fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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Editorial

This has been an interesting year for the Dunn School. We have watched (without too much sympathy) the expert demolition of the former Leslie Martin building, and the rapid construction of the Oxford Molecular Pathology Institute (OMPI) in its place. Our colleagues, who needed to decant to various parts of the South Parks site, have been magnificent in their capacity to adapt to makeshift laboratories and in their efforts to maintain a daily involvement in the life of the Dunn School. I am sure their patience will be well rewarded. We are now in the exciting phase of finding new inhabitants for OMPI, by filling three important vacant posts (the Chairs of Chemical Pathology and Experimental Pathology, as well as a Readership in Experimental Pathology) and we look forward to that challenge and opportunity.

Our Thursday lecture series have been as good as ever, and the highlight has been the 2010 Heatley lecture given by the Nobel Laureate, Elizabeth Blackburn. Not only was Dr Blackburn a wonderfully accommodating guest, but so too was her lecture full of new information in uncharted territory. Most spectacular was her observation that meditation had beneficial effects on telomere length, and stress the opposite. This gives me hope that the University and Divisional administrations now have a simple way of measuring the impact of their activities on the well-being of our population.

We are delighted to welcome Bass Hassan back into the fold, and are also delighted that Fumiko Esashi has joined us as a CRUK senior fellow. Congratulations too to Fiona Powrie who was appointed to the Truelove Chair of Gastroenterology , a remarkable achievement for a non-medical scientist. Finally, I am sure that many of you will be pleased to know the important contribution that George Brownlee and Ervin Fodor have made to enabling new types of influenza vaccine. It is very gratifying to us that their foresight has had such an important impact on human health, as in the recent epidemic of swine flu.

Herman Waldmann

'Elegance' by Adrian Gray, one of the winners of the Dunn School photographic competition 2009

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Closing the loop on cancer

Bass Hassan

My laboratory moved to the Dunn School during the Summer of 2009, but this is not my first residency within the department – I am privileged that the circular nature of life seems to return me here. Following clinical training in Oxford and a variety of junior hospital jobs that exposed me to the molecular basis of disease, I began life as a researcher in the Dunn School in 1991. At this time, Henry Harris was the Head of Department and ran a formal ship. I vividly recall the offer of a seat in one of the famous green leather chairs (now to be found in the library in the EPA building). Following an informal discussion, I knew my relationship with the department would be long and happy! With the support of Henry and the Wellcome Trust, I joined Peter Cook's lab to undertake a DPhil. I spent a very challenging and enjoyable three years at a time when Peter and his group started to move towards imaging nuclear functions. During this exciting time, we identified replication and transcription factories with Pavel Hozak and Dean Jackson. Working at the Dunn School was so inspiring that I was now hooked on science.

A return to clinical work at Addenbrookes' Hospital in Cambridge followed this and from there I went to work on curable cancers (such as lymphoma and testicular cancer) in the CRC unit in Southampton. In 1997, I returned to Oxford as a CRUK Senior Clinical Research Fellow, working exactly 100m away from the Dunn School in Chris Graham's lab in Zoology. Here, my interest in mouse genetics, imprinting and the IGF2 (Insulin-like growth factor 2) protein and the IGF2 receptor began and with it, the establishment of my own group. In 2003, I took up a Professorship in Oncology at Bristol University, before returning once again to Oxford in 2006 with a position at the Weatherall Institute of Molecular Medicine.

I am thrilled to be returning to the Dunn School. It is a privilege, nearly two decades and a few grey hairs later, but it comes with high expectations, not least from myself. With my ongoing clinical work, I hope to gradually introduce a further perspective to the department enabling greater interactions between students, scientists and clinicians, exposing all to broader experiences across the University.

"...I am privileged that the circular nature of life seems to return me here"

The work of my laboratory, over the last ten years, concerns the regulation of tumour growth. Current interests centre on the interaction between the growth-promoting function of the IGF2 ligand and its non-signalling receptor, the IGF2 receptor (IGF2R). Genetic models of the IGF system have established the key role of this signalling pathway in tumour growth control, in particular the function of the IGF2R as a specific inhibitor of IGF2. This information provided an excellent basis to study how tumour progression develops *in vivo* from pre-malignant changes to established tumours and how this information can be translated to human cancer prevention, diagnosis and treatment.

Structural and functional studies of the IGF2 receptor were carried out in collaboration with Yvonne Jones (STRUBI, Wellcome Centre, Oxford) and Matt Crump (Department of Chemistry, University of Bristol) and have led us to a basic understanding of ligand /receptor interactions. Characterisation of the protein-protein interactions has been the basis of extending the focus to the interactions of mannose-6-phosphate containing ligands, such as lysosomal enzymes, with other domains of the same receptor.

By using mutagenesis and surface plasmon resonance (BIAcore) we analysed the interacting amino acids and general stereochemistry involved in evaluation of these ligand/receptor interactions. These data have been used to develop a novel ligand trap for IGF2, having previously validated that the action of IGF2 in murine tumours genetically-predisposed to cancer is inhibited when bound to a soluble receptor. We have now generated novel forms of sIGF2R based on domain 11 of the receptor through collaboration with Cancer Research Technology. We aim to exploit these molecules for development and testing in early phase clinical trials run by my clinical group in the Oxford Cancer Centre.

The genetic aspects of the work now extend to interactions with loss of tumour suppressor gene function (Apc, Pten, Cdh1 and p53). In particular, we aim to define the Igf2 genetic dependency of tumours that arise from common somatically-acquired mutations in humans. For example, almost all human tumours are associated with loss of function of either p53 or Pten and we have recently shown that both phenotypes are modified by Igf2 gene dosage.

The group has been blessed with a Cancer Research Trust Programme Grant and this has allowed us to adopt new approaches. For example, despite decades of experimentation with IGF and insulin ligands, we lack detailed knowledge of ligand specificity with respect to gene expression signatures. We have utilised genetically-defined material that conditionally express different doses of IGF2 to study the temporal influence on the transcriptome of a gradually-accumulating dose of IGF2. Key transcriptional "hubs" have been identified and will be validated. Finally, the function of IGF2R still remains a particular interest and a number of novel transgenic strains with domain specific modifications at this locus are now being characterised.

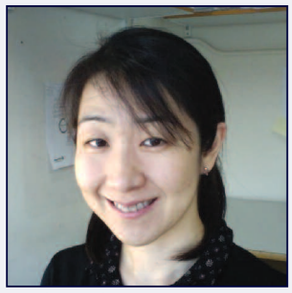
It now turns out that the IGF pathway is also a key regulator of human cancer growth, in particular tumours of bone, muscle and soft tissues (sarcomas). The lab is now intimately linked to translational research in sarcomas. Sarcomas are rare tumours that often occur in young people aged <25. The 5 year prognosis following treatment is around 50-60% survival and these treatments are often associated with side effects, such as infertility, secondary tumours and mutilating surgery. We are studying the IGF pathway biomarkers that may influence treatment decisions and the effects of therapeutic molecules that target the pathway. These will be tested in specifically designed clinical trials with the group and EuroBoNet, the first European network of excellence dedicated to bone tumours. The latter development has required a great deal of effort, but completes the bench-to-bedside ambition of my science in the Dunn School, thereby closing yet another loop on cancer.

An Interview with Fumiko Esashi

Tell us a little about your background and what led you into science as a career.

I grew up in an ancient Japanese city, Nara, surrounded by beautiful natural landscapes and old temples. I had no active encouragement from my parents or from schoolteachers to study science, but as a child I spent a lot of time observing small creatures in the rice fields or exploring the countryside with my older brother after school. Perhaps partly because my father was a sociologist and had a large collection of books at home, I also loved reading books and writing essays. The series of '*Souvenirs entomologiques*' was one of my favourites, and I tried to 'examine' similar insects' behaviour around my house. I guess these were my founding scientific experiences.

