during cell division. Crucially, the central part of the centrosome, called the centriole, is also essential for the formation of cilia: primary cilia are found on most mammalian cells and are required for correct embryonic development and adult tissue homeostasis, while motile cilia can move cells or move fluid over cells. As a consequence of their many roles in our cells, abnormal centrosome and cilium function is associated with a variety of developmental disorders as well as pathologies such as cancer, obesity and macular degeneration. An important aim of our group's research is to shed light on how cells normally maintain the correct number of centrosomes during cell division, and what mechanisms might misregulate centrosome numbers and therefore act as underlying causes of disease.

A centrosome consists of two centrioles surrounded by pericentriolar material, a cloud of proteins that are involved in microtubule nucleation and cell cycle signaling. The centrioles are barrel shaped microtubule-based structures, that are each a few hundred nanometers long and they are usually found attached to each other in a strict orthogonal arrangement. Centrioles serve as the platform for pericentriolar material assembly, so the number of centrioles within a cell usually determines the number of centrosomes.

At first glance, it may seem surprising why each centrosome contains a pair of centrioles instead of just one — especially as we now know that only one of these is actually involved in organizing the pericentriolar material of the whole centrosome! The answer to this puzzle lies in the special mechanism that cells use to ensure that when they divide, they duplicate their only centrosome once and only once and so deliver a single centrosome to each of the two daughter cells. Just as the two strands of DNA act as templates during chromosomal replication, so the two centrioles of the centrosome each act as templates for centriole duplication during the cell cycle. Thus, having a pair of centrioles per centrosome ensures that the right number of centrioles, and therefore centrosomes, is maintained in generation after generation of dividing cells.

Centriole duplication, just like DNA replication, is strictly constrained by several control mechanisms to ensure that it can be initiated only once per cell cycle and can only produce a single copy of each template. The accurate control of both processes relies on a layered mechanism. The first layer is always a 'permission' — the potential of a template to be copied sometime in the future. In the DNA replication field this has been termed the replication *license*, and due to the extensive analogies between the two duplication processes the centriole field had also adopted this term. The second layer is an activity that can act on a licensed template, but by doing so also disables the license permanently for the remainder of the cell cycle. The key to efficiently regulating templated duplication and prevent over-replication is to achieve licensing and activation sequentially, and never at the same time.

The license that makes a centriole competent to start a new round of duplication is the separation (or "disengagement") of the two centrioles, which occurs at the end of mitosis. At the time the license is given, the cell puts on hold the production of its key centriole components, so centriole assembly cannot start while licensing is happening. When the cell enters replication phase, the rapid production and activity of centriole components allows a new "daughter" centriole to grow on the side of each licensed template "mother" centriole. As soon as its first daughter centriole starts to form, a mother centriole loses its duplication license until the end of the cycle. Thus, by restricting licensing and daughter centriole assembly to different stages of the cell cycle, cells ensure that only one daughter centriole is formed by each mother centriole in any one cycle.

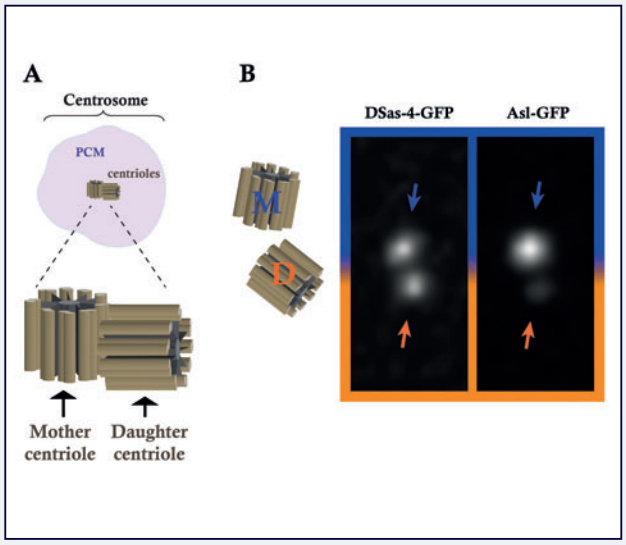

Figure 1. (A) Schematic of a centrosome. (B) The images show that while DSas-4-GFP (similarly to most centriole components) localizes equally to mother and daughter centrioles just after they disengage, Asl-GFP is strongly asymmetric: the mother centriole always contains much more Asl-GFP than the disengaged daughter centriole.

Now, here comes the key difference of the centriole cycle and DNA replication: in contrast to the complementary strands of DNA, the two centrioles within a centrosome are *not* licensed by identical mechanisms. While centriole disengagement allows older centrioles to duplicate again, this event is insufficient to license newly-formed daughter centrioles — in fact some research suggests that daughter centrioles don't require this step at all to allow their very first duplication! Instead, newly-assembled daughter centrioles acquire a certain 'modification' when they pass through their first mitosis — a mystery "primary license" that is required for the very first round of centriole duplication and, once acquired, is never lost. During my DPhil research I became really interested in what this primary licensing step was and how it completes our current understanding of the control mechanisms that enforce correct centriole and centrosome numbers.

To try to identify this primary license, I started to assay the localization of the few proteins that we knew were essential for centriole duplication in the fruit fly *Drosophila melanogaster*. Even though centrosomes contain hundreds of different proteins, centriole duplication remarkably only requires a handful of these, and each of these proteins has a clear homologue in humans. To visualize these proteins in living fly embryos, the gene of each protein was fused with the gene encoding green fluorescent protein so that I could follow the behavior of each individual protein on a confocal microscope.

I noticed that one of these proteins, called Asterless (Asl), stood out from all the others. While the other key centriole replication proteins were present in nearly equal amounts in mother and daughter centrioles just after they separated, Asl showed a very strong asymmetry: there was always far more Asl on mother centrioles than on newly-disengaged daughter centrioles. I showed that this was because no Asl gets incorporated into daughter centrioles while they grow and are engaged with their mother centriole; Asl is only incorporated once daughters disengage and are ready to start their own duplication. Knowing that Asl was essential for centriole formation but was not built into forming centrioles was exciting, as it meant we had potentially found the 'missing molecule' that is required for duplication but that is only built into new daughter centrioles at around the time they are converted into mothers. Could Asl be the elusive primary license that allows new-born centrioles to duplicate for the first time?

To test this idea I needed to block the incorporation of Asl into daughter centrioles that have just separated from their mothers and were ready to start their own duplication. This was very tricky, but by injecting an antibody into the embryos, which specifically bound to the part of the Asl protein needed to anchor it to centrioles, I was able to prevent its association with new centrioles. I had therefore generated disengaged new centrioles that had passed through mitosis (the period when licensing normally takes place) but now lacked Asl protein. Would these disengaged centrioles that lacked Asl be able to duplicate? As a readout of centriole duplication I chose another one of the essential centriole components, DSas-4 — a protein that I had earlier shown to be built into growing daughter centrioles from the very early stages of their formation. When assaying DSas-4-GFP accumulation during the time of daughter centriole assembly, I found that the disengaged new centrioles that lacked Asl couldn't acquire any additional DSas-4, in contrast to the Asl-containing centrioles. Thus, the presence of Asl is required to allow new centrioles to template their first daughter centriole.

Why don't older centrioles also rely on Asl recruitment in addition to daughter centriole disengagement to reset their duplication license? I found that a proportion of Asl molecules get permanently incorporated into the centrioles so, once a centriole has incorporated Asl for the first time, it no longer relies on the arrival of new Asl molecules to duplicate again. This makes Asl a special license as it is not reset every cell cycle but is acquired once and, as far as we can tell, then lasts for the lifetime of the centriole. Once Asl is permanently acquired by a centriole the presence of a daughter centriole becomes a possibility, and so disengagement takes over as the licensing mechanism. Nonetheless, Asl incorporation is crucial, even if needed only once, to set off a potentially long string of duplication cycles!

My current work now focuses on the mechanisms that govern the timely arrival of Asl at centrioles. I was able to show that DSas-4 is the most likely receptor of Asl within the centriole, yet DSas-4 is built into centrioles far before the arrival of Asl. My main interest now is what is stopping these molecules interacting during most of the cell cycle, and what is the change that suddenly allows the interaction at the time when centrioles are first licensed to duplicate.

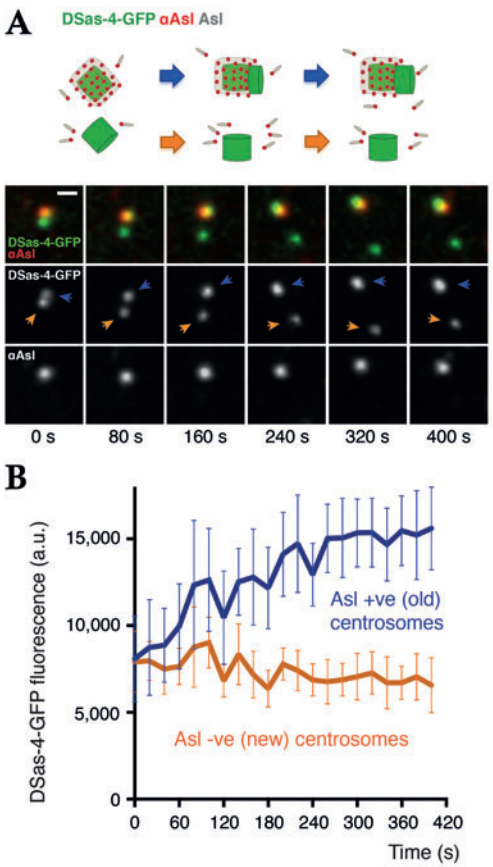

Figure 2. (A) The schematics and images show that the amount of DSas-4-GFP (centriole marker) does not change at a centrosome in which the template centriole is devoid of Asl. In contrast, the amount of DSas-4-GFP increases at the centrosome that contains Asl, as a daughter centriole is growing within. (B) The graph also shows how Asl-positive centrosomes increase their amount of DSas-4-GFP as a consequence of daughter centriole formation, while the same cannot be seen in Asl-negative centrosomes.

Rad51: An essential recombinase and beyond

Fumiko Esashi

How genetic traits are maintained and passed from one generation to the next is a fascinating question. A major conceptual breakthrough was made in 1927 when Nikolai Koltsov proposed a "giant hereditary molecule made of two mirror strands that would replicate in a semi-conservative fashion using each strand as a template". Through the identification of deoxyribonucleic acid (DNA) as the hereditary molecule by Oswald Avery and his coworkers in 1944, and the double-helical DNA structure model by James Watson and Francis Crick in 1953, it became clear that DNA is the central hereditary substance, and is inherited through the mechanism that was predicted by Koltsov. It was in the 1980s that I first encountered the DNA molecule at a scientific exhibition during my first year of secondary school and was completely captivated by its beauty.

I now run a research group in the Dunn School, and am studying how genomic DNA is protected from various stresses that are encountered in living cells. Double-stranded DNA (dsDNA), with its distinctive base-paired structure, is surprisingly stable in pure form. However, in living cells, dsDNA is unwound when genetic codes are read (transcribed) prior to protein production, and when DNA is copied (replicated) prior to cell division. Single-stranded DNA (ssDNA) that is inevitably generated during these processes is more vulnerable to mechanical stress, and more reactive with chemicals, such as those produced by cellular metabolism. Furthermore, ultraviolet and other radiation from the sun, earth and space, and chemicals from food, water and the atmosphere can all attack DNA, therefore it is almost inescapable to have DNA breakages. With broken DNA, cells stop functioning correctly, and ultimately fail to divide when required. Consequently, faulty repair of DNA is closely linked to a number of human genetic disorders encompassing developmental deficiency, premature aging, immunodeficiency and cancers.

Rad51, an essential enzyme that is highly conserved from bacteria to the human species, plays unique roles in repairing broken DNA and protecting vulnerable DNA structures (Figure 1). This enzyme is described as a recombinase, since it plays a central role during the process of homologous recombination (HR). HR provides a mechanism for the faithful recovery of missing genomic information by copying the corresponding region of the sister chromatid, which maintains an intact version of the missing DNA. To initiate this process, the broken end of dsDNA is first processed to ssDNA which is coated with Rad51, and then searches for a matching (homologous) sequence within duplex DNA. Once this has been achieved, the ssDNA engages with the homologous strand of the template dsDNA, copies the missing genomic information and fills the gap in the broken chromosome. In some circumstances, such, as when HR is hyperactive, inappropriate engagement of ssDNA with dsDNA may occur, resulting in genomic instability.

How is HR precisely regulated in human cells to prevent genome instability? Genome size has increased enormously during evolution (such that the human genome is around 1000 times bigger than those of bacteria), and the set of genes that regulate HR has evolved in line with this increased complexity. In the human population, mutations of these genes are closely linked to various genome instability

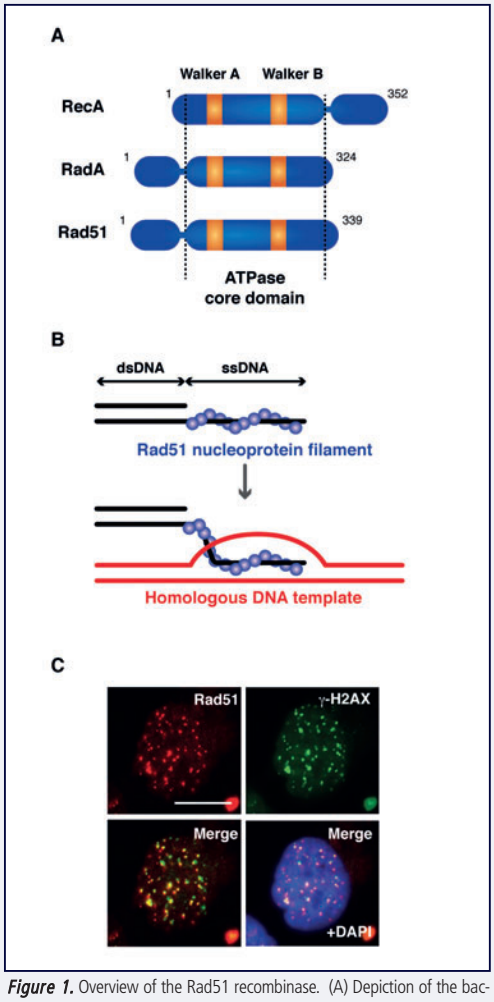

Figure 1. Overview of the Rad51 recombinase. (A) Depiction of the bacterial RecA, archaeal RadA and human Rad51 recombinases, sharing an ATPase core domain. (B) The molecular mechanism by which Rad51 mediates the initial step of HR. Rad51 assembles on ssDNA to form nucleoprotein filaments, which in turn mediates the invasion of ssDNA into the homologous region of the template dsDNA. (C) Immunofluorescence analyses showing the accumulation of Rad51 at sites of damaged DNA, marked with γ-H2AX, in irradiated human cells (4Gy, 4 hours).

syndromes, including hereditary breast and ovarian cancer syndrome (HBOS) and Fanconi anaemia (FA). BRCA2 (breast cancer, 2), also known as FANCD1 (Fanconi anaemia, type D1), plays a vital role in the regulation of HR. BRCA2 contains two distinct Rad51 binding motifs (BRC motifs in the central region and an unrelated TR2 motif in the carboxy-terminal region), and also binds directly to ssDNA; together, these features help BRCA2 to recruit Rad51 to sites of DNA damage and so promote HR. Interestingly, we found several years ago that BRCA2 is phosphorylated by cyclin-dependent kinases (CDKs), central drivers of cell proliferation, allowing fine-tuning of HR during the cell cycle [1].

