PHENOTYPE

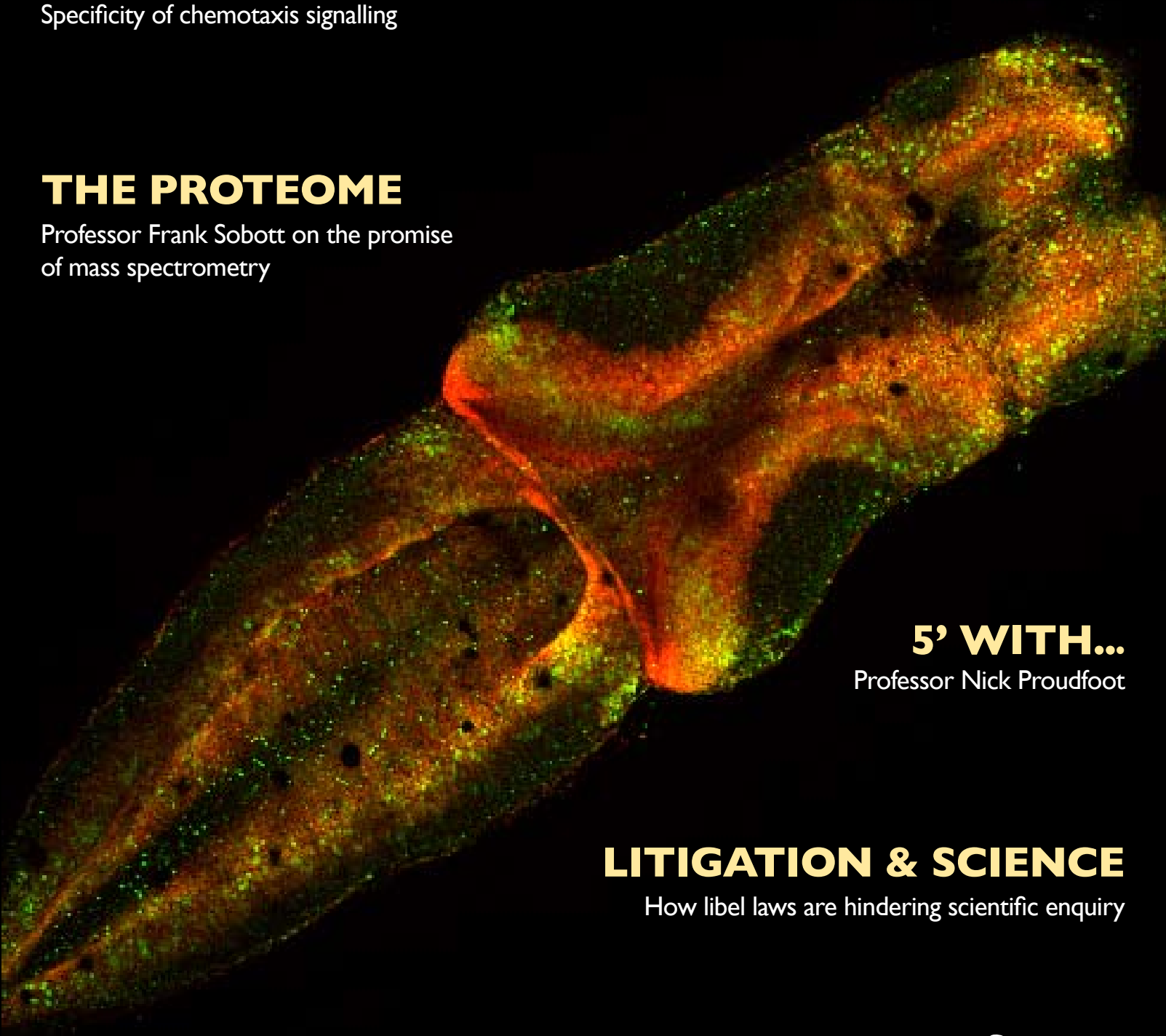
Issue 6, Trinity Term 2010

RESEARCH HIGHLIGHTS

Conformational changes in DNA PolI
PDMR9 and meiotic recombination
Specificity of chemotaxis signalling

OPTOGENETICS

Shining a light on brain research



THE PROTEOME

Professor Frank Sobott on the promise of mass spectrometry

5' WITH...

Professor Nick Proudfoot

LITIGATION & SCIENCE

How libel laws are hindering scientific enquiry

PLUS...

Decoding the Ribosome
Biotechnology & Innovation
10 Years of the Human Genome Sequence

OUBS

EDITORIAL

Welcome to *Phenotype* 6. This is the last issue of the academic year and, I hope you'll agree, the most exciting and diverse yet.

This term, OUBS is fortunate to host talks by two of the 2009 Chemistry Nobel Laureates. Our *Featured Seminar* article explores the pioneering work of Professors Venkatraman Ramakrishnan and Ada Yonath, whose research groups determined the first high resolution crystal structures of the 30S ribosome. These seminars are definitely not to be missed!

It's been nearly a decade since the first draft human genome sequence was published, but we still know relatively little about the function of much of our DNA. Penny Sarchet reviews the progress in genomic research in the last 10 years and discusses future directions, as well as the implications for understanding human diseases. One of the major challenges is to relate the genome to the proteome. In his *Feature* article, Professor Frank Sobott discusses the role of mass spectrometry in studying the proteome and how protein interactions give rise to complexity at the systems level. Remarkably, our ability to manipulate DNA sequences now allows researchers to control the behaviour of model organisms. Carinne Piekema tells us about the emerging field of optogenetics, a technique that uses light and genetic modification to target specific neural circuits.

This term's *Snapshot* competition sees the previous dominance of the Dunn School of Pathology overturned by Dr Leah Herrgen from the Pharmacology Department with her stunning image of a *Xenopus* brain. However, Pathology retains a strong presence in *5' with* Professor Nicholas Proudfoot, our first interview with an academic outside the Biochemistry Department.

There are two new additions to *Phenotype* in this issue. The first is *Science and Society*, with articles on the recent Simon Singh libel case and public engagement with science in Oxford. We also now have a book review section. You can win one of the textbooks reviewed in this issue, kindly provided by Wiley-Blackwell, in the crossword competition.

I would like to thank everyone who has contributed to *Phenotype* in the last year. The writers, editors and designers have worked tirelessly to bring you the first three printed issues of the magazine. We are extremely grateful to our sponsors, especially the Medical Sciences Division, RCUK and MRC Harwell.

We're looking forward to receiving your ideas and contributions for the Michaelmas '10 issue.

David Yadin

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Issue 6

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OUBS SEMINARS

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Main Meeting Room, New Biochemistry Building

Friday 21 May 16:00 - 17:00
Professor Venki Ramakrishnan
Winner of The Nobel Prize in Chemistry 2009, MRC Laboratory of Molecular Biology, Cambridge
What we have learned from structures of the ribosome
Martin Wood Lecture Theatre, Physics Department
Special Nobel Laureate Lecture

Tuesday 1 June 16:00 - 17:00
Professor John Sedat
University of California, San Francisco
What is on the horizon for 4-dimensional optical microscopy for live biology?
Main Meeting Room, New Biochemistry Building

Thursday 3 June 16:00 - 1700
Dr Antonella Riccio
MRC Laboratory for Molecular Cell Biology, University College, London
Chromatin remodelling mechanisms in neuronal development and plasticity
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Monday 7 June 16:00 - 17:00
Professor Gene Robinson
University of Illinois at Urbana-Champaign
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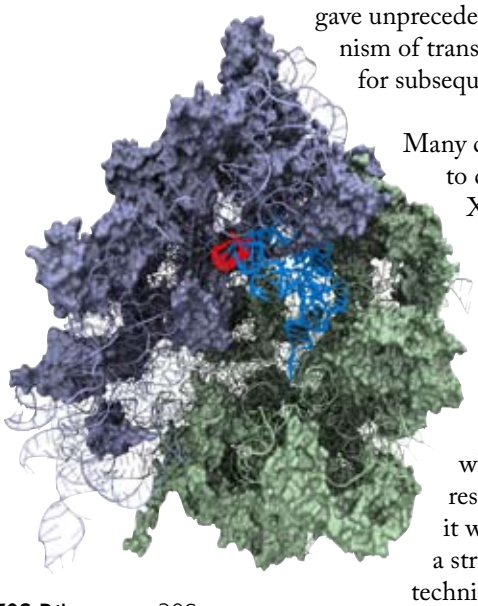
Tuesday 13 July 16:00 - 17:00
Professor Ada Yonath
Winner of The Nobel Prize in Chemistry 2009, Structural Biology Department, Weizmann Institute of Science, Rehovot, Israel
Title to be confirmed
Special Nobel Laureate Lecture; more details to follow soon.

DECODING THE RIBOSOME

OUBS Featured Seminars

In May and July this year, OUBS is privileged to host talks by Professors Venkatraman Ramakrishnan and Ada Yonath. David Yadin reviews the extraordinary achievements in studies of ribosome structure.

Ribosomes are large, complex macromolecular machines found in every living cell. They are responsible for reading the genetic code in the form of a messenger RNA transcript and translating it into a protein sequence with speed and accuracy. The 2009 Nobel Prize in Chemistry was awarded to Venkatraman Ramakrishnan, Ada Yonath and Thomas Steitz "for studies of the structure and function of the ribosome". Many people had worked for decades in this area, but these three were singled out for their achievements. They were the first to determine high-resolution atomic structures of ribosomal subunits, which gave unprecedented insights into the mechanism of translation and laid the foundations for subsequent work.



