fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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UNIVERSITY OF OXFORD

Exploring the Dunn School Archives

Innovation in Medical Teaching

Honouring Charles Fletcher

Focus on Pathogens

The Cryo-EM Revolution

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Editorial

2016 was a special year for the Dunn School. It was the 75th anniversary of the first trial of penicillin in humans, and we celebrated accordingly. But 2017 is also special, as it marks the 90th anniversary of the opening of the Dunn School. The Oxford Journal Illustrated, dated March 16th 1927, headlined Oxford's new home of research, modernised by a generous gift of £100,000. It described how "so convinced were the trustees that the surest and safest road to knowledge lay in research" that they had gone to the Court of Chancery to amend the terms of Sir William Dunn's will to support a research department, instead of making a gift that was more directly medical. Their aim was a "continual and sustained supply of men and women whose minds lend themselves to the stress and strain of research work - resourceful, keen, and adaptable minds". I like to think that, 90 years later, the trustees would feel that their gift had fulfilled both of those goals. Top men and women scientists resourcefully researching major biomedical challenges and, with antibiotics, the bonus of having developed a treatment that has saved hundreds of millions of lives: one of the greatest examples ever of the value of philanthropy.

We celebrate the past but must put our effort into the future by focusing on what's next. Recent recruitments highlight our continuing ability to attract the very best. Pedro Carvalho is the new EPA Professor of Cell Biology. He joined us this year from one of Spain's top research institutes in Barcelona, where is has been a rising star in European cell biology. His focus is on quality control of proteins in cells, and the molecular mechanisms that regulate how the identity of cellular organelles is established and maintained, a programme of research he outlines in detail in this edition of *Fusion*. This is the kind of fundamental biology that underpins many disease mechanisms and it epitomises how pathology in the 21st century depends on molecular and cell biology. We have also just recruited Monika Gullerova as an Associate Professor in cancer biology. Monika has been at the Dunn School as an MRC-funded Career Development Fellow for the last four years and was appointed from a strong field of applicants from around the world. Her research, likewise described later in this edition, focuses on a new mechanism of repairing damaged DNA, which she discovered. Again, a fundamental piece of science of clear and direct relevance to disease. We are very grateful to the trustees of the EPA Research Fund and the Lee Placito Memorial Fund for their generosity in endowing these two posts.

As non-British Europeans, both Pedro and Monika also illustrate a significant concern that we must tackle over the next few years. Of course, I speak of Brexit. While acknowledging that my column in *Fusion* is perhaps not the place for a politically-charged polemic, I am unable to hide my own personal frustration and disappointment about the outcome of the EU referendum, and my concern about its potential effect on British science. But our job now is to react in a way that ensures that the Dunn School can continue to be a truly world-leading research and teaching department. We have, of course, been a fully-international department for 90 years and that will not change. Long before the EU was dreamt of, the Dunn School was recruiting the best from around the world and supporting the best science. Indeed, the first three heads of the Dunn School were not British (Dreyer, Florey and Harris), nor was Chain, nor many of the other scientists whose photographs adorn the walls of the department – a lovely tradition that we maintain to this day. If you take a look at the homepage of the Dunn School's website you will see a ringing endorsement of our determination to remain welcoming to all, from wherever they come, and whatever the current political situation throws at us. The excellent recruitments mentioned above exemplify my confidence that we will succeed: by the time of our centenary, I predict we will be as international as we are now, with equally outstanding science.

Let me finish by saying, as I do each year, how much we value staying in touch with our former members and friends. We greatly appreciate hearing your news and meeting you when you visit, and are especially grateful for the support that the extended Dunn School family provides in so many ways. Please do stay in touch and keep an eye on the news pages of the website, where we post stories about a wide range of Dunn School events and achievements. In the meantime, we very much hope you will enjoy reading about the many aspects of life within the department, celebrated in the pages of this year's edition of *Fusion*.

Front cover image:
We are grateful to Simon Shelley for allowing us to feature a piece of his artwork on the front cover. Please see the interview with Simon for further details.

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News

Fusion is 15 years old!

The inaugural issue of Fusion was published in Michaelmas term 2002, an initiative first introduced by Herman Waldmann and edited by Susan Harrison, the Dunn School's Development Officer at the time. The first edition included details of George Brownlee's contribution to the treatment of haemophilia B, an interview with newly-appointed group leader, Keith Gull, and details of the completion of the EPA Building a year prior to its official opening by Nobel Prize-winner Dr Tim Hunt. Since its modest beginnings, Fusion has been published annually and distributed to many hundreds of friends and alumni across 27 countries, from Sri Lanka to Argentina and from Luxembourg to Taiwan, but has also evolved with the times: full colour printing was introduced in 2009, a dedicated front cover was added in 2011 and the magazine is now made available for download as a pdf file from the departmental website.

Over the years, Fusion has documented the arrival of many group leaders who continue to work at the Dunn School including Chris Norbury, Quentin Sattentau, Susan Lea and Oreste Acuto as well as more recent recruits, of which Pedro Carvahlo's appointment is showcased in this issue. It has also celebrated the work of many key people who, upon retirement, have left a significant scientific legacy to the Dunn School including Gordon MacPherson, Siamon Gordon, Neil Barclay, George Brownlee, Simon Hunt and previous Head of Department, Herman Waldmann. Furthermore, it has served as a forum to explore and celebrate the rich history of the department and the colourful characters that have worked here over the years. Most importantly, however, the editors frequently receive letters from readers sharing their reminiscences of the years they spent in the Department which invariably occupies a particularly fond place in the hearts and memories of many who worked here. We look forward to receiving many more such letters over the next 15 years!

Corrigendum

In the last edition of *Fusion*, we reported how Elizabeth Robertson had been awarded the Royal Medal for her many contributions to developmental biology and was one of a number of distinguished members of the Dunn School over the years who had likewise been honoured for their contributions to science. One name that was inadvertently omitted from the list of those who had received the Royal Medal was that of James Gowans, whose pioneering work on lymphocyte recirculation was recognised in 1976.

The Editors apologise for this oversight.

Susan Lea Elected to the Academy of Medical Sciences

On 28th June 2017, Susan Lea, Professor of Microbiology at the Dunn School, was formally admitted as a Fellow of the Academy of Medical Sciences in recognition of her exceptional contributions to the field of infection and immunity. The Academy is an independent body in the UK with a mission to advance biomedical and health research and to facilitate its translation into the clinic. This year, 46 new Fellows were elected, of which 15 were women, the highest proportion so far elected by the Academy.

Susan's laboratory is renowned for its work on biological structures with specific medical significance in two important areas: the complement system and bacterial secretion systems. Her recent research has focussed on the activation and regulation of the alternative pathway of complement by both membrane bound and soluble proteins. Studying the hijack of complement components by microbial virulence factors for both complement evasion and cell entry may influence the design of antigens for new vaccines based on these proteins, work that is now being developed with Novartis. Furthermore, her group has made major advances in defining the architecture of bacterial secretion systems, including the pathogenic type III secretion system of *Shigella flexneri* and the major integral membrane component of the TAT system. Her election to the Academy of Medical Sciences formally recognises the quality of her contribution to these important areas of medical science.

The Academy of Medical Sciences

Jeremy Farrar Delivers the 2016 Norman Heatley Lecture

Against the impressive backdrop of the newly-refurbished Weston Library, the 2016 Norman Heatley lecture was delivered by Professor Jeremy Farrar OBE, FRS, Director of the Wellcome Trust, one of the world's largest and most influential medical research charities. The occasion provided an opportunity to celebrate the 75th anniversary of the first systemic administration of penicillin in man which heralded the antibiotic era. The event drew an audience of several hundred which included many current members of the Dunn School as well as numerous people with personal links to the penicillin story. These included members of the Heatley family who continue to support the annual lecture founded in 1992 to commemorate the vital contribution made by Norman Heatley to the purification of penicillin that made its subsequent clinical use possible. The lecture was also attended by Mark Fletcher, son of Charles Fletcher, the physician responsible for treating the first patient with penicillin and who subsequently went on to become the nation's first TV doctor, responsible for bringing medical education to many of the British public.

Jeremy Farrar proved a very fitting choice of speaker for such a significant occasion since he has previously served as Professor of Tropical Medicine and Global Health at Oxford and Director of the Oxford University Clinical Research Unit in Vietnam. With a long-standing interest in the control of infectious disease, Professor Farrar spoke at a time when outbreaks of MERS were threatening Asia, a pandemic of influenza virus was top of the government's risk register and the WHO had recently declared the antibiotic era to be over and drug resistance to be one of the greatest threats to the future of human health. He outlined how drug resistance is evident not only among common bacterial pathogens such as *Staphylococcus* but also among the malaria parasite *Plasmodium* which has recently rendered the artemisinin derivatives largely obsolete in some parts of the world. Using the recent outbreak of Ebola in Sierra Leone in 2014 as a sobering example, Professor Farrar made the case that interconnectivity of travel across the globe poses an unprecedented threat to human health: "What happens in Freetown today", he commented, "affects us here in Oxford tomorrow." Drawing an important parallel with the development of penicillin, Professor Farrar commented that clinical aspects of drug development commonly take 10-20 years before approval is granted: in contrast penicillin made the journey from mouse to man in less than a year. In a call to arms to today's medical researchers, Professor Farrar argued that new paradigms are required that work in parallel on different aspects of drug discovery in order to make more rapid advances to meet future threats from infectious microorganisms.

The lecture was followed by a celebratory dinner at Exeter College.

And the Winner is ... Antibiotics!

On 15th June 2016, BBC2 broadcast *Britain's Greatest Invention*, a live event from an aircraft hangar near Swindon, which serves as a storage facility for some of the Science Museum's largest exhibits.

A number of well-known celebrities championed a wide range of inventions from the jet engine to concrete and from television to the humble fridge. But journalist and former news reader, Angela

Rippon was on a personal mission: owing her very survival as a child to the use of antibiotics, she made an impassioned case in favour of penicillin. In support of her arguments, she visited the Dunn School to interview the department's archivist, Eric Sidebottom, and reported on her findings from the iconic bridge overlooking the Bridge Café. Her estimate that 200 million lives have been saved by antibiotics since the advent of penicillin appeared to strike a chord with the general public who voted overwhelmingly that antibiotics should be considered Britain's greatest invention!

Following the publication of an article about Art at the Dunn School in last year's edition of* Fusion*, the Editors received an email from alumnus Simon Shelley who, since leaving Oxford, has pursued parallel careers in art and science. Intrigued,* Fusion *dispatched a reporter to find out more…

Interview with Simon Shelley

What is your connection with the Dunn School?
I was a DPhil student on the top floor in the Leslie Martin Building, or 'new building' as it was more commonly called, of which George Brownlee was the head. My supervisor was Francisco Ernesto (Tito) Baralle. I started as a research technician in Tito's lab in 1982 and was subsequently a DPhil student from 1983-86, based at Magdalen College.

How has your career developed since leaving the Dunn School?
When I left the Dunn School in October 1986, my first postdoc position was at Children's Hospital, part of Harvard Medical School, in Boston, Massachusetts. I then did additional postdoc stints at the Harvard Medical School institutions of the Center for Blood Research and Massachusetts General Hospital (MGH). In 1991 I was appointed Instructor in Medicine and in 1995 Assistant Professor of Medicine at Harvard Medical School. While at MGH, I held sabbatical appointments at the Institut d'Hématologie, Hôpital Saint-Louis, in Paris and the Institut de Recherches sur le Cancer de Lille in France. In 2008 I moved to La Crosse, Wisconsin to take up the position of Director of the Kabara Cancer Research Institute. During this time, I was appointed faculty at Viterbo University and the University of Wisconsin, La Crosse. In 2014 I was appointed Adjunct Clinical Professor of Medicine at the University of Wisconsin School of Medicine and Public Health, but in 2015 I moved back to Boston to found the biotech firm Leukemia Therapeutics, LLC. In addition to being President and CEO of this company, I continue to be involved in academic collaborations with researchers in Britain, France, Italy, Germany, Japan, the USA, Australia, and Nigeria. I am also actively involved in the Cancer Genome Atlas (TCGA) program of the US National Cancer Institute.

What are your recollections of the time you spent in the department?
It was probably the best time I have had as a scientist! Great people, extremely supportive, motivated, engaged, inspiring, creative, hard-working: you knew what you, and those around you, were doing was world-class and cutting edge. I have so many recollections of the years I spent at the Dunn School that it is difficult to single out any one in particular, however, the Dunn School was the place that I first drank coffee. My breaks in the coffee room felt a little short with just a can of orange juice which could be downed in a couple of seconds. The answer was, of course, the free instant coffee which was hot and took longer to drink. I hated the taste and added five large spoons of sugar for masking purposes. But then after a few weeks with too much coffee and far too much sugar and my hands starting to tremble, I was forced to go cold-turkey on the sugar, which I have maintained until this day.

I well recall taking two weeks to synthesize and purify a single oligonucleotide (the normal time frame in those days!), picking hundreds of colonies in triplicate for a cloning screen while watching the test match on a small black and white TV, and melting glass micropipettes over a Bunsen burner and pulling them to make a fine hollow strand capable of loading an acrylamide gel. Tito's office provided the best light for holding up x-ray film at an angle to the window and at arm's length to try to imagine a signal!
And then there was Kris Jenner, Marshall Scholar, lab mate and friend confessing over a farewell pint in the King's Arms that for the first six months he knew me he just smiled and nodded when we spoke, since he couldn't understand a word I said through my Yorkshire accent!

When did you first develop an interest in art?
My mother is an artist: a sculptor and potter and an art teacher. She went to Goldsmiths, University of London and Batley School of Art and was heavily influenced by the arts-and-crafts movement, the potters, Bernard Leach and Shōji Hamada, and the sculptors Barbara Hepworth and Henry Moore. My father is an amateur artist. Consequently, art supplies were always around the house. Furthermore, my mother took me out with her students on 'plein-air' drawing expeditions and made sure I was exposed to art from a very early age, from watching art shows on the TV (eg Kenneth Clark's Civilization), to visiting art museums both in my home town (Huddersfield) and on trips to London. On these expeditions, I realized I liked the shared visceral passion of Medieval and Modern art. Both were making strident statements about existence that I thought was often lacking in the centuries between. Being an adolescent during the Punk era also helped! I loved the later works of Turner and considered him, rather than Cezanne and the French Impressionists, as the father of modern art.

How would you describe the art form you have developed?
The term 'outsider art' was coined when I was a boy and really spoke to me as someone with a complex ethnic background. My father was born and raised a Muslim in India: his father an Indian Muslim, his mother a German Jew. His mother's father won the Iron Cross in the First World War: he wore his medal in public next to the Star of David

but 'disappeared' during WWII. Our family narrative was, therefore, of a Muslim in Hindu India with the traumas of Partition and of a Jew in Hitler's Germany.

My childhood was the product of a mixed marriage in working class Yorkshire in the 1960's which led me to witness violent school-yard racism and my father being carried off a cricket field, the only member of the factory team targeted by body-line fast bowling. These experiences made me intrinsically distrustful of the mass and the mob: my brother and I were always the outsiders. And then there was Punk and the Pistols and New Wave and Joy Division and Francis Bacon and Mark Rothko and Bosch and the Flemish Primitives and suddenly I felt at home!

