FUSETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

ISSUE 18 · MICHAELMAS 2019

Structural Biology

Interview with

Richard Gardner

Focus on



Spotlight on Spin-Outs

Cell Fate Decisions in the Early Embryo

> A Brief History of Macrophages



Welcome

In the last edition of *Fusion*, I wrote about the celebration of penicillin we held when we were awarded a national blue plaque for the Dunn School building. This time I want to focus on the future. Changes are afoot in Oxford science and I believe these will place the Dunn School in an even stronger position globally. Important science is increasingly interdisciplinary, and I have been involved in long discussions about how Oxford should respond to this evolution, moving away from narrowly-defined subject areas. Two new initiatives promise to define a new ethos for Oxford research: the establishment of a new institute and of a brand new college.



At first glance, these projects are unlinked, but actually they represent a significant shift in Oxford's thinking. The vision of the new institute, the Dorothy Hodgkin Centre, is to integrate life and physical scientific approaches to reveal how cells work and the underlying mechanisms of disease. Unlike earlier developments in South Parks Road, this new institute, housed in a large, state-of-the-art building adjacent to the Department of Biochemistry, will not belong to a single department. Instead, outstanding people from many departments including the Dunn School, but also Chemistry, Physics, Biochemistry and others, will work collaboratively. The last few years have seen the convergence of new physical and biological techniques and this initiative provides an extraordinary opportunity to revolutionise our understanding of human disease.

The second new development, Parks College, has no official links with the Dorothy Hodgkin Centre, and was the product of different thought processes. Among other ambitions, it aims to provide more college associations for postdocs, many of whom do not currently have the opportunity to experience one of Oxford's defining characteristics. Strikingly, it is making interdisciplinarity the core of its mission, focussing initially on three broad themes: the environment, artificial intelligence, and cellular life. It will be located in the old Radcliffe Science Library at the end of South Parks Road. The relevant message here is that, like the Dorothy Hodgkin Centre, Parks College reflects the new Oxford philosophy of removing all boundaries between disciplines.

Neither one of these developments is uniquely associated with the Dunn School but both should have direct and indirect positive consequences for us. Perhaps most importantly, they will enhance the scientific environment in which we operate: the additional recruitment, the increased concentration of equipment and scientific facilities, the spirit of integration and collaboration - these will all help to ensure that the Dunn School continues to recruit the world's best at all levels, and do the best science. More college places will also be a direct benefit to members of the Dunn School, particularly postdocs and young faculty without current college links.

Focusing closer to home, I am excited to report that the Dunn School is planning a major recruitment drive over the next year. We will be in the unusual position of having three endowed senior professorships to fill, as well as substantial new space - as we recover a floor of OMPI that has for the last few years been lent to another department. It is rare to offer three statutory chairs simultaneously, and I look forward to writing to you about these substantial new appointments over the next year or two. This year we said goodbye to Professor Kevin Maloy, who was head-hunted by Glasgow University. We will miss him but I am pleased to announce that his replacement will be Dr Sumana Sanyal, who is currently a group leader and rising star at the University of Hong Kong. Sumana works on the cellular

response to viral infection and will be an excellent fit with many of our other groups.

Among many achievements by members of the Dunn School this year, I would like to single out the award of two international prizes to Tanmay Bharat, one of our early career group leaders (see opposite). Tanmay was the recipient of the Sir John Kendrew Award from the European Molecular Biology Laboratory, as well as being the only non-US recipient of a Vallee Scholar Award, recognising both his achievements and future promise, and accompanied by a major grant.

Finally, I am very pleased to report that the Dunn School has launched a new summer studentship programme for undergraduate students who want to gain experience in top labs. This programme, currently funding about ten students a year, aims not only to educate and inspire the next generation of biomedical scientists, but to extend the Dunn School 'family' even further, while identifying promising future DPhil students. The studentships are possible because of philanthropic support by the EPA Research Fund, to whom we owe much gratitude.

I always sign off by saying how much we enjoy staying in touch with the widespread family of Dunn School alumni and friends. Professor Keith Gull, who is about to retire after 20 years in the department (10 concurrently as Principal of St Edmund Hall) has offered to trace as many of our old members as possible, so please let him know if you are aware of people who may have been lost from our network. And of course, let me repeat the open invitation to all our friends and alumni: do stay in touch, and please consider visiting us to meet current members of the Dunn School, see how the department has changed, and learn more about our exciting future plans.

Contents

Welcome.	Matthew	Freeman2

NE\	NS
-----	----

The Truth about Antibiotics3
<i>Tanmay Bharat Celebrates a Year of Successes</i> 3
Greg Winter Delivers the 27 th Annual Norman Heatley Lecture4
Symposium in Honour of Keith Gull 4
<i>Gillian Helstrom Retires after 20 Years of Service</i>
In Memoriam: Satish Keshav5
Interview with Annemieke Kok7

IN CONVERSATION...

Celia Bungay and Ines Alvarez Rodrigo. A shared experience, 60 years apart 8 Interview with Sir Richard Gardner 8 SPOTLIGHT

STOLIGITI

A Short History of iotaSciences12

Junn School Biolmaging Facility Image
Awards 201814

RESEARCH INSIGHT

Dissecting Cell Fate Decisions in the Early Mammalian Embryo15

FOCUS ON STRUCTURAL BIOLOGY

A Closer Look at the Centrosc	ome:
Harnessing the Predictive Power	r of
Structural Studies	.19
Caught in the Act: Snapshots	<i>of</i>
Host-Pathogen Interactions	.21
ADP-Ribosylation: The Devil Rem	<i>ains</i>
in the Detail	.23

TECHNOLOGY FEATURE

The Power of Many: 'Super Resolution' Imaging Flow Cytometry......25

RETROSPECTIVES

A Life	e with	Mici	robe	S		
Keith	Gull.				 	27

A Brief History of Macrophage Research at the Dunn School30

HISTORICAL PERSPECTIVE

Unsung Heroes: The Dunn School Technical Staff through the Ages 33
<i>Celebrating the Dunn School</i> <i>Centenary</i> 35
From the Fusion Archives

News The Truth about Antibiotics



In January 2019, the BBC broadcast a science documentary series discussing everyday issues that affect us all, from sleep to getting fit and from healthy eating to the menopause, all presented by well-known celebrities. Angela Rippon is no stranger to the Dunn School having visited the department on numerous occasions in 2016 to build a case for penicillin as Britain's greatest invention, a case which ultimately persuaded the general public who overwhelmingly voted it the winner. However, this time Angela Rippon returned to uncover '*The Truth about Antibiotics*', briefly charting their initial development through the pioneering work of Florey, Chain and Heatley, before



investigating why so many common infectious agents are now becoming resistant to the antibiotics currently in use. Through discussions with experts in antimicrobial resistance, she assessed the risks of a return to a pre-antibiotic era, during which even the most trivial of infections could prove fatal. After interviewing Susan Lea, a group leader at the Dunn School, Angela Rippon's journey took her to the habitats of wild alligators and a rather less glamorous sewage works in search of new and innovative ways to fight bacterial infection. The fascination with antibiotics and their possible



demise was also top of the agenda for BBC Radio 4's *The Inquiry* which asked the question '*How did we mess up antibiotics?*' for which the Dunn School's Eric Sidebottom served as an expert witness. Whatever the future may hold for the treatment of infectious disease, the legacy of penicillin will undoubtedly continue to occupy an important place in the history of medicine.

Tanmay Bharat Celebrates a Year of Successes

For Tanmay Bharat, the 2018-19 academic year will surely stand out as a year noteworthy for its many successes. Tanmay joined the Dunn School in 2017 as a Group Leader and a Sir Henry Dale Fellow of the Royal Society and Wellcome Trust and has established a vibrant laboratory focussed on the dynamics of bacterial biofilms. His pioneering work using cryo-EM to investigate biofilm structure led to his being named a Vallee Scholar by the Bert L. and N. Kuggie Vallee Foundation which seeks to foster originality, creativity and leadership within biomedical research and medical education. As one of five Vallee Scholarships named in 2018, Tanmay was the only scientist from a non-US institution to be recognised with such an award. And as if such recognition were not sufficient endorsement of the quality of his research, Tanmay was subsequently selected to receive the European Molecular Biology Laboratory's (EMBL's) 2019 John Kendrew award. This

award honours the first Director of EMBL, Sir John Kendrew, by seeking to support promising young scientists in the early stages of their career who either worked or studied at EMBL in Heidelberg, a



criterion amply fulfilled by Tanmay who pursued his PhD in John Briggs' laboratory. Tanmay expressed his gratitude to those who had helped him achieve such recognition: 'I am grateful to our Head of Department, Matthew Freeman, for nominating me for these awards. Being at the Dunn School and in the spotlight has certainly helped!' The prize money from either award will help support Tanmay's ongoing programme of research.

Greg Winter Delivers the 27th Annual Norman Heatley Lecture



The Dunn School's success in attracting eminent speakers for the annual Norman Heatley lecture continued this year with the delivery of the 27th annual lecture by Sir Greg Winter who had been awarded the Nobel Prize for Chemistry earlier in the year. The University Museum of Natural History provided a strangely appropriate backdrop for this year's lecture: famed for hosting the most significant debate in the history of evolutionary thought between Huxley and Wilberforce in 1860, Sir Greg spoke to a capacity audience about harnessing evolutionary principles to create novel pharmaceutical drugs. He presented his work, conducted over

several decades in Cambridge, on phage-display technology for the design and generation of monoclonal antibodies, a technique that selects antibodies *in vitro* thereby by-passing the need for *in vivo* priming, clonal expansion of antigen-specific B cells and affinity maturation. Sir Greg reminded the audience that 6 of the top 10 drugs are currently monoclonal antibodies one of which, Humira, used for the treatment of rheumatoid arthritis, was responsible for revenues of \$16.1bn in 2016 alone. Illustrating the power of phage-display, he outlined how 60 phage antibodies are currently in clinical trials, suggesting that the technology will provide a pipeline of new drugs for many years to come. When guestioned by members of the audience about the philosophy that had driven him to succeed, he put it down to his 'total immersion in science' which, he admitted, would fall short of today's expectations for an optimal work-life balance!

Symposium in Honour of Keith Gull

On 22 March 2019, nearly 200 colleagues, former lab members, collaborators and friends gathered at the Dunn School to celebrate the career of Keith Gull. The scientific symposium programme reflected the breadth of Keith's pursuits over the years and wide-reaching influence, from his early work as a fungal biologist to his work on the cytoskeleton and many fundamental discoveries in the cell biology of parasites. Each of the speakers looked back on a particular stage of Keith's journey from London and Kent via Manchester to Oxford and Ghana. Stephen Beverley (USA) and Gloria Rudenko (London) started the day with talks on new discoveries in parasitology, touching on many productive interactions with Keith and his lab over the years. By the end of the first session we were reminded that with Keith in the room there is never a dull moment: Julius Lukes (Czech Republic) presented Keith with a preserved piece of tapeworm, which surely

must represent the highest token of appreciation from one parasitologist to another! Philippe Bastin (Paris) interspersed science with amusing and poignant anecdotes reminiscing about his own time as a postdoc in the Gull lab in Manchester, complete with photographic evidence. Doubtless, anyone who has ever presented their work at a Gull lab meeting would have connected with those memories. The success of so many ex-Gull lab members in running their own labs is testament to the lasting impact of this rigorous training as exemplified by the talks given by Sue Vaughan (Oxford) and Johanna Höög (Sweden). As the day progressed, the audience was treated to a broader scope of science, with talks from Iain Hagan (Manchester), Roy Quinlan (Durham), Andre Schneider (Switzerland) and Dick McIntosh (USA), all illuminating a different facet of Keith's ability to influence, challenge, inspire, and "make things happen". In the final

session Mike Ferguson (Dundee) looked to the future of parasitology and Gordon Awandare (Ghana) showed the impact of Keith's engagement in capacity-building in Africa. Finally, Neil Gow (Exeter) and Mick Tuite (Kent) returned to the early beginnings, sharing more anecdotes and photographs. The symposium was followed by a dinner and party at St Edmund Hall where Keith was Principal until his retirement in 2018. This memorable day was a moment to celebrate and say thank you for all that Keith has done for science and for so many people over 40+ years of tireless engagement. From what we have learned on that day and over the years, there is no doubt that Keith will remain an influential and inspirational figure for many years to come.

Eva Gluenz (Eva was a postdoc in Keith's lab from 2004-11 before setting up her own group in the Dunn School).



Gillian Helstrom Retires after 20 Years of Service

Gill Helstrom will be familiar to many members of the Dunn School, both past and present, through her long and faithful service to the department over a 20-year period. Gill joined the Dunn School in 1999 and worked as PA to Herman Waldmann, Head of Department at that time, until his retirement in 2013. Gill continued as PA to Matthew Freeman for a short period before being appointed to the position of Trust Administrator for the four trusts associated with the department - the EPA, Guy Newton, Cephalosporin and CIU Trusts - in which role she has overseen the award of grants to numerous groups to further the aims of the trusts in supporting basic research in the biomedical sciences and its downstream clinical translation. Gill plans to use her time in retirement on handicrafts and the varied demands of family and allotment. Her retirement leaves a significant void which has been amply filled by the appointment of Rachel Faulkes who moves to the Dunn School from Research Services, bringing with her a wealth of experience in the management of grants.



In Memoriam: Satish Keshav at the Dunn School 1987 - 1996

Siamon Gordon

Our dear friend and colleague Satish died tragically and unexpectedly on the 23 January, 2019, aged 56 (Figure 1). There has been an outpouring of grief in Oxford, South Africa, his country of origin, and from many colleagues and friends throughout the world who had been touched by his warmth and humility, as a gentle family man and dedicated clinician and scientist. In this appreciation, I would like to provide a few details of his life in South Africa, leading to his stay at the Dunn School, his doctoral research, and the making of his career as a gastro-enterologist. His medical colleagues have commemorated elsewhere his clinical skills and humanity.

I first met Satish at the Johannesburg airport, on my way back from a family visit to Cape Town. He had been elected a Rhodes Scholar and came highly recommended by Wieland Gevers, a prominent University of Cape Town medical scientist who had himself been a Rhodes Scholar at Balliol, where Satish was headed. He was eager to complete a DPhil as quickly as possible in order to return to South Africa. As fellow countrymen, we immediately established a rapport which lasted throughout his life. Our last meeting in 2018 was at the Manor Hospital in Oxford where we bumped into each other; by then, he was a consultant gastro-enterologist who had remained in England after falling in love with Oxford. We had a quick, lively discussion about Paneth cells and macrophages, which had become the other passion of his life since his student days at the Dunn School.

