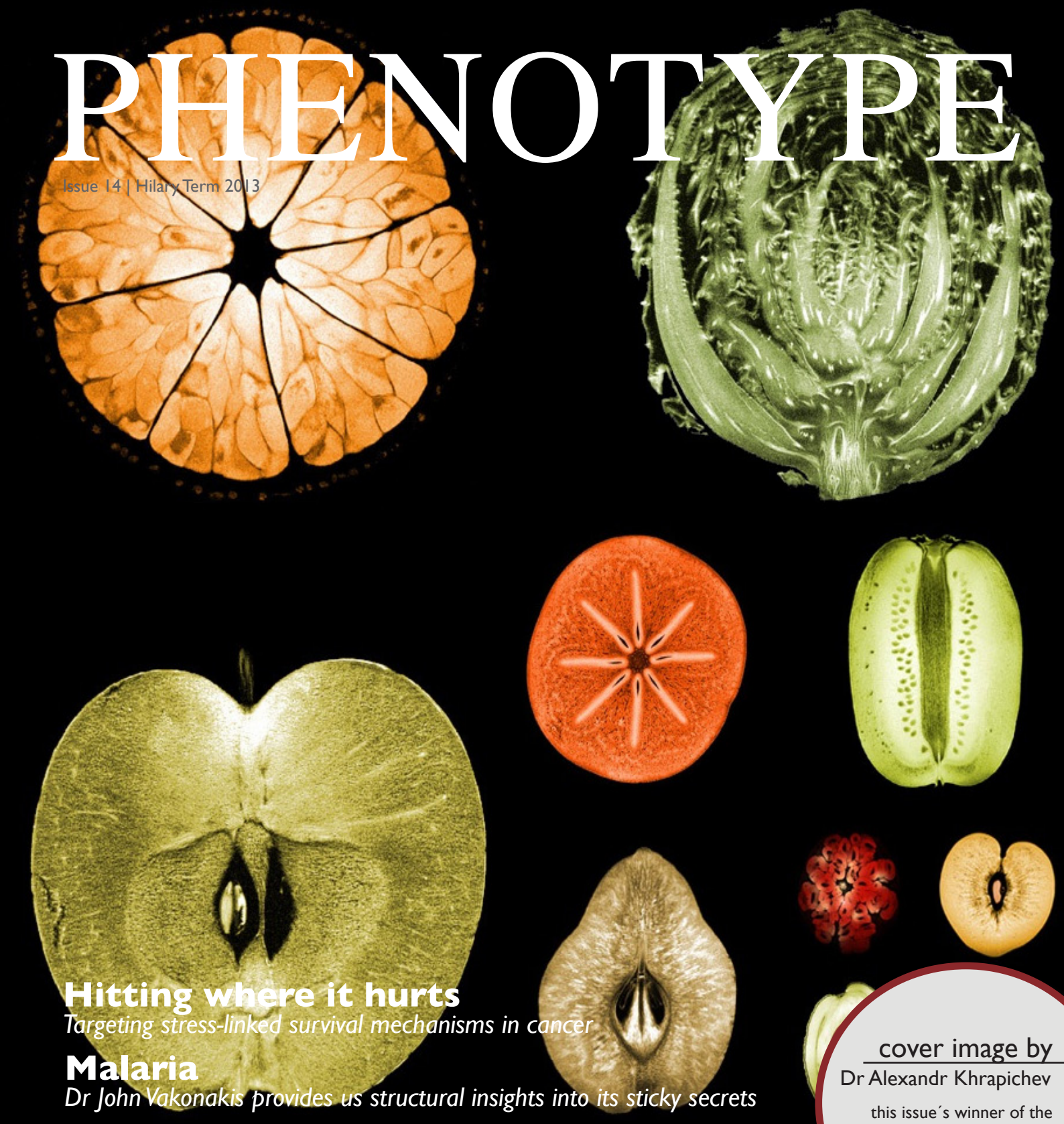
PHENOTYPE

Issue 14 | Hilary Term 2013



Hitting where it hurts
Targeting stress-linked survival mechanisms in cancer

Malaria
Dr John Vakonakis provides us structural insights into its sticky secrets

Schizophrenia and Bipolar Disorder
When mood is dependent on epigenetic DNA changes

cover image by
Dr Alexandr Khrapichev

this issue´s winner of the SNAPSHOT scientific image competition
page 31

Are women losing out in the world of science?

What makes a good scientist?

5´ with... Dr Monika Gullerova

What can life be like after science?

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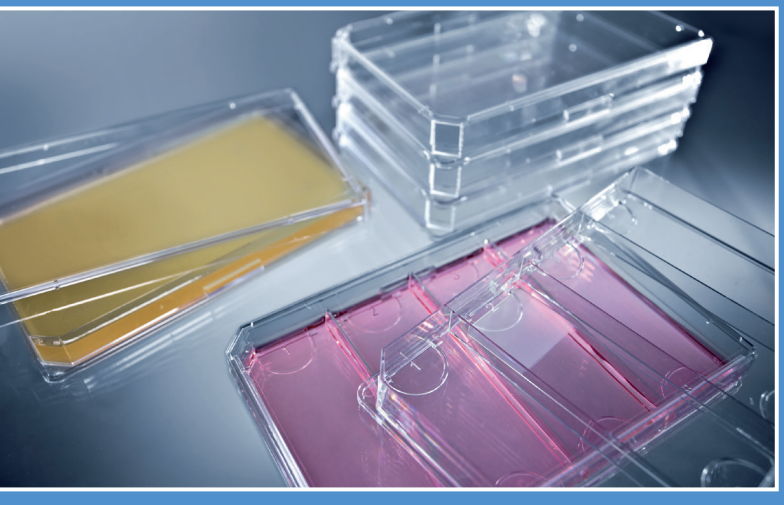

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EDITORIAL

Welcome to the fourteenth issue of *Phenotype* magazine! This issue is packed with our traditionally diverse and exciting articles contributed by PIs, research staff and students from across the University.

Dr John Vakonakis introduces us to the way the most lethal species of malaria parasite endows infected red blood cells with adhesion characteristics that lead to tissue damage, and describes work done in his laboratory to understand how parasite and host proteins organise in cytoadherence. We also spend five minutes with Dr Monika Gullerova, who is starting her own lab in the Sir William Dunn School of Pathology, and tells us about her journey in science and her enthusiasm for writing.

This issue we highlight a variety of human diseases: Aarthi Subramanian tells us about the latest research on the genetic cause of dystonia and treatment options; Maria Mogni focuses on recent epigenetic research in schizophrenia and bipolar diseases, pointing to commonalities between these diseases; and finally, Arsalan Azad introduces us to the mTOR pathway and explains how his lab has uncovered a regulating mechanism that could be targeted for cancer therapeutics. Still on the human topic, our research comic prodigy Richard Wheeler is back, decoding the ENCODE project!

Beyond the research, this issue also focuses on the researchers. Lars Hanssen provides food for thought on what makes a good scientist, by analysing last year's Nobel Prize winner Sir John Gurdon's life course. Sophie Fleurdipine reflects on attitudes based on gender in the academic world and challenges us all to uncover our implicit biases. Finally, Anna Boleininger gives us insights from the corporate world on how the skills developed in research can be applicable to other areas.

On the biotechnology side of research, Susan Graham reflects on the controversial challenges in regenerative medicine research and the way in which companies and research institutes are collaborating to develop personalised therapies. If you are curious about what it takes to start up a biotechnology company, Stuti Mehta's interview of PCR Biosystems' founder Alexander Wilson will provide you with some insight.

OUBS will be hosting Prof Mark Buttner later this term. Read more about his vast work on *Streptomyces* with a focus on clinical applications particularly regarding antibiotic production, presented by Isobel Steer.

Congratulations to senior research fellow Dr Alexandr Khrapichev, the winner of last issue's **SNAPSHOT** competition. Our cover is colourfully graced by his cross-section images of fruits obtained by Magnetic Resonance Imaging. Further details on his work can be found on page 31.

Wrap your head around our cryptic molecular biology crossword and give it try! If you can crack the crossword you could win one of the Wiley-Blackwell textbooks reviewed in this issue. If you were puzzled by last issue's crossword, the answers are also available on page 32.

If you enjoy science communication and publishing, why not join us in our sixth year of existence as a writer, editor or designer. Contact us on oubs@bioch.ox.ac.uk! While you are at it, why not enter the Oxbridge Biotech Roundtable science writing competition and stand a chance at winning a £500 prize. The competition opens in February, so keep an eye on www.obrreview.com.

Lastly, a very warm thank you to the highly motivated and creative *Phenotype* team of post-docs and students, whose hard work and enthusiasm make this wonderful publication possible!

Clara Howcroft Ferreira
Department of Physiology, Anatomy and Genetics

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Phenotype is also available online: www.bioch.ox.ac.uk/oubs/phenotype.php

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This issue is supported by:

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TEAM

OUBS SEMINARS

All seminars are held in the Main Meeting Room, New Biochemistry Building from 4 to 5 pm, unless stated otherwise.

Featured Seminar:

Monday, 28th January
Prof Mark Buttner, *John Innes Centre.*
"The Control of Apical Growth and Branching in *Streptomyces*"

For a full list of the Monday seminars, please check the OUBS website: http://www.bioch.ox.ac.uk/oubs/

OUBS Featured Seminar: Prof Mark Buttner

by Isobel Steer

This term, Oxford University Biochemistry Society (OUBS) welcomes Prof Mark Buttner from the John Innes Centre in Norwich.

Prof Buttner heads a cutting-edge team in the Department of Molecular Microbiology, and his lab has been publishing prolifically since 1998. His research uses *Streptomyces* bacteria to explore antibiotic production, morphological differentiation and stress responses. This work pioneers the use of innovative techniques in functional genomics.

In 2010 Prof Buttner's team was instrumental in resolving the 20-year-old problem of how bacteria trigger resistance to the antibiotic vancomycin, a drug commonly used as the last line of defence against methicillin-resistant *Staphylococcus aureus* (MRSA) (1). A vancomycin-resistant strain of MRSA emerged in 2002, which has the potential, if widespread, to regress hospital wards back to a pre-antibiotic era. This strain induces resistance through expression of a three-gene cassette: vanH, vanA and vanX. This expression is controlled by a two-part regulatory system involving a receptor histidine kinase, VanS, and a response regulator, VanR. The identity of the VanS receptor ligand had been previously unknown and mired in controversy.

Using a synthesised vancomycin photoaffinity probe, Prof Buttner and colleagues definitively showed that vancomycin directly binds *Streptomyces coelicolor* VanS, and that this binding is correlated with resistance and required for vanH, vanA and vanX gene expression. By identifying such an alarm system, they enabled the potential development of new antibiotics that could circumnavigate the bacterial sensing system.

The team has recently received a grant from the BBSRC to continue research into the complexities of the regulation of antibiotic biosynthetic pathways. Prof Buttner's laboratory is investigating how bacteria regulate the export of synthesised antibiotics from the cell, focusing particularly on: *Streptomyces antibioticus*' transcriptional repressor protein SimR, responsible for this process; and two antibiotic-responsive transcription factors encoded in the simocyclinone biosynthetic cluster. This research aims to establish the role that antibiotics play in regulating self-resistance and their own biosynthesis. With the ever-growing problem of resistance, this kind of fundamental research is vital.

The group's recent work has focused on the bacteria *Streptomyces*' branching growth form, which occurs by tip-extension and branch formation behind the tips. The evolutionarily-ancient form of apical growth exhibited by *Streptomyces* is directed by a polarisome-like complex that involves the essential protein, DivIVA. Prof Buttner and co-workers discovered that the bacterial polarisation machinery is regulated by a eukaryotic-type serine-threonine protein kinase, AfsK, which localizes to hyphal tips and phosphorylates DivIVA (2). The laboratory has also been working to construct a quantitative model of a novel mode of hyphal growth regulation that may be widely employed due to the ubiquity of this ancient growth mode (3). Furthermore, Prof Buttner and colleagues also elucidated the role of chaplin, a hydrophobic sheath protein in the aerial spores of *Streptomyces* (4).

Prof Buttner is also collaborating with the Oxford Department of Biochemistry on previously uncharacterised Zinc-containing Anti-Sigma factor (ZAS) proteins and their complexes with sigma factors. Recent, unpublished work in the Kleanthous lab on the ZAS protein RsrA suggests that the complex formed with its cognate sigma factor σR possesses an unprecedented mechanism enabling it to act as a disulfide-stress sensor in the bacterial cytoplasm. Disruption of such protein-protein interactions is not only an *in vivo* method of probing function, but could also lead to the development of novel antibiotics.

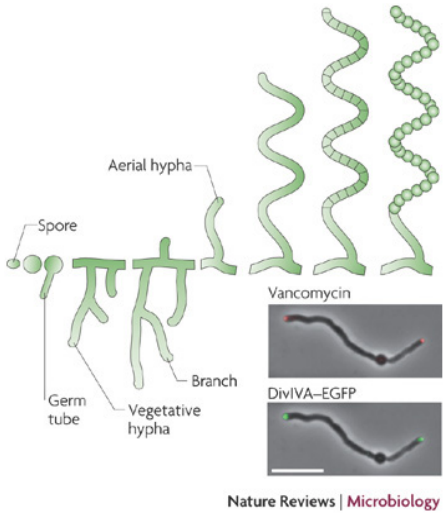

Figure 1: Developmental life cycle of *Streptomyces coelicolor*. Schematic representation of the germination of a spore, emergence of a germ tube and development into vegetative and aerial hyphae. The micrographs depict the polarized growth of two vegetative hyphae emerging from a spore with the apical zones of active cell-wall assembly stained with a fluorescent vancomycin conjugate (red) and the localization of the polarity determinant protein DivIVA tagged with enhanced green fluorescent protein (EGFP; green). Sourced from (5).

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RESEARCH HIGHLIGHTS

by Madeleine Pope

Dunsch A, *et al.* (2012) *Journal of Cell Biology* 198(6):1039–1054

Dynein light chain 1 and a spindle-associated adaptor promote dynein assembly and spindle orientation

The cytoplasmic dynein motor is a key component of the mitotic machinery of cells. During mitosis, dynein accumulates asymmetrically at only one pole of the cortex and is crucial for correctly positioning the mitotic spindle. Whilst many players in this process have been identified, the roles of different dynein complexes are not fully characterised. Dunsch and colleagues investigated the role of a specific dynein chain, dynein light chain 1 (DYNLL1) in spindle formation.