70S Ribosome: 30S in blue; 50S in green; tRNA (A and P site) in red; mRNA in bright blue (1) (Rendered by Alastair Stewart).

Many challenges had to be overcome to determine these structures by X-ray crystallography - the only technique capable of solving structures of very large macromolecules at atomic resolution. In the early 1980s, Ada Yonath was the first to obtain crystals of a bacterial 50S (large) ribosome subunit, which diffracted X-rays at low resolution. However, at the time it was not possible to determine a structure because of significant technical difficulties with data collection and analysis. Meanwhile, other groups pursued alternative approaches, including biochemical methods, electron microscopy and neutron scattering. This work furthered the understanding of ribosome architecture and function, but precise models of the structure-function relationship were lacking.

Several advances paved the way for ribosome structures. The first was the use of ribosomes from thermophilic archaebacteria, resulting in more stable crystals. Additionally, cryo-crystallography allowed diffraction data to be collected for longer and with higher intensity X-ray sources. Carrying out diffraction experiments at low temperature (100 K) and in the presence of cryo-protectant reduces the radiation damage caused by X-rays. One of the biggest challenges was determining the phases of the diffracted X-rays, necessary to compute the electron density, using which structural models were built. This was helped by the development of tunable synchrotron X-ray sources, which enabled phasing using anomalous scattering. Advances in computer technology and automation of data analysis also played a large role.

Following years of work, high-resolution structures emerged in 2000. The Yonath and Ramakrishnan groups independently published structures of the 30S (small) subunit from *Thermus thermophilius* at better than 3.5 Å resolution and the Steitz group published a structure of the 50S subunit from *Haloarcula marismortui* at 2.4 Å resolution. Their impact was huge. Finally, it was possible to directly visualise what had been previously deduced from countless other experiments. The architecture and arrangement of the protein and RNA components were revealed in spectacular detail. The structures also vastly increased the amount of information about RNA three-dimensional structure. Most significantly, it was confirmed that RNA components of the 50S subunit are solely responsible for catalysing peptide bond formation - there are no proteins at the active site.

Many mechanistic insights soon followed as structures were obtained of ribosomes in complex with accessory factors. Ribosomal subunits were co-crystallised with initiation, elongation or termination factors and messenger and transfer RNA (mRNA and tRNA) molecules. Of particular note, Ramakrishnan's group published a high-resolution structure of the 70S ribosome (30S bound to 50S) in complex with mRNA and tRNA in 2006. These models showed the binding sites of these molecules on the bacterial ribosome, as well as the associated conformational changes. Other structures showed the binding sites of antibiotics, which explained how they disrupt ribosome function.

What next for ribosomes? The amount of structural and functional information is increasing rapidly. While eukaryotic ribosomes perform translation in a similar way to bacterial ribosomes, they are much more complex, requiring many accessory factors. There is no crystal structure of a complete eukaryotic ribosome. Combining results of studies employing different techniques, especially electron microscopy, will provide an integrated picture in future. Furthermore, understanding the differences between eukaryotic and prokaryotic ribosomes will be essential for designing antibiotics that specifically target bacteria. All of this would not be possible without the pioneering efforts of Steitz, Yonath, Ramakrishnan and many others who pushed the limits of X-ray crystallography to determine the first high-resolution ribosome structures.

References:

1. Selmer M, Dunham CM, Murphy FV 4th, Weixlbaumer A, Petry S, Kelley AC, Weir JR, Ramakrishnan V (2006) *Science* 313:1935-42.

Further Reading:

Ramakrishnan V and Moore PB (2001) Atomic structures at last: the ribosome in 2000. *Curr Opin Struct Biol.* 11:144-54.

Rodnina MV and Wintermeyer W (2010) The ribosome goes Nobel. *Trends in Biochemical Sciences* 35:1-5.

GelRedTM & GelGreenTM

Environmentally safe and ultra-sensitive nucleic acid gel stains for replacing EtBr

EtBr has been used for nucleic acid gel staining for decades because of its low price and sensitivity. However, EtBr is a highly mutagenic material and the costs associated with waste disposal can ultimately make the dye expensive to use. Thus alternative gel stains, such as SYBR® dyes, have become available. Although these alternatives have reduced mutagenicity, they often sacrifice on other aspects such as sensitivity and stability. SYBR® dyes also enter cells rapidly to stain mitochondria and nuclear DNA, making it more likely for the dyes to be toxic at high concentrations.

To make GelRed & GelGreen safe, scientists at Biotium used a very simple concept: reducing genotoxicity by preventing the dyes from entering living cells. We engineered the chemical structures of GelRed & GelGreen such that the dyes are incapable of crossing cell membranes. The Ames test confirmed that GelRed & GelGreen are non mutagenic at levels well above their working concentrations.

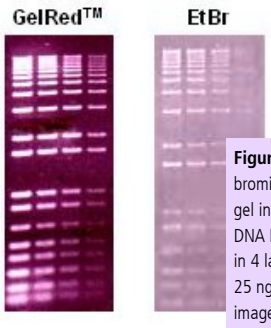


Figure 1. Comparison of GelRed™ and ethidium bromide (EtBr) in post gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder from Invitrogen were loaded onto each gel in 4 lanes in the amounts of 200 ng, 100 ng, 50 ng and 25 ng, respectively, from left to right. Gels were imaged using a 300 nm transilluminator and photographed with an EtBr filter and Polaroid 667 black and white print film.

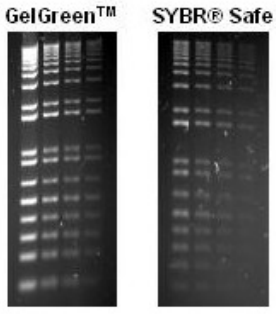


Figure 2. Comparison of GelGreen™ and SYBR® Safe in post gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder from ng, respectively, from left to Invitrogen were loaded onto each gel in 4 lanes in the amounts of 200 ng, 100 ng, 50 ng and 25 right. Gels were imaged using a 254 nm transilluminator and photographed with a SYBR filter and Polaroid 667 black and white print film.

Designed primarily for use with a 312/302 nm UV transilluminator, GelRed is more sensitive than EtBr, and brighter than SYBR® Gold. GelGreen is developed for researchers who use a 488 nm laser-based gel scanner or a Dark Reader that uses a visible blue light for excitation. GelGreen is spectrally similar to SYBR® Safe.

Another major advantage of GelRed & GelGreen is their remarkable stability. You can handle the two dyes the same way you do with EtBr. This means that the dyes are perfectly stable in water at room temperature for long-term storage, and they can be microwaved. Both dyes are also very photostable, permitting their use under normal room light.

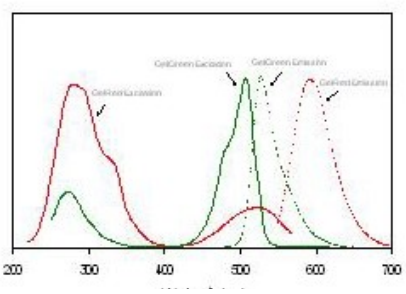


Figure 3. Normalized excitation and emission spectra of GelGreen™ (green) and GelRed™ (red) in the presence of dsDNA in PBS buffer.

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Figure 4. HeLa cells were incubated with 1X of SYBR® Safe, GelGreen™ or GelRed™. Images were taken following incubation for 30 min. SYBR® Safe entered into cells rapidly as evident from the bright green nuclear staining. However, GelRed™ and GelGreen™ were unable to cross cell membranes as shown by the lack of any fluorescence staining.

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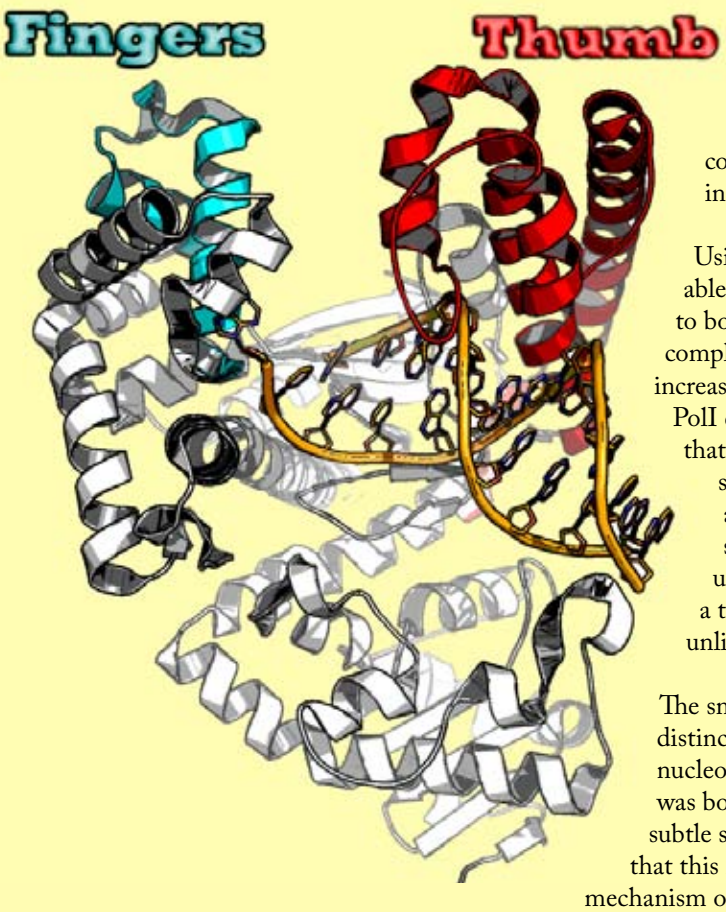
Conformational transitions in DNA polymerase I revealed by single-molecule FRET
Yusdi Santoso, Catherine M Joyce, Olga Potapova, Ludovic Le Reste, Johannes Hohlbein, Joseph P Torella, Nigel DF Grindley, and Achillefs N Kapanidis (2010) *Proc Natl Acad Sci USA* 107:715-720.