Phosphorylation of BRCA2 by CDKs occurs at multiple residues within both terminal regions of this large protein. Our efforts to understand the functions of these BRCA2 phosphorylation events recently showed that CDK-mediated BRCA2 phosphorylation at threonine 77 (T77), which has been found to be mutated in some breast cancer patients, triggers its direct binding to another cell proliferation driver, polo-like kinase 1 (Plk1) (Figure 2). Remarkably, BRCA2 binding to Plk1 begins during the late phase of DNA replication, and reaches a peak when cells enter mitosis. Furthermore, we found that Plk1 directly phosphorylates Rad51 at serine 14 (S14) within its amino-terminal region, and this occurs most efficiently when BRCA2 is present. On the basis of these findings, we propose that BRCA2 acts as a molecular platform to facilitate Plk1-dependent Rad51 phosphorylation, and that Plk1, which is generally thought of as a mitotic driver, is also involved in HR regulation [2].

This observation led us to an important and challenging question — what is the role of Plk1-mediated Rad51 phosphorylation? Our studies have so far failed to reveal a direct impact of this phosphorylation event on the known biochemical activities of Rad51 in HR. Instead, we found that Rad51 S14 phosphorylation triggers a second phosphorylation event at the adjacent residue, threonine 13 (T13), by the acidophilic kinase casein kinase 2 (CK2). T13-phosphorylated Rad51 binds to a DNA damage sensor protein, Nbs1, the product of the gene mutated in a chromosomal instability disorder, Nijmegen breakage syndrome. This highly dynamic and elaborate mechanism allows Rad51 to accumulate at sites of damaged or stressed DNA during replication, repairs and/or protects DNA, and hence promotes genome stability (Figure 2) [2, 3]. It is important to note that, when expressed at a high level in cells, Plk1 can directly phosphorylate Rad51 with little assistance from BRCA2. We suspect that such conditions, found in many cancers, may be linked to the resistance of BRCA2-defective cancers to widely used anticancer drugs that cause DNA damage.

We are now beginning to understand the mechanism by which human cells protect Koltsov's 'giant hereditary molecule' through the highly sophisticated process of HR. However, there are numerous mysteries still to be solved. In contrast to bacteria, which trigger the expression of RecA (the orthologue of human Rad51) when DNA is damaged, human Rad51 is expressed regardless of DNA damage and is essential for normal cell survival. Human cells with faulty Rad51 die after S phase (when most DNA is replicated) and before mitosis, suggesting that Rad51 is important not only for repair of broken DNA but also for as-yet-unknown events before cells divide. We are posing the simple question: what does Rad51 really do in perturbed and unperturbed human cells, and what are the regulatory mechanisms? I anticipate that answering this naïve question will be useful for various practical applications. For example, excessive HR can induce genome instability, contributing to phenotypic heterogeneity among cancer cells, a major obstacle to cancer treatments. In this context, targeting the HR pathway would be an effective strategy to treat and prevent cancer recurrence. Another interesting avenue is to exploit knowledge of the HR pathway to improve gene editing technology, as described elsewhere in this issue by Andrew Bassett, head of the Gene Editing Facility in the Dunn School. I expect that modifying the pathways that control HR could increase the chance of HR-mediated gene targeting. My childhood curiosity about the beautiful DNA molecule has brought me this far, but the finish line of my journey still seems to be a long way off.

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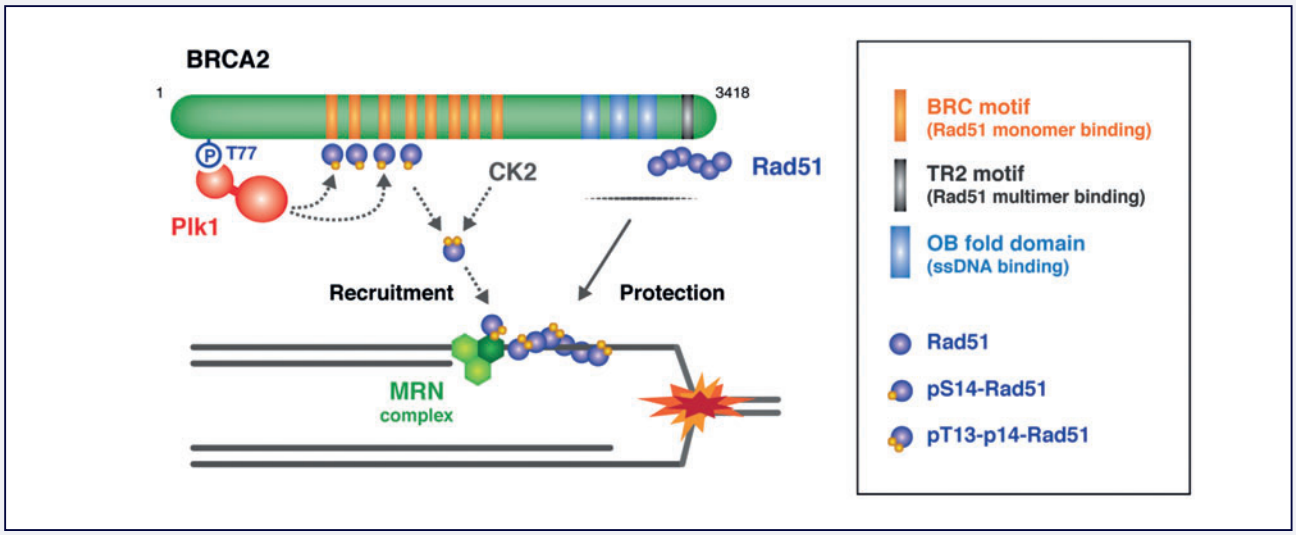

Figure 2. Architecture of BRCA2, and its proposed functions in recruiting Rad51 to stressed DNA. BRCA2 binds monomeric Rad51 through its BRC motifs and multimeric Rad51 through a TR2 motif. It also binds Plk1 following phosphorylation at threonine 77 (pT77) by CDKs, and assists Plk1-mediated Rad51 phosphorylation at serine 14 (pS14). This triggers subsequent phosphorylation at the adjacent threonine 13 (pT13), which in turn mediates binding to Nbs1, a subunit of the MRN (Mre11-Rad50-Nbs1) damage sensor complex, facilitating Rad51 accumulation at sites of stressed DNA. The BRCA2 carboxyl-terminal region, which binds ssDNA and Rad51 multimers, stabilises the Rad51 nucleoprotein filament and hence protects vulnerable DNA structures at stressed DNA replication forks.

Focus on virology: Interview with Jeff Almond

Having led a distinguished career at the forefront of virology, Jeff Almond recently took early retirement from Sanofi Pasteur and now holds a visiting Professorship at the Dunn School.* Fusion *caught up with him to find out more about his past research interests, the tensions between his parallel careers in academia and industry and his vision for the future of vaccinology.

Perhaps, you could begin by telling us a little about your background and your career to date.

My first degree was in biochemistry and microbiology combined Honours from Leeds. I then moved to Cambridge University Department of Pathology to study a PhD in Virology. I first joined the Pharmaceuticals Industry immediately afterwards as a post-doctoral fellow, to work on influenza cold adapted vaccines (the current FluMist vaccine) at Sandoz Forchungsinstitut in Vienna. After 2 very enjoyable years I spurned an opportunity to stay in Industry and moved back into academia, joining Leicester University to start my own research group, where I worked on a range of viruses including polio, rhinovirus, Coxsackie, Hepatitis A and HIV. I also worked on archaebacteria and prions. In 1985 I won the Society for General Microbiology's Fleming Prize, an annual award that recognizes outstanding research in any branch of microbiology by a scientist in the early stages of his or her career, and was awarded a Lister Institute Fellowship. After six years at Leicester I was offered the chair of Microbiology and Head of Department at the University of Reading. In addition to expanding my research group, I became involved in a range of other activities; for example I was a member of the Government's Spongiform Encephalopathies Advisory Committee (SEAC) during the height of the UK 'mad cow' crisis; I was an expert witness for the first Biotechnology patents to be examined by the English legal system, and was retained as advisor to counsel through the Court of Appeal and up to the House of Lords; I was International Secretary for the Society for General Microbiology and Chairman of the Virology Division of the International Union of Microbiological Societies (IUMS). During this phase of my academic career I also spent a one year sabbatical working for Ciba-Geigy in Basel, Switzerland developing drugs in their HIV and Oncology division.

My industrial career started seriously, however, when I joined Sanofi Pasteur (then Pasteur Merieux Connaught) at their Lyon site in 1999. I was appointed as Vice President R&D France with a role to manage and develop a portfolio of projects on human vaccines, including therapeutic vaccines against cancer. My role became more global through the early 2000's and I was promoted to Senior Vice President for Global Research with responsibilities for all early phase (up to end of Phase 1) R&D projects in the company, incorporating research at our Toronto and Swiftwater Pennsylvania, and eventually Cambridge Massachusetts sites. I also took responsibility for finding and establishing collaborations with groups in academia and the biotech sector where the work was relevant to our interests. Last autumn, after almost 15 years in the role, I took early retirement, easing out via this "sabbatical" period at the Dunn School. Since 2008, I have served as a member of Council of the Medical Research Council. In 2007 I was elected to a Fellowship of the UK Academy of Medical Sciences and one year later was co-opted onto its council where I served for two years. In 2013 I won the Colworth Prize of the SGM for "recognition of outstanding work in the field of human virus vaccines".

What first inspired you to study virology and what would you consider to be your most important contribution to the field?

As a young microbiology student I witnessed the growth in molecular biology techniques during the 1970's and realised that understanding the full structure and function of a "life" form would start and be easier with the smallest entities; hence my attraction to virology. The 1970's and early 80's were an incredibly exciting period during which we witnessed the first full genome sequences of numerous important viruses as well as some 3-D structures at high resolution. We also began to gain insights into mechanisms of pathogenesis. My own contributions to the discipline at that time included the first identification of a gene controlling the host range of influenza virus and determination of the first complete genetic map for the avian influenza virus in 1977. In 1979, Stephen Inglis and I obtained the first evidence for the existence of overlapping genes in any RNA genome (influenza virus). At this timem, I also completed the first comprehensive analysis of proteins encoded by influenza B virus. In the early 1980's when DNA sequencing was still in its infancy, we determined the complete nucleotide sequence of poliovirus and identified major antigenic structures on the surface of the virus particle. Perhaps our biggest achievement of that era, however, was the complete genetic analysis of the attenuation phenotype of a human vaccine (the Sabin poliovirus vaccine) working in collaboration with scientists from NIBSC. This work illustrated key features of the poliovirus genome that were required for neuro-virulence and, therefore, gave insights into how to construct improved attenuated vaccine strains. We also produced the first complete nucleotide sequence of a rhinovirus in 1984 and a Coxsackie virus shortly thereafter. Through this period, we turned our attention to the details of the pathogenesis of these viruses including, for example identifying cellular receptors such as the decay accelerating factor (CD55) for Echovirus 7 and trying to understand how interaction with this complement regulator influenced immunity and pathogenesis. We then pioneered the use of these viruses as vectors for foreign antigens.

What has brought you to the Dunn School and what do you hope to achieve during your sabbatical here?

A combination of factors brought me to the Dunn School. First is my longstanding friendship with Keith Gull who encouraged me to write to Matthew to request a visiting Professorship, but I was also very well aware of the reputation of the Dunn School in virology with scientists such as Quentin Sattentau, Ervin Fodor and William James and before them George Brownlee and Geoff Smith. I was also aware of excellent work in vaccinology going on more broadly in Oxford, particularly through the Jenner Institute. Although my objectives for the year have been to read and learn generally, my main aim has been to bring an industrial perspective to academic research and to provide guidance to academics who are trying to translate their findings into practical applications. I have had numerous meetings and discussions with researchers who are searching industrial collaboration or who are interested in starting companies. I have also joined several Scientific Advisory Boards (e.g.

Public Health England, The Jenner Institute, The Pirbright Institute, NIBSC, Institut Pasteur, CIRI, JDRF, etc.) to provide an industrial perspective on the work they are engaged with. Here in Oxford, I have also worked with Isis Innovation to help them assess the value and strength of their patent portfolio in the vaccines area and with their patenting strategy. I have been a member of MRC Council for the past 6 years and one of my observations is that UK medical science is in excellent health but scientists could do much more in terms of enterprise creation to provide a better "return on investment" for the nation. Producing papers in *Nature* and *Science* and other high impact journals is regarded as a good measure of success by scientists, but politicians and the general public want to see wealth creation via products that improve people's lives. If I can encourage and provide guidance to those wanting to try entrepreneurship while I am in Oxford, then my sabbatical will have been a success.

Having led a very distinguished career as a virologist, both in academia and industry, how would you compare the challenges and opportunities that either setting brings?

