fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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UNIVERSITY OF OXFORD

Athena Swan and Equal Opportunities

Focus on Cancer

Sir Henry Harris: A Life in Science

Intercellular Signalling and the Rhomboid Family

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Editorial

Many readers of* Fusion *will, by now, have noticed our new Dunn School logo which was recently adopted following an internal competition that attracted numerous entries. Various innovative designs were received but the simplicity and subtle connotations of the logo designed by Richard Wheeler finally won the day. Many of you will also have discovered our new website. It went live in June and it is a major overhaul: most of the text and all of the structure is new. The previous version was showing its age and we decided that it was time for a complete renewal. We are grateful to the EPA Research Fund for supporting this project that was, perhaps inevitably, more complex than we originally imagined. I would also like to express by personal thanks to Professor Chris Tang for taking the lead.

I hope you will agree that the product of all this labour is excellent: showing that the Dunn School is a vibrant, successful, world class centre of science. In the end, of course, it is the people that make the department, and the new website seeks to acknowledge this with a greater emphasis on both the individual group leaders but also the postdocs and students who are so much the drivers of our research success. We also have new pages devoted to teaching – our other raison d'etre – including some teaching videos. Of course the history of the Dunn School is also covered in a variety of ways.

We'd love to hear from you with any feedback about the website. If you have good ideas about how to improve it further, do tell us. We already have plans for further development including, for example, a page devoted to alumni, more videos, and more content generated by the students and postdocs. We have also provided links from each group leader's page to any personal websites, and now have templates to make it easier for busy PIs to generate a professional-looking page.

This all matters! Websites are now the primary interface we have with the external world. When students are thinking about where to do a doctorate, postdocs are wondering about whether the Dunn School would be a good host department, and even at more senior levels, the image we project is important. Also in more indirect ways, funders, the central University, and policy makers will all learn about us from our website. Of course, our main strategy will always be to continue to build our scientific strength – and I'm pleased to report that things are going very well on that front – but in what is currently a tight and very competitive funding environment, everything that we can do, even if indirect, is valuable.

Let me finish by saying that the work that has gone into the website has made me think about how we engage not just with the outside world, but also with Dunn School alumni and supporters. It's always nice when people come and visit us, but I'd like to do more to develop the network of people who are interested in our progress. Maybe by the next edition of *Fusion*, I will have a bit more to report on that front but in the meantime, I'd be interested to hear from anyone who has ideas. The photos of past Dunn School researchers that adorn the walls of the department make me realise what an amazing group of people have worked here over the years, and it would be nice to think about how to strengthen this extended family.

Matthew Freeman

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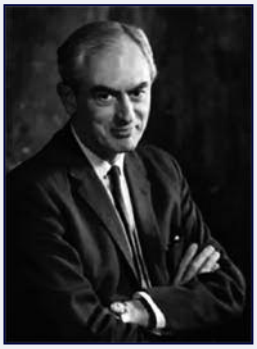
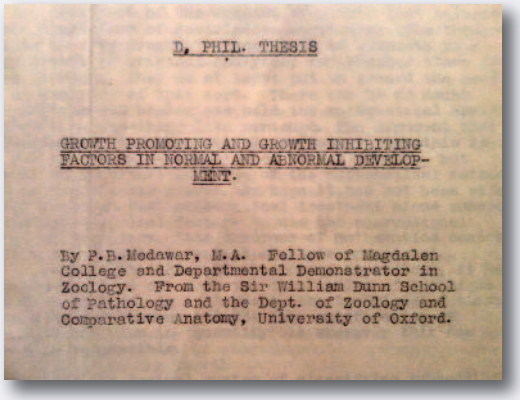
The cover shows a mosaic depicting the process of chromosome replication which was produced by Catarina Gadelha, a former post-doc in Keith Gull's lab whose work has featured in previous editions of Fusion *[please see* Fusion *8 (2009)]*

News

Celebrating the Life and Works of Sir Peter Medawar



This year marks the centenary of the birth of Sir Peter Medawar, Nobel Laureate and one of the Dunn School's most illustrious alumni. Although originally based at the Department of Zoology, where he was awarded a first class degree in 1935, he moved to the Dunn School at the invitation of Howard Florey to complete his DPhil where he was able to take advantage of the Department's new state-of-the art tissue culture facilities, on which his research depended. An original copy of Medawar's thesis entitled 'Growth promoting and growth inhibiting factors in normal and abnormal development' may still be seen on display in the Dunn School library.

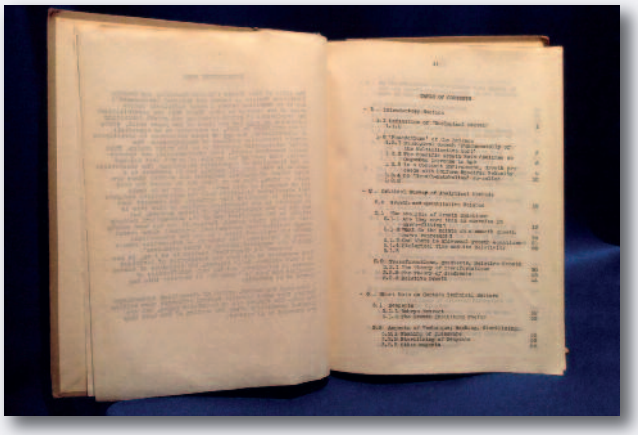


D. PHIL. THESIS

GROWTH PROMOTING AND GROWTH INHIBITING FACTORS IN NORMAL AND ABNORMAL DEVELOPMENT.

By P.B. Medawar, M.A. Fellow of Magdalen College and Departmental Demonstrator in Zoology. From the Sir William Dunn School of Pathology and the Dept. of Zoology and Comparative Anatomy, University of Oxford.

Medawar is best known for his contributions to understanding the mechanisms of allograft rejection, inspired by his first-hand experience of the need for skin grafting of pilots badly burned during World War II. But it was his later demonstration that the immune system could be fooled into tolerating foreign tissues, now referred to as immunological tolerance, which earned him wide acclaim as a scientist and the 1960 Nobel Prize for Medicine or Physiology. This work contributed significantly to establishing immunology as a discipline in its own right and helped elucidate the scientific basis for whole organ transplantation that continues to benefit so many today. In addition to his many accomplishments as a scientist, Medawar was passionate about conveying his excitement about science to the general public: in 1959 he delivered the prestigious BBC Reith lecture and authored many articles and popular science books including his autobiography *Memoirs of a thinking radish*.

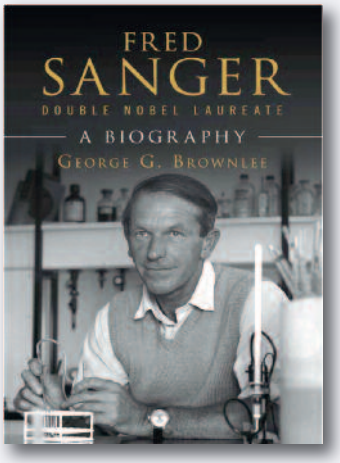


The centenary celebrations were held on 2nd March in the Department of Zoology and included a series of lectures by Henry Bennett-Clark, one of Medawar's former students, on his role as a teacher and mentor, Paul Fairchild, on Medawar's scientific legacy and from Richard Dawkins, who spoke about Medawar's literary genius.

George Brownlee Publishes the First Biography of Fred Sanger

Last November, George Brownlee, Emeritus Professor of Chemical Pathology at the Dunn School, published a biography of Fred Sanger, one of only four people worldwide ever to have been awarded two Nobel Prizes. Sanger, who died in 2013, was widely revered as 'the father of modern genomics'. Known for his pioneering methods for the sequencing of proteins, RNA and DNA, Sanger's work paved the way for much of modern molecular biology and the human genome project.

As a former PhD student and colleague of Sanger, George gained valuable first-hand insight into the man and the scientist, putting him in an unparalleled position to write his biography. Indeed in 1992, he conducted an extensive interview with Sanger, published in the Biochemical Society Archives, which covered both his life and research and outlined the qualities Sanger considered essential for a scientist to be successful: the book features a largely unabridged transcript of this interview.



George's book 'Fred Sanger: Double Nobel Laureate' was recently reviewed for the International Society for Computational Biology. In her review, Christiana Fogg described the book as being '…filled with scientific details that will delight a biochemist, (are) accessible to lay readers, and will certainly inspire budding scientists and established academics alike'. The publishers, Cambridge University Press, have kindly offered readers of *Fusion* a 20% discount, valid until 1st February 2016, which can be obtained by applying the discount code FUSIONSANGER when purchasing a copy at the following dedicated webpage: www.cambridge.org/fusionsanger.

Few would doubt the need for scientists to convey the importance of their work to the general public and to inspire the next generation to pursue science as a career. But to do so can prove rewarding in surprising ways, as members of Chris Tang's laboratory discovered recently when they took time out of a busy schedule to share their fascination of bacteria with primary school children.

Reaching the Next Generation of Scientists

Mariya Lobanovska and Gareth McVicker

Earlier this year, a team from our group comprised of a Professor (Christoph Tang), two award-winning demonstrators (Rachel Exley and Gareth McVicker), and a Masters student (Mariya Lobanovska) decided to take a daring step out of the lab and visit Elm Wood Primary school in Middleton to teach bacteriology to nine- and ten-year old children as part of their "History of Science" module. Most outreach programmes focus on teaching final year GCSE or A-level students, so we decided to try developing an outreach day for Year 5 pupils, as it is equally important, if not more so, to introduce children to research at a young age. Our goal was to teach the class about microbiology and engage them in discussions about science (as well as disprove the general perception that scientists are mad-men in white lab coats!).

Given the seminal contributions of research at the Sir William Dunn School of Pathology to the treatment of bacterial infection, we had no shortage of interesting topics to include throughout the day, and inspirational stories and material with which to engage the children. We aimed to explore the notion of the scientific breakthrough, so we set up four stations, each with a group of around six pupils for half an hour at a time (Figure 1). Crucially, two activities centred on major advances in infectious disease research, with a selection of tasks designed to bring history to life. At one station, children learned about the "animalcules" seen for the first time by Antonie van Leeuwenhoek through the lenses he had crafted by hand. The pupils also had a chance to investigate and handle the different tools that we use in the microbiology laboratory (such as petri dishes, culture flasks, and sterile loops). Another station was designed to teach the class about the history of penicillin and its contribution to human health. It was here that the children's attention was drawn to the microscope used by Lord Florey, and one of the original bedpans that was used to grow *Penicillium* at the Dunn School in the 1940s. This emphasised the key contribution of researchers at the Dunn School in the development of penicillin and the photographs of soldiers demonstrated the impact of penicillin on the war effort between 1942 and 1945.