Santoso *et al*. used single molecule Fluorescence Resonance Energy Transfer (smFRET) to examine the conformational changes within the Klenow fragment of *Escherichia coli* DNA polymerase I (PolI). The crystal structure of DNA polymerase I (Klenow fragment) has been previously determined and has been compared to that of a hand. Furthermore, structural studies showed that upon addition of the complementary dNTP to the polymerase-DNA complex (Pol-DNA), a transition from an open to closed state occurs and a binding pocket is formed around the nascent nucleotide. This has been described as the fingers-closing step, as the mobile 'fingers' subdomain moves toward the 'thumb' subdomain (see figure).



In this study, PolI was labelled with a donor fluorophore (Cy3B) at the fingers subdomain and a complementary acceptor fluorophore (ATTO647N) on the thumb subdomain, thereby maximising the interfluorophore distance change upon fingers-closing. As FRET measurements are dependent on interfluorophore distance, this technique allows changes in protein conformation that result in distance changes to be measured for individual proteins.

Using smFRET with alternating-laser excitation, Santoso *et al*. were able to detect the expected distinct molecular species corresponding to both the open Pol-DNA complex and the closed Pol-DNA-dNTP complex. The proportion of molecules in the closed conformation increased as a function of dNTP concentration. Interestingly, unliganded PolI displayed a highly heterogeneous FRET distribution, indicating that the molecule is rapidly fluctuating between the open and closed states. Using fluorescence correlation spectroscopy (FCS), including assays performed in 5% non-denaturing polyacrylamide gels to slow rates by approximately 3-fold, the authors concluded that unliganded PolI fluctuates between the open and closed state on a timescale of ≈3 ms. This is a novel finding as crystal structures of unliganded polymerases have all been in the open state.

The smFRET measurements also suggested that PolI forms a unique distinct conformational species in the presence of a mismatched nucleotide. Another apparently distinct species was observed when PolI was bound to an rNTP rather than a dNTP. This is the first time that such subtle structural changes have been observed in the enzyme and it is hoped that this information will aid future studies aimed at understanding the mechanism of DNA polymerase specificity.

Drive against hotspot motifs in primates implicates the PRDM9 gene in meiotic recombination
Simon Myers, Rory Bowden, Afidalina Tumian, Ronald E Bontrop, Colin Freeman, Tammie S MacFie, Gil McVean, Peter Donnelly (2010) *Science* 327:876-879.

Recombination hotspots are sites in the genome at which meiotic crossover events cluster. The locations of hotspots differ greatly between species. Humans and chimpanzees, for example, share almost 99% identity at aligned bases but appear to share few, if any, meiotic hotspots. Recent research identified a 13 bp motif which is apparently overrepresented in human hotspots (it has been implicated in the activity of 40% of hotspots). In this study, Myers *et al*. investigated whether this 13 bp hotspot motif is also active in chimpanzees. Chimpanzee genetic variation data at 22 loci, where there was both human-chimpanzee sequence conservation of the 13 bp motif and an inferred hotspot at the orthologous location in humans, was obtained from 73 chimpanzees. The

statistical software package LDhat was used to estimate recombination rates in each species. The results indicated that the 13 bp motif does not recruit hotspots in chimpanzees, implying differences between the recombination machinery of the two species.

Based on an extended region of sequence specificity surrounding the 13 bp motif, with a 3 bp periodicity of influential bases, the authors previously suggested that this motif may be bound by a C2H2 zinc-finger protein. Using a computational algorithm, Myers *et al*. identified 5 putative human C2H2 zinc-finger proteins for which the 13 bp motif was present in the corresponding predicted protein binding sequence. *In silico* analysis was then used to compare the predicted tolerated sites of degeneracy of the protein binding sequence with the empirical degeneracy pattern found within the 13 bp hotspot motif. This analysis identified PRDM9 as the most likely candidate for the 13 bp motif-binding protein.

Comparisons to chimpanzee PRDM9 revealed that the chimpanzee protein was dramatically different to the human protein at the residues predicted to contact DNA. In fact, compared to all 544 C2H2 zinc-finger human-chimpanzee orthologue pairs, PRDM9 is the most diverged. The authors observed that the PRDM9 sequences in five other mammals are also significantly diverged. Another study, published in the same issue of *Science*, demonstrated that PRDM9 can indeed bind directly to the 13 bp motif *in vitro*.

These findings imply a role for PRDM9 in determining hotspot locations and the authors suggest that the rapid evolution of this protein may explain the lack of hotspot conservation between species.

Using structural information to change the phosphotransfer specificity of a two-component chemotaxis signalling complex

Christian H Bell, Steven L Porter, Annabel Strawson, David I Stuart, Judith P Armitage (2010) *PLoS Biology* 8 e1000306.

Two-component signalling pathways are used in bacteria, archaea and some eukaryotes to regulate cellular behaviour in response to environmental stimuli. These pathways utilise a sensor histidine kinase (HPK), which phosphorylates a cognate response regulator (RR) in response to a specific environmental cue. The RR can then direct the appropriate response within the cell. As some bacteria have over 150 such two-component systems, it is essential that specificity between HPK and RR pairs is tightly controlled to prevent 'crossed-wires' between signalling pathways.

Bell *et al*. investigated the structural determinants of specificity in a CheAY two-component signalling system from *Rhodobacter sphaeroides*, involved in chemotaxis. The chemosensory pathway of *R. sphaeroides* is significantly more complicated than that of *Escherichia coli*, containing four CheA homologues (the HPK) and six chemotaxis RR components. Thus, this bacterium is a useful system for investigating specificity between cognate CheA and CheY pairs.

In this study, the authors solved the crystal structures of CheY6 in complex with either the unphosphorylated or phosphorylated Hpt domain of CheA3. In CheA HPK proteins, the initial phosphorylation occurs at a histidine-containing phosphotransfer (Hpt) domain. This is the first structure of a CheA Hpt domain in complex with its RR. Based on these structures, Bell *et al*. identified three residues (M13, L16, Y17) on the surface of CheY6 predicted to be involved in interactions with CheA3, leading to phosphotransfer. Subsequently, these three residues were mutated and the resulting proteins showed both decreased binding-affinity for CheA3 and decreased phosphotransfer activity, supporting the group's structural predictions.

Bell *et al*. then engineered multiple non-cognate RRs (*R. sphaeroides* CheY1, CheY3, CheY4, CheY5, and *E. coli* CheY) that are not normally efficiently phosphorylated by CheA3 with the aim of allowing phosphotransfer. Although these proteins only share between 30% and 33% sequence identity with CheY3, introduction of amino acid substitutions at A12 and M13 was sufficient to allow (or greatly enhance) phosphotransfer from CheA3. This indicates that these residues play a major role in determining phosphotransfer specificity of RRs.

It is hoped that this work will facilitate the rational engineering of two-component circuits, with customisable kinetics, for applications in synthetic biology.

10 Years of the Human Genome Sequence:

EQUINOX GRAPHICS

What we now know, or know we don't know...

Penny Sarchet

2010 marks the 10th anniversary of the announcement of the completion of the draft human genome sequence. Eagerly anticipated and fiercely fought for, our full genome sequence turned out to be a surprise. Not the easily decipherable human blueprint that we had expected, the last decade of genomic research has struggled onwards in an effort to understand how something as complicated as humans can be put together using only as many pieces as there are in a London Transport bendy bus.

The rationale behind sequencing the human genome was simple. We are built from proteins, and these proteins are encoded in regions of DNA called genes. Malfunctioning of our proteins causes disease, and James D. Watson, co-discoverer of the structure of DNA and the first leader of the publicly funded Human Genome Project, argued that not sequencing the human genome as soon as possible would be "essentially immoral". Competition came from the company Celera Genomics, which sought to patent the human DNA sequence, and the victory of the HGP was hailed as a clear triumph of international cooperation and publicly funded science. The meaning of the genome sequence itself, however, was less clear. Only 1.5% was found to encode protein, and we have fewer genes than rice. Rather than unlocking the secrets of life, the following decade of genomics research was forced instead to confront the issues of "junk DNA" and what, if anything, the human genome sequence can actually tell us with our limited understanding of its function.

The first studies to use the human genome produced numbers rather than answers. A look at chromosome 21, the culprit in Down's Syndrome, found that it contains 225 genes, but did not reveal anything about how they cause the disease. It was the publication of the mouse genome in 2002 which enabled Down's Syndrome researchers to start making genomic progress. Studying chromosome 21 sequences in Down's Syndrome mice enabled them to identify regions of DNA, and even individual genes, which contribute to specific Down's Syndrome traits. However, the functions and interactions of most genes on chromosome 21 remain to be determined, and a genomic therapy for the condition is at least a decade away.