Although I was (and still am) always curious about how life arose and how it functions, I didn't formally study biology at high school. When I was asked to choose only two science classes, I vaguely felt that studying chemistry and physics might provide a strong foundation that would eventually allow me to understand biology in depth. I read Animal Science at Kyoto University as an undergraduate simply to fulfil my scientific curiosity, but started to consider a scientific career seriously when I was accepted as a PhD student in the laboratory of Prof. Mitsuhiro Yanagida, one of the world pioneers of fission yeast genetics. When I started, I didn't know much about him except his intensive research on the cell cycle and chromosome segregation. What I found unexpectedly enjoyable, however, was that he had many close personal interactions with leading scientists from Europe and from the US, so we had quite frequent visitors from abroad in the lab who provided a lot of stimulating inspiration. On these occasions, he often asked students to give the visitor a guided tour of Kyoto, and also arranged meals together. From these pleasurable experiences, I naturally developed a desire to study science abroad when I finished my PhD.



Fumiko Esashi

My PhD studies addressed biological processes primarily using a genetic approach, which at times leaves the underlying molecular mechanisms unexplained. For my post-doc research, I wanted to learn something different. Despite my poor English skills at that time, I was lucky to be given an opportunity to work as a post-doc at Cancer Research UK, Clare Hall Laboratories in the laboratory of Dr. Stephen West, who is an expert on protein biochemistry in DNA repair. He employs a reductionist approach to understanding biological processes, which was exactly what I was hoping to learn. All my experiences at Clare Hall were eye-opening and refreshing for me; everyone was extremely respectful of each other's research style, and I gradually developed my own identity as a scientist during my six years post-doc research supported by personal fellowships from the Human Frontier Science Program and the Japanese Society for the Promotion of Science.

In the last year of my post-doc, I was privileged to be awarded a Cancer Research UK Career Development Fellowship, which is annually granted to 2–4 people in the UK. This Fellowship allowed me to run a small group for a period of 6 years, and I started the group in the Weatherall Institute of Molecular Medicine in 2007. I am very fortunate to have two very enthusiastic research staff in the group, and a new DPhil student joined the group last year. Having relocated to the Dunn School last December, I am excited and thrilled to have distinguished scientists around who run fore-front research groups in various fields.

Where did you first develop an interest in the cell cycle and why does it have such a fascination for you?

I don't exactly remember how I first developed my interest in the cell cycle, but I remember that I immensely enjoyed the International Exposition held in Tsukuba in 1985, which my brother and I attended without my parents. I remember that I insisted on queuing for hours to watch the world's first 3D-computer graphics movie shown in the Fujitsu Pavilion. The movie, 'The Universe', showed how living organisms are created from atoms to cells, and from cells to human beings. I was especially impressed by the beautiful architecture of the DNA molecule and became very curious about how DNA molecules totalling 2m in length can be packed inside a nucleus 10μm in diameter. That's equivalent to 2km of string being packed into a 1cm ball – and all the DNA is copied and segregated into two daughter cells without tangling during the cell cycle – how amazing is that! While I was studying for my PhD, I developed an interest in the molecular mechanism of DNA repair in actively-dividing cells. The cycle of cell division is so beautifully organised, and even if DNA is damaged at times, cells can fix it and resume the same beautiful cycle again. It is just so elegant and is simply fascinating.

What, for you, has been the most exciting discovery you have made so far in science?

The feeling of 'excitement' can be influenced by experience and by expectation at the time, but I think my most exciting and unexpected results were found during my PhD study, which addressed the involvement of cyclin-dependent kinase (CDK) in DNA damage responses. CDK is a master regulator of the cell cycle, and it was well known by that time that activation of CDK is blocked in the presence of broken DNA. This mechanism, known as the DNA damage checkpoint, explained how dynamic cell cycle events such as cell division can be delayed while broken DNA is being repaired. My PhD study addressed the role of a fission yeast checkpoint protein, Crb2, identified by a former student in the lab. Fission yeast lacking the *crb2+* gene showed clear problems in checkpoint activation and DNA damage repair, but I was curious how Crb2, as a protein, functions in cells. We knew that Crb2 protein was highly phosphorylated after DNA damage, and contains a perfect target site for Cdc2, a fission yeast CDK. My PhD supervisor casually suggested that I should analyse a Crb2 point mutant at the Cdc2 site and, to our surprise, the mutant showed striking sensitivity to DNA damage. Damage-induced Crb2 hyper-phosphorylation was also significantly reduced by this single point mutation. Interpretation of this phenotype was rather puzzling however, as what we had found was that a Cdc2-dependent phosphorylation event is important for a function of Crb2 under circum-stances where Cdc2 was considered to be inactive as a result of the checkpoint response. It is still not clear precisely how this phosphorylation is involved in the normal DNA damage response, but later studies from other groups confirmed that CDK activity is indeed required for damage responses in various organisms including budding yeast, fission yeast and human cell lines.

Tell us a little about your current research interests: what questions do you hope to address over the coming years?

I am still extremely curious about how CDKs control DNA damage responses positively in some respects, and negatively in others. Is CDK activity differentially regulated spatially and temporally in cells after DNA damage? If so, what is the underlying molecular mechanism? Is there a simpler and more elegant explanation that we haven't yet uncovered? I find this extremely fascinating because of the inherent apparent contradiction. At present, I am particularly interested in CDK-dependent regulation of homologous recombination, which is used to repair broken DNA after DNA replication. We primarily focus on molecular regulation of the breast cancer susceptibility protein BRCA2, which regulates homologous recombination by its direct interaction with the evolutionarily-conserved recombinase Rad51. BRCA2 is highly phosphorylated by CDK during the cell cycle, and we speculate that these phosphorylation events may play important parts in cell cycle-dependent homologous recombination. It is worth noting that hyper-recombination is known to be one of the major causes of genome instability, which often underlies cancer development. As CDK de-regulation is often found in actively-dividing cancer cells, I hope my research will also address a molecular mechanism of cancer progression.

What do you find most frustrating about science?

I never find science itself frustrating, as every single result tells us something new, and negative results give me further motivation to design other strategies to address the question. It sometimes feels like digging holes here and there to find a treasure box, but I love

new challenges and feel that science is a most rewarding activity. If I could be very honest, however, hunting for research funding is frustrating. In an ideal world, there would be greater recognition that many great discoveries in the past were made, not as the result of strategic approaches, but through scientists' simple curiosity.

How have you found the transition to the Dunn School from the Weatherall Institute of Molecular Medicine?

Thanks to generous help from Prof. Waldmann and the support teams in the Dunn School, I found the transition extremely smooth. Despite our off-site lab location in the Rodney Porter Building, we met most people in the School within a few months, and our research is moving ahead well. I am very pleased to find many like-minded scientists in the School, and am very much looking forward to developing my research in the coming years by interacting with new colleagues here.

A model for all genomes:
the role of transcription factories

Peter Cook

Human chromosomes are arguably the largest and most important biomolecules. Here, Peter Cook summarizes a model for their structure that has emerged from his long-term research into nuclear structure.

Human chromosomes are arguably the largest and most important biomolecules, but what their structure might be – and how that structure affects function – remains one of the major challenges of our age. We proposed a model for their organization; active transcription units scattered along a chromosome cluster into "factories", to loop the intervening DNA (Fig. 1). Although loops are found in many other models, here a promoter distant from a factory is unlikely to be transcribed; instead, that promoter only fires once it has diffused to a factory and bound to transcription factors and polymerases concentrated there. This creates a new loop, and transcription drives the organization; loops appear (and disappear) as factors bind (and dissociate), and polymerases initiate (and terminate). Consequently, the structure constantly changes, and statements about it are necessarily probabilistic. This model is general in the sense that it can be applied to all genomes, including those of bacteria.

Transcription factories play a central role in this model. A "factory" is defined in The Oxford English Dictionary as *'a building or range of buildings with plant for the manufacture of goods.'* In our case, each one contains at least two (often more) polymerases and associated plant active on at least two (often more) different templates. The *raison d'être* of all factories is the same: to enhance production by concentrating relevant machines and raw materials in one place. For example, the nuclei of HeLa cells contain a 1mM pool of RNA polymerase II, but essentially all its transcripts are made in factories where the local concentration is ~1,000-fold higher. A second important feature is the immobilization of engaged RNA polymerases; an enzyme fixed in a factory reels in its template as it extrudes its transcript. These two features have prevented acceptance of the model, simply because they run so counter to what we were taught – namely that individual active enzymes track like locomotives along their templates (Fig. 2A,i). But like so many other cherished beliefs, this one has little support.