The group employed state-of-the-art imaging techniques alongside molecular assays to investigate the role of dynein-DYNLL1 complexes at the mitotic spindle of cultured cells. Initially, complexes isolated by immunoprecipitation were analysed by mass spectrometry to identify binding partners for DYNLL1. Two novel components were found: hyaluronan-mediated motility receptor (HMMR) and spindle protein CHICA. These proteins formed a novel complex at the spindle that did not contain other previously described binding partners of DYNLL1.

Using siRNA technology, the authors showed that depletion of either HMMR or CHICA resulted in reduced DYNLL1 at the spindle and spindle misalignment, suggesting that the novel HMMR-CHICA-DYNLL1 complex is required for correct spindle orientation. When cells were depleted of CHICA, HMMR or DYNLL1, the asymmetric localisation of dynein was lost. Furthermore, depletion of DYNLL1 resulted in an accumulation of dynein at the cortex, which suggested that DYNLL1 normally inhibits the interactions of dynein with cortical proteins.

Based on their results, Dunsch and co-workers propose that the novel HMMR-CHICA-DYNLL1 complex provides information on spindle position to dynein and regulates spindle orientation in dividing cells. They suggest a model for the asymmetric accumulation of dynein at the cortical pole furthest from the spindle: dynein associated with the spindle-bound HMMR-CHICA-DYNLL1 complex inhibits the binding of free dynein to cortical proteins, thus resulting in less dynein at the pole closest to the spindle. Dynein therefore generates asymmetric pulling forces to rotate the spindle.

Burke C, *et al.* (2012) *Nature* 492(7429):433–437

Layered reward signalling through octopamine and dopamine in *Drosophila*

Octopamine has historically been synonymous with reward signalling in insects and more recently, dopamine has also been linked to reward reinforcement. Flies lacking octopamine cannot form appetitive memory, but the precise role of the molecule is still elusive. Burke and colleagues investigated the role of octopamine release in the *Drosophila* brain and its relationship with dopamine signalling in appetitive memory.

The group tested whether octopamine neurons were required for appetitive olfactory conditioning by selectively blocking them during conditioning with sugars. When tested with sucrose, no memory defects were seen. However, when flies were tested with arabinose, a sweet but non-nutritious sugar, memory impairment was observed, which could be rescued by supplementing the arabinose with nutritious sorbitol. This suggested that octopamine only conveys reinforcing effects of sweet taste, and nutrient value is relayed via an octopamine-independent mechanism.

The investigators then showed that artificial stimulation of octopamine neurons could provide sufficient reinforcement to form robust memory for up to 30 minutes. This process was dependent on octopamine, because artificial stimulation of the same neurons in flies unable to synthesise octopamine prevented memory formation. Likewise, stimulating octopamine neurons in flies lacking the dopamine receptor DopR impaired memory formation, and activation of selected dopamine neurons allowed memory formation in flies lacking octopamine. This suggested that a functional dopamine system is required downstream of octopamine in the formation of appetitive memory. Using flies lacking specific octopamine receptors the authors showed that the OAMB receptor was essential for octopamine-dependent memory, whereas the OCTβ2R receptor was only required in satiated flies, with the memory impairment restored by food deprivation. This indicated that signals transmitted via different octopamine receptors may be integrated with additional signals of state, such as hunger.

Together, this data indicates that octopamine release provides reinforcement for the formation of appetitive memory by modulating dopamine neurons, with dopamine acting as a reinforcing signal.

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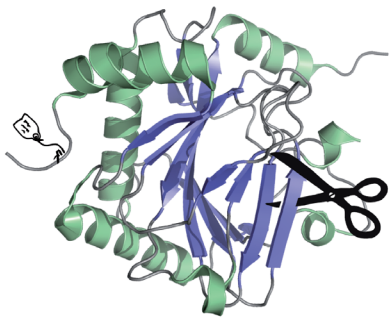

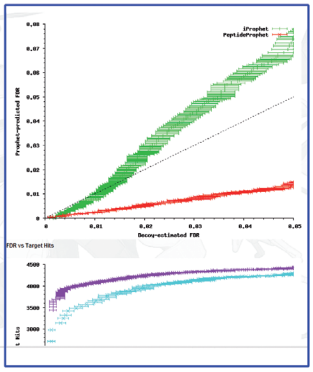

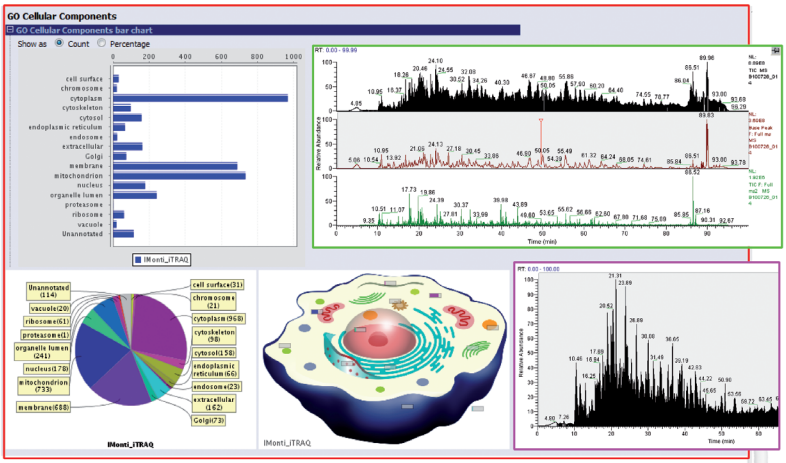

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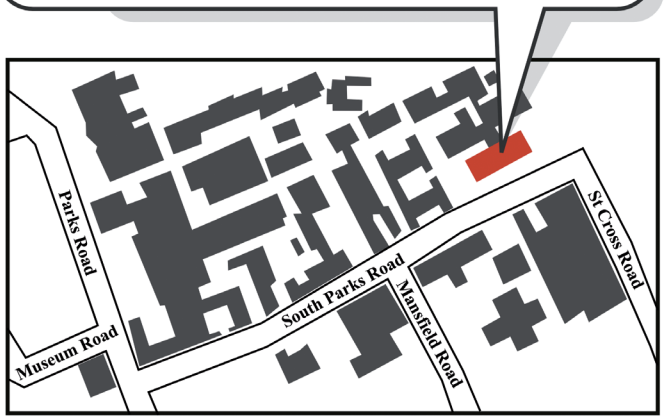

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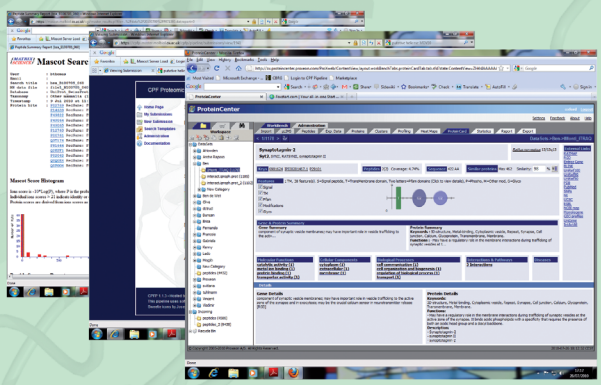

Structural insights into malaria cytoadherence

by
Dr John Vakonakis

The most lethal species of malaria parasite, *Plasmodium falciparum*, endows infected red blood cells (iRBCs) with adhesion characteristics. This unique cytoadherence property helps the parasite escape clearance by the spleen; however, at the same time it causes the accumulation of iRBCs in the microvasculature, thereby blocking blood flow. The resulting tissue damage is a major contributor to *P. falciparum* lethality. My group uses structural biology and biophysical tools to understand how parasite and host proteins organise into assemblies necessary for cytoadherence.

Malaria, cytoadherence and the human response

Malaria as a disease needs little introduction: its global reach with approximately one third of the world's population at risk of infection, the over 200 million documented cases per year and the annual death rate of approximately one million, ensure that the threat of malaria is understood and appreciated even by non-experts. Despite the need and ongoing efforts, no effective vaccine is yet available, and parasite resistance to even the most recent antimalarial therapies is emerging fast. Deservedly, understanding the molecular mechanisms of malaria is, along with HIV and tuberculosis, at the forefront of research priorities for charity foundations and public bodies alike (1). From the perspective of the human host, malaria begins with a bite from an infected female mosquito and the subsequent release of parasites, termed sporozoites, in the host. The sporozoites travel to the liver where they quickly multiply and transform to the next parasite stage, merozoites. Thousands of merozoites are released in the blood circulation, where they infect erythrocytes and each multiplies into 8 to 24 new merozoites over a 36 to 48 hour period. The first malaria symptoms appear between 8 and 25 days after the initial mosquito bite, by which time the blood stage of the disease is well established.

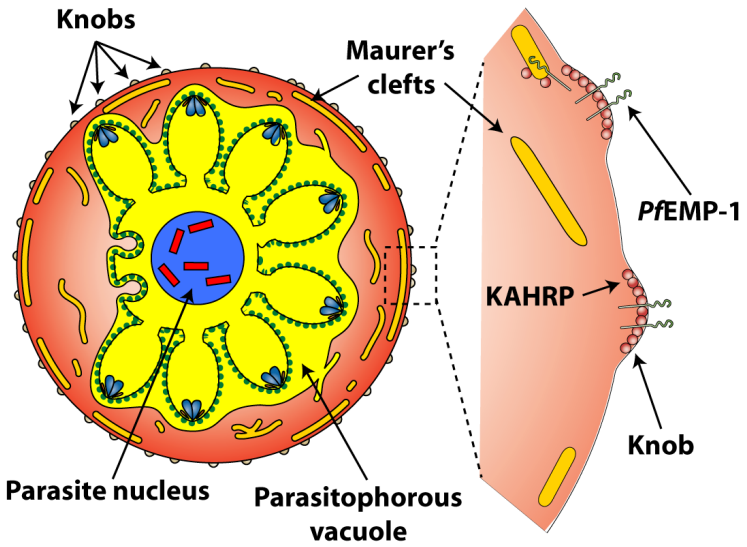

Figure 1: Schematic diagram of an iRBC. The malaria parasite lives inside the parasitophorous vacuole, a membrane compartment created upon infection, and exports approximately 200 proteins to the host cell. A subset of these proteins creates regularly spaced protrusions on the iRBC surface (knobs), where the main parasite adhesion receptor, PfEMP-1, localises. The parasite KAHRP is essential for knob formation and also localises therein. PfEMP-1 and possibly KAHRP are transported to the iRBC surface through novel membrane compartments created by the parasite, termed 'Maurer's clefts'.

P. falciparum exports approximately 200 proteins to the host iRBC (2). Some of these proteins take care of housekeeping duties: pumping in essential nutrients and, in resistant strains, pumping out drugs. Others establish membrane compartments in iRBCs, necessary for protein trafficking, digestion of haemoglobin and isolation of unwanted metabolites. A third set creates protrusions on the iRBC surface known as 'knobs' (Figure 1), which mediate cell adhesion between iRBCs, uninfected red blood cells and endothelial cells. The parasite benefits from cytoadherence as it prevents the normal circulation of iRBCs, and so reduces clearance by the spleen. At the same time, cytoadherence causes clumps of infected and uninfected erythrocytes to stick in the microvasculature, thereby obstructing blood flow (3). The resulting tissue damage is a major contributor to severe malaria cases that often end in death. Of the five parasite species causing malaria in humans, cytoadherence exists only in *P. falciparum*; this one species is responsible for over 90% of malaria deaths.

Exactly which parasite proteins contribute towards cytoadherence is still unclear; however, two main players have been identified. First is the main adhesion receptor family, *P. falciparum* Erythrocyte Membrane Protein 1 (*Pf*EMP-1), which binds surface molecules on endothelial cells. The *Pf*EMP-1 family comprises 60 copies with variable extracellular segments (4) which provide binding pluralism and an ever-changing antigenic surface on iRBCs that hampers the host immune response. *Pf*EMP-1 clusters on knobs (Figure 1), and it is believed that this clustering provides the mechanical force necessary to attach iRBCs to the endothelium against blood flow. However, the molecular details behind *Pf*EMP-1 clustering, and how adhesion forces are transmitted to the iRBCs, remain open questions in the field. A

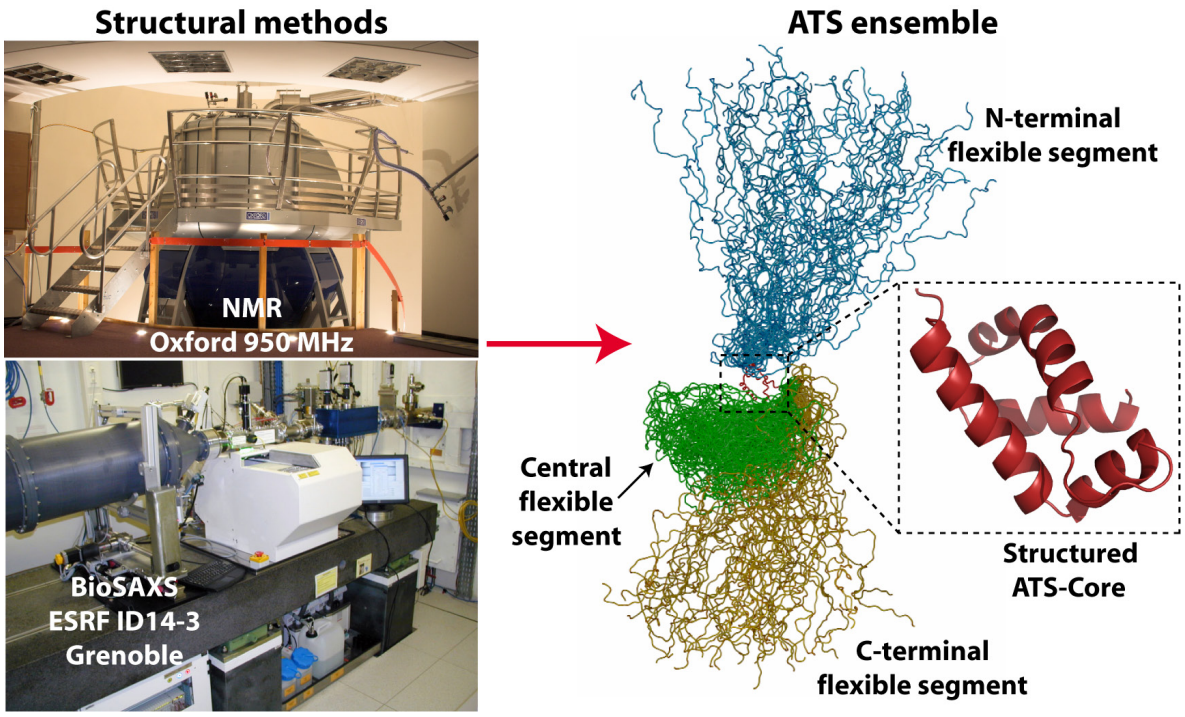

Figure 2: Characterising a dynamic system. Flexible proteins are hard to analyse by conventional tools. We used a combination of NMR and SAXS methods to compute an ensemble of possible conformations for ATS. We found that a small core in the protein is structured, while the flexible segments could be studied in isolation with no loss of functionality.

second critical player is the parasite Knob Associated Histidine-Rich Protein, or KAHRP. Surprisingly for such an important protein, we actually know very little about KAHRP beyond its sequence and that it is absolutely required for knob formation (5). Similar to *Pf*EMP-1, KAHRP also localises in knobs (5); however, earlier suggestions that these two proteins interact directly have not been upheld by biophysical experiments (6). KAHRP possesses amino residue sequence repeats that are important for its *in vivo* role (5), but we currently lack a basic understanding of how KAHRP functions.