Early Life in South Africa

According to my main source, his younger sister Nita, the Keshav family originated from India. Their father was a teacher, then headmaster, spending two periods between 1967 and 1977 in rural Zambia. Satish, born near Johannesburg in 1962, and Nita had magical childhoods in Zambia enjoying Gujarati delicacies and walks in nature. A younger brother, Nilesh, was born during a stay in South Africa from 1972-74. Satish attended primary school



Figure 1. Satish Keshav 1962 – 2019

and Indian classes, excelling at both. He loved reading and made a name for himself through a newsletter, debating and chess. He was modest and unassuming even then, with a wicked sense of humour, becoming the best matriculant in the Transvaal. He chose to study medicine at the University of Witwatersrand and took a gap year to study for a BSc, the beginning of an interest in science. In 1986, he graduated head of his class in medicine, leading to the Rhodes award. He remained close to his family, with regular visits, especially after the murder in Johannesburg of Nilesh in 2007 and the death of his father a year later. According to Nita, Satish 'lived a principled life, serving selflessly in a state of grace', naturally caring, a devoted son, brother, husband to Camilla, his English wife, and as father of three English-born sons. Throughout his life he had a gift for friendship, forming close ties with fellow students. An early mentor during his medical studies was Professor Nerina Savage, a physiological biochemist, studying blood leukocytes of the baboon.

The Dunn School

Satish spent nine years in my macrophage research laboratory, first as a Rhodes Scholar, then as Staines and Nuffield research fellow at Exeter and Corpus Christi colleges, respectively. His thesis dealt with the *in situ* analysis of macrophage activation in the mouse by BCG, the tuberculosis vaccine, and the secretion of lysozyme and TNF α , important in adaptive immunity to infection¹ (Figure 2). It was then, while examining lysozyme expression in the uninfected small intestine that he had his eureka moment, falling in love with Paneth cells, specialized epithelial cells at the base of the crypts of



Figure 2. Staining of BCG granulomas in mouse liver with the macrophage-specific monoclonal antibody F4/80 (A). In situ hybridization for lysozyme¹ (B).

Lieberkühn, which contain secretory granules intensely labelled with an anti-sense probe for lysozyme^{2,3} (Figure 3). While famous for their role in innate immunity to bacterial infection in the gut, these cells also nurse epithelial stem cells in the intestine⁴. This epiphany induced a long lasting and successful career in fundamental and clinical gastroenterology. During the course of his further research, he demonstrated a secretory phospholipase in Paneth cells⁵ and contributed to the first description by Michael Stein, his compatriot and close friend, of an alternative, Th2-dependent, pathway of macrophage activation⁶. This pioneering paper is widely cited as the initial spark for numerous studies of M2-type polarization of macrophage effector functions.

Satish had broad intellectual interests, natural scientific curiosity, and enthusiasm for research. He was always helpful to others whilst being modest about his achievements and academic success. Michael Stein became a companion in and outside the laboratory, gracious about always losing to Satish at chess: together they established a long term relationship with Tom Schall, an American visiting biotech entrepreneur and expert on chemokines. Genevieve Milon, a visitor from the Pasteur Institute, was another close colleague. During long experiments in the lab, his sense of fun sustained morale, as reported by a fellow student, Matt Collin: 'Satish will always be remembered for his ability to find humour in any situation. When research was tough, he used to joke about performing the 'Great Macrophage Experiment'; it had no hypothesis and no aims, but it just existed in a Platonic sense as something that



Figure 3. In situ hybridization in the mouse small intestine. (A) $TNF\alpha$ (B) Control (C) Lysozyme²³.

would one day be realized and bring enlightenment to all the mysteries of biology. I am sad that we'll never get the chance now'.

Satish's stay in the lab coincided with the loosening of the grip of Apartheid in South Africa and the transition to a non-racial government. Although a natural opponent of Apartheid, he strongly believed in non-violence and the use of reason to change society. At times, he contemplated returning to South Africa, but this was not to be. Lynn Morris, a fellow student who did return to South Africa, sensed that 'this impulse died in Satish after the tragedy of his brother's murder, followed by his father's death'. This was reinforced by the needs of his wife and their three sons, Vijay, Ajay and Sanjay.

I end with a quote from one of his subsequent clinical colleagues, Alison Simmons: 'Re Satish, I would say he was a wonderful clinician, much loved by his colleagues and patients, who is sorely missed. His work on NOD2 directing control of anti-microbial peptides in Paneth cells was massively insightful and ahead of its time. His early work paved the way for further studies in this area internationally, which have cemented a role for Paneth cell signaling defects in driving inflammation in Crohn's (disease). It's possible the true potential of these observations has yet to be realized as therapeutics targeting this axis are currently beginning to be trialed'.

Acknowledgements

I would like to thank Nita Keshav for providing details of Satish's early life in South Africa.

References:

- 1. Keshav S et al. (1991) J Exp Med 174:1049-1058
- 2. Keshav S et al. (1990) J Exp Med 171:327-332
- Chung LP, Keshav S et al. (1998) Proc Natl Acad Sci USA. 85:6227-6231
- 4. Clevers HC, Bevins CL (2013) Annu Rev Physiol 75:289-311
- 5. Keshav S et al. (1997) Cell Prolif 30:369-383
- 6. Stein M, Keshav S et al. (1992) J Exp Med 176:287-292



Annemieke Kok is one of a rare breed of scientists whose interests span both creative arts and sciences. Annemeike discussed her interest in designing jewellery with Fusion and how her background in science has been instrumental in its success.

Interview with Annemieke Kok



How important is art to you and when did you first develop an interest in expressing your creativity in this way?

I never thought about myself as an artist (I can't draw well at all, and that seems to be a prerequisite for getting a foot on the ladder to becoming an artist), but *craft* has always been a big part of my life, from building souvenir cardboard model castles and windmills, to very elaborate St. Nicholas 'surprises' (gift packaging specific to the recipient, with an accompanying poem...). I also dabbled a bit in making jewellery, such as bead bracelets and earrings to coordinate with my outfits, but not so seriously that I had the proper tools and training, until I found myself feeling rather lonely as a postdoc in Dublin, and signing up for a 10-evening course at the local bead shop.

What form of art have you developed and how would you describe your work?

From there my interest in making jewellery really blossomed. At the end of my time in Ireland, I enrolled in a week-long summer course at the Irish National College for Art and Design, where I was definitely the least accomplished artist on the course. The tutor gave us free The piece and the report were then evaluated by external examiners. It was amazing: for the first time in my life I was taught proper art foundations (mark making, colour theory, elements of design etc), and a design process for generating ideas.

Has your training as a scientist prepared you for your artistic endeavours?

Definitely for the manual dexterity and the trust that muscle memory will build up with repetition. Also the patient, planned, organised way of working: the sequence of actions is of utmost importance for the success of each fabrication – you can't take shortcuts or skip a step. Mistakes are very hard to undo, often meaning you have to start again from scratch. In addition, some of the materials are just as scarce and precious as, for example, a blood sample from a very ill patient. Furthermore, skills can be taught, although artistic and scientific creativity and brilliance probably not...

Where do you find inspiration for your artwork?

Always in nature - very few of my pieces don't have that link. And in my childhood love of myths, legends and fairy tales.

What is your favourite piece of jewellery and what is the story behind it?

It is my Mermaid necklace, because it is a real multi-media piece, combining store-bought metal ribbon mesh, stitched-on pearls, beads, and charms, with my cast seahorse charm and my hammered mussel shell pendant (see Figure, top left). It was also made in, and in reference to, Ireland, land of fairies and legends, where I loved living so close to the sea, dreaming of selkies, mermaids and other mythical beings – how unscientific...!

reign, showing us some basic techniques, such as sawing shapes out of metal, sanding and polishing, and the dreaded soldering (luckily he was happy to help out with the tricky bits), and suggesting what sort of pieces we could make. I ended up making three rings, my first pendant (an ivy leaf shape with a garnet mounted on it, which looks like a ladybird; see Figure, bottom left), a cast silver seahorse charm, and my first 3D piece since sculpting clay in school: a silver hammer-shaped mussel pendant with mounted pearl.

Forward many years, to a period of unemployment here in the UK. The town where I lived had an excellent arts centre, where jewellery making was taught one evening a week. After doing a few freewheeling project-based courses, I was able to enrol on a City and Guilds certificate programme. This was a whole different kettle of fish: we were given a brief to design and fabricate a specific item of jewellery, using at least the specified techniques, and were required to plan, cost and document the entire design and manufacture process.



Celia Bungay completed her DPhil at the Dunn School in 1964. She recently returned to the department to meet Ines Alvarez Rodrigo, a current graduate student in the same laboratory where Celia had worked.

IN CONVERSATION...

Celia Bungay and Ines Alvarez Rodrigo discuss their shared experience as graduate students, 60 years apart

Heather Jeffery

Since the Sir William Dunn School of Pathology opened in 1927 there have been substantial changes, in terms of both infrastructure and life within its walls. Celia Bungay, a DPhil student at the Dunn School from 1958 to 1964, met with Ines Alvarez Rodrigo, a final-year graduate student, to discuss how life in the Dunn School has been transformed over the past 60 years.

On first sight of the lab where she had worked, Celia remarked upon the physical changes. Both Celia and Ines have worked in the original Dunn School building, known for its traditional red brick external walls and imposing wooden staircase. However, the interior has undergone rather dramatic changes, with the merging of two labs into one, where Ines now works. Two research groups still share the space so in spirit it's not changed all that much. Celia commented, 'things change and it is a long time ago, but being back here it doesn't seem it. Seeing those doors, you just travel back in time when you see something just as it was'.





Figure 1. A meeting of minds: Celia and Ines in the Raff lab where both have worked

Their topics of research are quite diverse, Celia having worked on bacteriology and virology with John Watkins and Gareth Gladstone, and Ines working with Jordan Raff on cell biology in the fruit fly *Drosophila*. However, both bacteriology and virology are still being pursued by other labs in the department, although, the methodology

has changed a lot, with lots of high-tech equipment having been developed in the interim. Within the labs, bench space is now mostly assigned to specific people or techniques rather than being multi-purpose as it was in the past. The concept of shared equipment facilities and the 'push for facility-managers' also appears to be new, with the current microscopy and flow cytometry facilities being unimaginable in the 1960s. In general, there seems to be a lot that can be taken for granted nowadays, for example, the ability to have 'fun' decorations on the lab walls would not have been allowed in Celia's time: scientific posters may have been permissible but definitely not Giant Microbe toys!

Fortunately, the working environment seems to have survived the test of time. Both Celia and Ines have enjoyed informal relationships with their supervisor, both having been able to approach them with problems, and having been invited to house events. Celia made lasting friendships, particularly with Gladstone's 'wonderful technician, Jimmy Smith, who I'm still in touch with and his wife', which bodes well for when Ines leaves the department. Some other things also seem to have persisted, not least among which are the long hours that can be associated with biomedical research.

The DPhil experience is not, however, identical to what it used to be. In addition to research, Celia was a 'Departmental Demonstrator' which meant she gave lectures (her first being on gonorrhoea) and ran practical classes. Despite, being 'warned to expect some fun from the boys' as a young female lecturer, Celia remarks that 'they were never a problem'. Demonstrating in practical classes now takes a rather different form: assisting with a group of practical sessions for a couple of hours at a time which is no longer a formal job title. The amount of preparation work for practical classes has also changed dramatically. If Celia had wanted to use antibodies for the students to use in practicals, 'I would immunise the rabbits' she recalls, whereas nowadays this would inevitably be out-sourced. This does mean, however, that there is no weekly delivery of 'two big sacks of rabbit droppings to put on the garden' she remarks! Alternatively, there are now many other opportunities to take science out of the lab, something Ines has embraced by being actively involved in public engagement events with local schools.



Figure 2. Celia and lnes beneath the photograph of Celia that still hangs on the wall of the main Dunn School building

One of the main differences that became evident in conversation was the size of the Dunn School, both in terms of buildings and number of people. Over time, the diversity of researchers has also increased, with many more nationalities now represented. Furthermore, while Celia had been the *only* female DPhil student, 62% of the students in the 2017 intake were female. Regarding socialising, the concept of going for coffee as a lab group has been preserved, however, the increased size has led to more organised social events including Christmas and summer parties. Also, the coffee is no longer consumed in the lab and there is now more communication between students and staff. Another major difference was in the approach to health and safety. Of course, lab coats have always been required, however, the use of gloves is a relatively new addition, although there was 'plenty of handwashing' Celia recalls, which comes as a big surprise as glove use is now very widely ingrained in laboratory work.

On a personal level, these two individuals are connected not only by their research at the Dunn School, but also their experience of becoming engaged during that time. Even this is period-dependent though, with Celia having changed her surname and become a 'Mrs', while Ines plans to retain the title 'Dr' and will keep her current surname. Regardless, for both, this adds extra excitement to their time at the Dunn School and remarkably Celia even recounts giving birth to her first child the day after submitting her DPhil thesis!

Both Celia and Ines come from non-science backgrounds and Celia was 'over the moon' to have the opportunity to study here. 'Being able to [perform research] as a female was superb' as 'not many, either male or female, did'. They have both enjoyed their research time in the department and found the Dunn School to be a supportive environment. As lnes approaches the time to write her thesis, she can be reassured by the fact she will not have to handwrite the entire document and ask someone to type it up for her!

For all DPhil students, Celia's advice is as applicable now as it would have been 60 years ago: 'Enjoy it and don't leave things to the last minute' and when discussing future paths, she advises that 'change is nearly always possible'.



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. Richard Gardner has had a long and varied association with the Dunn School, most recently as the Chair of Trustees of the Edward Penley Abraham Trust. Fusion caught up with him to find out more about his background and the philosophy of science that has underpinned his exceptional career.

Interview with Sir Richard Gardner



How did you come to work for six years in the Dunn School? I left Cambridge for Oxford in 1973 to take up a lectureship in the Department of Zoology. Henry Harris was a member of the panel that interviewed me for the post and he continued to take an interest in my career thereafter. I recall having a moan to him one day about how difficult I was finding it to maintain my very hands-on research in the face of departmental and college teaching and other commitments. He told me not to make any hasty decisions about moving on without consulting him. Then one day he summoned me to his office to ask whether I had any objection to his putting my name forward for the Henry Dale Research Professor of the Royal Society which Jim Gowans had relinquished to become Secretary of the MRC. To my astonishment I was offered this highly-prestigious professorship despite not being a Fellow of the Royal Society at the time. This appointment depended on my being offered suitable space and facilities for my research. Since Zoology could not make such commitment because it was still in the throes of finding a new Linacre Professor, Henry kindly invited me to join the Dunn School where laboratories were made available for me on the ground floor of the main building with its wonderful high ceilings and elegant brass fittings. One of the rooms I took over was occupied hitherto by Norman Heatley who with his typical kindness and generosity welcomed me with a wonderful gift, one of his precious 'penicillin bed-pans'.

What was the focus of your research during the years you spent in the Department?

There were two principal themes to my research during this period. The first was to extend clonal analysis of mouse development by transplanting single cells between blastocysts of different genotypes. Here the objectives were to elucidate cell lineage and commitment and, in collaboration with Mary Lyon at MRC Harwell, try to establish when X-chromosome inactivation occurred in female embryos. The second line of investigation that was carried out in conjunction with Martin Evans and other colleagues, was to study the behaviour of murine embryonal carcinoma (EC) cells following their introduction into blastocysts. We were able to show that such cells could, to a limited extent, participate in normal development despite the capacity of individual cells to yield highly malignant teratocarcinomas in histocompatible adult hosts. The findings stimulated Martin Evans and Matt Kaufman to isolate embryonic stem (ES) cells directly from blastocysts. I had the privilege of being the first person to inject such cells into blastocysts and thus witness their remarkable ability to participate in normal development.

In what capacity have you been associated with the department since moving to Zoology in 1986?

