fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

ISSUE 12 · MICHAELMAS 2013

UNIVERSITY OF OXFORD

Focus on Molecular Biology

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Bioimaging at The Dunn School

Editorial

In the last edition of* Fusion, *I described how excited I was about my impending move to the Dunn School. I have now been in post for about six months and, although slightly overwhelmed by the amount I have to learn, I am having a great time! My colleagues have been welcoming, I'm discovering how good the science is, and plans are well underway to take maximum advantage of the outstanding facilities that we now have — thanks to Herman Waldmann's ambition and vision.

The recruitment of new group leaders into one of the empty floors of OMPI has been my main focus so far, and I think that by the end of 2013 we may have as many as eight new groups at the Dunn School. My overall vision for the Department is to build an internationally leading position in the cell biology of human diseases. It's hard to know exactly how the founders of the Dunn School defined 'pathology', and our name has always been confusing to outsiders: after all, I don't think that the Department has ever provided clinical pathology services. In practice, the Dunn School has always focused on disease mechanisms and therapeutics; in the 21st century this broadly translates into the cell biology that underlies human disease.

My own work is located at the basic end of the research spectrum but I much admire the successful history of translation at the Dunn School, and one of my goals is to maintain that basic/translational balance. Not only is it a powerful combination scientifically but, on a more worldly note, it places the Department in a strong position to take advantage of current political and funding pressures. Lest this be misinterpreted, however, as being too focused on the current vogue of 'impact', let me be clear that I firmly subscribe to the idea that the biggest long-term impacts are likely to arise from fundamental research.

Although recruitments are still ongoing, I can now report on four new members of the department. Dr Ulrike Grüneberg has been appointed a University Lecturer, and is currently moving from the Biochemistry Department, where she has held a Cancer Research UK Career Development Fellowship. Ulrike studies mechanisms of cell division and how it is regulated. Dr Ivan Ahel is moving his group from the Patterson Institute in Manchester, where he has been researching the molecular mechanisms underlying DNA damage and repair.

Dr Dragana Ahel also moves from the Patterson Institute to establish a group working on DNA helicases in genome stability and cancer. In addition to these new faces, Dr Monika Gullerova, until last year a post-doc in Nick Proudfoot's group, is now establishing her own group at the Dunn School, having been awarded an MRC Career Development Award. You can learn more about these new members of the Department in this edition of *Fusion* and from the Dunn School website; the only comment I will make here is that I am proud that we can attract people of such outstanding calibre.

We also say goodbye to a number of people this year. Simon Hunt has been at the Dunn School from his days as a student in the 1960s, and his impending retirement will, therefore, represent a huge change: a loss for the Department and no doubt a period of significant adjustment for him. Peter Cook and Herman Waldmann are also formally retiring, although they both have research projects to complete and will, in practice, remain in the Department for a little longer: our gain. Of course, turnover and renewal are essential, but the Department will take some time to get used to the retirement of three such prominent members. We marked the occasion with a dinner at Lincoln College, which was attended by many alumni and friends of the Department.

There are many other developments at the Dunn School, and they will be reported in subsequent issues of *Fusion*: for example a new website is underway, there will be further recruitments, and we are preparing for the 'REF', the major governmental assessment of research quality that will have major financial implications for our next five years. In the meantime, you can stay in touch via our website, or indeed follow our new departmental Twitter account @Dunn_School.

Matthew Freeman

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Front cover: *The Japanese Garden is a recent, notable addition to the Dunn School, ably captured in all its glory by Tim Davies.*

Interview with Ivan Ahel

Ivan Ahel has recently moved with his family to Oxford from the Patterson Institute in Manchester.* Fusion *caught up with him in the midst of his move, to find out more about his background and plans for the future.

Perhaps you could tell us a little about your background and how you first became interested in science.

As a child I was always curious about nature and living organisms and was, therefore, always keen to become some sort of field biologist. During my studies at the University of Zagreb in Croatia, I realised about the exciting complexity of life at the cellular and sub-cellular level and directed my interests more towards molecular biology. I remained in Zagreb for my PhD, investigating how microorganisms sense and respond to DNA damage. Although during the course of my PhD, the limited capacity of Croatian science to carry out high calibre research was a big challenge, it also served as a stimulus for me to develop my own career. I therefore took the opportunity to join Professor Dieter Söll's laboratory at Yale University as a postgraduate research associate working on the function and evolution of aminoacyl-tRNA synthetases – the proteins ensuring the faithful translation of genetic information. For my postdoctoral investigations I joined Dr. Stephen West's laboratory at Clare Hall Laboratories, London Research Institute, UK, which took my research back to the field of DNA repair. After a successful post-doc at Clare Hall, in 2009 I was appointed to a position at the Paterson Institute for Cancer Research as a junior group leader.

What is currently the main focus of your research and how do you see it evolving in the future?

The general interest of my laboratory centres on the cellular mechanisms underlying genome stability and how these processes link to human disease. This is a wide field of research and my particular focus at the moment surrounds the cellular pathways regulated by a family of proteins called poly(ADP-ribose) polymerases (PARPs). PARPs are enzymes that, in response to different stimuli, modify proteins by a peculiar type of post-translational protein modification made of chains consisting of repeating ADP-ribose nucleotide units. In this way, PARPs regulate many key processes involved in the maintenance of genome stability such as DNA repair, transcription, cell division and apoptosis. Our goal is to understand how poly(ADP-ribosyl)ation signalling is regulated in human cells and also the exact roles for proteins that are targeted by this signalling. This knowledge should greatly increase our understanding of how defects in the PARP-dependent pathways contribute to the development of human disease, in particular neurodegenerative disease, cancer, immunodeficiencies and developmental disorders. Furthermore, since in recent years drug-targeting of DNA repair enzymes has become a promising strategy in cancer treatment (for example inhibitors of PARPs are used to treat certain types of hereditary breast and ovarian cancers), we are investing a part of our research into the development of small molecule inhibitors against novel enzymes involved in PARP-dependent pathways.

In your opinion, what has been the most exciting discovery you have made to date?

Our most exciting discoveries have been the elucidation of molecular functions for several novel proteins involved in DNA repair pathways, which also shed important light on the molecular mechanisms underlying human genetic disease. For example, we have elucidated the molecular function and reaction mechanism for Aprataxin, a DNA repair enzyme that is deficient in the human neurological disorder Ataxia with Oculomotor Apraxia type 1. We also recently described an enzyme responsible for the reversal of cellular protein poly(ADP-ribosyl)ation called TARG1/C6orf130 and showed that deficiency of this protein leads to severe neurodegeneration.

What advice might you give to graduate students contemplating pursuing a career in science?

The excitement of making new discoveries and understanding how life works gives a highly rewarding feeling, especially when it is combined with the obvious benefit for health and society in general. Also, it is never boring working in science, in fact I have found that it is certainly one of the most fun jobs you can imagine. I would advise students to go bravely into their research and try to enjoy it without worrying too much about the future and competition. It is important to remain open-minded, think critically, be responsible and possess a willingness to learn. Never be shy to seek advice and stay on top of your project!

Moving to the Dunn School is bound to be a big transition: what hopes and fears do you have about your move?

Generally, I have a great feeling about the move. My main fear is how my family is going to settle in Oxford, but after we go through that stage I believe living and working in Oxford will be a joy. Oxford is a lively, vibrant place; there is so much great science in a relatively small area which gives lots of opportunities for interactions and collaborations. My interests fit well within the Department, but at the same there is a great diversity in research here. The balance between clinical and basic research is also optimal for my liking. So altogether, I anticipate successful and exciting times ahead. My current feeling is that I could potentially remain in Oxford for the duration of my career.

Focus on Molecular Biology: Big news from small genes

Shona Murphy

My laboratory has a long-standing interest in the control of gene expression at the level of transcription and RNA processing. Most of the work in these fields focuses on expression of protein-coding genes, from which messenger (m)RNAs are transcribed. Instead, our laboratory studies the expression of the genes for non-coding small nuclear (sn)RNAs, whose RNA products are involved in processing other RNAs. For example, the ubiquitously-expressed U1 and U2 snRNAs are required for splicing the introns out of pre-mRNA to make a functional mRNA that can be translated into protein. The U1 and U2 snRNA genes are transcribed by the same multisubunit RNA polymerase as protein-coding genes, RNA polymerase II (pol II), but are different in several respects (Figure 1). The promoter sequences that recruit pol II are different and are recognised by a different set of transcription factors, snRNA genes are very short and simple, with no introns in the transcript, and 3' end formation of snRNAs is directed by a 3' box rather than the polyadenylation (polyA) signal, present in most pre-mRNAs (1). The snRNA genes are, therefore, a good model system to tease out the fundamental mechanisms underlying control of expression of all genes transcribed by pol II, while at the same time working out how the specific features of each type of gene are utilized.

In both protein-coding and snRNA genes, transcription and RNA processing are tightly coupled and our most recent work has focused on understanding the mechanics of this connection. It turns out that the pol II itself is a key player in this coupling and can profoundly influence how factors are recruited for transcription and RNA processing. The largest subunit of pol II has a very unusual structure at the C-terminus called the carboxyl-terminal domain or CTD, with 52 repeats of the consensus heptapeptide tyrosine/serine/proline/-threonine/serine/proline/serine or $Y_1S_2P_3T_4S_5P_6S_7$ for short.

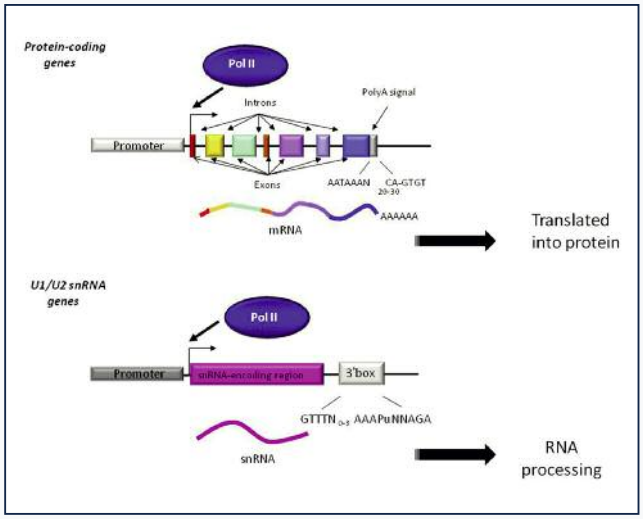

Figure 1. Protein-coding and snRNA genes have different structures. The forward arrows on the schematics of a typical protein-coding and snRNA gene indicate where transcription starts. The consensus sequences for the polyA site and 3' box are noted below the schematics.

Intriguingly, each of the amino acids can be modified; tyrosine, serine and threonine by phosphorylation and proline by isomerisation (Figure 2) and all modifications have been identified in living cells (1). There is, therefore, the potential for a very large number of different combinations of modifications along the CTD. For example, there are already more than 20 different combinations if up to 3 different phosphorylations can co-exist on one single repeat, and this can be multiplied by the 4 different combinations of proline isomerization state and then by 52, the total number of repeats. In reality, the number of different combinations is likely to be restricted by incompatibility between modifications and the availability of the enzymes that carry out the modifications.

CTD modifications are known to orchestrate the sequential recruitment of transcription and RNA processing factors during the transcription cycle and a complex interplay of enzymes that either add or remove modifications, results in changes in CTD modification during the transcription cycle. For example, phosphorylation of S_5 predominates early in transcription of both protein-coding and snRNA genes, while phosphorylation of S_2 predominates towards the end (1). It has been suggested that the many different potential combinations of CTD modifications produces a code that is read by the factors recruited for transcription and RNA processing (1). Good progress is being made towards a full understanding of how this code is written and read and, interestingly, some CTD modifications appear to play a specific role in expression of snRNA genes.