This is a great pity, for nature points to the cytoadherence system as a way of combatting severe malaria. In areas where the disease has been historically endemic, such as India, the Middle East, the Mediterranean and sub-Saharan Africa, malaria led to the selection of abnormal haemoglobin types in human populations (7). The sickle-cell genetic trait and α- or β-thalassemias severely reduce the life expectancy of people homozygous for the alleles, but confer tolerance to malaria in heterozygous hosts. A major mechanism behind this tolerance appears to be the disruption of knobs and of *Pf*EMP-1 transport to the iRBC surface by formation of abnormal haemoglobin filaments inside the erythrocyte (7). The subsequent lack of iRBC sequestration in the microvasculature, and the higher rates of iRBC clearance by the spleen, lead directly to reduced parasitaemia and protect tissues from damage.

Cytoadherence proteins as structural components

Can we reproduce this natural protection of abnormal haemoglobin types with artificial means? How do we disrupt knob formation, *Pf*EMP-1 localisation or the structural linkages that resist adhesion forces? To address these questions we first need to understand the cytoadherence system at a mechanistic level.

Luckily, protein chemistry, structural biology and biophysics methods are now more powerful than ever, and Oxford has cutting-edge facilities in all these areas. We recently started studying cytoadherence components by looking first at the intracellular segment of *Pf*EMP-1, termed the acidic terminal sequence (ATS). ATS is highly conserved among all *Pf*EMP-1 members, and it forms a vital link in the cascade between extracellular receptors and the iRBC interior. To our surprise, this sizeable protein fragment (~45 kDa) proved to be highly dynamic in solution (Figure 2), as shown by both Nuclear Magnetic Resonance (NMR) and small-angle X-ray scattering (SAXS) methods. Flexible proteins similar to ATS are widespread in eukaryotes including *P. falciparum* (8), and they commonly serve as hubs for protein-protein interactions (9). At the same time, flexible proteins constitute difficult targets to work with, as they are prone to degradation, they cannot be crystallised for X-ray diffraction, and their NMR spectra are highly degenerate.

What did we learn from our ATS studies? Only a small part, approximately one fifth of the protein forms a structured unit, which we call the ATS-Core (Figure 2). ATS-Core then delineates three flexible segments of approximately equal length, at the ATS N-terminus, C-terminus and centre. Crucially, the biophysical properties of these flexible segments are identical, whether one studies them in isolation or as part of the complete ATS. Furthermore, the amino acid sequence conservation among *Pf*EMP-1 variants is equally strong in the ATS-Core and in the flexible regions, suggesting that the dynamic segments retain important functions. Therefore, we postulated that the flexible ATS parts feature protein interaction epitopes, and we set about finding what these might be. To our aid came a large body of high-throughput proteomic and interactomic data from *P. falciparum*

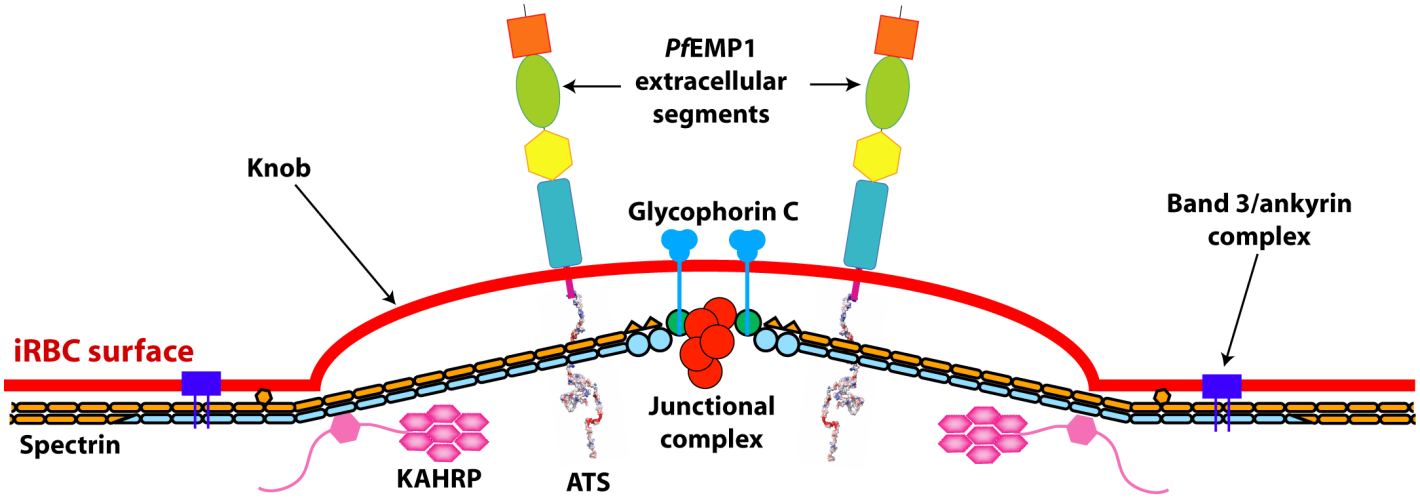

Figure 3: Current model of the 'knob' structure. Our findings suggest that KAHRP and ATS both interact with the iRBC cytoskeleton. We believe that the KAHRP-spectrin interaction centres the knob on the spectrin-actin junctional complex, and that it induces a cytoskeletal rearrangement that distorts the membrane and creates the knob protrusion. The ATS–spectrin interaction is responsible for PfEMP-1 localisation and it mediates adhesion forces from the cell exterior to the rigid cytoskeleton.

for data-mining, as well as novel computational methods that attempt to predict protein-protein interactions (6).

Our analysis has uncovered two such interactions with major implications for knob structure and cytoadherence. Firstly, we found an interaction between the ATS *N*-terminus and spectrin, the major component of the iRBC cytoskeleton (Figure 3). In erythrocytes the cytoskeleton forms a continuous lattice just under the cell membrane which allows red blood cells to withstand mechanical forces as they pass through thin vessels. Thus, the ATS-spectrin interaction would anchor *Pf*EMP-1 to the toughest mechanical component of erythrocytes, thereby ensuring that adhesion forces can be resisted. Furthermore, this interaction would place ATS close to the junction of the spectrin lattice, creating a natural clustering of *Pf*EMP-1 molecules.

Similar to ATS, we determined that KAHRP also contains highly flexible segments over approximately 50% of its length. We found that a specific region in those segments interacts again with spectrin, but at a position that would place KAHRP far from the cytoskeletal junction (Figure 3). Given the known knob diameter (5), we believe that a ring of KAHRP molecules defines the knob rim, and it functions to centre the protrusion with respect to the cytoskeletal lattice. Although we currently lack a high-resolution structural view of the KAHRP-spectrin interaction, its location cannot be a mere coincidence. Our current hypothesis is that KAHRP induces a cytoskeletal rearrangement that 'pushes' the iRBC membrane outwards, thereby generating the knob protrusion. Together with our ATS-spectrin data, we are now in a position to propose the first molecular model of the knob structure (Figure 3).

Why is this information important? We believe that both the ATS-spectrin and KAHRP-spectrin interactions may be essential for successful cytoadherence. In collaboration with cell biology laboratories in the UK and Switzerland we are now trying to test this hypothesis by creating parasite variants that would lack these interactions and, hopefully, cytoadherence properties. Should this be the case, the ATS and KAHRP interactions may be suitable targets for medical intervention. At the same time, ATS and KAHRP are just two of many proteins exported to iRBCs; much work remains to find out what the roles of other components are, and which of these provide us with the best therapeutic targets.

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Hitting where it hurts: Targeting stress-linked survival mechanisms in cancer

Tumour cells need to meet the metabolic requirements of increased cellular growth and proliferation, often in stressful conditions (1). Oncogenic mutations in pathways critical to nutrient and growth factor sensing are acquired, causing a disconnection between physiological signals and metabolic regulation that facilitates adaptation to cellular demands.

by
Arsalan Azad

Interestingly, recent work from our lab has highlighted a novel mechanism on the surface of internal nutrient-rich compartments, primarily the late endosomes and lysosomes (LELs; Figure 1), which is involved in regulating the nutrient-sensitive mTORC1 signalling hub, particularly during cellular stress (2). Our data suggest that this mechanism may be altered in cancer cells, allowing them to survive and grow better than adjacent normal cells in adverse (low nutrient and oxygen) conditions, which often favour adaptive mutation and further progression of tumour cells.

Background: the mTOR pathway

As its name suggests, the identification of mammalian or mechanistic target of rapamycin (mTOR) was intimately linked to the discovery of rapamycin. Rapamycin is a macrolide produced by a soil bacterium found in and named after Rapa Nui (Easter Island). There has been a lot of interest in rapamycin due to its profound anti-proliferative and immunosuppressant capacity. mTOR is an atypical serine/threonine protein kinase and acts in conjunction with several other proteins in two distinct complexes: complex 1 (mTORC1) and complex 2 (mTORC2).

A remarkable feature of the mTORC1 pathway is the breadth of major pathways under its control. This is perhaps understandable given the diversity of upstream signals it integrates. The regulatory inputs that control the mTORC1 pathway include, but are not limited to: growth factors, energy status, stress, oxygen and amino acids. Active mTORC1 controls growth, metabolism, cell cycle progression, autophagy and macromolecule biosynthesis.

It will not come as a surprise therefore that defective mTORC1 signalling has been linked to a variety of major human diseases: obesity, type 2 diabetes, neurodegenerative diseases, cancer and it is even thought to have an effect on ageing. Blocking the mTORC1 pathway in the treatment of cancer has gained significant momentum, to a point at which many rapamycin analogues (rapalogs) are under phase III clinical trials. One such rapalog, Temisrolimus (CCI-779), was approved by the Food and Drug Administration (FDA) in 2007 for the treatment of renal cell carcinoma. However, the effectiveness of rapalogs has been hampered by two major problems. Firstly, the presence of a number of feedback loops suppressing their effects, and secondly, their strong side effects, presumably through inhibition of globally active mTORC1 signalling. Our lab has been particularly focused on gaining a context-specific understanding of how to modulate mTORC1 activity in a more selective manner to mitigate against these problems.

mTORC1: 'sensing' amino acids

Studies as early as the 1980s posited a critical role for amino acids in the regulation of mTORC1, particularly leucine and arginine. Since then, intensive research has led to our current understanding that RHEB (a well-characterised activator of mTORC1) can only promote mTORC1 signalling in the presence of an amino acid sensitive complex comprising a heterodimeric pair of RAG proteins and the trimeric Ragulator complex (3).

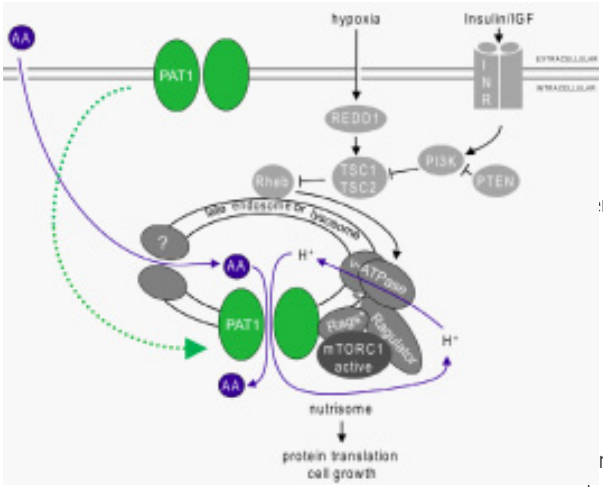

Figure 1: PAT1 is a key component of an amino acid-sensing complex or 'nutrisome' located on the surface of late endosomes and lysosomes (LELs) that activates mTORC1. PAT1-nutrisomal engines are suitably placed to respond to changes in amino acid levels in both the extracellular environment and also within LELs lumens, and appear to become particularly important under stress conditions. We are investigating whether this mechanism could be targeted to selectively impact on cancer cells.

Figure 2:
Flies lacking the PAT transporter, *pathetic* (upper fly), are much smaller than normal flies (lower fly).