At the other stations, groups learned about other important medical discoveries including John Snow's classic investigation of the London cholera epidemic of 1854 that identified the water pump on Broad Street as the source of infection. Furthermore, as there is currently increasing interest in the role of the microbiome in human health and disease, the children also explored the concept of "good" and "bad" bacteria, by reading several microbes' 'Facebug' profiles, before deciding whether or not to accept their friend request.

Perhaps even more important than the discussion of science and history was the question and answer session we had with the class and staff at the end of the day. The children had the opportunity to share their impressions of the activities and recall what they had learned during the day. They asked a series of questions that were interesting and probing, such as "Who inspired us when we were younger?" and "What was our latest discovery?". There were a couple of very entertaining questions as well; for example, one of the boys asked Chris how he became the boss and another one asked if it is really necessary to do all the homework they are set in school in order to become a scientist!

Figure 1. Four stations were set up to teach the class about different scientific discoveries. Clockwise from the top: Gareth showing the class the microbiology lab equipment; Mariya telling pupils about the 1845 cholera epidemic in London; Chris showing pupils the model of a bacterial cell; Rachel teaching the children how to use a microscope.

Our goal was to give the pupils first-hand exposure to research, and hopefully help them realise that they themselves could join the world of research and become the scientists of the future. However, the outreach had a further impact on all of us who were involved: we learned how to explain complex material to a very non-specialised audience, and enjoyed the feedback received from the children, which was an indication that we had successfully conveyed our own enthusiasm to them. It was also exciting to see that even a one-day microbiology class could inspire any child's passion for science, irrespective of their background. We were thankful to the teachers and the headmaster who helped prepare the activities and classroom, and supported us throughout the day.

While we are used to dealing with a laboratory full of undergraduate medical students, facing a class full of 9 and 10-year olds was a new challenge. We hope that our visit will grow into a long-term partnership with this and other schools, with even greater opportunities for schoolchildren to be involved. And if even one of them feels inspired enough to take up a career in science, we will have achieved our objective…

Interview with Susan Lea

The Dunn School was recently awarded an Athena SWAN bronze award in recognition of its efforts to promote gender equality and a family-friendly working environment. Susan Lea, who chaired the committee responsible for the successful bid, spoke to* Fusion *about the award and why it is a significant milestone in the life of the Department.

What exactly is Athena SWAN and what does it seek to achieve?

Athena SWAN is a national scheme which recognizes a commitment to supporting and advancing women's careers in science, technology, engineering, maths and medicine (STEMM) in higher education and research. Applications can be made at three levels, bronze, silver and gold and these awards reflect an ongoing commitment to actively monitoring and seeking to improve the way in which a department or university facilitates career progression among women.

Having been responsible for coordinating the Department's successful bid for the bronze award, perhaps you could give us a flavour of what the application process involved.

The application for the bronze award is essentially a process of collection and analysis of data – statistics on everything from gender breakdown of applications, shortlisting and appointments to the department at all levels (from graduate students to personal chairs), looking at take up of parental leave and return from leave to collecting data about people's experiences of working in the department. These data were collected both by our Human Resources Department and also by everyone participating in cross-departmental surveys or offering opinions to members of the working group preparing the application. We then looked closely at the data to see if there were issues that surprised us, or were of concern and discovered that for academic appointments (in contrast to appointments at all other levels), we had very few women applying to the Department. We then thought about what the survey had revealed about the culture of the Department and were delighted to discover that the vast majority of departmental members were very happy to be working in the Dunn School and would definitely recommend it to their friends! However, the survey also suggested that we could work harder at communicating how the Department is run and how decisions are made. We then brainstormed to identify potential ways of addressing these issues and set these as targets in the bronze application. These targets, together with the data analysis, were written up and sent to Athena SWAN for assessment. Of course, we then had to implement the changes we had suggested and begin collecting data about their effectiveness in preparation for submitting our Silver application at Easter!

What impact have the principles of Athena SWAN already had on the working environment of the Dunn School?

Apart from the rather controversial change to the timing of departmental seminars (proving you can never make everyone happy!) I think the changes Matthew has made to postdocs by bringing mentoring of their careers to the fore has been a great outcome. Matthew might well have done this without the influence of Athena SWAN, but the introduction of more opportunities for postdocs to explicitly think about career choices, combined with the introduction of annual reviews, during which supervisors and postdocs sit down together to think back over the past year and plan for the future, seems a very good thing to me. I'm sure many supervisors were already doing this, but it's great to know that everyone now has access to this time to focus on their career, rather than always thinking about the next experiment!

What changes do you envisage being necessary in the future to make the Department more accommodating of family life?

At some level I think it's steady as she goes – Fran, our Human Resources Manager, is doing a great job making sure we now have all the data available to be able to accurately monitor our progress and continues to ensure that all group leaders are updated on a regular basis about training opportunities for learning how best to manage people. We have a series of senior recruitments to make and Matthew is very proactive about seeking out excellent candidates. Finally, the recent adoption of shared parental leave by the University as a whole and the enthusiastic support this has been given within the Dunn School, makes me very hopeful that things are getting easier for all scientists trying to combine workloads with the demands of young families – we just need to ensure that this emphasis on family life is reflected in the continued expansion of childcare facilities in Oxford!

Having pursued a very successful career as a scientist yourself, what do you consider to be the greatest challenges for women in science today?

I'm not sure that the challenges are especially gender-segregated: with a few exceptions, I think academic science is mostly in a very good place to allow able women to have satisfying careers and compete on an equal footing. Hopefully, the Athena SWAN initiative will deal with the few remaining problems and lead to a better work place for all scientists, irrespective of their gender. For those who choose to have children, childcare continues to be expensive and not always simple to access – but generally there is a solution, although at times it may not seem that way!

What would be your advice to young women aspiring to develop a career in scientific research?

The same as to young men – do it because you love it, do it as well as you can and always remember there are plenty of other things you can do with your life. If you aren't enjoying it anymore, go and do something else that you do enjoy! If you are enjoying it, the chances are you're making good progress, so don't give up!

Focus on Cancer:

Cancer Research in the Dunn School: A Brief History

Eric Sidebottom

It is probably true that the Dunn School is not generally regarded as one of the leading cancer research centres of the world. Indeed, as a medical student studying here in 1960, the lectures I attended on cancer were delivered by a visitor to the department, Dr R. J. C. Harris. He also wrote the chapters on cancer in Florey's highly respected* Textbook of Pathology *which was based on the pathology course taught in the Department. I believe that this 'gap' in the Dunn School staff at the time influenced my later decision to research and teach in this area of pathology. Perhaps surprisingly in the circumstances, I believe a case can be made that two of the most important advances in understanding cancer have come from research conducted at the Dunn School.

First, from 1938 until 1948, Isaac Berenblum worked here and developed his 'two-stage' hypothesis of cancer: 'initiation and promotion' involving mutation and mitogenesis. During this time he published at least 34 papers on the subject. On leaving the Dunn School he went via the National Cancer Institute in the USA to The Weizmann Institute in Israel, eventually becoming its Director. During his long life (from 1903 to 2000) he published many research papers, wrote several books and received many awards, including the Israel prize and the Alfred P. Sloan award.

While in Oxford he wrote "there is a romance in science which stimulates the mind and satisfies the soul; it also happens to be the surest approach to one's understanding of the truth". Over thirty years later he said "I do not feel I have changed much. I still experience some sense of adventure every morning when I set out to work, just as I did on the first day, close to 50 years ago, when I began my life as a scientist. Trying to discover the unknown is still exciting, even though the methods may have changed over the years". I find these words rather inspiring. It would be good if they were better known amongst young people considering a future career in science.

My second important advance comes from the work of Henry Harris and colleagues in Stockholm and Oxford. That work was first published in *Nature* in 1969 under the title "The suppression of malignancy by cell fusion". The paper provoked a wide range of responses from frank disbelief via amazement and excitement to admiration. It ultimately led to the identification of anti-oncogenes – or tumour suppressor genes as they are now known - and a new understanding of the genetic changes in malignant cells. Harris continued with this line of work for the rest of his life. His conclusions were often controversial but he continued to do experiments long after his official retirement and, even after he gave up his laboratory in the Dunn School, his daughter, Ann, working in America, continued the search for factors influencing the suppression of malignancy. Their last joint paper was published in 2013. It is also notable that in 2008, Harris published a re-translation of Theodor Boveri's seminal work "Concerning the origin of malignant tumours" published in German in 1914.

It has just occurred to me that Berenblum and Harris had much in common in their backgrounds. Both were from Jewish families that had to emigrate to avoid persecution, Berenblum, aged 3 from Poland, via Belgium to England and Harris from Russia aged 4 to Australia and thence Oxford. Both had a burning passion for scientific research and continued to work almost to the ends of their long lives. Berenblum died in 2000 aged 96, Harris in 2014 aged 89.

I am optimistic that a further important advance in our understanding of cancer will result from current work in the Dunn School. Several research groups work on various aspects of the control of gene expression. Such work may, either directly or indirectly, feed into the control of tumour growth. A good example is provided by Chris Norbury's research into the post-transcriptional regulation of gene expression in cancer cells (see page 9). In addition, Bass Hassan splits his time as a clinical oncologist between hospital clinics and the Dunn School where his group studies the structure and function of genes that regulate tumour phenotypes. The following articles provide a taste of such ongoing research within the Department that might one day form the next breakthrough in the fight against cancer.

Double Agent Caspase: "What doesn't kill you makes you stronger"

Alberto Baena

According to my grandfather, the second part of the title of this article became one of the most common proverbs in post-civil war Spain. In the face of constant food shortages, new sources of nourishment were sought as the most basic priority. Under such circumstances, the regular intake of expired food caused not only the death of many through food poisoning, but favoured the survival of others. But, rest assured, this text is not intended as part of an articulated campaign for the consumption of rotten food products. Nor does it correspond to the start of a spy novel set in Spain at the beginning of the last century. Nevertheless, the combination of both worlds is a good metaphor for the research topic in my laboratory. Through this article, I hope to explain the relevance of uncovering the role of a key protein family called 'caspases' during normal and pathological conditions.

At the Origin

Proteins are specialized workers within cells and individual genes within the genome provide the template for each protein. Related proteins can be grouped into families that vary in amino acid sequence, spatial folding, and ability to interact with other cellular components. All these features ultimately define protein function.