So a good short-hand for my work is 'outsider art'. As far as technique is concerned, it could be described as sculptural or 3D decoupage, mixed media using found materials such as newspapers, magazines, catalogues, scientific journals, advertising, clothing and toys, acrylic artist paint, household paint, automotive spray paint, soil and wood stain.

Where do you find inspiration for your artwork?

I find inspiration in the chaotic and ephemeral nature of life around us every day: the daily struggle, banal as it often seems, but which embodies the nobility of striving to do better, to know more, to be wiser.

Art done well is the other side of the same coin as science done well. Both require imagination, hard work and the production, exhibition and publication of something new, something novel. Both art and science build on the past to press forward, groping in the darkness to new knowledge and insight. An artist, like a scientist, strives to be relevant and impactful. There is no beginning, no end, only the process, the act of doing. A finished piece of art is like a scientific publication, a milestone in a journey that never ends, a transient fleeting moment of accomplishment with which the artist and the scientist become bored, dissatisfied, frustrated at the very instant of completion but, nevertheless, lays the groundwork for the next step; inspiration for the next new and exciting chapter of the story; the next chapter that will again become boring, frustrating and imperfect but inexorably drives the scientist and artist forward.

What are the challenges of maintaining two very different careers in parallel?

When I was a very young boy I was asked like all children 'what do you want to be when you grow up'. Instead of the expected answer of fireman or policeman, my family was dismayed by my answer of a monk! I think I must have seen something on TV about a monk's life being filled with artistic (illuminated manuscripts) and scientific (Mendel) pursuits and this looked great to me. Later at 16, after "O" levels and deciding on "A" levels, I had to make a choice: art subjects or science subjects. I reasoned that I could do art in my spare time but not science in my spare time. So I chose science and went to Imperial in London as an undergraduate. I also felt I did not want to have a career at the mercy of subjective, artistic judgements of what is good and what is bad and have to be a showman to be successful. Success in science seemed to me to be based on objective peer judgements instead. I understand now that this was probably a little naïve!

So I chose as a profession to be a scientist with the intention of being an artist in my spare time, free of the subjective whims of art critics. But what spare time? Science can consume 25 hours a day and it did so for me for decades. In the end I realized that if I was really going to be an artist I had to deliberately and consistently carve out space, both physically in the form of a studio and psychologically by being more disciplined, efficient and focused as a scientist. So I rented space in a warehouse as a studio and made myself go to it in the evenings and at the weekends. A bit like making myself drink coffee for the first time at the Dunn School! Like coffee at the Dunn School, over time going to the studio became an easy, familiar habit, not a strange and bitter wrench. When I do art, it frees my mind to dwell on science ideas; a kind of meditation. When I do science, the images in journals, in westerns, northerns, southerns, *in vivo* imaging, immunohistochemistry etc drive artistic ideas. For me, there is cross-fertilization between art and science which are different sides of the same coin.

Simon Shelley exhibits his artwork under the pseudonym Michael Metcalfe. His works of art can be viewed at http://michaelmetcalfe.studio.

A Year in the Life of the Dunn School Graduate Student Association

The GSA Committee

We are lucky to have a large and diverse student community in the Dunn School, which currently comprises over 65 graduate students from all over the world. The Dunn School Graduate Student Association (GSA) makes an important contribution to student life, by actively promoting a social and supportive environment for students in the department.

The GSA is run entirely by a committee of student volunteers, who meet regularly to share ideas and organise a wide range of social, scientific and career-related events. Since being founded by Patty Sachamitr in 2012, the GSA has flourished, and currently has a record number of 14 committee members (Figure 1). We are very grateful for the enthusiastic backing and generous financial support we receive from the department, which enables us to organise a wide range of events. Here, GSA members summarise some of the exciting events and initiatives we have led in the past year, giving you an insight into 'a year in the life' of the GSA.

Figure 1. Some current members of the GSA committee.

Welcome events for new students *Mariya Lobanovska*

GSA welcome events are designed to introduce new students to the Dunn School and help them make a smooth transition into their DPhil. Two years ago, the GSA created a 'buddy' system, where each new student is paired with a more experienced student from a different lab. Buddies can be a great source of advice, helping to prepare new students for their first year. The GSA organises social evenings (Figure 2) and a tea party in the first week of term for the freshers and their buddies, which provides an excellent platform to meet students from all year groups and find out about the GSA. This year, following the tea party, the GSA led a tour around the department for the new students. They explored the departmental facilities, learned about the history of the Dunn School and found out more about our research themes. We plan to make this tour a yearly event, giving new cohorts of graduates an early flavour of the vibrant and inclusive research community at the Dunn School.

Medical Sciences Careers Day *Jessica Hardy*

In September 2016, we hosted our largest and most ambitious event yet – the Medical Sciences Careers Day. Over 150 students and post-docs attended, and were able to gain an invaluable insight into

Figure 2. A social evening with board games and pizza.

a diverse range of post-DPhil career pathways. Speakers included pharmaceutical scientists, a medical writer, a management consultant and a BBC producer, amongst others. A particular highlight was the lively discussion panel based around transitions in the academic career ladder (Figure 3). In addition, networking sessions over lunch and tea gave participants the chance to approach the speakers directly and chat with them in more detail. We received overwhelmingly positive feedback from participants, and owing to this success, we plan to run similar large-scale events once a year. The next Careers Day will take place in 2018, while this year's event, taking place on Thursday 28th September, will feature an exciting range of talks and discussions around the theme of 'The Future of Science'.

Speed Science *Jessica Valli*

In April, the GSA hosted a Speed Science Evening sponsored by the Medical Sciences Division. This event aimed to promote Dunn School inter-lab relationships by allowing students and postdocs to find out about their colleagues' research. A broad range of research areas were represented, and each participant had one-on-one conversations with every other participant (Figure 4). Each conversation lasted 5

Figure 3. Discussion panel at the career's day.

minutes, within which time both members had to summarise their research with the help of graphical abstracts. This event provided invaluable practice in clear and concise communication, and received excellent feedback from all of those involved. At the end of the evening, each participant nominated their favourite conversation.

The runner up prize went to Bernadeta Dadonaite, and first prize was won by Derek Xu, whose *Drosophila* illustrations left a lasting impression.

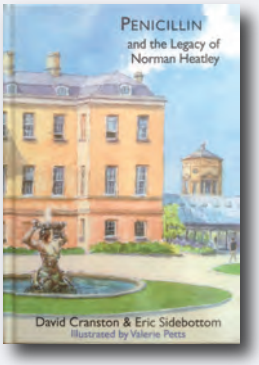
Figure 4. Speed science event.

Promoting contact with Dunn School alumni *Felicia Tan*

It comes as no surprise that graduates of the Dunn School pursue ambitious and exciting careers after completing their DPhils, often achieving success not only in academia, but also in industry, publishing, government, and the corporate world. Last year, the GSA invited several Dunn School alumni to speak at our Careers Day, allowing students to draw from their experiences and gain inspiration and insight into a variety of possible career paths. This year, we hope to continue connecting with alumni not only for the benefit of current students, but also to encourage alumni to maintain links with the Dunn School and each other. If readers of *Fusion* are interested in helping current students by sharing experiences and advice, or would like to reconnect with old friends or make new contacts, the Dunn School Alumni Facebook and LinkedIn groups are looking for new members. Alternatively, readers are welcome to contact the GSA at graduate.studies@path.ox.ac.uk.

Book review: Penicillin and the Legacy of Norman Heatley

by David Cranston and Eric Sidebottom



Annemieke Kok

If you have been at the Dunn School for any length of time, you are likely to know about Norman Heatley and his role in developing penicillin as a therapeutic agent, under the leadership of Howard Florey, then Chair of Pathology and Head of Department. However, this story is not well known outside of Oxford's medical and scientific community. This book, by two authors who knew Norman Heatley during his life in Oxford, includes a foreword by Professor Matthew Freeman, current Head of Department, and is the first biography on his life and work.

Eric Sidebottom completed his DPhil at the Dunn School, but after retirement has become a keen medical historian. So the book starts with an interesting summary of the history of penicillin, starting from early evidence (as far back as ancient Egypt) on the application of moulds to treat wounds and infections, to the discovery and early attempts at isolating penicillin by Alexander Fleming and his colleagues, and ending with the invitation of Norman Heatley onto the team of Florey and Ernst Chain, who were interested in developing penicillin as a therapeutic antibacterial agent, and who famously were honoured, along with Fleming, with a Nobel Prize for their work. Norman Heatley did not share this Nobel Prize, but after reading this book many will agree he should have.

Despite the title, the book covers the whole of Norman Heatley's life - building a picture of how he became such a valued scientist and academic, and of his kind and charitable personality - from his early years in Suffolk and at boarding school in Kent, his pre-WWII undergraduate and postgraduate studies in Natural Sciences at Cambridge, to his main scientific career and later life in Oxford. The authors have spoken to Norman's family and friends, and also had access to Norman's own observations, frequently cited in the book, which he recorded in his many laboratory notebooks and personal diaries (we are told these have been preserved for posterity in the archives at the Wellcome Trust). Through these sources, and the authors' sympathetic writing style, we get a real feeling for Norman's inquisitiveness, ingenuity, practical skills, and warm personality, almost as if we were able to meet the man himself.

This biography also illustrates poignantly how science needs more than just great thinkers and visionary scientific leaders with extensive networks. In order to make, and exploit, scientific discoveries, it also needs the makers, the developers of equipment and methods, who, with determination and tenacity, endeavour to make the ideas work in practise. This book positions Norman Heatley as a kind of patron saint of that particular strain of scientist.

I highly recommend this beautifully produced little book. It can be read in its entirety in one or two evenings, by lay persons as well as scientists. And I would predict that a fair few readers will enjoy going back for a second reading, to savour the exquisite little details, detours, and asides that bring the main story to life.

Penicillin and the Legacy of Norman Heatley by David Cranston and Eric Sidebottom in available from Amazon ISBN: 978-1-909075-46-7.

Chris Horton, a former FHS student in Paul Fairchild's lab, and Tom Kirk, an Engineering Masters student, discuss how they are aiming to keep medical students interested and engaged in anatomy by developing a mobile and desktop application designed for the Oxford Medical Course.

Innovation in Medical Teaching: Harnessing Technology for the Teaching of Anatomy

Chris Horton

Currently at Oxford, all medical students have access to an online library of anatomy modules, designed and written by the medical school, which aims to support lecture-based and tutorial teaching. Modules are closely aligned to the Oxford pre-clinical course syllabus and take the form of detailed notes, diagrams, dissection photographs, videos, interactive quizzes and references to experimental and clinical matters of interest.

In recent years, the Anatomy Section of the Medical School has run an Anatomy Fellowship programme, offering students the chance to become involved in the design and development of such teaching material. The major aims of the Summer Anatomy Fellowship, supervised by Mr Tom Cosker, Director of Human Anatomy at Oxford, are to investigate how and why current pre-clinical medical students use the medical school's WebLearn Computer Assisted Learning (CAL) material for anatomy and identify common recommendations to further improve provision. With this information, we aimed to review and revise a CAL anatomy eModule, whilst investigating other ways in which anatomy could be taught at the medical school. We therefore designed a comprehensive online survey, to be completed by current pre-clinical medical students. Questions were selected to assess how and why students use CAL material, which modules they found most and least helpful and how they think modules could be further improved. Students were able to give honest and anonymised feedback of the current provision in the free response sections at the end of the survey.

The results of the online survey, taken by 76 first and second year pre-clinical medical students, led us to produce an entirely revised Gastrointestinal Tract module on WebLearn. However, a number of common student recommendations could not be easily implemented using the current WebLearn (Magnolia) software. 63% of students said that it was not easy to quickly find information that they were looking for within the CAL material, and around 50% indicated that the current self-check questions could be made more relevant and useful to mimic examination-style, multiple choice questions in order to identify gaps in knowledge. A frequent comment in the "free answer" section of the survey was that it would be useful for anatomy modules to be available in a mobile app format, allowing students to study in an interactive way without the need for internet access and/or a computer. Others mentioned that some information was quite unorganised, recommending the use of clearer subsections and a more natural progression through the eModules. As such, a pilot module covering the Shoulder Joint anatomy was written and designed using eLearning course designer Articulate Storyline, incorporating as many suggestions made by students as possible. The aim was to provide students with the opportunity to study discrete, syllabus-aligned anatomy modules in an interactive manner, on both desktop and mobile platforms.

The intention of the Anatomical Walkthrough app, now known as Operation: Anatomy, is to build up the student's knowledge of an anatomical structure from the inside-out, progressively increasing the complexity of anatomy until the entire structure is understood. In the case of the shoulder joint, the app begins with the basic bony articulations, which then progresses to stabilising structures, blood supply and innervation, musculature acting across the joint, movements possible and finally clinically relevant information. Simple, digitally edited public domain Gray's Anatomy diagrams were used throughout the module, highlighting important features discussed in the first-year course syllabus. In addition, joint movement animations were included to help students visualise the actions and positions of various muscles.

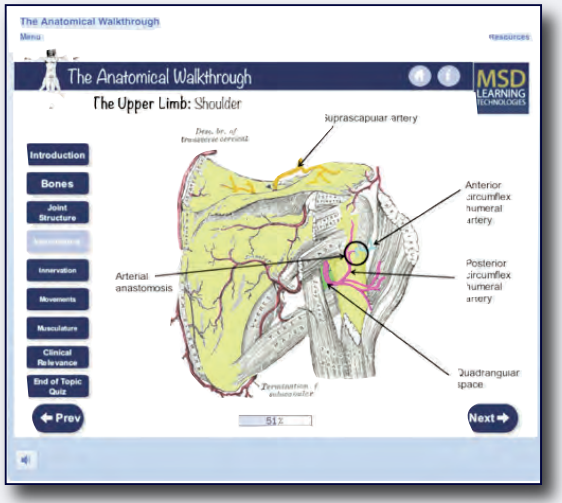


Figure 1. *Digitally edited Gray's Anatomy diagrams were included throughout the pilot app*

Each subsection contains a set of mid-topic check-up questions, written in a variety of short-answer, multiple choice, drag-and-drop and "clicking the named structure" style questions. If not answered correctly, students can readily navigate back to previous sections to find the relevant information. To ensure ease of navigation, a drop-down menu with a search bar function is available for students to type in key words, whilst relevant lecture slides and links to other webpages can be easily accessed by clicking the "Resources" tab.

Figure 2. *University resources, including lecture notes and WebLearn pages can be readily accessed through the app's "Resources" tab*

At the end of the module, understanding is checked by an end-of-topic quiz, which mimics the styles of questions encountered in Oxford's end of year examinations. One of the key features of the app is the option to run the module as a "Walkthrough". This means students cannot progress to the more complicated parts of the anatomy before satisfactorily completing previous sections and mid-topic check-ups. If run in this mode, the entire joint can be taught in the space of around 40 minutes. If students are looking to quickly access specific information, the module can also be run in "Revision Resource" mode, allowing students to freely access all of the module's resources without the need to complete each subsection.