I consider myself very fortunate to have had two successful careers. My academic life was a buzz from start to finish and I enjoyed every minute of it but I can also say the same about my industry life. The reality is that both environments provide great opportunities, challenges and rewards and I have met impressive people who have done well in both settings. It is of course truly rewarding as an academic to generate new knowledge and insights on a favourite topic but it is at least as rewarding for an industrialist to develop new products that impact society. My advice to academics is to familiarise themselves with industry through visits, discussions and collaboration and to learn what goes on there. There are many challenges and job opportunities that lie just behind the cutting edge of their discipline, in areas such as regulatory affairs, quality control or process development. These jobs are challenging and rewarding and often provide an excellent team-working environment.

There has been much speculation in the popular press about the emergence of new viral pathogens and their likely virulence. How great a threat, in your opinion, does this pose in an age of globalisation?

I usually say that the propensity for a virus to evolve and spread is in part related to the size and density of its host population. For viruses that infect humans there is a juicy 7 billion of us for them to get their teeth into, an increase of almost 3-fold in my lifetime! Add to that the fact that we move around and cross continents like never before, and that we have rapid climate change which may extend the range of virus vectors, and you have a situation ripe for the emergence of virulent new viruses: witness HIV, SARS, MERS and West Nile Virus among others in the last few decades, and we have all been shocked by the severity of the latest re-emergence of Ebola virus in Africa. There is also an ongoing threat from pandemic flu and I am not one who talks down the risk of emergence of highly-virulent H5, H7 or other sub-types that could evolve to facilitate rapid human spread. Everyone talks about the 1918 Spanish flu that killed an estimated 40 million people but even a strain as virulent as the Asian flu of 1957 would have a dramatic effect in today's world (and not only on health but also politically and economically). All that said, I think bodies like WHO, national governments, and industry are much better organised than in the past. The control of SARS was impressive and worked, after an initial hiccup, through good inter-governmental cooperation and sensible quarantine, although perhaps we were lucky that it was not a highly transmissible virus. We are working hard on building surge capacity for pandemic flu vaccine production and the anti-viral drugs portfolio at our disposal has broadened impressively in the last couple of decades. So, although the threat is real and constant, I believe that medical science is gradually reducing the severity of the hazard. But certainly, politicians and policy makers need to be aware that there is much more work to be done.

Although there have been many past successes in the development of vaccines for viral pathogens, vaccine development appears to have reached something of a plateau. What do you see to be the future of vaccination and where the next big breakthroughs might come?

You see a plateau? I am not sure that I do. The last few years has seen the development of very effective vaccines against human papilloma viruses, pneumococcus, rotavirus, meningitis B, and a new Dengue vaccine is on its way. Improved versions of existing vaccines and better combination vaccines are continually being produced and we have also had some encouragement recently in the field of malaria and HIV. But I agree that the remaining human pathogens are difficult, mainly because of the tricks they elaborate such as antigenic variation and immune-modulation. For some, such as HIV, or recurrent HSV, we don't know what an immune status looks like so we don't know exactly what we are trying to achieve with a vaccine in terms of immune responses. I am convinced that further progress on these difficult pathogens depends on academia and the vaccines industry working closely together. Improving our knowledge of the natural biology of pathogens improves our prospects of developing effective vaccines against them. Personally, I think that the application of transcriptomics and proteomics to better understand natural infection, especially where that infection leads to protective immunity, will be very informative. I also anticipate progress in allergy vaccines and cancer therapeutic vaccines as we learn how to better control immune responses via manipulations at the numerous immunological checkpoints that we have learned about in recent years.

How do you envisage the field of virology evolving in the future and what are the key challenges in the field that still need to be addressed?

That's a huge question! Perhaps it stems from a sentiment around in the 1990's, as molecular techniques advanced rapidly and the study of more complex life forms became tractable, that virology was a dying discipline. However, now, as then, it is important to think of virology as more than just the study of virus structure and replication. The study of viral pathogenesis at the molecular level remains a rich and fertile area where virology, immunology, physiology and biochemistry meet. Beyond that we have vaccinology and antiviral pharmacology. I remind you that today we have no cure for AIDS, we struggle to control recurrent HSV, we have little against CMV and EBV, and we all suffer regularly from respiratory infections caused by influenza, para influenza, respiratory syncytial virus, coronaviruses and rhinoviruses. Some of these are a real problem in COPD patients and severe asthmatics. We then have diarrhœa-causing viruses such as noroviruses and rotaviruses and I haven't even started to mention the viruses of animals and plants that cause problems in agriculture, and occasionally wreak havoc in wild populations. Viruses display huge diversity in their interaction with their hosts: some have evolved hit and run strategies, others may become latent or on slow burn and yet others may induce cancerous changes in the cells they infect. Working out the immune evasion and decoy strategies that the viruses have evolved to outwit their hosts can provide insights into how our

immune systems work. Recently I was fascinated to learn that there are RNA sequences in human cells that seem to have no corresponding DNA sequences. Potentially they are RNA replicons that are on an evolutionary path to becoming transmissible viruses. This reminded me that our understanding of the origins of viruses is still rudimentary, as is our knowledge of the role of viruses in ecology and the way changes in their numbers and fitness may cause fluctuations in the populations of their hosts. The study of the microbiota of humans has surprised us all in the last few years with the discovery that there are hundreds and perhaps thousands of different species of bacteria that live in us and on us and no doubt influence our health and behaviour. It is highly likely that they all carry specific and even mutual bacteriophages that contribute to their phenotypes and influence the behaviour of the bacteria in which they live and perhaps their relative population sizes. Some may even act as guardians against hostile bacteria.

So I am convinced that virology is far from a dying subject. There is much still to be learned and new avenues to explore.

What would your advice be to anyone contemplating embarking on a career science?

Put simply: "do it". Science provides a fascinating and very rewarding career. But more than that, it provides a philosophically sound way of thinking. It is a way of knowing.

Alien genes: The unusual molecular biology of influenza viruses

Ed Hutchinson

Viruses are molecular parasites and, as such, must take over the biochemical resources of a host cell in order to reproduce. However, the host cell may not be able to do everything that a virus needs, as the molecular biology of many viruses includes features never normally employed by cellular life. The influenza viruses, the focus of our group's research, are a good example of this principle. They entirely lack a DNA genome, having instead evolved with a genome consisting of short segments of single-stranded, antisense RNA. This RNA genome is so utterly different from anything in normal cell biology that influenza must make its own arrangements for the transcription and replication of its genes.

How this works on a molecular level is fascinating in its own right, but as influenza viruses are serious human and veterinary pathogens there is also an urgent clinical need to understand how they operate. Every winter, influenza causes epidemics of a highly contagious respiratory disease, leading to substantial mortality in high risk groups such as the elderly. In addition, the virus has an unusual facility for cross-species adaptation, which has caused repeated influenza pandemics in the past and which threatens more in the future. Currently, vaccines only offer short-lived protection and, although two classes of antivirals have been approved for treating the disease, one of these has been discontinued due to drug resistance and the efficacy of the other class, neuraminidase inhibitors such as Tamiflu, is the subject of heated debate. As a result, new targets for antiviral chemotherapy are needed, and we and others hope that influenza's distinctively viral mechanisms of RNA transcription and replication may prove to be its Achilles heel.

These mechanisms, as well as wider details of the influenza virus replication cycle, have been studied in the Dunn School through the close collaboration of groups led by Ervin Fodor, Frank Vreede and George Brownlee (now retired). Together, we have worked to clarify the basic molecular mechanisms that control the replication, and hence the pathogenesis, of influenza viruses.

As with any problem in molecular biology, we have gained considerable mechanistic insights through reductionist approaches. An influenza virus particle contains up to eight rod-shaped ribonucleoprotein complexes (RNPs) (Figure 1(A)), each of which consists of a single

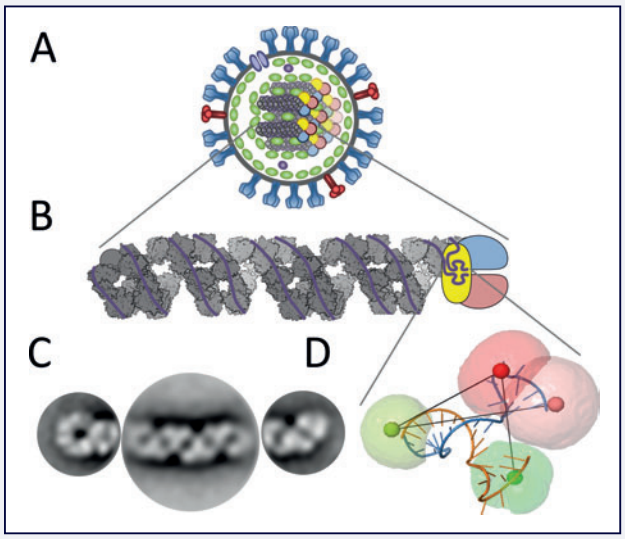

Figure 1. The influenza virus ribonucleoprotein (RNP). Schematics of (A) an influenza virus particle and (B) an RNP containing viral RNA (continuous line), NP (grey) and the RdRp (pink, yellow and blue). Structures of (C) a replicative intermediate — an RNP containing postitive-sense RNA — determined by electron microscopy (from York *et al.* (2013) PNAS 110 (45) E4238–45) and of (D) the RNA promoter sequence, based on the location of bound fluorophores depicted as spheres (Robb and Tomescu, unpublished data).

strand of RNA, corresponding to a portion of the genome, as well as multiple copies of a scaffold-like nucleoprotein (NP) (Figure 1(B)). As antisense RNA can neither be transcribed nor replicated by host proteins, RNPs must also contain the trimeric viral RNA-dependent RNA polymerase (RdRp). Each RNP functions as an independent replicative unit, and RNPs can, therefore, be reconstituted in cells by expressing their constituent proteins and a suitable RNA from plasmids. This approach, combined with *in vitro* polymerase activity assays, has allowed us to perform detailed mutational analyses of the RNA sequences that define the viral promoter and polyadenylation signals, as well as of residues in the RdRp required for obtaining mRNA cap sequences. By expressing additional viral proteins in these assays, we have shown that influenza can modulate its own replication, transcription and splicing. A key breakthrough in reductionist studies of the virus was the development by Ervin Fodor in 1999 of a 'reverse genetics' system, which allowed fully infectious virus to be generated from plasmids. This allowed studies of individual RNPs to be extended to studies of complete viruses; in addition, the technology is now used for the rapid development of influenza vaccines.

RNPs are dynamic, and by combining functional assays with biochemical techniques we have begun to understand how their assembly and function is regulated. We have seen how the separate nuclear import of subunits of the RdRp confines RNP assembly to the nucleus (Figure 2(A)), and how the rod-shaped helix of an RNP is formed by the directional oligomerisation of NP, with a loop on a free NP monomer inserting into a groove in the assembling RNP. For an RNP to replicate, its single-stranded RNA must be copied into a complementary intermediate and back again. As well as characterising the dynamics of this process, we recently showed that this complementary intermediate has a similar structure to the template RNP (Figure 1(C)) and that its replication requires the action of a second, unbound RdRp — though exactly what this second polymerase is doing is unclear.

Structural information can also provide enormous mechanistic insight. To understand how the RdRp acquires mRNA cap structures we undertook a collaborative study to solve the structure of an endonuclease active site within the RdRp, and a separate collaboration is nearing completion to solve the structures of the RNA promoter using single-molecule technologies (Figure 1(D)). We are also involved in an ongoing collaboration to tackle the substantial technical problems associated with solving the structure of the complete RdRp trimer.

Viruses are often seen as very simple biological systems, which makes reductionist studies of their biology particularly appealing. But the idea that viruses have only a handful of genes at their disposal is false. Most of the activities relevant to a virus' replication are performed not by virally-encoded proteins but by the proteins of its host, and further host proteins act to antagonise the viral replication cycle. We have, therefore, tried to place our studies of RNP function in the wider context of their host's molecular biology. For example, we have shown that the virus benefits from interactions with host factors such as Hsp90, RanBP5 and CCT, but is restricted by other factors such as RIG-I. We and other groups have found that nuclear import of viral proteins proceeds via interactions with specific importins (Figure 2(A)), with adaptation to mammalian hosts linked to differential importin usage. Within the nucleus, we have shown that RNPs associate with the C-terminal tail of host RNAPII, where they can efficiently shut off host transcription and facilitate their own transcription by 'snatching' cap structures from host mRNAs and attaching them to their own transcripts (Figure 2(B)). In addition to its nuclear localisation, we found that one of the RdRp subunits is targeted to mitochondria and enters the mitochondrial matrix (Figure 2(C)). We have shown that this mitochondrial localisation is linked to host adaptation and virulence, although why this should be the case is a problem we are still trying to address.

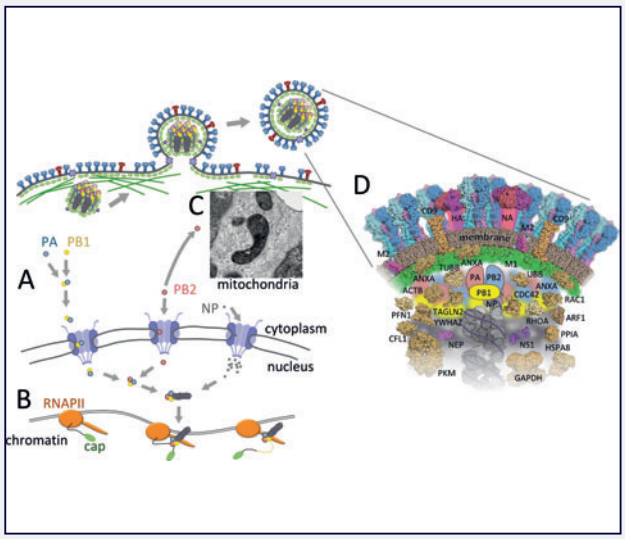

Figure 2. Host proteins in the viral replication cycle. Schematic of an infected cell, showing (A) the transport of RdRp subunits into the nucleus, with complete trimer assembly only occurring after nuclear import, (B) the binding of a newly-assembled RNP to the C-terminal tail of host RNAPII, followed by 'snatching' the cap structure from a nascent host mRNA and attaching it to viral mRNA (yellow), (C) a mitochondrion containing a subunit of the RdRp, labelled with a tag that causes dark staining (Long and Johnson, unpublished data) and (D) the viral proteins (bright colours) and host proteins and lipid (brown) incorporated into a virus particle (Hutchinson, unpublished data).