This story began ~35 years ago on the top floor of the old building: after lysing HeLa cells in Triton and

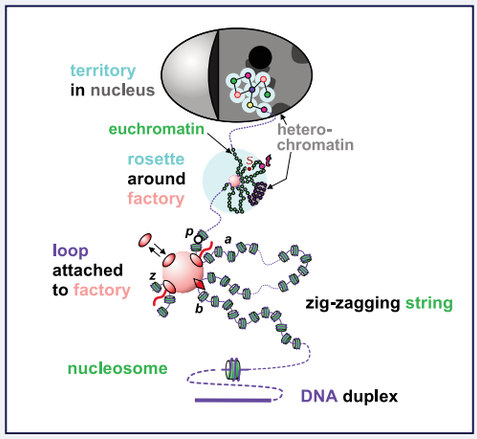


Figure 1. A model for all genomes. *In man, DNA is coiled into the nucleosome, and runs of nucleosomes form a zig-zagging string looped by attachment to factories through transcription factors (diamond) and engaged polymerases (ovals). In HeLa cells, the average contour length of a loop is 86 kbp (range 5-200 kbp), and the core of a nucleoplasmic factory has a diameter of ~90 nm and a mass ~10 MDa. The promoter, p, has just initiated, and a fixed polymerase is reeling in its template as it extrudes its transcript; the polymerase will soon transcribe (a). Components in a factory exchange continually with the soluble pool. About 16 such loops (only a few are shown) form a rosette around a factory; half the attachments are mediated by active polymerases, half by transcription factors. Distal nucleosomes in long loops tend to be static and acquire a (heterochromatic) histone code that spreads down a fibre; they also aggregate on to the lamina, nucleoli, and chromocenters. A string of 30 –180 successive rosettes forms a territory (the general path of DNA is shown). Different factories (circles of different colours) specialize in transcribing different sets of genes. Here, active transcription units that are near neighbours form a rosette (e.g., a and b), but the structure can be more complex; for example, z may be distant from x on the genetic map (which would generate a giant loop), perhaps even on a different chromosome. As promoter s lies closer to the factory than t, s is more likely to collide with a polymerase in the factory and initiate.*

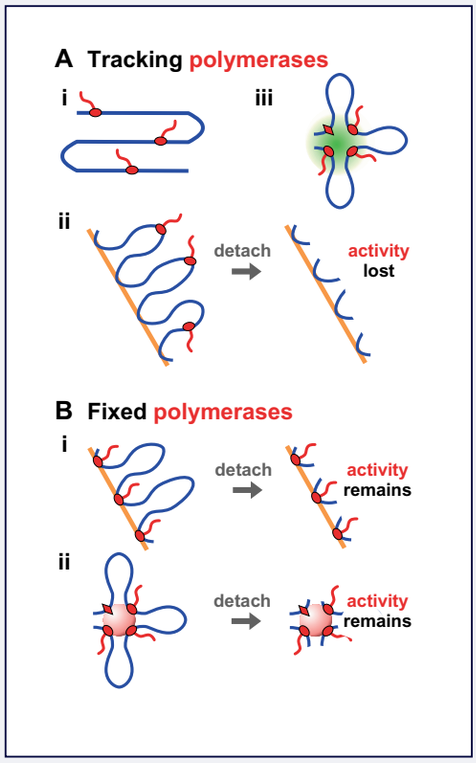


Figure 2. Models for the organization of transcription.

A. Tracking polymerases.

(i) The traditional view: genes are transcribed by tracking polymerases wherever those genes might be. (ii) Loops are attached to the nuclear sub-structure, and active polymerases transcribe as they track round the loops; nuclease treatment should detach active polymerases. (iii) An unspecified force field (green) keeps tracking polymerases in a cluster.

B. Immobilized polymerases.

(i) Active enzymes are bound to the sub-structure; they loop the fibre, and resist detachment. This was our original model. (ii) Active enzymes are bound to the surface of factories (as in Figure 1), and resist detachment. This is our current model.

2 M NaCl, Iris Brazell and I found that the now-naked DNA was supercoiled. As supercoiling can only be maintained in a linear DNA molecule if the ends were tied down in some way, this meant that the linear chromosomal fibre was organized into loops. The obvious next step was to map which DNA sequences were attached to the substructure, so Dean Jackson's experiment was to trim away most of the loop with nucleases and then see which DNA sequences were left. We all expected he would detach the active genes, to leave some conserved and repeated sequence attached to the substructure (Fig. 2A,ii). To our surprise, essentially all polymerizing activity remained; it looked as if active polymerases were the molecular ties that maintained the loops (Fig. 2B,i). These results could (rightly) be criticized on the grounds that our extraction buffer induced active transcription complexes to aggregate, so we confirmed them using a "physiological" buffer. We now know that our imagined repeats are unlikely to exist (as the genome projects would surely have uncovered them), and that immobilized enzymes are powerful enough molecular motors to act in the required way.

The next big step forward also came as a surprise, although with hindsight, it shouldn't have done! Now in the middle floor of the old building, we permeabilized HeLa cells, and allowed the still-engaged polymerases to "run-on" by ~40 nucleotides in BrUTP; the resulting nascent BrRNA was not diffusely spread throughout the nucleus (as in Fig. 2A,i or Fig. 2B,ii) but concentrated in a few discrete foci – the factories (Fig. 2B,ii and Fig. 3A). Fig. 3B illustrates what is currently our highest resolution image of a nucleoplasmic factory. We are now isolating factories (the trick is to release them from the sub-structure with caspases) and examining their contents by mass spectrometry; we are also seeing which DNA sequences share which factories (in particular, those involved in transcribing genes 10 min after inducing an inflammatory response with tumour necrosis factor α).

Models should be useful. Fortunately, intuition suggests that a promoter tethered close to a factory will be more likely than a distant one to collide with that factory and initiate (compare promoters *s* and *t* in the rosette in Fig. 1). Computer simulations confirm that this is so, and it is then easy to imagine that proximal and distal promoters will be active and inactive respectively, and that regulatory motifs act by tethering target promoters more or less closely to factories.

This model is still controversial – people find it difficult to give up their cherished beliefs and accept the idea that active polymerases are both immobilized and clustered. As a result, they are now proposing models like that in Fig. 2A,iii, where still-tracking polymerases somehow cluster together through the action of ill-defined forces. (This becomes our model, if a factory replaces the green force field!) But the tide seems to be turning, especially as techniques like "chromosome conformation capture" and RNA fluorescence *in situ* hybridization are confirming that active transcription units and their transcripts cluster together in the way we imagine. Moreover, we will soon know if the details of our model are correct. For example, the new "deep" DNA sequencing technologies should allow us to determine the relative frequency with which every base in a genome interacts with a polymerase, transcription factors, and every other base (through looping), while "super-resolution" microscopes should permit individual polymerase molecules to be imaged in living cells. And there is much more to do. For example, the model allows us to predict how active a gene might be when inserted at different positions in a loop – an essential requirement if the dreams of gene therapy are ever to be fulfilled.

Acknowledgements

None of this work would have been possible without that very special group of people we call the Dunn School; I also especially want to thank the E.P. Abraham Research Fund. Work in my lab has also been supported by the BBSRC, Cancer Research UK, MRC, and the Wellcome Trust.

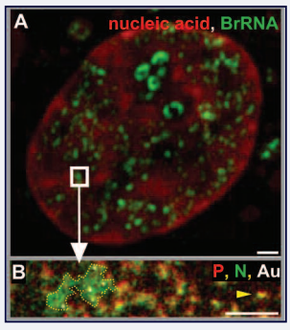


Figure 3. Factories in HeLa cells.