The pilot module of this app was accepted for an oral presentation at The Anatomical Society Winter Conference 2015 in Cambridge. Following the talk, a number of senior anatomy lecturers from around the UK, including the University of Cambridge, University of Warwick and Royal College of Surgeons of Edinburgh provided very positive feedback.

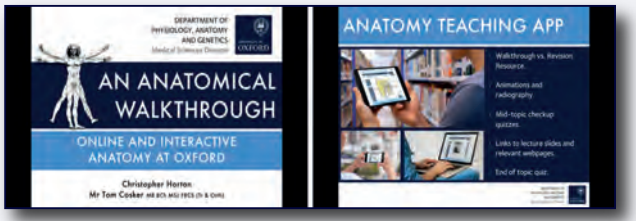


Figure 3. *- The application was presented at The Anatomical Society Winter Meeting (2015) at Magdalene College, Cambridge*

The app has been trialled with a number of medical students who commented on its ease of use, comprehensiveness and direct relevance to their course. Comments included: "It's by far the best anatomy resource on the web or in our books" and "I think the questions at the end of every section are really useful in reinforcing what you have learnt because there have been too many instances when we read through large sections of text only to not remember anything. Having questions at the end really helps to highlight what we may have missed out / not remembered".

Due to the positive responses received from students, the pilot module was submitted to the 2016 OxTALENT Competition run by Oxford University IT Services. We were thrilled to be awarded the prize of best "Student IT Innovation". The judges considered *The Anatomical Walkthrough* to be an excellent example of an initiative to produce a valuable learning and revision resource that can benefit future cohorts. They particularly liked the simplicity in the structure of the learning materials and the flexibility of the navigation, which offers a choice between a directed walkthrough or free access to individual resources for revision purposes.

Following on from the pilot module and OxTALENT competition, we have worked to develop the app in our spare time and have undergone a bit of a rebrand. We have recently decided to code the app from the ground-up ourselves, without the use of costly third-party software, as this offers us more flexibility to add features students would like in an anatomy learning resource. New additions include an anatomy glossary, a dynamic question bank that regularly updates itself based on the user's answers and a central webserver on which the app can be hosted and accessed.

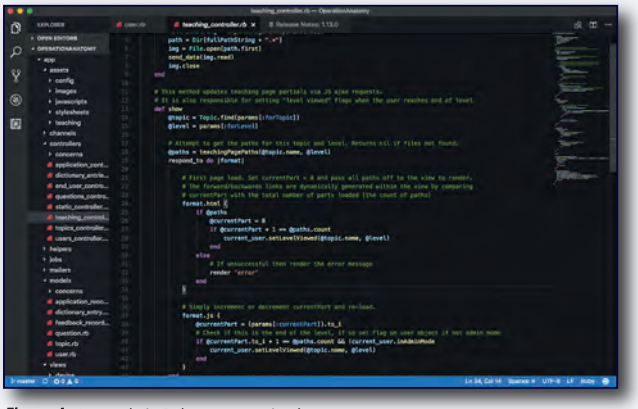


Figure 4. *App code in Ruby programming language.*

We hope that other students would be interested in getting involved with writing, designing and inputting new anatomy modules onto the app over the next few years, using the pilot module code as a template. The hope is to gather a team of pre-clinical medical students during the long vacation to begin work on subsequent modules. In this way, a comprehensive, interactive anatomy resource can be made, whilst allowing students themselves to learn and revise anatomy by making and reviewing the modules. Further modules are currently being designed, which include further musculoskeletal modules, in addition to cardiovascular, respiratory and renal anatomy. We hope these will be available for use by the new cohort of pre-clinical medical students beginning their studies in October 2017.

We have thoroughly enjoyed working on this project so far and would actively encourage others with similar interests in undergraduate education and technology to give it a go. We've learned a great deal from the material writing and app design process and hope that ultimately students will benefit from our modules. Perhaps in the future, if this project proves to be of use to students, modules for other topics covered in the medicine course, including physiology, pharmacology and pathology, may be developed. Should you have any suggestions or comments, or would like to get involved in the project, we would be delighted to hear from you!

Figure 5. *The app has undergone a recent rebrand, has been re-written in Ruby code for added flexibility and is now hosted on a dedicated web server*

SPOTLIGHT

Monika Gullerova was recently appointed to an Associate Professorship within the Dunn School. Here we cast a spotlight on the ground-breaking research for which her laboratory is already known and which she intends to pursue within the Department.

Transcription and DNA Damage: Friend or Foe?

Monika Gullerova

Every living organism consists of minimal functional units or cells. Cells have "bodies" called cytoplasm and "brains" called nuclei. The whole genetic information, essential for the life of every cell, is stored and encoded in a single type of molecule, DNA, which is located in the nucleus. DNA is a very long, double stranded helix-shaped molecule which contains coding regions, genes, which are interrupted by non-coding parts. Genes are transcribed into RNA molecules, which are in turn translated into proteins. Proteins are functional units which perform various tasks essential for cell maintenance, chemical processing, energy management and other functions in the nucleus and also outside in the cytoplasm. Therefore, DNA and its transcription determines the fate of a cell. Unfortunately, DNA is continuously exposed to a multitude of environmental factors which can cause DNA damage. If this is not immediately and efficiently fixed, a damaged cell can die or become cancerous. The study of DNA repair mechanisms is, therefore, of fundamental importance. Not only do we learn about how this process works, but, in doing so, we also develop knowledge that can help the design of future therapeutic strategies.

So far, most DNA repair research has focused on the functions of the various proteins involved. How do they recognize the damaged sites? How do they work to fix them? The primary research interest of my lab is the novel role which transcription plays in DNA damage repair. We have discovered that as soon as damage occurs in some sections of DNA, nascent transcription is initiated, resulting in production of relatively short non-coding RNA molecules. These molecules are transcribed from both strands of DNA and due to their complementarity, they hybridize and form double stranded RNA (dsRNA) molecules. Normally, such dsRNA is very dangerous for a cell, because it can stimulate the interferon response. Indeed, the interferon pathway was originally developed as a protection mechanism against many viruses, as lots of them have their genomes encoded in a form of dsRNA. Once dsRNA is sensed in a cell, interferons activate apoptosis ultimately leading to cell death. So how can DNA damage lead to *de novo* transcription and formation of dsRNA without triggering the interferon response and subsequent apoptosis? We actually found that damage-induced dsRNA is quickly processed into small effector RNA molecules called DDRNA (damage derived RNA) by an enzyme called Dicer, which works like scissors. Dicer is mostly localized in the cytoplasm of cells, but upon DNA damage it is modified. This modification is called phosphorylation and it occurs on one particular amino acid within Dicer's protein structure. This modification triggers Dicer's re-localization from the cytoplasm into the nuclei, where Dicer finds its dsRNA substrates, close to DNA damaged sites, in order to process them into small RNA molecules (Figure 1).

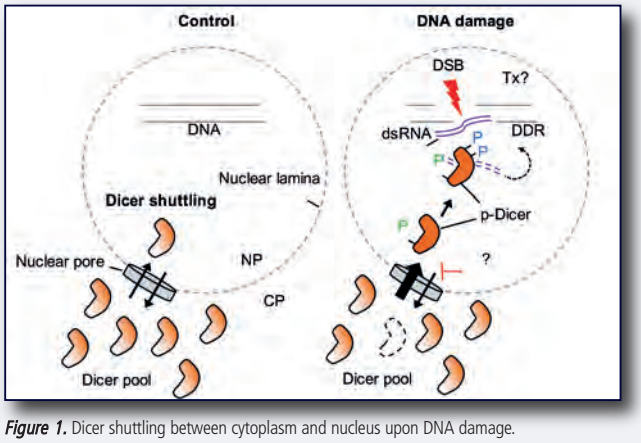


Figure 1. Dicer shuttling between cytoplasm and nucleus upon DNA damage.

We are now very interested in why these small RNAs are important for efficient DNA repair, how they function and which proteins they bind to. We employ state-of-the-art experimental and bioinformatics techniques in order address these questions. RNA-dependent DNA damage repair (DDR) is a novel concept, which is currently not well understood.

Being based at the Dunn School, we are privileged to use its facilities to dissect the molecular mechanisms of DDR, leading to efficient DNA repair. In particular, we are interested in the interplay between, transcription, RNA and the RNA interference pathway upon DNA damage. These factors possess canonical primary functions in gene expression and gene silencing. However, within physiological concepts they act as the key players in DDR, exhibiting their non-canonical functions in processing of endogenous dsRNA, antisense transcription and mediation of DDR.

We therefore study RNA-dependent DDR on four connected levels:
1. Regulation: What regulates transcription at double strand breaks (DSBs)?
2. RNA: What are the features and function of newly synthesized transcripts around DSBs?
3. Processing: What factors are important in damage derived RNA processing?
4. Function: What is the role of RNA in the DDR pathway?

Overall, we aim to provide mechanistic insights into novel RNA-dependent DDR and its role in effective DNA damage repair. Our research may unravel new insights into DDR and the maintenance of genome stability which are fundamental to a better understanding of the molecular mechanisms that cause cells to become cancerous.

RESEARCH FEATURE

Quality Control of Membrane Proteins

Pedro Carvalho

In cells, proteins are everywhere and participate in virtually all cellular activities. Synthesized as linear, spaghetti-like molecules, proteins become functional only upon acquiring defined three-dimensional structures. To ensure that this folding process is successfully executed, cells evolved exquisite protein quality control processes. Like in any modern production line, quality control mechanisms operating during protein biogenesis identify aberrant molecules and promote their degradation. Defects in these protein quality control systems are frequently linked to pathologies, such as cystic fibrosis and protein aggregation diseases, highlighting their impact on normal cell physiology.

A major interest of our laboratory has been the characterization of protein quality control mechanisms and their contribution to overall cellular homeostasis. In particular, we have focused on proteins embedded in the lipid environment of cellular membranes, and whose biogenesis takes place in a dedicated cellular compartment, the endoplasmic reticulum (ER). After folding in the ER, membrane proteins can eventually traffic to their final cellular destination to function in a wide range of processes, from lipid synthesis to signaling or communication in and between cells. However, not so rarely, molecules fail to fold correctly and become substrates of a dedicated quality control process called ER-associated protein degradation or ERAD[1]. This multistep process encompasses recognition of the faulty molecules, their removal from the membrane and tagging for degradation in the cytoplasm (Figure 1).

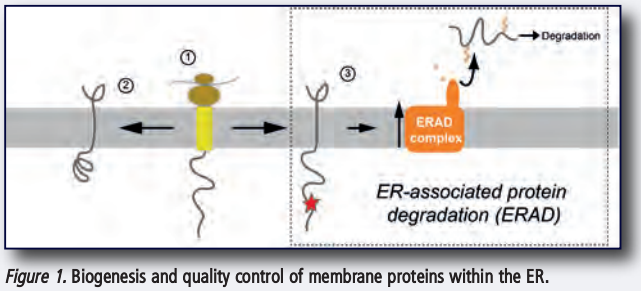


Figure 1. **Biogenesis and quality control of membrane proteins within the ER.** Newly synthesized proteins are inserted in the membrane of the ER in an unfolded state [1]. Upon successful folding, proteins remain in the ER or traffic to their final destination [2]. Misfolded proteins are recognized by ER-associated degradation (ERAD) that promotes their extraction from the membrane into the cytoplasm for degradation [3].

Although ERAD was first discovered in human cells, most of its components were identified in *S. cerevisiae*, the common baker's yeast. This unicellular organism shares many features with human cells and its facile manipulation has led to a wealth of information on a variety of basic cellular mechanisms. Over a decade ago, during my postdoctoral training using yeast, we came to the realization that the various steps of ERAD, from substrate recognition to degradation, were coupled in well-defined protein assemblies (or protein complexes).[2,3] Importantly, we found that cells contain distinct flavors of these complexes, each with specificity for different types of misfolded proteins. Now, a major question that we are addressing in the lab is how this specificity for substrates is achieved. How do these complexes detect and recognize their substrates while all other proteins in the crowded environment of the ER membrane are spared from degradation.

Aside from a canonical role in protein quality control, it was long known that ERAD could also target a specific folded protein. This was HMG-CoA reductase (HMGR in mammals and Hmg2 in yeast) required for the biosynthesis of sterols, lipid molecules with essential cellular functions and linked to common diseases. Curiously, the degradation of HMG-CoA reductase by ERAD is dependent on the levels of specific sterol metabolites in cells: if low levels of these metabolites were present, HMG-CoA reductase was stable; however, if the levels rose to a certain threshold the protein was quickly degraded. This metabolite-induced degradation of HMG-CoA reductase establishes a feedback mechanism required in maintaining sterol homeostasis. Moreover, HMG-CoA reductase degradation by ERAD involves a specific substrate adaptor which is not necessary for the recognition of misfolded membrane proteins. These findings suggested that ERAD could potentially have a more general role in the turnover of properly-folded ER proteins and consequently regulate ER functions beyond protein quality control.

To investigate how general was the role of ERAD in the degradation of folded proteins we took a global approach using mass spectrometry in our favorite model system, baker's yeast. By comparing whole proteomes of ERAD mutant and control cells we identified several endogenous substrates of the different ERAD complexes. For example, we found that a second sterol biosynthetic enzyme, squalene epoxidase (SQLE in mammals and Erg1 in yeast), was also degraded by ERAD.[4] Interestingly, the degradation of squalene epoxidase was also controlled by a sterol metabolite, however distinct from the one involved in HMG-CoA reductase regulation. Moreover, the degradation of squalene epoxidase and HMG-CoA reductase was mediated by distinct ERAD complexes. Subsequently, we and others showed that ERAD-mediated degradation of these two sterol enzymes was conserved from yeast to mammals which is quite remarkable considering that a billion years of evolution separates these organisms (Figure 2). Thus, ERAD has a central and ancestral role in sterol homeostasis.[1]

Regulated degradation of HMG-CoA reductase and squalene epoxidase in response to specific sterol metabolites implies that cells have the means to sense and measure the concentration of those metabolites in membranes. However, how this is achieved is not known and is an area of great interest in our lab. Insights on the molecular mechanisms of these sensing processes may allow us to interfere with sterol homeostasis and eventually provide therapeutic strategies for hypercholesterolemia.

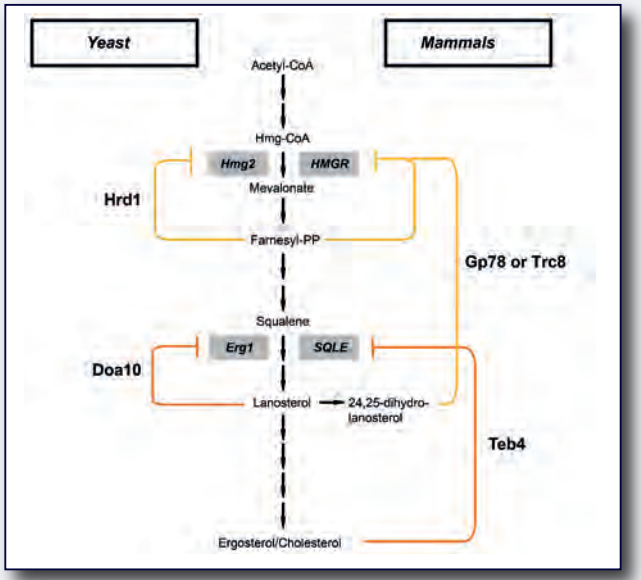


Figure 2. **A central role for ERAD in sterol homeostasis.**
Schematic representation of the feedback inhibition systems required for sterol homeostasis in yeast (left) and mammals (right). ERAD complexes are in bold and the enzymes targeted by ERAD regulated degradation are enclosed in grey boxes.