Comparisons between human and mouse sequences in Down's Syndrome research also produced a surprising result. The most evolutionarily unchanged chromosome 21 sequences were found to lie in non-coding regions of DNA termed "conserved non-genic" (CNG), rather than in functional genes. These CNGs were found to be remarkably similar in all mammals, and their constancy throughout 150 million years of evolution suggests they have an important function. Whilst the role of CNGs remains a mystery, findings like these indicate that the 98.5% of our genome termed "junk" is likely to have some function. We now know that, in addition to proteins, the genome also encodes molecules called microRNAs. These molecules control the expression of specific genes, determining the kinds of proteins made in a cell. We are also beginning to understand the importance of epigenetics. The structural arrangement of our DNA – something which cannot be detected by genome sequencing – has a direct impact upon a gene's activity.
The most significant progress in genomic research of the past decade has been the development of faster and

cheaper sequencing technologies, known as "next generation sequencing". In 2008, next generation techniques sequenced an entire genome – that of James D. Watson – in only two months, suggesting that personalised genome sequencing for medical purposes may not be too far away. Next generation sequencing methods have also revolutionised the field of transcriptomics, the study of gene expression through time and space. New sequencing technologies enable researchers to sequence and identify all the main genes being expressed in, for example, a developing heart or a failing liver, without requiring any prior knowledge of potentially important genes. The power of these new techniques was shown in 2009 with the sequencing of the genome of a malignant melanoma. The study identified 33,345 mutations in the cancer and identified new genes which may play a role in cancer formation. Sequencing was also used to examine the relationship between gene expression, mutation frequency, and DNA repair. The use of genomics in cancer research is likely to provide insights into the processes and patterns of mutation that occur in an individual years before a cancer starts to cause symptoms.

Next generation sequencing also gives us the tools to ask what makes us human, a question that could not be answered by the human genome sequence alone. To identify genes which were vital to the evolution of *Homo sapiens*, we need to look at other human-like species, such as our extinct cousin, *Homo neanderthalis*. At the start of the decade, the analysis of fossil DNA seemed impractical. Ancient DNA is damaged, low in concentration, and easily contaminated by the DNA of scientists and even, as it has transpired, the chicken sandwiches they ate for lunch. The parallel repetitions and computational statistics of next generation methods, however, are ideal for solving these problems.

The last two years have seen a flurry of Neanderthal genome sequencing, and comparison with our extinct cousin has the real potential to highlight key genes in our evolution. The first applications of Neanderthal genomics have been to address one continuing debate: did Neanderthals really die out, or did they interbreed and assimilate with us? DNA evidence so far shows no evidence of interbreeding. Whilst it is possible that there was a low, undetectable level of intermingling, there is no evidence of extensive gene exchange between the two species. With this question semi-answered, the next phase of Neanderthal genomics will be to use whole genome comparisons to detect genes which underwent evolutionary change in the estimated 600,000 years since the separation of the two species.

As the cost of genome sequencing falls, it is fast becoming affordable for laboratories worldwide. Medical research of the next decade should soon be able to start deciphering the genetic causes and changes involved in disease ontogenesis. The 1000 Genomes Project, launched in 2008, is currently using next generation technologies to sequence the genomes of 1000 individuals from a range of ethnic groups. Due for completion in 2011, this ambitious step-up from the human genome sequencing effort of the previous decade will determine the extent to which genome sequences can vary, and this variation should provide a useful tool in tracing the evolution of our genes. As more and more genomic data is collected, it will be the job of research institutions like the University of Oxford's Wellcome Trust Centre for Human Genetics to develop methods for identifying the links between genome sequence variation and the causes of disease. Worldwide, departments like Oxford's MRC Functional Genomics Unit are now researching the evolution of regulatory RNA-encoding and non-coding DNA sequences with the aim of developing new treatments for illnesses like Parkinson's, Alzheimer's and motor neuron disease.

When the human genome sequence was published in 2001, it raised more questions than it answered. The subsequent genomic research of the Noughties failed to unlock the secrets of human evolution, disease and what makes us so special, but it has revealed the gaps in our knowledge. Now that we know protein-coding genes cannot be the whole story, the next decade will look at the roles of non-coding regions and other molecules beyond the "one gene, one protein" dogma of genome function. Research in these new fields is young, and the next 10 years could bring about a whole new understanding of how DNA functions. Whilst uncovering the human genome sequence failed to be the answer to all things human, it has shown us how fascinatingly complex the role of our DNA really is.

Penny Sarchet is a second year D.Phil student in Dr Angela Hay's laboratory in the Department of Plant Sciences, University of Oxford.

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The deadline for article submissions is 5 July 2010 • We accept articles on any aspect of biological sciences research, books or science education • Articles can be either 700 or 1400 words
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Mass spectrometry and the proteome

Professor Frank Sobott

Major advances in our understanding of the molecular processes of life have been brought about by the advent of new experimental methods. These methods, which have often originated in the physical sciences, have revolutionised the way in which we ask questions of biological systems and the hypotheses which we can form based on the answers. Mass spectrometry is one of these methods – it can do a lot more than confirm masses and identify proteins on gels. Here we to take a look at the diversity of the proteome as well as recent methodological developments in this exciting field.

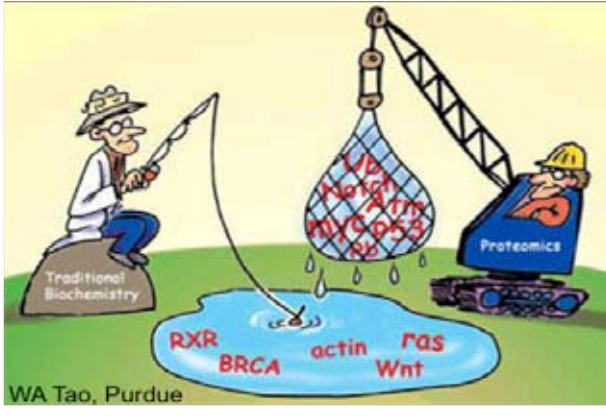
From genomes to systems

Genetic determinism, just like Newtonian mechanics almost a century earlier, is in urgent need of updating. Human life does not propagate from DNA alone but via reproduction of a cell, a complex microcosm. The 'Selfish Gene' (1), is not the master which controls the organism, but is instead part of a system and subject to modification and control (2). This paradigm shift has been embodied by the emerging field of epigenetics (3) and has monumental implications for our understanding of biological systems, as well as our ability to interfere with them for the purpose of curing disease.

As Penny Sarchet described in her article on p.8, we now know that the genome is not the sole determinant of an organism's phenotype. Rather than looking for a monocausal, one-directional flow of information, we should adopt a more holistic view of biological systems where mutual interactions between molecules and structures define the current state as well as the fate of a system. This is no mean feat, but has anyone ever claimed that adopting Systems Biology approaches would make scientists' lives easier?

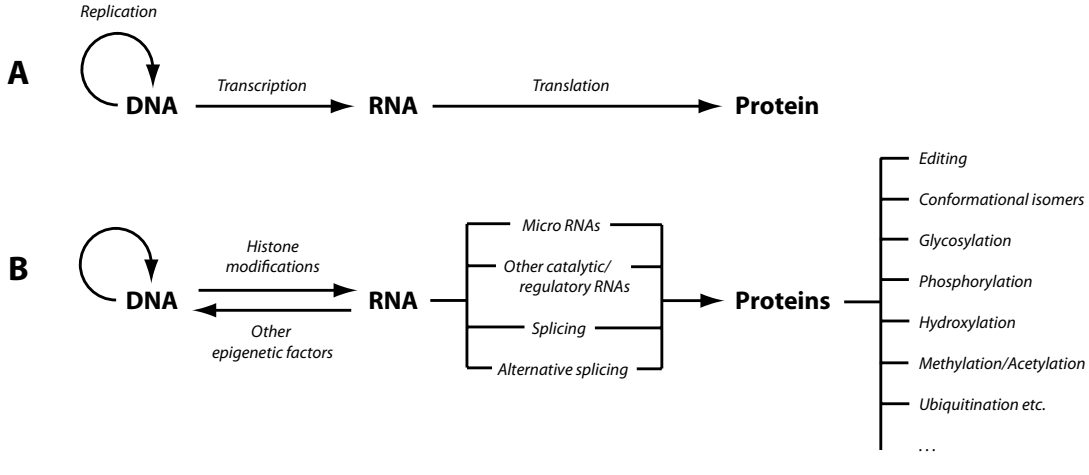
What makes humans more complex than nematode worms?

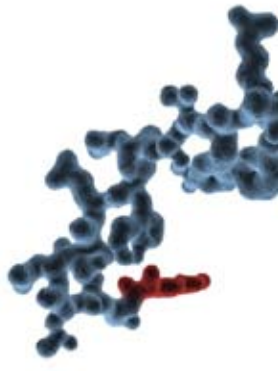
It is not just the genes, that is for sure – humans and nematodes have around the same number. The jury is still out on how many protein-encoding genes we call our own, but the figures will probably settle at around 20,000-25,000 – quite a manageable number as far as sequencing and bioinformatics efforts are concerned. That number is similar for one of the biologist's favourite pets – the nematode worm *C. elegans* (4). So what exactly does make us (arguably) more complex than the humble worm?