I returned to the Zoology/Experimental Psychology building in 1985 to serve as Honorary Director of a Developmental Biology Unit set up by the Imperial Cancer Research Fund (now Cancer Research UK) on the top floor of the building. There, studies were undertaken on Drosophila, Zebrafish, frogs, chicks and mice. However, I continued to collaborate with members of the Dunn School, notably Herman Waldmann and Paul Fairchild who were interested in my work with Frances Brooke in which we devised a way of obtaining ES cells in hitherto intractable strains of mice. Obtaining such cells from CBA/Ca inbred mice enabled the derivation of dendritic cells as an initial step towards trying to manipulate the immune response. I also continued to be engaged with the Dunn School in another way, namely as a Trustee of the Edward Penley Abraham Trust following Edwards death in 1999. I took over as Chair of the Trust When Dame Louise Johnson stood down in 2003. Holding this position has enabled me to gain an appreciation of the increasing scale and scope of research within the department.

In what ways has the Dunn School changed over the years?

When I joined the Dunn School in 1979 there was just a single building behind the main one that essentially housed Jim Gowan's Cellular Immunology Unit on its lower floor and Edward Abraham's Chemical Pathology laboratories on its upper one. Members of staff were encouraged to take tea in what was then the pathology museum in the main building, a tradition dating back to the days of Lord Florey, for which table cloths, tea pots and cups and saucers were provided. I recollect Edward Abraham, very much a gentleman in the best sense of the word, once wistfully observing that the ladies used to bake cakes for such teas, a hint that was received with barely disguised irritation by one of my female colleagues!

It was, however, easy to get to know most members of staff in those days and have at least some idea of the thrust of their research. Henry recruited a succession of distinguished retirees to help with the teaching of bacteriology and virology, among them Sir David Evans and John Tobin. They invariably provided most stimulating conversation. James Porterfield was the most senior member of staff actively engaged in basic research in microbiology.

The Dunn School was the first laboratory I worked in where the head of department not only knew where my laboratory was but also took an obvious interest in my research. Henry would not infrequently drop by for a chat before going home and invariably had interesting matters to discuss. For a head of department well into their 50s still to be heavily engaged in benchwork and to admit to being kept awake at night by the excitement of what they were doing was something that I had not encountered before.

The period in question was one with emphasis on studies at the cellular level, molecular approaches still being largely in their infancy. As the scope of molecular biology increased, larger research teams became necessary, and this was reflected in a very significant increase in the size of the Dunn School during Herman's subsequent tenure of the Chair of Pathology. This inevitably meant a change in character of the institution from the fairly close knit one with which I was acquainted.

What do you anticipate to be the challenges facing the Dunn School in the future?

I think the distinctiveness of the remit of different biological sciences is inevitably becoming blurred since much of the research undertaken in one such department could be considered equally appropriate for another. During my time in the Dunn School, some basic research into what could properly be termed traditional pathology was still current. Nowadays, while medical students still have to be taught bacteriology and virology, the research in these subjects from a medical perspective seems increasingly to take place in a clinical setting. Hence, a major challenge for the Dunn School is to retain its identity as a centre of excellence for research in what one might call abnormal physiology and development, whether extrinsic or intrinsic in origin.

What impact do you envisage Brexit having on British science? Are members of the Dunn School right to be concerned?

While finding certain aspects of the governance of the EU irritating, I am unable to think of a single respect in which British science will be better off outside it. The potential loss of the benefits we have enjoyed in terms of access to grants, collaborations and exchange of personnel should be of grave concern to all engaged in research.

How have the rewards and pressures of academic science changed during the course of your own career?

When I started in mammalian developmental biology the field was relatively small and one knew essentially all those involved, both

within the UK and abroad. One could freely present unpublished findings at meetings with little fear that others would take advantage of them. It was also the norm when contemplating a move into an area someone else was investigating to ensure that this did not conflict with their plans. Moreover, becoming conversant with the relevant literature was not too onerous a task. With time, the number of people working in this field, as in others, has expanded vastly so that competing for grants, keeping up with the literature and avoiding needless duplication of effort is becoming increasingly challenging.

What advice would you give to young scientists aspiring to be group leaders of the future?

The prime consideration in choosing a problem to research should be its intrinsic interest to you rather than its topicality or likelihood of securing funding. If what you are doing really fires you with enthusiasm you are more likely to manage inevitable setbacks or periods of stagnation with the determination necessary to achieve eventual success. It is also important for the success of research not to dwell excessively on matters relating to it. This depends on having a hobby that is sufficiently engaging to drive all other concerns from your mind. The great benefit of this is that it offers you the prospect of approaching problems with a fresh outlook. In my own case, I found painting in watercolours an ideal solution since, having to work from light to dark necessitates a degree of care in planning and execution that demands unremitting attention.

It is important to cite the original work of others in a fair and unbiassed way. Lord Florey recognised two categories of researchers, 'innovators' and 'colonisers'. Harold Varmus did likewise but used more colourful terms, namely 'tree shakers' and 'jelly makers'. It is not unknown for colonisers to try to promote themselves as innovators by grudging reference to work of the latter or by opting to cite secondary rather than primary sources. This is obviously a deplorable practice that seldom if ever succeeds in obscuring the truth. However, above all else, it is vital to enjoy what you are doing and to limit the size of your group to one that can be managed comfortably, particularly if you wish to continue being actively engaged in benchwork.



SPOTLIGHT

iotaSciences is spin-out company founded by Peter Cook, Alex Feuerborn, and Ed Walsh that is developing technologies for miniaturizing biomedical workflows. The name comes from the smallest letter in the Greek alphabet, representing an infinitesimal amount.

A Short History of iotaSciences

Peter Cook

iota's tag-line – '*shaping fluids'* – sounds silly, but it encapsulates exactly what iota's tech allows one to do: push, pull, and carve fluids.

In the late 1960s (before automatic pipets were invented), I was a graduate student in the Dunn School developing a micro-assay. So I asked an expert (Norman Heatley) about the assay for penicillin he had developed ~25 years earlier – an assay so micro that standard doses of antibiotics are measured in millions of units. He taught me to make constriction micro-pipets, and soon I was handling volumes down to a microliter. This is still the practical limit, which is extraordinary given how detection sensitivities have improved.

Fast forward to 2013. I was about to retire (after working mainly on chromosome folding and its effects on transcription) when I met Ed Walsh, an expert on fluid flows in jet engines and microfluidic 'chips'. I knew engineers built 'chips' for biologists, but I (and most others in the Dunn School) don't use them. Why? Reasons include: they are expensive, they require a PhD in plumbing to operate, and they aren't made with cell-friendly materials'. Ed wanted to do microfluidics in drops, and soon we (plus Alex Feuerborn – a post-doc in my lab, and now iota's CSO) were collaborating.



Figure 1.

In the typical Dunn School way, we began by taking more steps backwards than forwards, but the 'eureka' moment eventually came. In the everyday world, gravity is powerful, and water is always contained behind solid walls – otherwise it drains away (think of leaking dams and water pipes). In the micro-world, gravity is irrelevant and interfacial forces dominate; these raindrops (Figure 1) defy gravity, and are shaped by interfaces between grass, air, and water. We could escape from the tyranny of gravity and the need for solid walls by confining micro-volumes behind fluid interfaces!

How? 'Circuits' (Figure 2) are built much like writing freehand on paper (and take roughly the same time to make)². A 'pen' held by a



Figure 2.

robot 'prints' media in a desired pattern on a standard 6cm dish, an immiscible fluorocarbon (FC40) is then overlaid to prevent evaporation, and dyes are pipetted into drops to aid visualization. FC40 is a liquid relative of Teflon that is transparent, bio-inert (it has been used as a blood substitute), and permeable to O_2 plus CO_2 (so cells grow under it in CO_2 incubators). The aqueous phase is held in place by interfacial forces; it is confined by liquid walls of FC40! Conduits have heights down to ~10µm, and flows can be driven through them by external pumps (FC40 walls spontaneously seal around connecting tubes). However, flows are driven here (colored arrows) *without* pumps by exploiting properties of fluid walls; drops with small radii of curvature harbour higher Laplace pressures that automatically drive flow. In **A**, if there were cells in the 'sink', they would be exposed to 8 different dyes (drugs); in **B**, 'cells' in drops *a*, *b*, *c*, and *d* would see dye/drug 1, 1+2, 1-4, and 1-8, respectively)².





'GRIDs' (Figure 3) are made differently; now FC40 is put where wanted (and not media)³. The bottom of a dish is covered with media, FC40 overlaid, and a Teflon 'stylus' lowered to the dish; as FC40 wets stylus and dish better than media, FC40 runs down to the dish. Next, the stylus 'carves' lines that create FC40 walls between aqueous chambers. GRID **A** could be used in a combinatorial drug screen involving 16 drugs in all pairwise combinations³. Carving more lines creates smaller chambers; **B** has a chamber density ~1,000x that of a 96-well plate3!

GRIDs are used like microplates, except that one pipets through FC40, not air.

Cristian Soitu, a DPhil student, should be congratulated here. In February 2019, he introduced this technology to 5,500 conferees in Washington DC. His talk (out of 140 by scientists at all career stages) won the '*The SLAS Innovation Award*' for "*exceedingly innovative research*" with the citation: "*This transformative approach to manipulating small volumes eliminates the need for expensive equipment and expertise*"⁴.

iota's first product is the 'isoCell' printer (Figure 4). It made the circuits/GRIDs shown here, and adds/removes nanoliter volumes. It also won an award on its debut⁵, and has been used to miniaturize many workflows in cell biology (e.g., cell feeding, replating, cloning, cryo-preservation, lysis for RT-PCR, transfection + genome editing, fixation + immunolabeling)³.



Figure 4.

Fluid walls bring many advantages^{2,3}. They are incredibly strong yet flexible (difficult to believe until you think of water striders skimming over ponds). They morph above unchanging footprints when liquids are added/removed (Figure 5). They are built accurately and quickly from media, standard dishes or glass slides, and arguably the most bioinert immiscible liquid known (FC40). The resulting circuits/GRIDs fit seamlessly into current workflows because sterile 'hoods', microscopes, plate-readers, etc., already accept dishes/slides.



How was the company spun out? The University has established a pipeline to help. It owns the IP of its employees, and one of its departments – OUI (Oxford University Innovation) – files patents on their behalf. OUI provided early research funding, helped create (in 2016) a shell company (iota), granted exclusive use of founders' patents to iota, and introduced iota to OSI (Oxford Sciences Innovation) – a private company established by The University to bring its world-class research to the market. We then pitched to OSI, who subsequently led successful 'seed' and 'Series A' investment rounds in 2016 and 2018.

iota was born in what was the old library, but has now moved to the University's Science Park at Begbroke. iota wouldn't be alive today without help and support from top to bottom of the Dunn School – thanks to all. As some kind of recompense, I hope its technologies will soon be accelerating research in the department. I also hope Norman would be pleased.

Disclosure: Peter Cook owns shares in iotaSciences, and receives fees and royalties from iotaSciences and Oxford University Innovation.

References

- 1 Sackmann EK et al (2014) Nature 507:181-189
- 2 Walsh EJ et al (2017) Nat Commun 8:816
- 3 Soitu C et al (2018) Proc Natl Acad Sci USA 115:E5926-E5933
- 4 https://www.slas.org/media/news-center/2019-news-releases/ cristian-soitu-university-of-oxford-recognized-with-innovationaward-at-slas2019/
- 5 https://www.youtube.com/watch?v=QRbtu8R2J_A.



Dunn School Bioimaging Facility Image Awards 2018



Top, left.	Charlotte Melia (Bharat lab): Gross Fireworks. A false-coloured SEM image of a Pseudomonas aeruginosa biofilm.
Top, right.	Derek Xu (Baena lab): Cellular Nebula. Confocal image of a fluorescently labelled Drosophila wing disc.
Middle, left.	Andriko von Kügelgen (Bharat lab): Crystalised protein. Cryo-electron tomography and sub-tomogram averaging of
	crystalised protein filaments.
Middle, centre.	Ita Costello (Robertson and Bikoff labs): At the heart of it. A 3D projection of an early mouse embryo gut tube and
	developing heart.
Middle, right.	Sophia Fochler (Gluenz lab): Microtubule flower. Composite TEM image a Leishmania axoneme.
Bottom, left.	Flavia Moreira-Leite (Gull Lab): Screaming Leish. TEM image of a negatively-stained Leishmania parasite.
Bottom, centre	. Anna Caballe (Raff lab): Mexican wave. Fluorescently-labelled centrosome dividing in an early Drosophila embryo, imaged
	on a confocal microscope.
Bottom, right.	Sonia Muliyil and Clemence Levet (Freeman lab): Cookie monster. SEM image of an adult Drosophila fly.

RESEARCH INSIGHT

Dissecting Cell Fate Decisions in the Early Mammalian Embryo

Liz Robertson

Shortly before implantation, the early blastocyst stage mammalian embryo is comprised of three distinct, lineage committed cell sub-populations. The outer cell layer, the extra-embryonic trophectoderm, has an essential role in allowing the embryo to attach and invade into the maternal uterine environment. The so-called 'inner cell mass' contains progenitors of both the extra-embryonic primitive endoderm, destined to form the protective yolk sac, as well as the pluripotent epiblast, the cells that give rise to the embryo proper. Over the next few days, following implantation into the uterus, these three cell populations rapidly proliferate giving rise to the relatively simple radially symmetrical egg cylinder stage embryo. Subsequent expansion and diversification of these progenitor cell populations within the safety of the maternal environment drives the earliest phase of embryonic development, resulting in establishment of the basic body plan (Figure 1).

expression in the early epiblast signals to the adjacent primitive endoderm to induce formation of a specialized sub-set of cells, termed the AVE, that in turn instruct the adjacent epiblast to maintain an anterior identity. Nodal simultaneously activates genes in the overlying extra-embryonic ectoderm to promote posterior cell fates. These early studies, performed collaboratively with my close friend, the late Rosa Beddington, a highly talented embryologist (and incidentally an alumnus of the Dunn School who did her DPhil studies here in Richard Gardner's lab), revealed that the extra-embryonic tissues, discarded at birth, do not merely provide a supporting role for the developing fetus, but are also the source of essential instructive signals that pattern the early epiblast and trigger the process of gastrulation¹.



Figure 1. At the blastocyst stage (5.3 days of development) the embryo is composed of the trophectoderm, primitive endoderm and inner cell mass. After implantation by embryonic day 5, these three cell lineages expand to give rise to the extra-embryonic ectoderm (containing progenitors of the placenta), the endoderm of the yolk sac, and the epiblast which gives rise to the embryo proper. Some 4 days later (E9.5) the final body plan of the embryo is apparent.

My lab has exploited mouse genetics to investigate the signalling pathways and downstream transcriptional networks controlling temporally and spatially correct patterns of gene expression that direct cell fate decisions in the developing mammalian embryo. Some years ago, during a recessive screen for essential genes guiding early post-implantation development, we discovered that functional loss of the TGF β -related growth factor, termed Nodal, severely disrupts patterning of the early mouse embryo. Nodal

The onset of gastrulation is marked by the formation of the primitive streak, when cells on the prospective posterior epiblast undergo a process of epithelial to mesenchymal transition (EMT) in response to dose-dependent Nodal signals, to break the radial symmetry of the embryo. Nodal activity initially elicits the formation of mesodermal sub-sets including the cardiovascular cells that give rise to the heart. At later streak stages, prolonged Nodal signalling subsequently induces progenitors of the midline and gut endoderm.