We found that phosphorylation of S_7 is not required for expression of protein-coding genes but is required to recruit the large multisubunit Integrator complex specifically to snRNA genes (2). Integrator recognises the snRNA gene-specific 3' box RNA processing element (Figure 1) and cleaves the transcript to produce the 3' end of the snRNA. This was the first gene-specific function for a CTD modification to be described. Subsequently, we showed that phosphorylation of S_7 on one heptapeptide plus phosphorylation of S_2 of the next heptapeptide creates a double mark that is recognised by the Integrator complex (Figure 2 inset), emphasizing that the CTD modifications that are read by binding proteins need not be restricted to one repeat. Our most recent work has demonstrated that phosphorylation of S_7 also helps to recruit an enzyme, RPAP2, that removes the phosphate from phospho-S_5 specifically on snRNA genes. This finding suggests an snRNA gene-specific cascade where S_5 is phosphorylated early in transcription and subsequent phosphorylation of S_7 in turn recruits the enzyme to dephosphorylate S_5, contributing to the changing pattern of CTD modification during the transcription cycle of snRNA genes. Clearly, a full understanding of the CTD code and how it is read is critical to working out how expression of the genes in our genome is regulated during normal development and in diseased cells.

Interestingly, while studying expression of the human U1 snRNA genes, Dawn O'Reilly made the unexpected discovery of a whole new class of snRNA genes; the variant (v)U1 snRNA genes. It has been known for some time that multiple copies of the U1 snRNA gene exist. Since they contain numerous base changes within regions known to be required for expression and/or function, it was thought that the majority of these U1 snRNA gene copies were non-functional. Consequently, they were termed U1 snRNA pseudogenes and classified as 'junk' DNA; evolutionary remnants of our past. However, Dawn was astounded to discover RNA copies that match the sequence of many of these so-called pseudogenes in human cells. These RNA copies are particularly present in some human cancers and human embryonic stem cells (hESC) and are subject to negative regulation upon differentiation. This was a very important finding, considering the pivotal role U1 snRNA plays in our cells. U1 snRNA interacts closely with two major protein complexes, the Spliceosome and Polyadenylation complex, which play fundamental roles in processing pre-mRNA. During transcription, the 5' end of U1 snRNA forms base pair interactions with complementary sequence elements, known as splice donor sites, located at exon/intron boundaries at the 5' end of our introns. In so doing, U1 snRNA marks the regions within the pre-mRNA for the Spliceosome to catalyse the removal of introns and joining together of exons.

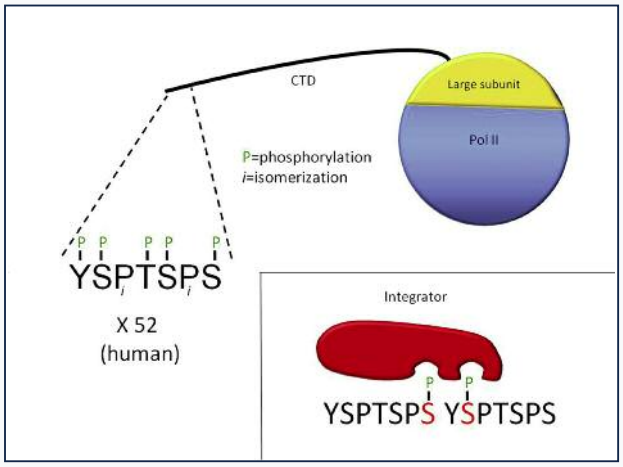

Figure 2. The pol II CTD heptapeptide can be modified at every residue. The CTD of the large subunit of pol II is shown schematically as a "tail" comprising 52 repeats of the YSPTSPS heptapeptide, with the potential modifications noted on the enlarged repeat. ***Insert: The Integrator complex recognises a new double mark on the pol II CTD that straddles two repeats.*** A schematic of the Integrator complex is shown with pockets to allow recognition of the phosphate modifications on two serines (coloured red) in two different repeats.

Depending on where U1 snRNA binds, some exons can be skipped or introns included such that the final mRNA can give rise to a completely different protein (Figure 3A, *U1 snRNA*). Furthermore, for an mRNA to be translated into a protein, it requires a polyA tail (Figure 1). The polyadenylation complex adds the tail after recognising a specific sequence in the RNA known as the polyA site (Figure 1). As most pre-mRNAs contain numerous polyA sites, choosing the right one can often be problematic. This is where the second major function of U1 snRNA comes into play. In addition to splice donor sites, U1 snRNA also base pairs with complementary sequences, located within intons and exons of the pre-mRNA. U1 snRNA binding close to internal polyA sites blocks their recognition, ensuring that premature polyadenylation of the pre-mRNA is avoided in favour of proper polyadenylation at the end.

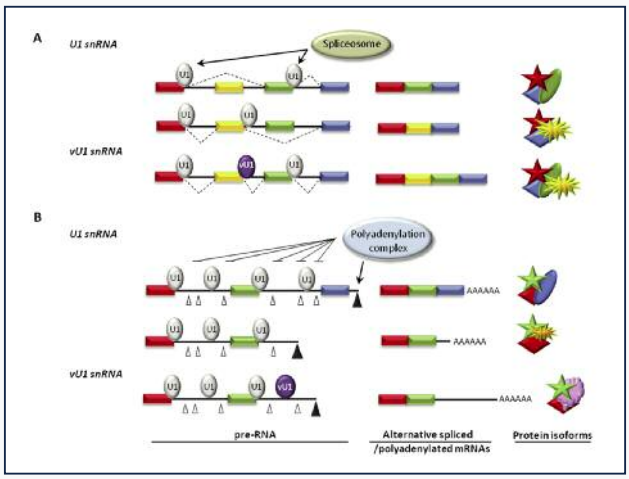

Figure 3. U1 and variant (v) U1 snRNAs regulate splicing and polyadenylation. Schematics indicating the role of U1 and vU1 snRNA in RNA processing events, including alternative splicing (A) and alternative polyadenylation (B). A single gene can generate multiple protein isoforms depending on where U1 and vU1 snRNAs associate with the pre-mRNA transcript.

Thus, in addition to splicing, U1 snRNA also participates in controlling the length of the final mRNA (Figure 3B, *U1 snRNA*). These processing mechanisms are termed alternative splicing and alternative polyadenylation, respectively, and together are fundamental to generating protein diversity in all our cell types. Failure to control these processes results in disease due to the appearance of the wrong product in the wrong place and /or at the wrong time.

Consequently, we were keen to establish whether vU1 snRNAs could function in a manner analogous to U1 snRNA. Our recent work confirms this to be the case, as deregulation of one of the vU1 snRNAs (vU1.8) in a human cancer cell line causes dramatic changes in the RNA processing of a specific subset of pre-mRNAs (3). This result suggests that the lack of sequence conservation, which labelled them 'junk' DNA, could in fact be key to their function, enabling vU1 snRNAs to contribute to alternative processing events through recognition of specific sequence elements across pre-mRNAs (Figure 3A and B, *vU1 snRNA*). Moreover, with our collaborators in the US, we now have evidence that failure to downregulate vU1 snRNA expression during differentiation is associated with defects in motor neuron function, suggesting that vU1 snRNAs are key regulators of cell fate. We are currently using the latest sequencing techniques and innovative induced pluripotent stem cell (iPSC) technologies, in collaboration with Sally Cowley and William James here at the Dunn School, to identify the pre-mRNA targets of individual vU1 snRNAs and establish how de-regulation of their expression could lead to defects in stem cell function. A full understanding of the mechanisms that control cell fate decisions is central to unlocking the full therapeutic potential of stem cells in regenerative medicine.

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Focus on Molecular Biology: Studying signaling systems with systems biology

Omer Dushek

'People who wish to analyze nature without using mathematics must settle for a reduced understanding.' **Richard Feynman**

Molecular biologists have, in recent decades, focused on identifying proteins involved in various cellular processes. The next major challenge is to understand how these proteins produce these processes. The new discipline of systems biology promises to tackle this challenge: precisely how remains controversial, but most practitioners agree that some non-trivial mathematics is going to be required. As an immunologist with training in mathematics, I have been awarded a Sir Henry Dale Fellowship, jointly funded by the Wellcome Trust and the Royal Society, to start a laboratory focused on using systems biology to study T cell activation.

T cells are a critical component of our immune system. They patrol the body in search of the molecular signatures (or 'antigens') of infection and cancer, which they recognize using their T cell antigen receptors. The recognition of antigen by T cells is not only responsible for helpful immune responses that allow T cells to kill infected and cancerous cells, it is also responsible for unhelpful immune responses against endogenous and harmless antigens, which lead to autoimmune and allergic disorders, respectively. Given its central role in immune responses, it is no surprise that the T cell antigen receptor has been under intense investigation since it was discovered in the early 1980s. The many signaling proteins that relay information from the receptor (at the cell surface) to changes in the expression of genes (in the nucleus) have been identified. But as in other areas of molecular biology, what has emerged is a complicated signaling network whose relation to the process of antigen recognition remains difficult to understand.

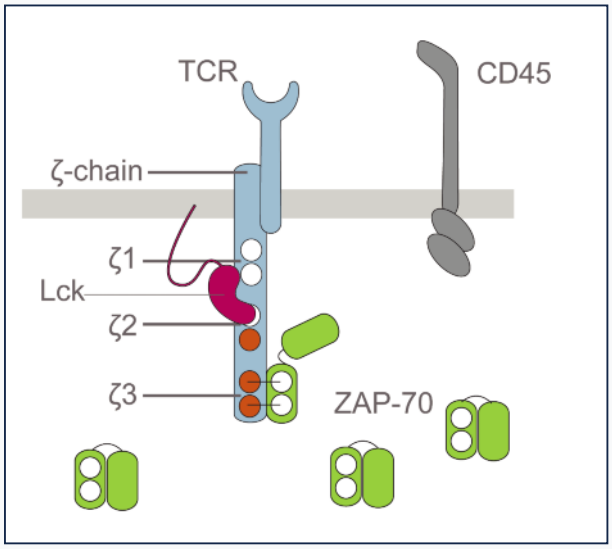


Figure 1. Schematic of T cell receptor signaling. Shown is the T cell receptor ζ-chain, which contains 6 phosphorylation sites distributed on 3 conserved motifs known as immunoreceptor tyrosine-based activation motifs (ITAMs). These ITAMs are thought to be phosphorylated sequentially (membrane-distal to membrane-proximal) by the tyrosine kinase Lck, and dephosphorylated by the tyrosine phosphatase CD45. When an individual ITAM is fully (doubly) phosphorylated it serves as a docking site for an effector (ZAP-70) that, upon binding, is able to activate downstream signaling proteins that may activate the T cell and initiate an immune response.

Consider a specific example involving four signaling proteins: the receptor, two enzymes, and an effector molecule (Figure 1). The receptor has a signaling chain (ζ-chain), which has six tyrosine phosphorylation sites. When antigen binds to the receptor these sites become phosphorylated. These sites are thought to be

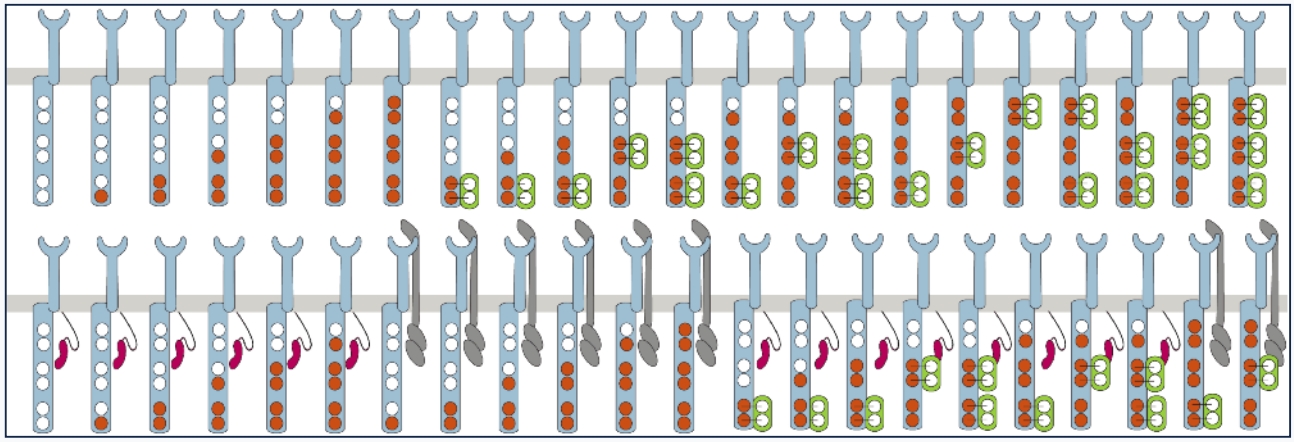


Figure 2. Combinatorial complexity in complexes. Shown is a schematic of a subset of the chemical states (or complexes) that can be formed with the four signaling molecules shown in Figure 1.

sequentially phosphorylated (from the site furthest to the site closest to the membrane) by a kinase enzyme (Lck) and dephosphorylated by a phosphatase enzyme (CD45). When the signaling chain becomes phosphorylated it provides multiple docking sites for an effector molecule (ZAP-70) that is normally found diffusing freely in the cytoplasm of the cell. The effector is known to bind to the phosphorylated sites with different binding strength (affinities): it is lowest for the site furthest from the membrane and highest for the site closest to the membrane. When bound, the effector activates downstream signaling proteins that may lead to T cell activation and consequently, to an immune response. For this reason the amount of bound effector is thought to be tightly regulated.