A. *Cells were permeabilized, nascent transcripts extended by ~40 nucleotides in BrUTP, cells cryosectioned (100 nm), resulting BrRNA immuno-labeled with FITC (green), nucleic acids counterstained with TOTO-3 (red), and a confocal image collected. Newly-made BrRNA is concentrated in factories in the cytoplasm (made by mitochondrial polymerases), nucleoplasm, and nucleoli. The bar represents 1μm. Image courtesy of Ana Pombo.*

B. *Image of nucleoplasmic factory in an unstained section obtained using an EM with a special filter. Cells were permeabilized, nascent transcripts extended in BrUTP, and resulting BrRNA immunolabeled with 5nm gold particles; after sectioning (70 nm), images of endogenous phosphorous (red) and nitrogen (green), plus immunolabeling gold particles (white), were collected and merged. Five particles mark BrRNA in a nitrogen-rich factory (perimeter indicated). Absolute numbers of N and P atoms within this perimeter (and so the molecular mass) can be calculated using nearby nucleosomes as references (arrowhead). Bar: 100 nm. Image courtesy of Christopher Eskiw.*

Structural Biology in the Dunn School Today

Susan Lea

The use of atomic structures to inform biology has been a longstanding interest in the Dunn School. From the early structures of penicillin and cephalosporin through the work of Penny Handford and George Brownlee, which defined the structure of epidermal growth factor like domains, to Dale Wigley's work to define structures associated with replication of the genome, there has always been a significant interest in the fundamental architecture underlying function. In recent years, there has, however, been a significant increase in the number of structures arising from the Dunn School, some of which continue to flow from collaborations with external scientists but many of which follow on from the arrival of the my group in 2006.

Apart from that basic desire to "know what something looks like" how does structural knowledge inform and drive the scientific process? I will attempt to answer that question using examples from our own work since our arrival in the Dunn School. The interest for me, in relocating the group to the Dunn School, was to embed ourselves amongst other scientists interested in pathogenesis and immunity, rather than to define our physically-closest colleagues by use of shared techniques. This greater sense of shared focus means that, in addition to the projects in which I have historically taken an interest (see for example Figure 1), we are now working with many of the other groups within the Dunn School to help them answer questions using our methods. Prior to moving to the Dunn School we had solved the structure of a molecule identified in Siamon Gordon's group, EMR2, a member of the EGF-TM7 family of proteins, but the first collaboration after our move was with the newly-appointed Professor of Microbiology, Stephen Bell. This collaboration was not actually to determine a structure (Stephen had many pre-existing, successful structural collaborations) but to use another biophysical technique called electron paramagnetic resonance to distinguish between two hypotheses regarding the allosteric mechanism controlling the activity of an archeal helicase, by demonstrating that activity is regulated by interactions between, rather than within, the subunits that make up this ring-like structure.

Another recent structure from our group arose from a long term interest in the molecules used to regulate a part of our innate immune system, the complement regulators. In particular we have been interested in a process common to many bacterial pathogens, where the invading bacteria scavenge host complement regulators and coat themselves in these host-derived molecules to provide protection from this arm of the host's immune response (Figure 2). This structure, solved in collaboration with Prof. Chris Tang (Imperial College, London) was of one of these scavenging proteins in complex with a portion of the host regulator and revealed that the bacterium uses a protein to bind the regulator in a chemically similar manner to that in which the regulator would usually bind to the surface of host cells via interactions with sugars. Whilst this was interesting from the basic science perspective of trying to understand how protein chemistry is used to mimic sugar chemistry, the key result of this work was the demonstration it provided of minor changes to the bacterial protein ablating binding of the regulator. The scavenging protein was derived from *Neisseria meningitidis* and the ability to design antigenically similar versions of this protein that do not bind the complement regulator has proven of great interest to those trying to design vaccines against *Neisseria meningitidis* strain B, to which no established vaccine exists. This work has now been licensed by Novartis and we are developing our ideas for novel vaccines as part of a research agreement which includes a commitment to determine several related structures to further refine our ideas.

Figure 1. Shigella flexneri *type three secretion machine.*

In the last few months we have been working with Neil Barclay's group, who have been interested in the structure of cell-surface proteins for a long time, to bring their structural work "in house" to the Dunn School. The first structure we have investigated in collaboration with them – one member of a group of proteins called CD200 receptors – has now been solved and we are just entering the exciting phase of working with Neil, Deborah Hatherley and others to determine what this structure means. This is proving a very strong validation of my idea that locating the group amongst people interested in the same biological questions would be an exciting place to work. With structural research occurring either directly or via collaborations with more than half of the groups in the Dunn School, the next few years should be an exciting time to be thinking structurally in this part of South Parks Road.

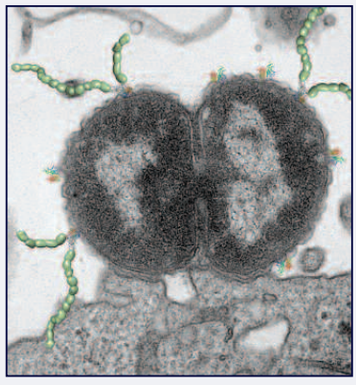


Figure 2. Neisseria meningitidis *scavenges host immune regulators.*

Decision Making In The Intestine — The Importance of Going With Your Gut

Andrew Johnson

Fiona Powrie was recently elected to the Truelove Chair of Gastroenterology in recognition of her achievements in the field of mucosal immunology. Here Andrew Johnson, a DPhil student in Fiona's laboratory, reviews work which has influenced our understanding of the way in which the decision to mount an immune response in the gut is made.

The complex environment in the intestine poses difficult questions for our immune system to answer. Fundamentally, it must continually assess the level of danger and 'decide' whether to *tolerate* a stimulus e.g. food or commensal flora or to launch an inflammatory response e.g. against pathogens. This balance between tolerance and inflammation underlies many inflammatory conditions not just of the intestine but of disparate tissues and may even influence metabolic diseases such as obesity. Therefore, determining how inflammation is regulated in the intestine is of increasing importance and offers sizeable therapeutic benefits.

A small population of myeloid cells known as dendritic cells (DCs) are crucial in initiating and regulating immune responses. They sample the tissue environment looking for foreign material which may be evidence of an infection. This material is carried to the draining lymph nodes where the DCs present fragments of the antigen to T-cells. If the material presented is truly non-self then the T-cells will become activated and the immune response is initiated (Figure 1).

However, the intestines contain a huge amount of foreign material which is *not* evidence of infection. It is simply derived from the diet or from the commensal flora with which we have a largely symbiotic relationship. Therefore, it is undesirable that we should launch inflammatory responses against all foreign material, but rather the immune system must distinguish between the "good" and the "bad" in more subtle ways. In order to manage this problem, the DCs of the intestine are specialised so that they actively promote *tolerance* of the harmless material while still remaining able to instigate protective responses against opportunistic infections. Broadly speaking this is achieved in two main ways. Firstly, DCs differentiate into distinct subsets which are more or less predisposed to act in an inflammatory manner. Secondly, these subsets are modified by the local environment so that they have the ability to become pro-inflammatory or equally to promote tolerance depending on the context in which they encounter foreign material.

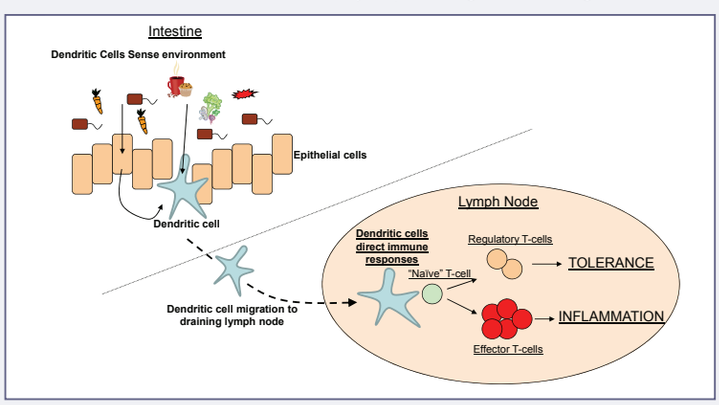


Figure 1 | *Dendritic cells and the initiation of immune responses.*

Intestinal Dendritic Cell Subsets

DCs in the lymph nodes and spleen can be readily identified by high expression of CD11c and MHC class II. Application of these markers to the intestine identifies a population of cells which can be further separated into two main subsets (summarised in Figure 2).