As well as interacting with host proteins, viral proteins themselves participate in the full biochemical complexity of the cell, undergoing extensive post-translational modifications. We recently identified phosphorylation sites in all the viral proteins, and we are now investigating their regulatory functions. For example, we have found that the assembly of RNPs is regulated by phosphorylation of the NP-NP interaction site, and that viral replication relies on the recruitment of specific cellular phosphatases to the polymerase.

The final stages of viral replication involve packaging new RNPs into virus particles (Figure 2(D)). Using the Dunn School's proteomics facilities we have begun to characterise these particles in detail, showing that they incorporate substantial amounts of host proteins as well as viral proteins, and that, as a result, the structure of the virus particles that spread infections is strongly determined not just by the virus, but also by the type of host cell it has infected.

Influenza is not a new problem — the name dates back to early modern fears of malign external 'influences,' and the disease itself probably dates back to the domestication of animals. But despite the apparent simplicity of the virus — to say nothing of eighty years of intensive laboratory study — some of the key details of its molecular biology are only just starting to emerge. We know that they will be fascinating, and we hope that they will prove useful in tackling the virus in time for the next influenza pandemic.

Working to understand and eradicate HIV-1

Quentin Sattentau

The human immunodeficiency virus type-1 (HIV-1) is a major human pathogen, which, since its discovery in the early 1980s, has been responsible for an estimated 36 million AIDS-associated deaths. This virus has a formidable array of immune evasion strategies that allow it to persist in the infected host lifelong, even in the presence of suppressive antiretroviral therapy. Despite 25 years of significant effort, we still do not have an effective vaccine. HIV-1 is predominantly a sexually transmitted disease, and our inability to prevent new infection or cure existing infection means that pandemic viral spread continues.

Antigens

My laboratory has been interested in aspects of HIV-1 infection and spread for almost 30 years, in fact since only a few years after the virus was first isolated in 1983. Our early work concerned virus-receptor interactions, and helped define the binding site for CD4, the primary virus receptor, on the viral surface glycoprotein, gp120. This led to attempts to inhibit the CD4-gp120 interaction, and parallel analyses of the mechanism of action of neutralizing antibodies isolated from immunized mice and infected patients. An obvious application of neutralizing antibody function is the development of a vaccine that might elicit such antibodies by active vaccination. However, the extensive antigenic variation exhibited by the target of neutralizing antibodies, the envelope glycoproteins (Env) gp120 and gp41 (Figure 1), has made this very difficult. Additional problems relating to use of Env as a vaccine antigen include extensive glycan coverage masking the protein surface from B cell recognition, conformational instability reducing B cell receptor (BCR)-epitope engagement, and steric interference with BCR and antibody binding to Env (Figure 1). A recent breakthrough that has revitalized an otherwise rather depressed field is the isolation of so-called broadly neutralizing antibodies (bNAb) that recognize highly conserved features on Env, enabling neutralization of a very broad spectrum of viral strains. The current challenge, which is by no means trivial, is to translate the knowledge of bNAb paratope-epitope interactions into useful immunogens for eliciting similar antibodies by vaccination [1]. We are contributing to this by: i) attempting to increase the B cell immunodominance of bNAb epitopes relative to other regions of Env using targeted semi-synthetic glycan insertion (in collaboration with Ben Davis, Chemistry); ii) eliminating the conformational instability of Env using chemical cross-linking approaches; iii) synthesising glycopeptide mimetics of regions of Env to act as mini-immunogens.

Adjuvants

A second research thread relating to HIV-1 vaccine design concerns adjuvants. Vaccines that rely upon subunit antigens such as HIV-1 Env need extrinsic adjuvantation to trigger and modulate the immune response. Currently available adjuvants are either sub-optimal for this purpose (eg. alum), or are proprietary and not available for research or exploratory clinical use. We therefore set out to discover new adjuvants that would be potentially suitable for eliciting bNAb against HIV-1 and could be used for research and potentially translated to clinical use. We discovered two classes of adjuvant. The first is a family of anionic polymers termed carbomers with potent B and T cell activating properties. In fact, it turned out (after some attempted patent filing with Isis) that this class of molecule is already in use in some veterinary adjuvants, but this had not been published. Nevertheless, a number of groups are now actively engaged in developing carbomers for human vaccine use. The second type of adjuvant is a cationic polymer called polyethyleneimine (PEI), well know to many as a transfection reagent. PEI has robust innate and adaptive immune stimulating properties via both mucosal and systemic administration routes, and drives strong antiviral neutralizing antibody responses [2]. We are actively pursuing the use of PEI in man for HIV-1 and other vaccine purposes.

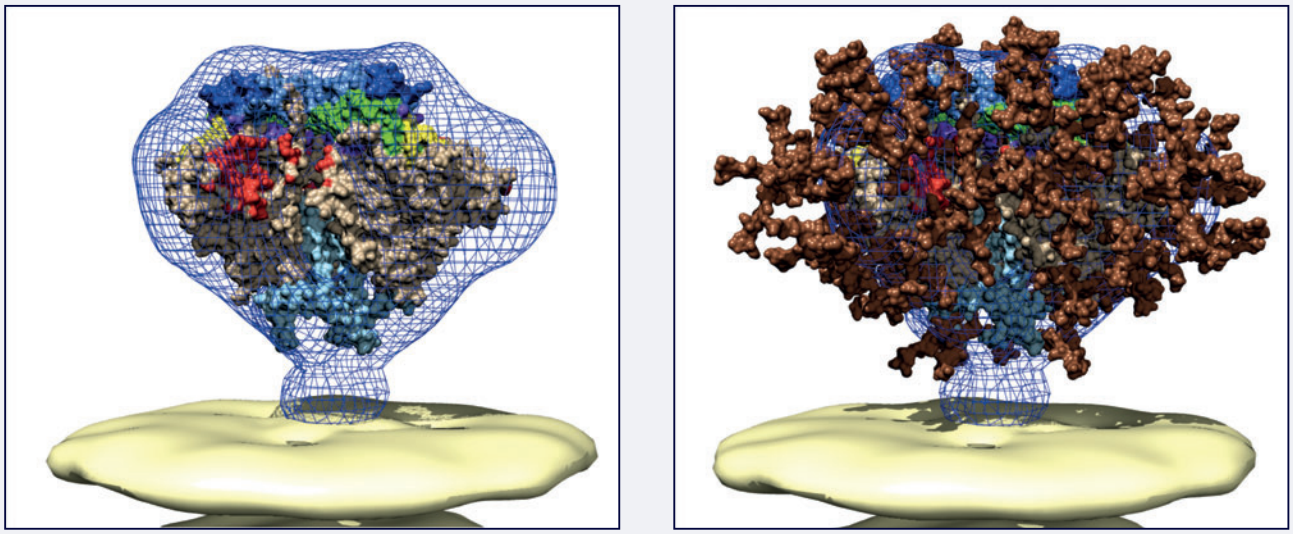

Figure 1. *The HIV-1 envelope glycoprotein (Env) spike — entry machine and target of antiviral antibodies.* Left panel, *protein core of Env lacking the glycan shield comprised of an* in situ *electron cryo-tomographic reconstruction of the spike (blue mesh) into which have been integrated the atomic-level structural features of the transmembrane glycoprotein gp41 (light blue), the outer envelope glycoprotein gp120 (tan), the gp120 CD4 binding site (red), the coreceptor binding site (yellow) and the hypervariable loops V1V2 (dark blue) and V3 (green).* Right panel, *the Env spike with the predicted N-linked high-mannose and complex glycans modeled in brown. Courtesy of Torben Schiffner.*

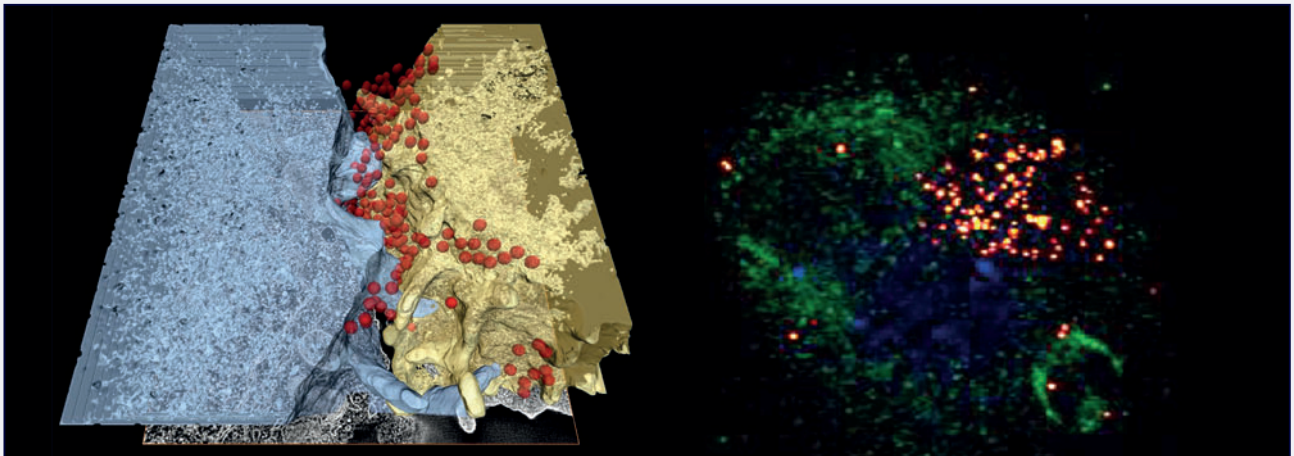

***Figure 2.** Virological synapses. Left panel, 3D reconstruction of a virological synapse in which HIV-1 (red spheres) is moving from an infected T cell (left cell, light blue) across a synaptic cleft, into a receptor-expressing target T cell (right panel). Courtesy of Sonja Welsch. Right panel, a macrophage infected with HIV-1 imaged using super-resolution STED microscopy. The image shows HIV-1 virions mostly contained within a compartment termed the 'virus-containing compartment', with an adherent $CD4^+$ T cell in the process of becoming infected (bottom right). Red = viral core (Gag); green = CD4; blue = early endosome marker EEA1. (Courtesy of Jakub Chojnacki)*

Pathogenesis

HIV-1 virions released from infected cells target receptor-bearing cells for further infection. However, this mode of fluid-phase diffusion is slow and inefficient, and the vast majority of virions become inactivated before they reach a permissive cell. An alternative mode of HIV-1 dissemination between infected and receptor-bearing cells is across a small gap, or synapse, present between contacting cells [3]. We first described the virological synapse for HIV-1 spread between T cells in 2004, and have gone on to characterize the cell biology and virology of this interaction. We have more recently shown that HIV-1 can also spread most efficiently via synapses between T cells, the dominant HIV-1 target cell in the body, and macrophages, the second most important target cell (Figure 2). The existence of synaptic viral spread has implications for antiviral therapy and vaccine design. Antiretroviral therapy (ART) is dosed to prevent onward spread of virus in its cell-free form. However we and others have shown that synaptic transmission of the virus leads to dramatically reduced efficacy of ART, and that the drugs would need to be used at 10–100-fold higher doses to achieve complete suppression of this mode of spread. Similarly, neutralising antibodies are substantially less effective at reducing HIV-1 infection by synaptic compared to cell-free transmission, implying that higher titres of such antibodies may need to be elicited to afford complete protection from cell-associated viral infection.

The Future

Our work to date has revolved around using *in vitro* systems to interrogate HIV-1-host cell interactions. We will continue in the quest to understand these interactions in more detail and at higher resolution, using, for example, super-resolution light microscopy and 3D electron microscopy (Figure 2). Whilst we strive to use primary immune cells (rather than immortalized cell lines) for our work, this nevertheless has the usual caveats relating to *in vivo* relevance. We would, therefore, like to move towards more sophisticated systems that better recapitulate the complexity of the immune system. These systems may include *ex vivo* human tissue explants and artificial human lymphoid tissue mimics, and *in vivo* studies using human immune system-reconstituted mice and intravital imaging. My hope is that we can continue to try to understand the fundamental biology underlying HIV-1 infection of target cells and its inhibition whilst translating elements of this work into products with potential biomedical use.

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3. Sattentau (2008) *Nature Rev Microbiol* **6:**815–826

Making a gift to the Dunn School

The Dunn School owes its existence to a philanthropic gift, from the Trustees of Sir William Dunn, and over the years has been the beneficiary of many acts of philanthropy, not least from those who have worked here. Any gift made to the Dunn School helps to further research here, whether it is made to support a specific initiative such as the ones described in this newsletter, or at the discretion of the Head of Department.

If you would like to make a gift to the Department this year, please use the gift form enclosed with this edition of *Fusion*. Please make sure that you have completed a gift aid form so that we can reclaim tax on your gift, and note that if you are a higher rate tax-payer, you can also set your gift against your tax liability for the year. All gifts made to the Dunn School from the USA are also fully tax-deductible, when made through the University's 'giving vehicle' there, the *Americans for Oxford, Inc* organization.

Stem Cells, Macrophages and HIV-1: An Experimental Threesome

Kenny Moore

Research into macrophage biology has been a staple of the Dunn School for over sixty years and currently there are numerous laboratories independently investigating the role of macrophages in a variety of diseases, from atherosclerosis to *Leishmania*. Our laboratory's interest in macrophages arises from their susceptibility to HIV-1 infection, for which they act as long-lived viral reservoirs, continually producing virus and enhancing viral dissemination to the major target cells, $CD4^+$ T cells.

HIV Entry of Macrophages

The James lab's focus has been to understand the molecular events that are required for HIV-1 infection of macrophages, with a particular emphasis on the role of endocytosis. This work was originally performed using inhibitory drugs to elucidate the precise requirements for HIV-1 entry, culminating in the view that a specialised but previously uncharacterised cholesterol-dependent macropinocytosis-like pathway was involved in HIV-1 entry into macrophages, which required the activity of dynamin, Rac1 and Pak1 [1]. However, this type of study is limited by a dependence on either chemically-differentiated monocytic cell lines that have limited capacity to accurately recapitulate macrophage biology or blood monocyte-derived macrophages, differentiated *ex vivo*, which are non-dividing, genetically intractable and suffer from donor-to-donor variability.