So how do these signaling proteins regulate the amount of bound effector? And what are the roles of multiple phosphorylation sites, their sequential phosphorylation, and the different effector binding affinities in this process? To offer plausible answers we have constructed mathematical models. These models contain nothing more than binding and catalytic (enzyme modification) reactions. The novelty is that instead of focusing on just two interactions, as one typically does in experiments to study binding or enzymatic activities of proteins, we include every necessary interaction without any simplifying assumptions. In principle, this is a simple task, but in practice, it is complicated. Until a few years ago such models were not possible. And here's why.

Consider the interactions we are interested in studying (Figure 1). These four molecules can form many different complexes or chemical states. For example, the receptor can be phosphorylated at different positions and the kinase and/or phosphatase and/or effector can be bound or unbound to each phosphorylated state of the receptor (see Figure 2 for an illustration of a subset of these chemical states). The explosion in the number of chemical states formed by just a few interactions is called 'combinatorial complexity'. This complexity is a challenge because, mathematically, we are required to write down an equation for each chemical state, specifying every possible reaction between states. In our example, there are 53 distinct chemical states and 168 reactions. In order to model all twenty phosphorylation sites on the T cell receptor we would need to include an equation for at least 2^{20} (or just over 1 million) different chemical states.

It is difficult to manually enumerate 53 equations, let alone over 1 million, without making just as many mistakes. I learned this the hard way when, as a graduate student, I failed to model these interactions. The solution only came in the last five years when the systems biology community decided that automated tools are necessary. These tools are now able to generate the large set of (nonlinear coupled) equations from a small number of user-specified interactions. It is likely that user-friendly versions of these tools will be released in the next few years. Nevertheless, I have already begun teaching group members to use these tools and only a few complaints have been filed so far.

Returning to the question we originally posed: How is the amount of bound effector regulated? Using a mathematical model that includes all ingredients previously mentioned, we calculated the amount of bound effector as the concentration of kinase was

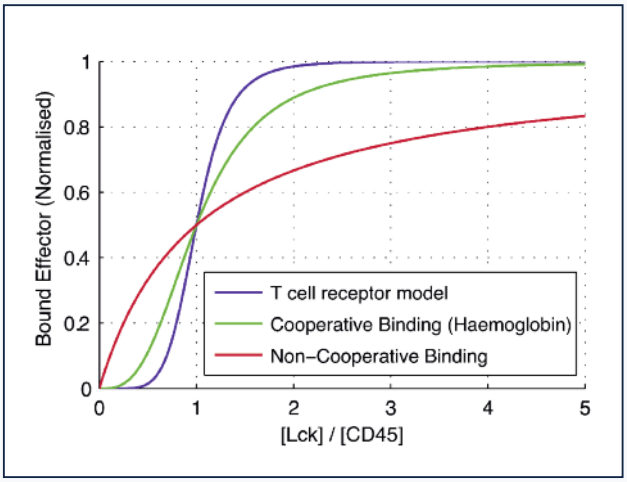


Figure 3. Switch-like response in the amount of effector bound to the T cell receptor. The amount of effector bound to the T cell receptor (y-axis) is shown as a function of the relative concentration of active kinase (Lck) to phosphatase (CD45) (x-axis) based on a systems model that includes all ingredients described in Figure 1. The model unexpectedly predicts a switch-like response, whereby small changes in Lck (or CD45) can lead to very large changes in the amount of bound effector. This switch-like response is compared to ligand binding to a non-cooperative and cooperative (e.g. haemoglobin) receptor. Note that the axes for the two binding curves are not shown but would read ligand concentration (x-axis) and bound ligand (y-axis).

increased (Figure 3). To our surprise, the model predicted that *small changes* in the kinase (or phosphatase) could lead to very *large changes* in the amount of bound effector. This switch-like response is reminiscent of the high sensitivity that haemoglobin exhibits to oxygen binding and is contrasted by the low sensitivity (or gradual response) that a non-cooperative receptor exhibits. Moreover, the model predicted that this switch-like response relied on all model ingredients, so that removing multiple phosphorylation sites, sequential phosphorylation, or different effector binding affinities abolished the switch. This emergent switch provides a plausible explanation for these intriguing observations and is likely to have a role in discriminating antigen. Over the next several years, we aim to test these predictions.

A key aim of this work is to generate mechanistic models that can be used to guide immune therapies. For example, a promising new therapy aims to re-direct T cells to kill infected or cancer cells by engineering them to express a novel chimeric antigen receptor that signals through the ζ-chain. Modulating the efficacy of these therapies has focused on modifications to the ζ-chain and is based largely on an educated guess-and-check approach. Even in these early days, we are able to use our models to understand these therapeutic receptors and recently have predicted some novel synthetic ζ-chains that we will test in the laboratory. Ultimately, we hope to provide the community with tools that can be used for the rational design of novel T cell therapies with improved clinical efficacy.

Systems biology is a discipline in its infancy. There are presently no standardized mathematical or experimental methodologies to relate protein interactions to cellular processes. For this reason a healthy scepticism is needed to ensure that the discipline matures into a useful enterprise that provides novel biological insights that we can ultimately utilize for the benefit of human health.

Focus on Molecular Biology: Switching on the immune system: Disulfide bonds offer a novel way of controlling the immune response

Clive Metcalfe

Communication between individual cells and between cells and their environment is paramount for the correct operation of all systems of the human body. This is especially true of the immune system, where cells have to constantly discriminate between our own components and foreign material from bacteria, viruses and other pathogens. Cell subsets express a distinct set of proteins on their cell surface that are used to sense their environment and transduce signals through the plasma membrane into the cell, where these signals are processed instigating an appropriate cellular response, a process termed signalling. There are two mechanisms through which signalling occurs. Direct cell-cell contact allows proteins on the surface of one cell to interact with complementary receptors on the surface of another cell which initiates specific signals depending upon the function of the interacting proteins. Indirect signalling occurs when one cell secretes messenger molecules (e.g. chemokines or cytokines) which are then sensed by receptors on the surface of a distant cell. The 'strength' of the signal will depend upon the abundance of the proteins that are interacting but it is becoming more evident that some receptors can be in an inactive non-signalling state i.e. switched off. One recently-characterised method of regulation of receptors is through the cleavage of disulfide bonds.

Disulfide bonds are covalent bonds between the sulphur atoms of distant cysteine residues within a protein (Figure 1). They are well known for their roles in maintaining protein structure but some of them can be broken and change both the shape of a protein and its activity,

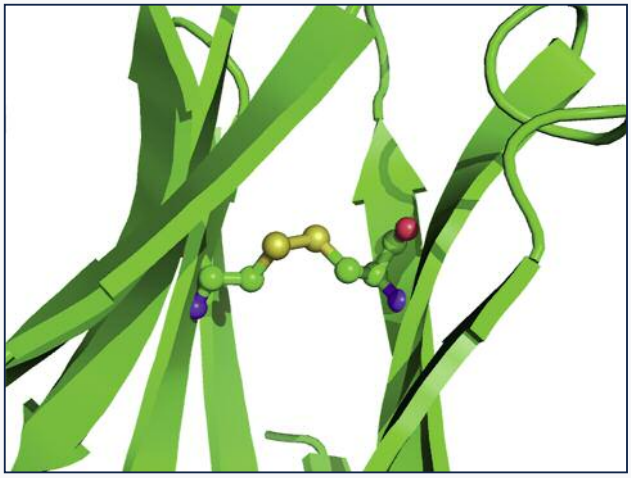


Figure 1. *A disulfide bond (shown in yellow) is a covalent bond between the sulphur atoms of two cysteine side chains (ball and sticks). The cysteines can be far apart in the primary sequence but, when the bond is formed, two areas of the protein are locked together.*

thus acting as a switching mechanism. Enzymes, called reductases, were identified which, when secreted by cells onto their surface, could catalyse the making and breaking of disulfide bonds and allow the switching on and off of proteins. Examples of this are in platelet activation and HIV-1 infection of $CD4^+$ T cells. When vascular damage occurs, platelets become activated and secrete an enzyme called Protein Disulfide Isomerase (PDI) onto their surface which breaks disulfide bonds in the platelet integrin allowing it to cross link many platelets together to cause a blood clot, halting the loss of blood from the wound. HIV-1 entry into $CD4^+$ T cells requires disulfide bonds in gp120, one of the protein components of the viral envelope, to be broken by thioredoxin, an enzyme secreted by T cells. This causes a change in the surface topology of the virus, allowing it to fuse with T cells and infect them.

Neil Barclay has long been interested in the structure and function of proteins on the surface of immune cells and his studies highlighted many disulfide bonds in proteins which were not required for maintaining structure. Furthermore, cells analysed from sites of inflammation had increased levels of 'free thiols' (broken disulfide bonds) on their surface compared to resting cells, suggesting switching was occurring during inflammation. I joined the lab in 2008 and with a background in protein structure and function I set about searching for proteins on immune cells which may undergo disulfide bond switching. I utilised the Dunn School's investment in mass spectrometry-based proteomics and designed a screen where we can 'freeze' all the disulfide bonds in the proteins on the surface of a cell in their conformation at a given point in time (1). We then used mass spectrometry to determine which proteins the disulfide bonds are from and whether they were formed or broken at the time their state was frozen. By treating the cells with the enzymes known to break disulfide bonds under conditions that mimic inflammation, we were able to determine which disulfide bonds are likely to be acting as switches: we term these disulfide bonds 'labile'. We identified around 80 proteins among the 400 known to be expressed on the surface of a T cell hybridoma that potentially contain labile disulfide bonds. The range and function of the proteins was diverse and included antigen receptors, cytokine receptors, adhesion molecules and transporters. This suggests many cellular processes could use disulfide bond switches as a method of protein regulation. Furthermore, we screened primary cells from mice with induced septic shock, revealing similar repertoires of proteins to our *in vitro* studies.

The current focus of our research is determining the functional significance of these labile disulfide bonds and the regulation mechanisms that control how they are manipulated. We have decided

to concentrate on T cells as two very important molecules involved in T cell activation emerged from our screens. CD132, or the common gamma chain, is a key component of cytokine receptors in the interleukin-2 (IL-2) family (indirect signalling). Other components of the receptor assemble around it and when IL-2 is bound, a signal is transduced inside the T cell, telling it to proliferate. Mutations of CD132 are responsible for X-SCID, a disease where young males are severely immune compromised because they lack the entire T cell compartment and are susceptible to infection, often dying young. The disulfide bond that we identified as labile in CD132 was also one of the mutation hotspots in X-SCID. Previous analysis showed that mutants lacking this disulfide bond could no longer respond to IL-2. We showed that if this disulfide bond is reduced, the receptor does not respond to IL-2 and activated T cells no longer proliferate. When we removed the reducing conditions, the T cells continued to proliferate as normal (2). As inflammation produces a local reducing environment, we hypothesise that this may be a feedback mechanism built into the immune system as a way of limiting T cell proliferation and inflammation.