Figure 2. Gastrulation establishes the three germ layers of the embryo. (A) The primitive streak is initiated on the prospective posterior side of the epiblast and is the site where mesoderm and then endoderm progenitors arise. Ectoderm is specified by the actions of the AVE. (B) Cell growth and morphogenesis that accompany gastrulation deploys these three sets of progenitors to establish the anterior-posterior axis of the early embryo. (C) Confocal image of a day 8.0 mouse embryo showing the emergence of the headfolds (the future neural tissue of the head), the gut endoderm cells in green on the ventral surface of the embryo, and the embryonic midline cells labelled in red, that separate the left and right sides of the body axis.

Cells on the opposite side of the epiblast in contact with the AVE are instructed to maintain anterior identity. These so-called ectoderm cells are destined to give rise to the neural tissue of the head. Gastrulation thus converts the epiblast into the three future "germ layers" of the embryo, namely the ectoderm (future brain and skin), endoderm (the gut and its associated organs including the liver and pancreas) and the mesoderm, which gives rise to all of the muscles, bones and numerous organs including the kidneys and reproductive system. Over the next few days, the 3D cellular template of the body plan becomes morphologically recognizable (Figure 2).

Nodal binds to cell surface receptor complexes that subsequently activate the closely-related downstream intracellular effectors Smad2 and Smad3 that translocate into the nucleus to regulate target gene expression. Smad2 and Smad3 expression domains mostly overlap at early embryonic stages. However Smad2 is uniquely expressed in the primitive endoderm. Consistent with this, Smad2 and Smad3 mutants display strikingly different phenotypes. Smad2 null embryos fail to induce the AVE, display early patterning defects and die at mid-gestation. By contrast, Smad3 mutants develop normally and adult homozygous mice are viable. Smad2 and Smad3 are functionally redundant, and elevated expression of Smad3 can rescue the Smad2 mutant phenotype². However, interestingly, as for Nodal mutants, Smad2/3 double mutant embryos entirely lack mesoderm, fail to gastrulate and die shortly after implantation.

To learn more about their underlying cellular and molecular defects, Anna Senft, a Wellcome Trust sponsored DPhil student, together with Ita Costello, a post-doctoral fellow, exploited double mutant embryonic stem cells (ESCs) lacking both Smad2 and Smad3 in combination with *in vitro* differentiation protocols. Using genome-wide ATAC-seq to monitor chromatin accessibility changes together with RNA-seq analysis, they were able to show that Nodal dependent Smad2/3 signalling is essential for epiblast cells to become 'lineage primed' prior to mesoderm and endoderm specification, explaining the profound early block in Nodal deficient embryos during gastrulation³.

We discovered a few years ago that the Tbox transcription factor Eomesodermin (Eomes) is a key immediate downstream target activated by Nodal/Smad signals in the early epiblast and extra-embryonic ectoderm. Eomes plays multiple essential roles during gastrulation including specification of both cardiac and gut endoderm progenitors⁴. *In vivo* in the context of a wild type embryo, Eomes mutant ESCs are incapable of contributing to the developing heart tube and gut tube. Likewise, during *in vitro* differentiation, Eomes mutant ESCs lack the ability to activate the cardiac programme and also fail to form endoderm progenitors.

Eomes-expressing cells in the early epiblast rapidly upregulate expression of Mesp1 and Mesp2, two closely-related and tightly-linked basic helix-loop-helix transcription factor genes, previously shown to act as master-regulators of cardiac development. Mesp1/2 double mutant embryos entirely lack the ability to form cardiomyocytes. Our chromatin-immunoprecipitation (ChIP) experiments demonstrated that Eomes directly binds to multiple T-box containing enhancers in the vicinity of the Mesp1/2 promoters⁴. Activation of Mesp1/2 expression by Eomes is necessary and sufficient for specification of cardiovascular progenitors. Claire Simon, our former DPhil student, recently used next-generation Capture-C, in collaboration with our colleague Doug Higgs at the WIMM, to show that the Eomes locus lies within a pre-formed 500 kb compartment of the genome, and is poised for activation via Smad2/3-dependent enhancer elements in response to Nodal signalling⁵.

We also characterised Eomes functional contributions at slightly later stages of gastrulation during endoderm specification. The Lim-homeodomain transcription factor Lhx1 is selectively expressed in progenitors of the midline and gut. In its absence, decreased numbers of endoderm cells display an abnormal cellular behaviour that results in defective formation of the midline and tissue



Figure 3. Blimp1 expression identifies the primordial germ cells. (A) At E6.0 the first PGCs form on the proximal side of the epiblast opposite the AVE, and can readily by identified by confocal imaging of embryos carrying a Blimp1Venus reporter. (B) Some 36 hours later the PGCs have proliferated to form a tight cluster of Venus+ cells at the base of the forming allantoic bud (which gives rise to the umbilical cord).

disturbances affecting establishment of the left/right body axis. Eomes directly binds to an enhancer region located immediately adjacent to the Lhx1 promoter to activate expression. Next, to learn more about Lhx1 functions, Ita used proteomic approaches to identify Lhx1 interacting proteins. She discovered that Lhx1 physically interacts with two other transcription factors called Foxa2 and Otx2, both of which were previously shown to have important roles during formation of both endoderm and midline cells⁶. These three DNA binding proteins likely play both unique and collaborative roles controlling the transcriptional output of many hundreds of down-stream target genes that co-ordinately govern endoderm specification, their migratory behaviour and subsequent patterning of the emerging gut tube.

We are also interested in understanding functional contributions made by the zinc-finger transcriptional repressor transcription factor Prdm1/Blimp, originally described as a master-regulator necessary and sufficient to drive plasma cell terminal differentiation. We originally decided to study Blimp1 because of its robust early expression in the primitive endoderm and AVE. Disappointingly, our experiments demonstrated that Blimp1 is dispensable for early axis formation but surprisingly we discovered that Blimp1 is essential for specification of primordial germ cells (PGCs), the highly specialised cell population that ultimately gives rise to the mature gametes, namely the eggs and sperm of the adult⁷.

Blimp1 expression is induced in response to signalling by the TGF β growth factor BMP4, within a few proximal posterior epiblast cells prior to formation of the primitive streak. These cells expand and cluster together at the base of the allantois during gastrulation before migrating along the hind gut to colonize the nascent gonads

(Figure 3). Blimp1 activity within these PGC precursors is thought to repress the somatic cell programme thereby insulating them from the Nodal signalling environment that would otherwise cause them to adopt a mesodermal cell fate. Very recently, Anna and Ita, examining the contributions made by different components of the TGF β signalling pathways, including Smad2 and the BMP-regulated Smad1 during PGC specification, were able to demonstrate that crosstalk between the Nodal and BMP4 signalling pathways co-ordinately regulates both the precise location and absolute numbers of PGCs in the early post-implantation embryo⁸.

We originally reported that Blimp1 null embryos die at mid-gestation due to defective placental morphogenesis⁷. Growth and survival of the early mammalian embryo within the maternal uterine environment is critically dependent on formation of this specialized organ which grows in concert with the embryo to support its growth. Arne Mould, a post-doctoral fellow, found that Blimp1 expression is required for the specification and differentiation of the so-called spiral artery trophoblasts that remodel the maternal arteries that invade the forming placenta thereby increasing blood flow necessary to promote efficient exchange between the maternal and fetal circulatory systems⁹ (Figure 4).

To learn more about Blimp1 contributions during maturation of functionally-distinct trophoblast cell types Andy Nelson, a post-doc, working together with Arne, performed the first single-cell RNA sequencing analysis of Blimp1 expressing cell populations at the maternal fetal interface¹⁰. Clinical studies have shown that defects in placentation in humans are associated with pathological defects including pre-eclampsia and intra-uterine growth restriction. Our experiments provide a better understanding of the formation of this uniquely mammalian and highly specialized organ, necessary to promote maternal-fetal exchange, provide an immunological barrier, and produce hormones that systemically influence maternal physiology during pregnancy.

Female mammals produce milk to feed their newborn offspring before teeth develop permitting the consumption of solid food. Intestinal enterocytes dramatically alter their biochemical signature during the suckling-to-weaning transition. Using a conditional inactivation strategy, we characterized Blimp1 requirements in the developing gut. Loss of Blimp1 expression in developing enterocytes has no noticeable effect before birth. However Blimp1 loss severely compromises post-natal survival. Transcriptional profiling experiments demonstrate that Blimp1 is required to repress the adult signature prior to birth, and its down-regulation at post-natal stages subsequently results in induction of the adult enterocyte programme necessary to digest solid food¹¹. Recent ChIP-seq experiments demonstrate that Blimp1 acting as a "gate-keeper", also prevents premature activation of the MHC class I pathway necessary to maintain tolerance in the neonatal intestine¹².

Our studies, focusing on two master regulators, the Tbox transcription factor Eomes and the zinc finger transcriptional



Figure 4. Blimp1 is required for formation and growth of the placenta. (A) Cross section through the developing placenta. Blimp1Venus expression (green) identifies the wave of invasi endovascular trophoblasts migrating into the uterine stroma. (B) In situ hybridization experiments show loss of Blimp1 (-/-) results in failure to specify the invading cells that are characterized by the expression of a set of distinctive marker genes (+/+). (C) Blimp1+ cells induced in the early extra-embryonic ectoderm preferentially give rise to terminally-differentiated trophoblast sub-sets that form the outer spongiotrophoblast compartment of the mature placenta.

repressor Blimp1/PRDM1, suggest that their diverse cell type specific functional activities probably reflect a high degree of selectivity during target site selection. Besides their DNA binding activities, both Eomes and Blimp1/PRDM1 contain additional structurally distinct domains likely to mediate functional partnerships. Indeed, it is well known that the ability of Blimp1/PRDM1 to recruit co-repressors such as LSD1, G9a, and HDAC 1/2/3, that cooperatively re-model the chromatin landscape, plays an important role during repression of target gene expression. We are just beginning to learn something about Eomes associations with its transcriptional partners including Smad effector proteins. Our longer term ambition is to better understand the 3D structural features that specifically enable Eomes and Blimp1/PRDM1 to regulate diverse transcriptional programmes controlling cell fate decisions during early mouse development.

Acknowledgements

I would like to thank the Wellcome Trust for their long term support of our research programme and those members of the lab whose work I have discussed.

References

- 1. Beddington R, Robertson E (1999) Cell 96:195-209
- 2. Dunn NR et al. (2005) Genes Dev 19:152-163
- 3. Senft AD et al. (2018) Cell Rep 24:1977-1985
- 4. Costello I et al. (2011) Nature Cell Biol 3:1084-1091
- 5. Simon CS et al. (2017) Development 144:1249-1260
- Costello I et al. (2015) Genes Dev 29:2108-2122 6.
- 7. Vincent SD et al. (2005) Development 132:1315-1325
- 8. Senft AD et al. (2019) Nature Commun 10:1089
- 9. Mould A et al. (2012) Genes Dev 26:2063-2074
- 10. Nelson A et al. (2016) Nature Commun 7:11414
- 11. Harper J et al. (2011) Proc Natl Acad Sci USA **108**:10585-10590
- 12. Mould A et al. (2015) PLoS Genet e1005375



Andv Nelson

Anna Senft

Arne Mold

Ita Costello

FOCUS ON STRUCTURAL BIOLOGY

A Closer Look at the Centrosome: Harnessing the Predictive Power of Structural Studies

Laura Hankins

Centrioles are cylindrical, nine-fold symmetric structures within all cells. These enigmatic organelles are often neglected in undergraduate lectures; however, they are of crucial importance for the organisation of centrosomes, which are formed when a pair of centrioles recruits a cloud of protein called the pericentriolar material (or PCM), surrounding themselves in this matrix. The PCM expands in preparation for mitosis, in a process known as centrosome maturation¹ (Figure 1).



Figure 1. Schematic illustration of the dramatic PCM expansion that occurs in preparation for mitosis. Cnn, Spd-2 and Polo are key players in this process.

These centrosomes are the major microtubule organising centres in animal cells. This means that they nucleate and coordinate microtubules (protein filaments that are formed by polymerising smaller units of a protein called 'tubulin' into long chains). The most familiar purpose of this activity is the segregation of genetic material into two daughter cells at the end of mitosis, or cell division. During this process, microtubules radiating from two centrosomes at opposite poles of the cell attach to the chromosomes that have lined up in the middle of the cell. Once all the chromosomes are correctly attached, the forces delivered by these microtubules, and motor proteins that move along them, pull the chromosomes apart, dragging precisely half the DNA into each nascent daughter cell. So how are centrosomes able to perform this feat? The answer lies somewhere deep in the PCM. This matrix of protein, which greedily expands on entry to mitosis, contains γ -tubulin ring complexes (γ -TuRCs). These protein complexes catalyse microtubule nucleation. However, it is unclear how these γ -TuRCs are brought to the PCM. Indeed, the even more fundamental question of how the PCM itself is recruited and expanded in mitosis is still under investigation. Members of Jordan Raff's lab are currently exploring the mechanism of mitotic PCM expansion using structural biology, an approach whose strong predictive power has been combined with *in vivo* studies in the early fly embryo.

Spd-2 and Cnn are key players in PCM expansion

Centrosomes were discovered as early as the nineteenth century, but the question of how the PCM assembles and then organises microtubules, remains unanswered. Amongst the hundreds of proteins that form part of the PCM, two stand out in the literature: Cnn and Spd-2. The latest in vivo work from our lab has shown that Spd-2 is recruited to the centriole, where it is phosphorylated². This modification allows Spd-2 to recruit a kinase called Polo to the centriole. Spd-2 also recruits Cnn. Once at the centriole, Cnn is phosphorylated by Polo and, in turn, is able to stabilise Spd-2^{2,3}. This results in amplification, with the stable Spd-2 recruiting even more Cnn. These proteins can then both expand: in flies, this process involves Cnn and Spd-2 being brought to the centriole and then fluxing outwards from the centriole to form a matrix. Spd-2 and Cnn are then able to act as a dynamic duo to recruit other PCM proteins. This recruitment is dramatically reduced if Cnn and Spd-2 are knocked out, demonstrating their crucial role in PCM expansion⁴.

The predictive power of structural studies

We therefore have a better handle on how these important PCM proteins reach the centriole, and the events that seem necessary for them to start forming a matrix. However, the lab was also interested in the interactions that are important for building this matrix from a structural perspective, since no direct molecular interactions had been demonstrated to explain how Cnn might interact in order to assemble into a matrix. Could we find important binding sites that allow them to come together? We had a hint from *in vivo* work that two domains within Cnn, known as LZ and CM2, might be involved in matrix formation. Taking a structural approach in collaboration with Susan Lea's lab, these two regions were purified *in vitro*⁵. This involved hijacking the protein production machinery of bacteria and using these single-celled organisms to pump out the fly proteins for us. When mixed together, two LZ and two CM2 domains come together to form



Figure 2 Structure of the LZ:CM2 tetramer.

what we call a 2:2 tetramer. To determine the structure of this tetramer, the team embarked on some crystallography. This involved mixing and concentrating the domains, before leaving them for three days to form crystals. These crystals were then subjected to an X-ray beam at the Diamond Light Source facility at the Harwell Science and Innovation Campus. Analysis of the diffraction patterns produced was used to model the structure.