The first subset expresses the integrin CD103 and has relatively low expression of macrophage markers such as F4/80 ($CD103^+$ DCs). The $CD103^+$ DCs differentiate along the same pathway as conventional DCs and migrate to the lymph nodes carrying antigen. The Powrie lab and others have shown that, in the absence of inflammation, $CD103^+$ DCs preferentially promote regulatory T-cells which are crucial for maintaining tolerance. This property of $CD103^+$ DCs was dependent on transforming growth factor (TGF)-β and was enhanced by their production of retinoic acid. Furthermore, $CD103^+$ DCs produce fewer inflammatory cytokines than their $CD103^-$ counterparts indicating that they are more tolerogenic in nature.

The second subset does not express CD103, but instead has very high expression of the fractalkine receptor, CX3CR1. Although resembling DCs in many respects, it is now clear that $CX3CR1^+$ cells do not migrate, remaining resident in the intestine instead. Furthermore these $CX3CR1^+$ cells in fact differentiate from monocytes in a manner more akin to that of a macrophage. Therefore, the precise classification of this subset is a matter of debate with many preferring the vague classification of lamina propria cell ($CX3CR1^+$ LPC) until they are better understood functionally. It is clear that these cells are more inflammatory in nature than $CD103^+$ DCs although this does not routinely result in excessive inflammation. The unique ability of $CX3CR1^+$ DCs

to extend processes into the lumen of the intestine has favoured a hypothesis whereby these cells are specialised to sense the environment and act in a more innate manner to prevent infection. However, the function of these cells in the steady-state remains largely speculative and a role in promoting tolerogenic T-cell responses in the tissue remains possible.

A partitioning of function between CD103$^+$ DCs (which migrate to lymph nodes) and CX3CR1$^+$ LPCs (which remain resident in the tissue and interact more closely with the bacteria) is one way in which the complex environment of the intestine is managed. In this model the adaptive immune system remains largely unexposed to the bacteria present in the lumen. Furthermore, any antigen presentation in the lymph node is coupled to the more tolerogenic CD103$^+$ DCs favouring induction of regulatory T-cells and tolerance. However, opportunistic infection is prevented locally by the more inflammatory, yet tissue-resident, CX3CR1$^+$ LPCs.

DC Conditioning by the Intestinal Environment

The tolerogenic phenotype of CD103$^+$ DCs in the intestine does not necessarily hold true for CD103$^+$ DCs identified in other locations such as the liver, skin and spleen. In addition, the retinoic acid producing capability of CD103$^+$ DCs in the lymph node is restricted to those which have migrated from the intestine rather than those which differentiate and reside within the lymphoid tissue. Therefore, it is clear that the phenotype is in part determined by the local intestinal environment. Conversely, during an immune response CD103$^+$ DCs lose their tolerogenic phenotype and become more inflammatory.

Exactly which signals in the intestine modulate CD103$^+$ DCs in the steady-state is still unclear. Such factors have been postulated to derive from the diet, such as specific lipids, the commensal flora and signals from the epithelial cells, such as TGF-β. Intriguingly, retinoic acid itself can be derived from dietary vitamin A reinforcing the link to environmental factors. During inflammation, it is likely that innate signals, such as inflammatory cytokines, can convert CD103$^+$ DCs into more inflammatory cells. Alternatively, the breakdown of the intestinal barrier during inflammation may increase exposure to microbial products overcoming any existing limits to DC maturation. The identification of environmental factors which modulate intestinal DCs is likely to offer potential therapies to both dampen inappropriate inflammation and to improve adjuvants for oral vaccines.

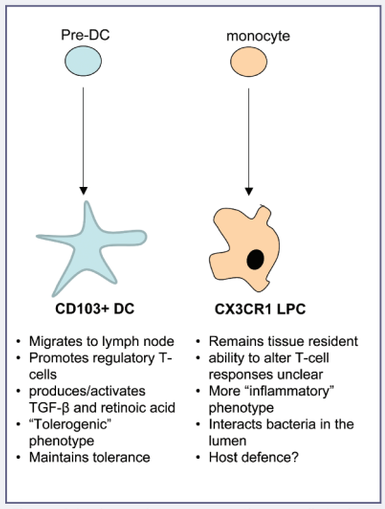


***Figure 2** | Subsets of CD11c$^+$MHC Class II$^+$ cells in the intestine.*

In summary, the intestine represents a complex and difficult environment full of foreign material. This material is mostly harmless but there remains the very real threat of pathogenic infection. The characterisation of the intestinal DCs indicates how this environment might be managed through distinct subsets and local modification. However, there are many questions still to address. Uncovering the function of CX3CR1$^+$ LPCs in their new context as non-migratory cells and the pathways modifying CD103$^+$ DCs represent two areas which are of high importance.

News, Honours, Prizes

March 2010: Neil Barclay awarded programme grant

Neil Barclay has been awarded a 5 year programme grant of £1.8M by the MRC to continue the analysis of the leukocyte cell surface and in particular the class of proteins called paired receptors.

April 2010 Eva Gluenz features in recent ASCB news publication

Eva Gluenz is featured in a recent ASCB news publication following her talk on 'using "virtual labs" in African Workshops' at the ASCB Meeting in December in San Diego.

Eva Gluenz

April 2010 Keith Gull chairs report on teaching in Universities

A new report from the Academy of Medical Sciences draws attention to the need to value teaching in UK universities. The report comes from a committee chaired by Professor Keith Gull. Comments have been published on the *New Scientist* website

May 2010 Royal Society University Research Fellowship for Janet Lovett

Our congratulations go to Janet Lovett (Lea Group) who has been successful in obtaining a Royal Society University Research Fellowship to move to the University of Leicester. We wish her well in her future career.

Establishment of CIU Trust for Immunology and Pathology

A new charitable trust has been set up to aid research and education in pathology and immunology. It has been established using royalty income and provides a simple method of utilising these funds with tax advantages. The trust has been set up so that particular aims can be prioritised according to the donor's wishes. If you are interested in donating royalties or other funds to this new venture, please contact ciutrust@hotmail.co.uk

A Letter from America

Siamon Gordon

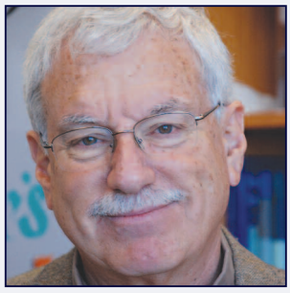


Figure 1 *Alan Sher*

Figure 2 *Giorgio Trinchieri*

Siamon Gordon retired from the Dunn School almost two years ago and has recently returned from a year's sabbatical in the United States from where he took time out of a hectic schedule to write of his recent exploits.

In the run-up to my official retirement in September 2008, after 32 years at the Dunn School, I was loath to give up my laboratory and research group, though not sorry to gain freedom from Home Office rules and grant applications. It was, therefore, welcome when Alan Sher (Figure 1) and Giorgio Trinchieri (Figure 2), immunologists at the National Institutes of Health, invited me to spend a year in Washington. President Obama was about to take office and expectant optimism was in the air, although the economic downturn was still gathering pace. My job description was vague (come and talk to people about myeloid cells, somewhat neglected in a highly T cell-centric environment). My wife readily agreed, since she was finishing a book on Emily Dickinson and would enjoy privacy and ready access to the Library of Congress and research libraries at Yale, Harvard, Brown and the New York Public Library. So, we left our older daughter with partner in our Oxford home and set off to enjoy freedom and stimulation in the District of Columbia. In the spirit of Alistair Cook, I was pleased to be asked to write a letter from America, with reflections on the time we have spent here.