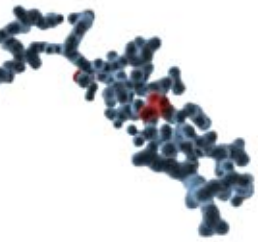
'omics methods, such as mass spectrometry, enable us to go on fishing expeditions 'in bulk'.

The Central Dogma of Molecular Biology (5) has changed a lot over the last 50 years. There is no one-way street for genetic information anymore, but rather a complex web of (epi-) genetic factors which control transcription and translation; there is also differential splicing. Most importantly, proteins are not just defined by their amino acid sequences, but equally by the presence of





Evolution of the Central Dogma over the last 50 years.



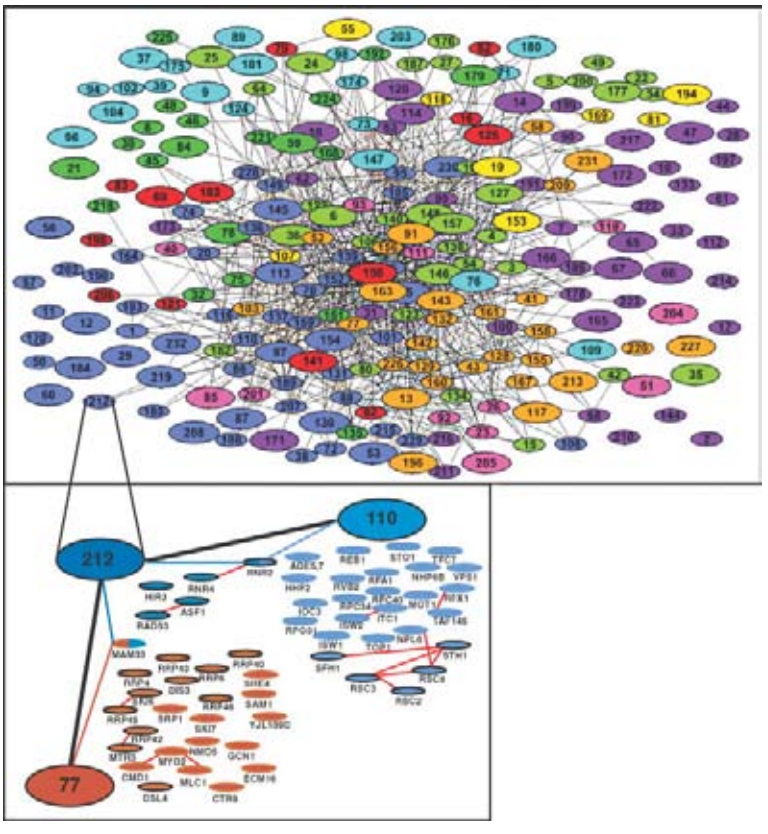
post-translational modifications (PTMs) and by their conformational state. The complexity is in the proteins rather than in the genes. Over 20,000 genes are believed to give rise to well over a million different, distinct human proteins. And they all have a specific place and role to play.

So what has mass spectrometry got to do with it?

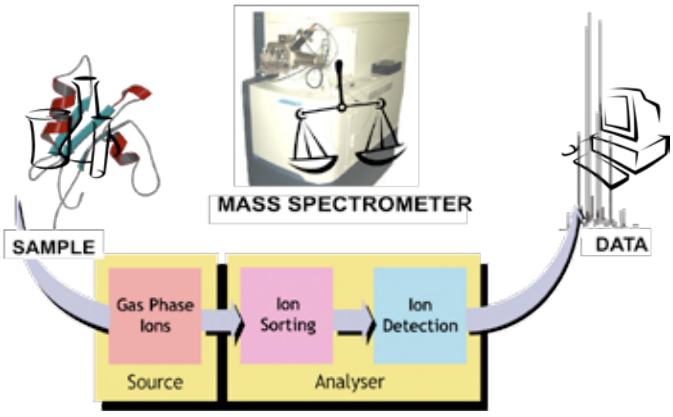
If we want to understand the entirety of proteins in a cell or organism (the proteome), we need a method to identify its constituent parts – preferably in a fast and automated way. Mass spectrometry in conjunction with 1 or 2D separation methods, gel-based or chromatographic, now provides the routine approach to this high-throughput problem (6). In addition, it has become the method of choice for PTM mapping of histones as well as kinase substrates. The new 'kid on the block' of mass-spec approaches is Ion Mobility, which under certain circumstances can be used to characterise conformational states of proteins (7) – an approach we are developing here at Oxford.

Social networking for proteins

This is not the whole story. We might have millions of different proteins but the real complexity of life comes at another level. A million eremites do not make a society! Rather, the various types of interactions (and the resulting activities) of the individuals, do that. For example, we are all part of a family group, have housemates or neighbours, study or work colleagues, friends, and are members of clubs. At some stage we encounter the same people again who we know from elsewhere, bringing the interaction-web full circle. A zoomed-in view of a protein interaction network is not fundamentally different from a Facebook interaction map, with some six degrees of separation between individuals.



Functional organisation of the yeast proteome by systematic analysis of protein complexes (Figure from reference 8). Each protein, identified by a number, interacts with other proteins, which interact with others, and so on.



A mass spectrometer 'converts' samples into spectral data, via gas phase ions which are sorted and detected according to their mass-to-charge ratios.

Proteins have relationships: some are exceedingly sociable (for example, Hsp90), others only 'hang-out' with their buddies (like subunits in protein nano-machines). Some interactions are stable, others rather fleeting. When we analyse protein-interaction networks, an important task is to find the 'socialites' of the protein world, the nodes or hubs which provide the platform for the assembly of many complexes. These key players are often very talented individuals, performing a range of diverse regulatory or structural functions.

Molecular sociology under the mass microscope

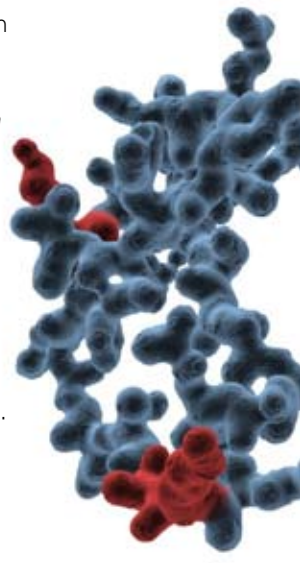
A lot of effort is now spent on detecting new interactions between old acquaintances. Demonstrating a crucial link between two prominent pathways is always worth a good *Nature* or *Science* paper. And mass spectrometry comes into play here as well. After capturing the initial complex in the cellular context, for example by immuno-precipitation of tandem-affinity purification (9), the isolated assembly is analysed with respect to its constituent parts after digestion with the protease trypsin. While this approach is pretty good at catching all the individuals who were present at the 'crime scene', the odd innocent bystander might get caught as well.

Crucially, we cannot get a good picture of the actual stoichiometry, shape and structure of complexes in this way. This brings us to more traditional biological methods, where we try to reconstitute and study them in isolation, and test their function. The gold standard for structures is X-ray crystallography, but we cannot realistically always expect this approach to succeed, so we need alternatives. Low-resolution methods have recently started to make significant contributions, based on fluorescence but also on a combination of electron microscopy and 'native' mass spectrometry (10). Indeed, it is possible to 'fly' non-covalent complexes intact in the gas phase in a mass spectrometer, and investigate their composition and stoichiometry (using

the mass) as well as their shape and size (using the charge distribution and ion mobility). This exciting and novel approach was originally developed at the former Oxford Centre for Molecular Sciences by Professor Carol V. Robinson's group, who have recently returned to Oxford. It also works for membrane protein complexes embedded in micelles.

My guess is that we have only really scratched the surface of this important aspect of the 'society' of molecules. More and more complexes are being characterised structurally – we now have a detailed of protein-RNA nano-machines as large as the ribosome and begin to understand how some protein assemblies travel along, cut, splice, and condense DNA strands. But we are only starting to look at factors controlling how these complexes assemble so specifically in a crowded, compartmentalised environment, and how we could intervene in these interactions using small molecule modulators to the benefit of our health and environment.

Frank Sobott has recently been appointed to a Professorship for Mass Spectrometry at the University of Antwerp, Belgium, where he is also director of the Centre for Proteome Analysis and Mass Spectrometry. He is currently a visiting scientist in the Department of Biochemistry.



References:

1. Richard Dawkins (1976) The Selfish Gene. *Oxford University Press.*

2. Denis Noble (2006) The Music of Life: Biology beyond Genes. *Oxford University Press. A riposte to Dawkins' original work, published 30 years later by his PhD examiner.*

3. Bird A (2007) Perceptions of Epigenetics. *Nature* 447:396-398.

4. *Polychaos dubium* may have the largest genome known for any organism, consisting of 670 billion base pairs of DNA, which is over 200 times larger than the human genome (see McGrath CL and Katz LA (2004). *Trends in Ecology and Evolution* 19 (1):32-38).

5. Crick F (1970) Central Dogma of Molecular Biology. *Nature* 227:561-563.

6. Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. *Nature* 422:198-207.

7. Clemmer DE, Hudgins RR, Jarrold MF (1995) Naked Protein Conformations: Cytochrome c in the Gas Phase. *JACS* 117 (40):10141–10142.

8. Gavin AC *et al.* (2002) Functional Organization of the Yeast Proteome by Systematic Analysis of Protein Complexes. *Nature* 415:141-147.