In the early 1990's, H. Robert Horvitz and his colleague Junying Yuan found that the protein encoded by the ced-3 gene in worms (*Caenorhabditis elegans*) had similar enzymatic properties to the mammalian protein known as interleukin-1β converting enzyme (ICE). At the time, Horvitz had little idea that he would receive the Nobel Prize for Physiology or Medicine in 2002 for this discovery and the pioneering work describing the genetic mechanisms that lead cells to die. Currently, we know that ced-3 and ICE are both part of a protein family of ***c***ysteine-***asp***artic prote***ases*** that have been given the generic name 'caspases'.

Caspases as "Biological Scissors"

Caspases are evolutionarily conserved proteins, which perform very similar functions in eukaryotic cells (protists, plants, animals) and prokaryotes (bacteria). Why has nature allowed these proteins to exist and evolve in all these organisms? To answer this question we first need to consider their molecular properties and biological functions.

The two distinguishing features defining caspase function are: 1) the ability to physically interact with a wide range of proteins, and 2) a well-characterized sequence-specific protease activity (Figure 1a). Both properties enable caspases to specifically bind to, as well as efficiently cleave, target proteins (substrates) after recognition of cysteine-aspartate sequences (Figure 1b). At the cellular level, these actions often earmark proteins for degradation, but can also irreversibly modify their structure, thus changing their function. It is not surprising, therefore, that caspases are considered one of the most relevant "biological scissors" operative within cells.

The Public Life of the Sniper: "The Killer"

In military terms, snipers are soldiers publicly known for having been trained in precision shooting at distance to eliminate selected targets. Caspases are synthesized as inactive enzymes that are only switched on when necessary within cells. Intracellular caspase activation at high levels commonly causes cell death (Figure 2). The possibility of providing a molecular explanation for cell death captivated the attention of researchers soon after the initial discovery of this protein family. Subsequent studies in a wide variety of organisms including humans, have concluded that caspases can orchestrate a cascade of events, which ultimately degrade the essential proteins maintaining the structure and functionality of cells.

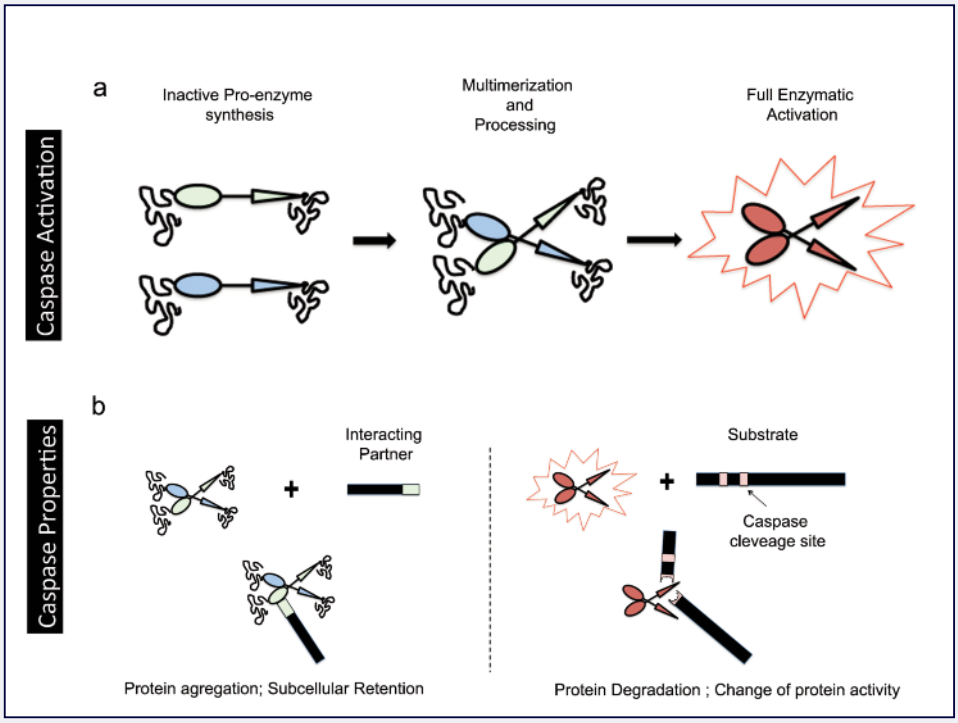


Figure 1. Schematic summarizing the mechanisms of caspase activation (a) as well as the main molecular properties of caspases (b). Inactive caspases are represented in green and blue, while the enzymatically active version is coloured red.

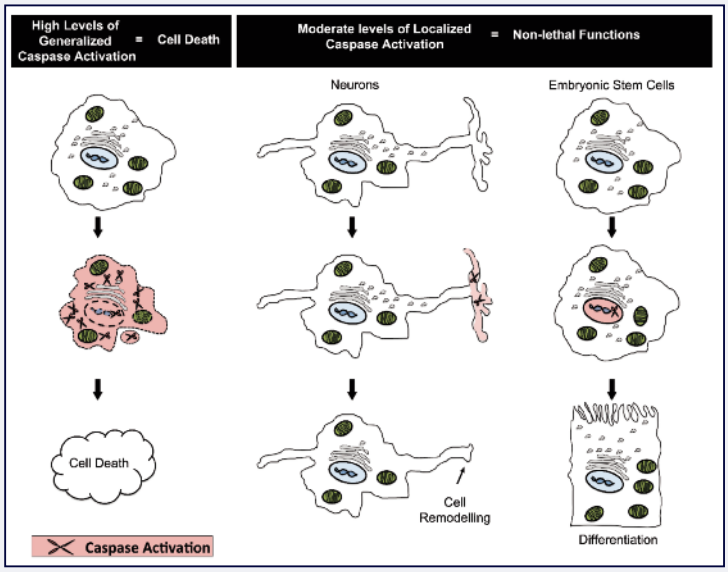


Figure 2. Consequences of caspase activation in apoptotic and non-apoptotic contexts. Notice the differences in levels of caspase activation, and corresponding subcellular localization.

The immediate consequence of this degradation process is the precise elimination of defective and/or unnecessary cells, through a version of programmed cell death known as 'apoptosis' (Figure 2). Consequently, caspases are the key regulators for one of the major killing systems inbuilt within cells. Importantly, malfunction of caspases, or of apoptosis in general, commonly leads to diseases, either by accumulation of undesirable cells or their unscheduled elimination. The biological relevance of these findings and their pathological implications have, since then, inspired efforts to identify the genetic mechanisms that regulate the different modes of cell death (e.g. apoptosis, necrosis and autophagy), as well as the functional characterization of caspases in many other cellular contexts.

The "Private" Life of the Sniper

Beyond the ability of precision shooting at a distance, snipers are highly valued in the army for obtaining information about the enemy without being detected. Similar to the sniper analogy, recent data have uncovered unexpected roles for caspases that do not induce cell death. These new non-apoptotic functions are involved in the regulation of "living processes" (control of cell proliferation, cellular differentiation and migration), proving that caspases are not just efficient killers. A low level of caspase activation, localized into well-defined regions of the cell, is what distinguishes these non-lethal functions (Figure 2). The next two examples are intended to illustrate these properties.

The communication and connection between neurons depends on cellular extensions called axons and dendrites. These cellular projections are naturally remodelled (interrupted, extended or consolidated) in response to a variety of environmental and developmental cues. Caspases are involved in the remodelling of neural circuits on at least two fronts. On the one hand, they appear to modulate signalling between neurons in regions of contact (synapses); on the other hand, they facilitate the retraction of neuronal projections. In both cases, caspase activity is restricted to the neuronal extensions and never triggers cell death (Figure 2).

Embryonic stem cells are pluripotent and, as such, have potential to differentiate into any cell type after receiving the appropriate set of genetic orders. Recently, the non-lethal, enzymatic activity of caspases has been found to regulate the function of genetic factors implicated in the differentiation of stem cells. In this case, contrary to the previous example in neurons, caspases act in the cell nucleus to mediate the cleavage of transcription factors (proteins involved in the control of gene activation and silencing) (Figure 2).

Nowadays the list of examples where caspase function is required for "living processes" is expanding rapidly. However, the molecular mechanisms regulating caspase activity in these non-lethal contexts, as well as the interacting caspase partners required, remain largely unknown. Importantly, elucidation of this biological question goes beyond pure scientific curiosity, because deregulation of these non-lethal functions appears to have a strong impact on the origin and progression of metabolic diseases, neurological and immunological disorders, sterility, cancer and metastasis.

The Story of Intimate Enemies: Caspases and Cancer

There is growing evidence suggesting that the aberrant activation of caspases underlies the initiation or maintenance of multiple diseases, including cancer. Cancer originates when proliferating cells ignore the signals that normally regulate their growth within tissues. This disconnection allows tumour cells to proliferate in a non-controlled manner, which ultimately compromises the physiological properties of the host tissue. In principle, caspases are at the forefront of preventing the expansion of undesirable cells, including tumour cells. However, tumour cells fight against the caspase action, either by increasing the expression of proteins that prevent their activation (inhibitors of apoptosis, IAPs) or by reducing caspase levels, although mutations in caspase genes are rarely detected in tumours. Therefore, apart from their ability to proliferate excessively, cancer cells are very efficient at evading apoptosis. In this sense, cancer and caspases could be considered mutual antagonists. Nevertheless, recent investigations indicate that the

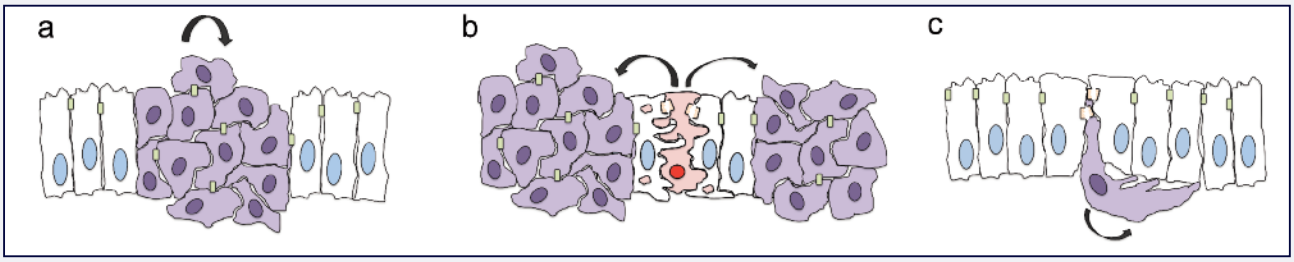


Figure 3. Presumptive tumorigenic roles of caspases. (a) Expansion of tumour cells due to defects in cell death or cell differentiation. (b) Cell proliferation stimulated by persistent signalling at a distance coming from long-lasting caspase-activating cells (red). (c) Cell migration and metastasis facilitated by sustained caspase activation in tumour cells. In all panels, tumour cells are coloured purple. Black arrows indicate cells directly affected by changes in caspase activation. Notice that caspase activation and the subsequent effects occur in tumour cells in panels (a) and (c). In panel (b), caspase activation happens in different cells from those undergoing tumorigenesis.

relation between these two intimate enemies could be more complex, and not necessarily antagonist- or cell death-dependent. The next three examples aim to illustrate the potential tumorigenic role of caspases.