We are also investigating the effect of a labile disulfide bond in the T cell antigen receptor complex (TCR). The TCR is the primary receptor for antigen on T cells and engagement with foreign antigen is the first step in T cell activation. The TCR is a complex of 8 proteins and is interesting in that antigen binding and signal transduction are carried out by different proteins in the complex. We can completely switch off the TCR's response to antigen by reducing disulfide bonds in the complex. This switching does not appear to effect antigen binding or TCR formation, it is merely signal transduction that is switched off. Preliminary data suggest that it is a disulfide bond between the small ζ chains, the proteins that perform signal transduction, which is responsible for this switching.
This is intriguing because several other classes of receptors use these small adapter proteins to signal their engagement with ligand. Most of these are disulfide-linked homodimers and we plan to investigate whether the switching seen in the TCR might be common across all of the adapters.

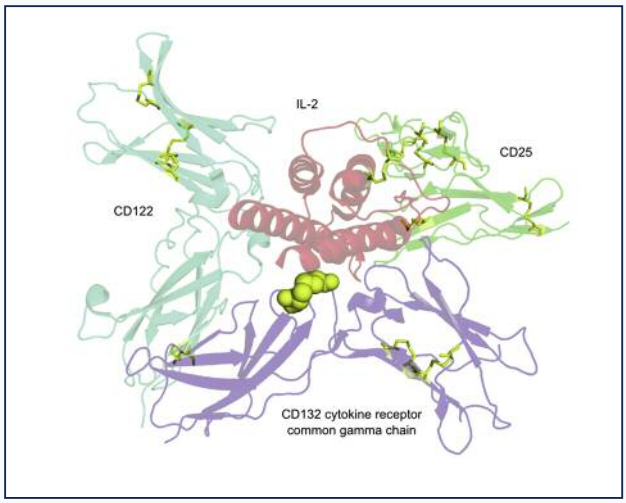


Figure 2. *The IL-2 receptor complex contains a labile disulfide bond (yellow spheres) that is close to the IL-2 binding site. When this disulfide bond is broken, the receptor is switched off.*

We have only just scratched the surface regarding disulfide bond switches in the immune system and I am sure there will be many exciting discoveries over the next few years. Nevertheless, our work in the Dunn School has attracted the attention of some prominent international laboratories. Peter Cresswell, Howard Hughes Professor of Immunology at Yale Medical School spent a sabbatical here working on disulfide bond switching and Philip Hogg, Director of the Lowy Cancer Institute in Sydney has a long standing interest in disulfide bond switching and recently spent a month in our laboratory. We also hope to organise a meeting in the near future, dedicated to disulfide bond switches, so watch this space…

References

1. C. Metcalfe, P. Cresswell, L. Ciaccia, B. Thomas, and A. N. Barclay (2011) *Open Biol.,* **vol. 1,** Nov. 2011.
2. C. Metcalfe, P. Cresswell, and A. N. Barclay *Open Biol.,* **vol. 2,** no. 1, Jan. 2012.

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.

Window of opportunity: *Imperfections in the window panes of the old Dunn School Building provide unexpected beauty in their distorted reflections. Photographs by Paul Fairchild.*

Bioimaging: more than meets the eye

Errin Johnson and Alan Wainman

The Dunn School Bioimaging Facility gives researchers access to state-of-the-art preparation, imaging and analysis instrumentation and the expertise to use it. Errin Johnson and Alan Wainman run the electron and light microscopes respectively and here provide an update on recent bioimaging developments, projects and future directions.

The Bioimaging Facility (BIF) is the hub of microscopy in the Dunn School and was established with funding provided by the Wellcome Trust, EPA Research Fund and the Dunn School. The BIF is governed by an in-house steering committee, consisting of Keith Gull (co-chair), Jordan Raff (co-chair), Peter Cook, Eva Gluenz, David Vaux, Chris Norbury and ourselves. The facility comprises a slide scanner, two epifluoresence microscopes, two confocal laser scanning microscopes, a spectrofluorimeter, a transmission electron microscope (TEM) and a scanning electron microscope (SEM), together with a laboratory dedicated to sample preparation for electron microscopy. We are also affiliated with both The Micron Advanced Bioimaging Unit in the Department of Biochemistry and the Oxford Particle Imaging Centre in the Division of Structural Biology (STRUBI), which opens up to our users the exciting fields of super-resolution microscopy and cryo-electron tomography, respectively.

Approximately two thirds of our users are members of the Dunn School, although usage is rising from external departments, including Zoology, STRUBI, Plant Sciences, the John Radcliffe Hospital and Physics. There are currently over 70 microscopy projects in progress, covering an extremely diverse and exciting range of science, from fundamental cell biology and medicine, through to ecology and bio-manufacturing. The composite figure (page 13) shows some examples of how the BIF is facilitating such varied research. Particulate specimens ranging from proteins, viruses and parasites, to model organisms such as *C. elegans, Arabidopsis, Drosophila* and mice, are all currently being imaged in the BIF using a range of different microscopy techniques.

In the light microscopy (LM) area, brightfield microscopy is most often used for imaging stained histological sections, which can be prepared using the tissue and pathology service in the Dunn School. Epifluorescence and confocal microscopy are extensively used in the BIF across a wide range of applications. Several projects are employing targeted fluorescent dyes to track organelle, membrane, ion or DNA dynamics in live cells. However, the majority of researchers are using fluorescent fusion proteins (eg: GFP and variants) to investigate the localisation, movement and interactions of their protein(s) of interest in live cells or immunocytochemistry to localise proteins in fixed cells. Unlike a fluorescence microscope, the confocal uses lasers to excite the fluorophore(s) in a specimen (eg: 405 nm for DAPI, 488 nm for GFP and 561 nm for Cy3). Coupled with the use of a pinhole that excludes

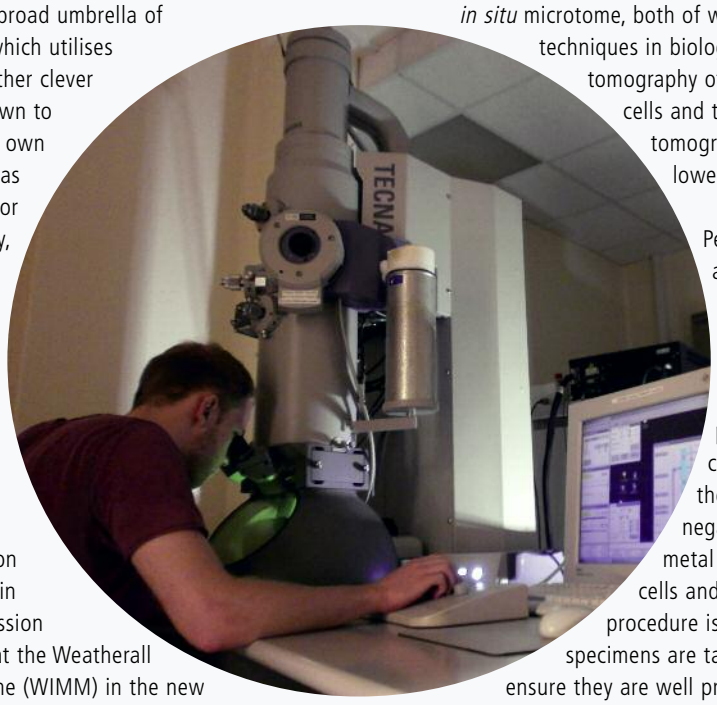
signals from outside the focal plane, this allows thicker samples to be imaged at higher resolution (eg tissue sections), and even optically sectioned to create 3D images. Our most popular confocal, the Olympus FV1000, is also equipped with a fluorescence lifetime imaging module (FLIM), which takes very accurate fluorescence recovery energy transfer (FRET) measurements for the analysis of protein-protein interactions. Our second confocal, the Zeiss LSM5, is mainly used for live cell time-lapse imaging, and is furnished with an environmental chamber to keep mammalian cells happy for a few hours (eg for cell spreading assays by the Vaux lab) or for up to a week (eg organoid growth by the Hassan lab).

There are times when the resolution limit of conventional LM (approx. 200nm) can pose a problem. However, over the past decade several new fluorescent imaging techniques have been developed that fall under the broad umbrella of super-resolution microscopy, which utilises advanced algorithms and/or other clever tricks to achieve resolution down to 20nm. Each technique has its own advantage and application(s), as well as specific requirements for specimen preparation. Happily, several initiatives across the University have recently brought these cutting-edge super-resolution techniques within the reach of Dunn School members. There are several OMX microscopes for Structured Illumination Microscopy (SIM) and direct stochastic optical reconstruction microscopy (dSTORM) housed in Micron, plus a Stimulated Emission Depletion (STED) microscope at the Weatherall Institute for Molecular Medicine (WIMM) in the new Wolfson imaging centre. This certainly is an exciting time for light microscopy at Oxford, as super-resolution imaging becomes increasingly accessible to researchers here.

That said, electron microscopy (EM) will always be the gold standard in the resolution department! Using an electron beam improves the resolution to below 1 nm, due to the extremely short wavelength of accelerated electrons (which is up to 100,000x shorter than that of light). The electron beam is manipulated using electromagnetic lenses and the sample can be magnified thousands of times without sacrificing resolution.

In TEM, the image is formed by electron interactions with the specimen as the beam transmits through it, enabling small particulate samples (eg proteins, viruses and bacteria), internal cellular components and tissue architecture to be visualised at high resolution. While the bulk of projects in the EM division use the 120 kV Tecnai 12 TEM to investigate either purified proteins or cellular ultrastructure, there are a growing number of projects employing more advanced techniques, such as protein localisation using immuno-gold labelling and electron tomography. The latter is facilitated by the capability of our TEM to acquire automated tilt series, which allows ultrastructural components to be reconstructed and modelled in 3D (eg the synapse between HIV-infected macrophages and uninfected T cells, studied by the Sattentau lab).

SEM works differently, the image being formed instead by scattered electrons that result from interactions between the electron beam and the specimen as the beam is scanned across its surface. This permits specimen topography to be imaged in exquisite detail and is ideal for morphological characterisation of mutant or drug-treated cells, tissue or even whole organisms (eg *Drosophila* and *C. elegans*). Our JEOL-6390 SEM is extremely user-friendly and we are eager to increase usage of this microscope. In future, we hope to upgrade to a higher resolution SEM capable of 3D imaging via focussed ion beam milling or an *in situ* microtome, both of which are exciting emerging techniques in biological microscopy that enable tomography of much larger areas (eg whole cells and tissue slices) compared to TEM tomography (although at a slightly lower resolution).



People are often daunted by the admittedly more complex specimen preparation required for EM, but there is no need to be put off by the prospect. Indeed, preparing small particulate samples for TEM can be as simple as applying the sample to a filmed grid, negatively staining with a heavy metal for contrast and drying it. For cells and tissues, the preparation procedure is more labour intensive, as specimens are taken through multiple steps to ensure they are well preserved, stable in the vacuum (which is required in EMs to ensure integrity of the electron beam), conductive (more important for SEM), well contrasted and extremely thin (TEM only). This process is facilitated in the BIF EM lab by the availability of dependable protocols, up-to-date instruments and a staff member always on hand to help. Indeed, we are actively working to ensure that EM is readily accessible, by training increasing numbers of users to self-sufficiency in the lab and on the microscopes, and continuing to develop our specimen preparation capabilities (eg cryo-preparation methods).

Another area in active development is correlative microscopy, which nicely ties together the LM and EM sides of the BIF. Using this method, the same region of a specimen is imaged using two different techniques, commonly confocal and TEM. In this way, proteins and/or other sub-cellular components can be imaged on the confocal using fluorescent probes and then placed into ultrastructural context at the EM level. Although the specimen preparation involved is more demanding, this is an extremely powerful bridging technique and we look forward to assisting more correlative projects in future.

Insert: *Bioimaging in action: Joshua Long (Fodor lab) using the Tecnai 12 TEM to investigate mitochondrial localisation of an influenza viral protein.*

Montage Figure Legend

Row 1, L-R

Fly brain, *by Carolina Rezeval (Goodwin Lab, DPAG) on the Olympus FV1000 confocal: Adult female brain showing neutrophil, stained with nc82 (blue), and octopaminergic neurons, labelled with anti-tyrosine decarboxylase (red). This is part of a project investigating the neural mechanisms that underlie sex-specific behaviours in the fruit fly* Drosophila melanogaster.