Using this crystallography approach, the lab solved the structure of the tetramer, finding that it is composed of a helical dimer of LZ and a helical dimer of CM2 (Figure 2). This was where the predictive power of structural approaches came into play. Solving the structure allowed the group to predict which residues would, when disrupted, affect the interaction interface of the tetramer. To investigate the importance of these residues in vivo, the lab made use of the early embryo of the fruit fly Drosophila. A firm favourite of the Raff Lab, this model system is particularly advantageous for the study of centrosome biology since the embryo's centrosomes sit close to the cortex and hundreds can be imaged simultaneously. The group injected mRNA for Cnn-GFP that contained point mutations at these key residues. With confocal microscopy, they were able to observe the effects of these mutations on matrix assembly. Excitingly, mutagenesis of any one of three conserved amino acids in the CM2 interface perturbed matrix assembly, producing a similar phenotype to that of a complete CM2 deletion.

Similarly, mutation of predicted key residues in LZ produced a phenotype that was comparable to the LZ deletion mutant phenotype observed by the lab previously.

To be continued

This study is an example of how, through a combination of structural and *in vivo* work, the Raff and Lea Labs are gaining insight into the assembly of the mitotic PCM. The working model posits that Cnn forms dimers in the cytoplasm, but the LZ and CM2 interaction domains are not exposed. Phosphorylation at the centriole destabilises these Cnn dimers, allowing unwinding that permits interaction with other, compatriot dimers. Looking to the future, we have begun a collaboration with the Hyman Lab at the MPI-CBG in Dresden, who work on PCM assembly in the nematode worm *C. elegans*, where protein homologues of Spd-2, Cnn and Polo are also essential for mitotic centrosome assembly. Through this work, we hope to continue applying structural techniques to gain a deeper insight into the centrosome.

References

- Conduit PT, et al. (2015) Nature Rev Mol Cell Biol 16:611-624
- 2. Alvarez-Rodrigo IA, et al. (2018) bioRxiv 380907
- 3. Conduit PT, et al. (2014) Dev Cell 28:659-669
- 4. Conduit PT, et al. (2014) eLife **3**:e03399



Caught in the Act: Snapshots of Host-Pathogen Interactions

Katharina Braunger

A person in disguise. A distracted guard. An ominous syringe. Strike. The victim is dead.

What could be a scene from a thriller movie is constantly happening on a molecular scale when our bodies suffer from bacterial infections by pathogens such as *Salmonella* or enterohemorrhagic *E. coli*. The bacterial syringe used to inject toxins into the target cell is a multiprotein complex known as a type three secretion system (T3SS).

In total, nine different types of bacterial secretion systems have been described to date. Each has its own fascinating mechanism to increase the pathogenicity of the bacteria in which it is expressed. Not all of them comprise extensive extracellular structures, such as the large needles of the above-mentioned injectisome T3SS. However, all of them form pores in the bacterial membranes, delivering effector proteins from the cytoplasm, or in some cases the periplasm, to their site of action in the extracellular space or inside the eukaryotic target cell.

The human immune system has diverse strategies to detect and eliminate invading pathogens. One of the earliest barriers against infection is the complement system. Consisting of over thirty, mostly soluble proteins present in serum, it can sense the presence of pathogens early on. Complement activation triggers a proteolytic cascade which, within hours, results in a potent inflammatory response and formation of a lytic pore in the pathogen membrane. Consistent with the powerful consequences of its activation, dysregulation of the complement system is linked to severe diseases such as systemic lupus erythematosus, atypical haemolytic uremic syndrome, rheumatoid arthritis or age-related macular degeneration. Numerous pathogens have evolved mechanisms to 'sneak past the guard'. These include expression of inhibitory proteins that block the assembly or enzymatic function of complement factors but also of proteins that mimic eukaryotic regulators of the complement system and attenuate the host response. In the Lea lab, we use an integrative structural biology approach to investigate host-pathogen interactions. We characterise bacterial secretion systems, with a focus on type three (T3SS) and type nine (T9SS) secretion systems. Another priority of the lab lies in studying the activation of the human complement system and the identification of inhibitory molecules aiding pathogens' attempts to evade it.

The CirpT family – a novel class of complement inhibitors

The terminal steps of the complement cascade are initiated by the cleavage of the complement factor C5 into the potent anaphylatoxin C5a and the pore initiating C5b. Therefore, preventing binding of C5 to its convertase or inhibiting its cleavage is an effective pathogen evasion strategy. In addition, C5 is a promising target for therapeutic agents in complement-related diseases. For example, the complement inhibitor OmCI (marketed under the brand name CoversinTM) which has been functionally and structurally characterised by our group¹ is currently undergoing Phase III clinical trials for the treatment of the severe renal diseases Paroxysmal Nocturnal Hemoglobinuria and Atypical Uremic Syndome. Despite its pivotal role, the molecular mechanisms behind C5 activation remain poorly understood. In our most recent work, we have identified and characterised a novel family of C5 inhibitors from tick saliva, which we named the CirpT family². A 3.5Å cryo-electron microscopy (cryo-EM) structure of human C5 in complex with a member of the CirpT family, as well as the previously-characterised tick inhibitors OmCI and RaCI,



demonstrates that the CirpT family proteins occupy a novel C5 binding site, sterically blocking access to the convertase (Figure 1). Considering the molecular weight of the complex being just above 200kDa, the structure also exemplifies the tremendous developments in the field of cryo-EM over the past decade. Complexes of this size would have been considered far beyond the scope of the

Figure 1. Inhibiting the complement cascade at C5 level attenuates inflammation and prevents pathogen lysis. Our cryo-EM structure reveals simultaneous C5 binding of the inhibitors OmCl, RACI and the novel inhibitor CirpT to different surface areas demonstrating that multiple interacting surfaces play a crucial role for successful C5 conversion.

technique just a few years ago. By solving a crystal structure of CirpT1 with its C5 interaction domain, we could, furthermore, get a molecular understanding of the binding process. Taken together, our results contribute to a more comprehensive view of the C5 conversion process.

A new twist to T3SS

The T3SS not only comprise the above-mentioned injectisomes, but also exist as a second class which instead of a pointy needle, assembles the highly flexible bacterial flagellar machinery. In general, flagellar and injectisome T3SS share a common architecture. However, conflicting results have been reported regarding the stoichiometry of their export apparatus subunits. The export apparatus is part of the T3SS basal body, which traverses the bacterial inner and outer membranes. Structural and functional studies indicated a cup like export apparatus structure in the inner bacterial membrane, formed by five proteins, hereafter referred to as P, Q, R, A and B (according to the corresponding protein names FIIP, FIIQ, FIIR, FIhA, FIhB in the flagellar system). However, the arrangement of subunits and the functional mechanism of the export apparatus within the T3SS remained controversial. We isolated P, Q, R assemblies from a range of flagellar and injectisome systems³. Subjecting them to native mass spectrometry revealed variable amounts of Q, but more importantly, demonstrated a common core P5:R1 stoichiometry conserved across both classes of T3SS. Furthermore, we determined a 4.2Å cryo-EM structure of a P5:Q4:R1 export apparatus from *Salmonella* Typhimurium (Figure 2, left). It assembles into a pseudo-hexameric helical structure with a central pore, suggesting that the export apparatus could be crucial to accomplish the switch from circular symmetry in the T3SS translocation pores to the helical array observed in the needle or flagellar portions. Furthermore, based on our collaborators' crosslinking analysis and docking of our structure into a previously reported basal body reconstruction, we propose that the location of the export apparatus is not within the inner membrane plane. Instead, we suggest it is positioned in the periplasmic space in agreement with its proposed role of nucleating filament assembly.

T9SS - Two gates to a one-way street

Our latest study of bacterial secretion has focused on T9SS – the most recently-discovered class of bacterial secretion systems. They form an outer membrane translocon, capable of transporting fully-folded substrates of several hundred kilodaltons in size. To do so, they must form membrane pores of large diameters and devise



Figure 2. Left: Cryo-EM structures of flagellar and injectisome T3SS export gates reveal a common architecture and suggest how the export apparatus might serve as a switch from circular to helical symmetries. Right: The T9SS forms a large beta-barrel in the outer bacterial membrane which is gated by distinct accessory factors on the periplasmic and extracellular sides to maintain selectivity and prevent leakage.

mechanisms to prevent leakage from or into the cell while not engaged in translocation. However, until recently, only very limited structural data had been available to get mechanistic insights into T9SS architecture and function. We are collaborating with Ben Berks' group to isolate building blocks of T9SS and characterise them using cryo-EM. We succeeded in determining the first high resolution structure of the T9SS translocation pore in two different states in complex with distinct sets of accessory subunits⁴ (Figure 2, right). We identified two previously-unknown interacting factors and gained important insight into the translocation mechanism. Our data imply that T9SS employ alternate gating on the extracellular and periplasmic sides of the pore to maintain selectivity and prevent leakage.

From structure to function

Whether we study bacterial secretion or complement-related processes, our goal is to shed light on the underlying mechanisms at molecular detail. We achieve this by combining in-depth structural characterisation with biophysical and functional studies. Our results improve our understanding of microbial infections and complement related immunity, sometimes even contributing to the development of novel therapeutic options.

References

- 1. Roversi P, et al. (2007) J Mol Biol 369:784–793
- 2. Reichhardt MP, et al. (2019) bioRxiv 647354
- 3. Kuhlen L, et al. (2018) Nature Struct Mol Biol 25:583-590
- 4. Lauber F, et al. (2018) Nature 564:77-82

ADP-Ribosylation: The Devil Remains in the Detail

Antonio Ariza and Johannes G. M. Rack

All organisms are in constant contact with their environment, which forces them to adapt to either changes in their surroundings, for instance alterations of the ambient temperature or nutrient availability, or transitions between environments, as in the case of a microbial pathogen entering the body of a host. On the molecular level, one important and universally employed strategy in this adaptation process is the posttranslational modification (PTM) of proteins by covalent linkage of moieties or whole polypeptide chains to target residues. PTMs allow an organism to fine-tune or repurpose protein functions by, for example, altering their activities, localisation or interaction networks. Consequently, the function of PTMs can be conceived as expanding the limited genome encoded proteome – typically only a few thousand proteins – to millions of distinct protein forms.

Most PTMs are the result of enzymatic activity and are performed by proteins adding (writing) their modification onto target proteins after recognition of a specific sequence motif within a defined cellular context. Auto-modification of writers is hereby a common

arginine-link ether-link PARPS ARTCs polybranch sirtuins PARPs point Target Target Targe Protei rotein roteir ARHs PARG macrodomains (ARH3

Figure 1. Schematic summary of the ADP-ribosylation cycle. All known transferases can catalyse the initial mono-ADP-ribosylation, but vary in the preference for the chemical nature of the target residue (most commonly encountered modifications are depicted in the boxes). A subset of PARPs can extend the initial modification to form linear or branched polymers. PAR chains are degraded predominantly by PARG and, to a lesser degree, by ARH3. The terminal ADP-ribose moiety is removed by mono-specific hydrolases of the ARH or macrodomain families.

regulatory feature of PTM signalling pathways. Furthermore, PTMs are highly dynamic and precise control over the activity of the modified proteins is often achieved by enzymatic reversal (erasing) of the modification.

So far, over 200 different modifications have been identified and in Ivan Ahel's lab we are interested in the formation, regulatory function and removal of one particular PTM: ADP-ribosylation. It was first discovered in isolated nuclei in the early 1960's and we now know that it is an ancient and complex modification found in organisms from all kingdoms of life, as well as some viruses.

In contrast to other modifications, advances in the study of ADP-ribosylation developed slowly as progress was hampered by technical difficulties after the initial discovery. However, recent developments in mass spectrometry, non-radioactive detection, structural biology and others have greatly helped the development of the field. ADP-ribosylation entails the transfer of an ADP-ribose

> moiety from NAD⁺ onto a target protein combined with the release of nicotinamide. Several families of enzymes act as protein ADP-ribosyl transferases (writers), including poly ADP-ribosyl polymerases (PARPs), cholera toxin-like transferases (ARTCs), and sirtuins. The complexity of ADP-ribosylation arises from (1) the variety of different residues it can be attached to – modification of residues containing acidic (glutamate/aspartate), basic (arginine/lysine), hydroxyl (serine), and thiol (cysteine) moieties have been described – and (2) the fact that some writers such as PARP1, PARP2, and tankyrases (PARP5a/b) can extend the initial modification known as mono(ADP-ribosyl) (MAR) modifications and create linear or branched ADP-ribose polymers known as poly(ADP-ribosyl) (PAR) modifications (Figure 1).¹

In eukaryotes, ADP-ribosylation regulates a wide variety of processes including transcription, ageing, cell replication, the unfolded protein response (UPR), metabolism, immunity, development and cell death, but is probably best known for its involvement in the early DNA damage response (DDR). In the DDR, PARP1, a large multi-domain transferase, recognises single or double strand breaks via its zinc finger domains, which leads to a general compaction and context specific activation of the protein. Once activated, PARP1 is a prolific writer that catalyses the formation of PAR chains on itself and on proximal target proteins at serine sites within the vague KS-motif.² These polymer chains are recognised by a number of proteins (readers) and act as an initial recruitment scaffold to attract repair factors. However, equally important is the rapid turn-over by poly(ADP-ribosyl) glycohydrolase (PARG), as it was shown that PAR removal is required for an efficient assembly of the repair machinery.

The specific details of ADP-ribosyl signalling even in this context are still under investigation, but from a structure-function point of view two observations in this process are especially remarkable: firstly, biochemical investigations had long shown that PARP1 preferentially modifies acidic residues such as glutamate and aspartate in vitro. However, utilising novel mass spectrometric approaches we could show that in vivo DNA-induced ADP-ribosylation is nearly exclusively linked via the hydroxyl moiety of serine. Our group identified histone PARylation factor 1 (HPF1), an auxiliary PARP1 interactor, as crucial for this shift in target residue specificity, but its molecular mechanism remains elusive.^{3,4} Secondly, disassembly of the bulk of PAR modification is mediated by PARG, which removes ADPr units from PAR chains, one at a time, with the exception of the initial MAR modification, thus effectively converting one signal into another. Since PAR and MAR modifications have specific readers associated with them, it is still an open question whether this change of signal has functional consequences rather than being a short-lived intermediate. Whatever the answer, the complete reversal to the unmodified protein state requires a different set of hydrolases depending on the nature of the terminal ADP-ribose linkage to the target protein. Ester bonds found in modified acidic residues are cleaved by macrodomain-type hydrolases such as MacroD1, MacroD2 and TARG1. The ether bond found in the modified hydroxyl moiety of serine is lysed by (ADP-ribosyl)hydrolase 3 (ARH3). The N-glycolytic bond found in the modified basic residue arginine is in turn lysed by ARH1.1,5

The very fact that the specificity of MAR hydrolysis depends on the chemical nature of the protein-ADPr linkage allows for an additional layer of regulation, but also raises questions regarding the underlying mechanism(s). For example, PARG belongs to the family of macrodomains, but unlike the other macrodomains that cleave MAR ester bonds, it cleaves PAR ribose-ribose bonds, which are chemically closer to the ethers than the esters. Understanding the structural basis for these activities, including substrate recognition and turn-over, is of great importance not only to gain further insights into the details of ADP-ribosylation signalling, but also to devise strategies for the pharmacological manipulation of these enzymes for scientific study and ultimately disease therapy.