As described, caspase activity is required to promote stem cell differentiation. Consequently, deregulated caspase activation in these cellular contexts can fuel the expansion of stem cell populations, thus propelling the progression of various cancers such as leukaemia, bone cancers and colon cancer (Figure 3a). Unlike the previous example, there are situations where caspase-activating cells are not the direct beneficiaries of such activation. Dying cells normally send signals to stimulate the proliferation of healthy neighbours in order to ensure their replacement. This process guarantees the integrity and continuity of tissues under all circumstances. However, if cells committed to die are not eliminated in a timely manner, the continuous delivery of proliferative signals to healthy neighbours can trigger their uncontrolled growth (Figure 3b).
Besides the stimulating role of caspases, recent investigations have detected higher cell motility in caspase-activating cells. Among other consequences, caspase activation loosens the contacts with normal cells in order to facilitate the elimination of the cells undergoing apoptosis. In comparison to normal cells, tumour cells display non-lethal but elevated caspase activation, which have been correlated with the spreading of primary tumour cells towards other tissues (metastatic behaviour). This phenomenon eventually has fatal consequences, since it increases the probability of forming secondary tumours in other organs (Figure 3c).

All these examples put the emphasis on the negative sense of the proverb "what doesn't kill you makes you stronger", since tumour development appears to be sustained by the action of caspases. However, at the same time, they are a reminder of all the positive roles associated with caspase function in normal cells, as key regulators of death, proliferation and differentiation. Finally, they suggest that the versatility of caspases could be useful for therapeutic intervention in a broad repertoire of diseases.

At the End

I hope, by now, to have introduced the caspase family of proteins and the need to further understand their functions, not only during apoptosis, but also in non-apoptotic contexts. The challenge ahead is to shed light on the molecular mechanisms that "make us stronger". An important part of this challenge will be decoding the contact network of the double agent caspase.

Further Tricks of the RNA Tail

Chris Norbury

RNA molecules may be modified at their ends in ways that are often crucial to their function. For example, modification of messenger RNAs (mRNAs) by the addition of non-templated poly(A) tails at their distal (3') ends can promote their nuclear export, stability and translation into proteins. My fascination with this area dates back to my undergraduate years, when a young lecturer called Nick Proudfoot first introduced me to the finer points of gene expression (Nick, working with George Brownlee, had identified the hexanucleotide motif that directs endonucleolytic cleavage and polyadenylation of pre-mRNAs just a few years earlier). I was intrigued too by the way in which vesicular stomatitis virus mRNAs are polyadenylated as a result of stuttering by the viral RNA polymerase, a mechanism subsequently found to be used by the influenza RNA polymerase studied at the Dunn School by Ervin Fodor's group. I pursued these interests further as a graduate student at the Imperial Cancer Research Fund (now CRUK) laboratories in London, where my PhD work involved an investigation of the two alternative cleavage/polyadenylation sites in the polyomavirus early region. I found that the two sites were used to different extents as the lytic cycle of the virus progressed, and that this was linked to alternative splicing of the early region transcripts, allowing the virus to fine-tune expression of the three early region tumour antigen products to maximise viral replication. After a six-year interlude as a postdoc with Paul Nurse, investigating the regulation of the cell cycle by cyclin-dependent kinases, and subsequently as a junior group leader looking at the relationship between cell cycle checkpoint arrest and apoptosis, a genetic screen in the fission yeast *Schizosaccharomyces pombe* marked the start of what, in retrospect, seems an inevitable return to RNA 3' ends and their modification.

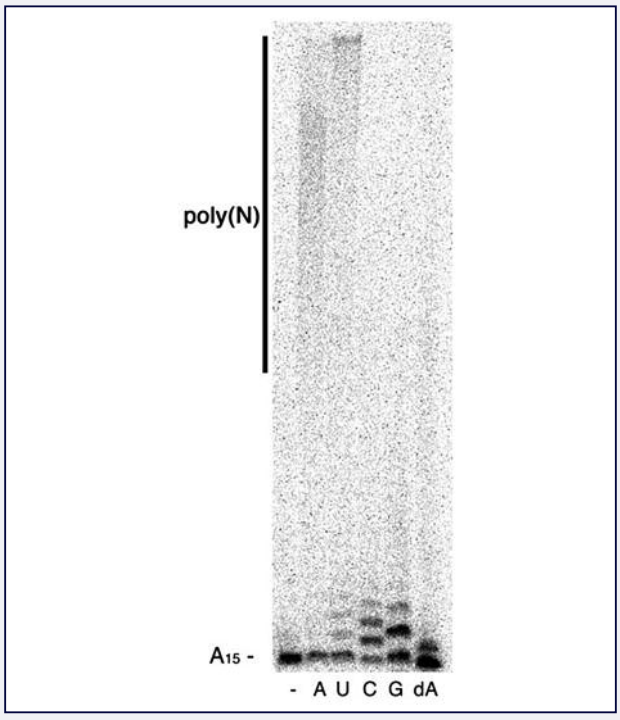


Figure 1. The first indication that Cid1 can uridylylate RNA. A [^{32}P] end-labelled A15 RNA substrate was incubated for 30 min at 30°C with 400 ng of recombinant Cid1 and ATP (A), UTP (U), CTP (C), GTP (G), dATP (dA), or no added nucleotide triphosphate (–). Products were separated by denaturing polyacrylamide gel electrophoresis and were detected by autoradiography. Note the high molecular-weight smear of products generated in the presence of ATP and UTP; poly(A) and poly(U) respectively. From Read *et al.* (2002) *PNAS* **99**: 12079–12084.

The screen, carried out by postdoc Shao-Win Wang in 2000, was designed to identify genes that reinforce cell cycle arrest following exposure to the anti-cancer drug hydroxyurea, an inhibitor of deoxyribonucleotide synthesis in the presence of caffeine, which inhibits the checkpoint arrest that normally results from exposure to hydroxyurea. This admittedly arcane screen identified a previously uncharacterised gene we called cid1 (for caffeine-induced death suppressor), the sequence of which showed it to encode a member of the DNA polymerase beta (Pol β) superfamily, an ancient and diverse group of nucleotidyl transferases. By 2002, I had taken up my current position at the Dunn School, and DPhil student Rebecca Read (now a surgeon back in her native Australia) had established that Cid1 was not, as we had first suspected, a novel DNA polymerase, but instead specifically modified RNA 3' ends, similarly to another member of the Pol β superfamily, the canonical nuclear poly(A) polymerase. We found, however, that the Cid1 protein is cytoplasmic, suggesting a distinct function in RNA metabolism. Cytoplasmic polyadenylation had been described previously by Joel Richter as an important level of gene regulation in *Xenopus* oocytes, and Cid1 indeed had poly(A) polymerase activity *in vitro* when presented with an RNA substrate and ATP. But we were unable to detect changes in mRNA poly(A) tail length on deletion or over-expression of cid1, and for a couple of years it seemed we would never have a clear view of its physiological function. The breakthrough came following the arrival in the lab of Olivia Rissland, a Rhodes Scholar from Brown University with interests ranging from Roman poetry to RNA (as well as coxing the University women's 2nd VIII). Olivia picked up on the observation that, in Rebecca's *in vitro* assays, Cid1 had been as happy to use UTP as ATP as a nucleotide co-substrate. Through ingenious *in vitro* competition assays, Olivia showed that UTP was, in fact, the preferred nucleotide, even when ATP was in ten-fold molar excess, as it would normally be *in vivo*. Post-transcriptional RNA 3' uridylylation was not thought to be widespread at that time, though it had been detected on U6 snRNA (a component of the nuclear RNA splicing machinery) and on the products of microRNA-directed cleavage in plants. It was a great surprise, therefore, when Olivia sequenced mRNAs she had circularised using RNA ligase and found that a substantial proportion of all polyadenylated mRNAs in *S. pombe* were modified by the addition of short U tails at the 3' ends of their poly(A) tracts. Her further studies showed that Cid1 was responsible for most of this mRNA uridylylation, and that U tails were added as a prelude to removal of the 5' cap structure, as part of a major pathway of bulk mRNA turnover. Following postdoctoral work with David Bartel at the Whitehead Institute, Olivia now heads her own research group in Toronto.

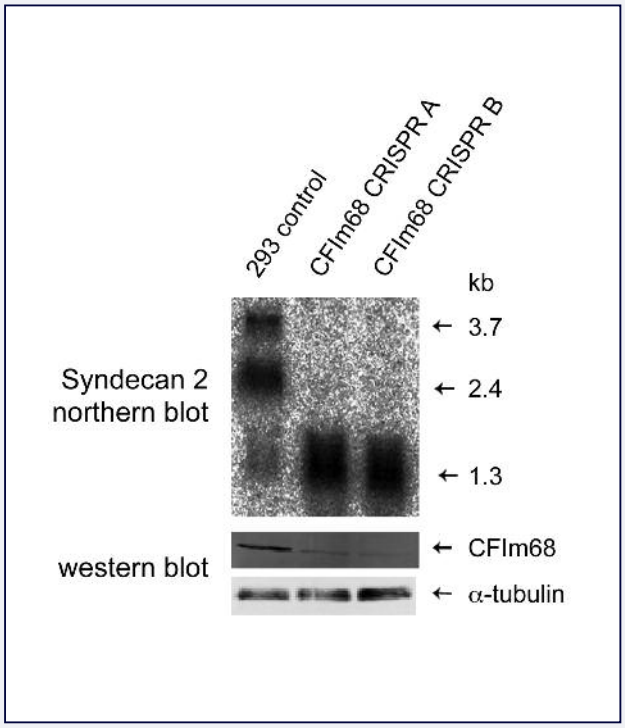


Figure 2. Down-regulation of the 68 kDa subunit of CFIm causes a shift to promoter-proximal alternative polyadenylation in human HEK293 cells. Primary transcripts of the syndecan-2 gene are processed by alternative cleavage/polyadenylation to generate mature mRNAs of 1.3, 2.4 and 3.7 kb, which have identical protein-coding capacity but which differ in the extent of their 3' untranslated regions. Two clones of HEK293 cells, in which the gene encoding CFIm68 was targeted for CRISPR/Cas9-mediated deletion, exhibit a marked shift to promoter-proximal cleavage/polyadenylation (J. Goodchild, A. Bassett and CJN, unpublished).