Coccolithophore, *by Errin Johnson/Maeve Eason-Hubbard (Rickaby Group, Earth Sciences) on the Tecnai 12 TEM: Maeve is a PhD student investigating the ultrastructure of the pyrenoid, the site of carbon dioxide fixation within the chloroplast, across several species of these ecologically important unicellular marine algae. This cell is approximately 3µm in diameter.*

Blood monocyte and macrophage, *by Errin Johnson/Bonnie Van Wilgenburg (James Lab, Dunn School) on the JEOL-6390 SEM: As part of her recently completed DPhil, Bonnie investigated the effect of growth media on the ultrastructure and morphology of stem-cell derived blood cells.*

Fly cilia, *by Janina Baumbach (Raff Lab, Dunn School) on the Olympus FV1000 confocal: The third antennal disc from a* Drosophila *pupa expressing the basal body marker PACT-GFP (green dots at the base of the sensory cilia), with DNA stained using Hoechst (red) and sensory hairs exhibiting yellow autofluorescence. The Raff Lab studies centrioles and their function in diseases, such as ciliopathies and cancer.*

Row 2, L-R

Dendritic cell, *by Errin Johnson/Simon Hackett (Fairchild lab, Dunn School) on the Tecnai12 TEM: This is a large dividing immature bone marrow-derived dendritic cell (BMDC), approximately 8µm across. As part of his DPhil, Simon is characterising ultrastructural and morphological differences between mouse immature and mature BMDCs, and comparing them with DC differentiated from induced pluripotent stem cells.*

Fly cilia, *by Metta Pratt (Raff Lab, Dunn School) on the OMX Structural Illumination Microscope (SIM) at Micron: Super-resolution image of a basal body (green, 300 nm in diameter) and cilia (red) from* Drosophila *spermatocytes. The tube-like structure of the cilia has not previously been observed, due to the resolution limitation of conventional light microscopy.*

DNA tetrahedra, *by Aiman Entwhistle (Tuberfield Lab, Physics) on the Zeiss LSM5 confocal: DNA tetrahedral, with Cy3 (red) and Cy5 (green) labelled nucleotides, in mammalian culture cells with nuclei stained with Hoechst (blue) and lysosomes stained with Lysotracker. The Tuberfield group use DNA to engineer 3D structures, such as tetrahedra, that are capable of holding cargo or delivering drugs.* ***Hepatitus B vaccine,*** *by Errin Johnson/Nicky Green (Oxford Clinical BioManufacturing Facility, CBF) on the Tecnai12 TEM: Negatively stained vaccine particles (approx. 22nm in size), which consists of the viral envelope protein and hepatitis B surface antigen. The CBF is using TEM to assess the quality of their preparations of such virus-like particles.*

Row 3, L-R

Mouse Intestine, *by Claudia Buehnemann (Hassan Lab, Dunn School) on the Olympus FV1000: Mouse intestinal section labelled with lysozyme to detect Paneth cells, β-Catenin (red) and E-Cadherin (yellow). β-Catenin is bound to the plasma membrane-localised cell adhesion molecule E-Cadherin and can translocate to the cytoplasm and nucleus upon activation of the Wnt signalling pathway.*

Migrating cells, *Asharf Malhas (Vaux Lab, Dunn School) on the Zeiss LSM5 confocal: This is a still taken from a timelapse movie of BRCA1 transfected cells in a wound healing assay and is part of a project examining the role of BRCA1 in the regulation of breast cancer cell spreading and motility.*

Brain capillary, *imaged by Andrew Douglas (Wood lab, DPAG) on the Tecnai 12 TEM: Andrew is characterising the ultrastructure of the blood-brain barrier in mouse models of Duchenne muscular dystrophy.* ***Worm embryos,*** *by Erica Namigai (Scheibel Lab, Zoology) on the Olympus FV1000: Part of a project on the evolution and development of simple marine organisms, these four-cell stage* Pomatoceros lamarckii *polychaete embryos show microtobules labelled with anti-tubulin (red), actin stained with phalloidin (green) and nuclei stained with DAPI (blue).*

Row 4, L-R

Whole-mount cytoskeleton, *by Samuel Dean (Gull Lab, Dunn School) on the Tecnai 12 TEM: Immunogold labelling of the flagellum transition zone component (FTZC) protein at the proximal part of the flagellum in the procyclic form of* Trypanosoma brucei, *the parasite responsible for human sleeping sickness.*

Human intestine, *by James Ussher (Klenerman Group, Nuffield Department of Medicine) on the Nikon Coolscope slide scanner: Lipopolysaccharide staining of lamina propria cells in the gut of HIV-infected patients. The Klenerman group studies the role host immune responses play in the outcome of viral infections.*

Myoblast cell, *by Khadijeh Pakzad (Clarke Group, DPAG) on the Olympus FV1000: Fixed cell with mictochondria stained with the Mitotracker dye (red) and nuclei with DAPI (blue), part of a study on the metabolic control of gene expression in muscle cells.*

Leishmania, *imaged by Eva Gluenz (Dunn School) on the JEOL-6390 SEM: Amastigote form of* Leishmania mexicana *at 3700x magnification. Eva's group uses electron and light microscopy to characterise both the molecular cell biology of this devastating parasite and the function of the flagellum during the host-parasite interaction.*

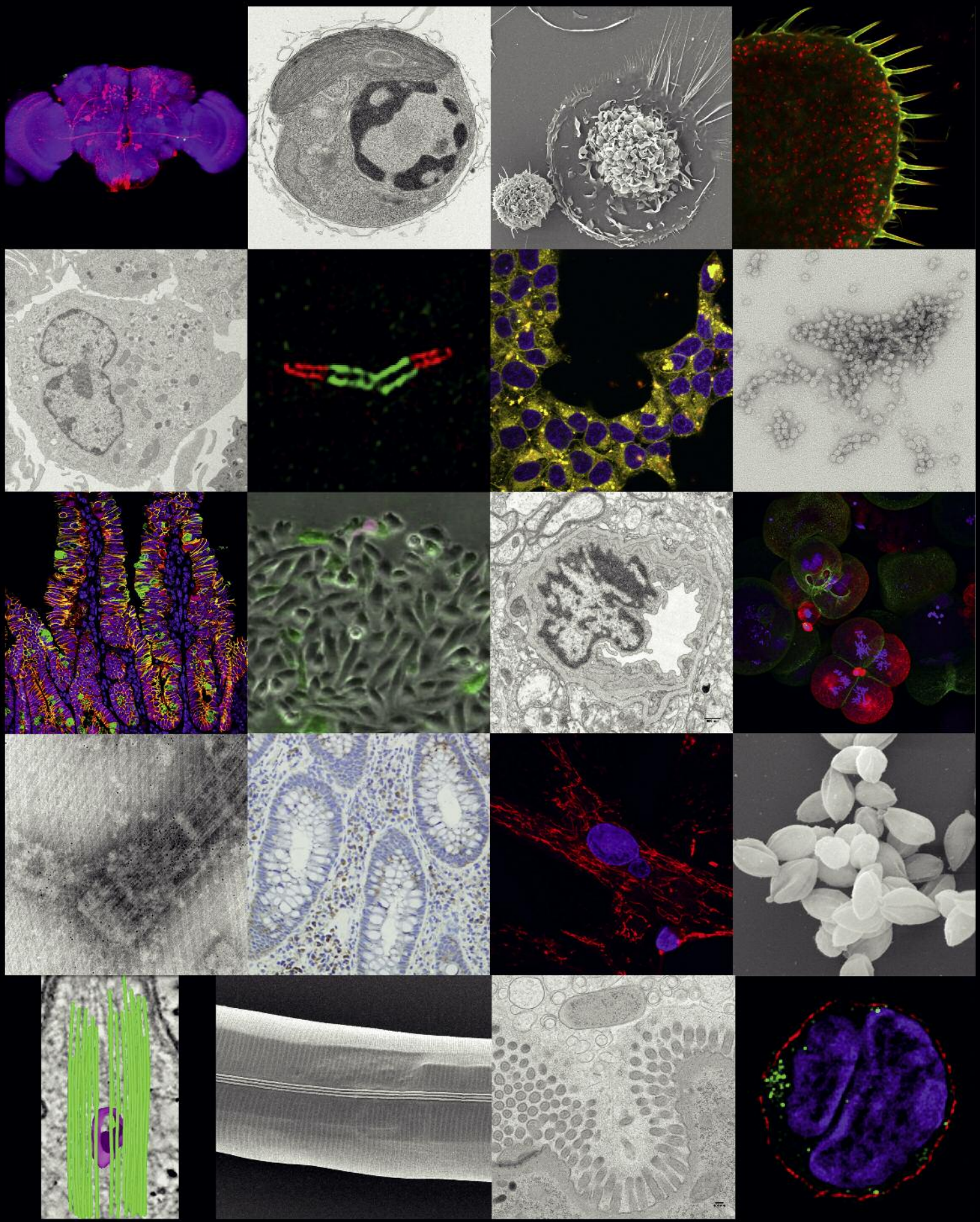
Row 5, L-R

3D Centriole, *imaged by Helio Roque (Raff Lab, Dunn School) on the Tecnai12 TEM: Electron tomography of mutant* Drosophila *centrioles, with microtubules modelled in green and an anomalous central structure in pink.*

Worm cuticle, *by Errin Johnson/Aileen Moloney (Vaux Lab, Dunn School) on the JEOL-6390 SEM: Outer surface morphology of wild-type* Caenorhabditis elegans *at 1200x magnification.*

Worm gut, *by Errin Johnson/Aileen Moloney (Vaux Lab, Dunn School) on the Tecnai12 TEM: Bacterium in the gut of cryo-fixed* C. elegans. *Aileen is characterising the ultrastructure and morphology of* C. elegans *mutants, as well as the localisation of amyloid proteins, as part of the Synaptica-funded neurodegeneration project.*

T cell, *by Konstantina Nika (Acuto Lab, Dunn School) on the OMX SIM at Micron: Super-resolution image of a human $CD4^+$ T cell, showing the plasma membrane (red), a cytoplasmic protein (green) and the nucleus (blue). This project is examining the regulation of TCR-triggered signalling in the immune response.*



How to make two out of one

Ulrike Grüneberg

Ulrike Grüneberg holds an MRC Senior Non-Clinical Research Fellowship and was recently appointed a University Lecturer within the Dunn School. Here she outlines her research interests in cell division and the questions her laboratory intends to address.

The mystery and beauty of cell division has fascinated scientists for more than 130 years, ever since Walther Flemming described the events of nuclear division, or mitosis, in salamander cells in 1879. He discovered that cells contain a thread-like material that thickens into visible units (the chromosomes, as we now know them) which split apart longitudinally during mitosis and are segregated to opposite poles of the cell.

Since these early observations, we have come a long way in understanding how cells segregate their chromosomes and divide the cytoplasm, but many questions still remain unanswered. For a start, it may be evident that cells have to divide to generate and maintain an organism, but it is not easy to comprehend how it is ensured that both daughter cells reliably obtain a complete set of chromosomes containing the full complement of genetic material. Furthermore, for a fertilized egg to develop into a fully grown organism, a startling number of cell divisions have to occur, and even once that has been accomplished, millions of cell division events continue to take place every second, replenishing the supply of red blood cells, skin cells and the lining of the gut. Yet diseases that are a consequence of errors in cell division, such as cancer, are relatively rare, and mostly occur in old age, suggesting that there are rigorous control mechanisms enforcing the correct segregation of the genetic material. Consequently, an accumulation of mitotic errors sufficient to give rise to cancerous progeny builds up only after many cell divisions. When errors in mitosis occur, they often result in the mis-segregation of chromosomes, a condition called aneuploidy. Aneuploidy has long been considered a driving force for tumorigenesis. The significant incidence of aneuploidy in cancer cells was already noted by the German physician David von Hansemann in 1890 but the molecular insight into how aneuploidy arises is only now emerging. Work in my laboratory is aimed at understanding how the faithful division of the duplicated sister chromatids is accomplished and regulated, and how aneuploidy may be created. Mutations in proteins involved in regulating cell division have the potential to promote aneuploidy and tumorigenesis, and we are particularly interested in identifying novel cell cycle regulators with these characteristics. Before discussing in more detail the research questions that my group is interested in answering, I will give some brief background to our work: the mechanics of cell division.