Other folded proteins not related to sterol metabolism were also identified as ERAD substrates. While the characterization of those substrates is still ongoing, the contribution of ERAD in defining the functional ER proteome is becoming increasingly clear.

The proteomic analysis revealed an interesting set of substrates that did not follow any of the known ERAD complexes, suggesting the involvement of some previously-unidentified components. This was indeed the case and through a number of experiments we identified these elusive ERAD components. Unexpectedly, these were acting exclusively on the inner nuclear membrane (INM), a specialized region of the ER facing the interior of the nucleus and involved in nuclear organization, such as chromosome positioning or transcriptional control.[5] To carry out these specialized functions, the INM has a unique set of membrane proteins distinct from the rest of the ER, even if the two membranes are continuous. Thus, it turns out that a major function of this eccentric ERAD branch is to safeguard the unique composition of the INM by degrading ER proteins fortuitously mislocalized to this specialized membrane region (Figure 3). These findings support the

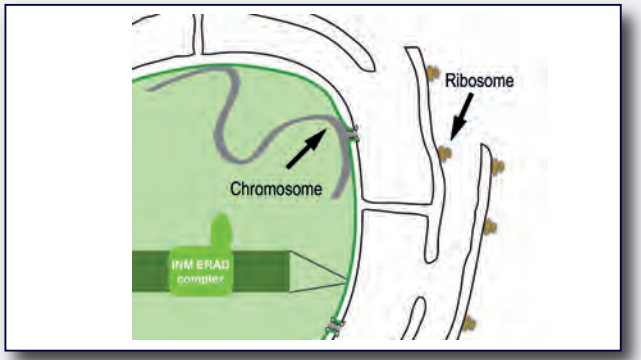


Figure 3. **Protein quality control by ERAD at the inner nuclear membrane (INM).**
The INM (in green) is continuous with the rest of the ER membrane (grey) but contains a unique protein composition. An INM-specific ERAD complex (see inset) contributes to the unique INM proteome by promoting the degradation of mislocalized ER membrane proteins.

notion that cells carry out distinct forms of quality control based not only on the folding state of proteins but also their localization inside cells. A challenge for the future will be to understand how spatial cues are encoded in proteins and deciphered by quality control processes, such as ERAD. Another major priority in the lab is to identify the components of INM quality control in mammalian cells and understand its contribution to organelle homeostasis.

In early days, the concept of protein quality control was perceived to deal exclusively with the folding state of proteins. The picture now emerging places protein quality control processes, such as ERAD, in a broader context. These same pathways, by controlling the activities of folded proteins both in space and time, appear as major contributors to overall architecture and organization of organelles and cells. By defining the principles guiding these protein quality control processes we expect to learn about basic mechanisms of cell physiology. We believe these insights may eventually shed light on a variety of common diseases linked to defects in protein homeostasis.

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Photograph courtesy of Paul Fairchild

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.

FOCUS ON PATHOGENS

Infectious diseases remain a significant contributor to the global burden of disease. In this edition of* Fusion, *we showcase on-going research by different groups at the Dunn School into three distinct classes of pathogen: viruses, bacteria and eukaryotic parasites.

Influenza and Host Transcription: A Love-Hate Relationship

David Bauer

While it might not be the 'hottest' virus of the year or feature in the latest round of Hollywood disaster movies, influenza remains one of the top challenges to global health. Despite substantial public health infrastructure, the annual number of deaths attributable to seasonal influenza in developed countries (37-174 deaths/million population in the United States) is only a single order of magnitude behind the number of deaths during the 2014-16 Ebola outbreak in West Africa (480 deaths/million population). These statistics do not include periodic global pandemics of new influenza strains, which have historically produced much higher mortality rates (~300 deaths/million population in 1968, 290 deaths/million in 1957, and 10,000-30,000 deaths/million population in 1918).[1]

The molecular architecture of the influenza virus explains why the virus remains difficult to combat. The virus is a (-) sense, single-stranded RNA virus, with a genome divided into 8 segments, each containing 1-2 major genes. This segmentation allows "reassortment" of a given segment between two different viruses that happen to co-infect the same cell. Evolution, in effect, converged again upon a type of sexual reproduction to increase diversity in the overall population of influenza viruses. On a single-virus level, the origin of genetic diversity lies in the error-prone replication of the viral genome. Like most RNA viruses, influenza encodes its own viral RNA polymerase that is responsible for carrying out both transcription and replication.[2] The lack of proofreading by the polymerase during RNA synthesis leads to the generation of multiple mutations throughout the course of an infection, and rapid changes to the structure of the antigens that are present on the surface of the viral particle.

Overall, the influenza RNA polymerase is a major determinant of the virulence and host range of influenza viruses, and is a key regulator of the adaptation of avian influenza viruses to mammalian hosts. The Fodor group at the Dunn School – where I am a postdoc – studies the molecular mechanisms of how the polymerase replicates the viral RNA genome and how it transcribes viral genes into mRNA in the context of various virus-host interactions in an infected cell.

Host-Shutoff and Transcription

Viruses employ a variety of mechanisms to achieve "host shutoff" with the aim of increasing the amount of resources available to the virus and preventing the host cell from mounting an effective response to the invading pathogen (Figure 1). Since the generation of an effective antiviral state fundamentally requires a change in gene expression by the host, viruses have evolved mechanisms that target host mRNA at nearly all points in gene expression – from transcription, processing and export from the nucleus to mRNA translation and stability in the cytoplasm.[3]

The most obvious strategy for a virus that encodes its own RNA polymerase might be to prevent host gene expression in preventing transcription from initiating in the first place. Both (+) sense RNA viruses such as polio and (-) sense viruses in the Rhabdoviridae family degrade or inhibit transcription Factor II D (TFIID), which is responsible for recognising the TATA box of promoters and recruiting RNA polymerase II.[4,5] Similarly, (-) sense viruses in the Bunyaviridae family target TFIIH, the helicase responsible for unwinding DNA to allow transcription bubble formation.[6]

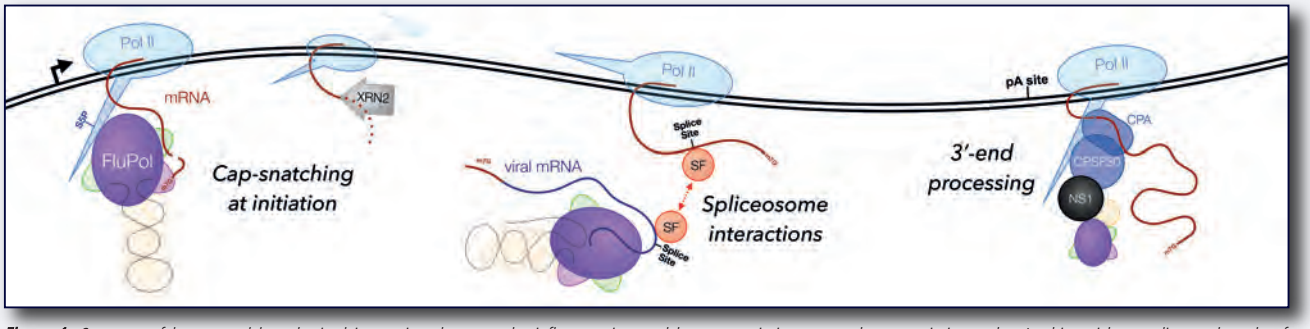

Figure 1. *Summary of known and hypothesised interactions between the influenza virus and host transcription across the transcription cycle. In this article, we discuss the role of cap-snatching by the viral polymerase (FluPol) at host RNA Polymerase II (Pol II) transcription start sites and the role of the host nuclease Xrn2. In the body of genes, interactions have been proposed to be mediated by splicing factors (SF) mutually required for host and viral mRNA splicing. Lastly, at the poly(A) site, the viral non-structural protein (NS1) can target host 3'-end mRNA processing by direct interaction with the CPSF30 subunit of the cleavage and polyadenylation (CPA) complex.*

Neither mechanism, however, is readily available to influenza. The virus, which transcribes and replicates its RNA genome in the nucleus, relies on the host RNA polymerase to generate 5′-capped RNAs that the viral polymerase then co-opts to prime its own mRNA transcription. This process of "cap-snatching" is carried out in a coordinated manner between the three subunits of the influenza polymerase,[7] which bind the 5′ cap of the nascent host transcript, cleaves the transcript 10-15 nucleotides downstream of the cap, and feeds this fragment into the polymerase active site to prime transcription,[8] to yield transcripts that appear to be "normal" host transcripts at their 5′ ends. The price the virus must pay for this advantage is that it cannot completely shut off host transcription. Indeed, the virus must balance two competing needs during the early stages of infection: to keep the host RNA polymerase active and supplying 5′-caps, while suppressing host gene expression.

A Tale of Two Polymerases

Over 10 years ago, our lab showed that the viral polymerase directly binds the host RNA polymerase.[9] This interaction is the result of a specific interaction with the long, unstructured C-terminal domain (CTD) of the host polymerase, which consists of 52 heptad repeats with the sequence (Tyr-Ser-Pro-Thr-Ser-Pro-Ser)52. The CTD normally acts both as a platform for the assembly and recruitment of various components of the transcription complex, and as a master regulator of Pol II activity via specific phosphorylation marks on the amino acids of the CTD.[10]

We have recently shown that the viral polymerase binds to Pol II specifically when the fifth serine residue of the CTD is phosphorylated (Ser5P), and that this binding requires a fully assembled viral polymerase: the 3 individual subunits alone are insufficient.[11] This specificity provides an explanation for how the viral polymerase manages to efficiently sort through the ~80,000 Pol II molecules in each nucleus[12] in order to find those with nascent capped mRNAs available for cap-snatching: the Ser5P mark is uniquely associated with Pol II that is in the initial stages of transcription at the start of genes,[13] while unassembled viral polymerase subunits alone do not compete for host Pol II binding.

Once the viral polymerase has successfully obtained capped RNA from the host Pol II, the host polymerase is effectively redundant as far as the virus is concerned. Recently, we have employed mammalian native elongating transcript sequencing (mNET-seq) that provides single-nucleotide resolution information of the dynamics of Pol II.[14] The results generate a "snapshot" of the location of host Pol II and its various phosphorylated isoforms across the genome during influenza infection. From these data, we can examine behaviour on individual genes, as well as create more generalised "metaprofiles" of Pol II across the entire genome. This recent work – a collaboration between the Fodor, Murphy and Proudfoot groups at the Dunn School – has shown that viral infection leads to a depletion of Pol II from the gene bodies, downstream of transcription start sites. We have found that this depletion is likely specific to active viral transcription, and not just the presence of the viral polymerase alone or other viral proteins. This result suggests that the very act of cap-snatching and RNA cleavage leads to suppression of host transcription elongation and, therefore, full gene expression. We are currently exploring the exact mechanism by which displacement of Pol II occurs. One intriguing coincidence is that the normal process of transcription termination at the polyadenylation site (pAs) of a gene begins with the cleavage of the RNA transcript from the elongating Pol II in order to be passed to the 3′-end mRNA processing machinery.[15] The product of this cleavage reaction is an RNA polymerase that contains a short piece of uncapped RNA trailing behind that can be recognised by the cellular exonuclease Xrn2, which chews back this RNA and can then "torpedo" Pol II off the chromatin to terminate transcription.[16] It is possible that viral cap snatching therefore induces premature termination – in this case just a few hundred bases downstream of the transcription start site – by the same mechanism.

This process of premature termination comes with an added bonus for the virus: in addition to preventing the host from transcribing any activated antiviral genes, the displaced Pol II is now free to re-initiate transcription and feed viral polymerase transcription in the process. By the same token, the dependence of the virus on the host's own transcription initiation processes is a ripe target for new antiviral agents against influenza that act by targeting the viral polymerase and the host's transcriptional machinery itself, an approach that we are now exploring.

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Host Pathogen Interactions: A Circular Argument

Jessica Martyn and Christoph Tang

A small step for bacteria - a giant leap backwards for mankind

Our bodies are teeming with microorganisms. Many cannot be grown in the laboratory but the full catalogue of our microbial passengers has become apparent by molecular methods to detect their presence through their tell-tale DNA sequences. Advances in sequencing technology have enabled us to look deep into ourselves and at the microbes we carry, and appreciate the scale and diversity of our bacterial passengers.

In most instances, our microbial flora is completely harmless to us. The organisms that make up our flora are called commensals, stemming from the word 'to eat at the same table'. They are part of us and take a share of our food. In return, they synthesise vitamins that we absorb as nutrients, and contribute to innate immune defence against the incursion of other microorganisms. There is compelling evidence that bacteria in the intestinal tract stimulate our immune responses, locally at mucosal surfaces, and also beyond to prime our systemic immune system.

However despite our mutually-beneficial coexistence with the vast majority of microbes to which we are exposed, there is a small group of bacteria, the pathogens, which have the capacity to inflict harm. Pathogens can establish themselves in niches usually devoid of commensal microbes, breaching anatomical barriers and evading immune defences. The distinction between pathogens and our flora can be blurred; there are several bacterial species that can be found colonising healthy people yet can cause disease in others.

In contrast, other pathogens are highly virulent, and have emerged from harmless bacteria in a single genetic step. A remarkable example is *Shigella*. To all intents and purposes, *Shigella* is identical to *Escherichia coli* which is a ubiquitous component of the flora of the mammalian intestinal tract. However the four species of *Shigella* have arisen from harmless strains of *E. coli* between 500 and 20,000 years ago by the uptake of one piece of circular DNA called a plasmid. This plasmid contains all the genetic information needed for the bacterium to invade human cells, including cells of our immune system, elicit inflammation, and cause dysentery. The small step of acquiring a plasmid has led to a quantum leap in evolution and allowed the bacterium to exploit new niches in the human body. As a result, *Shigella* now causes over 100 million cases of dysentery per year, with around 700,000 deaths among children living in impoverished circumstances, and eclipsing malaria as a cause of mortality.

Plasmids: going around in circles

Plasmids are circular, double-stranded DNA molecules that are distinct from a bacterium's chromosome. Often, genes carried by plasmids provide their host with fitness advantages, such by conferring virulence or antimicrobial resistance (AMR). Resistance plasmids, R-factors, were first characterised in the 1950s as mediators of antimicrobial resistance in bacteria such as *Salmonella*. There then followed a period of intense investigation into and modification of plasmids that allowed them to be tamed as tools for the laboratory. Plasmids are exploited as vectors to clone, transfer, and manipulate genes. Then, because bacteria divide rapidly, plasmids are used as nano-factories to make DNA fragments in large quantities.