In the Ahel lab we use a multidisciplinary approach that brings together researchers from a variety of scientific backgrounds such as cell biology, biochemistry, genetics and structural biology. These highly complementary skills allow us to study ADP-ribosylation from different angles and answer scientific questions that a single discipline might struggle with. We use structural biology to reveal the 3-dimensional shape of the enzymes we study and to characterise the interaction architecture of the complexes we are interested in. This is often the only way to uncover the structural/chemical basis of their specificity (for substrates, interaction partners, inhibitors, etc), to gain sufficient detailed insights to elucidate their reaction mechanism and to support our search for inhibitory compounds.

The primary technique we employ in our lab is X-ray crystallography, which we use extensively to gain highly-detailed insights into our target proteins. However, crystallography comes with its own set of limitations and we utilise other structural methods such as NMR, SAXS and cryo-EM to overcome these. The latter in particular allows us to study molecules that due to their size, complexity or slight degree of structural variability are nearly impossible to examine near the atomic level using other techniques. It is a relatively quick and flexible technique, whose attractiveness is increasing as access to the required equipment has become easier and the resolution of the structures it produces are improving steadily.



Figure 2. Differences in ADP-ribose coordination amongst the hydrolases is an important aspect of their catalytic mechanisms. Upper panel: In PARG, ADP-ribose (green) adopts a strained conformation, which supports the acid mediated hydrolysis. Lower panel: In ARH3. ADP-ribose (green) adopts a relaxed conformation with the protein-ribose linkage tightly coordinated by the catalytic magnesium ions (cyan).

ovarian cancer in 2014. This is currently followed up in clinical trials for other cancer types with an associated low survival rate. In addition, inhibitor development for MAR and PAR hydrolases, most prominently PARG, has recently come into focus and, in this context, structural data from our research will allow us to carry out in-depth search for potentially new classes of PARG inhibitors.9

genetics, and cell biology has allowed us to successfully unravel the different substrate binding and catalytic mechanisms of enzymes such as PARG, TARG1, ARH1 and ARH3 (Figure 2).6,7,8 This information greatly improves our general understanding of how protein ADP-ribosylation is regulated and how defects in this process are linked to human disease.7 Crucially, we are also using structural studies to examine the basis for various inhibitor affinities to accelerate real world applications of our findings. Olaparib, a potent PARP1 inhibitor, was approved by the FDA for the treatment of BRCA-deficient breast and

Using structural biology

Here we have illustrated the importance of ADP-ribosylation in the DDR, but the range of cellular pathways regulated by ADP-ribosylation is vast. This, together with recent advances in technology leading to new insights, has resulted in our field of research experiencing a surge of interest in recent years – exemplified by an increase in the number of groups working in this particular area of research. Alongside challenges to understand the implications of ADP-ribosylation on a functional level, the journey into its atomic heart has just begun. Many aspects of the mechanistic regulation, target selection and organisation of complexes containing writers, readers and erasers still remain elusive. Finally, we are faced by the challenge of bringing together our findings on the atomic, molecular and cellular level to achieve a true understanding of ADP-ribosyl signalling.

References

- 1. Cohen MS & Chang P (2018) Nat Chem Biol 14:236-243
- 2. Leidecker O *et al.* (2016) *Mol Cell* **12**:998-1000
- 3. Gibbs-Seymour I et al. (2016) Mol Cell 62:432-442
- 4. Bonfiglio JJ et al. (2017) Mol Cell 65:932-940
- 5. O'Sullivan J et al (2019) Nat Commun 10:1182
- 6. Sharifi R et al. (2013) EMBO J 32:1225-1237
- 7. Slade D et al. (2011) Nature 477:616-620
- 8. Rack JGM et al. (2018) Cell Chem Biol 25:1533-1546
- Min W & Wang ZQ (2009) Front Biosci (Landmark Ed.) 14:1619-1626

Further Reading

1. Brosey CA et al. (2017) Methods Enzymol 592:417-455

TECHNOLOGY FEATURE The Power of Many: 'Super Resolution' Imaging Flow Cytometry

Stephen Cobbold

Imaging Flow Cytometry combines many of the advantages of conventional flow cytometry with those of single cell imaging. It is unique, however, in being able to provide an unbiased and quantitative analysis of the features that define the representative or 'average' cell image and to identify and exclude any outlier images. Although the resolution of individual images is limited by optical diffraction, we can use interpolation during the 'averaging' process to estimate distances between features of interest to provide 'super resolution' imaging flow cytometry (SR-IFC) for certain co-localisation applications.

Imaging flow cytometry is available in the Dunn School Flow Cytometry Suite as a fully specified ImageStream (Amnis/Merck) instrument, an overview of which was given by Michel Maj in the last issue of Fusion¹. It is similar in principle to a conventional analytical flow cytometer but rather than using detectors (PMTs) it has highly sensitive cameras to capture images (at up to 5,000 images per second) in a maximum of 10 independent fluorescent channels. Dedicated image analysis software (IDEAS) can then extract and quantitate many thousands of parameters or features that describe and characterise each individual image. The IDEAS software can then perform statistical analysis across all the features for every image within each sample, present them as histograms and scatter plots, or export them for further analysis such as the "Super Resolution" method discussed here. In comparison, although conventional flow cytometry also rapidly acquires data on many thousands of cells, it usually only measures a single intensity value for each fluorescence channel and provides only limited information about the size and shape of the cell using light scatter.

Limitations of conventional and confocal imaging for co-localisation studies

One important application for cell imaging is identifying whether two different stains are co-localised within the image. This might be to determine whether two different proteins are both located within the same cellular location, structure or organelle. It can also be used to determine whether fluorescently labelled pathogens, particles or metabolic precursors are taken up and internalised and to indicate where they distribute within the cell. Such studies have usually relied on conventional or confocal fluorescent imaging of a small number of selected individual cells that the observer considers to be a representative sample. This sampling can be problematic as the observer has no statistical information about the variability within the population on which to base this selection and will likely choose images with the 'clearest' fluorescence or the 'best images' for the markers of interest, which may not be truly representative. The techniques used to improve the resolution of confocal microscopy also mean that fluorescence intensity measurements are rarely quantitative. Some of the more recent super-resolution imaging technologies also image only very restricted parts of the cell, and can take a very long time to acquire, potentially exacerbating sampling and fading issues. Imaging flow cytometry overcomes these issues by very rapidly acquiring large numbers of images for statistical analysis, and by using the extended depth of field (EDF) option, each image is of the whole cell in a single plane of focus, to provide accurate quantitation.



Figure 1.

Exploiting 'the power of many' to achieve super-resolution Let's illustrate the principle of SR-IFC with an example². Suppose we have two different monoclonal antibodies (mAbs) against different forms of the intracellular kinase Lck and wish to determine whether they stain primarily at the plasma membrane and co-localise with CD45. We perform two colour immunofluorescence labelling (plus DAPI for the nucleus) and acquire many thousands of images on the ImageStream. We can first select a representative subset of images (Figure 1) within each sample by gating on single, live cells forming a tight cluster of double positive cells in a fluorescence intensity scatter plot. In the 3-colour overlays shown, pixels where Lck (in green) and CD45 (in red) potentially co-localise show up as yellow. On visual inspection, it is clear that the distribution of all stains both within and between images is very heterogeneous and that pixilation is limiting the resolution, but perhaps there is a hint of more red pixels present at the surface in some of the images stained with Lck-mAb1 compared to more yellow pixels in Lck-mAb2. Preliminary analysis in IDEAS confirmed that red and green pixels

were indeed more highly correlated across all the Lck-mAb2 images but what we really wanted to know was what proportions of the stains were co-localised specifically at the cell surface in the 'average' image.

To do this we needed to reduce the complexity of our fluorescence intensity measurements (ie. many cells, each in 3 dimensions) down to a single, representative measurement in 1 dimension for each feature of interest. We can do this by stripping off a series of concentric, single pixel layer masks from each cell image and measuring the total fluorescence intensities within each mask, and then taking the median values at each distance (0.3µm per pixel) from the cell surface (in

this case using the bright field channel) across all the images. We can then plot these relative intensities against distance and fit two (or more) component dispersion curves to these data to interpolate peak heights and positions and to test whether the two curves are significantly different

(Figure 2). From this analysis, it is clear that Lck-1 peak staining (red line) is separated from the peak of CD45 where we would expect the actual position of the plasma membrane to be (blue line). In contrast, in the Lck-2 sample, the red and blue lines are completely superimposed. The Lck-1



stain also has two peak components – one at 0.44+/-0.08 μm inside the CD45 peak - and a smaller component (about 26% of

the Lck-1 stain) distributed across the rest of the image, probably in the cytoplasm.

We can then illustrate the clear differences between the two samples by using the 1D measurements to reconstruct the 2D 'average' image at a 'super-resolution' of $\sim 0.1 \mu m$. These data confirm that, like CD45, the Lck protein as stained by mAb2 is mostly distributed at the plasma membrane, but does it really co-localise with CD45? We can go one step further in our analysis by interpolating the proportion of red and green pixels that are correlated across the radial projection. Integrating across these plots gives the total proportion of stain that is correlated with CD45 and further shows that this correlation peaks only at the cell membrane for both mAbs (Figure 3). This suggests that a proportion (16.7%) of mAb1 genuinely stains the Lck associated with CD45 in the membrane, compared to 45% for mAb2, but that mAb1 also stains something else – perhaps immature Lck protein in the ER or a cross reaction with another



Figure 2.

protein (Fyn has been suggested). The example used here demonstrates the analytical power of SR-IFC, but a similar approach could be used for many other applications, two obvious examples including how fluorescently-labelled pathogens or metabolites are taken up and distributed within cells and their organelles, and where signalling proteins are phosphorylated in the immune synapse.

References

Maj M (2018) *Fusion* **17**:22
Cobbold S (2019) In preparation

RETROSPECTIVES

After almost 40 years of faithful service, Keith Gull retired from science earlier this year but took time to reflect on his very successful career with its many twists and turns...

A Life with Microbes

Keith Gull

Early Days

It's not clear where my interest with microbes started. Certainly, it was nurtured by an interest in natural history and focussed by the influence of teachers. In the brave new world of the 1960s it seemed old-fashioned to just study biology, zoology or botany and a few Universities (not including Oxford!) had diversified their degrees to include specialisations such as microbiology. I left North Yorkshire for the University of London and the world of microbes – as well as, the 'swinging sixties'.

I veered towards an interest in eukaryotic microbes and after my undergraduate degree stayed on to do a PhD on the mode of action of an antifungal antibiotic, griseofulvin. Although widely used for dermatophytic infections griseofulvin's mode of action was unclear. It produced bizarre morphological effects on the fungal hyphae. So, although I used much biochemistry it was the use of combined electron and fluorescence microscopy that led to my discovery that griseofulvin inhibited mitosis by affecting the polymerisation and functioning of microtubules. This discovery set the scene for the rest of my career! Science and careers in science are not always about defined pathways; chance plays an important role.

A lab of my own!

I had started planning a move either to Oxford to take up a post-doctoral position in the Botany Department, next door to the Dunn School, or to the USA – but in the early third year of my PhD a few mentors suggested that it was not too unrealistic to apply for lectureships in a set of new UK Universities established during the expansion of UK higher education. Hence, missing the post-doc stage, I moved straight from my PhD to a brand-new Biological Sciences Laboratory at the University of Kent. The first year involved setting up the electron microscope unit and getting a lab established, in addition to teaching. The department was focussed – only biochemists and microbiologists – and interactions and collaborations soon emerged. We developed methods for purifying microbial tubulin and the *in vitro* polymerisation of microtubules. This enabled us to demonstrate differences between the tubulin of microbes, plants and worms, and that of mammalian cells. Basic studies of microtubule biochemistry were revealing both the target and selective action of a group of agriculturally-important anthelmintics, herbicides and fungicides.

Purifying tiny amounts of tubulin from microbes for these studies was demanding. To maximise our chances, we moved from filamentous fungi to two other microbes – the slime mould *Physarum polycephalum* and the protozoan *Trypanosoma brucei* (Figure 1). In



either case, we studied the tubulin biochemistry, effect of inhibitors and the cell biology of the diversity of microtubule organelles. *Physarum* is a fascinating model system that played a central role in the understanding of the cell cycle in eukaryotes. The plasmodial form is a syncytium – a macroscopic, multinucleated single cell. Indeed, in one

Figure 1. Trypanosoma brucei

terrific experiment performed when I was on sabbatical in the USA we studied periodic tubulin synthesis and mitotic spindle construction in a 10 hour cell cycle by taking "pizza slices" of a plasmodium grown to over 30cm in diameter (Figure 2) – I suspect a world record for a single cell experiment! By the mid-1980s we had a wonderful understanding of the multiple tubulin genes, post-translational modifications and microtubules in both Physarum and trypanosomes. However, *Physarum* proved impossible to transform effectively, meaning that the community studying this model organism was unable to use reverse genetics for functional studies. I regretfully closed that aspect of the lab's work with the knowledge that molecular genetic interrogation of the microtubule cytoskeleton was increasingly key to understanding function. The work on trypanosomes continued apace however, moving from studies of tubulin biochemistry to cell cycle control, cellular morphogenesis, shape determination and the construction of the flagellum.



Figure 2. Plasmodium 'pizza'

Sabbaticals and moving institutions

In 1989, I moved to the University of Manchester having spent a long, very productive period in Kent. Importantly, in that period I had taken two sabbaticals. The first was at the long-term research institute set up in Vienna by the Swiss pharmaceutical company Sandoz under the leadership of Jurgen Drews. This institute's approach to leadership and management to facilitate innovative research, discovery and translational science was extremely influential. I often say that it was during this time that I learned 'how to stop doing things' by observing how commercial research projects were managed. Similarly, my second sabbatical to Bill Dove's lab in the McArdle Laboratory in Madison, Wisconsin was massively influential, socially, intellectually and scientifically. One of the major benefits of an academic career is the opportunity to up-sticks and work in another country for a year on sabbatical. Yes, it means an upheaval for the family and oneself but the benefits for all are immense. I find it slightly depressing that young scientists frequently do not take this opportunity, or if they do they stay in their own institution. Having time to observe, think, discuss and do in a new environment is hugely beneficial throughout the rest of one's career.