It was a good experience, although inevitably I accomplished little of a formal nature, a review or two, some previous research papers, sundry talks, both in and outside the NIH campus, a snow-disrupted conference and rather less tourism in DC than anticipated. Nevertheless, we renewed old friendships, having lived almost 10 years in New York before moving to Oxford in 1976 and made some new friends… I barely knew the NIH itself, though had known several contemporaries over the years. In this brief, informal account I reflect on my impressions of a privileged, national research enterprise which surpasses, in both size and scope, the biomedical research activities in Oxford and at Rockefeller University, where I had been one of the first foreign research students. Since I was outside the system, yet made welcome, the atmosphere was relaxed, open and interactive, although there are several dispersed sites in Bethesda, Frederick (about an hour away, near Civil War battlefields), Twinbrook, Baltimore and even Rocky Mountain. Although the downside is that groups and individuals easily become separated, the flip side is that regular Retreats bring colleagues together: since many research groups are small, collaborations are encouraged, as is the sharing of facilities and expertise. Nevertheless, it seems a pity that bacterial infectious disease and immunology, for example, are not as integrated as virology and parasitology. With my interest in macrophages, I was lucky to be attached to Alan Sher's group at Bethesda (parasite immunology, rich in biologic interest) and to the innovative programme on Inflammation and Cancer led by Giorgio Trinchieri at Frederick. Bethesda and Frederick are worlds apart; the one busy, a never-ending stream of seminars and academic traffic, the other in peaceful, small-town America. The support staff, administrative services and security officers in Bethesda are interracial and very international; those in Frederick tinged by the South, are friendly but with a cordon of stern perimeter guards, perhaps because of its close proximity to Fort Detrick, an earlier military base with Category 4 pathogen containment facilities. Although tiring at times, I ended up enjoying the commute by shuttle or with Joost Oppenheim, a leukocyte biologist steeped in anecdotes and my guide to the institution.

I savoured my contacts with the postdocs and the few doctoral students, some of whom are in the NIH Oxford-Cambridge programme. It was endearing to be greeted unexpectedly by Dunn School Alumni such as Xiangping Yang from Oreste Acuto's lab, or Katie Graef, who worked with Ervin Fodor. Through them I got to know their NIH supervisors, John O'Shea, a talented musician and epigeneticist, and Kanta Subbarao, an interactive influenza virologist. My own student, Willie Siu, was completing his last year of research and his supervisor Bob Nussenblatt, an ophthalmic immunologist, literally opened my eyes to this privileged organ.

So as not to give too rosy an account, the bureaucracy involved in obtaining a badge to enter the NIH campus and laboratory was second to none, confirming the adage of the bigger the organisation, the less efficient, perhaps because I belonged to two independent administrative centres. Another trial was my over-hasty subscription to the Immunology e-mailing list, which brought with it a flood of urgent requests for reagents, proof of open collaboration in daily action.

So, does the NIH provide value for money, a point raised by many U.S. scientists competing for tight funding in the extra-mural programme, whilst the "fat cats" bask in stable, more secure intramural support. The groups I saw at closer quarters, are in the top flight internationally. Apart from cutting edge research, the NIH covers rare diseases as part of their clinical translation of basic science, and as such serves as an international referral

centre. There is also the feeling that they are expected to react to global medical crises, such as HIV/AIDS and swine/avian influenza epidemics. Their international and outreach programmes are generous and accessible, in spite of Federal Government control. And yet, there is soul searching on the part of senior administrators and staff to do more for health through programmes on problems such as obesity, drug abuse and adolescent health. The scientific diversity reflects the rich natural history of human disease.

My abiding memories will remain the campus in Spring, not only during Cherry blossom time, the lunchtime concerts, while staff enjoyed hot soup in *Le Bon Pain*, the sense of a community which employees could expect to enjoy for decades. These attributes are a far cry from the poverty of parts of Washington, an ivory tower away from the strident clamour of Tea Party politics. Inevitably, the Obama honeymoon was short-lived, with fierce protests and, dare I say, intolerant speech by opponents of a "socialist" health care bill. With the change in the Administration, stem cell research is no longer such a doctrinal issue, and it was reassuring to hear a reasoned, articulate defence of American society, even if greeted with noisy divisions between the two nations that comprise the United States.

The Broader View

Don Mason

After a long and distinguished career at the forefront of immunology, Don Mason retired from the Dunn School in 1999. Since then, he has been involved in the work of four charities and served as an official prison visitor at HMP Grendon. Recently, he published a book entitled Science, Mysticism and Religious Belief and provides here a brief taster of its contents…

We experience the world at two distinct levels: there is the world of common experience and the world which is known to us only as individuals – our personal world. Only the first of these provides the material for those sciences that are regarded as exact. In these no scientific fact is established unless the observations on which it rests are precisely repeatable by independent workers. For the world of personal experiences no such repeatability is possible and here what is true for one may be heresy for another. I shall return to this point later but first I shall examine the limitations of science in its provision of explanations of the world of common experience.

The Physical World

It is a widely held view, not only among the general public but also among many scientists, that science provides us with the understanding of natural phenomena. The origin of this misconception in readily found: science has been amazingly successful in explaining, for example the fact that the planets in the solar system rotate around the earth in elliptical orbits and that mutation-induced differences in DNA between parent and offspring give rise to phenotypic differences between the generations. One could, of course, quote literally hundreds of other examples. The reason that these explanations do not lead to a comprehensive understanding of the physical world is that they are essentially superficial – they require that so much is 'taken as read' before the offered explanation is developed. Although this limitation seems to be either ignored or not even recognised at all by many scientists, there are some notable exceptions.

It seems to be one of the fundamental features of nature that fundamental physical laws are described in terms of a mathematical theory of great beauty and power, needing quite a high standard of mathematics for one to understand it. You may wonder: Why is nature constructed along these lines? One can only answer that our present knowledge seems to show that nature is so constructed. We simply have to accept it. One could perhaps describe the situation by saying that God is a mathematician of a very high order, and He used very advanced mathematics in constructing the Universe.

Paul Dirac

Dirac, a Nobel laureate, was, of course one of the most outstanding scientists of the 20th Century. His mathematical theory of the electron led to the prediction of the existence of anti-matter before it was discovered experimentally. The above quotation is taken from something he wrote when he was over sixty; in his earlier years he was a strident atheist. The above quotation might be paraphrased by stating 'the world is the way it is because that's the way it is'. Put in these terms the idea that at a fundamental level science explains anything, is exposed for the fallacy that it is. Albert Einstein made a similar point, much more elegantly when he wrote:

I am not an atheist and I don't think I can call myself a pantheist. We are in the position of little children entering a huge library filled with books in many different languages. The child knows someone must have written those books. It does not know how. It does not

understand the languages in which they are written. The child dimly suspects a mysterious order in the arrangement of the books but does not know what it is. That, it seems to me, is the attitude of even the most intelligent human being towards God. We see a universe marvellously arranged and obeying certain laws, but only dimly understand those laws. Our limited minds cannot grasp the mysterious force that moves the constellations. My religiosity consists of a humble admiration of the infinitely superior spirit that reveals itself in the little we can comprehend of the knowable world. That deep emotional conviction of the presence of a superior reasoning power, which revealed itself in the incomprehensible universe, forms my idea of God. The most beautiful and most profound emotion we can experience is the sensation of the mystical. It is the sower of all true science. He to whom this emotion is a stranger, who can no longer wonder and stand rapt in awe, is as good as dead.

Albert Einstein

A striking feature of these quotations is that both scientists readily recognise the limitations of the human intellect to 'explain' the physical world. In passing, one may note that not all scientists display such humility. However, it may be recalled that there are thought to be more stars in the universe than there are grains of sand on all the world's beaches and the oceans of this world alone contain countless drops of water – but man cannot make one grain of sand, nor one drop of water. A degree of humility would seem to be appropriate.

The whole modern conception of the world is founded on the illusion that the so-called laws of nature are the explanations of natural phenomena.

Ludwig Wittgenstein

The World of the Individual

If we have difficulty in comprehending the world of common experience how much more is this so of our own personal experiences. In the western world, where the materialistic paradigm is dominant, individuals are naturally cautious about recounting their own experiences that call into question its validity. This is particularly so where an experience may be described as religious, spiritual or mystical and those who defend the materialistic paradigm sometimes do so with remarkable vigour.