Subsequent work by my group and others has shown that mRNA uridylylation is not a peculiarity of fission yeast; just as in the case of cell cycle regulation, *S. pombe* turned out to be hugely informative about the equivalent processes in human cells, where uridylylation also promotes mRNA turnover. As might be expected, the situation in mammalian cells is more complex than that in the fission yeast model; there are two mammalian Cid1 orthologues, and, as well as mRNAs, their substrates include further cytoplasmic RNAs, notably the precursors of tumour suppressor let-7 microRNAs. Indeed, inhibition of expression of a human Cid1 orthologue ZCCHC11 in cancer cells can block some aspects of the cancer phenotype *in vivo*, and ZCCHC11 over-expression predicts disease progression in breast cancer. Furthermore, the 3´-5´ exonuclease DIS3L2 was shown by others to be responsible for the degradation of uridylylated pre-microRNAs and mRNAs in mammals and *S. pombe,* respectively. Human germline DIS3L2 mutations are the underlying cause of the foetal overgrowth characteristic of Perlman syndrome, and predispose to the development of Wilm's tumour. These findings suggest that targeting the RNA uridylylation pathway may be of therapeutic value in a variety of cancers; with this in mind we are now working with our structural biology collaborator Robert Gilbert to identify small molecule inhibitors of RNA uridylyltransferases via high-throughput screens.

Like the polyomavirus early region (where we started), many human genes possess two or more alternative sites of cleavage/polyadenylation, and can hence generate alternative mRNAs that have identical protein coding capacities but differ in the extent of their 3' untranslated regions. Shorter mRNAs generated by polyadenylation at promoter-proximal sites often lack target sites for inhibitory trans-acting factors, and are, therefore, more efficiently translated, for example during T cell activation or in cancer cells. The molecular basis for the shift to favour upstream polyadenylation, and hence promote cell proliferation, is not yet known, but current DPhil student Jessica Goodchild is testing the hypothesis that modification of the cleavage factor I (CFIm) complex is central to this process. This project, examining post-translational modification of proteins in synchronous human fibroblast populations, echoes several aspects of my postdoctoral work on the cell cycle, though happily the technology now at our disposal is way beyond anything we ever dreamed of in the 1980s. So in some respects the trajectory of my research so far has been quite circular; with luck, as T S Eliot wrote, "the end of all our exploring will be to arrive where we started and know the place for the first time".

How to Replicate a Genome

Conrad Nieduzynski

Virtually every living cell contains a complete copy of its genome – the genetic blue print for life. Maintaining genome integrity requires accurate replication of the whole genome prior to each cell division. Replication errors give rise to diversity and fuel evolution, but can also cause the mutations that lead to genetic diseases, including cancer. Therefore, each time a human cell divides, the ~6 billion base pairs that make up our genome must be accurately copied in a process that takes just a few hours. Famously, the double helical structure of DNA suggested a solution: 'It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.' [1]

Watson and Crick's understated 'copying mechanism' has stood the test of time and we now have a molecular understanding of how DNA is replicated in bacterial and eukaryotic cells. However, in addition to accurate DNA replication, cells must also coordinate DNA replication with cell division. It is essential that the whole genome is replicated once and only once in each cell division cycle. Two pathological scenarios must be avoided: no region of the DNA should be replicated more than once and no region should be left unreplicated. My research aims to understand how cells ensure that DNA replication is completed before cell division takes place.

The sheer size of eukaryotic genomes requires that the process of DNA replication must start simultaneously at thousands of sites. These initiation sites, called DNA replication origins, are specialised DNA sequences that assemble the proteins required for copying the DNA. Each replication origin gives rise to two bidirectional replication forks that proceed away from the origin to replicate the flanking DNA. Therefore, complete genome replication requires the activation of sufficient and appropriately distributed replication origins in every cell cycle. Failure to activate sufficient replication origins is associated with genome instability, including chromosome breakage and rearrangements.

Members of my research group have identified the location and activity of replication origins in model organisms. Primarily, we work with baker's yeast (*Saccharomyces cerevisiae*), since this is safe, cheap and ethical, but most importantly all the steps of genome replication are conserved between *S. cerevisiae* and humans. Consequently, advances we make working with yeast will be informative for future studies in human cells and ultimately may help treat human diseases. A major advantage of working with *S. cerevisiae* is that it was the first eukaryote to have its genome completely sequenced and it has been at the forefront of genomics research ever since.
When a region of the genome replicates it changes from one to two copies per cell. Recent advances in genome sequencing essentially provide a precise counting machine, allowing us to accurately determine the time at which each region of the genome replicates (Figure 1A). In this way we have been able to identify replication origins as the earliest DNA sequences to replicate and then track the movement of replication forks as the cells complete genome replication [2]. Not all replication origins activate simultaneously, rather they activate in a characteristic order such that different regions of the genome are replicated at different times (Figures 1B and 2). A further complexity is that, of the many origins available, only a subset is active in each cell division cycle, referred to as the Jesuit model: 'Many are called but few are chosen'. Therefore, to understand how genome replication is completed we have to determine not only the location of replication origins but also

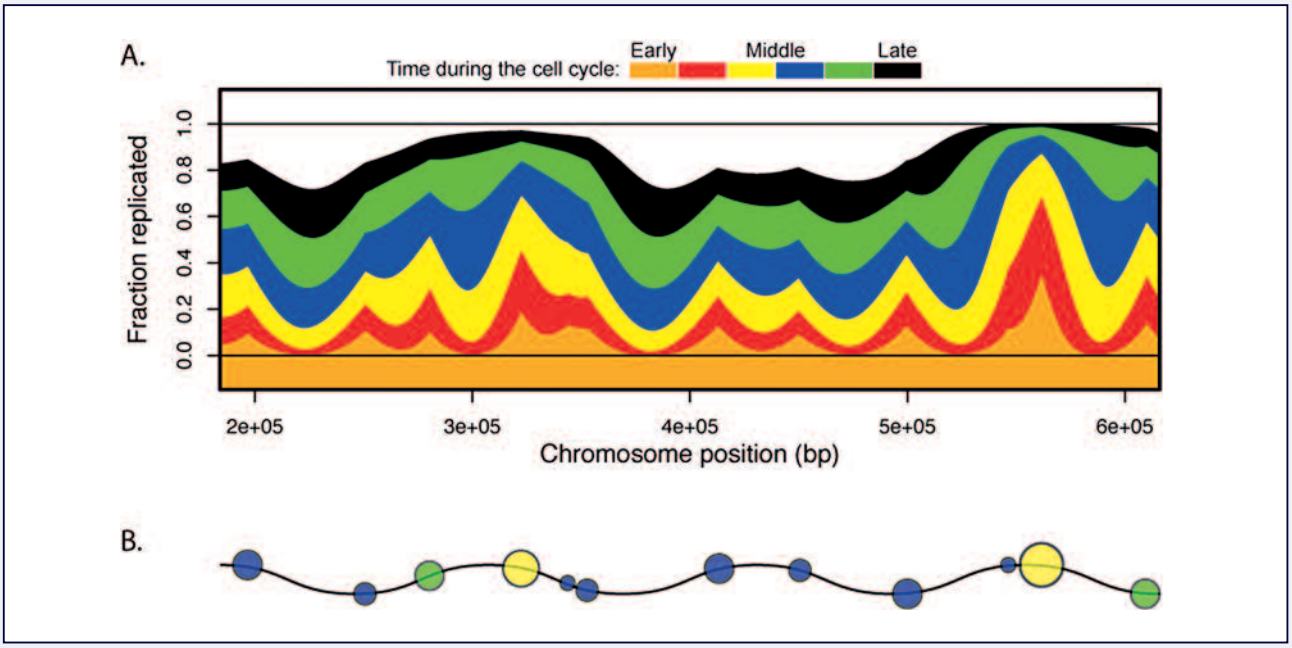


Figure 1. *The dynamics of genome replication. (A) Deep sequencing measures the extent of DNA synthesis at each chromosomal location and time points through the cell cycle. Replication origins are the earliest sequences to replicate and therefore generate peaks, the larger and earlier peaks corresponding to greater activity. (B) A cartoon characterising the location of replication origins (circles), when they are active (yellow is early; blue later) and how active they are (circle size).*

understand what determines the differences in origin activity. What makes some replication origins highly active, whereas others are rarely active? An appropriate number of active origins is critical: too many and the cell can't supply sufficient precursors (dNTPs) and replication errors are made, too few and genome replication may not be completed on time. One of our approaches, known as comparative genomics, involves comparing genome replication in a range of yeast species. Using this approach, we discovered that replication origin activity is generally evolutionarily conserved: if an origin is highly active in one species the equivalent origin is similarly active in other species [3]. However, just occasionally, the activity of a replication origin differs between species and this offers us the opportunity to discover what is responsible for the difference in activity.

In an attempt to study the consequences of insufficient replication origin activity we turned to a species of Archaea, *Haloferax volcanii,* for which our collaborator, Thorsten Allers (University of Nottingham), developed genetic tools. The *H. volcanii* main chromosome has just four replication origins; sufficiently few that we could try to delete most of them. Unexpectedly, we found no detriment to deleting origins and in fact the more origins we deleted the faster the cells grew. Eventually we were able to delete all of the origins from the main chromosome with no obvious defect and accelerated growth [4]. These findings challenged our assumptions about the requirement for replication origins. We now have to determine exactly how *H. volcanii* survives without replication origins and whether similar pathways exist in other organisms or in cancer cells.

In 2014, I made the decision to relocate my research group to the Dunn School from the University of Nottingham. The Dunn School's combination of outstanding laboratory facilities and expertise in cell biology offers us exciting research opportunities and a springboard to future success. There remain fundamental questions about the regulation of genome replication that we will answer in model organisms. However, the Dunn School offers us an opportunity to take our findings one step further and to start to understand how genome replication is completed in human cells. Insufficient replication origin activity contributes to genome instability and human diseases, including cancer. Therefore identifying the mechanisms that act in healthy cells will be a critical first step to understanding what goes wrong during disease development and how it might be treated.