Macrophages from Stem Cells

As an alternative source of macrophages for studies into HIV-1 infection, we have developed an efficient method to derive functional macrophages from embryonic stem cells or, more ethically justifiable, induced pluripotent stem (iPS) cells [2]. The process involves aggregating stem cells into embryoid bodies, which are then "encouraged" to differentiate along the haematopoietic linage. This process results in adherent "factories" that release monocytic cells, which are harvested and differentiated into macrophages using physiologically relevant cytokines. This technique overcomes the main limitations of blood monocyte-derived macrophage research, as the stem cells are self-renewing, genetically-tractable, and of defined origin. Thus, experiments can be reproducibly repeated on cells of the same genetic background. Furthermore, specific genetic modifications can be made at the stem cell stage and their impact measured down-stream in the generated macrophages, allowing the complex pathways involved in viral infections, such as HIV-1 or Influenza (Figure 1), to be studied without additional complications, such as the off-target effects associated with drugs. Our technology has now been employed by numerous groups around the globe wishing to produce iPS cell-derived macrophages, from the Cambridge Sanger Centre to Florida and Pretoria.

Macrophages — not all created equal

Siamon Gordon's work at the Dunn School was key in demonstrating the incredible diversity and functional plasticity of macrophages. However, recently it has become apparent that they also have numerous origins. Adult blood monocytes arise from haematopoietic stem cells within the bone marrow and circulate around the body until, upon inflammatory

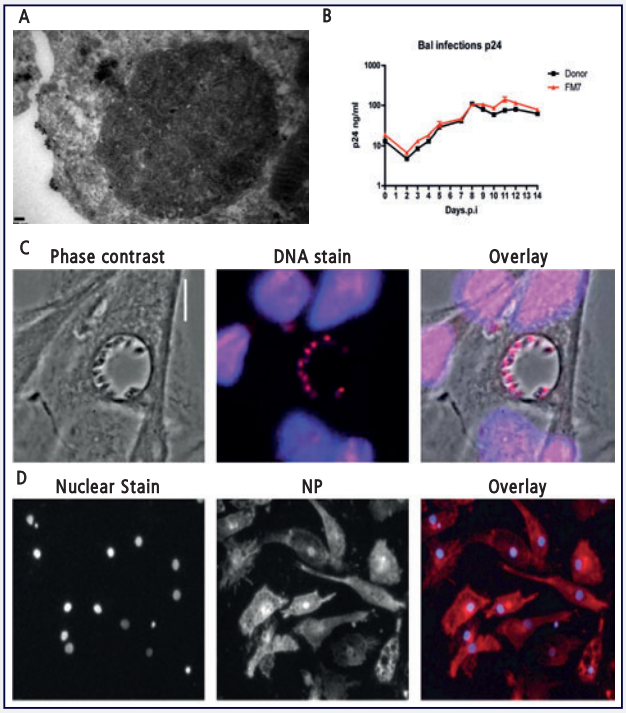

Figure 1. *Stem cell-derived macrophages can be infected by a variety of pathogens. (A) Electron micrograph of an HIV-1 infected cell, viral assembly observed by Gag immunogold labelling. (B) Similar HIV-1 growth kinetics, as shown by p24 ELISA, between donor-derived and iPS cell-derived (FM7) macrophages. (C) Leishmania-induced parasitophorous vacuole within an iPS cell-derived macrophage. (D) Influenza infection of iPS cell-derived macrophage shown by immunostaining for viral NP protein.*

signals, they infiltrate into tissues and differentiate into macrophages. In contrast, the majority of tissue resident macrophages (e.g. Kupffer cells of the liver and microglia of the brain) are derived from primitive macrophages generated in the embryonic yolk sac, and alveolar macrophages are derived from foetal monocytes during the primitive wave of haematopoiesis — further complicating the macrophage developmental landscape.

How the location and origin of a macrophage impacts the role it may play in disease is currently unknown. Most work with HIV-1 infection has focused on blood-derived, terminally-differentiated, monocyte-derived macrophages, representing infiltrating inflammatory macrophages. However, for HIV-1 some of the most important macrophage interactions that occur do so during the very early stage of infection, at the site of transmission, and are, therefore, most likely to be with tissue resident macrophages of the genital mucosa. Additionally, HIV-1 infection can lead to neurological complications due to replication of the virus within the brain, the tissue resident macrophages (i.e. microglia) being the most readily available target cell. Thus, modelling tissue resident macrophages of different developmental origins, is important to understanding not only HIV-1 transmission events but also the course of the disease.

Within our stem cell factories it is currently unclear what type(s) of macrophage are produced and how closely their generation resembles the *in vivo* macrophage developmental programs. What we know is that they are anti-inflammatory and can undergo a limited amount of proliferation, aligning them more closely with the self-renewing, homeostatic tissue-resident macrophages. Work in the laboratory, using a transcriptomics approach, is now on-going to fully establish the nature of the macrophages produced by our factories, their temporal stability (the factories can continue to produce macrophages for up to a year) and what impact these features have on their suitability as a macrophage model of human disease. Additionally, we are investigating the plasticity of our factories in an attempt to more closely replicate the developmental cues to generate tissue-specific macrophages, e.g. microglia as part of the EU IMI StemBANCC initiative to investigate their role in neurodegenerative diseases.

GMMs: Genetically Modified Macrophages

The stem cell-derived macrophages developed by our lab have furthered our understanding of HIV-1 entry and infection by allowing us to carry out experiments previously impossible in authentic, terminally-differentiated primary cells. Using stable RNA interference targeting CD4, alongside exogenously expressed mutant CD4 molecules, we have demonstrated the importance to efficient HIV-1 infection of the specific location of CD4 within the membrane with respect to lipid rafts, areas of the cell membrane rich in cholesterol that undergo active endocytosis [3]. By exogenously expressing Lck, a tyrosine kinase that maintains CD4 at the cell surface of T cells, we were able to show an increase in CD4 at the macrophage surface, an increase in viral fusion, but no concomitant increase in viral infectivity, also suggesting that the location of virus fusion is paramount to a successful infection event (Figure 2). Using the same model we have also been investigating the GTPase requirements for the endocytic uptake route that HIV-1 favours for infectious entry in macrophages, as well as the role of dNTP-regulating enzymes (e.g. SAMHD1) in protecting the macrophage from HIV-1 infection.

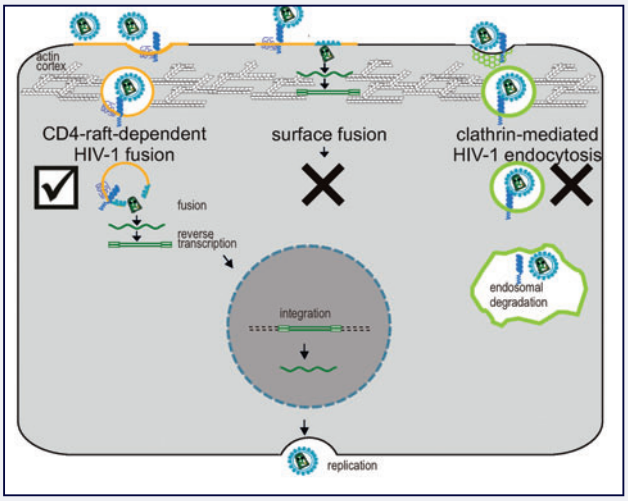

Figure 2. *Efficient HIV-1 infection of macrophages occurs through an endosomal pathway requiring lipid raft association of CD4.*

CRISPRs: A new era

Although transgene expression (lentiviral transduction of a gene under the control of a strong promotor) and gene knockdown (lentiviral delivery of shRNA to reduce the level of target mRNA) are useful tools to investigate the role of particular genes, they are often insufficient. Our lab has had a long-standing interest in novel genetic manipulation tools, and through a collaboration with Dr. Ulrich Siler at the University Children's Hospital Zurich and a StemBANCC work-package led by Sally Cowley, who heads the James Martin Stem Cell Facility which is aligned with our laboratory, we have been developing methods to cleanly correct genetic mutations, namely those responsible for Chronic Granulomatous Disease and those associated with Parkinson's disease, respectively. For most of this work we have focused on CRISPR/Cas9 technology as an efficient method to introduce or correct mutations within the human genome (Figure 3(A)). This technology, coupled with our ability to differentiate human pluripotent stem cells along numerous lineages, has opened up a new era in human genetics. In the past, knockout screens have been constrained to model organisms such as yeast and mice, now, with the advent of CRISPR/Cas9 technology we can begin to perform similar screens in our human pluripotent stem cell lines to identify genes essential to numerous developmental and cell-specific processes. To that end, in collaboration with Dr. Andrew Bassett (Head of Genome Engineering Oxford, based at the Dunn School), we have created a human iPS cell pooled library containing CRISPR-mediated knockouts of every gene in the genome. With suitable selection screens we intend to use this iPS cell line to uncover genes essential to HIV-1 infection — a world first in a physiologically relevant cell type — influenza infection (in collaboration with Dr. Ervin Fodor and Dr. Edward Hutchinson), as well as genes involved in basic stem cell maintenance and macrophage development (Figure 3(B)). Just a small glimpse into the potential of these combined technologies.

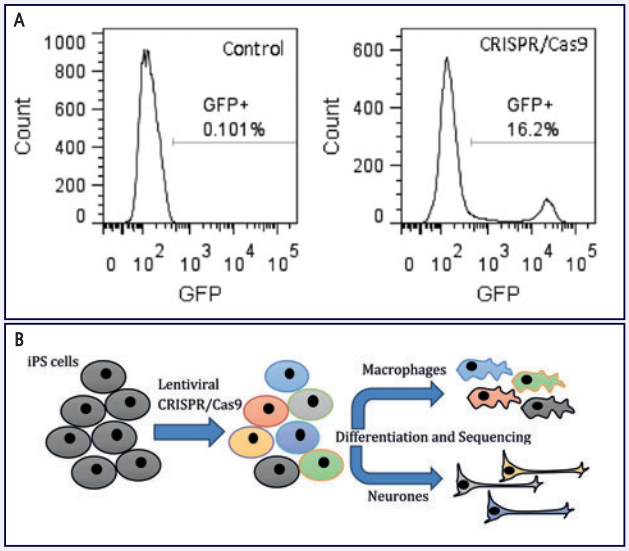

Figure 3. *CRISPR/Cas9 aided genetic manipulation. (A) Gene conversion of BFP to GFP using CRISPR/Cas9 technology to enhance the efficiency of homologous recombination in iPS cells. (B) Genetic screens using a genome-wide knockout approach using a pool of lentiviruses to identify genes involved in various processes, e.g. differentiation of macrophages.*

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Emerging Technology:
Genome Engineering: A new era of genetic analysis

Andrew Bassett

Genome engineering enables precise, targeted changes to be made to the genomes of essentially any organism, permitting genetic analysis and screening in systems previously refractory to such manipulations and greatly simplifying and speeding up the process in genetic model organisms. Genome Engineering Oxford (GEO), based in the Dunn School, provides a centre for collaborations, expertise, information and validated reagents to enable researchers to rapidly exploit these exciting new techniques to answer important biological questions.

The new era of genome engineering

The dream of any geneticist is to be able to simply and rapidly make desired alterations to the DNA sequence of an organism. Recent advances in the field of genome engineering have made this a reality and promise to revolutionise our approaches to studying gene function. The process of genome engineering involves the use of sequence-specific DNA binding factors to target genetic modifications to defined sites within the genome. Although this idea was first postulated several decades ago with the discovery of the modular nature of zinc finger DNA binding proteins [1], their practical application has proven difficult.

The answer to this problem, as with much of biology, stems from an unexpected source, namely studies on viral defense systems in bacteria. This led to the identification of the 'clustered regularly interspaced short palindromic repeats' (CRISPR) system, and the important discovery that bacterial immunity to viruses was mediated by an RNA-guided DNA endonuclease, the CRISPR-associated (Cas9) protein [2]. The Cas9 protein binds to short RNA fragments derived from the invading virus that are used as "guides" to target the destruction of viral DNA. The sequence of the guide RNA defines the specificity of the Cas9 nuclease in a highly predictable manner, based on Watson-Crick base pairing with the target DNA (Figure 1). This system was shown to be functional when transferred to human, mouse and zebrafish by three groups at the end of January 2013 [3–5], demonstrating its use in genome engineering. By reprogramming the Cas9 protein with a desired guide RNA, it can be used to generate targeted double-strand breaks in the DNA, resulting in mutagenesis at these sites.

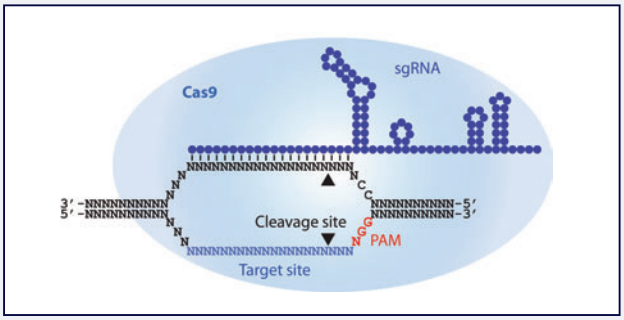

Figure 1. *The CRISPR/Cas9 system of mutagenesis. The Cas9 protein (blue oval) is recruited to a target site in the DNA by a 20 nt complementary sequence in the guide RNA (sgRNA, dark blue). Cleavage requires a protospacer adjacent motif (PAM (NGG), red) in the DNA, which does not appear in the sgRNA. A double strand break is made 3 nt from the PAM sequence on both strands of the DNA (cleavage site, black triangles).*

Since then, over 600 scientific papers have been published describing the use and development of this technique in organisms ranging from bacteria to cynomolgus monkeys. Importantly, this system has enabled genetic analysis in essentially any organism where the Cas9 protein and guide RNA can be produced or delivered. This means that many species where genetics were previously impractical or impossible are now amenable to genetic manipulation, such as many human and mouse cell lines, and non-model organisms such as bees, crop plants, pigs and monkeys [reviewed in 6].