The RAG-Ragulator complex drives mTORC1 translocation to LELs surfaces and synergises with RHEB. This links mTORC1 activation to the presence of amino acids. Moreover, further components of the RAG-Ragulator complex have emerged recently. The vacuolar-ATPase has been suggested to be involved in an inside-out model of amino acid sensing, whereby amino acid accumulation in the lumen of LELs initiates mTORC1 activation. However, the putative amino acid 'sensor' activating this mechanism has remained elusive, and how it would potentially interact with the RAG-Ragulator complex is of intense interest. As the RAG-Ragulator complex was being characterised, a parallel set of experiments in a completely different system had started to highlight amino acid transporters in the LELs as important growth regulators. Unexpectedly, *in vivo* screening of amino acid transporters for growth regulatory activity in Drosophila melanogaster in our lab highlighted the Proton-assisted Amino acid Transporter (PAT/SLC36) family as having a uniquely potent effect on amino acid-dependent mTORC1-mediated growth (4). These studies revealed that two members of the PAT family, PATH and CG1139, could drive cell growth when overexpressed and that PATH was required in flies for normal growth (Figure 2).

The lab turned to human cell culture to test whether this mechanism was conserved and found that the two ubiquitously expressed PATs, PAT1 and PAT4, which also promote growth in cultured human cells, are located on LELs and co-immunoprecipitate RAG proteins (5, 6; Figure 1 and Figure 3). To add to this, we have recently discovered that in serum-deprived conditions in which many cells fail to proliferate, HCT116 colon cancer cells become dependent on PAT-mTORC1 signalling for PI3K-dependent growth. This finding mirrors earlier work in *Drosophila* in which the growth-promoting properties of PATs were shown to be enhanced synergistically when PI3K signalling is up-regulated (6). These data suggest that hyperactivated PI3K makes cells more reliant on the PAT/mTORC1 signalling pathway and even perhaps 'addicted' to its use in the stress conditions when these cells become most dangerous.

Implications

In summary, our work raises the possibility that tumours with increased PI3K signalling may be more susceptible to inhibition of the PAT-mTORC1 axis. We are currently investigating the role of PAT-dependent growth regulatory mechanisms in HCT116 cells under different microenviromental stress (MES) conditions, and in xenograft models. These experiments should indicate whether there is an environmental context in which these putative amino acid sensors are suitable for targeting, and whether inhibitors might be employed in combinatorial therapies, i.e. radiosensitisation in MES (hypoxia and amino acid deprived) conditions.

Arsalan Azad is a Research Student under the supervision of Prof Adrian Harris (WIMM) and Dr Deborah Goberdhan (DPAG), having recently completed his Masters in Radiation Oncology at the University of Oxford.

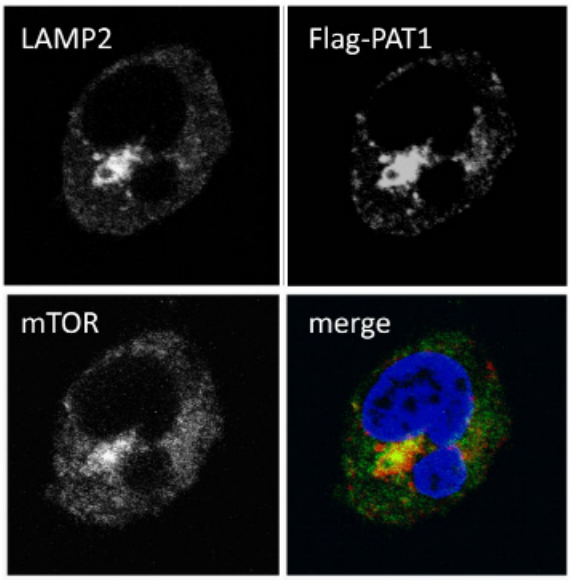

Figure 3:
Under amino acid-stimulated conditions mTOR re-localises to LELs containing PAT1 in HEK-293 human embryonic kidney cells..

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Schizophrenia and Bipolar Disorder: when mood is dependent on epigenetic DNA changes

Schizophrenia and bipolar disorder are mental disorders characterised by different symptoms. Recent data, however, suggest that they are related.

by
Maria Mogni

Schizophrenia and bipolar disorder are major psychiatric diseases. Schizophrenia is characterised by psychotic symptoms, including delusions, hallucinations and cognitive impairment, while patients suffering from bipolar disorder have recurrent mood swings consisting of cycles of mania and depression. Recently, there has been an increasing body of overlapping biological and genetic data underlying these two conditions, suggesting the disorders are related to each other and their onset might be caused by similar cellular, environmental and genetic events. However, the study of these two disorders is hampered by their non-Mendelian pattern of inheritance and a lack of disease-specific diagnostic biomarkers. Complex genetics in conjunction with allelic, locus and phenotypic heterogeneity makes the study of the role of candidate genes in psychotic disorders difficult. Nonetheless, *de novo* mutations in the *shank3* gene have been implicated in the onset of schizophrenia (1). Furthermore, environmental and epigenetic perturbations during the early development of the nervous system are thought to increase the chances of developing schizophrenia and bipolar disorder (2). Mice exposed to stress *in utero* displayed a similar behaviour to human patients suffering from schizophrenia and bipolar disorder (3). Therefore, early life stresses have been proposed to affect chromatin remodelling and thus transcriptional regulation in specific anatomical brain structures. Further studies suggest that DNA methylation events underlie the pathogenesis of psychotic symptoms.

Epigenetic regulation in neurons

A number of epigenetic regulation events occur in neurons, with histone deacetylation being implicated in synaptic plasticity and memory formation. DNA methylation is considered to be the most important epigenetic modification event involved in the regulation of transcription in the brain (4).

DNA methyltransferases (including DNMT1, DNMT3A and DNMT3B) coordinate DNA methylation in cytosines of CpG islands (regions of DNA rich in sites where a cytosine nucleotide occurs next to a guanine nucleotide). Functionally relevant differential methylation occurs at a small proportion of these islands during development, especially within cell-type specific regions. Specifically, CpG methylation is low at promoters and high between genes and in gene bodies, where it is thought that the binding of transcription factors not only protects sequences from methylation but also initiates active DNA demethylation. As cells differentiate, the locations of low-methylated regions migrate towards regions close to promoters that are transcriptionally active in the differentiated state. Furthermore, tissue-specific DNA methylation is more prevalent in the sequences surrounding CpG islands, defined as 'CpG island shore regions'.

It has been proposed that hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by the TET (ten-eleven-translocation) enzyme family is the first step in a demethylation mechanism in the brain. However, TET proteins were also found to bind and repress a set of CpG-rich promoters by interacting with the polycomb repressive complex 2 (PRC2) and the Swi-independent 3A complex (SIN3A). Thus, TET proteins may have a pleiotropic effect, activating transcription by facilitating demethylation, but also blocking transcription by participating in a repressor complex.

Both methylated CpGs and hydroxymethylated CpGs are sites for 5mC- and 5hmC-binding proteins that promote transitions between open and closed chromatin states, depending on further signals or regulators. For example, the GADD45β protein couples neuronal excitation with the regulation of DNA demethylation via interaction with a multitude of proteins involved in DNA repair, cell cycling, genome stability, stress and immune responses. A mechanism has been proposed, based on deamination of 5mC and 5hmC sites by complexes of the deaminase/glycosylase pair AID/

MBD4, or apolipoprotein B mRNA-editing, both of which are enzyme-catalytic enzymes that interact with GADD45 proteins.

Epigenetic regulation in psychiatric disorders

Initial studies to elucidate the causes of schizophrenia and bipolar disorder suggested that high levels of homocysteine are linked to the etiology of the diseases, for which a common polymorphism in the *MTHFR* (methylenetetrahydrofolate reductase) gene might be responsible (5). Further studies suggested a 'transmethylation' theory, where methylation of catecholamines would produce products such as dimethoxyphenethylamine, whose levels were found to be higher in schizophrenia sufferers. Recently, the study of mRNA expression levels coding for reelin (RELN), an extracellular matrix protein, and glutamate decarboxylase/glutamic acid decarboxylase 67 (GAD67), which is involved in the formation of the neurotransmitter gamma-aminobutyric acid (GABA), showed that both are consistently downregulated in patients suffering from schizophrenia and bipolar disorder. Therefore, the function of GABAergic neurons, such as CA3/2 hippocampal neurons, is disrupted, causing an inhibitory/excitatory imbalance in various brain circuitries that underlie the cognitive dysfunction seen in schizophrenic patients (2, Figure 1).

The genes coding for RELN and GAD67 have also been studied in the context of epigenetics. The *RELN* promoter was found to be embedded in a large CpG island, and its *in vitro* expression correlates with DNA methylation (6). Specifically, DNA methylation inhibitors were found to increase the expression of RELN mRNA levels in neuroprogenitor cells. Higher methylation levels in the *RELN* promoter were found in postmortem schizophrenic cortices compared to healthy ones. The *GAD67* promoter is also GC rich and likely to be regulated through changes in promoter methylation (7). GAD67 mRNA levels are reduced in schizophrenic patients, while the amount of methylation increases near the promoter. Moreover, increases in DNMT1 mRNA levels were found to parallel reduced levels of RELN and GAD67 mRNAs, suggesting an inverse correlation, where DNMT1 mRNA was found to co-localise with GAD65/67 protein and RELN mRNA in neurons. Thus, an inhibitory role for DNMT1 has been proposed, in which DNMT1's binding to unmethylated CpGs through the linker protein region is thought to prevent the CpGs from coming into contact with the active site of DNMT1. This prevents new methylation, supporting the idea that gene repression occurs in GABAergic neurons of schizophrenic and bipolar patients by a DNMT1-mediated mechanism.

In addition, genome-wide methylation studies using post-mortem human tissue found a variety of methylation differences in a significant number of genes between schizophrenic and bipolar patients versus control ones. The approach consisted of digesting unmethylated regions in control patients

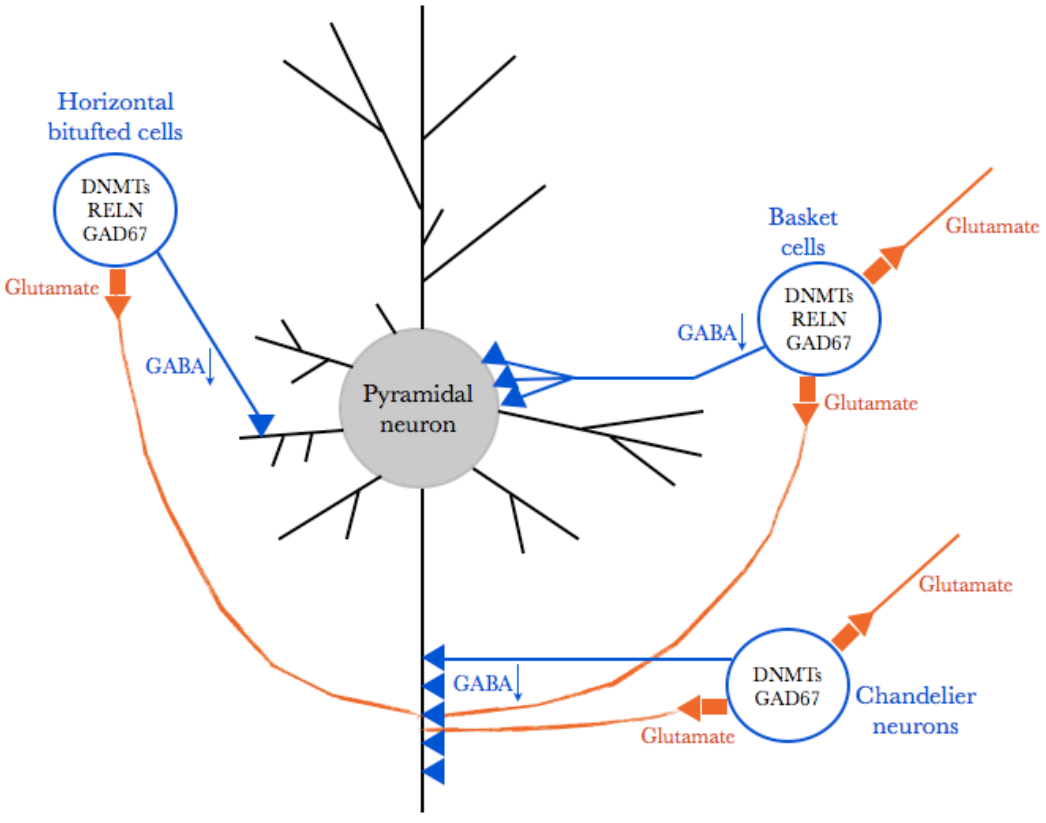

Figure 1: Principal neuronal circuits in the cortex showing the interaction between GABAergic innervation of pyramidal neurons, glutamatergic innervation and chandelier GABAergic interneurons. The expression of DNA methyltransferase (DNMT) in schizophrenic and bipolar disorder patients leads to the GAD67 and RELN mRNA downregulation in GABAergic neurons, increased methylation, and reduced GABA output. Adapted from (11).

using restriction enzymes sensitive to methylation, with subsequent DNA amplification. The amplified DNA was then hybridized to a CpG island microarray to identify DNA methylation. The microarray revealed psychosis-associated differences at a number of different loci for genes involved in neurotransmission (such as GABAergic signalling), brain development, mitochondrial function, stress response, and further pathways linked to the development of the diseases. Gender-specific differences were also identified. For example, the *VGLUT1* promoter is hypermethylated in schizophrenic female patients' DNA, but the corresponding mRNA is downregulated in both schizophrenic male and female postmortem human brain (8). Furthermore, methylation analysis of peripheral blood DNA from sets of twins revealed psychosis-associated differences in methylation patterns, such as for example hypomethylation of the *ST6GALNAC1* promoter (ST6GALNAC1 protein is involved in cell-cell interactions) in affected individuals (9). Also, a temporal evaluation of DNA methylation profiles in CpG islands revealed a number of genes in which DNA methylation increases with age. It is important to point out that DNA methylation studies are prone to high variability between results obtained from within individuals as well as between individuals. Indeed, different methodological approaches, drug treatments affecting methylation status, cellular heterogeneity of postmortem human tissues, and DNA modification approaches including bisulphite-modification can all lead to variability.

Finally, an increase in TET-1 mRNA has been observed in psychotic patients, with an increase in genome-wide levels of 5hmC, and specifically as well in the *GAD67* promoter. An accumulation of 5hmC could be due to the overall downregulation of genes, including cytidine-deaminating enzymes that convert 5hmC into 5hmU. TET-1 could also act as a repressor independent of its enzymatic activity. In this case, TET-1 would associate with co-repressor proteins to inhibit the transcription of particular genes.