There used to be spiritualism, there continues to be ESP (extra-sensory perception)… Where corruption of children's minds is at stake, I do not believe in the freedom of the press or freedom of speech. In my view, publishers who publish or teachers who teach any of the pseudo-sciences as established truth should, on being found guilty, be publicly horse-whipped, and forever banned from further activity in these usually honourable professions.

Ex-director of the U.S. Bureau of Standards
(Published in the *Bulletin of Atomic Scientists*)

(There is a famous historic precedent for this sort of reaction to new ideas. One recalls that a charge of corrupting the minds of the young was used by the establishment in ancient Athens to sentence Socrates to death. On a personal note I, like other scientists who chose to write on these matters, have been warned that I put at risk my own scientific reputation and indeed, as I write this article, I am aware that the editors of *Fusion* may decide not to publish it, lest it tarnishes the credibility of their publication.)

Consider the following quotation from the American psychologist and philosopher William James:

The sciences of nature know nothing of spiritual presences, and on the whole hold no practical commerce whatever with the idealistic conceptions towards which general philosophy inclines. The scientist, so-called, is, during his scientific hours at least, so materialistic that one may well say that on the whole the influence of science goes against the notion that religion should be recognised at all.

The word 'religion' is a difficult one in that it means different things to different people. Fortunately James gives us his definition.

…the feelings, acts, and experiences of individual men in their solitude, so far as they apprehend themselves to stand in relation to whatever they may consider the divine.

As I have indicated when quoting Dirac and Einstein, James' pronouncement about the materialist attitude of scientists is not universally applicable and as Poincaré has written:

The scientist does not study nature because it is useful, he studies it because he delights in it, because it is beautiful. If nature were not beautiful, it would not be worth knowing, and if nature were not worth knowing, life would not be worth living.

Despite these very weighty exceptions, one does recognise that for the most part James is correct in his assertion. However, the materialist paradigm does not deal comfortably with those human experiences that are most significant to those who have them. To illustrate this point I shall describe the experience of a small child, recounted in later life.

It was a summer day and I was playing at the back of the house, in an alley in the city where we lived. It was one of my happier days, when I had found playmates. A sudden storm came up and interrupted our play. I sat alone out there between garages behind the house waiting for it to end. It was near noon. The rain ended almost as soon as it came, and the sun shone hot and bright once more. All at once I felt as if I were seeing

everything for the first time. The light seemed like gold, the smell of the wet foliage was like perfume, with the rainwater shining and running about in little rivulets, the humming and the buzzing of insects and bees was pleasant to my ears. Everywhere I looked there was beauty. In that dirty alley wherever there was a leaf or a blade of grass it sparkled. I was filled with a sense of great comfort and peace. Now I watched a beetle going about its business, and then a small garden spider, and I was glowing with warmth. It was as if all that was outside of me, I felt to be part of it. Then a thought came. It said, "See! Everything is alive, everything lives. That insect, it has life, the grass, the air even." And then I felt joy, and with joy, love and then a feeling of reverence.

Such experiences are not as rare as one might suppose. In different surveys in the UK, China, Turkey and India approximately 50% of people reply in the affirmative to the question 'Have you ever been aware of or influenced by the presence or power, whether you call it God or not, which is different from your everyday self?' (1)

However one may choose to interpret these findings, one can say with conviction that experience of the mystical is as much a contemporary phenomenon as it is one found in historical accounts and religious texts. So, what do you think?

1. The Alister Hardy Centre, Department of Theological and Religious Studies, University of Wales, Lampeter, Ceredigion. SA48 7ED

Pipicilline *or* Waste not, Want not

Gilbert Shama

Gilbert Shama is a Senior Lecturer in the Department of Chemical Engineering at Loughborough University and has a long-term interest in the history of penicillin, which he has researched extensively over the years. In the second of the articles he has written for Fusion, Gilbert explores a little-known twist to the penicillin story…

What's the connection between events at the Hôpital de la Pitié following the Liberation of Paris in World War II, those activities that, according to Lord Byron, conspire to leave us only 'the summer of a dormouse,' and a spouted ceramic vessel manufactured by the Burslem firm of James MacIntyre? If you think you know the answer then perhaps you should consider putting yourself forward for BBC Radio 4's *Round Britain Quiz:* if, on the other hand, you haven't a clue, then read on…

The means by which news about penicillin came to be diffused throughout Continental Europe was described in an earlier edition of *Fusion*. In France attempts to make penicillin centred on the Pasteur Institute and also on the pharmaceutical company of Rhône-Poulenc, with which the Institute had had a history of successful research collaboration with those forerunners of antibiotics – the sulphonamides. Because the Institute possessed Fleming's strain of *P. notatum*, they were able to undertake the small scale preparation of penicillin beginning sometime in the autumn of 1943. By January 1944 researchers at the Institute had accumulated enough material to attempt the treatment of patients. One of the first of these (and possibly *the* first patient) to be treated with 'home-grown' penicillin was a 4½ month old baby suffering from pneumococcal meningitis. The infection had proven resistant to massive doses of sulphonamides – 7 g per day were being administered to a baby weighing only 6 kg! Penicillin therapy commenced in late January, 1944.

The physicians treating the child were to witness the powers of penicillin in eliminating – initially at any rate – the *Pneumococcus* from the baby's spinal fluid. But despite early success the young patient succumbed to an oedema and died on 16th February.

Researchers at the Pasteur Institute were working under conditions of extreme privation. Louis Pasteur Vallery-Radot (Louis Pasteur's Grandson), the author of a preface to a collection of articles on medical research carried out in France during the war years, attempted to give some idea of what life was like for scientists during this time:

'How could these men (sic!) *carry on with their work, under such seemingly impossible conditions? The equipment of laboratories was deficient, often the gas and electricity were cut off; to obtain the animals for necessary experiments was of the greatest difficulty.'*

Shortages engendered first by the war and then the Occupation, forced people in France wherever possible to seek to re-use and endlessly recycle *all* scarce resources. Researchers at the Pasteur Institute had a pretty good idea of what the fate of penicillin was once it had been administered, and they would have taken to extracting the antibiotic from the urine of patients receiving treatment. (Incidentally, award yourself two well-earned points if you were able to link 'urine' with Lord Byron's phrase about 'buttoning and unbuttoning').

Figure 1 *Intelligent design: from bedpan to culture vessel.*

Interestingly, this approach had also been employed in Oxford in February 1941 in the treatment of PC Albert Alexander with penicillin. Researchers at the Dunn School became engaged in a desperate struggle to save PC Alexander's life, but they lost the battle when the yields recovered became too low to be effective. Later studies in Britain and the United States were to show that some 50% of penicillin administered intravenously was eliminated into the urine within a period of one hour. Subsequent American work, conducted with dogs receiving penicillin, showed that the antibiotic could even be detected in their tears. Interestingly, those tears contained two of Alexander Fleming's great discoveries – penicillin *and* lysozyme!

One testament to the difficulties faced by the French researchers is the fact that, despite their heroic efforts, only some 30 persons received treatment with penicillin – they simply could not access the raw materials to produce more. Notwithstanding the difficulties involved, they took to publishing accounts of penicillin therapy whenever possible; the case of the hapless 4½ month old baby was the first to appear in print in a French medical journal. But most importantly, these *Pastoriens* had gained expertise in extracting every last unit of penicillin from urine, and they even went on to publish a paper on the subject only months after the end of the war. However, their expertise was to be called upon in a manner few could have predicted...

Following the Liberation, Paris became the centre for a number of Allied military hospitals. At this time, penicillin was still reserved exclusively for military use. However, the urine of wounded servicemen receiving penicillin was quite a different matter. An arrangement was brokered with American hospitals in which urine from such patients was to be made available to the newly-formed and grand-sounding French Military Penicillin Team under the command of '*Pharmacien-Capitaine*' Desbordes. There was apparently some initial resistance to the scheme by certain elements in the US Army Medical Corps on the grounds that the urine containers to be placed on each ward could themselves become sources of infection, and that the additional *va-et-vient* on the wards associated with a twice daily collection of the containers would disrupt normal ward procedures. However, these objections were brushed aside and the first hospital to participate became the *Hôpital de la Pitié*. (Award yourself another 2 points if you got that!)