The cell cycle

The cell cycle consists of a series of events that have to occur in a specific temporal order to have a successful outcome, e.g. DNA replication has to occur before chromosome segregation (see Figure 1 for illustration of the different stages of cell division). This temporal order is prescribed by the interlocking actions of kinases, most prominently cyclin-dependent kinases, and phosphatases. In addition, cell cycle checkpoints delay cell cycle progression if a certain event (e.g. DNA replication) has not been satisfactorily completed or until the problem is resolved and cell cycle progression can be safely resumed. Most mitotic problems can thus be fixed without consequence.

What are the main events that are required to make two cells out of one? Key early requirements are the duplication of the centrosomes, the mammalian microtubule organizing centres, as well as the chromosomal DNA. Importantly, during DNA replication, cohesion is established between the two sister chromatids and it is critical that this cohesion is maintained until the metaphase to anaphase transition when the sister chromatids are segregated to the two emerging daughter cells. DNA and centrosome replication are followed by the breakdown of the nuclear envelope and the condensation of the mitotic chromosomes. Concomitantly, the mitotic spindle is formed by microtubules nucleated from the duplicated centrosomes. These events can be visualized using live cell imaging or immunofluorescence analysis of fixed cells and give rise to images of spectacular beauty (Figure 1)! Mitotic spindle formation is driven by a search-and-capture mechanism, during which the microtubules emanating from the centrosomes explore the cytoplasm and become stabilized when they interact with the kinetochores, specialized proteinaceous structures formed on centromeric DNA in mitosis (red structures in Figure 1). A combination of forces generated though microtubule polymerisation and depolymerisation at kinetochores and motor protein action results in the alignment of the chromosomes on the metaphase plate (Figure 1, top). At this point, it is critical that sister kinetochores have attached to microtubules from opposite spindle poles for equal chromosome segregation to occur. The establishment of bi-orientation is monitored by the spindle assembly checkpoint (SAC), a signal transduction machinery that monitors both the formation of microtubule-kinetochore attachments as well as the establishment of tension, created by the combination of pulling forces from the two centrosomes and the cohesion holding sister chromatids together. Failure to generate bipolar attachment and thus tension is

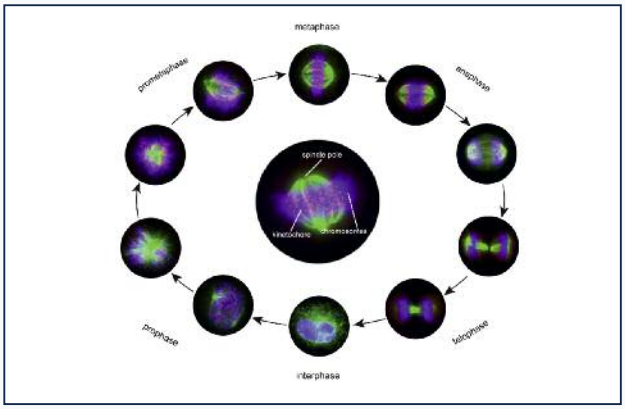


Figure 1: The mammalian cell cycle. *Different mitotic stages were imaged in HeLa cells. Microtubules are in green, DNA in blue and kinetochores in red. Note that the kinetochores are assembled at the beginning of mitosis and disassembled in late anaphase. Mitosis comprises five distinct stages, prophase (condensation of chromatin, migration of centrosomes to two distinct poles), pro-metaphase (capture of kinetochores by microtubules), metaphase (bipolar attachment of kinetochores to microtubules from opposite poles), anaphase (separation of sister chromatids and movement of DNA to poles) and telophase (nuclear envelope reformation, division of the cytoplasm by cytokinesis). The arrowhead indicates the metaphase plate, the arrow indicates the central spindle.*

detected by the SAC and results in the delay of anaphase onset until the problem is resolved. Once bipolar attachment of all sister chromatids has been achieved, the SAC is silenced and anaphase can ensue, resulting in the activation of separase, a protease which cleaves cohesin and initiates chromosome segregation. The final stages of cell division are directed by the central spindle, the microtubule structure that forms between the retracting DNA masses (Figure 1, telophase). The central spindle determines the site of physical cell division and also acts as a platform for the assembly of factors important for the final events of cell division, the physical cleavage of the two daughter cells, also called cytokinesis.

Microtubule-kinetochore attachments and the spindle assembly checkpoint

The formation of stable microtubule-kinetochore interactions is crucial for the success of chromosome segregation. In particular, it is essential that incorrect microtubule-kinetochore attachments (e.g. merotelic attachments where a single kinetochore is attached to microtubules from opposite poles, or syntelic attachments where both sister kinetochores have attached to microtubules from the same pole) are resolved and converted into amphitelic (bipolar), stable attachments. Over the last decade a large number of kinetochore and microtubule proteins have been identified that contribute to microtubule-kinetochore attachment formation and chromosome segregation but the precise biochemical properties conferred by these molecules are not fully understood. One focus of my laboratory is to assign biochemical activities to novel players involved in microtubule-kinetochore attachment formation using a combination of unbiased screens and targeted analysis of protein complexes. Our recent work in this area identified the microtubule and kinetochore localized heterodimeric astrin-kinastrin complex as a novel microtubule plus-end tracking factor required for efficient chromosome alignment and segregation. We have shown that the astrin-kinastrin complex aids the polymerization of microtubule plus-ends and that this activity is required for the efficient formation of stable microtubule-kinetochore attachments. In the absence of astrin or kinastrin, stable microtubule-kinetochore attachments cannot form, chromosome alignment is delayed and mitotic progression is halted because of spindle checkpoint engagement.

How does the spindle assembly checkpoint monitor the correct formation of microtubule-kinetochore attachments? Unattached kinetochores serve as a binding and signaling platform for key SAC proteins and trigger the activation of the spindle checkpoint. How exactly an unattached kinetochore is recognized by the SAC proteins and how the spindle assembly checkpoint is activated and maintained even when only a single kinetochore is unattached, yet promptly silenced once this last attachment has been made, is still one of the biggest questions in cell cycle research and one that is of particular interest to my laboratory. We are investigating how the spindle assembly checkpoint is activated and silenced, and we are particularly interested in understanding the role of mitotic phosphatases in these events.

Kinases and phosphatases

The ordered progression though mitosis is orchestrated by an array of mitotic kinases, including cyclin-dependent kinases, but also polo-like kinase 1 (Plk1), kinases of the Aurora family (Aurora A and B) and spindle assembly checkpoint kinases such as Mps1. The activation of these kinases at the right time and place is critical for successful cell division and a lot of progress has been made by many laboratories in the last few years in understanding the interplay and actions of these different kinases. Recently, it has also become apparent that the phosphatases opposing these mitotic kinases are of equal importance, however, for many mitotic phosphorylation events the regulatory phosphatases are still unknown. Through unbiased screening for phosphatases affecting cell cycle progression, my laboratory, in collaboration with Francis Barr's group in Biochemistry, has identified the multi-subunit PP2A family phosphatase PP6 as a novel regulator of mitotic spindle formation. PP6 regulates the activation status of the key mitotic kinase Aurora A by dephosphorylating the activatory phosphoThr288-site on Aurora A. Depletion of PP6 results in hyperactive Aurora A and consequently impaired spindle formation and chromosome segregation, ultimately resulting in aneuploidy and micronucleation where single chromosomes are not incorporated into the main nucleus (Figure 3). Interestingly, two recent studies identified mutations in the PP6 catalytic subunit, PPP6C, as driver mutations for UV-induced skin cancer. Analysis of the reported mutations in our laboratory revealed that all tumour-associated PPP6C mutations are loss of function and that a cell line carrying a homozygous PPP6C mutation displays strong genomic instability, consistent with the idea that loss of PP6 activity promotes tumorigenesis through the induction of aneuploidy (Figures 2 and 3).

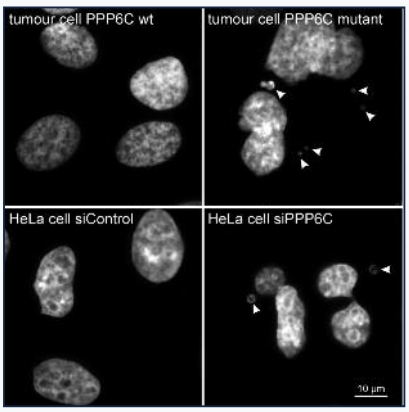


Figure 2: The PP2A family phosphatase PP6 is essential for faithful chromosome segregation. *Loss of PP6, either through homozygous mutation or through siRNA mediated depletion, leads to errors in chromosome segregation resulting in highly abnormal nuclear shape and micronuclei (arrowheads).*

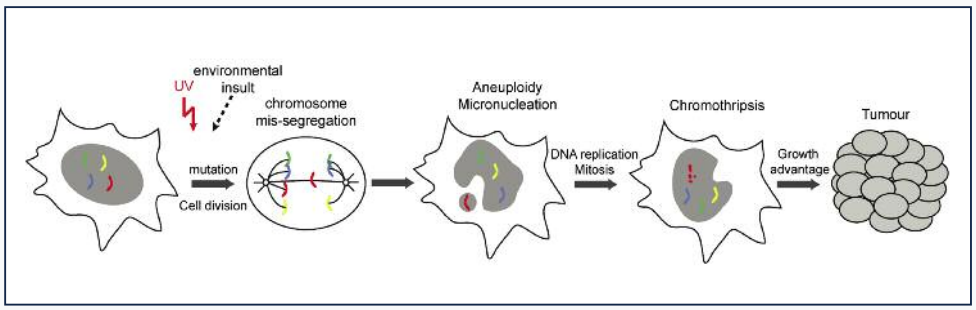


Figure 3: Model for tumour formation as a consequence of chromosome segregation errors. *Mutation of cell cycle regulators may lead to chromosome segregation errors resulting in aneuploidy or micronucleation. Micronucleation has recently been shown to induce chromothripsis (chromosome shattering). Chromothripsis results in massive local chromosome re-arrangements, facilitating the accumulation of multiple tumorigenic mutations in a single step.*

Aneuploidy and cancer

Recent evidence suggests that aneuploidy is a critical factor for the selection and survival of a tumour cell. In fact, aneuploidy and accompanying micronucleation have been implicated in the generation of so-called chromosome shattering also referred to as chromothripsis. Chromothripsis describes the substantial local re-arrangement of one or several chromosomes in cancer cells, a phenomenon that has been observed in 2–3% of all tumours. Because of the potential to introduce multiple tumour-promoting mutations or chromosomal re-arrangements in one event, chromothripsis can be considered a catalyst of tumour evolution (Figure 3). Further insight into how aneuploidy and micronucleation are generated and lead to chromothripsis is clearly required in order to be able to tackle tumour formation more directly or even prevent it.

Although aneuploidy appears to give emerging cancer cells a selective advantage, excessive problems with chromosome segregation will result in mitotic catastrophe and cell death. Spindle assembly checkpoint function is, therefore, expected to be of increased importance in tumour cell divisions with aberrant chromosome numbers and potential mutations affecting efficient chromosome alignment. An interesting concept is, therefore, the idea of reducing the tolerance for aneuploidy in tumour cells – potentially by impairing spindle assembly checkpoint function – and thus driving tumour cells into mitotic catastrophe. Drawbacks of this idea are the potentially detrimental effect of interfering with spindle checkpoint function in non-tumour cells. Ultimately, the goal is to gain a comprehensive understanding of the mechanics of chromosome segregation in tumour versus non-tumour cells and a thorough insight into the role of the spindle assembly checkpoint in these distinct situations so that we will be able to exploit the differences between aneuploid and euploid cells in a therapeutically valuable fashion.

Oxford to Cologne in 4½ years: An abridged chronicle

Akis Papantonis

***And still it moves!* — Galileo Galilei**

Early on, a biologist is taught that transcription involves a mobile enzyme, the RNA polymerase, which tracks along a gene to copy its sequence and produce an RNA transcript. Likewise, a researcher soon becomes accustomed to the idea that academic life equals 'mobility' — or does it?