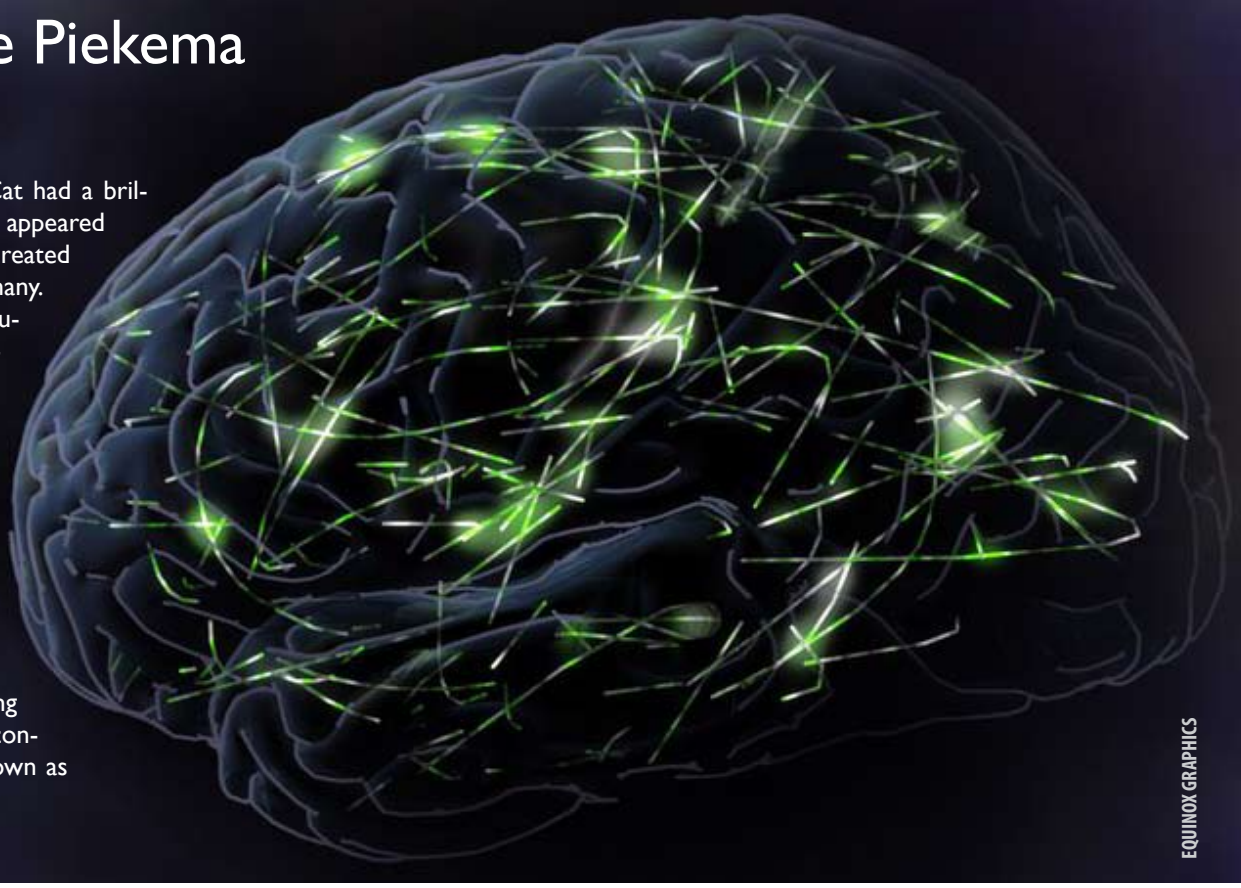
9. http://www.embl.de/ExternalInfo/seraphin/TAP.html

10. Robinson CV, Sali A, Baumeister W (2007) The Molecular Sociology of the Cell. *Nature* 450:973-982.

Illuminating the bright future of the brain

Dr Carinne Piekema

In the 1920s Felix the Cat had a brilliant idea and a light bulb appeared over his head; thus was created the signature of an epiphany. But recent advances in neuroscience leave you wondering whether in the future we will be more familiar with light bulbs actually driving our thoughts and inspiration rather than just being a visual metaphor. Gero Miesenböck, Waynflete Professor of Physiology at Oxford University, has been pioneering work that uses light to control brain cells, a field known as optogenetics.



EQUINOX GRAPHICS

Our brain consists of approximately 100 billion neurons that, as Miesenböck lyrically describes, form "an intricate tapestry". To understand how neuronal signalling drives our behaviour, he says, we need to tease apart the disparate contributions that each of the different populations of neurons make to our behaviour. Nobel Laureate Francis Crick remarked in a famous article in 1979 that one thing scientists have dreamed about is a tool that would allow them to selectively activate or turn off certain groups of cells while leaving others unaffected. Twenty years later, he suggested how this might be achieved: with light and molecular engineering. And this is precisely what optogenetics does.

To understand this technique we have to go back to the 1990s when German biologist Peter Hegemann discovered that green algae, commonly found in ponds, respond to light by wagging their tail. This behaviour was intriguing because algae are unicellular creatures without eyes. Hegemann discovered that when light photons hit the protein coils packed in the algae's cell membrane, a chemical reaction creates a tiny gap in the membrane, causing an ionic current to be produced and the algae's tail to wag. The protein that allows this reaction with light is called channelrhodopsin and is comparable to rhodopsins found in our own eyes.

Meanwhile, Miesenböck and his colleagues, working in New York and later at Yale, wondered whether they could exploit a similar mechanism to control brain cells. They took light sensitive proteins like the photoreceptors of our eyes, transplanted them into neurons and, by simply shining a light on them, the team was able to activate the modified neurons, a first step towards neuronal control.

To exploit the full power of this method, however, the researchers needed to discover a way just to excite or inhibit selected populations of cells, and with genetic engineering they were able to achieve this. By harnessing the cunning of viruses or by creating genetically-modified mice and flies, it was possible to make expression of the rhodopsin-encoding gene specific to particular neurons, meaning that only those neurons would become active when illuminated.

The road to success for optogenetics was not easy. The first difficult step was to find out whether they were able to transplant the rhodopsin-containing photoreceptors of flies to other cells in a culture and activate them with a flash of light. Once they succeeded in doing this, the second, even more complicated challenge was to move from changing neuronal activity in a cell culture to changing the behaviour of a living being, in Miesenböck's case the fruit fly. The promise became

initially clear when Susana Lima, Miesenböck's PhD student at the time, showed him the first baby steps taken by a fruit fly on command of light. Within 5 years, they had learned how to 'remote control' a fly.

The technique is now so advanced there is a large volume of work looking at how brain cells control behaviour. Last year in *Cell*, Miesenböck and his team exposed the learning mechanisms of a fly by creating false memories (1). They placed a fly in a narrow chamber, half of which smelled of an old tennis shoe, the other half of sweet fruit. By observing how much time the fly spent on either side, the researchers were able to work out which was the fly's preferred smell. When this location was later paired with a memorable, aversive signal – a painful electrical shock – the fly learned to avoid this location and spend more time on the opposite side of the chamber. From previous research, Miesenböck knew which neurons were involved in learning to associate the shock with an odour and could therefore directly target this system with optogenetics. By activating these cells with light when the fly was in the location of its preferred smell, Miesenböck's team was able to provoke identical avoidance behaviour even though no electric shock was given. Thus, the fly learned from an experience it never had.

Might we be able to use this technique to control our minds in the future? Miesenböck thinks that it will be a while before optogenetics can be used in humans: "You would have to express a foreign gene in a targeted fashion and this is where the show-stopper currently lies". While using this technique in humans may be a long way off, he does believe that optogenetic research in flies might nonetheless directly aid our understanding of the human brain because biology is generally conserved: "Nature rarely invents the wheel twice".

For now, Miesenböck thinks the field should focus on blurring the boundaries between work in whole organisms and fine-scale research in cell cultures. They could make use of the fact that tissue in a cell culture can be treated as if it was still part of a functioning brain by activating the cells with flashes of light – a use of optogenetics that is currently underappreciated. "There will be room for brain-free neurobiology, where optogenetics provides the interface to allow researchers to really talk to and feed artificial information into neuronal systems".

Miesenböck also advocates using light "to enable scientists to drive nervous systems outside their normal operating limits, because this is often where mechanisms reveal themselves". Miesenböck's team used this approach to investigate the origin of sex differences in flies. While male and female fly brains are very similar, they nonetheless display sex-specific courting behaviours. The gene that controls male courting behaviour is expressed in a very small number of neurons in the abdominal ganglia of the fly. By specifically targeting these cells with optogenetics and shining

Optogenetics: A new tool in neuroscience research.

light onto this circuitry, Miesenböck's team was able to produce male courting behaviour in all the flies, even the females (2). Thus, they were able to show that females possess a bisexual brain containing a motor programme necessary for male courtship behaviour, but do not activate it because the neuronal commands required for the behaviour are absent.

With the ability to dissect neuronal functioning in the healthy brain, optogenetics might also hold potential to help understand the exact mechanisms that cause neurological and psychiatric diseases such as depression and schizophrenia and even help treat them. For example, Dr Karl Deisseroth and his team at Stanford University in California published a study in *Science* last year that used optogenetics in rats to investigate directly how deep brain stimulation might alleviate symptoms of Parkinson's Disease, something that had previously been poorly understood (3).