The use of CRISPR techniques also greatly enhances genetic analysis in model organisms such as mice, where the Cas9 protein and guide RNA can be delivered in early zygotes, resulting in an ability to produce mutant mice within a single generation [7]. The efficiency of CRISPR-based mutagenesis is generally very high, so it is also possible to supply multiple guide RNAs at the same time, resulting in several mutations in a single step. We have shown that by injecting two guide RNAs into early *Drosophila* embryos, it is possible to simultaneously mutate two independent genes (Figure 2). Other groups have demonstrated that it is possible to make a triple mutant mouse, or up to five independent mutations in an embryonic stem cell line in a single step [7].

Figure 2. *Multiple mutations can be induced simultaneously in* Drosophila. *A* Drosophila *double mutant was generated by coinjection of Cas9 mRNA and two guide RNAs targeting the* yellow *and* white *genes. Mosaic* yellow *expression is observed as patches of lighter body colour in the abdomen and thorax, including the left wing. Tissue containing mutations in the* white *gene can be observed as white patches in the eyes. (Image courtesy of Nicolas Gompel)*

The fact that the specificity of the Cas9 nuclease can be altered in a predictable manner by changing a 20 nucleotide stretch of RNA makes assembly of constructs very simple. Several groups have taken advantage of this and used oligonucleotide printing technologies to create libraries of more than 60,000 guide RNAs [8–10]. When supplied to a cell line, these constructs can be used to mutagenise every gene within an organism in one experiment. This makes it possible for the first time to perform genetic screens in cell lines to investigate cellular phenotypes or to identify drug targets (for an example you may like to refer to the article by Kenny Moore in this issue of *Fusion*). Identification of the causative mutations is also facilitated by the fact that the guide RNAs supplied are known, and so the mutated genes can be easily determined.

Double strand DNA breaks produced by the Cas9 nuclease are repaired by the cellular DNA repair systems (Figure 3). This repair is often achieved by a process known as non-homologous end joining (NHEJ), where the two DNA ends are glued back together by a DNA

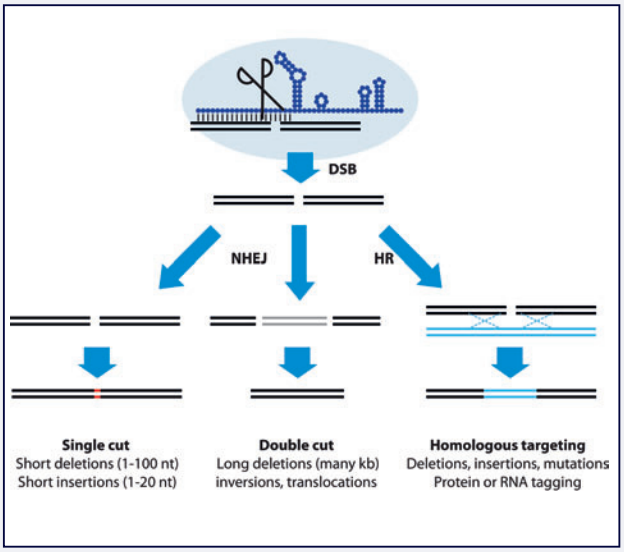

Figure 3. *DNA repair occurs by NHEJ and HR pathways. The double strand break induced by the Cas9 / guide RNA complex can be repaired by non-homologous end joining (NHEJ) or homologous recombination (HR). This can result in small insertions or deletions at the target site (left), deletions of larger genomic regions when two cuts are made (middle) or homologous repair with a desired template (right). This can be used to alter the genome in a variety of different ways (bottom).*

ligase. This process is somewhat inefficient, and often results in small insertions and deletions in the DNA. These can be used to generate frameshifts in protein coding sequences, resulting in a dysfunctional protein, but can also be used to remove other small functional elements within the genome such as transcription factor or microRNA binding sites. The repair can also be achieved by a homologous recombination (HR) based mechanism that uses a template DNA to precisely repair the lesion. If we supply an excess of a desired template for repair, this allows defined insertions, deletions and substitutions to be made in the DNA. This process has been used extensively in the production of transgenic mice, but is highly inefficient in most cell types. However, the ability to produce a targeted DNA break with the Cas9 protein now makes it possible to use this system to make defined genetic changes in most cell types. This has many applications, such as the repair or generation of point mutations (SNPs) to show that they are causative in a disease, or to tag genes within the genome for visualisation, purification or detection.

Interestingly, two point mutations in the Cas9 protein remove its catalytic activity completely (dCas9), resulting in a sequence specific DNA binding factor that can be used to recruit any protein or RNA domain to specific sites within the genome. This has already been used to interfere with the process of transcription, either positively or negatively, and to label specific DNA sites in living cells, but has the potential to be used for many novel experiments to uncover the mechanisms underlying gene regulation (Figure 4).

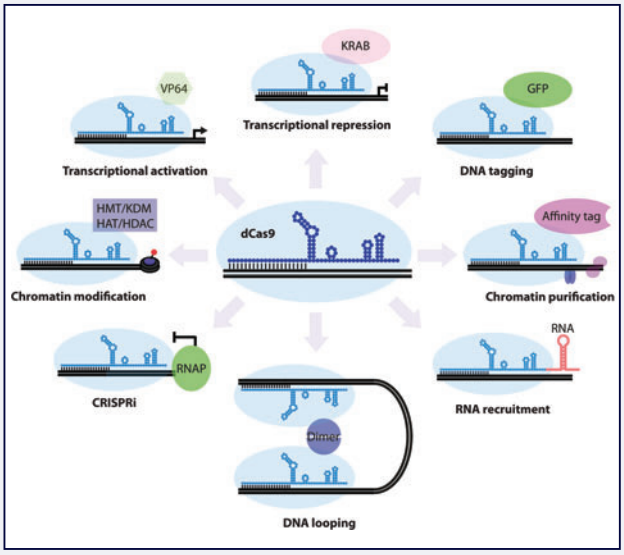

Figure 4. *Uses of dCas9 as a DNA binding factor. Nuclease deficient Cas9 protein (dCas9) can be used for multiple other functions including regulation of transcription by interference with RNA polymerase (CRISPRi) or fusion to transcriptional activation (transcriptional activation, VP64) or repression (transcriptional repression, KRAB) domains. Other possibilities include DNA tagging with GFP, chromatin purification, RNA recruitment, or altering DNA looping with dimerisation domains (DNA looping). Alterations in epigenetic states can be targeted by fusion to enzymes such as histone methyltransferases (HMT), acetyl-transferases (HAT), demethylases (KDM) or deacetylases (HDAC).*

Genome Engineering Oxford

Genome Engineering Oxford (GEO) is a joint venture between the Dunn School and the departments of Biochemistry, Pharmacology and Physiology, Anatomy and Genetics to enable researchers in Oxford to employ genome engineering technologies. It provides a service for generating experimentally-tested vectors, is involved in developing new CRISPR methods, and provides opportunities for collaborations on more extensive or challenging uses of CRISPR techniques.

Although CRISPR technologies can result in highly efficient mutagenesis, a substantial proportion of guide RNAs (around 25%) fail to produce mutations. Currently, this is not predictable, and an important role of GEO is to test mutagenesis efficiency of different CRISPR constructs by high resolution melt analysis (HRMA) in a cell line from the organism of choice (e.g. human HEK293T, mouse N2A, *Drosophila* S2R+). For a small fee to cover costs, GEO can provide advice on the optimal experimental strategy and vector choice, design optimal guide RNAs, generate vectors, and test these for mutagenesis efficiency. DNA for these vectors is supplied along with information on their cleavage efficiency and tested oligonucleotide primers to amplify across the deletion site for further validation or selection of mutant clones.

GEO can also provide experience, advice and assistance with the design and implementation of more complex projects involving genome engineering techniques that are currently not routine and may

involve development of novel methods. These include the design, manufacture and application of genome-wide or selective libraries to analyse cellular phenotypes. We currently are working with the genome-wide human CRISPR library from Feng Zhang at the Broad Institute, have obtained a similar mouse library from Kosuke Yusa at the Wellcome Trust Sanger Institute, and are developing libraries of our own in collaboration with several groups across Oxford. We are also working with other groups to make mutant organisms including mice, pigs, zebrafish and *Drosophila*, as well as developing novel expression vectors, selection systems and using homologous targeting to introduce or repair point mutations and incorporate tags to endogenous proteins. These projects will be charged to recover costs, and projects will be assessed on an individual basis. We are also happy to be involved in writing joint grant applications, where the expertise provided by the facility may be beneficial.

Genome engineering technologies will undoubtedly change the way that we design and perform genetic experiments and enable elegant genetic manipulations that would have been inconceivable a few years ago. There are already moves to exploit its obvious applications in gene therapy, and it represents another step towards making this a reality, with its associated benefits and ethical implications.

For more details, advice, information or any suggestions to improve GEO, please contact andrew.bassett@path.ox.ac.uk.

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In July, the Dunn School began a new tradition of a family fun day in the University Parks. The photographs provide a snapshot of the wide range of activities enjoyed by all who attended.

History Corner:

After completing his DPhil at the Dunn School in 1972, Simon Hunt remained in the Department throughout his scientific career where he taught immunology to countless cohorts of medical students until his recent retirement. Here, he reflects on his research career and some of those who have influenced its course over the years.

The longer perspective: fluke and choice

Simon Hunt

The pearly gate

Everyone remembers their interviews. Even if you don't have a clue which of many future possible 'Heavens' you'd prefer (after all, it really is a fluke which door you knock on) you do know that admission is determined by an interviewer guarding the Pearly Gate. In my case, I was a very fresh-faced biochemist from the final cohort[1], twenty-four of us in total, of Oxford undergraduates to be fledged by Sir Hans Krebs. He retired from the Whitley Chair in 1967, though not from his research on intermediary metabolism. During our course there was not a single mention of immunoglobulins or white blood cells. But when the name of the successor to Krebs, Rodney Porter, was announced during our final year, I did have the nous to recognise the fascination of Variable and Constant regions, newly reported at the 1967 Cold Spring Harbor Symposium. My interview with Rod[2] — just he behind his desk, and I — was dramatically un-pompous. He leaned back in his chair, delivered his questions with his 'hrrmmm' throat-clear engagingly disguising his speech impediment, and my answers had to navigate their way back to his ears past the soles of his shoes prominently facing me on the edge of his desk.

My DPhil with him began on the 4th floor of the Biochemistry tower. Before long I was a frequent commuter across to the Dunn School, which I had never heard about during my Biochemistry course, to collect rat lymphocytes (never come across them either) from Judy Coughlin, thoracic-duct cannulator extraordinaire, who was technician to a man called Gowans (oh dear; never previously heard of him either). He became my thesis supervisor. His classic 1957 and 1959 papers had incontrovertibly established the recirculation of small lymphocytes from blood to lymph to blood, round and round. In the early '60s he had demonstrated, along with Douglas MacGregor, Jonathan Howard and Susan Ellis, their role in the induction of both primary and secondary immune responses, including allograft reactions[3]. That it was *small,* non-dividing lymphocytes in which immune responsiveness was invested was crucial, since a long "lifespan" (a proportion of them have an intermitotic interval of several years in humans[4]) could explain lifelong persistence of protection by vaccination. In Jim Gowans' original experiments, small lymphocytes free from large ones, had been enriched and purified by differential apoptosis during overnight culture in medium 199. However, the Popperian principle of advancing knowledge by exclusion of all plausible alternatives[5], demanded that we isolate and test the large ones which are in rapid cell cycle. My thesis task soon morphed into developing a technique for separating the small (~8–10 μm diameter) from the large lymphocytes in thoracic duct lymph (12 – 15 μm), by simple velocity sedimentation over a few chilly hours in the terrestrial gravitational field of the cold room[6]. I confirmed that the small ones worked and the large ones essentially didn't[7]. It's still a view with which most present-day immunologists are content that long-term immunological memory *in vivo* is not invested in proliferating lymphocytes perpetually amplified by antigen, although for a period following an antigenic insult they help to expand the right clones. We would concede these days that minute amounts of persistent antigen on follicular dendritic cells in secondary lymphoid tissues are vital to remind B cells not to die, in contradistinction to kicking them into cell cycle.

Darwinian concepts and the recirculation solution

Niels Jerne's Natural Selection Theory of Antibody Formation (1955, re-invoking Ehrlich, 1901) and Burnet's formulation of the Clonal Selection Theory (1957) posed literally fantastic questions. If 'one cell–one antibody' was to be the basis of adaptive immunity, with antigen acting merely as a selecting agent not a moulding one, how could tens of millions of differing monospecific [8] lymphocytes be prophylactically created but not cause auto-reactive havoc? [Answer: V-(D)-J gene-segment rearrangements, plus a combination of central tolerance and peripheral T cell regulation]. Is all diversity generated before immunisation, or is there a contribution from post-immune refinement of an initially gimcrack specificity? [Answer: for B cells, the latter: somatic mutation provides a substrate for further refinement]. In one human body without previous experience of a particular antigenic threat, there are perhaps a thousand-odd lymphocytes with an approximately suitable receptor to deal with it, amongst the roughly one hundred billion lymphocytes in each of us. Time is of the essence in overcoming an invasion by a virus or toxin, so how can it be guaranteed that the most apt but vanishingly rare lymphocytes will meet one given antigen very promptly, wherever it may be? Answer: lymphocyte recirculation. Gowans himself, with Marilyn Smith, showed that the transient selection by antigen temporarily and specifically depleted

[1] Among whom also was Peter Cook, who pursued his DPhil with Henry Harris on RNA transcription in chicken erythrocytes reactivated within heterokaryons by cell fusion.

[2] at St Mary's Hospital where he worked immediately before Oxford

[3] "It is a sound rule that if a scientist has made discoveries of importance they can be summarised in a couple of lines; if he has not, a summary of his achievements may have to cover a couple of pages." (Gowans, in his memoir of Medawar). A telling rule indeed, and totally appropriate for its author's own scientific contribution.

[4] Best shown in human volunteers by the ingestion of a pulse of glucose, deuterated to serve as a marker.

[5] Very logical perhaps, but not a philosophy favoured by the funding agencies. Later in my time, I, with Alan Williams, eliminated the tiny quantity of immunoglobulin adherent to a T cell as a valid candidate for its antigen receptor; and I also disproved that loss of the DNA for the non-expressed light chain allele in a B cell is a tenable explanation for allelic exclusion in the rat kappa chain system. Karl Popper might have been pleased, but it's not the way to advance your research career.