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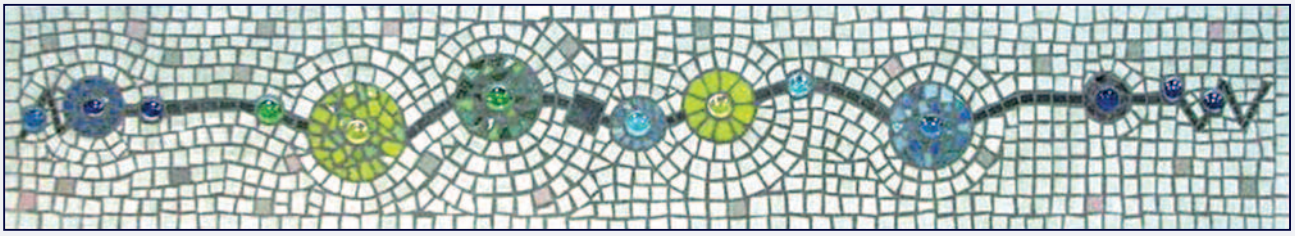

Figure 2. *Chromosome replication mosaic by Dunn School aluma Dr Catarina Gadelha. As in Figure 1B, the circles represent replication origins with the size and colour indicating activity.*

Eclipse: On 20th March 2015, members of the Dunn School witnessed the solar eclipse, some taking to the roof of the main building for the best view in town. This picture of the event was taken by Patty Sachamitr.

Research Focus:

Signalling, Cell Biology and Rhomboids

Matthew Freeman

The fundamental issue that my laboratory has been addressing since I set up my own group at the MRC Laboratory of Molecular Biology in Cambridge over 20 years ago, is how cells communicate with each other. Since most of the decisions that cells make are guided by signals from their environment, this question underlies much of biology. It is not directly medical, but understanding intercellular signalling provides a biological foundation for a myriad of future medical advances.

Like so many good stories, it starts with a fruit fly. For almost 100 years, *Drosophila* has been the model of choice for scientists wanting to understand cell and developmental biology of animals. On establishing my own group, my plan was to use *Drosophila* genetic screens to ask the question of how a rather small number of core signalling pathways can control a huge number of developmental decisions. The numbers imply that each pathway must control many different decisions, further implying that signalling must be regulated very precisely. Genetic approaches allowed us to ask how such precision is achieved without preconceptions.

Our focus was the *Drosophila* epidermal growth factor (EGF) receptor pathway, whose human counterpart is implicated in many diseases, most notably cancer. The results of years of our work can be outlined in three broad conclusions, all rather unexpected at the time. First, much of the control occurs in the cell that sends the signal; then, and even now, there is more focus on the receptors and downstream signalling events within the cell receiving the signal. Second, since many components of signalling pathways are membrane-embedded proteins, the cellular machinery that regulates the trafficking of membrane proteins is also implicated in the control of signalling. And third, the protein with the most important role in the control of EGF receptor signalling in flies was a completely novel membrane protein called rhomboid (for arcane fly genetic reasons) (Wasserman *et al.*, 2000). The problem was that rhomboid looked like no other known protein, despite being conserved in all organisms, from bacteria to humans, so we had no idea what it did.

To complete this historical survey, we did eventually figure out that rhomboid was the first of a completely new kind of enzyme – an intramembrane serine protease (Figure 1) (Urban *et al.*, 2001), an enzyme that cuts other proteins but, unusually, with its active site embedded in the lipid bilayer of cell membranes. Consistent with that intramembrane location, the sequences that rhomboids cut are transmembrane domains of other proteins. More than 50 years ago, proteases were the first class of enzyme whose catalytic mechanism was worked out, and the idea that they could work in a hydrophobic lipid bilayer was sufficiently heretical that scientists in the field took a while to accept that rhomboids were indeed proteases. Eventually, however, this all made sense (we were right!) and we were able to explain how rhomboid in flies controls EGF receptor signalling by releasing the active signalling ligands from the cells sending the signal (Figure 2).

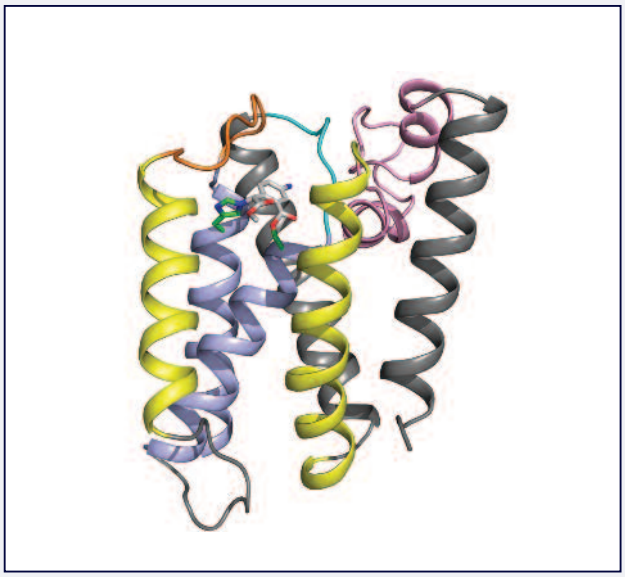

Figure 1. *Atomic structure of a rhomboid protease, viewed from the plane of the membrane in which it is embedded. The trans membrane helices surround the active site, which is highlighted in this structure by a bound molecule of an isocoumarin inhibitor. From Vinothkumar* et al., *(2010)*

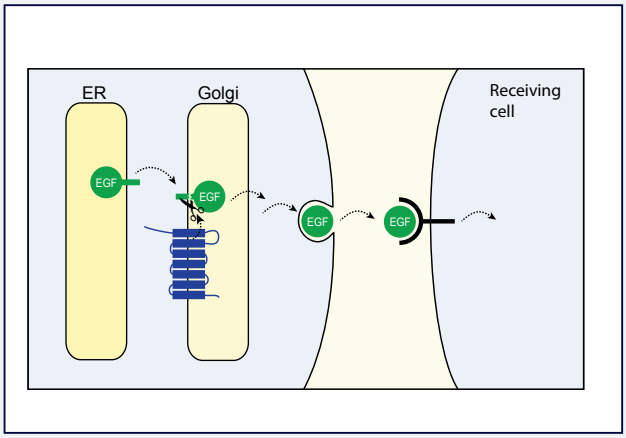

Figure 2. Drosophila *Rhomboid-1 (blue) cleaves EGF. EGF is synthesised as a membrane protein. As it moves through the secretory pathway, it encounters Rhomboid-1 in the Golgi apparatus, where the extracellular signalling domain is cleaved from the transmembrane domain, converting it into a soluble ligand. The active ligand is then secreted from the cell, to signal to a nearby cell.*

Rhomboid proteases, the bigger picture

Since rhomboids are found in almost all organisms, but EGF receptor signalling is restricted to animals, it was immediately clear that they must have functions beyond this one signalling pathway (Freeman, 2008). Their novelty and ubiquity led us to pursue them more broadly. We have now worked on rhomboid proteases in many contexts. We showed that all eukaryotes have a distinct mitochondrial rhomboid, which in humans is now believed to be relevant to several diseases including type II diabetes and Parkinson's Disease. We found that rhomboids in apicomplexan parasites (which include the malaria parasite, *Plasmodium*) may contribute to parasitic invasion of host cells. And we discovered that a bacterial rhomboid regulates a protein export channel called Tat, which can control aspects of bacterial virulence. In all these cases, our initial discoveries have seeded ongoing work by others but, being far from our own core expertise, we have chosen not to pursue them. Currently our focus on rhomboid proteases is to understand the role of the biggest class in mammals, which reside in the secretory pathway (like the one we originally discovered in flies) and which we believe also control signalling between cells.

Dead rhomboids – iRhoms and beyond

More recently our primary effort has shifted in an unexpected direction. It has become clear that rhomboids are just one branch of a much larger superfamily of distantly related proteins (Freeman, 2014); strangely, these relatives have lost their protease active sites during evolution. It is something of a mystery as to what well-conserved dead proteases might be doing, and this has become a major recent focus of my laboratory, with a particular emphasis on one specific sub-group called the iRhoms.

iRhoms clearly do several related things, so one of the current challenges is to develop a coherent explanation that integrates these superficially distinct roles. In one context iRhoms control EGF receptor signalling by regulating the stability of EGF ligands in the signal-emitting cell (Zettl *et al.*, 2011). In another context, and of particular medical relevance, iRhoms control the activation in mammals, including humans, of the primary inflammatory cytokine, TNF (Figure 3) (Adrain *et al.*, 2012; McIlwain *et al.*, 2012). This takes us into the realm of anti-inflammatory therapeutics, an enormously active pharmaceutical area. In yet another context, iRhoms regulate the release of growth factors that are implicated in cancer (Li *et al.*, 2015; Siggs *et al.*, 2014).

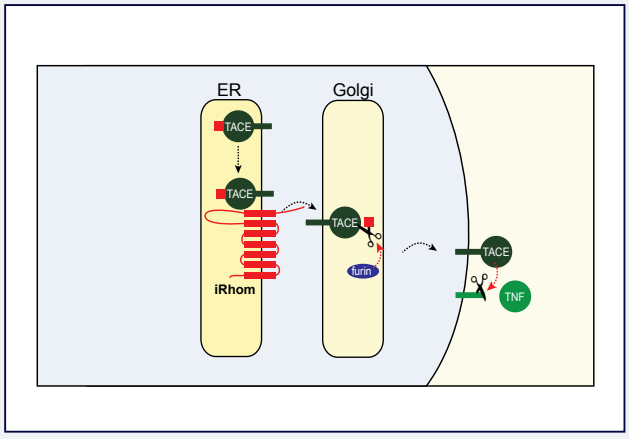

Figure 3. *In mammals, iRhom (red) is necessary for release of the inflammatory cytokine TNF. iRhom traffics the protease TACE from the ER, allowing its maturation and release to the cell surface. At the cell surface, TACE cleaves and releases TNF in response to inflammatory signals.*

Notably, although none of this work has yet led to medical advances, it lays foundations for future translational opportunities.