Thus, despite the difficulties in applying the method to humans, Miesenböck is hopeful: "With optogenetics we can really identify the players that are responsible for particular behaviour and that may give us knowledge for targets of more conventional treatment. Then conventional treatment can become more effective and cleaner."

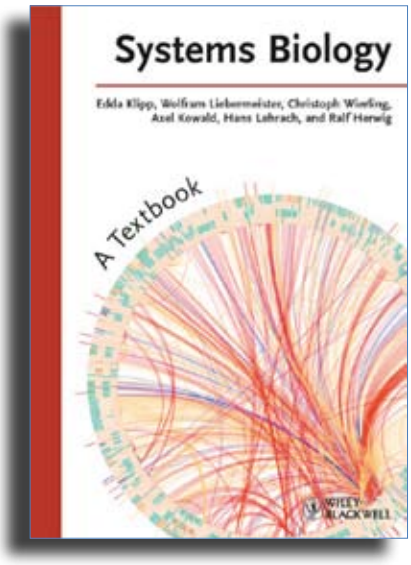
References:

1. Claridge-Chang A, Roorda RD, Vrontou E, Sjulson L, Li H, Hirsh J, Miesenböck G (2009) Writing memories with light-addressable reinforcement circuitry. *Cell* 139:405-415.

2. Dylan Clyne J, Miesenböck G (2008) Sex-Specific Control and Tuning of the Pattern Generator for Courtship Song in Drosophila. *Cell* 133:354-363.

3. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K (2009) Optical deconstruction of parkinsonian neural circuitry. *Science* 324:354-359.

Systems Biology: A Textbook. *By Edda Klipp, Wolfram Liebermeister, Christoph Wierling, Axel Kowald, Hans Lehrach, Ralf Herwig (Reviewed by Jennifer de Beyer)*
Published June 2009 by Wiley-Blackwell, 592 pages, £60.00



Systems Biology: A Textbook presents a summary of the integration of computational and experimental techniques catered to the investigation of cellular networks. This text is aimed at students of biology, biophysics and bioinformatics, but also serves as an introduction to the field of systems biology for senior scientists.

The main section of the textbook covers both theory and a how-to-model practical guide. The concepts behind the modelling of both biochemical systems and gene networks are dealt with in detail. Popular modelling techniques and methodologies in the analysis of the resulting models are discussed. These concepts are illustrated with several in-depth examples including glycolysis and cell cycle control.

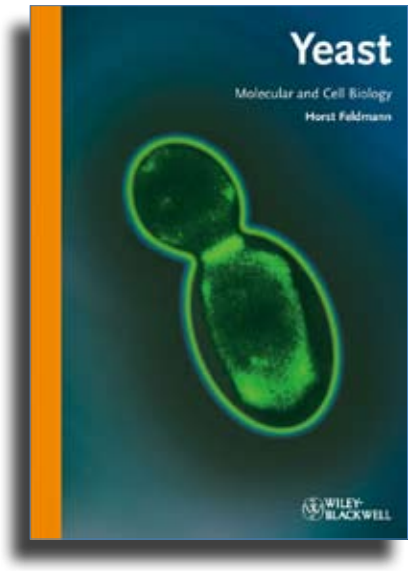
Modelling biochemical systems requires both knowledge of interactions between system components and the parameter values guiding these interactions, such as rate constants. Chapters explaining parameter selection and fitting and the high-throughput methods for data generation and analysis highlight this link between wet lab and computational work in systems biology.

The main text is followed by an additional eight reference chapters, supporting the diverse backgrounds of the intended readers. Chapters introducing mathematical and statistical techniques are sufficient for understanding the basic calculus and linear algebra in the main text. Cell biology and molecular biology techniques are also summarised. Finally, the available online databases of biochemical data and current popular modelling tools are presented. This information is invaluable for the new modeller, although it is likely to become out of date before the rest of the content due to the speed of software development.

Boxed summaries at the start of each subchapter and examples illustrated throughout the book highlight the key points and provide clarity. Most chapters conclude with a short problem set, summarising the basic concepts and prompting further thought.

This clear text is a useful starting point for anyone aspiring to solve a biological question using systems biology approaches.

Yeast – Molecular And Cell Biology. *By Horst Feldmann (Reviewed by Dr Mike Youdell)*
Published November 2009 by Wiley-Blackwell, 348 pages, £80.00

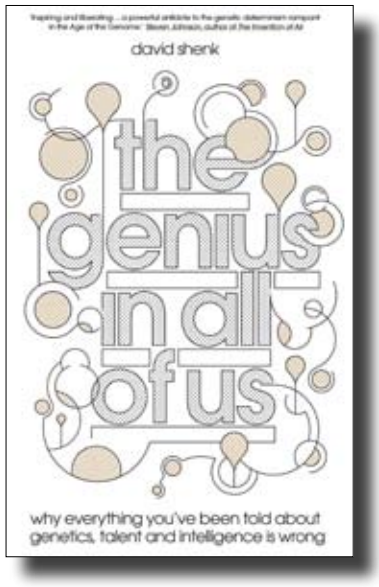


This book is a general overview of the biology of budding yeast, the model organism *Saccharomyces cerevisiae*. Any researcher looking to use *S. cerevisiae* as a model organism needs to be familiar with all the fundamental biological processes going on inside the cell to understand the context and implications of their work. This book provides the essentials in a simple and easy to understand way, suitable for use as a primer for researchers new to this highly studied microbe, especially if coming to yeast from another organism. The book covers a range of topics including the impact of the study of budding yeast on fields such as transcription regulation and metabolism, the basic workings of yeast cell biology and some insight on how to carry out your own investigations.

Early chapters are relatively basic (similar to those found in most detailed cell biology text books) and focus on the particulars of the *S. cerevisiae* system including chromosomal arrangement and features and methods of genetic manipulation. The book later discusses the majority of cellular and molecular mechanisms that dictate yeast biology in more detail, including transporter proteins, cell cycle regulation, cellular trafficking, metal ions, transcription, cell signalling and organelle function, which you would find in any classic *cerevisiae* text. The later chapters cover more modern aspects of yeast research, including regulatory networks and genome-wide studies, as well as the impact of yeast research in industry and on our understanding of disease.

Although it lacks much detail required for in-depth analysis of any of the topics covered, it is very well referenced, providing both classic seminal studies as well as more recent publications, making it a useful text for those starting out in the field. It is worth bearing in mind that any *pombe* fans should look elsewhere for an introductory text as *S. cerevisiae* is the sole focus of the author.

The Genius In All Of Us: A New Science Of Genes, Talent And Human Potential.
By David Shenk (Reviewed by Dr Amy Strange)
Published April 2010 by Icon Books, 307 pages, £14.99



Genius is often perceived as a mysterious quality, a gift bestowed by genetics to the lucky few. But David Shenk, author of *The genius in all of us*, believes the role of genes in the creation of genius has been aggrandised; instead, we should be looking to a more radical idea, termed "dynamic development".

In this empowering model, it is proposed that our genes interact with the environment to such an extent that alone they determine almost nothing. Thus, Shenk argues, genes are not limiting in the quest for genius and with the right environment everyone has the potential to achieve brilliant things.

Support for dynamic development is amassed using varied examples from biology, sociology and psychology. Through these vivid and eloquently described observations, Shenk identifies genius-founding characteristics and turns them into guiding principles for those hoping to inspire genius in themselves or others. The result is an unusual combination of science review and self-help manual, equally suited to the bookshelf of a philosopher, educator, or popular science reader.

The main criticism I have of the book is the presentation of the model, which at times feels feverish, and it becomes questionable whether a fully balanced view is presented. The argument for gene-environment interaction is continually substantiated but the plasticity of our genes, rendering them capable of responding to environment, is barely acknowledged as a genetic trait. Similarly, it seems unlikely that are we all equally capable of the extreme determination and dedication required to become exceptional; perhaps this capacity is also a genetic trait, but this point is also avoided.

The book does not assume a scientific background and those with one will undoubtedly disagree with some of the interpretations. Nonetheless, it is a quick and surprisingly compelling read and the take-home message is provocative; perhaps it is within everyone's grasp to reach the heights of genius, should they aspire to.

At the wrong end of the evidence stick?

Dr Blanka Sengerová

If I said a famous author was being sued by tobacco companies for warning about the health risks of smoking, you would laugh in my face, right? In 2008, author Simon Singh warned that chiropractors' claims of chiropractice being a cure-all are misleading, with research suggesting mixed results and in some cases even lethality. Responding, the British Chiropractic Association (BCA) sued for defamation.

At a preliminary hearing on meaning the judge ruled that the article "was fact" (not comment) and that it contained "allegation[s] of dishonesty and accuse[d] [the BCA] of thoroughly disreputable conduct". Singh would be defending the supposed allegation that the BCA were deliberately dishonest in promoting fake treatments. To me, this is unfortunate because although Singh suggested that chiropractors were "deluded and reckless", he did not appear to claim that they were "dishonest". It is also difficult to defend legally, as "showing that a treatment is not backed by evidence is one thing, but showing what was inside the mind of an association is another". Singh could go to trial to defend an almost indefensible case or settle out of court, still involving high costs and requiring an apology for the article whose message remained important. In October 2009, the Royal Courts of Justice allowed Singh to appeal the "meaning" ruling, hailed as the first step in the right direction of the process.