[6] of the then new, now demolished, "Leslie Martin" building whose ground floor housed the MRC Cellular Immunology Unit

[7] My thesis title, which was "The fractionation of lymphoid cells", gave enough reason on its own to fail it. The examiners, generous to a fault, should have immediately referred it back for amendment to "The fractionation of lymphoid cell populations". But they didn't even comment on the title. It took me quite a while afterwards, and on my own, to appreciate the very important difference.

[8] Necessarily and fascinatingly its receptor has to be specified by just one of the two alleles within each diploid lymphocyte — "allelic exclusion".

memory cells from the recirculating lymphocyte pool *in vivo*. His early-1960s DPhil student, Bill Ford[9], complemented this observation, showing how recirculation in a rat *ex-vivo* spleen perfusion system permitted selection of influenza-specific clones. Nowadays, we can peer a few millimetres deep into a living lymph node of a mouse using the visually-compelling technique of two-photon fluorescence microscopy. This shows directly the moment of arrest of specific lymphocytes from the peripatetic recirculating pool, giving a real-time action view of specific T cell detention by antigen-presenting dendritic cells.

Subdivision into T & B cells — what counts as a "subset"?

One shilling (5p) from those days is now worth the equivalent of more than one pound sterling[10] but monetary inflation is as nothing compared with inflation in the number of lymphocyte subsets. A common fable purports[11] that Jim Gowans was reluctant to accept the initial subdivision, into thymus-derived T and bone marrow derived B cell subsets. Trustworthy distinctive cell surface markers to distinguish subpopulations weren't available in the late '60s. Instead, he, with Jonathan Howard and me, exploited a still-unexplained differential uptake by freshly-isolated lymphocytes of radioactive uridine in half-hour culture. T cells incorporate into their RNA about ten times more than B cells on a per cell basis. In this way, we noted different T and B zones, by autoradiography of lymph node and spleen sections, (Figure 1). Martin Raff's use (1970, in Av Mitchison's lab) of "theta" murine alloantigen, nowadays called the Thy-1 or CD90 antigen, got surface antigenic markers going. That gave birth to the industrial-scale subsetting that cellular immunologists have very advantageously indulged in since then. I see that last year between 6000 and 30000 "phenotypic populations" of human lymphocytes have been identified in a mere 37-parameter analysis, by the new technique of Mass Cytometry — and that is just for Natural Killer lymphocytes alone, before taking T or B cells or polymorphic variations into account! Lanier has even speculated, in a fit of *reductio ad absurdum*, that each NK cell might uniquely define its own "subset". It's a sound rule for me that the sharpest scientists are those who recognise previously unremarked but valuable patterns in the variety they observe in the world around them. One cell does not a pattern make. Further, to denote as a "subset" a cell type that differs from a fellow cell type by some relatively temporary physiological or biochemical or cell-cycle-related state is muddled thinking. There has to be some continuity of the differentiated state through cell division.

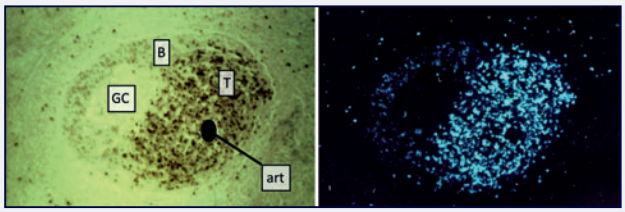

Figure 1. *Low-power autoradiograph of a spleen section following intravenous infusion with 3H-uridine-labelled syngeneic lymphocytes. (Left) brightfield and (right) darkfield illumination of the same area. The more heavily labelled T cells form a cuff around the central arteriole (art). The lightly-labelled B cells traffic to the primary follicle but not at this stage to the germinal centre (GC; secondary follicle).*

Extracurricular

Space does not permit me to recount all the stories of the fun we had. Three will have to suffice. Jim Gowans used to haul us off for occasional team-building weekends away to his Snowdonia cottage. He would "urge" on us a not-quite-voluntary march up the Pyg Track, then a traverse along the adrenaline-releasing arête of Crib Goch, to the summit of Snowdon. We did it! Bent Rolstad, visiting from Oslo, dived into Llyn Peris at the end of the day, exhibiting to us the Naked Norwegian way to celebrate still being alive after such an expedition. Then there was the time a certain Nobel-prizewinner visited with his team for a working lunch in the Cellular Immunology Unit. Jim Gowans devolved the menu arrangements to us juniors. The guest of honour was none other than his friend and colleague, Sir Peter Medawar, so Jonathan Howard and I thought a nourishing oxtail soup would be best — not out of a packet, of course, but from real ox tails bought that morning from the Covered Market. We brewed the soup in a giant stewpot to our own recipe over the lab Bunsen burners — enterprising, just possibly, but wholly disgusting to consume. Our most energetic and repeated skimming could not disguise the overwhelming preponderance of saturated fat that even premium ox tails release. My advice to the current generation: stick with what the Bridge Café provides when you're asked to fix up a lunch for a bigwig. Finally, what's the best way to dispose of the cotton-wool pad soaked in the diethyl ether that we employed to induce anaesthesia in rats undergoing cannulation or inoculation? Answer: simply discard it into the nearest plastic waste bucket, in which the flammable vapours can float and linger. Oops! A busy colleague down the bench is pulling glass Pasteur pipettes over a Bunsen to a fine point and chucking the still-hot tip into the same bucket. Kerwhoomph!

The bedrock

None of us in the Dunn School could have accomplished anything significant without the superb dedication of the technical experts. The roll-call of those who helped me is lengthy. Incomplete as it is, it must include Wendy Brownsill, Stan Buckingham, Mike Coates, Francis Cooper, Betty Dix, Don Etheridge, Marj Fowler, John Kirchoff, the two Strouds (Harry and Pete) — and above all, Jim Kent, (Figure 2), the doyen, past-master and patient tutor of animal research techniques[12]. In those pre-kit days, the Dunn School animal house, with its graceful balcony, housed goats, chickens, rabbits, guinea-pigs in addition to rodents, all of which he would immunise for us to prepare the necessary antibodies.

Figure 2. *Jim Kent*

It was my fortunate choice to pursue Darwinian questions about clonal selection for my scientific career, enabled by my astonishing fluke of inhabiting the extraordinary 'heaven' that is the Dunn School[13]. How undeservedly lucky I have been!

[9] Bill was the gentlest and most open-eared and receptive young medical scientist I knew in those times. His early death in a road crash, while he held the Chair in Immunology at Manchester, was a true tragedy. Not to be confused with Charles Ford, cytogeneticist, who also worked at the Dunn School.

[10] The value of the £ has fallen 21.05-fold since 1957.

[11] based on a properly sceptical comment he made at a 1969 conference.

[12] He'd been hired as a 14-year-old school-leaver by Florey in his pre-Oxford days, stayed with him through the penicillin experiments until Florey's retirement, and continued well into the Henry Harris suppression-of-malignancy era, inoculating platoon after platoon of nude mice.

[13] An account of my Pearly Gate interview with Henry Harris to get my lecturership will have to wait for another day.

S. N. 'Chubby' Arseculeratne, an alumnus of the Dunn School and Emeritus Professor of the Faculty of Medicine, University of Peradeniya, has recently written a detailed history of early penicillin research entitled **Lacunae in the conventional history of antibiosis and Penicillin.** ***Below is an abridged version of his recollections of the time he spent in the Department from 1962 to 1965 and some of the 'legends' he worked with during that period.***

Reminiscences of Oxford

S. N. Arseculeratne

During an exchange of views that included my memories of some notable people who I met during my post-graduate studies in the UK, an esteemed friend in the US suggested that I write them as a source of history — about these people who he called 'living legends'. It is because of the stature of the 'legends' that I reminisce now. This is not a comprehensive story of penicillin or life in the Oxford University's Sir William Dunn School of Pathology, but personal reminiscences of my 3 years there, the birth-place of penicillin, the therapeutic antibiotic that revolutionized the management of infections and introduced the antibiotic era.

There are four people at the heart of the penicillin story; Fleming, Florey, Heatley and Chain. Yet, underplayed and relatively unknown aspects of the penicillin story involve John Tyndall in England (1876), the Frenchman Ernest Duchesne who described the mould later known as penicillin in 1897 and Clodomiro Picardo Twight of Costa Rica who observed the inhibitory effect of *Penicillium* on staphylococci and streptococci; he is not mentioned in most books but data are available on the web. The better known Belgian, André Gratia, reported in 1924 the antibiotic action of *Penicillium glaucum.* But in the penicillin story, until recently, Fleming seems to have held the limelight. However, Macfarlane's books on Florey and Fleming have corrected the picture: indeed Fleming did not pay much attention to the use of penicillin in human disease.

Figure 1. *W.E. (Kits) van Heyningen pictured outside the front of the Dunn School*

Van Heyningen (Figure 1) was at the Dunn School during the penicillin years, and he met Fleming in his laboratory at St. Mary's. He stated (in *The Key to Lock-Jaw*) his opinion of the Nobel Prize that Fleming, Florey and Chain shared 'If I had my say in the matter, I would nominate Florey and Heatley'. Indeed van Heyningen also recollects of Fleming that 'He would frequently tell me that he did not deserve the Nobel Prize, and I could never bring myself to disagree with him over that'.

For me it all began in 1961 with my applying for places abroad for post-graduate training. It certainly helped that during my local probationary years, I had published seven research papers, some in international journals. My first stint abroad was in Manchester, under Professor H. B. Maitland, a pioneering virologist who had propagated virus *in vitro* in tissue culture. Maitland and Collard taught me on the Manchester Diploma course. Maitland appreciated my asking him searching questions, and it was perhaps his opinion of me that earned for me my second placing, at the University of Oxford, to which I applied, again on my own with, I gathered later, a recommendation from him to my future supervisor W. E. van Heyningen. I had an interest in bacterial toxins and 'van H', as we affectionately called him, was an expert on tetanus toxin that causes Lock-Jaw. But it was his chapter on bacterial toxins in the *Symposium on Mechanisms of Bacterial Pathogenicity* of the Society for General Microbiology that stimulated me and prompted me to seek him as my supervisor.

Professor George Feigen, a physiologist from Stanford, USA, and his wife Priscilla were in Oxford on sabbatical, and George was my laboratory room-mate. Another notable visitor to the department was Dan Campbell, an immunologist from the USA. Robert A. Webb, the immunologist eponymised in the "Dean & Webb optimum ratios" of antigens and antibodies in precipitation reactions, was also in the Dunn School but had no role in the penicillin story.

Figure 2. *The author and his wife with Ruth van Heyningen, Norman and Merci Heatley.*

When I arrived in Oxford the staff in the Sir William Dunn School of Pathology under Professor, Sir Howard Florey, Nobel Laureate and President of The Royal Society of London, included several members of the penicillin team, the self-effacing but technically brilliant Norman Heatley (Figure 2), Gordon Sanders, Edward Abraham and Margaret (Ma) Jennings, who was quite reserved and would simply say 'good morning' on passing me in the Dunn School corridors. It was these scientists and their colleagues who had battled against a lack of money and resources, and even against each other to develop the drug that would change the world. The team's first paper was titled *Penicillin as a chemotherapeutic agent* and had as its authors, in alphabetical order, E. Chain, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing and A. G. Sanders. I think it is noteworthy that this landmark paper that 'would change the world', did not have H. W. Florey as its principal author, a vivid illustration of the absence of ostentation in the great man. Indeed, it is recorded that Florey insisted that the authors' names be in alphabetical order.

It is quite regrettable and saddening that Heatley remained relatively unrecognized until, in 1990, fifty years later, he received a belated honorary D.M. from the University of Oxford. On that occasion, I sent him my congratulations and he replied: 'Thank you again for your very kind remarks about my 'honorary degree'; you may have heard that it was the only such to have been given by Oxford. That was what the officials of the University stated, but after a few weeks they found that this was

untrue. Someone had been awarded an honorary DM in 1907. I am, of course, immensely proud of this distinction. Warmest regards, yours sincerely, Norman Heatley'. Although Heatley was correct that he was not unique in receiving an honorary D.M., to this day, he remains the only non-medic to have received the award. Later Guy Newton came to the Dunn School: he did the pioneering work on the cephalosporins, with Edward Abraham; another of the Dunn School's great achievements.

It is a recurring experience that the truly great people are the least ostentatious and most helpful, and so were these penicillin-pioneering legends. Florey himself was quite unobstrusive, and I never heard his voice in the Dunn School. Van H wrote of his occasional rubs with Florey who, he also wrote, could be adamant. He used a simple old Vauxhall car that he drove himself. At his modest retirement party he walked up to my wife and me, shook our hands and apologized for not having met me at the laboratory more often.

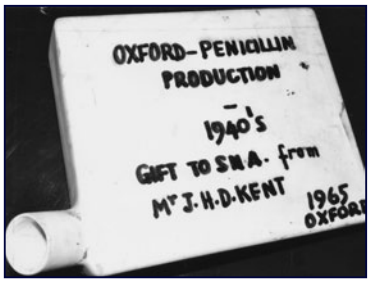

Figure 3. *One of the ceramic 'bed pans' used for the culture of* Penicillium.

Florey's chief technician Jimmy Kent was a gem of a man, simple and helpful, and is pictured in Lax's book helping Florey to inject a mouse. Kent gave me one of my most treasured possessions, a ceramic flask (Figure 3). This precious gift was one of Florey's original ceramic flasks for culturing the mould. They were first seeded on Christmas day 1940 and by February 1941 the new vessels had yielded enough penicillin for the first human trials. The second treasured gift I received in the Dunn School (from Mr Nat Smith, a genial and generous senior technician, father of Dame Maggie Smith, one of Britain's most famous actresses), was one of the original anaerobic jars invented by Sir Paul Fildes (Figure 4) and since then called the MacIntosh & Fildes jar.


Figure 4. *Paul Fildes and Howard Florey in celebration mode.*

In 1963, I was in great trouble with my research, for having proposed quite early, a mode of action of the bacterial toxin that I was working on. Although it impressed van H greatly, as it was a shrewd deduction from a curious phenomenon in a toxin-injected mouse, I spent a whole year without experimental confirmation of my idea; van H was very concerned and told me "Ratne, I am very worried about you". He arranged a discussion in London with Cyril Long the author of The Biochemists Handbook; Long, however, could add little to what I had already done. However my persistence and successive changes of methods ultimately gave me a method that worked and van H, the very humane being that he was, gave me a gentle kick with utter glee when I rushed into his room to inform him. It was the first demonstration of a bacterial toxin that has the enzymatic action that is found in the main toxin in snake venom — Phospholipase A.