I arrived in Oxford in November 2008, fresh out of my PhD, which I had defended in early October of the same year. What preceded this was Peter Cook pointing his finger at the audience of a FEBS conference in Athens saying: 'you have been taught wrong!'. To cut a long story short, soon enough I joined his laboratory for my first (and what later proved to be my only) post-doctoral placement, to study — what else! — RNA polymerases fixed in transcription factories. Obviously, the aspiration was nothing shy of convincing the world of a paradigm shift. Fast forward to today: as you are reading these lines I am at the Centre for Molecular Medicine of the University of Cologne, running my own research group, where I and three colleagues are trying nothing shy of convincing the world of that very same paradigm shift.

Between November 2008 and today, four-and-a-half years have elapsed, and therein lies a story. My Oxford experience involves a variety of memorable events: my very first publication as a post-doctoral researcher (a paper showing that RNA polymerases are fixed when active on human genes); my very first college affiliation, the Kemp Junior Research Fellowship for the Medical Sciences in Lincoln College (a college closely linked to the Dunn School via the E.P.A. trust); the birth of my first son, soon to be followed by the birth of my second; my first ever lecture to a lay audience, trying to explain to non-scientists why transcription factories are of any importance to them by making (in vain) analogies to Galileo's work (an event, Storytelling Science, hosted by — who else — a former Dunn School member, Catarina Amorim); the first (and most probably last) time coaching a basketball team, the Oxford Blues, rather than playing for one, with the rather satisfying record of 3–1. And in the end, my first independent group leader position, in the CMM Cologne, the foundation of which involved, yet again, a former Dunn School member, Jonathan Howard.

Returning to mobility. Much like an RNA polymerase immobilised in a factory, one can do quite a bit just by staying firmly in one place, or as yet another prominent Oxford figure, Lewis Carroll, would put it: 'You need to do all the running in the world to stay in the same place' — the Red Queen from Alice in Wonderland. It is difficult to adapt to a new place, a new role, a new lifestyle (the weather!). But then again, finding oneself in a new context is at the same time a scary challenge and a way to appreciate the context you've dwelt in for quite a few years — the Dunn School that is.

So, what lies ahead? Hopefully the right number of surprises; not too few, so as to keep things upbeat, but not too many, so as to keep things manageable. My laboratory is focusing on how chromatin folding changes in human nuclei during ageing, and on the rules governing this dynamic process. We aspire to be able to take advantage of such rules (once we understand them well enough) to manipulate gene expression in senescent human cells. Meanwhile, we haven't stopped trying to convince the world of the paradigm shift…obviously.

So back to where I started off, but perhaps that's exactly the idea here (again, the same way we envisage an RNA polymerase to work in a factory): moving to Cologne never really meant leaving Oxford behind. I carry those 4½ years with me, and would not have it any other way: obviously!

On 14th June this year, David Cameron announced the launch of the £1m 'Longitude Prize' to fuel scientific innovation which, he envisaged, would help stimulate the search for 'the next penicillin…'. In response to the announcement, the* Guardian *published an article discussing what lessons could be learned from the discovery of the 'first' penicillin 'by the Oxford Group, lead by Howard Florey and Ernst Chain'. Discussion of the history of the Dunn School among such lofty circles, illustrates the enduring legacy of penicillin and how the story of its development continues to inspire successive generations. Gilbert Shama, Reader in Applied Microbiology at Loughborough University, has a long-term interest in the history of penicillin and has been a frequent contributor to* Fusion. *On a recent fact-finding trip to the States, he uncovered surprising details of some of penicillin's earliest enthusiasts…


theguardian

How to discover an antibiotic: a historian's guide

Given our pressing need for new antibiotics, or a whole new class of antibiotic-like drugs, perhaps we ought to try learning lessons from the history of penicillin (it might even help someone win the new Longitude Prize!)

Can history teach us how to make new discoveries? Photograph: Radu Razvan /Alamy

Historians of science and medicine are often terrible killjoys when it comes to great stories about discovery and genius. We've been quick to point out that the apocryphal story of Fleming discovering penicillin mould 'by accident' when it blew in through a window and landed on a discarded petri dish is, well, apocryphal. We're less unanimous about the way penicillin became a drug. Although the 'Oxford Group', lead by Howard Florey and Ernst Chain developed the chemical extract as far as clinical trials, in the end it was American agricultural, pharmaceutical and military researchers who produced the first

Praise the Lord and Pass the Penicillin!

Gilbert Shama

Whilst searching through the United States National Archives at College Park, Maryland I came across a newspaper clipping from *The Herald Tribune* entitled 'Three Nuns Get Penicillin from Food.' The clipping contained a photograph showing a nun working in a laboratory. I was intrigued and decided to investigate further.

It transpired that the work was carried out at St John's, a Catholic University in Brooklyn, New York and founded in 1870. I also discovered that the news item had been widely syndicated, and a number of newspapers including the *Brooklyn Eagle* and the *New York Times* also carried the story. Accounts of this work also appeared in the *Catholic Herald* and *The Tablet*. From these and other sources, I was able to put together a fuller picture of the research.

The three nuns mentioned in *The Herald Tribune* article turned out to be Sister Immaculate, Sister Jean Agnes and Sister Anthilia. They were all teachers, and taught in various Catholic schools in Brooklyn. Sr. Immaculate wrote her Master's thesis on penicillin and the other two nuns appeared to have lent a hand with the work but wrote their theses on different topics. Research on penicillin had commenced at St. John's in April 1943 and Sr. Immaculate submitted her thesis in May, 1944. The thesis is simply entitled 'Penicillin, an Antibiotic Produced by Mold' and her work appears to have been primarily directed towards finding low cost medium constituents for the cultivation of penicillin-producing strains of *Penicillium notatum.* She also subjected mould cultures to various regimes of illumination and incubated them at different temperatures in order to establish optimum conditions for penicillin production.

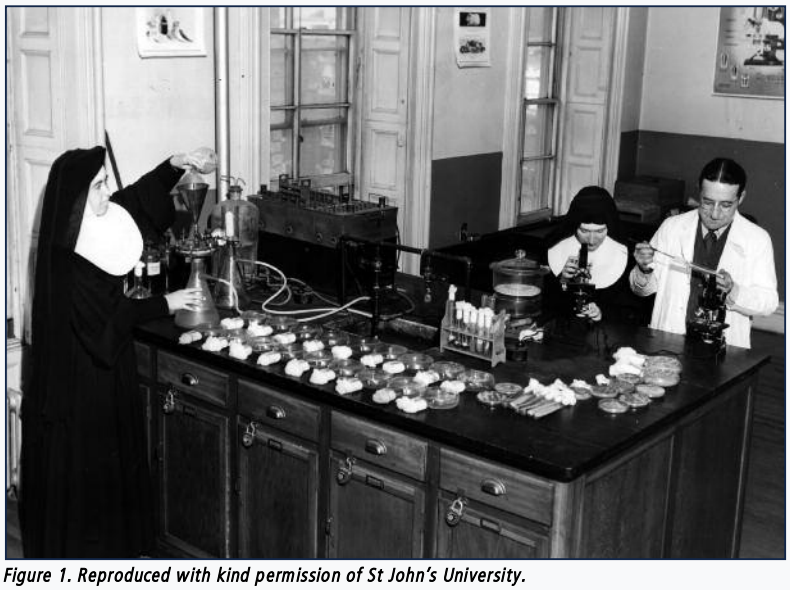


Figure 1. Reproduced with kind permission of St John's University.

A photograph, kindly made available to me by St John's University (Figure 1), shows the laboratory in which this work was conducted. Sr. Immaculate appears in the foreground and is in the process of filtering mould broth. Also depicted are Sr. Agnes and Professor Edward J Keegan, Chairman of the Biology Department at St. John's, and their research supervisor. In the foreground on the laboratory bench can be seen some twenty or so glass flasks for the surface cultivation of *P. notatum.* I attempted, so far without success, to establish the provenance of these flasks. Some of the newspaper accounts I came across referred to them as 'Kolle flasks', but these were originally developed for culturing micro-organisms on agar, and I am not therefore convinced that the description is accurate. One thing is certain, and that is that their design derives from the ceramic culture vessels first conceived of by Norman Heatley of the Dunn School.

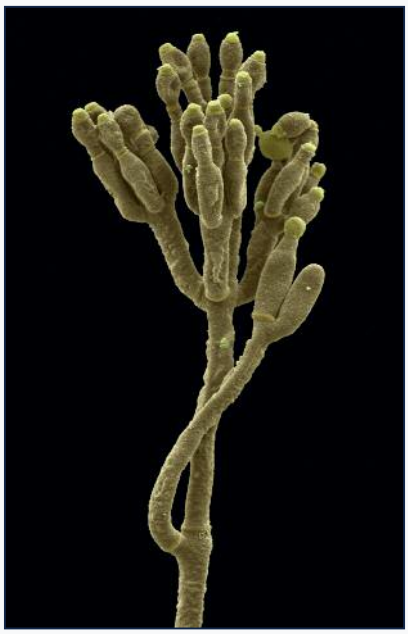


Figure 2. Penicillin conidiophore.
Courtesy of Errin Johnson

In her thesis Sr. Immaculate thanked the Pfizer Company for providing her with a strain of *P. notatum*, and Merck for supplying her with samples of penicillin for the purposes of standardisation. However, some appeals for information that she made were met with refusals on the grounds that the information requested was restricted as it could prove useful to the enemy. And indeed, Sr. Immaculate refrained for similar reasons from specifying the media formulations that yielded the highest penicillin titres. Whereas the newspaper reports make specific reference to the foodstuffs she worked with e.g. egg white and wheat flour, there is no mention of these ingredients in her thesis.

Inevitably inaccuracies crept into the newspaper accounts of this work, the *New York Times* informed its readers that 'Notatum are the reproductive bodies that cause a mold secreting the penicillin drug to grow.' Professor Keegan was quoted as saying that 'the addition of brandy and corn liquor to the foodstuffs acted in many cases as a catalytic agent, causing the penicillin-secreting mold to grow faster.' What Keegan almost certainly told the *New York Times* reporter was that the addition of corn *steep* liquor led to increased penicillin titres. It was researchers at the United States Department of Agriculture in Peoria, Illinois who had stumbled upon this particular finding. Corn steep liquor is in fact alcohol-free but contains a number of growth factors and, in particular, the amino acid phenylalanine, and it is this which leads to high penicillin titres. It might, of course, have been an innocent error on the part of a newspaper reporter not scientifically trained. But the reporter may have succumbed - or not been able to resist - the image of the nuns agonising over whether to tip the 'hard stuff' into the culture medium or whether to reserve it for other medicinal contingencies. A caution, therefore, (if one were needed), not to believe everything one reads in the newspapers!

Even in the days before mobile phones, the paparazzi managed to track Sr. Immaculate down by phone. She told a reporter from *The New York Times* that she and the other nuns 'preferred to let the university speak for them' but she did permit herself to confess that they all 'enjoyed their research immensely'.

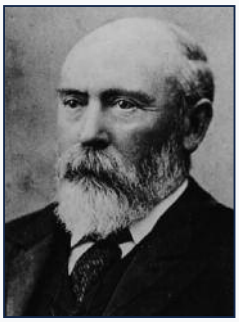
All of which reminded me of a passage I had come across in Robert Bud's book *Penicillin: Triumph and Tragedy* published in 2007. In it he made reference to research which showed that antibiotic usage tended to be higher in countries where Catholicism was the predominant religion. Epidemiological factors had apparently been ruled out as a possible cause leaving only cultural explanations. It is obviously important to understand these, as unnecessary dispensing of antibiotics is a factor in the development of disease resistance in bacterial pathogens. Bud went on to describe a comparative study of attitudes to disease and antibiotics conducted amongst the inhabitants of a Dutch town where the population was mixed Catholic and Protestant, and a Belgian town some 40 miles distant where the population was predominantly Catholic and where antibiotic use was considerably greater. The Dutch participants in the study tended to place greater reliance on nursing one's own illness without recourse to drugs in general and antibiotics in particular. This contrasted sharply with attitudes of the Catholic inhabitants from Belgium, where ailments such as colds and flus were more commonly referred to as 'bronchitis' and belief in alternative medicines was low. The revelatory conclusion to the study was that in the Catholic religion rituals with tangible sacraments play a central role, and as the authors put it, 'a pill could be seen as a secularised counterpart of the sacraments that can ensure salvation'. Quite how one changes such attitudes without shaking an individual's faith is a different matter altogether.