A notice that appeared at the 48th General Hospital (US Army) based at the Lariboisière hospital addressed to 'all ward personnel' informing them of the arrangements, and written by the Chief of Laboratory Services, Major Andrew Fodor, began thus; 'Seldom indeed does the opportunity occur to give with no material loss to the giver.' An attempt at rhetoric rendered somewhat bathetic in the French translation in which Major Fodor's first name appears as "Andrex"!

The first collections were made by metro, and *le bon Capitaine Desbordes* describes a scene when unwitting passengers were joined by one of his men carrying a wooden crate containing 30 litres of urine. But things were to look up for the Penicillin Team and they were soon to be properly staffed and equipped. The captain was assigned 12 men, one car, 3 small vans and various bottles, aluminium containers (probably milk churns!) and all necessary labels. In addition, there was to be a monthly allowance of 20 kg of chloroform and sundry other materials necessary for the actual extraction. There were soon to be no less than 14 participating hospitals. The urine thus collected was taken to an *atelier*, or workshop, in the south west of Paris made available by Rhône-Poulenc. It has not proved possible to access company records describing the precise nature of the extraction equipment, but it was evidently of the type that might be described as 'pilot scale'. It needed a minimum volume of liquid to enable operation to proceed, and early urine collections falling below this volume had to be poured down the drains to the obvious chagrin of all those involved in the endeavour.

Needless to say collecting and putting aside the penicillin-containing urine of wounded servicemen was not always a top priority for hard-pressed ward personnel who must have had other pre-occupations, particularly following wholesale evacuations of wounded soldiers from the forward battle areas to hospitals in Paris. The scheme started in January 1945, but by the middle of March of that year the Chief of Medical Services found it necessary to issue a memorandum pointing out that the collection of urine had become 'very lax' and ending with the slightly menacing 'It is desired that future reports received in this office [will] indicate a sufficient daily collection....' The memo evidently worked, and by mid April the daily 'harvest' exceeded 300 litres. It is estimated that 100,000 Oxford units of penicillin could be recovered from between 20 and 25 litres of urine. (Do the maths, but no points on this occasion!).

And finally: the Burslem firm of James MacIntyre? When Norman Heatley started thinking about scaling-up penicillin production at the Dunn School in 1941 he pressed all sorts of available containers into service. This included an enamel hospital bedpan with which he obtained very promising results. He soon set about designing a vessel based on this humble receptacle and the firm of MacIntyre's in Burslem manufactured a batch of spouted ceramic vessels with which the Dunn School were able to commence a steady output of penicillin (Figure 1). (Award yourself an additional 2 points if you knew that particular piece of trivia!).

History Corner

Immunology 100 years ago

Eric Sidebottom

The first custom-built pathology laboratory in the University of Oxford opened its doors in 1901 under the direction of James Ritchie.

From the earliest days, the work undertaken in the laboratory was heavily slanted towards the new science of immunity and immunology. Metchnikoff and Ehrlich were awarded the Nobel prize in Medicine in 1908 for their work on mechanisms in immunity but by then Ritchie had already submitted a review of the new subject to Edinburgh University for an MD degree (and had been awarded a Gold medal for it). The review had also been published as three articles in the Journal of Hygiene in 1902.

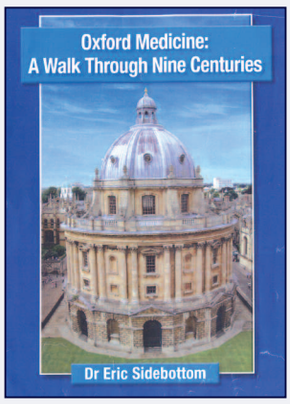
Ritchie had appointed Ernest Ainley-Walker (who had been a student in Oxford and attended one of Ritchie's earliest 'Path and Bact' courses) to a Lecturership in the department in 1903. After Ritchie's departure back to Edinburgh in 1907 Georges Dreyer, an anglophile Dane, was appointed as the department's first full professor. Dreyer and Ainley-Walker both worked on immunity and in 1909 they published two joint papers in the Journal of Pathology and Bacteriology entitled: "Observations on the production of immune substances" and "On the difference in content of agglutinins in blood serum and plasma", copies of which can still be seen in the library.

These century-old papers should make fascinating reading for those interested in understanding the background to the research they are now pursuing. The first paper discusses where in the body 'immune substances' 'internal secretions' or 'antibodies' are produced and also where 'complement' is made. In both cases the conclusion is that these are products of leukocytic activity. No specific mention is made of lymphocytes but mono-nuclear leukocytes do get a mention in the discussion. The reference list is fascinating in that it contains the names of several of the most famous scientists from that period, such as Pasteur, Metchnikoff, Bordet and Roux.

The second paper addresses the differences in the levels of agglutinins (antibodies) found in serum and plasma. This was, at the time, a controversial issue and Dreyer and Ainley-Walker claimed that they consistently found higher levels in plasma than in serum. They concluded, not unreasonably, that this must be due to non-specific absorption of the agglutinins in the formation of the clot.

On looking back at the early work in immunity and immunology, I find it very interesting to compare the rapid progress made in some areas, eg immunisation, with the almost insurmountable problems which still perplex us today, eg autoimmune disease. Let us hope that the current generation of scientists will surmount the current barriers.

Oxford Medicine: A Walk through Nine Centuries



One of our editors, Eric Sidebottom, has recently published a short guidebook on the history of Oxford Medicine. It is entitled "Oxford Medicine: A Walk through Nine Centuries". He has put a copy in the department library for inspection.

Eric is offering the book for £8, a discount of 20% on the publishers price of £9.99. If any readers are interested in learning more or buying a copy they should e-mail Eric directly at: eric.sidebottom@path.ox.ac.uk

Top Ranking: The Oxford Molecular Pathology Institute

Cecile Jenkins

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It is not often when people talk about the construction of a building that they can be positive about it both running on schedule and within budget. That is, however, exactly what the Dunn School can say about the new institute it is creating, the Oxford Molecular Pathology Institute, on the site of the former Leslie Martin Building.

Thanks to the immaculate budgetary planning, financial administration, and with a little bit of an advantage due to the economic times, the managers of this state-of-the-art research complex can be proud of the fact that this £30m institute is due to come in on schedule and under budget.

The Institute's core structure was completed with the last beam placed on top of the building in April, more than a month before schedule. The Topping Out ceremony was held at the end of May, in the same week that the 70th Anniversary of Penicillin was celebrated. Thought to have come from Scandinavia, this tradition places a leaf or branch on the topmost beam or, in the olden days, it would be a small fir tree, decorated with flags and streamers. This event, which was hosted by the Vice-Chancellor, celebrated the overall completion of the building's structure. Tradesmen, Dunn School staff, donors, architects and many others gathered on top of the building to toast the completion of the work thus far.

The Oxford Molecular Pathology Institute, or OMPI, will house existing programmes in immunology, molecular developmental biology, microbiology and cancer cell biology, and a new generation of leaders, for which the recruitment process has started. These will fill the currently vacant positions of the EP Abraham Professorship of Chemical Pathology (previously held by George Brownlee, FRS), the Glaxo Professorship of Cellular Pathology (previously held by Siamon Gordon, FRS) and the Readership in Experimental Pathology (previously held by Gillian Griffiths).

Providing the Dunn School with a distinct and competitive asset in the field, the new Institute houses 4,400m^2 of state-of-the-art laboratories, instrument rooms, containment suites and offices that are designed to offer flexible accommodation for the School's cutting edge molecular and cellular researchers and their teams.

The building is due to be completed in January 2011. And although one can never be sure in the building trade… it is fair to say that with the construction work being 1 month ahead of schedule and with only 4 months to go, it looks like we can say with a certain amount of confidence: OMPI will be open for business from January 2011.

A video clip showing the internal views of the Institute, including the labs, meeting rooms and offices, is available on the OMPI website at www.path.ox.ac.uk/Facilities/OMPI

Making a gift to the Dunn School

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

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

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