I then told van H that I'd like to start writing and typing my thesis and going back home to my wife and three kids. He, however, made a horse-deal with me: he would pay for getting the thesis typed and bound while I continued working; and I did continue, showing that my stubborn bug had another, yet undescribed, toxin which also proved to be an enzyme, now a Phospholipase B, also a first for a bacterium and a logical sequel to the Phospholipase A that I had already demonstrated. Phospholipase B acts on the reaction product, lysolecithin, of the action of Phospholipase A. As customary with research at the Dunn School, van H got me to make an oral presentation of my work before the 'legends'; it was quite intimidating although I cannot remember swallowing my words, as I have since been accused of doing. On my return to Oxford in 2001, Ruth his wife, and a biochemist, gave me a copy of van H's autobiography *The Key to Lock-Jaw.* I wish I had it when van H was alive, for there is much in the book on which we could have corresponded. Of his years in Dutch South Africa that bears close parallels with Ceylon, places his forbears visited in Ceylon including Ragama and Diyatalawa, the Boer War and the cemeteries in the island that had the graves of casualties of that war.

To return to terra firma, van H gave me a thick overcoat to keep me warm in Oxford's winter that was particularly severe in 1962. At a dinner with van H, my wife and our eldest daughter Sha (then only one and a half), Sha sat next to van H and insisted on holding his hand which he graciously gave her. We had our second child, a son in Oxford in 1963 and then my wife had to return home as she was expecting our third; when I told van H of our plans, he promptly said 'Ratne, you are breeding like a rabbit'; typical of the geniality and informality of the man. I stopped calling him 'Sir' and used his pet name 'Kits' after I left Oxford. He remained a firm friend till he died in the 1980s.

Florey retired one year later to be succeeded as Professor of Pathology by Henry Harris (later Knighted), who created the first human-mouse hybrid cell by cell-to-cell fusion. I was quite touched when Harris came down to meet me in G.P. Gladstone's room at the Dunn School when I visited the School again in 1980 when my student and colleague Malik Peiris (who later identified the SARS virus) was a DPhil student there; Gladstone had worked on staphylococcal toxins at the Dunn School, and was my examiner at the DPhil *viva vocé* examination with Irene Batty of the Wellcome Laboratories; Batty began by congratulating me on my persistence in solving the problem of the mode of action of that bacterial toxin, which had eluded me for one year. I was secure with the assumption that my DPhil was in the bag, and Christmas 1964 was approaching, so I drew a cartoon of van H as Santa Claus, giving me the DPhil as a Christmas present, shoved it in a mock-up stocking and hung it on his door!

Having spent three and a half years at Oxford, a major regret is that my work consumed nearly all my time with the unproductive one-year to boot, and left little for allowing me to browse around its famous museums, imbibing the great culture of a great city and a great University. However, it was not all work; I played for the Oxford University Staff Recreation Club's cricket team that played matches around Oxford; a cricket-ball blow on my lips required a visit to the Radcliffe Infirmary for stitching. Through cricket I met Jim Gowans, (later Sir James Gowans), the immunologist who discovered the circulation of lymphocytes. Furthermore, with Lankan friends, we made an occasional visit to the Trout Inn, by the River Cherwell; it was established in the 12th century and is, therefore, nearly as old as the great city of Polonnaruwa, our Island's capital from the 8th to the 12th centuries.

Origins of the Oxford "Path & Bact" Course

Eric Sidebottom

It might surprise many of our readers to learn that the origins of today's Principles of Pathology course for 1st BM medical students had its origins way back in the 19th century in the earliest days of the development of the germ theory of disease. The first formal notice of this teaching that I have found is in 1891, when Dr Menge of Munich gave four demonstrations on the new techniques of bacteriology. Earlier, Henry Acland, Regius Professor of Medicine from 1857 to 1894, made a collection of physiological and pathological specimens which formed the basis of his teaching about disease. John Burdon Sanderson (Figure 1), who was appointed in 1882 as Oxford's first Professor of Physiology and succeeded Acland as Regius Professor in 1895, had a background in Public Health and Pathology and Bacteriology and was responsible for the first organised courses in these subjects. He, with Acland's encouragement, recruited James Ritchie (Figure 2) as the first University Lecturer in Pathology in 1897 and together they planned and delivered the courses outlined below.

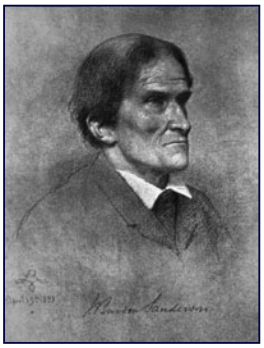

Figure 1. *John Burdon-Sanderson (1828–1905)*

Figure 2. *James Ritchie (1864–1923)*

The courses took place in the Regius' rooms in the University Museum of Natural History and there were between 6 and 10 students attending in the early years. One of these, Ewan Frazer, was so impressed that in 1899 he gave the University £5,000 towards the building of a new Pathology Department. This was completed in 1901 at a cost of £9,980. Another of these early students, Percy Temple Sutcliffe left behind a collection of about 300 microscope slides labelled 'Pathology Oxford', or 'J. Ritchie'. This collection was recently discovered by Sutcliffe's grandchildren in a flooded cellar in Cornwall and fortunately they were sufficiently curious about their origin to contact the Dunn School and bring the slides to Oxford as a gift. Some of them are remarkably well preserved and can still be viewed today (Figure 3). The contents of these slides provide an invaluable insight into the early teaching of Pathology.

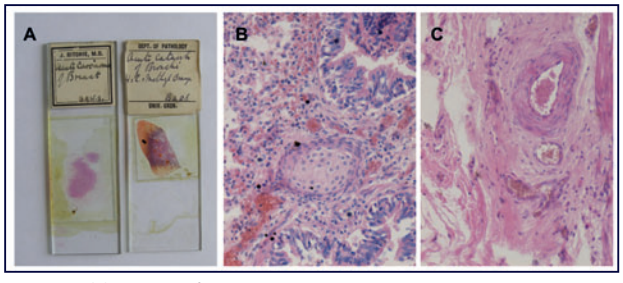

Figure 3. *(A) Examples of James Ritchie's microscope slides, now over 100 years old, recently recovered from a flooded cellar in Cornwall. (B) Light microscope image of the right-hand slide showing 'acute catarrh of the bronchi' stained with H&E and methyl orange. (C) Normal human appendix stained with H&E. (Photography courtesy of Tim Davies).*

I am currently studying the evolution of teaching in the Dunn School. My own experience has evolved from the 'Path & Bact' courses in 1960/1, as a student under Florey, via the introduction of Final Honours School teaching in the 1970's, to my current position as a demonstrator in practical classes: I hope to present my findings in a future edition of *Fusion*.

Courses of Instruction in Pathology, 1897-98.
MICHAELMAS TERM, 1897.
Course of Twelve Lectures on Elementary Pathology by Professor Burdon Sanderson.

Lecture I CONGESTION. Active Congestion; Collateral Hyperaemia; Passive Hyperaemia; Varix, Stasis, Transudation, and Diapedesis, etc.
Lecture II. DROPSY. Obstructive, Dyscrasic and Congestive varieties of Anasarca; Pulmonary Oedema; Physiology of Dropsy.
Lecture III. THROMBOSIS and EMBOLISM.
Lecture IV. INFLAMMATION Phenomena as described by J Hunter, Williams, Lister, Cohnheim, Metschnikoff, etc.
Lecture V. INFLAMMATION (cont.). Inflammation of non-vascular structures (Keratitis); Abscess ; Healing by the first and second intention.
Lectures VI and VII. ETIOLOGY of INFLAMMATION.¬ Aseptic Inflammation ; Phlogogenetic infective organisms (Staphylococcus, Streptococcus, etc.); Septicaemia and Pyaemia.
Lecture VIII. NEW GROWTHS. Pathological Mitosis; Granulation and Regeneration of Blood vessels; Cicatrisation; Regeneration of Epithelium; Surgical Grafting, etc.
Lecture IX. THE GRANULOMATA. Etiology and Histogenesis of Tubercle; the Syphilitic and Leprous Granulomata.
Lecture X. TUMOURS Their Histogenesis and Etiology.
Lectures XI and XII. FEVER. Pyrexia and Anti pyretics; Disorder of Nutrition and resulting Histological changes; Etiology.

Course of Practical Pathology. Michaelmas term 1897 by Dr. James Ritchie, Lecturer in Pathology.

Members of the class will prepare microscopic specimens illustrating the following general pathological processes, the naked eye appearances of which will be demonstrated by means of fresh specimens and by preparations from the Pathological Collection.

INFLAMMATION.
A. *Acute* Acute congestion. Leucocytosis. Connective tissue proliferation. Abscess formation. The process of repair.
B. *Chronic*. (a) The granulomata. Tubercle, syphilis. (b) Chronic interstitial inflammations of the heart, liver, kidney.
THROMBOSIS and EMBOLISM.
TISSUE DEGENERATIONS. Cloudy swelling of heart, liver, kidney. Fatty degeneration of heart, liver, kidney, Fatty Infiltration of heart and liver. Brown atrophy of heart.
Amyloid degeneration of liver, spleen, kidney, intestine.
Atheroma of arteries.
NEW GROWTHS. Fibroma. Myoma. Lipoma. Myxoma.

UNIVERSITY OF OXFORD

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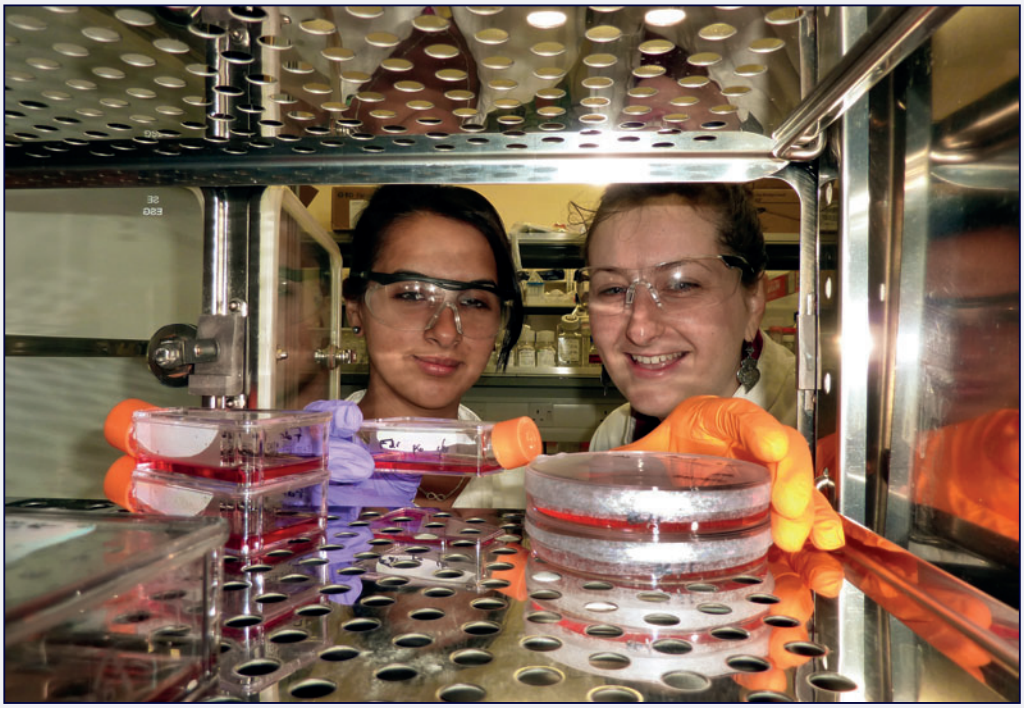
Naevus. Sarcoma (round celled, spindle celled, mixed celled, myeloid. Papilloma (epiblastic, hypoblastic). Fibroadenoma. Cystoma. Epithelioma. Malignant adenoma. Carcinoma. Melanotic sarcoma.

HILARY TERM, 1898.
Course of Regional Pathology.
Lectures and Demonstrations on the pathological processes affecting special organs will be given by Dr. Ritchie. Illustrative microscopic preparations will also be provided.

THE CIRCULATORY SYSTEM Pericarditis. Endocarditis. The Myocardium: (a) acute and chronic inflammations, (b) degenerations. Effects of disease processes on the structure and function of the heart.
THE RESPIRATORY SYSTEM. Diseases of the pleura: acute and chronic pleurisy, (a) simple, (b) infective. Diseases of the larynx, trachea and bronchi. Diseases of the lungs: acute inflammations, (a) croupous, (b) catarrhal, (c) septic. Chronic Inflammations: (a) infective (tuberculosis, glanders, etc.), (b) irritative. Emphysema. Collapse. Passive Congestion. Oedema. Thrombosis. New Growths.

THE ALIMENTARY SYSTEM. Diseases of the mouth, pharynx, and oesophagus. The Stomach: acute and chronic inflammations, passive congestion, 'simple ulceration, new growths. The Intestine: inflammations, ulceration (typhoid, tubercular, dysenteric), amyloid disease, new growths. The Liver: passive congestion, cirrhosis, degenerations (fatty, amyloid), new growths.
THE HAEMOPOIETIC SYSTEM.
THE GENITO URINARY SYSTEM The Kidneys: congestion, degenerations, acute and chronic inflammations (catarrhal, interstitial), new growths. The Bladder and Urethra: acute and chronic inflammations, new growths. The Testicles: acute and chronic inflammations, new growths.
THE NERVOUS SYSTEM. The Meninges: acute and chronic inflammations. The Brain: abscess formation, vascular degenerations and their effects. Nervous degenerations in Brain and Spinal Cord. New growths.
THE LOCOMOTORY SYSTEM. Diseases of bones and joints. Diseases of muscles.



This year saw the revival of a popular Dunn School tradition: the photographic competition. This year's theme, 'The Dunn School at work', generated many interesting and innovative entries. However, Tim Davies fought off all competition to win the prize with his composition 'How cells see us'.

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