But to return to Sr. Immaculate's work, it should not be forgotten that she was, in fact, engaged in what at the time was cutting edge antibacterial chemotherapy research. Her motivation for this work is conveyed in the following passage from the abstract to her thesis': 'Daily newspaper reports on the successful use of penicillin, coupled with the regret that the supply is so inadequate for military and civilian needs, together with a patriotic desire to further its progress as a contribution to the war effort, have been the inspiration of this investigation.' A similar sentiment was expressed in *The Catholic Herald* wherein researchers from St John's were quoted as saying that they looked forward to a time when penicillin would become widely available for civilian use and when it would 'cost as little to buy as insulin does at the present time'.

There is perhaps another factor that may have influenced researchers at St John's to investigate penicillin. Press reports of the time tended to refer to penicillin as a 'miracle drug' and the cures which it affected as 'miraculous' as indeed those early clinical applications of the antibiotic must have appeared. Those directly involved in this research must have believed that the age of miracles had not ended after all, and that they could play a part in bringing one particular miracle to pass.

Who was Sir William Dunn?

Eric Sidebottom



This is a question I asked frequently from the time I first entered the rather grandly styled 'Sir William Dunn School of Pathology' as a medical student in 1960 until 2005 when, not having received what I considered a satisfactory answer, I 'researched' him myself and finally published my own answers in the *Journal of Medical Biography* and *Scottish Field.*

Sir William (pictured) does not rate an entry in the *Oxford Dictionary of National Biography* and the reference book *Who was Who* has a rather dry version of his life that does not even mention his legacies. It records that he was born in Paisley on 1st September 1833, the son of John Dunn and Isabella Chalmers, shopkeepers. He married Sarah Elizabeth in South Africa in 1859, the daughter of the already-deceased businessman, James Howse, of Grahamstown, South Africa. He died on 31st March 1912 and was not survived by any children. In South Africa, where he emigrated in 1852 aged 19, he soon became a partner and then the owner of the trading company Mackie, Dunn, and Co. in Port Elizabeth. He subsequently set up W. Dunn and Co. in Durban and Dunn and Co.in East London.

In the early 1860's he returned to London and was senior partner in the banking and mercantile firm of William Dunn and Company, Broad Street Avenue, London. He was an Alderman, Cheap Ward, EC, and a director of Royal Exchange Assurance Co. and Union Discount Co. He was MP for Paisley from 1891 to 1906 and was created a Baronet in 1895. He had substantial properties in Kensington and Lakenheath Suffolk and was a member of many clubs including the Reform, the City Liberal, the City of London and the Farmers Club.

So much for the bare bones of his life but, rather more importantly, why is he remembered in the names of the professorships and buildings of some of Britain's most famous scientific institutions? The answer lies in his will and the Trust it created. In the will, dated 4th November 1908, the key clause was 'to advance the cause of Christianity, to benefit children and young people, to support hospitals and alleviate human suffering, to encourage education and promote emigration'.

His estate was valued at about £1.3 million. After providing annuities of £3000 for his wife and £1000 for his adopted niece Sarah, and stating that he had adequately provided for his interests in the Presbyterian Church and South Africa during his lifetime, he left: £1000 to the head clerk in Wm Dunn & Co; £300 to the other clerks with more than 20 years service; £100 to clerks with more than 10 years service; £200 to his coachman; £100 to his gamekeeper and assistant gamekeeper, and £100 to all domestic servants with more than 10 years service. He also left named sums (between £500 and £5000) to about 40 hospitals, orphanages, children's homes and similar charitable organizations, and also to institutions in Paisley, all of which amounted to about half the total money available. The remainder of his fortune he left in the hands of Trustees.

After Sir William's death, Lady Dunn contested the will, maintaining that at the time of their marriage her husband had said that he could look after her money better than she could and that she had, therefore, placed it in his care. Not surprisingly, she claimed, she was now entitled to a share of his fortune. The Trustees sympathized with her claim and advised the Attorney General accordingly: the court awarded her £170,000.
It was perhaps surprising to learn that the original Trustees included the 20 Directors of the Commercial Union Assurance Society, none of whom apparently was known personally to Dunn. Their chairman, Sir Jeremiah Colman, of mustard fame, undertook his role with great responsibility. After making grants of between £100 and £20,000 to more than 120 hospitals, nursing homes, orphanages and other institutions, and endowing a Lectureship in Pathology at Guy's Hospital, the Trustees decided that a larger project would have a better chance of being a permanent memorial to Sir William. A key event in implementing this policy was a dinner, hosted by Colman's friend Lord Knutsford, at which the President of the Royal Society, Sir William Hardy, and the Secretary of the Medical Research Committee (later called The Medical Research Council), Sir Walter Fletcher, were present and were asked for their views. Their clear advice was to support the new discipline of biochemistry, for which Sir Frederick Gowland Hopkins (1861–1947) was the leading British figure. Accordingly, in 1920, the Trustees made a grant of £210,000 to Cambridge. Sir Walter Fletcher became a key adviser to the Trustees and it was largely his influence that led to the second large grant made by the Trustees, this time of £100,000 to Pathology in Oxford in 1922, where Georges Dreyer (1873–1934) was Professor (and £3000 to convert the existing small Pathology Department for Pharmacology). At that time, Dreyer was a member of the Medical Research Council and apparently a personal friend of Fletcher. Fletcher had stressed the crucial importance of giving financial support to medical research rather than merely to those institutions that housed patients. He believed, fervently, that identifying the causes of disease would give far greater benefit to mankind in the long term. By this means the phrase 'for the alleviation of human suffering' would be amply fulfilled according to the benefactor's wishes. His beliefs have been vindicated. It is perhaps fitting that Oxford University awarded Fletcher an honorary degree in 1926 when the 'Dunn School' was in the final stages of building.

Special Grants made by the Trustees of the Sir William Dunn Estate to Science and Medicine between 1914 and 1929:

School of Biochemistry, Cambridge £210,000
School of Pathology, Oxford £103,000
Guy's Hospital, lectureship endowment £24,866
The London Hospital £23,000
University of Edinburgh Medical School £20,000
St Bartholomew's Hospital £10,000
St Thomas' Hospital Insulin Laboratory £10,000
Medical Research Council (£2000 for 5 years) £10,000
Christ's Hospital School £10,000
Nutritional Laboratory, Cambridge £6,000

A fitting tribute to Sir William's generous legacy to science and to mankind is to be found in the form of the marble memorial on the main staircase in the Dunn School of Pathology in Oxford (Figure 1). The Latin inscription reads: 'this building, completed in 1926, was made available by a munificent gift from the will of William Dunn, Baronet, for the relief of human suffering'.

UNIVERSITY OF OXFORD

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I think it is fair to say that the benefits accruing from the decisions of the Trustees have indeed been considerably greater than either they or their Chairman would have dared to hope in 1929, or that Sir William himself might have imagined before his death in 1912. After all, one might reasonably argue that the development of penicillin by Florey's team was the most important medical advance of the 20th century; and to the Nobel prize awarded to Florey (1899–1968) and Chain (1906–79) for that work might be added two more to graduates of the Oxford Dunn School (Sir Peter Medawar (1915–87) and Sir John Walker (1941–) and five awarded to scientists associated with the Biochemistry Department in Cambridge: from Sir Frederick Gowland Hopkins (1861–1947) in 1929, the first professor, whose reputation was instrumental in setting the Trustees on their philanthropic track, to Fred Sanger (1918–) who has been honoured twice (in 1958 and 1980). Graduates of Cambridge Biochemistry, A.J.P. Martin (1910–2002) and R.L.M. Synge (1914–94), shared a prize in 1952 and P.D. Mitchell (1920–92) was awarded one outright in 1978. Although the Cambridge Biochemistry Department received the largest grant given by the Trust, it has not retained the use of the 'Dunn' name in its title (although its head is formally known as the Sir William Dunn Professor) and so is not now widely perceived as being one of the 'Dunn School family' of laboratories. In Cambridge this recognition usually goes to the Dunn Nutrition Unit, which received a mere £6,000.

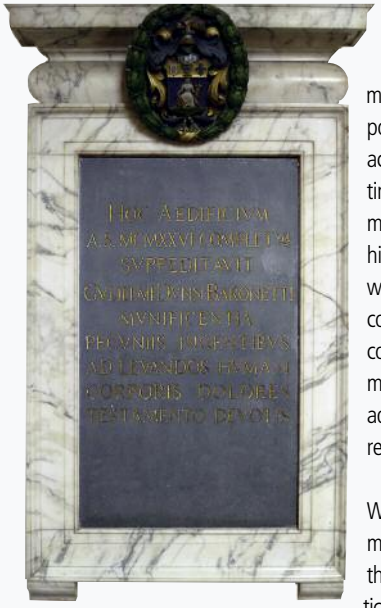

Figure 1. *The marble memorial to Sir William Dunn on the main staircase in the Dunn School of Pathology in Oxford.*

Lifetime benefactions

Throughout his life William Dunn was profoundly influenced by his firm Presbyterian upbringing and beliefs, and he was sympathetic towards many philanthropic causes. He gave substantial sums to the Presbyterian Church in Scotland, England and South Africa, and supported many needy causes in Paisley, including the gift in 1894 of an open space in the town centre, named Dunn Square, 'to be kept for the enjoyment of all the inhabitants'. In 1910 a fountain with a bronze statue representing Charity was erected in the square to commemorate Sir William's generous gifts to the town. The inscription on the drinking fountain statue in Dunn Square reads: *"The square was presented 23rd June 1894 to the town of Paisley by Sir William Dunn, Bart, MP for the Burgh 1891–1906. Erected to commemorate the gifts and services of Sir William Dunn, Bart, to the community of Paisley 1910"*.

An unpublished essay by Dr John Wylie, a well known Dunn School eccentric, which I found in the archives of the Oxford Dunn School, presents a totally different, extreme and entertaining view of Sir William's motives and mores but there is scant evidence for the highly critical view presented. Wylie's analysis groups Dunn with people, 'whose lives have been less than admirable in respect of commercial probity and hardly commendable as examples of social mores'. His attempted demolition of Dunn starts early with his marriage: "Dunn found it expedient, there was no damned nonsense about love, to marry and thereby extract the utmost benefit from contracting that particular civil state. He sought a spouse from the upper colonial class. His lot fell on one Sarah Howse. Her father was a man of substance both in terms of possessions and character" [he had actually been dead for seven years at the time of the marriage!] "It is, however, more than probable that Dunn schemed his way into what he must have known was a bereaved household and since he could, and often did, present a plausible countenance, no doubt secured his marital prize to his own great social advantage". (William and Sarah remained married for 53 years!).

Wylie makes many 'digs' at Dunn's commercial activities; "the precise nature of the businesses is still imperfectly authenticated and the obscurity was probably deliberately engendered and collectively covered a multitude of sins". "Like so many self-made men who amass large fortunes, Dunn was pathologically mean. He used to stay up late at the office checking and re-checking takings and being furious, obscene and blasphemous if the accounts were a farthing out".

The attempted character assassination continues with even greater vigour when Wylie turns to Dunn's political career; "successful men of business, when they enter Parliament, do so less for political than for social motives. Rightly or wrongly the House of Commons is regarded by many as a rather exclusive club and, as we have seen throughout this essay, William Dunn was the archetypal social climber. He was like so many of his genre in that once elected he took very little part in the business of the lower house."

I don't think we should take Wylie's views seriously. What we should be grateful for is the fact that a humble but ambitious Scottish youth became a very successful businessman who determined to give away most of his wealth to worthy causes and that he chose Trustees to administer his estate that sought advice from the country's leading scientists. And that one of those scientists was Walter Fletcher, a colleague and friend of Georges Dreyer, the Professor of Pathology in Oxford!

Fusion is produced by the Medical Informatics Unit, NDCLS, University of Oxford. Telephone +44 (0)1865 222746.
Ref: Fusion1013/800