Antipsychotic drugs targeting promoter methylation

Valproic acid (VPA), a drug used as an epilepsy anticonvulsant, has been tested for the reversal of DNA methylation in schizophrenic and bipolar patients. In mice, it was found that VPA increased the levels of acetylated histones H3K9 and H3K14 proximal to promoters, and increased subsequent promoter demethylation (10). Furthermore, VPA was found to increase GABAergic function by facilitating the increased expression of the *GAD67* gene and other genes expressed in GABAergic

neurons (10). The idea that VPA could work in conjunction with other antipsychotics such as haloperidol and clozapine to facilitate DNA demethylation is currently being explored. In the future, understanding DNA methylation in schizophrenia and bipolar disorder will provide a framework for the identification of new targets, which can be exploited for pharmacological intervention.

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Turning to genetics in dystonia syndromes research

Dystonia is a class of movement disorder characterised by involuntary, sustained muscle contractions that result in twisting, repetitive movements and abnormal posture.

by Aarthi Subramanian

Dystonias can be classified symptomatically, for example as focal dystonia, in which only one muscle or group of muscles is affected, or generalised dystonia, where the whole body is affected. Alternatively dystonias can be classified by etiology as either primary, in which dystonia is the only neurological symptom expressed, or secondary, in which dystonia occurs as a consequence of another underlying condition (1, 2). The cause of dystonia is currently unknown, although the consensus is that dysfunction in the basal ganglia of the brain may underlie the condition (3, Figure 1).

Some forms of dystonia are familial and inherited in a dominant manner. Other forms, however, are sporadic or idiopathic. In these cases the disease is caused by the interplay of genetic and environmental factors. Therefore, symptoms and disease progression vary widely in type and severity.

Genetic causes of dystonia

The recent identification of several genetic mutations which cause familial dystonia syndromes has provided important insights into the nature of this complex disorder. Such discoveries have permitted the development of animal models which recapitulate the cardinal features of dystonia. Research into the function of these genes has greatly enhanced our understanding of the cellular and molecular processes which may be dysfunctional in the disease.

Among the best-characterised dystonias is DYT1 primary syndrome, caused by mutations in the gene encoding TorsinA. TorsinA belongs to a family of ATPases with multiple cellular functions. It is thought to play a protective role in response to endoplasmic reticulum stress. When TorsinA is mutated, its buffering capacity is weakened, its ATPase activity diminished and its ability to bind to other proteins is increased (4). It is remarkable, and as yet unexplained, that dystonia is the only neurological manifestation of dysfunctional TorsinA, despite its widespread expression throughout the nervous system.

Dopamine-responsive dystonia is another familial form of dystonia, resulting from mutations in the gene *DYT5*. Onset generally occurs during childhood and patients experience difficulty walking. As its name suggests, this form of dystonia can be treated effectively with levodopa, a dopamine precursor.

Treatment options

Other forms of dystonia, however, are levodopa-insensitive and therapeutic development remains at the forefront of movement disorder research. The first-choice treatment for many forms of dystonia is botulinum toxin injection, often most effective in focal dystonias. Injection into affected muscles blocks release of the neurotransmitter acetylcholine, preventing muscle contraction and helping alleviate abnormal posture.

A recently developed treatment is deep brain stimulation in the basal ganglia, which provides effective symptomatic relief in DYT1 patients. Alternatively, drug therapies are available for some forms of dystonia, including anticholinergic agents such as trihexyphenidyl and benztropine and modulators of GABA signalling such as benzodiazepines (1).

In conclusion, a significant part of dystonia research focuses on familial dystonias. The study of genetic forms, particularly DYT1, has provided information on the molecular causes of dystonia and has enabled the development of animal models, which it is hoped will provide insight into the underlying pathophysiology of the disorder. Although the past decade has brought notable improvements in dystonia treatment, existing treatments provide only symptomatic relief. Investment into understanding the pathophysiology of the disease will facilitate the development of novel, more specific therapies which target the underlying causes of the condition. This would be an important step forward in the management of dystonia syndromes.

Figure 1: Mice with dysfunctions in the basal ganglia exhibit dystonia-like impairments. Reproduced with permission (3).

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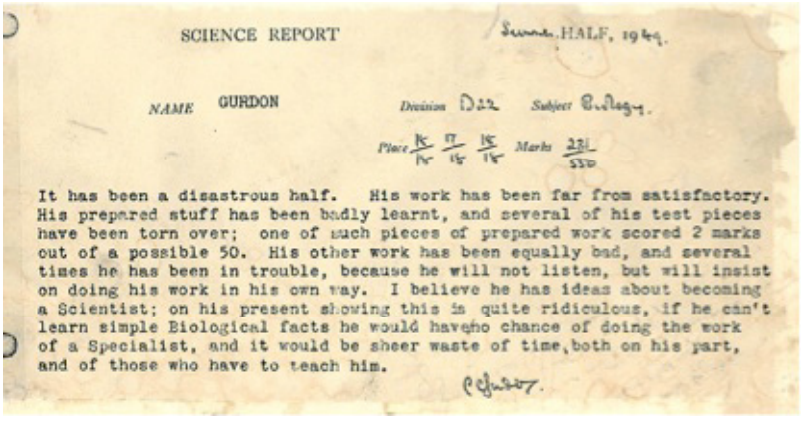
The Mind of a Scientist

by
Lars Hanssen

Earlier this year, the Nobel Prize for Medicine or Physiology was jointly awarded to the UK's Sir John Gurdon and Japan's Professor Shinya Yamanaka for their work on cell reprogramming. Gurdon's award wining research consisted of the nuclear transfer experiments published over 50 years ago.

Gurdon was the first to show that a somatic cell nucleus has the potential to regain pluripotency and produce a living organism, by transplanting the nucleus of a tadpole's intestinal cell into a nucleus-less frog egg. The somatic cell nuclear transfer technique he used, known as cloning, was later employed to produce Dolly the sheep. Gurdon was also the first to show that the genome of an adult cell contains all the information necessary to form an entire organism, which contrasted starkly with the main scientific view of the time that cellular differentiation was a one-way process.

At his press conference, Gurdon relished a story about an exceedingly negative school report he received from his Biology teacher at Eton College when he was 15 years old (Figure 1). The report states that Gurdon's ideas of becoming a scientist were "quite ridiculous" and that "… it would be a sheer waste of time, both on his part, and of those who have to teach him". Gurdon shared that he framed this report card, and keeps it in his office as a reminder that the schoolteacher may have been right when his experiments are not working, as so frequently happens in science. This story was extensively covered by the press, focusing on how a schoolboy deemed to be bad at science ended up winning the Nobel Prize. At the end of the sizeable list of faults attributed to Gurdon's work is an interesting statement in light of his later successes: "… (Gurdon) will not listen, but will insist on doing his work in his own way". The schoolteacher would have been interested to learn that the achievements Gurdon made in his later scientific career were defined by doing exactly this, going radically against the generally accepted scientific view of the time, and developing new methods.


SCIENCE REPORT

Summer HALF, 1949.

NAME GURDON Division D22 Subject Biology.

Place 15 17 15 15 Marks 231/550

It has been a disastrous half. His work has been far from satisfactory. His prepared stuff has been badly learnt, and several of his test pieces have been torn over; one of such pieces of prepared work scored 2 marks out of a possible 50. His other work has been equally bad, and several times he has been in trouble, because he will not listen, but will insist on doing his work in his own way. I believe he has ideas about becoming a Scientist; on his present showing this is quite ridiculous, if he can't learn simple Biological facts he would have no chance of doing the work of a Specialist, and it would be sheer waste of time, both on his part, and of those who have to teach him.

P.C.J.W.

Figure 1: The infamous science report card that Gurdon keeps above his desk as a humbling reminder.

Dwelling into Gurdon's hard time at Eton, it is striking that there is a stark contrast between what is expected of scientists-to-be during their years of training and after graduation. Whereas in secondary education, and even university, students do well if they are able to mainly understand, remember and reproduce existing scientific theory, this changes when they take their first steps carrying out their own research. Although it is still necessary to possess these qualities, carrying out and conducting their own research requires early stage scientists to also be critical and question existing theories. Gurdon's stubbornness and questioning of what he was being taught might have been disapproved of in the rigid secondary school system, but likely proved useful later on in his career. It allowed him to develop an original approach to address the questions he wanted to answer. Another interesting aspect of both Gurdon's and Yamanaka's work was that their research sought to disprove existing theories, rather than expanding on them. Gurdon disproved the generally accepted theory that cell differentiation was a one-way process. Similarly, Yamanaka showed that just four transcription factors are able to reprogram a differentiated cell to a pluripotent state, rather than the complex network of transcription factors present in the oocyte, as was then believed. In contrast, a lot of biological science research is aimed at confirming and expanding existing theory. The over-reporting of false positive results, as is currently observed in many preclinical studies, is a perfect illustration of the tendency of the human mind to observe that which fits with its beliefs (1). Although it is of course impossible to train everyone to be Nobel Prize winning scientists, valuing and stimulating those who are different is the least we can do.

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Lars Hanssen is a 1st year DPhil student in Chromosome and Developmental Biology.

Life after science

Anna held the *Phenotype* Editor role in MT'10 and HT'11. Since then she has finished her DPhil and left science behind to work for a leading strategy consultancy firm. In the hope of providing some clarity on the mysterious PhD 'afterlife', she addresses some of the most common questions she comes across relating to scientists making the leap into the corporate world.

Anna Boleininger

Oxford University DPhil alumna, currently working for Bain & Company.

Isn't it a waste to study for all those years and then do something non-science related?

This tends to be the first question people ask me, and every time I tell them that I could not disagree more. On bad days, this question sends me into a rant.

Firstly, I think studying science can never be described as a 'waste', no matter what you end up doing. To recall a quote that some of you might be familiar with: "Science is interesting, and if you don't agree, you can **** off!" (search YouTube for "Richard Dawkins brilliant quote"). All silliness aside, I think a society benefits greatly from scientists, whether they 'only' did an undergraduate degree, left after having completed a graduate degree or have dedicated their whole life to science. Be prepared to answer this question in an interview and don't forget that science is not a profession but a mindset.

Secondly, if you look at your next career step as an 'option A vs. option B' situation available to you due to your previous studies, leaving science will start to look a lot less like a 'waste' very quickly. You have probably discovered by now that the scientific career path is not as simple as building a tower out of Lego blocks, of which the undergraduate degree was the first building block and the PhD the second one, followed by the postdoc and then a lectureship or a prestigious industry position. When we have our mini-tower, by the time we finish the PhD we tend to have a very poor idea about when the next building block will 'arrive', what it will look like and where it will be. Taking into consideration the uncertainty regarding timings and final destinations, leaving science can be an attractive option simply because of the number and variety of options.

How can my skills be used in a business environment?

You might be surprised to see how much overlap there is between the skills required to be a good scientist and the skills you need to succeed in the corporate world. An analytical mind and a logical approach are invaluable, as is the ability to stand your ground, which you have hopefully acquired through stand-offs with your supervisor. If you have done a research degree, you will have developed independence of thought and critical thinking which constitute extremely valuable skills. Last but not least, if you were unfortunate enough to have had to deal with tricky lab politics, any lessons you will have learned from that will provide guidelines for many years to come.

Are corporate jobs boring/cutthroat/competitive/stressful?

I've heard a very wide range of adjectives used to describe corporate jobs, from boring, dull and repetitive to stressful and cutthroat competitive. If you cannot find a single adjective on the brief list above that you think doesn't, at least at times, also apply to doing a research degree, you are a very lucky researcher indeed! Did you ever spend weeks trying to express and purify a very temperamental enzyme, sabotaged by a broken fast protein liquid chromatography pump or a phage infection? No further comments needed.

Another thing to consider is that there is no such thing as 'the' corporate job. All areas are different, and in some areas there is even considerable variation among competing firms. This is no different from variations between research areas and research groups, and there is no reason why it would be.

My personal experience at a strategy consultancy has been that it is definitely neither boring, nor dull, nor repetitive, nor cutthroat, but that it can be very stressful. Even so, the type of stress is different. When I was a DPhil student, it was a long-term

Figure 1: Anna in the Chemistry Research Laboratory after her DPhil viva.

uncertainty that kept me awake some nights. I found it somewhat unsettling not to know whether all my 14-hour-days would ever give me any results at all, not to mention the results I wanted. With my current job, however, the stress tends to be very immediate and comes in bursts, at least at my level. The manager might ask you to turn around a piece of work within a couple of hours, using materials that you are not familiar with. You drop everything else (literally) and give it all you have, but time absolutely flies when you are very focused and it's over very quickly, with pretty much immediate feedback.

What skills are employers looking for in a scientist and what could be perceived as a weakness?

A weakness employers commonly quote is a lack of commercial awareness among science students. Commercial awareness is basically the current business context. How do you become 'commercially aware'? I have found numerous books claiming to teach commercial awareness on Amazon without any effort, but my guess is that they might be quite a boring read. I would suggest grabbing the Economist and having a read on a regular basis. Each time you don't understand something, you can either google it or ask one of your economics student friends (most of them will be very pleased to explain something to a scientist!).

Another thing to avoid if possible is a CV that says 'I spend day and night in the lab and don't do anything else other than labwork'. While employers' views on extracurricular activities might differ, a candidate that is heavily involved in non-academic activities will look more interesting in most cases, especially if these activities include interacting with other people. Nobody will doubt that you're incredibly smart if you have a PhD in Biophysics, for example. However, the stereotype of the socially inept scientist with no communication skills still exists, and you will want to distance yourself from that. Extracurricular activities are also a great way to find out if there is anything besides science that you are passionate about. With this in mind, I can confidently say that being involved in *Phenotype* was the best thing I did while doing my DPhil.

Will I be rich?

The range of salaries for entry-level jobs can easily be looked up in the target books one can find at career fairs, or at the careers service, and is currently £19,000 to £41,000. These numbers, however, always need to be viewed in context, because it's never just about the revenue (if you'll excuse me being a consultant for a minute!). It's also about the expenses. What I mean by this is that unless you actually do a proper calculation taking into account tax, rent, transport, etc., it will be hard to gauge how much money is left for fun, travel and savings by the end of the day. The other question is about what people want to spend their money on. Having enough to buy posh food? Very likely! Buying a mansion in Mayfair? Not quite that many zeros on my paycheque!