Plasmids have several interesting features. Their DNA must be duplicated within bacteria. For this, plasmids, which are inherently selfish genetic elements, hijack bacterial enzymes for their replication, similar to viruses in mammalian cells. DNA replication is carefully controlled to keep the number of copies of a plasmid inside a bacterium relatively constant from generation to generation. The number of plasmids in a bacterium can be in the hundreds, as for many plasmids used in the laboratory. However for plasmids that exist in the wild, more is often less. Plasmids can impose a significant burden on their bacterial hosts, so many plasmids involved in virulence and drug resistance are only found as a single copy inside each bacterial cell. And even this can be a heavy load for a rapidly dividing bacterium. For example, *Shigella* readily loses its plasmid in the laboratory, then grows at a far higher rate compared with a strain which has kept the plasmid (Figure 1).

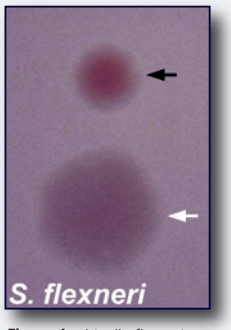

Figure 1. *Shigella flexneri grown on Congo red agar showing virulent bacteria (black arrow), avirulent bacteria (white arrow) which have lost part of their plasmid.*

Maintaining single copy plasmids presents a major challenge for bacteria to ensure that each daughter cell receives one of the two copies generated following replication. Replication of plasmid DNA must be co-ordinated with cell division, and then one plasmid must move into each daughter cell before division is complete. These aspects have been extensively studied in standard laboratory plasmids: segregation can be achieved by plasmid-encoded systems that either push (ParMR) or pull (ParAB) plasmids to the far ends of dividing cells. However, complexities abound. For instance, the *Shigella* virulence plasmid, pINV, surprisingly contains versions of both systems, a canonical ParAB sequence and an uncharacterised version of ParMR.

Coping with failure

But what happens if the systems to maintain a plasmid fail, and a daughter cell ends up without a plasmid? Plasmids even have a strategy in place to deal with this. As a selfish element, bacteria that are plasmid-free after division can be eliminated from a population through a process known as post-segregational killing (PSK) (Figure 2). PSK involves plasmid encoded toxin:antitoxin (TA) systems. In the normal state of affairs when a bacterium has a plasmid, a toxin is produced and neutralised by a specific antidote, the antitoxin. There are six different types of TA system depending on the nature of the antitoxin and mechanism of inactivation. Type II TA systems are abundant on plasmids, and are characterised by having both the toxin and antitoxin as proteins

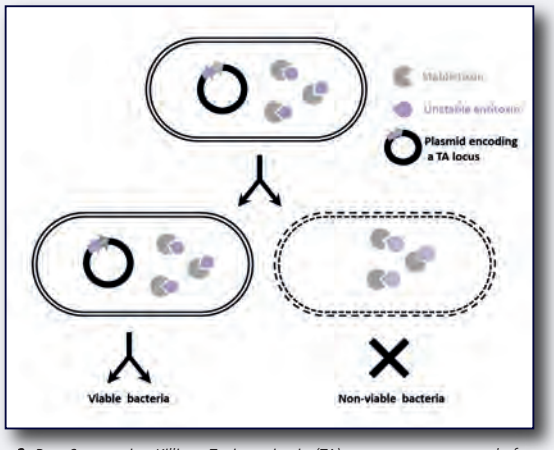

Figure 2. *Post-Segregation Killing. Toxin-antitoxin (TA) systems are composed of an toxin gene and an antitoxin gene. Presence of the plasmid ensures enough expression of the antitoxin to counteract the toxin. If the plasmid is lost, the antitoxin is degraded by proteases and its expression is not replaced by the plasmid. As there is no antidote antitoxin present, the stable toxin kills the plasmid-less cell.*

which form a complex resulting in toxin neutralisation. If a plasmid is lost, antitoxins are degraded in the cell as they are inherently unstable, allowing the more stable toxin to access its cellular target and arrest growth or kill the plasmid-less bacterium. In this way, bacteria lacking the plasmid (which would have a growth advantage) are kept in check.

Interestingly pINV from *Shigella* has three functional TA systems.[1] This seems like overkill as, for PSK, one TA system should be enough. However the TA systems operate under different circumstances. One system, MvpAT, helps to keep pINV in bacteria when they are grown at temperatures found in the human body; another (GmvAT) serves the same purpose but when the bacterium is in the external environment. Another role for the multiple TA systems on a single piece of DNA is that they can act to prevent loss of nearby sequences. MvpAT is near the origin of replication while GmvAT is located adjacent to a gene that controls the expression of virulence genes. So TA systems act locally as well as globally.

TA systems are not just interesting because of how they contribute to plasmid biology. There are a plethora of TA systems in bacteria. *E. coli* has 30 on its chromosome, where they mediate the transition of the bacterium into a dormant state (with the bacterium being viable but not growing), making them temporarily resistant to killing by antibiotics (which generally act on dividing bacteria).[2] Understanding how TA systems function could be exploited for the development of new antimicrobials. For instance, the toxin CcdB which is part of another TA system on pINV targets the same protein in the bacterial cell at precisely the same site as the fluoroquinolones, a major class of antibiotics. So what we learn from the activities of these toxins could be translated into finding novel antibiotics, which are desperately needed. GmvT encoded by the *Shigella* plasmid prevents protein translation in bacteria (an Achilles heel exploited by antibiotics such as streptomycin and aminoglycosides) by transferring a small chemical group to an as yet unknown molecule. We are currently studying how TA systems are controlled and how this could be used in biotechnology. Can we learn more about TA systems to make plasmids that are never lost from bacteria which would be valuable for vaccine design and gene expression studies? Can we find ways to inhibit plasmid maintenance systems and make them lose their virulence or resistance against antibiotics?

What goes around comes around

The rise in AMR in bacteria threatens to return certain areas of medical practice to a pre-antibiotic era in which there are no drugs to treat people with common infections. Resistance genes can cluster together on plasmids, which means that resistance against most classes of antibiotics can be acquired in a single genetic event.

There is a correlation between the global dissemination of *Shigella* and the acquisition of antimicrobial-resistance genes located on mobile genetic elements such as plasmids, transposons, and integrons. Stably-inherited extended-spectrum β-lactamase (ESBL)-producing plasmids are increasingly being recovered from *Shigella* in developing countries. Acquiring these plasmids, renders treatments with antibiotics ineffective, and promotes spread of bacteria within and between countries.[3] Plasmids are also prevalent in *Neisseria gonorrhoeae*, a bacterium that infects the genitourinary tract, causing the sexually transmitted disease gonorrhoea. There has been a steady rise in antimicrobial resistance in this bacterium, which has now been accorded 'Superbug' status. Although the incidence of gonococcal disease is highest in Africa, where it can act as a co-factor for the spread of HIV, remarkably little is known about strains of gonococcus from this continent. Through a collaboration with the Wellcome Trust Unit in Kilifi, Kenya, we have analysed a series of gonococcal isolates from over 70 sex workers. What was striking is that over 98% of strains in this population carry plasmids. We found two main plasmids: one plasmid (pTetM) is virtually ubiquitous and confers resistance against tetracycline, while the other plasmid (pBlaTEM) was found in around half of the strains, and makes bacteria untreatable with penicillin. While both plasmids have been found in gonococci before, this is the first time they have been seen at such high frequency. For example, only 4-5% of gonococcal strains in the UK and USA carry plasmids. Interestingly, pTetM can help the spread of pBlaTEM, so treatment of high risk patients with tetracycline (for other genital infections) can promote resistance against penicillin, which was previously the mainstay of treatment.

Perspectives

Plasmids are common in bacterial populations, and have a profound effect on how bacteria interact with our cells and our attempts to eradicate them. While many plasmids have been studied and are used in the laboratory, much is still to be learnt about plasmid biology in pathogenic bacteria. How do multiple plasmid maintenance systems function on a single genetic element? How are plasmid genes regulated and integrated in the transcriptional networks of new bacterial hosts? Can understanding TA systems lead to novel antimicrobials to kill bacteria or to disarm them? And can we wake up dormant bacteria in order to kill them with conventional antimicrobials? One thing is certain about the future, plasmids will be around.

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Jack Sunter joined the Gull laboratory in October 2011 and has thoroughly enjoyed working at the bench and in the department itself. He will soon set up his own group up at Oxford Brookes University taking this project with him and concentrating on understanding links between* Leishmania *cell shape, form and pathogenicity.

Leishmania – The Shape of a Killer

Jack Sunter and Keith Gull

It is an unfortunate truism that agents - as diverse as the spitfire fighter plane and the rattlesnake - that deliver injury and death often have a beautifully designed shape and form. This precise fitting of shape to function is seen in protozoal parasites causing devastating diseases such as malaria, trypanosomiasis and leishmaniasis. Such parasites have complicated life cycles in which they traverse significant transitions between vector and host and host and vector. Evolutionary forces have led to variations on a theme in the design of differentiated cells with shapes fitted to function – leading to beautifully "designed" infectious agents.

Leishmania and leishmaniasis

In the Gull laboratory, we are interested in eukaryotic microbial parasites. One strand of our research is focussed on understanding how the shape and form of the *Leishmania* parasite is fitted to its pathogenicity. We are not the first Dunn School researchers to study the *Leishmania* parasite; whereas we now concentrate on the parasite itself, in the 1970s David Bradley produced critical insights into the genetics of mouse host susceptibility to, and immunology of, *Leishmania* infection.

There are numerous different species of *Leishmania* that cause disease in humans, with the disease predominantly affecting people living in poverty in the less-developed countries of South and Central America, Africa and Asia. The various forms of leishmaniasis range from self-healing cutaneous lesions to severe and often fatal visceral leishmaniasis. The *Leishmania* parasite is spread by sandflies and, as with other vector borne diseases, the distribution of the parasite is completely dependent upon the range of the vector, which in turn is determined by the environment. Climate change is likely to significantly impact sandfly distributions and there have been recent rises in the number of cases of leishmaniasis in southern Europe.

Life cycle transitions are accompanied by dramatic shape changes

When an infected sandfly bites a human, infective *Leishmania* parasites termed metacyclics (Figure 1) are injected into the bite site. These metacyclic cells are taken up by macrophages where they differentiate inside a parasitophorous vacuole into the amastigote form. It is this form that then proliferates, spreading the infection to further macrophages. A circulating infected macrophage is then taken up with a blood meal when another sandfly feeds and, in the midgut of the sandfly, the amastigote forms differentiate into motile promastigote forms (Figure 1). These promastigotes traverse the sandfly digestive tract to a position close to the mouthparts where the parasite differentiates to the non-proliferating metacyclic forms, which are then competent to re-initiate the cycle.

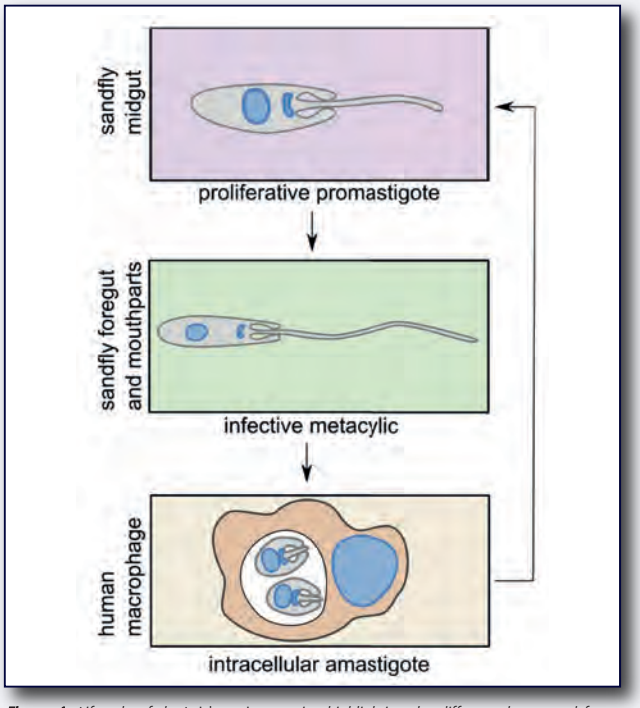

Figure 1. *Lifecycle of the* Leishmania *parasite, highlighting the different shapes and forms observed in the vector and the host. The proliferative promastigote is found in the sandfly midgut; this form then migrates to the sandfly mouthparts and differentiates into the infective metacyclic. The metacylics are taken up by the host macrophages after being injected into the host when the sandfly bites. In the macrophage, the metacylics differentiate into the intracellular amastigote form. (*Leishmania *cartoons courtesy of Richard Wheeler)*

A dramatic change in cell shape occurs when the *Leishmania* parasite transitions from the sandfly vector and infects the macrophage. In the sandfly, *Leishmania* cells have an elongated cell body with a long, motile flagellum that provides the propulsive force enabling the cell to move from the midgut to the mouthparts. After macrophage infection the *Leishmania* amastigote form has a short, non-motile flagellum that barely emerges from within the more spherical cell body. This flagellum is more likely to perform sensory functions pertinent to life inside the macrophage.

Understanding *Leishmania* shape

How are these different parasite cell shapes achieved and modulated? The key to understanding the parasite cell shapes lies in defining how the internal cytoskeletal architecture is built and how it influences the flagellum position and the flagellar pocket. The flagellar pocket is hugely influential in parasite biology: it is a plasma membrane invagination at the base of the flagellum that has a vase-like shape, bulging out at the bottom and narrowing towards the top with the neck sheathing the flagellum for a short distance (Figure 2). The

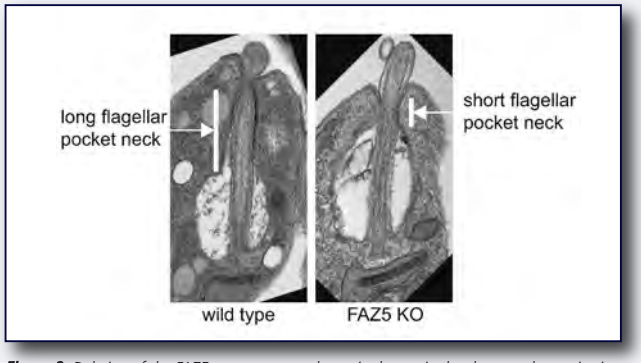

Figure 2. *Deletion of the FAZ5 gene causes a dramatic change in the shape and organisation of the flagellar pocket in the* Leishmania *amastigote with a large reduction in the length of the flagellar pocket neck.*

flagellar pocket is a critical interface between the parasite and its host, as it is the only site for the uptake and secretion of large macromolecules and is the likely location for critical surface receptors.

Insight into the cytoskeletal architecture of the flagellar pocket region in promastigote forms has come from using electron tomography, an electron microscopy technique that enables the generation of high-resolution 3D models. Despite first impressions, the *Leishmania* promastigote cell is not radially symmetrical and a key break in the symmetry of the cell is the attachment of the flagellum to one side of the neck region of the flagellar pocket. This attachment is mediated by a large cytoskeletal filament/membrane structure called the flagellum attachment zone. Significant changes to the flagellum attachment zone in the amastigote form seem to be associated with the remodelled cell shape and flagellar pocket.