A widening interest in trypanosomes

Throughout the 1990s in Manchester I was Head of Department of Biochemistry, the founding Research Dean of a large School of Biological Sciences and deeply involved in building an integrated life sciences school. The trypanosome work expanded. The microtubule cytoskeleton was still core but a greater focus developed on cell cycle controls, the flagellum and on how cell differentiations, associated with life cycle transitions, were achieved. A number of key advances were made in each of these themes. Epsilon and zeta tubulin were discovered, differentiation processes associated with infection and transmission defined, evolutionarily different cell cycle controls and a cytotactic mechanism involved in organelle inheritance demonstrated, amongst other insights. In the new environment in Manchester, I made a determined effort to see if we could use reverse genetics to study cytoskeletal function. However, although knocking out genes (thanks to excellent homologous recombination) was feasible I felt this was not going to be that useful since many cytoskeletal functions were likely to be essential - hence no mutant survival! One possibility was to use the concept of anti-sense RNA with an emerging inducible expression system in trypanosomes. Maybe this combination might produce a regulatable mutant phenotype? The

target was the paraflagellar rod - a massive structure of unknown function in the flagellum lying alongside the axoneme. My conjecture was that this was likely to have a function; however, since it was not present in all trypanosomatids it might not be essential. Putting the experimental intervention and target together seemed like a good approach to developing a technology for functional analysis in trypanosomes. Much work went into this without success until we noticed one transfection that was clearly different. The trypanosomes were immotile! Given the target, this seemed a logical phenotype. Perhaps, once again, a demonstration of Pasteur's comment at the Faculté de Sciences in Lille in 1854 about chance and the prepared mind.

But why this single clone from many experiments? Further analysis showed that the anti-sense RNA construct in this clone had been illegitimately inserted into the PFR target gene array (Figure 3). We repeated this targeting and reproduced the mutant phenotype easily showing that it worked on both loci in the diploid and so was not just a transcriptional block etc. We sent the paper to *Nature* defining a method for creating mutants in trypanosomes exemplified by the demonstration that the PFR was essential for motility. It was accepted without the genomic explanation, which we left in as a



comment that genome integration of the anti-sense construct (ie overlapping transcription on both strands) was critical and suggested some form of anti-sense interference with early processing. Two weeks later, the first RNA interference paper was published in Nature and it was clear that the phenomenon we had described, and also one that collaborators in Yale had discovered involving

Figure 3.

down-regulation of tubulin expression, were occurring through RNAi. These studies opened up a set of heritable and inducible RNAi approaches that have been incredibly useful in understanding trypanosome biology and pathogenicity.

The Dunn School

Although very well supported in Manchester, I felt one should move when ceasing leadership positions. My move to the Dunn School was facilitated by the award of a Wellcome Trust Principal Fellowship. I was initially encouraged by Jeff Errington and warmly supported by Siamon Gordon and Herman Waldmann.

The Dunn School's staff were superb and we were doing science within days. Two important studies came to fruition around this period. First, how the mono-allelic expression of the Variable Surface Antigen genes (over a 1000 VSG genes, many telomeric expression sites but only one active) was orchestrated in cell biological terms. We discovered a particular nuclear structure, the Expression Site Body which was associated with the Pol I-dependent transcription of the active expression site. Second, we provided a proteomic analysis of the trypanosome flagellum showing specialisations but also components deeply conserved through evolution to man.

Defining further components of the cytoskeleton was going to take yet more sophisticated Mass Spec proteomics. Neil Barclay and I worked together to develop funding that established excellent facilities in the Dunn School. This technology opened up novel avenues that have continued to provide component analysis of structures and organelles including the definition of the unusual kinetochores of the trypanosome spindle, the flagellum tip, basal body (the equivalent of the mammalian centriole) and flagellum/cell body filament systems controlling cell form and shape of the trypanosome.

We realised in the early 1990s that any experimental system without a sequenced genome was going to wither in experimental opportunity. Maintaining an effective study of protozoan pathogens meant becoming an advocate for their genome projects! This took a large amount of time on both sides of the Atlantic. All the excellent proteomics we were developing would be disabled if there was no high-quality genome information. We wrote the first grant to sequence a chromosome and, with more advocacy, the rest of the genome followed and was published in 2005. Looking back, this was a critical phase in the development of biological sciences - during that 10 year period, few would have prophesised that today we would be sequencing a trypanosome genome in the lab in days! The study of microbes has been at the forefront of the genome revolution, giving an evolutionary context as well as being important in their individual contexts. A high-quality annotated genome has perhaps been one of the most enabling tools in the study of pathogenic microbes.

A Dunn School colleague, Siamon Gordon, was influential in changing the direction of some of our science. Around 2007, he asked if I would write a chapter for a book that was reviewing macrophage-pathogen interactions. I felt I had little to contribute but, on reflection, agreed, so long as I could write on some unappreciated biology and open questions in the field. I focussed on the *Leishmania* parasite, which replicates in macrophages, and reflected on the sad lack of understanding of the cell biology of these organisms. Encouraging students and post-docs in the lab to tackle some of these outstanding issues led to many of them establishing their own labs working on *Leishmania* which have subsequently provided an understanding of cell differentiation, infection and motility in this interesting pathogen.

Throughout these scientific journeys with microbes I have had a long-standing interest in graduate education and the careers of young scientists in the UK and Africa. When I came to the Dunn School it was a pleasure to work with Anton van de Merwe to develop graduate education here, nearly two decades ago. In addition, my lab members and colleagues from around world have been excellent partners in assisting some very talented African scientists to develop a centre of excellence at the University of Ghana for the study of pathogens. I benefitted in my early career from a sense that people were willing to invest in youth and provide what I would call 'the freedom to succeed'. Putting something back into science and creating opportunities for young scientists seems only natural.

In this reflection, I have purposely not mentioned individual members of my lab. There have been very many graduate students, post-docs, undergraduates and even pre-University students who have contributed to some very successful science in an interesting and hugely collegiate community. I thank them all – their stories are legend! The Dunn School has been a very supportive and enjoyable home over almost 20 years. It is a remarkable collection of people and I want to thank all staff and Faculty for their friendship and support. Excellent departments do not come about by accident. I am very conscious of the amount of commitment needed, and for this I want to thank all Dunn School colleagues, Siamon Gordon for his help with my move here and continuing interest, Herman Waldmann for his leadership and Matthew Freeman for continuing to guide the Dunn School's rather special place in biomedical research.



Photograph Paul Fairchild. Those heady days of summer...

A Brief History of Macrophage Research at the Dunn School

Siamon Gordon and Annette Plüddemann

In 1965, I attended a lecture at the Rockefeller University in New York by Henry Harris, head of the Sir William Dunn School of Pathology, during which he described his dramatic studies with John Watkins on Sendai virus-induced fusion of different somatic cell types to form homo-and heterokaryons. One fusion combination was of macrophages with Hela cells. This experimental tour de force inspired me, a South African medical scientist, to undertake a doctoral research project under the direction of Zanvil Cohn, a macrophage guru. We planned to fuse primary mouse peritoneal macrophages with a melanoma cancer cell in order to gain insight into somatic cell differentiation and malignancy. This lead to studies on the fate of macrophage-specific phenotypic markers such as FcR, an immune phagocytic receptor, and lysozyme secretion, both extinguished by fusion with a different and malignant cell type. This training took me to an independent investigator position at the Dunn School from 1976 to 2008, and as Emeritus up until the present time. Investigation of macrophage-restricted gene expression using hybridoma technology guided much of my research group's studies, with discovery of differentiation antigens such as F4/80, implicated in immunologic tolerance, and a related adhesion G-protein coupled receptor (GPCR), EMR2, expressed on human myeloid leukocytes. Other valuable antibodies lead to the identification of Siglec1, a lectin-like receptor for sialylated glycoconjugates on haematopoietic cells, and Dectin-1, a glucan-specific phagocytic receptor for zymosan yeast particles and fungi (Figure 1). Towards the end of my group's research at the Dunn School, we identified a surface molecule, CD36, that was involved in macrophage IL-4-induced homokaryon formation and discovered novel phagocytic functions for multinucleated macrophage giant cells, compared with unfused cells in the same population (Figure 2).



Figure 2. IL-4-induced macrophage giant cell (photograph courtesy of S. Heinsbroek).

Throughout this period, members of my lab, some of whom are pictured (Figure 3), examined the role of these and other surface glycoproteins in various macrophage functions; for example, using specific antibodies for the collagenous scavenger receptors SRA and MARCO, we studied cell adhesion and migration, receptor-mediated phagocytosis of apoptotic and other dying host cells, endocytosis, and secretion of trophic growth factors and host defence products such as lysozyme, neutral proteinases, cytokines and reactive oxygen and nitrogen metabolites. Using mouse tissue macrophages and human blood monocyte-derived macrophages, we studied macrophage

interactions with a range of bacteria, viruses, fungi and parasites both in vivo and in vitro. Selective alteration of mannose receptor expression by cytokines of Th1 and Th2-type immunity was the clue to polarised gene expression signatures by interferon- γ (IFN- γ) and IL-4/IL-13 cytokines in classical and alternative activation of macrophages respectively. Gene expression studies revealed that transglutaminase 2, a crosslinking enzyme, was the most consistent marker of alternative macrophage activation in asthma. The induction of MARCO expression by Neisseria meningitidis, BCG and zymosan particles, enhanced phagocytic clearance on subsequent challenge; this represented an adaptive form of innate immunological "memory", which was antigen-dependent, but non-specific compared with lymphoid cellular immunity, as already described by George Mackaness, an alumnus of the Dunn School (Figure 4).



Figure 1. Cell surface antigens studied in the macrophage lab between 1976 and 2008 (see Taylor PR et al. (2005) Annu Rev Immunol 23:901-944).



Figure 3. Selected members of the Gordon lab together with Hugh Perry, one of our collaborators on microglia.

The F4/80 monoclonal antibody and F4/80-deficient mice, made it possible to map the presence and distribution of macrophages during embryonic development and throughout adult life of the mouse, in health and disease models, and, in combination with other surface antigens. This enabled us to illustrate their phenotypic heterogeneity and adaptation to different tissue microenvironments such as brain, gut, lung, endocrine and lympho-haematopoietic organs, in resting and various physiologic and pathologic states (Figure 5). These studies provide the foundation for a mouse and human macrophage tissue atlas based on gene expression analysis. In sum, the macrophages of the body represent a substantial dispersed and plastic organ, sensing and responding to physiologic and pathologic changes in their local and systemic environment, thus preserving homeostasis. strategy, funded by royalties from anti-macrophage antibodies, continues after more than 20 years.

Other macrophage studies at the Dunn School – then and now Howard Florey and his associates Robert Ebert, George Mackaness and Vincent Marchesi, as well as Jim Gowans and Alvin Volkman, provided important insights into monocyte migration (Figure 7), recruitment, ultrastructure, differentiation into tissue macrophages and immune interactions with BCG. Irv Weissman, also from the Gowans laboratory, has recently developed a strategy to treat lymphoid malignancies by enhancing phagocytic clearance of tumour cells, based on blocking 'don't eat me' signals, such as CD47.

Henry Harris, after his return to the Department, used macrophages to demonstrate that the majority of newly synthesised RNA molecules

When HIV and Mycobacterium tuberculosis brought about a catastrophic co-pandemic in South Africa, diseases in which macrophages play a major role, I felt obliged to contribute to study of their pathogenesis. In the absence of anti-retrovirals at the time, and the lack of a vaccine, I undertook a 'social vaccination' project of education together with Frances Balkwill, an immunologist, and Mic Rolph, a graphic artist, to produce and distribute a cartoon booklet, You, Me and HIV (Figure 6), with the help of a publisher at Cold Spring Harbour, John Inglis and a social worker, Linzi Rabinowitz, in South Africa. This prevention



Figure 5. Schematic showing components of the mononuclear phagocyte system (see Gordon S, Plüddemann A (2017) BMC Biol 15:53)



Figure 6. AIDS educational booklet written by Fran Balkwill (pictured), illustrated by Mic Rolph and published by John Inglis, Cold Spring Harbor Laboratory Press.

turned over within the nucleus and did not enter the cytoplasm. Although his theories were highly controversial at the time, he was ultimately proven to be correct. Gordon MacPherson and his group made important contributions to the early studies of dendritic cells (DC), related to, but distinct from macrophages. He demonstrated their migration into cannulated lymphatic vessels and activation of T lymphocytes in the rat. This contributed to the early acceptance in Europe of the then controversial claim by Ralph Steinman and Zanvil Cohn that DC were uniquely potent antigen-presenting cells for naïve T and B lymphocytes. MacPherson's work was in the Dunn School experimental tradition of combining *in vivo* manipulation with

microscopy. Alan Williams and Neil Barclay developed the OX antibody series to define rat leukocyte differentiation antigens, including several expressed on macrophages, CD45, CD200 and SIRP α , a receptor for CD47. Anton Van der Merwe's work on the immunological synapse is relevant to our understanding of the phagocytic synapse. Macrophage research at the Dunn School continues to benefit from the work on tolerance by Herman Waldmann, Steve Cobbold and Paul Fairchild.



Figure 7. Monocyte migrating through vascular endothelium during inflammatory recruitment (Marchesi and Florey. General Pathology Edited by Howard Florey 1970, 4th Edition).

David Greaves has established his own research group at the Dunn School to study the role of macrophages in inflammation and its resolution. Their interests include chemokines, antimicrobial peptides, foam cell formation and atherosclerosis. In earlier studies of transcription, he developed a CD68 promoter construct for macrophage-specific transgenesis in mice. William James, Sally Cowley and colleagues use iPSC technology to produce mouse and human macrophage cell lines with defined phenotypes, to study microglia in neuropathogenesis and complement longstanding interests in macrophage-viral interactions. Among other aspects of host immunity to HIV, Quentin Sattentau continues to investigate viral entry into macrophages. The Freeman and Robertson/Bikoff groups are beginning to exploit macrophages in their research into cell biology and development, respectively.

Conclusions

The macrophage is one of the best characterised cell types in the body, spanning a wide range of vertebrates and invertebrates, including humans and model organisms such as Drosophila, zebra fish and mice. As a distributed organ, these mononuclear phagocytes play an important role not only in innate and adaptive immunity, but increasingly, in development, organogenesis, physiology and pathology. They participate in trophic functions and repair, as well as host defence, in the recognition, clearance and disposal of damaged cells and molecules. In addition to their specialised functions, they provide a valuable resource for experimental cell and molecular biology, as primary cells from individual subjects, in situ and in cell culture. They are actively biosynthetic, and sensitive methods are now available for their genetic and pharmacological manipulation. They are present in all organs of the body and perform tissue-specific as well as generic functions, many of which remain to be discovered. Macrophage research will therefore always find a place in laboratories at the Dunn School.