Financially speaking, the long-term perspective of jobs outside of science, especially in management roles, tends to be brighter, as I'm sure you are aware. If, however, your lifetime aspiration is to be rich (which it probably isn't if you're doing science), you should remember that there is no such thing as a pre-carved path to becoming a millionaire.

Final message: Don't be fooled!

Remember that you are a scientist, don't be fooled by all those myths! Research your options carefully and make an informed career choice. It's far easier than writing up a thesis…

Write for *Phenotype*?

- The deadline for article submissions is Friday of 8th week, 8 March 2013.
- We accept articles on any aspect of biological sciences research, books or science education
- Articles can be either 650 or 1300 words.

If interested, please get in touch: oubs@bioch.ox.ac.uk.

Work for *Phenotype*?

If you'd like to get involved in editing, production or management of *Phenotype*, please get in touch: oubs@bioch.ox.ac.uk.

What does it take to start up a new company?

Young entrepreneurs Alexander Wilson and Mark Stevens set up PCR Biosystems, a company that specialises in various PCR-spectrum reagents, in 2004. We ask the ambitious founders their story, from conceiving the business idea to the practicalities of implementing it.

Stuti Mehta

Final year DPhil student in epigenetics working with Prof Jo Peters at MRC Harwell.

What is PCR Biosystems and what kind of products and services do you provide?

PCR Biosystems manufactures reagents for end-point and real-time PCR. We cover most applications that fall within the boundaries of these techniques. Our portfolio is a concise list of enzymes and mixes that includes various polymerases (routine application, hot-start, difficult and high-fidelity), plus 1-step and 2-step SyGreen and probe real-time PCR mixes and reagents for cDNA synthesis.

Who founded the company and what is the academic and professional background of the founders?

The founders are myself (Alexander Wilson) and Mark Stevens. We both worked for Bioline since 2004. Mark was a postdoc until 2006 when he was promoted to head of Research and Development. I worked in sales in the South West and after a brief spell with Eurogentec I was promoted to look after European third-party sales.

What made you think of starting PCR Biosystems?

I think it is something I have always wanted to do. Having met scores of company owners in the biotech sector I felt confident about my ability to start and run a company. So when the idea of PCR Biosystems arose it was a no-brainer.

Did it not deter you, entering a market that seems saturated from the outside and dominated by giants like Applied Biosystems and Bioline? What is the niche you are hoping to create?

We were not deterred at all. The PCR market is only approximately 20 years old; it is a baby when compared with other industries, and it has only experienced robust growth. Although young, the market is huge and it is still growing, according to market analysts, with a compound growth rate (CAGR; average growth rate over a period of several year) of over 10%. Plus, lately there has been a lot of consolidation in the market. That gave us confidence that there is room for one more.
We aim to sell high-quality reagents at affordable prices. Our core competency at the moment is PCR, and we are technically very strong around that. In the future, we are interested in exploring both upstream and downstream applications. For example, we have already launched a range of DNA markers. The PCR diagnostics arena is interesting in the medium term, but we do not have the resources for this yet.

What are the various steps involved in setting up a bio-product company?

Firstly you need a product that is saleable; secondly you need a sales channel for it. Mark was comfortable in looking after the first part and I was equally comfortable with the second part, so we make a good team. It was the middle part, logistics, that we underestimated. The labelling, boxing, shipping and accounting takes longer than you think, although we have people to help with this now!

What was the process of getting funding like?

It was surprisingly easy, although time consuming – there is a lot of talking to be done with explaining the model and setting realistic goals and expectations. But we had a strong proposal, and since the business is scalable, it makes it an attractive venture for angel investors (affluent individuals who provide capital for a business start-up). Compare entering a billion dollar industry that is growing with investing in a pub and I think the opportunity was easy to sell.

What is a typical day like in the life of a fledging Bio start-up?

Early on I splashed out on an expensive headset for use with Skype and it was money well spent. Mark lives in London and I work from my home in Oxford so we talk every day using it. There are also all the calls with distributors and potential customers.
I also spend time on the road. I have been repping around Oxford and the South West and I have been to a couple of international events in Germany, but I have been stuck behind a computer screen much more than I thought I would.

PCRBIOSYSTEMS

How big is your team now and what kind of talent would you be looking to employ?

We are still a small company. We try to maximise resources by outsourcing things such as graphic design, web design, consultancy, and accountancy. Some production is outsourced to our partner company and investor from Eastern Europe who develops and purifies the enzymes for us. We hope to employ a sales rep in the summer to help with direct sales here in the UK. We just won a grant worth 70,000 Euros for the research and development of new products, so watch this space!

What advice do you have for aspiring bio-entrepreneurs?

If you have a good idea, go for it. We did not stray from our comfort zone, so we fully understood the risks. So long as you fully understand the business you are entering, you only have to consider your own attitude to risk in relation to your life. Two pearls of wisdom my uncle handed down to me held some resonance: before we started, "Stick to what you know" and then after we started he said, "Manage your cash flow".

What future do you envisage for PCR Biosystems and for the PCR industry in general?

In 25 years nothing has come along to replace PCR, so we expect it to be here in the long term. It has changed somewhat with the advent of real-time PCR and now digital PCR, but these changes relate to sensitivity and method of detection rather than the fundamentals. The principal of the technique has remained unchanged and we expect it to remain so. PCR Biosystems will be a company with a Research and Development arm, so we hope to keep pace with, and even lead, any changes. The diagnostics market is yet to fully embrace PCR; however, this is changing very quickly. The reliability, speed and sensitivity of PCR make it ideal for point-of-care diagnostics. Also, PCR should have a role to play in the imminent arrival of companion diagnostics. But first we need to secure a firm foothold in the research market.

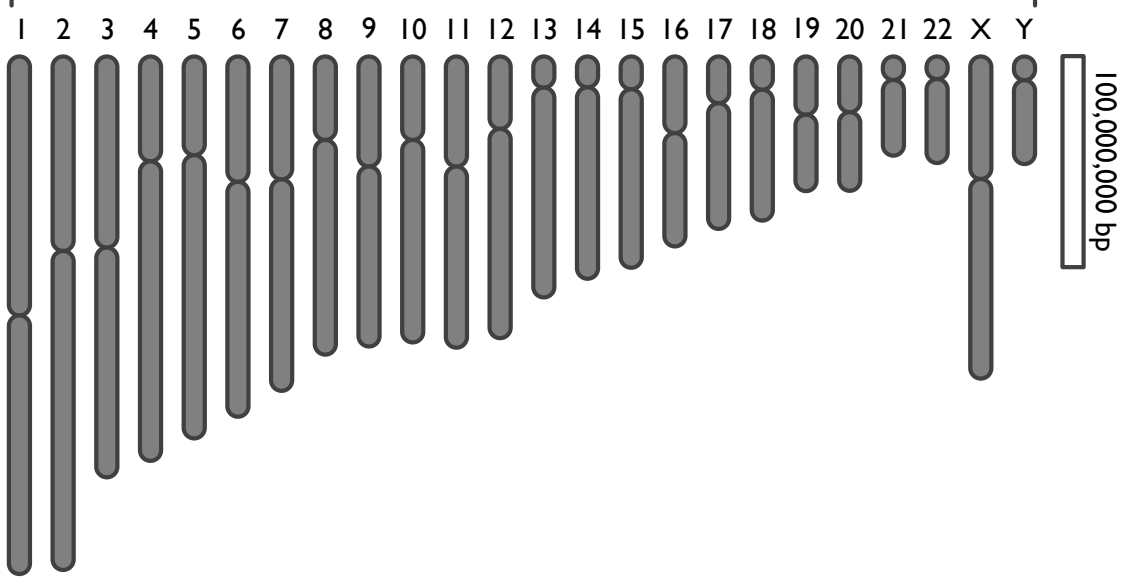
Please visit us at www.pcrbio.com!

Decoding the Encoding

The human genome is big. Very, very, very, big.

This is 200 base pairs of DNA, about 1 millionth of one human chromosome.

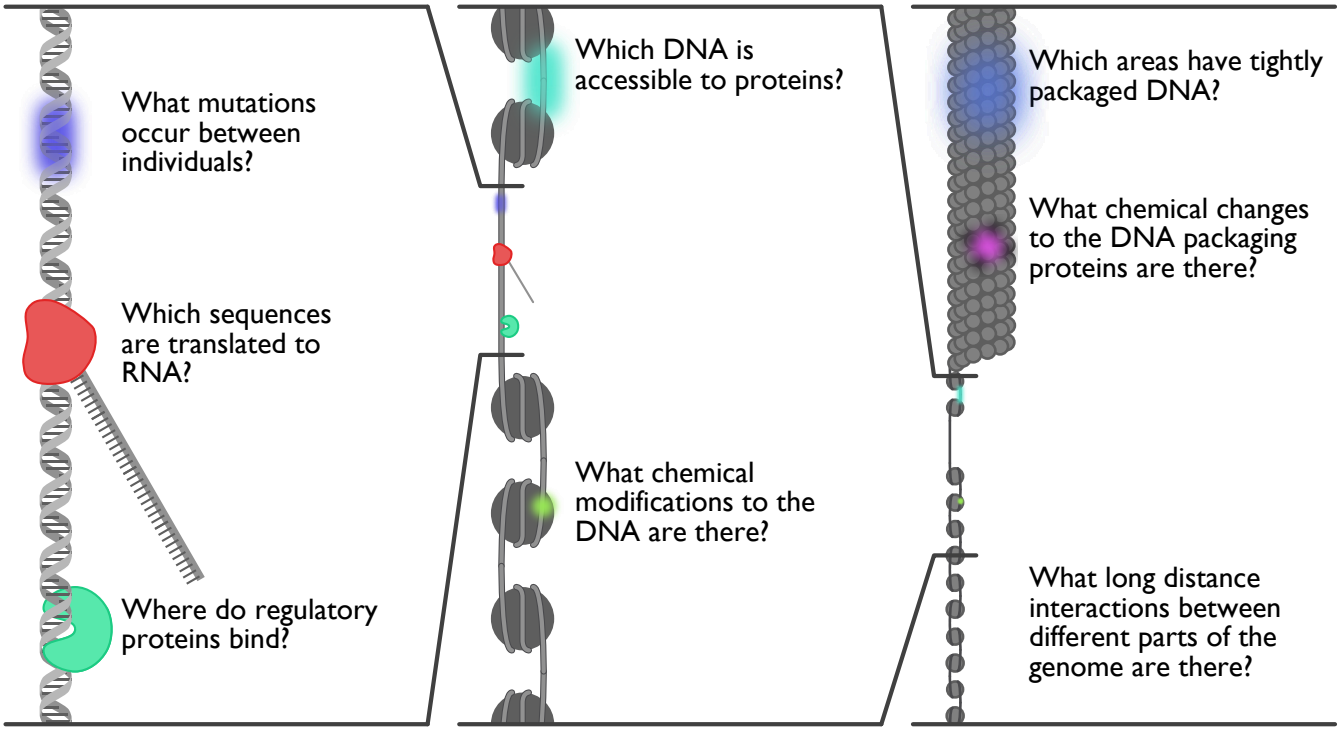
And these 3 billion base pairs, spread across 24 different chromosomes, make us human.



Unfortunately we only understand this much of that DNA well:

2% of all DNA, coding for about:
- 20,000 proteins
- 500 ribosomal RNAs
- 6,500 small RNAs

Originally the remaining 98% of DNA was viewed as junk. However, over the years we have learnt there is much more to human DNA than just its sequence:



The ENCODE project aimed to fill in some of these gaps, creating the most thorough map of the human genome ever...

442 researchers across **32** institutes in **5** countries.

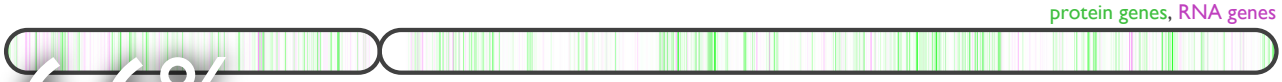
15 terabytes of data, **300** years of computer time and **741** wiki pages.

30 publications in **3** journals.

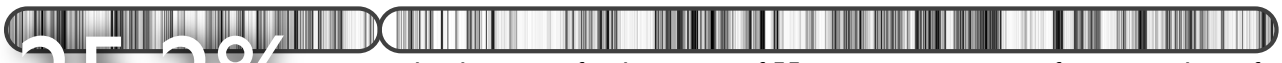
So what do we know now?

Let's take a look at *some* of the data from *only* chromosome 10, all 135 million base pairs of it! This is a summary of just 5% of the human genome, and about 0.01% of the ENCODE data set.

97% of the chromosome is sequenced to a high standard. Poorly sequenced regions are typically repetitive, like around the centromere.

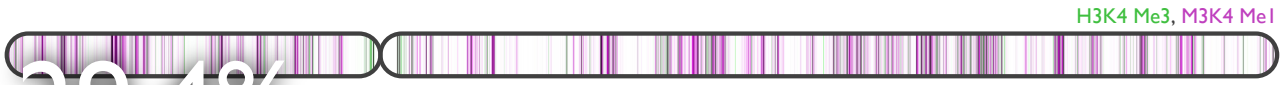


6.6% of the DNA sequence codes for proteins and RNAs, a coding that we already understand well.



25.2% is near a binding site of at least one of 55 major transcription factors, a class of protein which regulate translation of genes.

22.6% is translated into RNA. Some of this is expected (like transcription of protein coding sequences), some is not!



29.4% has chemical modifications to the DNA packaging proteins (histones) known to be associated with DNA sequences involved in control of gene expression.

8.4% is highly accessible to proteins (detected by high sensitivity to DNase1 degradation), meaning about 30% of DNA lies within 300 bp of easy protein access.

All in all we now know that about 80% of the human genome does something, although it is often not clear precisely what that function is. No matter how you look at it though, the majority of the genome is certainly not "junk DNA".

By Richard Wheeler 2012

Data from: genome.ucsc.edu/ENCODE & www.ensembl.org

Are women losing out in the world of science?

Gender biases have been extensively studied and the stereotype that women are 'communal' and men 'agentic' is still a part of modern day society. These stereotypes influence men and women's career options and choices, and science and technology are no exception.