Cell shape mutants and organisation

We have identified many of the protein components of the flagellum, the flagellum attachment zone filaments and the membrane attachments. Our hypothesis was that deletion mutagenesis of genes encoding some of these proteins may produce shape changes in flagellar pocket organisation, allowing us to interrogate specific links to pathogenicity. *Leishmania* are genetically tractable organisms and it is relatively facile to express fluorescently-tagged copies of specific proteins of interest and also to generate gene knockout mutants. After characterising the cellular positions of flagellum attachment zone proteins we produced a panel of knockout mutants.

As expected, the deletion of specific flagellum attachment zone proteins resulted in the loss of attachment of the flagellum to the cell body. But we noticed some unexpected consequences including a distinct change in the shape and organisation of the flagellar pocket (Figure 2). More surprisingly, we also observed a change in the overall shape of these parasites, with the *Leishmania* cell body becoming shorter and wider. Yet despite the changes observed, the cells were still able to proliferate in culture, suggesting that these significant changes to flagellar pocket structure were easily tolerated. However, we wanted to establish whether these changes had consequences in the process of infection, in both the sandfly and mammalian macrophage.

In collaboration with groups in the UK and Czech Republic, we looked at our mutants *in vivo* in established infection models. Early indications from these infections show that our mutants have much reduced infectivity in both sandflies and animals in comparison to wild type parasites and add-back controls in which those specific flagellum attachment zone proteins are present. There are a number of potential explanations for this loss of infectivity as the flagellar pocket region is a nexus for many processes in the cell. However, changing the parasite cell shape in a subtle way appears to have had dramatic effects on pathogenicity in both host and vector.

Photograph courtesy of Tim Davies

Earlier this year, Bernadeta Dadonaite, a DPhil student in Ervin Fodor's group, won first prize in the British Society for Gene and Cell Therapy (BSGCT) science writing competition. Here we reproduce her winning essay with kind permission of the Society

Gene Therapy against HIV: Fighting the Virus in Disguise

Bernadeta Dadonaite

The world around you is teeming with pathogens. Lucky, your immune system is well trained to protect and guard you from any unwanted invaders. Pathogens, such as viruses, are in a permanent arms race with our immune system, constantly evolving to evade our defenses. Perhaps the most successful pathogens are the ones which have learned to disguise their weapons. And what better disguise can there be than becoming a physical part of the target - just the kind of tactics used by HIV?

HIV, or human immunodeficiency virus, is a pathogen, which infects humans and causes AIDS (acquired immunodeficiency syndrome). Around 38 million people worldwide are infected with HIV and, while with appropriate treatment patients can live for many years, there is still no cure or vaccine against the virus.[1] The major treatment for people with AIDS is an antiretroviral therapy (ART). ART is essentially a cocktail of various drugs, which stop the virus at different stages in its life cycle. However, preventing the virus from replicating is only a part of the challenge, as HIV is very good at playing hide-and-seek. HIV primarily targets our immune cells - the very same cells which are meant to seek and destroy the invaders. When HIV encounters an immune cell it enters and becomes part of the cell by integrating into the cell's genome. Integration into a host genome is an essential part of HIV's life cycle and it is also a perfect Trojan horse strategy. When HIV becomes part of the cell's genome it is no longer recognized as a

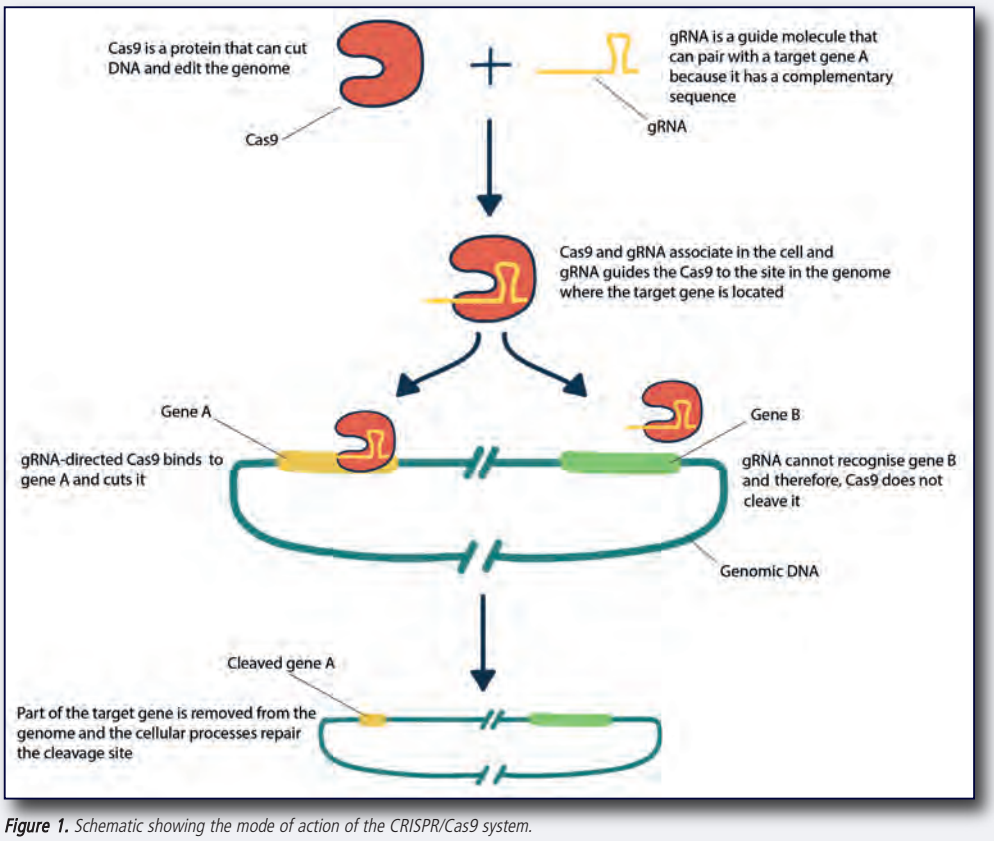

Figure 1. *Schematic showing the mode of action of the CRISPR/Cas9 system.*

foreign entity and can remain dormant there until the environment is safe to reveal itself. The ART drugs are only able to kill an actively replicating virus but the dormant virus can remain integrated into the genome for many years. Consequently, HIV patients have to use ART drugs every single day for the rest of their lives to prevent virus from re-emerging, killing the immune cells and leading to AIDS.

The challenges for treating HIV infections still remain great, however the incredible advancements in gene editing and therapy technologies are paving a way for a brighter future. Gene editing is basically an on-demand ability to change and modify any gene within a given genome. Because the human genome has thousands of genes, the targeting of specific genes, without interfering with the functions of

other genes, has always been a challenge. However, experiments using CRISPR (clustered regularly interspaced short palindromic repeats) technology (Figure 1) suggest that there is a way of eliminating the dormant form of HIV.

The dormant HIV virus, also called a provirus, is essentially a gene in disguise. The cell's surveillance system cannot distinguish the provirus from any other cellular gene. With the use of CRISPR, however, there is a possibility of cutting out this non-native gene from the genome (Figure 2). CRISPR can be specifically targeted to a single gene via a guide RNA molecule and the guide RNA can be made to recognise the HIV sequence in the genome. Once the CRISPR system has identified the site of the provirus, it can cut it out, which leaves the cell free of the HIV. While in laboratory experiments this strategy has been very successful,[2-4] the challenge has remained in being able to deliver the CRISPR system into a living organism. Recently, however, a combination of CRISPR and decades-long advancements in gene delivery methods showed the potential of this strategy in animals too. Adeno-associated virus vectors (AAVs) have for many years been used as treatment-delivery systems. AAVs can be made to carry almost any gene and target specific cell types. The genes carried by AAVs integrate into a genome and are expressed inside the cells like any normal gene. Last year the AAVs engineered to carry a provirus-targeting CRISPR have been used to remove an integrated HIV genome from mice.[5] As much as 90% of blood cells were successfully depleted of provirus in the mice, indicating the potential for the clinical use of this approach.

While gene therapy approaches to remove the provirus from a genome have still a long way to go, gene therapy methods that prevent the virus from entering a cell in the first place have already reached clinical trials. When HIV encounters an immune cell it has to bind a receptor on its surface. The receptor, called CCR5, acts like a lock into which HIV

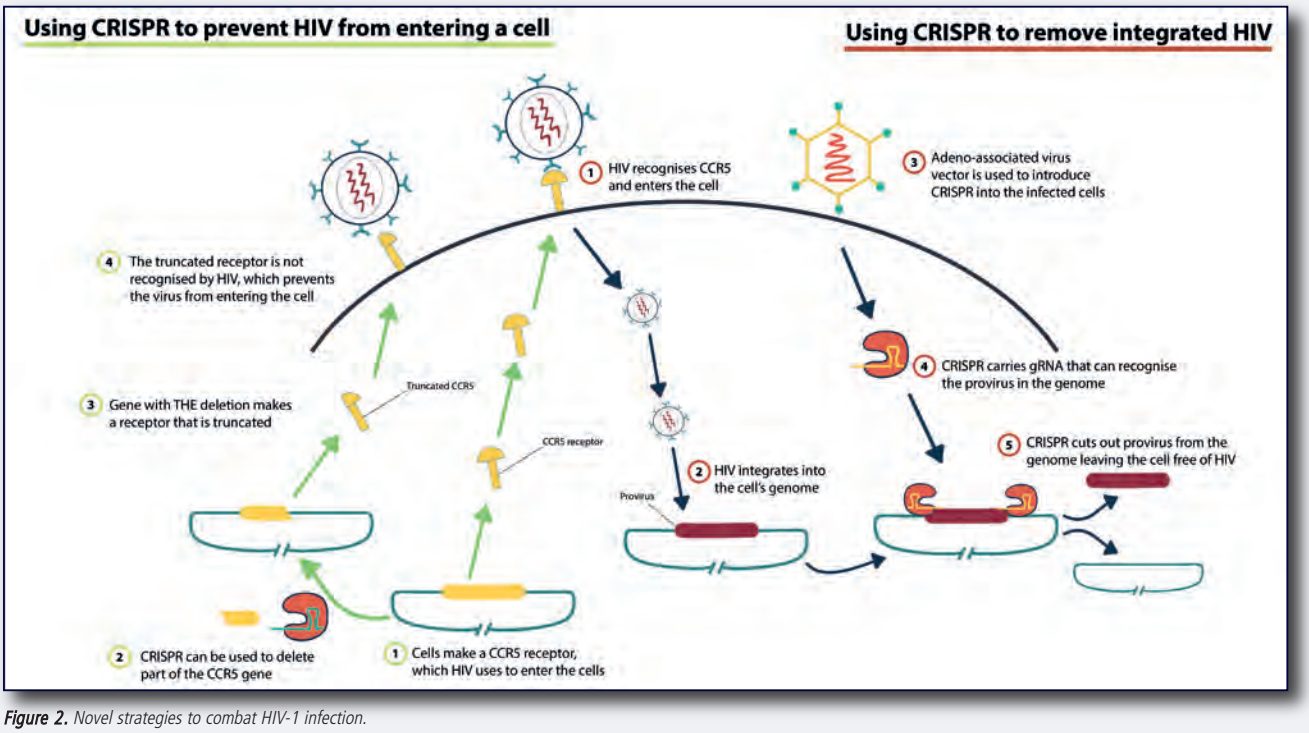

Figure 2. *Novel strategies to combat HIV-1 infection.*

inserts its key and opens the door into the cell. Just like with any lock, a slight change in the CCR5 can prevent the key from fitting. Interestingly, it has been observed that a small deletion in the CCR5 gene can prevent or attenuate HIV infection. 1% of Caucasians naturally carry the CCR5 deletion, indicating that it is not deleterious for humans. This observation suggested that engineering patient cells to carry the CCR5 deletion could be a potential treatment against the HIV. Indeed, experiments in a lab using CRISPR to introduce the CCR5 deletion have successfully produced immune cells that are resistant to HIV infection.[6] The first clinical trial exploring the same principles has also shown some promise. The patients whose blood cells where isolated and modified to carry the CCR5 deletion showed a much slower re-emergence of the virus in their blood in the absence of ART.[7]

Gene therapy has traditionally been used to treat genetic diseases, however, the advancements in gene editing technologies now enable the use of gene therapy to treat infectious diseases as well. Many challenges still remain for the use of CRISPR against HIV. HIV is renowned for its ability to change and evade many treatment strategies. Potentially, HIV could outsmart CRISPR by changing its sequence, so that CRISPR can no longer recognize it. Even more so, the use of CRISPR in humans has still many ethical and safety issues to be resolved. However, until effective vaccines against HIV are available, combination of ART and gene therapy approaches may be the only way of developing a cure against HIV.

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TECHNOLOGY FEATURE

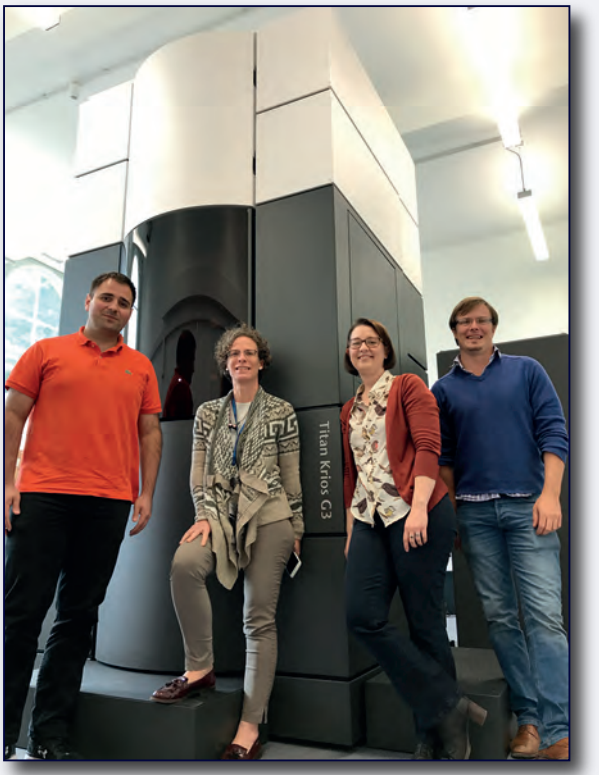
The New Ice Age: Cryo-Electron Microscopy Comes to South Parks Road

Errin Johnson

The field of structural biology has undergone a spectacular transformation in recent years due to the rapid rise of cryo-electron microscopy (cryo-EM) as a viable alternative to x-ray crystallography for protein characterisation. This was facilitated by a convergence of advancements in electron microscope design, signal detection, high performance computing and data acquisition/analysis software, which led to a significant improvement in the structural resolution achievable with cryo-EM. A wide range of proteins, as well as many other types of samples (eg: ribosomes, viruses, membranes and biological polymers), can now be imaged using cryo-EM and modelled at sub-15 Å resolution, and in some cases near atomic resolution, using single particle analysis and sub-tomogram averaging techniques. With the recent establishment of a state-of-the-art cryo-EM Facility in the Science area on South Parks Road, led by the Dunn School's Susan Lea, structural biologists across Oxford now have this powerful and cool (pun intended) technique at their fingertips.