Further Information

Readers may like to refer to the following articles in previous editions of *Fusion*:

Gordon MacPherson: Michaelmas 2008; Jim Gowans: Trinity 2005 and Michaelmas 2016; Henry Harris: Michaelmas 2015; Irving Weissman: Michaelmas 2016 (reflection on Jim Gowans); David Greaves: Michaelmas 2006; Quentin Sattentau: Trinity 2004 Readers may also like to listen to podcasts from Siamon Gordon, Gordon MacPherson and David Greaves available at the following link:http://podcasts.ox.ac.uk/series/sir-william-dunn-school-pathologyoral-histories

Bibliography

The following represents a selection of key publications from the Dunn School that have greatly influenced the field of macrophage biology:

- Austyn JM, Gordon S (1981) F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur J Immunol 11:805-15
- Brown GD, Gordon S (2001) *Immune recognition*. A new receptor for beta-glucans. *Nature* **413**:36-37
- Crocker PR *et al.* (1991) Adhesion receptors involved in the erythroblastic island. *Blood Cells* **17**:83-91
- Gordon S, Plüddemann A (2018) Macrophage Clearance of Apoptotic Cells: A Critical Assessment. Front Immunol 9:127
- Harris H et al. (1966) Artificial heterokaryons of animal cells from different species. J Cell Sci 1:1-30
- Harris H (2013) History: Non-coding RNA foreseen 48 years ago. Nature 497:188
- Hughes DA *et al.* (1995) Murine macrophage scavenger receptor: in vivo expression and function as receptor for macrophage adhesion in lymphoid and non-lymphoid organs. *Eur J Immunol* 25:466-473
- Lawson LJ et al. (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151-170

- Lin HH et al. (2005) The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. J Exp Med 201:1615-1625
- Martinez FO, Gordon S *et al.* (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000 *Prime Rep 6*:13
- Martinez FO et al. (2013) Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. Blood 121:e57-69
- Martínez-Pomares L *et al.* (1996) Fc chimeric protein containing the cysteine-rich domain of the murine mannose receptor binds to macrophages from splenic marginal zone and lymph node subcapsular sinus and to germinal centers. *J Exp Med* **184**:1927-1937
- Milde R *et al.* (2015) Multinucleated Giant Cells are specialized for complement-mediated phagocytosis and large target destruction. Cell Rep **13**:1937-1948

- Morris L et al. (1991) Macrophages in haemopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. Development 112:517-526
- Plüddemann A *et al.* (2009) SR-A, MARCO and TLRs differentially recognise selected surface proteins from Neisseria meningitidis: an example of fine specificity in microbial ligand recognition by innate immune receptors. *J Innate Immun* 1:153-63
- Rosen H *et al.* (1989) Exacerbation of murine listeriosis by a monoclonal antibody specific for the type 3 complement receptor of myelomonocytic cells. Absence of monocytes at infective foci allows *Listeria* to multiply in nonphagocytic cells. *J Exp Med* **170**:27-37
- Stein M *et al.* (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* **176**:287-292

HISTORICAL PERSPECTIVE

Unsung Heroes: The Dunn School Technical Staff through the Ages

Eric Sidebottom



Edwin Wheal

Georges Dreyer) until his untimely death in 1934. By then he had been rewarded by the University with an honorary MA degree (1926).

School, where he worked for Prof

One of my own technicians for many years, Steven Clarke, wrote an interesting piece in *Fusion* 11 (2012) on his retirement after 41 years in the Dunn School. *With no particular place to go* describes how he arrived for an interview for a technician's post in the Dunn School uncertain as to whether he actually wanted to be a technician. But he was offered the post, accepted it, thrived, engaged in further higher education, eventually achieving a DPhil himself, and ending his career by managing the animal facility at the Dunn School. It would be interesting to know how many Dunn School technicians have risen to be research scientists themselves.

More recently Simon Hunt wrote in *Fusion* 13, 'None of us in the Dunn School could have accomplished anything significant without the superb dedication of the technical experts. The roll-call of those who helped me is lengthy. Incomplete as it is, it must include Wendy Brownsill, Stan Buckingham, Mike Coates, Francis Cooper, Betty Dix, Don Etheridge, Marj Fowler, John Kirchoff, the two Strouds (Harry and Pete) — and above all, Jim Kent, the doyen. In those pre-kit days, the Dunn School animal house, with its graceful balcony, housed goats, chickens, rabbits, guinea-pigs in addition to rodents, all of which he would immunise for us to prepare the necessary antibodies'.

In the meantime *Fusion* has carried several interesting insights into the significance of our technical staff. One of my favourites is the article by Alvin Volkman in *Fusion* 8, in which he describes his first encounter with Nat Smith (Dame Maggie Smith's father):

'As I approached, I noticed a very elegant looking man in his late fifties or early sixties at the bottom of the steps. The white lab coat he wore was heavily starched, thoroughly pressed and exceptionally white. He was about to ascend the steps but paused when he noticed me crossing South Parks Road. As I drew near he smiled and extended his right hand in a cordial manner. "Ah," he said, "You must be new here. I am Nathaniel Smith. Welcome!"

As we shook hands I noticed that under his lab coat, Nat Smith, as I came to know him, wore a waistcoat spanned by a gold watch chain; his trousers were pressed to a knife-edge; a tasteful silk tie and a crisply starched collar topped his white shirt. Smith had an engaging

smile, and an impressive shock of straight white hair brushed backwards. To my surprise he invited me to join him for a cup of tea in his office and I accepted. He presided over tea while telling me about his work. He said that he had assisted the late A. Q. Wells in studies on the acid-fast Vole bacillus as a possible basis for a vaccine against human tuberculosis. Smith's name appears on several of Wells' publications. Wells had worked on this problem for a number of years but eventually vaccines prepared elsewhere from BCG became the choice internationally. Wells had died a few years earlier and Professor Florey had let Smith stay on.

Smith paused before pouring a second cup of tea; spout poised a few inches above the rim of my cup and asked, "Is my daughter, Margaret...Maggie, well-regarded in America?" I was lost for a moment. Why on earth should I know anything about his daughter, I asked myself. Maggie Smith? Then the penny dropped: I remembered from conversations with theatre-going friends that they had been impressed with an English actress named Maggie Smith and that she had received excellent reviews in her first appearance on Broadway in 1956. I myself did not follow theatre closely in those days and was unaware of her recent stage appearances. In 1961 most of the future Dame Maggie's great triumphs of stage and screen were yet to be. My skimpy knowledge about Maggie's standing in America seemed to disappoint Nat but not too many years would pass before he would no longer need to ask anyone about Maggie's accomplishments. Tea and conversation continued but I soon excused myself remembering that my own future lay behind another door in the Dunn School and it was late enough so that my arrival would not mark me as either compulsive or American.

Since that cup of tea with her father, I have seen Maggie Smith in films many times but unfortunately never on stage. When a close-up of Dame Maggie appears on-screen, however, I see something of her father in her face; a few memories flash by and then, in some mystical way, her performance, invariably good, becomes even better.

only about fourteen years old. Florey virtually recruited Kent on the spot thus beginning a remarkable relationship that lasted until Florey's death forty years later. By the time I arrived in Oxford, Mr. Kent, a short, cheerful and friendly man, seemed to be working as an overseer of technical services and a facilitator for the investigators. Kent, as Florey's technician, had been involved in animal testing during the early assessment of the antibiotic potential of penicillin. He clearly enjoyed telling and retelling stories about his role in these experiments. His favourite and mine, too, was about the day when eight experimental mice were infected with virulent streptococci; half the mice received penicillin and half did not. Detailed accounts of this key experiment have appeared in print many times with appropriate tribute to Florey and to Norman Heatley, a pivotal figure in the penicillin studies, but listening to Kent's evewitness version gave me a sense of, "You are there." He related it with fitting facial expressions ranging from doubt to wonder and with attempts to imitate Florey's voice and demeanour. Kent said that Florey had slept in the laboratory that night, checking the mice at intervals, and by about 3:00am the four mice that had not received penicillin were dead; the four protected mice had survived. Kent, who had been sent home at about six in the evening, returned the next morning (Sunday, May 26, 1940). As he continued, Kent would drop his voice an octave or so: 'So the Old Man says to me,' he would say, 'Kent, I think we've got something here'. Biographies and other accounts of what Florey said on this historic occasion quote him as having used the word, 'Miraculous'. Maybe so, hyperbole was certainly justified but I like to believe that Jim Kent quoted Florey correctly; a few understated and unpretentious words and the antibiotic era began.'

If one had to pick out one technician to characterize the importance of their work to the Dunn School, it would surely be Jim Kent. He, like Edwin Wheal was rewarded by the university with an honorary MA degree on retirement. Kent interacted with every new scientist, student and visitor to the Dunn School and guided them on the use of the facilities that the laboratory could offer. He essentially became a living



The penicillin team

There were others in addition to Nat Smith who added to the unique character of the Dunn School. I will mention only two. Someone I remember very well is Jim Kent. Prof Florey, I believe, told me that one day, in 1927, while working in a laboratory at Cambridge, he needed assistance. Florey noticed a small, teenage boy sweeping the floor and called him over. James Kent was the young man's name and he was

legend in his time in the lab.

Although Mike Simkins started modestly working at the bench with two scientists he eventually had the almost impossible task of succeeding Jim Kent as chief technician. He wrote in *Fusion* 10 (2011) about his 34 years in the lab. One group of technicians who deserve a special mention are the 'penicillin girls', Ruth Callow, Claire Inayat, Betty Cooke, Peggy Gardner, Megan Lancaster and Patricia McKegney. Ruth and Claire were the first to be employed from late 1940 or early 1941 and the others later in 1941. In 1943 Florey asked the MRC to increase their wages to £2.10s a week as 'they now have nearly 3

years' experience and are good technicians and enthusiastic about their work. They could easily be earning £3.10s elsewhere'. Rather surprisingly the MRC agreed to pay. It is a disappointment to those of us trying to fill in the history of the Dunn School that no-one apparently followed the careers of the penicillin girls and despite media interest little has been discovered since, nevertheless, their legacy will undoubtedly live on...

Celebrating the Dunn School Centenary

Keith Gull

The last edition of *Fusion* carried the announcement of the start of our planning to celebrate the centenary of the Dunn School. Established by a munificent £100,000 gift given by the Trustees of will of Sir William Dunn in June 1922, the original Dunn School building on South Parks Road was subsequently opened in March 1927.

We are now planning a series of celebrations of the Dunn School, its science and scientists. We will, of course, be recognising the many facets of its history and the different people who have worked and trained here. However, much of the focus will be on the future and how the Dunn School is positioned at the leading edge of research, teaching and discovery in the basic biosciences that underpin advances in medicine.

A central spine of the many events planned will be an annual, one day scientific discussion meeting, held around a topic of current interest. We will design these to be of general interest to alumni and friends as well as specialists in that area. These events will be held around Easter in each year from 2022 to 2026. Then, in 2027, the centenary of the opening of the Dunn School Building, we will organise a more extensive meeting coupled with a series of outreach and alumni events.

However, by way of a prologue to the centenary events we need to recognise the 75th anniversary of the award of the Nobel Prize in Physiology or Medicine 1945 which was awarded jointly to Sir Alexander Fleming, Ernst Boris Chain and Sir Howard Walter Florey 'for the discovery of penicillin and its curative effect in various infectious diseases'. Consequently, in late 2020 we will be holding a scientific discussion meeting on the theme of 'Inventing the Future: Discovering new anti-infectives and overcoming antibiotic resistance'.

To ensure that we are in touch with as many alumni as possible, please ensure we have your electronic contact details for future *Fusion* editions and event announcements. Also, please encourage others to register their interest for future Dunn School Centenary events by sending their contact details directly to myself (keith.gull@path.ox.ac.uk) with 'Dunn Centenary' in the subject line!

Privacy Notice

We recognise that during this forthcoming period celebrating the centenary of the Dunn School and beyond we will need to communicate by various means with many alumni, friends and supporters across the world. The Dunn School is committed to protecting the privacy and security of your personal information. The overarching purpose of collecting and processing your data is to provide you with the best possible experience of being an alumnus/alumna, donor or supporter of the Dunn School and the future events and projects we plan.

We will follow the University of Oxford guidelines on the collection and use of your personal data submitted to us online, by email, by paper or face-to-face, in accordance with the General Data Protection Regulation (GDPR) and associated data protection legislation. The details of these Privacy Notices can be found at:

https://www.path.ox.ac.uk/content/privacy-and-personal-information and https://www.campaign.ox.ac.uk/privacy-notice

To ensure that we are in touch with as many alumni as possible, please ensure we have your electronic contact details for future *Fusion* editions and event announcements. Also, please encourage others to register their interest for future Dunn School Centenary events by sending their contact details to myself (keith.gull@path.ox.ac.uk) with 'Dunn Centenary' in the subject line!



THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY is a department of the University of Oxford website: www.path.ox.ac.uk

CONTACTS: Matthew Freeman

Head of Department Sir William Dunn School of Pathology, South Parks Road Oxford OX1 3RE matthew.freeman@ path.ox.ac.uk

EDITORS Paul Fairchild Tel: (44) (0)1865 285751 email: paul.fairchild@ path.ox.ac.uk

Eric Sidebottom

Tel: (44) (0)1865 285751 email: eric.sidebottom@ path.ox.ac.uk

From the Fusion Archives...

The following is a selection of excerpts, chosen by the Editors from past editions of *Fusion*, that give a flavour of events and topical issues within the Dunn School during years gone by...

Five years ago...

Andrew Bassett discusses the emerging field of CRIPSR/Cas9-mediated genome editing and its likely future applications [*Fusion* (2014) **13**:16]:

The dream of any geneticist is to be able to simply and rapidly make desired alterations to the DNA sequence of an organism. Recent advances in the field of genome engineering have made this a reality...

Simon Hunt reminisces about his career as an immunologist, spent predominantly at the Dunn School [*Fusion* (2014) **13**:19]:

My interview with Rodney [Porter]...was dramatically un-pompous. He leaned back in his chair, delivered his questions with his 'hrrmmm' throat-clear, engagingly disguising his speech impediment, and my answers had to navigate their way back to his ears past the soles of his shoes prominently facing me on the edge of his desk.

Ten years ago...

Gilbert Shama discusses how information about penicillin was transmitted throughout Europe during the Second World War [Fusion (2009) 8:19]:

When Florey saw the startling results of his first experiments with penicillin his instincts as a scientist must have driven him to publish them immediately, notwithstanding the war. He may even have felt a moral imperative to do so – here, after all, was a novel compound with near miraculous properties. He soon came to see penicillin as offering the Allies a strategic wartime advantage...

Jordan Raff reflects on his appointment to a Personal Chair and Group Leader position at the Dunn School and the research on centrosomes he proposed to conduct [*Fusion* (2009) **8**:3]:

As a biomedical scientist in Cambridge, I was well aware of Cesar Milstein... He was a towering figure in immunology, and I feel fortunate and honoured to be the first holder of the Cesar Milstein Chair in Cancer Cell Biology in the Dunn School.

Fifteen years ago...

(

fusion

In an interview with *Fusion*, newly-appointed Group Leader Quentin Sattentau discusses how the twists and turns of his career inspired his continued fascination with HIV [*Fusion* (2004) **3**:10]:



I had the good fortune to do my first post-doc in Peter Beverley's department at University College London, collaborating closely with Robin Weiss, who has been a great role model for me and many other virologists. In 1985, AIDS offered a

> whole new disease and a brand new virus to study. Robin's work identifying CD4 as the HIV receptor had just been published and it was a very exciting time.

Eric Sidebottom provides a tribute to Norman Heatley who had died earlier in the year, marking the end of an era since he had been the last surviving member of the penicillin team [*Fusion* (2004) **3**:6]:

Norman Heatley died on 5th January, one week short of his 93rd birthday... We might speculate that he missed out on the Nobel Prize to Fleming, Florey and Chain, only because the rules of the Nobel committee restrict the number elected for an award to three. When it was suggested to him that he should have received a knighthood, Heatley's characteristic response was to shrug his shoulders and say: 'Oh well...'.

Front cover: Tatjana Terentjeva.