Sophie Fleurdipine

Post-doctoral researcher in Prof Chris Norbury's laboratory, Sir William Dunn School of Pathology.

Society sends the message to young girls that science and technology is not for them, and that women should be 'communal' (i.e. emotional, supportive and dependent), unlike men who should be 'agentic' (i.e. logical, decisive and independent). These pervasive messages don't just affect young girls and women directly; they shape society's attitudes, making it harder for girls and women who try to enter male-dominated professions. In a recently published article in *PNAS*, Moss-Racusin and co-workers showed that unfortunately these stereotypes still persist in science (1).

Moss-Racusin and colleagues contacted tenure-track faculty members working in Biology, Chemistry and Physics at six large American research universities and presented them with a cover story asking for their help in developing appropriate mentoring programmes for undergraduate science students. The faculty members were given a student's application for a laboratory manager position, supposedly randomly selected from a nationwide database. In fact, they were all given the same application written so as to correspond to an average student's application: promising, but not outstanding. The only difference was that half the faculty participants were given the application under the name of Jennifer and the other half under the name of John, names which had been shown to be equally favourable. They were asked to rate the candidate and provide honest feedback, not holding back on their answers, as if the student were to be working in their own lab.

The participants answered questions, such as, "Did the applicant strike you as competent?", ranking the application on a scale ranging from one (not at all) to seven (very much). In total, they answered three questions measuring the student's competence, three measuring the student's suitability for hire, and three to assess the extent to which they were willing to mentor the student. They were also asked to choose a starting salary for the applicant, and finally, to answer three questions indicating the extent to which they liked the applicant.

The results were surprising: the effect of the student name/gender was striking (Figure 1). The faculty members viewed the female student as less competent and less suitable for hire than the male student. They where also willing to offer the female student less career mentoring and a starting salary 12.3% lower than that offered to the male student. Remarkably, the faculty participant's own gender, scientific field, age and tenure status had no effect on these results. Moreover, rather than simply preferring males, the faculty members reported to like the female student better than the male student, apparently expressing sympathy towards emerging female scientists. However, this did not translate into positive perceptions of women's competence. As the authors state, "faculty members of both genders appear to be affected by enduring cultural stereotypes about women's lack of science competence that translate into biases in student evaluation and mentoring".

The authors argue that the experimental evidence from this study helps shed light on the on-going debate regarding the possible cause of gender disparity in academic science. The student applicants in this experiment were described as "adequately qualified but not outstanding". This description represents the majority of students and they are precisely the sort whose success in academic science is most likely to be affected by faculty support. Academia is thought to have an ideal of meritocracy, judging people on their individual abilities, yet these results suggest that people do not get equal opportunities to progress, based on their gender.

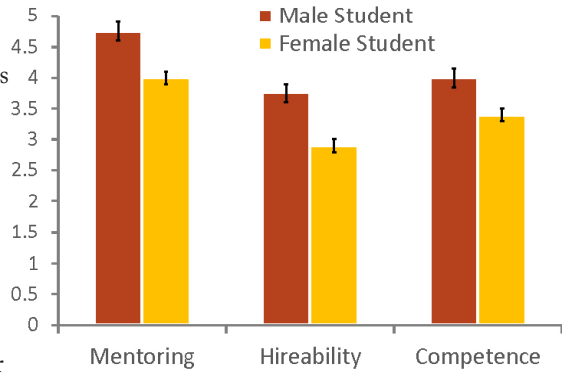


Figure 1: Graph showing faculty member response to questions about student employability. Adapted from (1).

It is interesting to note that a previous study has revealed another way in which implicit bias against women within academic faculty could harm women's careers. Madera and co-workers showed that qualities mentioned in letters of recommendation for women differ from those for men and conformed to the 'communal female'/'agentic male' gender stereotypes (2). This study also showed that a candidate described with communal characteristics in a recommendation letter was less likely to be selected for an academic position at a research university. Academic positions are high status occupations for which agentic qualities and leadership are valued, and it thus appears that there is a mismatch between attributes of gender and work roles that can affect women in the workplace.

How can gender stereotypes and biases in science be tackled?

Stereotypes are prescriptive and describe how it is perceived that people should behave. Stereotyping a group can have a negative effect on people's performance if confronted with it, a phenomenon known as stereotype threat. Stereotypes can also have an impact on people's self-assessment of their competence when they are performing a task that does not traditionally fit with the stereotype (for more information about stereotype threat and career ambition, see 3). Female students and women in junior positions in academia could benefit from programmes that present and discuss implicit bias and how stereotypes might affect their career planning. One such programme for women at an early stage of their scientific careers, designed by Isaac and colleagues (4), aims to increase leadership self-efficacy, described as "an individual's belief in his or her ability to succeed in the capacity of being a leader". The authors explain that "providing strategies to recognize and mitigate the impact of gender stereotypes is effective in increasing leadership self-efficacy". The participants reported that the course had a positive and sustained impact on their career.

In order to reduce implicit bias against women and improve their representation at all career stages, universities and research organisations should set up programmes aimed at both women and the gatekeepers in these institutions. Indeed, faculty members and principal investigators would benefit from training on implicit bias and how it could unintentionally affect decision-making and choice of leadership, and be detrimental to research and education. It is noteworthy that *Nature* has just published an editorial acknowledging that their editors might be affected by implicit bias and stating that they will implement a "gender loop" to improve the way they are reflecting women's contribution to science (5).

Why not explore your implicit biases by testing, among others, how easy (or not) it is to associate women/family and men/career compared to women/career and men/family? Warning: the experience can be saddening.
Visit the Project Implicit website (https://implicit.harvard.edu/implicit/) for more information

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Figure 2: An alternative solution? No, members of the French feminist activist group 'La Barbe' aim to highlight the countless areas of power almost exclusively controlled by men. (In French, "la barbe", translation of beard, is not only a hairy attribute of manhood but also an expression meaning "what a pain".)

Regenerative medicine: where will it grow from here?

Susan Graham

3rd year DPhil student in the Biomedical Ultrasonics, Biotherapy & Biopharmaceuticals Laboratory.

Regenerative medicine could hold the key to rebuilding bone and cartilage destroyed by cancer, letting a blind person see, keeping a failing heart beating and restoring the neurons lost after a stroke. But how much faith should we put in regenerative medicine, especially considering the harsh regulations limiting progress in research?

The hope

Regenerative medicine, using stem cell technology, could be key to the treatment of adults and children with a variety of failing organs. In 2010 a 10-year-old boy underwent pioneering treatment for a congenital tracheal defect, which made him unable to breath normally. His trachea was replaced with a donated trachea stripped of its cells and repopulated with the boy's own bone marrow stem cells. Two years on, he has a functional trachea and can breathe normally (1).

A UK-based company, ReNeuron, is trialling a regenerative medicine solution for stroke patients, which consists in implanting neural stem cells derived from foetal tissue, genetically engineered to remain conditionally immortal, directly into the brain. Interim data, from phase I clinical trial testing, suggest reductions in impairment and spasticity (2). However, the exact mechanism by which repair occurs is still unclear. Do the implanted cells form their own tissue separate from other areas of the brain, or do they interact with the body's own cells? These are the kinds of complex questions regenerative medicine poses.

Trouble ahead

Overcoming the challenge of mimicking healthy tissue *ex vivo* and reproducibly manufacturing it for treatment purposes would be a significant achievement in modern medicine. Logistically, however, transporting and storing live tissues presents major challenges requiring significant and novel engineering solutions. The regulatory and legal hurdles will be immense. Even if regenerative medicine can produce viable products, public acceptance is not guaranteed. Public concerns influenced the European Court of Justice's decision to ban the patenting of inventions incorporating human embryonic stem cells, unless they are for the purpose of aiding the embryo itself. Even though the number of patents applied for in this area has been steadily growing since 1991, the rate of patents being granted in the last six years has decreased (3).

All is not lost

A less trodden road of regenerative medicine is the use of test models out of the body, for drugs and therapies that will one day be used in the body. More than 70% of cancer and molecular biologists still use two-dimensional cell culture techniques to study complex biological phenomena of normal and cancerous tissues (4). Three-dimensional cell culture models on the other hand parallel the *in vivo* environment of tissues more closely, and can therefore more accurately represent normal and abnormal cell function. This new strategy has been adopted by pharmaceutical companies and research institutes to predict the efficacy and toxicity of drugs more accurately before testing them in animals and humans. In a further step aimed at developing personalised therapies, GE Healthcare Life Sciences, UK, and Beijing Genomics Institute, China are collaborating to develop drug assays that employ stem cells from multiple ethnic origins to understand differences in drug responses (2).

While replacement of diseased tissue remains the ultimate goal for stem cell therapy, the intermediate application in drug testing not only promises significant benefits for healthcare, but also avoids the pitfalls of clinical trial regulations and biocompatibility required for human use.

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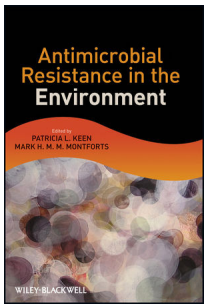
Oxbridge Biotech Roundtable (OBR)'s Oxford chapter will be hosting a talk on regenerative medicine on 6 February 2013, with Dr John Sinden, Chief Scientific Officer and co-founder of ReNeuron.

This article is co-featured on OBR's Review www.obrreview.com, a running conversation about science, business, and everything in between.

BOOK REVIEW

Antimicrobial resistance in the environment



Edited by Patricia L Keen and Mark HMM Montforts

ISBN: 978-0-470-90542-5, Wiley Blackwell (February 2012), Hardcover, 632 pages, £86.95

Reviewed by Jennifer de Beyer

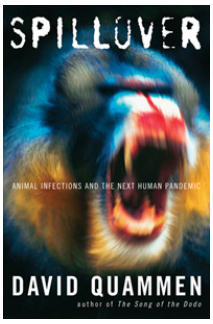
Antibiotics, the twentieth century's greatest defence against infection, may be failing us in the twenty-first. With the lack of new antibiotics, the precipitous rise in antibiotic resistance and the desperate arms race currently underway as Pharma tweaks our existing weapons into slightly more toxic versions of themselves: this picture will not surprise you. The popular view of how this situation has come to pass is straightforward. We use antibiotics in needlessly large quantities; we don't follow doctors' orders and forget to finish that full course; by not killing off every last bug in the current infection we create a survivable selection pressure and voila, resistant bacteria.

Antimicrobial resistance in the environment brings together current evidence suggesting this linear view is too simplistic. There exists a reservoir of resistance conferring genes within environmental microbes, collectively termed the resistome. Apart from the pathogenic bacteria whose resistance has been our primary concern to date, numerous other species of bacteria contribute to the resistome. PCR surveys suggest that only a fraction of these bacteria have been cultured, that genetic strategies for antibiotic resistance are incredibly diverse and, crucially, that resistance has arisen without the stimulus of manmade antimicrobials.

How the resistome interacts with the vast quantities of antimicrobials released into the environment through clinical, veterinary and agricultural practices, and how antibiotic resistance genes may jump from environmental microbes to human pathogens, are the main subjects of the text. A recurring theme is that selection is a one-way street, as once resistance has been recruited from the natural resistome and established in a pathogen or a community, removal of the selection pressure has very little effect on the spread of resistance.

This is an excellent publication, including primary data and detailed explanations of specific studies and techniques as well as review chapters. As both an introduction to a research area and a call for greater study and thought into how we regulate antimicrobial use in all spheres of society to limit further resistance, I highly recommend *Antimicrobial resistance in the environment*.

Spillover: Animal Infections and the Next Human Pandemic



David Quammen

ISBN: 978-0-393-06680-7, W.W. Norton & Company (October 2012), Hardcover, 587 pages, £20

Reviewed by Natasha Spottiswood

Spillover is a popular science book about zoonotic diseases, caused by pathogens, ranging from the lethal to the annoying, which migrated from their animal hosts into humans. It is a brisk and entertaining tour through a menagerie of baddies: from the very well-known (SARS, malaria, HIV, influenza), through the obscure (Ebola, Marburg, psittacosis), to the newly described (Hendra, Nipah).

Like a particularly grisly detective novel, each chapter usually begins with an unexplained dead body. With Hendra virus, for example, the body was of a racehorse trainer, dead along with many of his prized horses. The stage is set for a gripping whodunnit. What sort of pathogen is the killer? What is the reservoir host, the species that carries it with impunity and occasionally passes it onto humans? The fruit bat is identified as the reservoir host, raising another mystery: all the known victims worked with horses, while eccentric wildlife lovers who nursed injured bats for years never succumbed!

Spillover at times reads like a series of snapshot biographies, or a travelogue. Each pathogen is framed with fascinating and occasionally tragic character sketches of the scientists and lay people involved. Quammen thus shares his own trials as he interviews field biologists, lab scientists, and recovered victims; watches scientists trying to capture potentially SARS-infected bats or retraces the spread of HIV-1 group M by land and river.

Spillover's central, controversial, thesis is that zoonotic diseases are a growing threat to humanity because of changes in the way we interact with the environment. Quammen posits that our growing population and ecologically bad habits expose us to novel zooneses, and our behaviour increases the likelihood that they cause problems on a global scale. We disrupt habitats, mass-farm animals in unsanitary conditions and feed them antibiotics, and fly rapidly between crowded continents, facilitating global transmission of pathogens.

Quammen and the scientists he writes about are haunted by the idea of the Next Big One (NBO), the next lethal disease that goes global rather than killing a few people and fizzling out. Reading *Spillover*, one realises that we have been very lucky so far. Spillover is worth reading not just for the stories and the science, but also as a timely call to arms for the scientists, policy-makers, and citizens of this world.

Amino Acid Metabolism (3rd edition)

David A. Bender

ISBN: 978-0-470-66151-2, Wiley-Blackwell (August 2012), Hardcover, 478 pages, £75.00

Reviewed by Stuart Thomas

Amino Acid Metabolism is a compact but detailed account of the building blocks of life, from nitrogen to waste products. It is aimed at anyone with prior knowledge of the subject, derived from, in Bender's words, "the second year of a UK BSc course in biochemistry, nutrition or medical science". I remember using the previous edition of this book while I was an undergraduate, and it was already ancient (for a science textbook). It was last printed in 1985, so this 3rd edition is very welcome.