What is cryo-EM?

Proteins and other particulate samples, such as viruses, DNA, exosomes and liposomes, can readily be imaged with transmission electron microscopy (TEM) using the negative stain technique, which involves drying samples in a thin layer of heavy metal stain (such as uranium salts) on a TEM grid, so that the particles are visible in the TEM as white against the dark background of the stain. In this way, particle size, behaviour (e.g. ability to aggregate) and purity (e.g. amount of background protein) can quickly be assessed. However, negative staining induces numerous artefacts, chief of which is the significant distortion to particle structure which occurs as the sample is air dried in the stain. Cryo-EM avoids these problems, as it allows particles to be imaged fully hydrated and in their near native state. This is achieved by the process of vitrification, where the sample is applied to a TEM grid, blotted to remove excess solution and then frozen by immediately plunging it into liquid ethane, such that the particles are suspended at random orientations in a thin layer of amorphous ice within the foil holes of the grid. All subsequent handling and imaging (using specialised cryo-TEM holders) is done at cryogenic temperatures, which involves numerous technical challenges. For instance, if the grid warms above -155°C, crystalline ice will be formed, which distorts structure, as does exposure to the electron beam itself. Cryo-EM samples are, therefore, imaged under low electron dose conditions, which, in the absence of heavy metals to scatter the electrons, means that contrast in cryo-EM images is low. To overcome the low contrast, 3D models are generated using many tens of thousands of particles. Every particle needs to be correctly identified in the original image then the orientation with respect to the three dimensional object established. Combining signal from all the particles can then allow calculation of a model of the object imaged. This is computationally challenging as, in addition to the large size of the data sets, issues of heterogeneity (sample or conformational) also need to be resolved.



The 300 kV Titan Krios cryo-TEM at the Old Observatory and part of the team who helped with the installation. From left to right: Alex Buzduga (FEI installation engineer), Susan Lea (Director of COSMIC), Errin Johnson (COSMIC & Dunn School EM Facility manager) and Joe Caesar (postdoc in the Lea group and computing wizard).

Cryo-EM in structural biology

Cryo-EM was first developed in the early 80s, though it is only in the last 5 years that it has been increasingly applied to structural biology, as multiple technical, analytical and computing limitations have been overcome synergistically. A new cohort of microscopes, spearheaded by

the 300 kV FEI Titan Krios, with brighter, more stable electron sources, improved optics and higher thermal stability, allow data to be collected automatically over several days, with the help of new automated acquisition software. Arguably the biggest game-changer for cryo-EM has been the development of a completely new generation of cameras, called direct electron detectors, with higher sensitivity and much faster acquisition rates than CCD cameras. Instead of taking one frame per exposure, direct electron detectors acquire hundreds of frames per exposure under very low dose conditions (e.g. 50 electrons/Å^2 for a 50s exposure); these frames are then either summed into an integrated image or recorded as a movie. Improved image processing algorithms are then used to correct for the motion blurring that occurs when the particles are exposed to the electron beam, resulting in an improved resolution. At the same time, high performance computing has become more powerful and less expensive, which has made it more feasible to handle the huge data output of these cameras (e.g. 10-20 TB for 1 experiment) and subsequently perform the computationally-intensive data analysis. Taken together, these advancements make it possible to model macromolecules from the MDa range down to ~200kDa in size at resolutions where it is possible to construct atomic models with cryo-EM. Although it is still challenging to resolve structures using this technique, it does open up possibilities for systems where the protein cannot be crystallised, as this is not required.

The Central Oxford Structural Microscopy and Imaging Centre (COSMIC)

The critical need for a high resolution cryo-EM facility sited on South Parks Road was recognised by Susan Lea and Matthew Freeman at the Dunn School who, together with new Dunn School recruit Tanmay Bharat, formed a cross-departmental consortium with scientists from Chemistry (Carol Robinson) and Biochemistry (Francis Barr, Matthew Higgins, Simon Newstead) to establish one. This was successfully achieved with funding from the Wolfson Foundation, the Wellcome Trust and the EPA Research Fund, and the backing of the Medical Sciences Division. Commissioning of the Central Oxford Structural Microscopy and Imaging Center (COSMIC) began in the summer of 2016 with delivery of the first two 200 kV cryo-EMs and has now been completed by the recent successful installation of the third TEM, a 300 kV FEI Titan Krios. COSMIC comprises a fleet of high end instrumentation and computing for every stage of the cryo-EM workflow:

- FEI Tecnai T12 TEM (in association with the Dunn School EM Facility) for screening samples using negative staining
- FEI Talos 200 kV cryo-TEM with STEM detector for screening cryo-grids and for cryo-tomography and cryo-STEM tomography
- FEI Arctica 200 kV cryo-TEM with FEI Falcon 3 direct electron detector for high throughput screening of cryo-grids and single particle analysis
- FEI Titan Krios 300 kV cryo-TEM with FEI Falcon 3 and Gatan Bioquantum direct electron detectors for single particle analysis, cryo-tomography and cryo-STEM tomography
- Four FEI Mark IV Vitrobots for preparing cryo-EM grids
- High performance computing for data storage and processing

These microscopes will be flagship instruments of the new Dorothy Hodgkin Institute for Physical approaches to life-science research, due to be completed in 2020. In the meantime, they are currently located in the Dunn School and at the Old Observatory. The good news is that COSMIC is now open to users and we are looking forward to facilitating an exciting and diverse range of structural biology research at Oxford with cryo-EM. We are also keen to push the boundaries of what is possible with our new instrumentation and analysis tools by developing new capabilities (e.g. in cryo-STEM and cryo-correlative microscopy) and breaking new resolution limits on a wide array of biomolecules.

Further reading

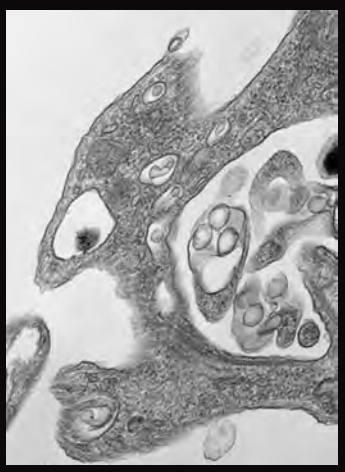
If you are interested in finding out more about COSMIC and the Dunn School Bioimaging Facility, please see our website for more details and contact information: www.dunnschoolbioimaging.co.uk

The following articles are a good starting point for learning more about cryo-EM:

- Milne JL *et al.* (2013) Cryo-electron microscopy – a primer for the non-microscopist. *FEBS Journal* **280**:28-45
- Bai XC *et al.* (2015) How cryo-EM is revolutionizing structural biology. *Trends Biochem Sci*, **40**:49-57
- Lučić V *et al.* (2013) Cryo-electron tomography: the challenge of doing structural biology *in situ*. *J Cell Biol* **202**:407-419

Photograph courtesy of Thomas Dunwell

Dunn School Bioimaging Facility Image Awards 2016



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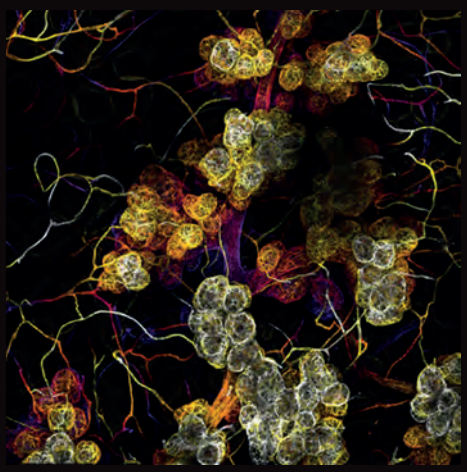

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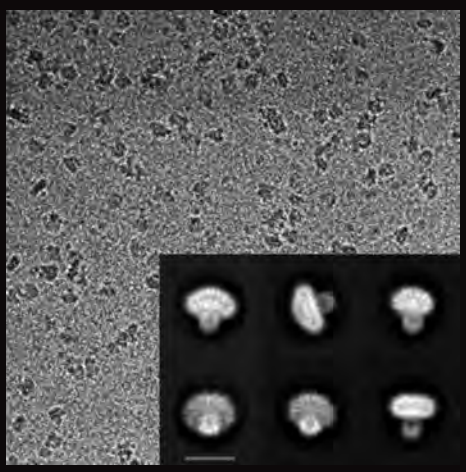

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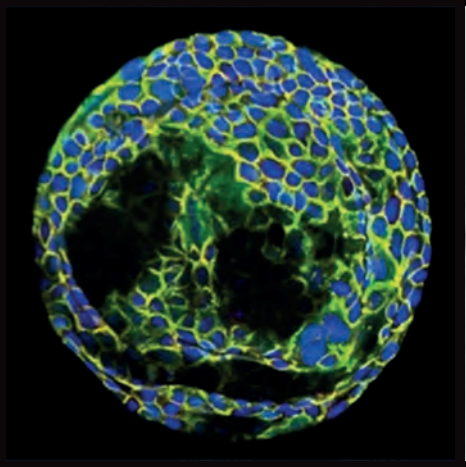

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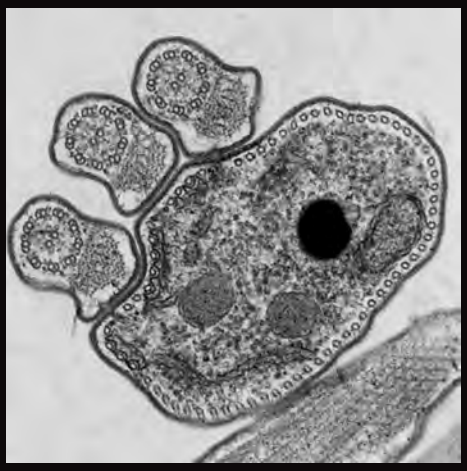

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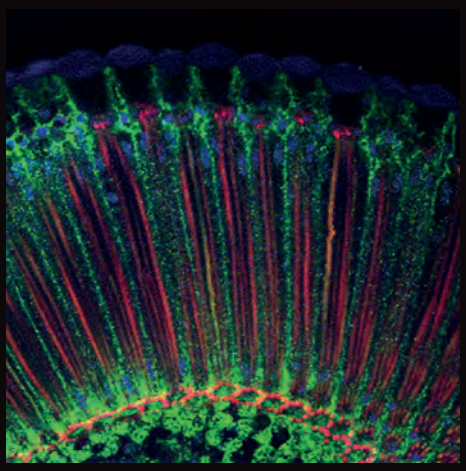

F

A: Runner-up, Most Humorous - Screaming *Leishmania* by Flavia Moreira-Leite (Gull lab) on the FEI Tecnai 12 TEM.

B: Winner, Light Microscopy - Day 14.5-of-pregnancy whole mount mouse mammary gland expressing membrane tomato by Salah Elias (Robertson-Bikoff lab) on the Olympus FV1000 confocal.

C: Winner, Electron microscopy - Cryo-EM of a bacterial membrane protein complex and the corresponding class averages created by particle alignment (inset) by Justin Deme (Lea lab) on the FEI Talos 200c TEM.

D: Winner, Most Humorous - Jack Cellington by Marvat Shurhi (Hassan lab) on the Olympus FV1000 confocal.

E: Runner-up, Electron Microscopy - Three adjacent flagellar profiles with flagellar attachment zones on a mutant *Trypanosoma brucei* bloodstream form cell by Jessica Valli (Gluenz lab) on the FEI Tecnai 12 TEM.

F: Runner-up, Light Microscopy - Horizontal view of a *Drosophila* adult retina by Sonia Muliyil and Clemence Levet (Freeman lab) on the Zeiss 880 Airyscan confocal.

HISTORY CORNER

The Dunn School Archive at the Bodleian Library

Charlotte McKillop-Mash

Carefully gathered by Eric Sidebottom over decades, the archive of the Sir William Dunn School of Pathology has now been transferred to the Bodleian and is being catalogued as part of the Wellcome Trust-funded project '75 Years of Penicillin in People'. The project has not only historical but scientific and social aims: from the full genetic sequencing of two isolates of the antibiotic fungi *Penicillium* and *Cephalosporium*, to sending Dunn School volunteers into local primary schools to encourage children to consider a future in science. And for the next couple of months, science writer and biographer Georgina Ferry will be conducting oral history interviews with current and former members of the department in an effort to capture the experience of being a scientist in the modern era, and to fill the historical gap between the well-recorded penicillin and cephalosporin period and the present day.

On the archival side, the material gathered by Dr Sidebottom, which derives from four main sources, will be made available to researchers via four separate catalogues. The first describes a four-box personal archive, a collection of degree certificates and family photographs belonging to Peggy Pickles, who received her medical training in Oxford in the 1930s and 40s, and went on to work as a clinical pathologist in the Radcliffe Infirmary. She later married Alastair Robb-Smith who, in 1937, at the fairly astonishing age of 29, became the head of pathology at what is now the Nuffield Department of Medicine.

The next catalogue covers Gwyn Macfarlane's working papers for his 1985 biography Alexander Fleming, The Man and the Myth which, as the title suggests, went a long way towards correcting the record on Fleming's contributions to the development of penicillin. What makes this material particularly rich is the candid correspondence with people who helped develop penicillin or knew people who did, including Norman Heatley, Edward Abraham, and Dorothy Hodgkin.

Hodgkin was awarded the 1964 Nobel Prize in Chemistry for her work in identifying the molecular structure of organic compounds through X-ray crystallography, and high among her achievements was her work on penicillin. In 1945 she was able to confirm E.P. Abraham and Ernst Chain's hypothesis that penicillin had a β-lactam ring, which made synthesising the drug practically impossible. In a letter to Gwyn Macfarlane written on 26th December 1982, she neatly sums up her contribution: "As the x-ray analysis of a chemically unknown structure of some complexity, the penicillin analysis was something of a land mark and plotted essential stages which have been followed since" [MS. 12387/3].

The third catalogue lists administrative papers, and these will join an existing collection of the Dunn School's papers in the Oxford University Archives, which takes care of the administrative records of the University.

Figure 1. They don't make invoices like they used to. [Oxford University Archives PT 60/2].

There are intriguing stories to be found here, including early invoices (Figure 1) and personnel records with evocative notes like 'left to get married' and 'left for national service' or 'emigrated to Canada'. The papers also reveal a little-known fact about the Dunn School: it had in-house instrument makers. Stephen Bush, the Consulting Mechanic to the Dunn School from 1927 to 1952, enjoyed a slightly odd arrangement in which he took a salary and had the use of two workrooms and an office in the School, but was required to fit those out himself and to employ his own mechanics. His first responsibility was to fulfil the Dunn School's apparatus needs and to take care of its electrical and mechanical plant (as the Dunn School's workshop still does) but he was also free to tout for business, taking orders for items like clamps and syringes from institutions across the country, among them Liverpool's School of Tropical Medicine and Oxford's Radcliffe Infirmary.