The iRhoms represent only one of several branches of the rhomboid-like superfamily and, given the significance of both branches studied so far (the active rhomboid proteases and the iRhoms), we are now pursuing some of the more distant relatives. Beyond just wanting to tick off as many rhomboid-like proteins as possible, which could be construed as mere stamp collecting, we want to understand the underlying functional theme that relates them all: they all evolved from a single ancestral protein in the last universal common ancestral organism, so we expect them to have related functions. There is not yet an answer, but a current guess is that the rhomboid-like protein fold – the core domain they all share – recognises transmembrane domains of other proteins. When the rhomboid also has an active site, as in the protease branch of the superfamily, they recognise and cut their substrates. In the cases that have lost the active site, binding a transmembrane domain leads to other consequences.

Final thoughts

iRhoms and other dead rhomboids illustrate an intriguing common principle. Genome sequences show that most classes of enzymes have inactive homologues. That these are often well conserved, implies that they are more than evolutionary junk – they must have evolved a function. In a recent survey (Adrain and Freeman, 2012), it became clear that in almost all cases where the role of an inactive enzyme cognate has been investigated, it is functionally related to the ancestral enzyme. In other words, dead enzymes often regulate the same processes as their active counterparts. This leads to the prediction that these relatively understudied proteins may be a rich source of new regulators of well-described cellular processes.

Finally, the medical potential of manipulating intercellular signalling is already well established – many existing drugs interfere with specific signalling pathways. The more specific question about the medical significance of the rhomboid-like superfamily is less well answered; it's too early to be sure about the specific opportunities. But the early indications are clear and many are being pursued. Rhomboid-like proteins have been discovered to have roles in cancer, parasite invasion, metabolic processes relevant to diabetes and Parkinson's Disease, and the life cycle of infectious bacteria; together, these add up to a good bet on the future medical relevance of the rhomboid-like superfamily. And as in so many prior cases, fruit flies led the way.

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Neil Barclay first came to Oxford to study biochemistry as an undergraduate but stayed to develop a very productive career at the cutting edge of immunology research. Here, he reflects on his career path and achievements as he now approaches retirement.

Life at the Surface of Leukocytes

Neil Barclay

When I was invited to write this article I was shocked that it is 37 years since I came to the Dunn School. What can one do for 37 years in one place? The answer is a simple one: doing research allows you to find out new things which, in many professions, is simply not possible. Furthermore, the Dunn School is one of the best research laboratories in the world. Add Oxford to the mix as a great city to live in and it all seems quite simple. It all started in the Biochemistry Department where I was studying biochemistry and, in particular, a project on the structure of immunoglobulins on the fourth floor in the unit headed by Rodney Porter. After a few weeks, Rod won the Nobel prize and took us off to the Kings Arms to celebrate – science was exciting! Now I was fixed on research. I chose to do a research project with Alan Williams on one of the few cell surface proteins expressed by lymphocytes and neurons that were known at the time, called Thy-1; key factors in this choice were firstly, I thought it was a very interesting scientific question and secondly I thought there was a scientific approach that might make it work. I was already fascinated by the cell surface and that interest continued throughout my academic career. It struck me that the surfaces themselves were where the key decision making went on and determined what cells did. Nowhere is that more important than in the immune and nervous systems.

Thy-1 and OX2

When I look back, it is remarkable how different science was then. We spent lots of time thinking about how to tackle a problem and even more time making reagents such as polyclonal antibodies. Now it is so easy to obtain reagents commercially the very next day. With Thy-1 the difficulty was in purifying it in sufficient quantities in order to perform biochemistry. Thy-1 was the first molecule to be biochemically characterized from the lymphocyte cell surface. We thought the sequence would be interesting but my attempts to sequence it failed. Whilst I worked in Sweden for two years, Alan Williams finally cracked the sequence and initiated the 'Immunoglobulin Superfamily' concept that changed the way we think about the structure and evolution of cell surface proteins. Alan joined the Dunn School in 1978 as director of the MRC Cellular Immunology Unit to replace Jim Gowans, who had moved to run the MRC. From the outset, Alan had recognized the potential of monoclonal antibodies (mAb) and following his successful collaboration with César Milstein, set about making his own mAb. So started the production of the OX series of mAb: OX1 recognized the leukocyte common antigen (CD45), raised against purified protein, while OX2 was raised against a pool of glycoproteins from thymus and was probably the first new protein to be identified by mAb technology. In October 1978 I rejoined Alan and Don Mason who was already in the Unit (Figure 1). One of my first projects was on OX2: from that point onwards, my career tended to follow OX2, now known as CD200. Firstly, we investigated where it was expressed, using the new technique on immunoperoxidase staining of tissue sections. But then the biochemistry suggested it might have similarities to Thy-1 - could it be related to immunoglobulins as well? The biochemistry was difficult as the protein was much less abundant than Thy-1. However, with the advent of the molecular biology era, I set up cDNA cloning to determine the amino acid sequence of membrane proteins. OX2 was the first protein to be sequenced this way, followed by OX1 (CD45) and then several more leukocyte proteins.

Figure 1. *Don Mason, Alan Williams and Neil Barclay in the 1980s, the heyday of the Cellular Immunology Unit.*

Making recombinant proteins and interactions

Clearly one was limited in obtaining membrane proteins in sufficient amounts to perform good biochemical analysis but we obtained an expression system from Celltech which gave us up to 400 mg/L in the early 90's that transformed our study of protein-protein interactions. I found out about the BIAcore apparatus shortly after it was introduced. This allowed us to study the interaction of proteins in real time, at high sensitivity using a detection system called surface plasmon resonance. The interactions between the cell surface proteins were very low affinity and this made identification of new

interactions difficult. The answer to this was in developing a bead assay where the protein was immobilized on beads to make it multimeric and a number of interactions were identified, including the OX2 (CD200) receptor. Although the science was going well, this period was marked by the death of my long term colleague and close friend Alan Williams, who was elected to the Chair of Pathology at the Dunn School but unfortunately died in 1992 before taking up the post. Following his death, there followed a period of difficult relations with the MRC but strong support from my colleagues Don Mason and the Head of Department, Herman Waldmann.

Structural analysis

I tried very hard to get a structure of full length CD4 membrane protein using many approaches but good diffracting crystals were elusive, although the structure for two of the four domains was eventually characterised. More recently, a full characterization of CD200 binding its receptor (CD200R) and also another interaction which shows similarities, namely CD47 binding SIRPα, were determined. This has given insight into how these receptors interact and how they might have evolved under the influence of pathogens. There is considerable interest in blocking both of these interactions to provide therapeutics in the fast moving area of immunotherapy for cancer. Finally this year, we solved the structure of Thy-1 (Figure 2) which tantalizingly has come up with some more interesting puzzles in addition to the expected identification of an immunoglobulin-like domain.

Disulfides and the future.

Looking at proteins and their amino acid sequences provided the stimulus to investigate the role played by disulfide bonds in membrane proteins and, in particular, whether they might be broken during immune responses thereby changing the activity of the membrane proteins. This project has proven technically challenging and very difficult to fund. Although not trendy yet, it is clear that membrane protein activity is altered by the redox changes that occur in inflammation which may have relevance to the study and treatment of immune disorders and cancer.

Industrial connections - Everest Biotech and Absolute Antibody

My interest in industry started with my DPhil student Nick Hutchings. We were bemoaning the lack of antibodies for our research but Nick talked to his friend from Nepal who suggested that since there were 6 million goats in Nepal, we could make antibodies against all the proteins of the human genome. My initial response was to laugh but then we thought why not give it a go and about a year later we obtained some funding and the company went into production in 2000. The company had to contend with political crises, electricity cuts, earthquakes and variable sales. The long-term future for polyclonal antibodies is limited as one would obviously much rather have the clearly defined specificity of monoclonal antibodies. Nick Hutchings persuaded me to take part in another venture, Absolute

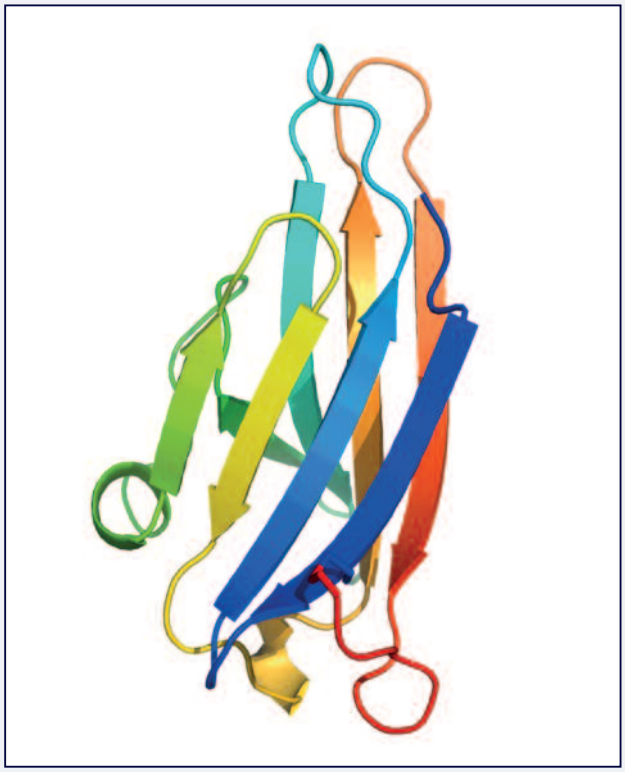

Figure 2. *The immunoglobulin-like structure of Thy-1*

Antibody. This is completely different in that it makes very high-quality reagents using recombinant DNA technology and was founded in 2013 with Geoff Hale from the Dunn School, a pioneer in the application of therapeutic antibodies, and Tim Bernard, former chief executive of Serotec. This new company specializes in the cloning of antibodies and their expression using transient high-level expression methods. In addition to making chemically-defined reagents it makes modifications such as switching the constant regions with those from different species and also making different fragments, such as Fab and F(ab')$_2$.

Conclusion

It has been a great pleasure to work at the Dunn School with so many good colleagues and friends that I don't have space to acknowledge them all but Figure 1 shows my close colleagues Alan Williams and Don mason in the 1980's and I have mentioned the long-term support offered by Herman Waldmann. I should also mention my close colleagues in the lab for more than 15 years, Debbie Hatherley, Marion Brown and Anton van der Merwe. And so to the future? The upside will be not having to write grants that aren't funded and the worries of running a lab. There will be more time for the things I never managed to get done. The biggest void will be the end to planning research projects, getting experiments done, interacting with my group and sharing the enthusiasm of students starting out on their research career.