Bender writes succinctly and clearly, in a manner which serves well for quick referencing or for reading whole chapters at a time. The chapters are well organised and arranged logically. The book begins with a detailed, if lengthy, chapter on nitrogen metabolism which summarises fixation and metabolism into amino acids, nucleic acids and waste. A general chapter on amino acids follows, which contains information on protein turnover and human nutrition, as well as essential and non-essential amino acids. This is a particularly interesting chapter, full of general metabolism. The third chapter, ostensibly about vitamin B6, is really about the reactions in which this molecule is a cofactor: transamidation, decarboxylation and racemisation.

The rest of the book details the metabolism behind specific amino acid groups. The amino acids are not grouped based on functionality (polar, uncharged etc.), but rather on structural features that are complementary during metabolism. For example, glycine and serine appear together due to their contribution to one-carbon metabolites; glutamine, proline and arginine are all metabolised from glutamate; lysine, methionine, cysteine and threonine are all involved in the aspartate pathway. This is a very logical way of studying each amino acid in relation to the wider metabolic vista.

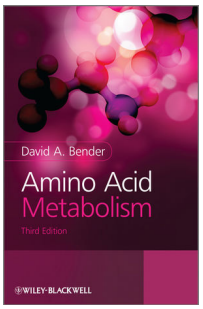
This latest edition has been extensively rewritten in parts, with a chapter on amino acid transport broken up and spread throughout the text. The whole text has been updated with new information and there is considerably more detail on physiology and genetics, due in part to the discoveries of the last 27 years. Bender's detailed prose is accompanied by diagrams of metabolic pathways throughout the book, however non-reaction diagrams are sparse. The book could benefit from additional related images, such as enzyme structures, which would be welcome to a textbook that functions as a go-to guide or readable primer for amino acid metabolism.

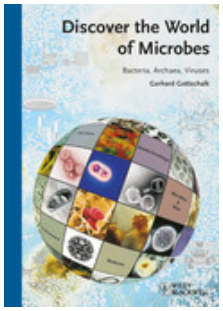
Discover the World of Microbes: Bacteria, Archaea, and Viruses

Gerhard Gottschalk

ISBN: 978-3-527-32845-1, Wiley-Blackwell (November 2011), Paperback, 388 pages, £45.00

Reviewed by Hua Wang





This book is an insightful read, in a down-to-earth style, that provides an alternative and engaging approach to understanding microbiology. The book has 32 short, comprehensive chapters that cover a broad range of topics from the existence of bacteria to interbacterial relationships, and antibiotics to bioinformatics. Ten additional sections act as study guides for specific topics with in-depth explanations of areas such as bacterial membranes and energy metabolism.

Gottschalk acts as a narrator by including a short introduction to each chapter and then presenting material in a question-and-answer format, providing answers using examples, analogies and contributions from prominent scientists. For example, "Chapter 6: Life in Boiling Water" begins by introducing the discovery of the bacterium *Thermus aquaticus*, which lives in a 70°C environment. It is a bacterium most molecular biologists recognise instantly since Taq polymerase, commonly used in the polymerase chain reaction, was originally isolated from it. Gottschalk goes on to describe the discovery of other bacteria that grow from 95°C to 110°C, finally pushing the limit with *Pyrolobus fumarii*, which grows at 113°C! He also quotes Karl Stetter, who first reported the possibility of life above 100°C. Gottschalk poses legitimately reasoned questions that anyone with or without a scientific background might ask, such as "it is hard to believe: on the one hand, there are hard-boiled eggs with completely denatured protein, and on the other hand, there are living creatures that grow vigorously under such condition. Does anyone know why there are such stark differences? Why are archaea but not bacteria found in boiling water?" The dialogue style in each chapter is engaging and kept my attention word after word, and to the bottom of each page, as though I were watching an action-packed film!

Throughout the book, Gottschalk transitions smoothly between topics by splitting them into short chapters, which avoids overwhelming readers with too many facts. For example, any book on microbiology has without a doubt a section on antibiotic resistance; in this book, the topic is divided between two chapters: "Chapter 22: Antibiotics" and "Chapter 23: Plasmids and Resistance".

I would highly recommend this book to anyone seeking to investigate the world of microbes, or perhaps looking for a teaching tool. The chapters are concise and complete, and include unique and captivating quotes to draw the reader in.

5' with... Dr Monika Gullerova

Dr Monika Gullerova completed her undergraduate work in Bratislava and her PhD at the University of Vienna. After a highly successful stint as a post-doctoral researcher, Monika is now starting her own lab in the Sir William Dunn School of Pathology through the support of an MRC Career Development Award. She shared her reflections on her career so far.

Interviewed by Daniel Scott

When did you first decide you wanted to be a scientist?

I actually grew up in a family of medical doctors and was focused on medicine and science from high school onwards. Despite that, it actually wasn't until the third year of my undergraduate degree that I decided to become a research scientist. I was given the chance to run a project on gene therapy as a treatment for cancer and was fascinated by the possibilities it revealed. Unfortunately gene therapy hasn't quite fulfilled its potential yet, but I'm still hopeful!

If you weren't a scientist, what would you be?

I've always wanted to be a fiction writer, right from a very young age. I actually applied to the Academy of Arts Conservatorium for a screenwriting course at the same time as applying for a science degree, so if things had happened slightly differently, who knows?

If you're not in the lab, you are . . .

Going to the gym a few days a week or playing badminton. I'm also thinking about writing a book about the experiences of being a young scientist abroad, and have been working on a children's book based on my pet rabbits – I'll keep you posted!

What has been the most important finding of your career so far?

While working on convergent gene pairs in fission yeast, I found that their overlapping transcription in the G1 phase of the cell cycle is able to drive the deposition of heterochromatic marks, including cohesin, across the genes during the S phase. In the subsequent G2 phase, cohesin is localised to the non-coding region between the genes and prevents transcription into this region, forcing the use of otherwise cryptic proximal poly(A) sites. This discovery demonstrated a whole new path for the cell cycle-dependent regulation of transcription termination.

In your opinion, what makes a good scientist?

You need to have a passion for science, and to be willing to stay late on a Friday night because you really want to see that result on the gel or under the microscope. We certainly don't do science for the money, so passion is essential!

Looking back at your career, what advice would you give to a young scientist just starting theirs?

Don't try to compare yourself to others, in terms of progress or papers or anything of the sort. Concentrate on your own work, make sure it's good science and that you enjoy doing it. There will always be other amazing scientists out there – don't let them faze you.

How do you think that the representation of women in academia could be improved?

I think that the support for women in science is definitely improving, especially as more women reach positions of power and are able to facilitate change, but there is still a lot that could be done. I think the biggest change that could be made would be to support women after the end of maternity leave, for example by establishing childcare facilities in research institutions, and also by the ability to request childcare support in research grants alongside a salary.

How do you imagine biological research will change over the next 20 years?

I think that the 'big science' trend we're seeing now, with the rise of genomics/proteomics and large-scale sequencing-based studies, is going to continue. However, I still think that a lot of the most interesting science being done involves detailed understanding of individual genes. I'm also hopeful that gene therapy can make a comeback!

SNAPSHOT Research Image Competition

This issue´s winner is...

Dr Alexandr Khrapichev

Alexandr Khrapichev is a senior research fellow in Dr Nicola Sibson's group at the Cancer Research UK/ Medical Research Council funded Gray Institute for Radiation Oncology and Biology (Department of Oncology), and a research fellow at Wolfson College.

Alexandr's image was produced using Magnetic Resonance Imaging (MRI), a technique for analysing the distribution of water molecules within a tissue. The image shows a collage of cross sections through multiple different fruits (such as a clementine, apple and kiwi). Normal MRI images are black and white, so the images have been coloured to represent the fruit in question.

The Experimental Neuroimaging Group, of which Alexandr is a member, is interested in developing imaging techniques for the detection of early stage brain cancers, in particular, secondary cancers from a primary tumour elsewhere in the body. The techniques currently available have limitations and are only able to detect secondary cancers in the brain at a very late stage, by which time few treatment options are available and the prognosis is poor. Alexandr and the rest of his lab hope the development of new diagnostic techniques will allow the early detection of tumours and result in patients having prompt access to the treatment they need.

The MRI technique used here is known as multi-sliced spin-echo imaging. The multi-sliced aspect means that several slices of the tissue (in this case, fruit!) are scanned simultaneously to produce multiple images. Spin-echo refers to the technique used to form the MRI signal. Briefly, samples are placed in a high field magnet and the protons within the tissue, which have spin, align along the magnetic field of the machine. Applying a radio-frequency pulse turns the spins perpendicular to the magnetic field, which causes their rotation around the main magnetic field. However, due to imperfections of the field, the protons' spins begin to rotate at slightly different frequencies and lose their coherence with each other, producing what is called a free induction decay. A second radio-frequency pulse can be applied to flip the spins, and initiate their refocusing. This leads to the formation of a 'spin echo', which can be detected.

Alexandr produced these images as part of an open day for his department. The idea was very simple: to use a complex medical technique to image everyday objects as a way of introducing these techniques to the public. As we can see, the result was very effective and the images have proven to be a great tool for public engagement, promoting questions and intrigue!

Win a £50 book voucher kindly provided by Oxford University Press!

Do you have an image from, or inspired by your research? Why not enter it in SNAPSHOT?
We are now accepting entries for pictures to be featured on the cover of *Phenotype* TT 2013. To enter, send images to oubs@bioch.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images. There is no limit to the number of entries per person.
The deadline for the competition is 8 March 2013.

PHENOTYPE crossword

Enter the competition by sending your answers to oubs@bioch.ox.ac.uk or leave a paper copy in a sealed envelope in the OUBS pigeonhole at the New Biochemistry reception. Entries received by 30 March 2013 will be entered into the prize draw.

Our resident cryptographer, Homarus (www.homaruscryptic.com), challenges *Phenotype* readers to crack this cryptic crossword on the theme of molecular biology.

The winner will receive their choice of three books reviewed in this issue, generously provided by Wiley-Blackwell.

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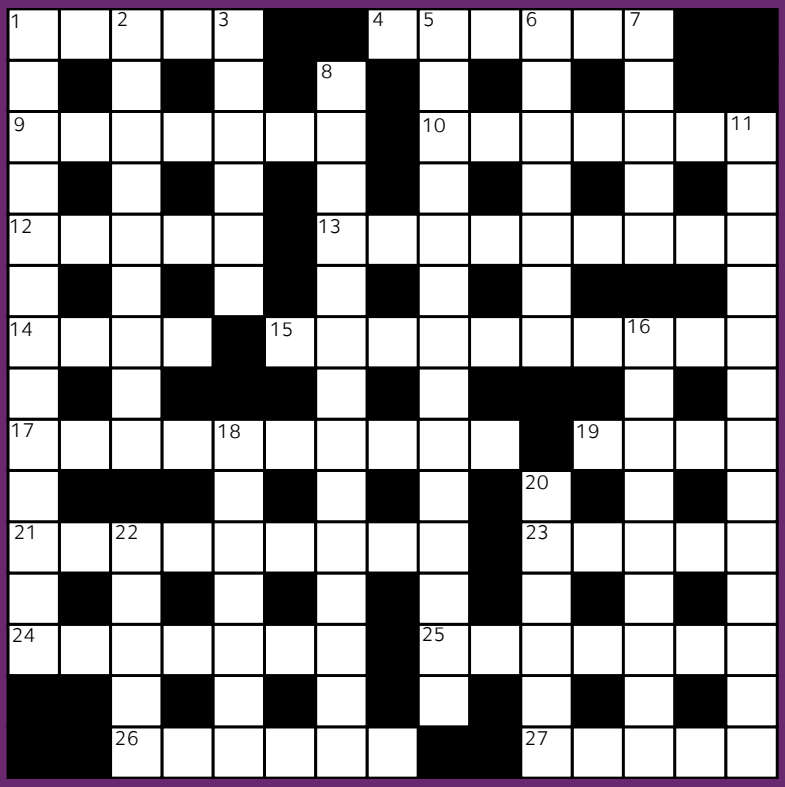

Congratulations to Stuart Thomas from the Department of Biochemistry who won the Michaelmas '12 crossword competition.

Answers to Michaelmas '12:
Across 2 nuclear bomb; 9 insurrectionary; 11 excavation; 12 acne; 14 rehash; 15 milfoil; 18 treacle; 19 lessee; 22 alma; 23 cytochrome; 25 cocktail sausage; 26 synonymised.
Down 1 toiletry; 4 egesta; 5 retrofit; 6 oboe; 7 Branchiostoma; 8 oxygen; 10 Saccharomyces cerevisiae; 13 after hours; 16 Illyrian; 17 Genevese; 20 talcum; 21 dorsum; 24 stun

Across
1 Indium acquired as metal bar (5)
4 see 8
9 Resin to disappear around alkyl group (7)
10 Annoyed at start of round (4,3)
12 Dismiss return of potato, say (5)
13 Torture: stuff between thong and ring ... (9)
14 ... measures real ales evenly (4)
15 Deep cow noise proposed (3-7)
17 Rodent sounds like the opposite of a scarf! (10)
19 Auntie loses one and a turn to pay (4)
21 On red planet, hydrogen mist conceals Romeo - a green amphibian (5,4)
23 N-rich group sees regular banzai idle (5)
24 Four-winged flier in the Navy? (7)
25 Ransom a tickler held bodily (7)
26 Royal house puts donkey between nitrogen and gold (6)
27 Glasses of liquor cause visions, but no energy (5)

Down
1 Confused imam converted by 'sign' (8,5)
2 Fish biscuit? (9)
3 Dehydrated, losing sulfur in place of zinc (6)
5 Student of man sees defender grasp crushed thorn (14)
6 Seer (or not?) has sixth sense in River Island (3-4)
7 Pesto may conceal pore (5)
8,4 Mad arch-enemy splits a plot of moss (14,6)
11 The Dutch middlemen follow loud lavatory death of dykes, say (5,8)
16 Someone lacks cell line consumed by Harry (9)
18 Sounds like a fallen woman among the beds of babies, all of similar ages (7)
20 Battles over small sea heated up (6)
22 Italian citizen of the right sultanate (5)