The fourth catalogue covers the historical archive, which includes 16mm cine film, audio recordings, and thousands of photographs of staff and students. The archive ranges from a mid-century score book of the Dunn School cricket team (good record against Zoology; crushed by Dyson Perrins) to a Department of Pathology ink stamp. Notoriously, the Dunn School was so strapped for cash in the 1930s that then-professor Howard Florey had to ban the use of headed stationary as a money-saving measure, and all outgoing letters were stamped instead. It is pleasing to imagine that the exquisitely-tooled stamp in the collection is Florey's famous penny-pincher…but it seems more likely to have been the Dunn School library stamp.

Many of the cine films date back to Florey's era and can be grim viewing for the squeamish, with more than one featuring open and gangrenous war wounds in the process of being treated, all in glorious colour. But (look away modern ethicists) there's light relief too in the form of a little film parody of the MGM studio's famous roaring-lion logo, made with a yawning lab rat. The photographs, meanwhile, form a priceless record of the Dunn School's Heath Robinson-style penicillin factory, and can be paired productively with resources like a 1977 recording of a Norman Heatley lecture about penicillin.

The Dunn School archives will be available in the Weston Library, to get a reader's card please see https://www.bodleian.ox.ac.uk/using/getting-a-readers-card. The catalogues will be found online at http://www.bodley.ox.ac.uk/dept/scwmss/wmss/online/online.htm

Although seldom recognised for his pioneering work, Charles Fletcher is central to the story of penicillin, having been responsible for its first systemic use in patients. Here we reproduce an excerpt from Max Blythe's book 'Pioneering Physician: The Life of Charles Fletcher 1911-1995', with kind permission of the author, in which Charles Fletcher describes in an interview, his own involvement in these historical events. We are indebted to his son, Mark Fletcher, for allowing us to reproduce the pictures of his father.

The First Systemic Use of Penicillin: A Personal Account by Charles Fletcher

Figure 1. *Charles Fletcher (circa 1953)*

How I came to conduct the pilot clinical trials on penicillin is a remarkable example of being in the right place at the right time. I have had this kind of good fortune on several occasions. Sheer coincidence drew me into the penicillin story in January 1941. I knew little of the penicillin project until, on a mid-January day, I called at Witts' office with some paperwork and was invited in to meet Howard Florey, the project's leader.

The small team of scientists that Florey led at Oxford's Sir William Dunn School of Pathology had made its wartime quest the isolation of penicillin, the strongly antibiotic substance secreted by the mould *Penicillium notatum*, with hopes of it proving of massive therapeutic value. Wonderfully innovative and resourceful, his team had overcome major early difficulties in extracting penicillin (a highly unstable beta-lactam) from the culture medium of the mould into which it was secreted in miniscule quantities. Using scarce early supplies, the team had shown its amazing effectiveness in combating life-threatening streptococcal infection in mice. Since then the challenge had been in acquiring sufficient supplies of the antibiotic for clinical tests. For this, the laboratory had needed to transform into a factory culturing the mould in hundreds of bedpan-like vessels, biscuit tins originally, just to accumulate the necessary few grams of antibiotic for pilot testing on cases of severe streptococcal and staphylococcal infection.

The conversation between Witts and Florey that I interrupted was about getting these tests under way at the Radcliffe Infirmary. Witts had been asked to take this on. Knowing that I was short of a research project he quickly decided that I should. I had recently come to the end of my house job on his firm and was back as a Nuffield research student in his Department considering research options. "Here's your man", he told Florey. "Fletcher can do the testing." Becoming involved was as simple as that.

Witts cannot have envisaged the historic role that he was giving me. Not even Florey could have envisaged the full magnitude of what I was to reveal.

The first essential clinical test was that of proving that the penicillin being produced at the Sir William Dunn School was safe to give to patients. Side effects were a serious and feared possibility and could even have been fatal. Who to give it to was a major ethical dilemma. It was Florey and Witts who decided that the initial test should be on a patient with limited time to live who agreed to take the risk in the interests of medical research. I chose a woman (Elva Akers) who was dying of inoperable cancer, with only a month or so to live, and she agreed to be injected with the antibiotic. There were no ethical committees then and I needed no other permission.

Records show that on 17 January 1941 I gave her an injection of 100 milligrams of penicillin intravenously, while Witts and Florey looked on. Unfortunately, the antibiotic was not pure enough and she had a sharp rise in temperature and a rigor (a shivering attack) about an hour and a half later, showing that there were still pyrogens in the penicillin sample used. A rather unhappy Florey returned to his team at the Sir William Dunn School of Pathology to get more rigorous purification of the antibiotic under way.

With purer supplies produced, I set about testing the best way of administering penicillin. Giving it by mouth was soon ruled out. It did not survive passage through the stomach. Tests of intravenous, intramuscular and rectal routes showed intravenous injection to be the best way.

By then we were ready to treat a severe case of bacterial infection and early in February I found one of remarkable severity in the Hospital's septic ward. All major hospitals had septic wards then for victims of severe septicaemia (bacterial infection of the blood), many of them with grimly discharging abscesses. Mainly sufferers had dangerous staphylococcal infection causing great pain. Septic wards were places of misery.

Florey had advised that I treat the worst case that I could find and this was of severe staphylococcal and streptococcal infection in a 43-year-old Oxford policeman (Albert Alexander). The infection had begun in a small scratch on his face due to contact with a rose bush and had led to numerous facial abscesses. This was a time when such scratches could have fatal consequences. He had been on the septic ward for several weeks and treated with massive doses of the best anti-bacterial drug, sulphapyridine, without any effect. When I saw him on the 12th February 1941 his eye had recently been removed due to the spread of infection, which had now reached his right shoulder and lungs. I judged that he had only days to live.

I began giving him intravenous injections of penicillin without delay, beginning with a dose of 200 milligrams, the largest that I had given, based on Florey's calculation of an appropriate first injection for a man

Figure 2. *Charles Fletcher talking with Norman Heatley on the front steps of the Dunn School building. Heatley is widely credited with having solved the difficulties of purifying penicillin from culture medium and patients' urine.*

of his stature. This was followed by 100 milligram injections three hourly. The nearest I ever came to seeing a miracle was in witnessing this patient's dramatic improvement. After 24 hours he looked considerably better and was able to sit up and eat. By the fourth day he looked on the way to full recovery. Lazarus being raised from the grave could not have been much more impressive. But regrettably our penicillin supplies ran out on the fifth day and about a week later he relapsed and never recovered. Treatment had not completely eradicated the infection by the time it prematurely ended.

Supplies of penicillin were a critical limiting factor. The penicillin laboriously extracted from about a thousand litres of mould culture medium was only enough for a day's injections. Although Florey's team at the Sir William Dunn School supplemented the scarce supplies available by laboratory reclamation of every milligram of penicillin in the patient's urine each day, we ran out too soon. We all shared bitter disappointment at the patient's death, but elation also that penicillin had proved vastly more effective than we had dared to hope.

I have vivid memories of cycling from the Radcliffe Infirmary to the Sir William Dunn School of Pathology each evening with bottles of the patient's urine for penicillin extraction for re-use, and Florey and his colleague Ernst Chain anxiously asking how the patient was progressing. Florey, a laconic Australian, contained his delight well as penicillin revealed its colossal therapeutic potential. "That's good" he would comment, while Chain was excitedly jubilant, in his mid-European way.

Over the next four months (February-June 1941) I treated a further seven patients with penicillin. Most were children to whom I could give smaller doses of the antibiotic and so maximise the limited supplies that Florey's team laboriously produced.

The first child that I treated was a young teenage boy of 15 who was desperately ill with septicaemia resulting from an infected surgical hip wound (following pinning of the hip). Sulphonamides had not relieved his condition and I decided to test whether penicillin would. Again, the result was astounding. Within two days of starting the treatment (on 22 February 1941) he was feeling much better and remained so until the hip pin that had caused the infection was removed a month later.

Equally remarkable results followed the treatment of a man with a gigantic carbuncle on his back (from 3-10 May, 1941). We don't see carbuncles nowadays: clusters of boils usually with numerous pus-discharging heads, resulting from severe localised staphylococcal infection of the skin. This one was about four inches wide and causing great pain. Typically, recovery was expected to be slow, over at least a month of hospital care, with various dressings being applied, but I decided to test the effectiveness of penicillin and the difference was recovery in a week. Daily, one watched the carbuncle shrinking. The most appalling case that I treated with penicillin was of a boy of four who was dying of cavernous sinus thrombosis. I still remember the grim, cat-like cries that he was wailing. Infection that had begun locally on the face had spread to the cavernous sinus, a cavity behind the eye ringed by numerous thin-walled veins, and resulted in a blood clot there. Watching this small boy recover from a virtually fatal condition was among the most memorable moments of my career. This was in the second half of May (from 13-22 May 1941).

Tragically this boy died of a brain haemorrhage a week later, due to the rupturing of a weakened blood vessel. But from the post-mortem examination we had confirmation that the infection had been completely eradicated.

By then the amazing potential of penicillin was indisputable. I remember saying to Florey "Sir, it must be marvellous to have achieved a medical breakthrough like this" and his typical no-frills reply "Yes, it doesn't happen to everybody and I don't suppose I'll do anything like it again, but it is nice to have happened once in a lifetime". Unfortunately, the magnitude of penicillin's potential was not grasped by the Medical Research Council and leading drug firms in Britain, who largely resisted Florey's requests for help in speeding penicillin research and technology towards large-scale production. He ran into enormous myopia in trying to promote its development, with key figures in research-funding and heads of pharmaceutical companies unable to perceive the importance of penicillin to the war effort, particularly its capacity to heal war wounds and get troops back into action more swiftly than ever before. Sir Edward Mellanby, my father's successor as head of the Medical Research Council, has to be black-marked for his failure to recognise the importance of penicillin production. It was the great black spot on an otherwise illustrious career. The lack of British interest in penicillin production led to Florey seeking American collaborators and the antibiotic's first commercial production being in the USA, not Britain, with consequent colossal loss of revenue to this country.

In addition to the tests of penicillin that I have mentioned, I also treated a urinary tract infection in a baby and several cases (4) of acute eye infection at the Oxford eye hospital, all successfully. But by June 1941 penicillin production in Florey's department developed problems. Pressures of penicillin production had exhausted the team and supplies and my involvement ended.

I was dismayed and delighted to be named on the landmark penicillin research paper published by Florey and his team in the *Lancet* a short while afterwards. My part had been so minor that I questioned my inclusion, but Florey insisted on the publication being by the whole team: Abraham, Chain, Fletcher, Gardner, Heatley, Jennings, Florey. And later, through Florey's generosity, my name was included in the list of penicillin pioneers in the memorial garden established at Magdalen College, Oxford.

Max Blythe's book (ISBN: 978-1-909075-42-9) is available to buy online at www.wordsbydesign.co.uk at the recommended retail price of £15.

The Curious Tale of Cows, Quarantined Islands and Saddam's WMD

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Few people will remember Roy Vollum as I do. He arrived in Oxford as a Rhodes Scholar from Canada in 1921, the same year as Florey arrived from Australia, likewise a Rhodes Scholar. Vollum was my examiner in the Pathology and Bacteriology BM exam in 1961. In those days all candidates had practical exams and vivas and my own viva concentrated almost exclusively on *Rickettsia* and their diseases, not generally considered a major contributor to human infectious disease, and, I thought, rather below the belt as a major topic for a viva grilling. Vollum was also the senior bacteriologist in the Radcliffe Infirmary in 1965 when I was a Resident Pathologist starting my professional training. If we were presented with a CSF sample at night for suspected meningitis, we had to phone Vollum and he usually arrived in the lab, whatever the time, because he feared we novices might contaminate the sample. He was not an easy man!

THE TIMES TUESDAY AUGUST 9 2005

Saddam's germ war plot is traced back to one Oxford cow

By Dominic Kennedy

A BRITISH cow that died in an Oxfordshire field in 1937 has emerged as the source of Saddam Hussain's "weapons of mass destruction" programme that led to the Iraq war.

An ear from the cow was sent to an English laboratory, where scientists discovered anthrax spores that were later used in secret biological warfare tests by Winston Churchill.

The culture was sent to the United States, which exported samples to Iraq during Saddam's war against Iran in the 1980s. Inspectors have found that this batch of anthrax was the dictator's choice in his attempts to create biological weapons.

The discovery has angered some British politicians. Austin Mitchell, the Labour MP for Great Grimsby, has renewed his call, supported by 126 MPs in the last Parliament, for a UN investigation into whether Washington broke a weapons control agreement. "It just makes them look more hypocritical than ever," he said.

The odyssey of the Iraqi anthrax was unravelled by Geoffrey Holland, a politics student and antiwar campaigner at the University of Sussex. The exact batch chosen by Saddam was disclosed in the CIA report by Charles Duelfer, the former UN weapons inspector, last autumn.

"Iraq declared researching different strains of *B. anthracis* but settled on the American Type Culture Collection strain 14578 as the exclusive strain for use as a BW," Mr Duelfer said.

A congressional investigation into Gulf War syndrome by Don Riegle had already uncovered invoices showing that this batch was shipped from the United States between 1986 and 1988.

The ATCC is a private, non-profit-making collection of cultures of living micro-organisms, viruses, plants and human and animal cells, stored in Virginia.

Its catalogue shows that batch 14578 consists of "bovine anthrax", isolated by R. L. Vollum, a professor of bacteriology at Oxford University during the 1930s. It is named after him.

Martin Hugh-Jones, who co-ordinates the World Health Organisation's Working Group on Anthrax Research and Control, said: "We have traced

DEADLY SPORES

- Anthrax is caused by the bacterium *Bacillus anthracis*
- The spores can survive in soil for years
- Herbivores are most vulnerable. Humans get it from contaminated flesh
- The word comes from the Greek for "coal" because victims develop black skin lesions
- Contaminated mail was used to attack US Congress in 2001. Two postal workers died

Nevertheless, though not medically qualified, he was a highly-regarded bacteriologist and had an important role in planning the new building for the Sir William Dunn School of Pathology which was completed and opened in 1927. He became an important member of the teaching staff at both the Dunn School and the Radcliffe Infirmary from that time onwards.

In the late 1930's he received an ear from a cow that had died of Anthrax and the culture he isolated was submitted to The American Type Culture Collection in Virginia, America. It was catalogued as 14578 'Vollum'. In 1942 the British Government authorised biological weapons tests using this strain of Anthrax on Gruinard Island, off the Scottish coast. Sheep were infected and subsequently died, requiring the island to be quarantined for 48 years! However, more recently and rather more significantly, on 2nd May 1986 a sample of strain 14578 was shipped from the ATCC in Virginia to the Iraqi Higher Education Ministry and a CIA report on 30th September 2004 confirms that Anthrax strain 14578 was Saddam's exclusive choice for bioweapons research. Perhaps rather fortunately, Roy Vollum himself could never have known this story since he died in 1970, at the age of 70. Who could possibly imagine that the Dunn School played a role, however minor, in Saddam's ambitions for WMD?

Photograph courtesy of Paul Fairchild

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