Early career scientists in the Dunn School are encouraged to find out about the opportunities and challenges of conducting research in other parts of the world to place their own studies in a broader context. Anna Senft is a final year DPhil student in Liz Robertson and Liz Bikoff's laboratory and recently travelled to Madagascar with the alumni of the Life Science Lab in the German Cancer Research Centre. Here she reflects on her trip and the work of a PhD student there for whom the relevance of her research is uncomfortably close to home…

Fleas for Plague Research — a visit to the Institute Pasteur Madagascar

Anna Senft

The fleas jump, but cannot escape. The basin is too deep. With a rasping sound Adélaïde slides a hard-bristled brush through the fur of a dead rat at the bottom of the pale-coloured basin (Figure 1). Using a pump she transports the fleas to a glass jar. It contains sterilized rice bran and food for the flea larvae (Figure 2). To feed the adult fleas, Adélaïde tested multiple methods. Currently, she has to rely on putting a living young mouse into the jar. The blood source is short-lived: it has to be replaced weekly to keep the fleas alive.

Figure 1.

Adélaïde Miarinjara is a PhD student at the Institute Pasteur in Antananarivo, Madagascar. She is researching methods of bubonic plague prevention. The life-threatening disease has been eradicated in most countries, but not here. In 2012, Madagascar accounted for 67% of the ~2000 worldwide plague cases recorded that year. The bacterium responsible for plague, *Yersinia pestis,* is transmitted via fleas, which live on rodents. The Malagasy government tries to contain the disease by killing the fleas with the insecticide Deltamethrin. "One possible explanation for the recent outbreaks is the emergence of insecticide resistance in fleas," Adélaïde says. During the course of her PhD, she would like to understand whether different flea populations in Madagascar's plague areas display variation in susceptibility to Deltamethrin and, if so, why.

Figure 2.

Poverty and cultural practices impact on the incidence of plague in Madagascar, which is restricted to the highlands of the island above an altitude of 800m. In rural areas, rats live in close proximity to humans. Additionally, people in remote villages often prefer traditional healers to health centres and thus delay antibiotic treatment. With field trips to remote villages inaccessible by off-road vehicles (Figure 3), the Institute Pasteur Madagascar (IPM) and Adélaïde are working at the coal face of the problem.

Founded in 1898, during the French colonial era, the IPM was built just eleven years after the main site in Paris (Figure 4). Today, it is part of an international network of 32 Pasteur institutes. Since Madagascar's independence in 1961, the institute has been the subject of a convention between the Malagasy government and the Institute Pasteur Paris. Originally founded to treat rabies and smallpox, the IPM still devotes itself to diagnosing, preventing and treating infectious disease like plague, malaria and tuberculosis as well as viral and other parasitic diseases. The IPM is the largest research institute in the country.

Figure 3.

Adélaïde is one of 30 PhD students at the institute. "It is a great opportunity to do a PhD here", she says. "We get the best lab materials and technology in Madagascar. I enjoy working in an environment of specialized national and international scientists where everybody is supportive." She also profits from a grant paid to PhD students and has free access to opportunities ranging from training in bioinformatics to courses on geographic information systems.

This exceptional environment is part of the strategy of institute director Christophe Rogier. "Madagascar's science has to become competitive", he says. He promotes publications and international exchange whenever he can. But Madagascar's geographical isolation and history make simple steps like scientific communication in English or international collaborations difficult. The IPM therefore provides English courses and travel stipends. Adélaïde has just applied to attend an international conference of the Indian Ocean Commission held in Mauritius. The focus is on epidemiological monitoring and its impact on public health. "I hope to have the opportunity to encounter international research outside of Madgasacar to bring our work forward".

Adélaïde's project connects research with medical improvement. Madagascar is one of the poorest countries in the world. The IPM offers diagnostic services to supplement the Malagasy health system. Roughly 400 people make use of these services each day. In a country, where people are still dying from diseases, such as bubonic plague, that can normally be treated, medical diagnostics has priority over curiosity-driven research. For her flea study, Adélaïde collaborates with the ministry of health and local authorities to collect rats from plague areas.

Her experiments, recently published in the research journal *Public Library of Science One* (*PLoS One*), show that only 2 out of 32 tested flea populations are susceptible to Deltamethrin and therefore suggest that the current insecticide strategy used by the government is ineffective against fleas. She supports a move to stop using Deltamethrin and to change instead to a more effective strategy. She is currently screening alternative insecticides to find the most effective substance for the national flea control program and to elucidate the mechanism of resistance. Her work is going well: "We are submitting our next manuscript this month", Adélaïde says.

Figure 4.

Adélaïde's research might contribute to preventing further outbreaks of plague in Madagascar and also in other parts of the world. It might be a step towards eradicating a disease that has troubled humanity for hundreds of years. Her work, thus, reaffirms the three values of the Institute Pasteur: for research, for health, for our future.

Photographs courtesy of Adélaïde Miarinjara (Institute Pasteur Madagascar) and Johannes Schmidt (University of Oxford).

Sir Henry Harris was Professor of Pathology and Head of the Dunn School from 1963 until his retirement in 1993. His scientific legacy has been immense: indeed it is his work on cell fusion to which this newsletter owes its very name. Sir Henry died suddenly but peacefully at home in Oxford, on 31st October 2014. Here, Eric Sidebottom reviews his extraordinary life.

Sir Henry Harris: A Life in Research

Eric Sidebottom

Henry first set foot in Oxford in April 1952 as a Travelling Scholar of the Australian National University. His first impressions of Oxford are set out vividly in his autobiography "The Balance of Improbabilities". He was to spend most of the next 62 years in Oxford and so, during that time, he became the most familiar figure in the Dunn School. He continued to visit and indeed work in the department until shortly before his death.

Harris was born on 28th January 1925 in Russia, the son of Sam and Ann Harris. The family emigrated to Sydney, Australia, when Henry was 4. He was educated at various primary schools but then at the prestigious Sydney Boys High School and Sydney University where he graduated in Modern Languages in 1944 before studying medicine and graduating in 1950. In 1952 he came to Lincoln College Oxford to study for a DPhil under Howard Florey, Professor of Pathology at the Dunn School until 1963. He completed his DPhil in 2 years, graduating in 1954.

Harris then became Director of a small British Empire Cancer Campaign Research Unit. Here he declared his independence from Florey by not following his mentor's advice on what research projects to pursue. He became interested in RNA turnover in cells and his work convinced him that the then current hypothesis about messenger RNA as set out in the Nobel prize-winning work of the Frenchmen Jacob and Monod was not entirely correct. Not surprisingly this work was controversial and ensured that Harris's name became well known. After a short spell as a visiting scientist at NIH in the USA, Harris returned in 1960 as Head of the Department of Cell Biology at The John Innes Institute in Bayfordbury, Hertfordshire. In 1963 he was elected to succeed Florey as Professor of Pathology on the latter's decision to relinquish the Chair and accept the invitation to become Provost of Queen's College.

As Head of the Dunn School, Harris was able to broaden his research programme and attract considerable numbers of high quality graduate students, 'postdocs' and senior visiting scientists. This constant flow of scientists ensured that the laboratory was a lively environment fostering high quality research. Soon after taking the chair, his discovery (with John Watkins) of artificial cell fusion hit the headlines and strengthened his reputation as an innovative and courageous scientist. This work was to have far reaching consequences in cell biology, genetics and cancer research. Harris himself, collaborating with George Klein, demonstrated the 'suppression of malignancy' in hybrid cells; work leading to the later identification of tumour suppressor genes. Again this work was controversial but it certainly stimulated a great deal of discussion and much further

experimentation. Indeed, exploring mechanisms of tumour suppression remained Harris's abiding interest until the end of his life.

In 1979 Harris was elected as Regius Professor of Medicine, Oxford University's senior medical chair. Once again this was a controversial appointment but it confirmed the opinion that Harris was not only an outstanding scientist but an efficient leader and administrator. Harris only agreed to accept the chair so long as he could retain the Headship of the Dunn School, the base for his experimental work. This was agreed but Harris, like his mentor Florey, was known not to be a great admirer of clinical research and, it is rumoured, was opposed to the creation of a chair of General Practice and the founding of the Institute of Molecular Medicine (IMM) on the John Radcliffe hospital site. There is a certain irony in the fact that the first holder of the chair in General Practice was his own GP and that the IMM has now been renamed the Weatherall Institute of Molecular Medicine after David Weatherall, his successor as Regius Professor of Medicine.

His tenure of the Headship of the Dunn School was extended, following the untimely early death of Alan Williams who had been elected to succeed him as Professor of Pathology in 1992 but died before taking up office. This meant that Harris was actually Head of the Dunn School for 30 years. Indeed he was a regular presence in the Department for 62 years, continuing until shortly before his death.

In addition to planning and carrying out experiments, Harris enjoyed writing. His style was simple, clear and unambiguous. He expected the same high standard from his students and collaborators. Early drafts were often returned covered in red ink with suggestions that Fowler's Modern English Usage should be consulted.

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His first book 'Nucleus and Cytoplasm' was published in 1968 and attracted much critical attention since it discussed several of the most controversial issues of the time. Its prologue from Robert Frost's poem 'The Road Not Taken' explains Harris's scientific philosophy rather well:

'Two roads diverged in a wood, and I
I took the one less travelled by,
And that has made all the difference.'

His second book *Cell Fusion*, published in 1970, derives from The Dunham Lectures delivered at Harvard University and covers perhaps the most productive period of his research. His later books include an autobiography, *The Balance of Improbabilities*, a collection of short stories, *Remnants of a Quiet Life* — fictional but mischievous, based on sharp observation of colleagues at work and play — and several historical works on cell biology. His output of high quality scientific papers was considerable and continued to the end of his life.

Not surprisingly for someone of his distinction, Harris served on many local, national and international committees and advisory boards. He received many honours, including degrees, medals and prizes. He was elected to Fellowship of the Royal Society in 1968 and was knighted in 1993. As Professor of Pathology, he held a Fellowship at Lincoln College and as Regius Professor of Medicine, a Studentship at Christ Church. Both these attachments gave him much pleasure and he was able to share his wide scholarship with colleagues in both colleges.

His extensive papers have recently been catalogued by the Wellcome Library. The announcement entitled 'Sir Henry Harris: a life in research' is a fitting epitaph.

In 1950 Henry married Alexandra Brodsky, a marriage that survived almost 64 years. They had three children, Paul, a lawyer, Helen, a linguist and author, and Ann, a scientist. All survive him. As a scientist, Henry Harris was a public figure but as a family man, he was much more private.

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.

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