The Singh case exemplifies problems with English libel law. Firstly, it is easy for someone to sue and leave the burden of proof on the defendant. As lawyer David Allan Greene explains, "once the claimant has established they have a reputation in England, and that there is a defamatory statement, they have an automatic right to bring legal proceedings without having to show any damage has been suffered". Secondly, the costs of English libel cases are huge, meaning that those faced with libel claims are often forced to back off and apologise. This effect was observed by the charity Sense About Science (SAS), who discovered that the media avoid some issues for fear of litigation. When informing about dodgy product claims and celebrity pseudoscience, SAS met with lawyer-checking delays before the media reported them.

Other worrying cases include that of cardiologist Peter Wilmshurst, who was sued by NMT Medical for criticising their heart implant device at a US conference, and Henrik Thomsen, who was being sued by GE healthcare for suggesting that their product may be dangerous to patients with renal failure. On 18 January 2010, the case against Thomsen was withdrawn as he threatened to countersue GE for calling him a liar. SAS have joined forces with the charity English PEN and Index on Censorship to form the Coalition for Libel Reform. In December 2009, these organisations, with support from performers, writers, poets, patient groups, legal experts, broadcasters, journalists, and others, launched the National Campaign for Libel Reform, claiming that "England's libel laws are unjust, against the public interest and internationally criticised - there is urgent need for reform".

Since this article was first written, as you may have gathered from the national media, the BCA have dropped the case after Singh was given leave to appeal the meaning ruling. However, victory in this case does not mean that others are not being silenced by the restrictive libel laws. As Singh said after BCA dropped their case, "My victory does not mean that our libel laws are okay, because I won despite the libel laws - we still have the most notoriously anti-free speech libel laws in the free world".

Covering Singh's case, *Guardian* columnist Ben Goldacre said that "it is possible in healthcare to do great harm, while intending to do good, and so medicine thrives on criticism: this is how ideas improve, and therefore how lives are saved". If doctors, journalists and members of the public fear litigation when publicising the dangers of treatments, there must surely be a problem with the law and we must campaign for change?

References:

http://www.senseaboutscience.org.uk/index.php/site/project/340/

http://jackofkent.blogspot.com/

More details about the National Campaign for Libel Reform can be found on http://www.senseaboutscience.org.uk/index.php/site/project/333/ and you can join the campaign at http://www.libelreform.org/

When heading out of Oxford by bus, I have always noticed a colourful building advertising...

SCIENCEOXFORD

Using the internet, I discovered that this organisation does what we scientists often don't allow ourselves time for: engaging with the public, enthusing younger generations about science, and showing how much science is part of our everyday life.

In their quest to excite and inspire the younger generations, *Science Oxford* has recruited over 250 STEM (Science, Technology, Engineering and Maths) ambassadors. These men and women of all ages and backgrounds share their own enthusiasm for the science they encounter in the workplace in an effort to inspire young people and inform them about the many career opportunities science has to offer.

Since 2005, this organisation has opened the doors of the colourful building that used to catch my eye, *Science Oxford Live* on St Clements. Several times a month you can go here to visit the Exchange Zone, where you can listen and participate in discussions on topics ranging from ethical issues surrounding electronic patient records to how hedgehogs can save the world. In the Discovery Zone, you can experience science hands-on by playing with magnetism, frozen shadows and much more and, although this zone is specifically for children, it is highly enjoyable for everyone's inner child.

If that has not satisfied your appetite, you can also look around the permanent 'Science in Oxford' exhibition or just have a drink on sofas in the Science Lounge.

You can access *Science Oxford* online (http://www.scienceoxfordlive.com/) to learn about upcoming events, download podcasts of past events and find lots of information on any scientific topic you might be interested in. So, next time you're on the bus heading out of Oxford and that building catches your eye, log on and discover the world of science in Oxford.

Dr Carinne Piekema

Wow! How?

Have you ever made your own fossil? Or seen levitating magnets? How about extracting DNA?

Perhaps many of us have done the last experiment but all these activities were open to everyone, of all ages, at this year's *Wow! How?* science fair held at the University and Pitt Rivers Museums. On Saturday 13 March I joined over 90 volunteers drawn from the museum staff, university students, scientists and local science enthusiasts who ran 29 stalls showcasing hands-on science fun.

My table explored the variety of leaves and their functions in nature. Although it was sometimes difficult to compete with the liquid nitrogen demonstrations and live cockroaches which were also on offer, our table had a steady stream of visitors, probably lured by the ever-popular carnivorous plant display. Children were encouraged to spray water on huge water-proof banana leaves and touch the gel-like insides of a cut-open aloe vera leaf. On craft tables, kids made leaf identification keys and their very own Venus Flytrap. Their enthusiasm was infectious and before I knew it, we had spent six hours engrossed in science.

This year, the seventh year of *Wow! How?*, attracted 2700 attendees and I think a large measure of that success derives from its location in the museum. Most stalls were nestled in amongst the museum taxidermy display cabinets, making an intriguing juxtaposition between a scientific 'end-product' and active demonstrations. It was an interesting experience to show off cactus spines next to a stuffed albatross!

Many families come every year to *Wow! How?* and I know that I will be returning as a volunteer. Years of bench work during my PhD and post-doc had lulled me into thinking perhaps only scientists may be interested in science. Volunteering at the *Wow! How?* science fair reminded me that everyone can share in the excitement of scientific discovery.

Dr Sarah McKim

5' WITH...

Professor Nick Proudfoot

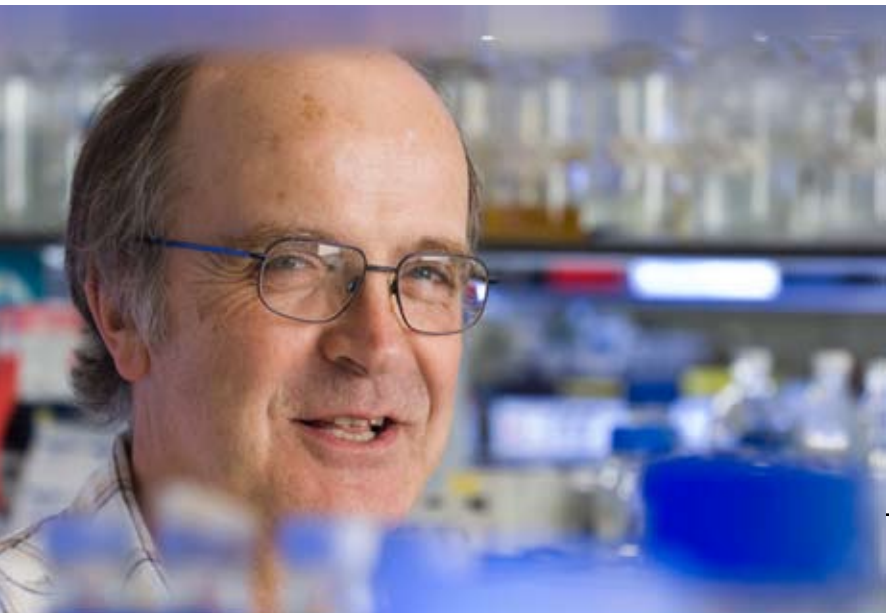
This issue's 5' with features Professor Nicholas Proudfoot FRS, Professor of Molecular Biology in the Sir William Dunn School of Pathology.

As he pointed out, '3' with' would have been a more appropriate name for this interview, as his research interests lie at the 3' end of genes. His group works on the mechanisms and function of transcriptional termination by RNA Polymerase II, its interplay with mRNA processing, and the role of polyadenylation in the regulation of gene expression. Professor Proudfoot is a fellow of Lincoln College.

When did you realise that you wanted to be a scientist?
I drifted into science. I enjoyed being a student so after my undergraduate degree at London I decided to do a graduate degree. I was lucky and by chance secured a place in 1973 as a PhD student at the Laboratory of Molecular Biology (LMB) in Cambridge and graduate member of King's College (I lived in college all three years as a student there). Being in arguably the best place in the world to do molecular biology at that time, I got the research bug. Even though I couldn't aspire to the levels of some of the science going on, I certainly enjoyed the buzz of both my own science and that which was going on around me. LMB Cambridge then boasted an amazing group of senior scientists all with Nobel Prizes then or subsequently: Fred Sanger, Cezar Milstein, Sydney Brenner, Francis Crick, Max Perutz, Aaron Klug and John Walker. Some of the more junior scientists were also phenomenal e.g. Liz Blackburn, Tom Maniatis, Gerry Rubin, and Joan Steitz.

If you were not a scientist, you would be...
I was a reasonable musician (French horn and bass voice). At 18, I toyed with the idea of trying to get into music school but figured that making a career in science would be easier than in music. After all, you can keep up music as a hobby, but not science.

If you are not in the lab you are...
Sadly, nowadays I am not at the lab bench. I spend as much of my time as possible discussing, interpreting and planning scientific research with my group as well as writing papers, grants and endless peer reviews. I also spend a lot of time travelling to meetings and conferences.



Worst disaster in the lab?
Hard to say! Possibly the worst accident was years ago when a post-doc in my lab managed to spill a lot of phenol onto his lap while working at the bench even though he was wearing a lab coat. Mercifully only his upper thighs were burnt but he had to have extensive skin grafting. He was always keen (though unsuccessful) to show off his scars especially to female lab members.

What has been the most important moment of your career so far?
Getting an FRS a few years back was a great honour.

Any memorable findings?
I was lucky and made my biggest scientific break at LMB Cambridge as a graduate student. There I discovered the mammalian polyA signal AAUAAA. I have been working on this on-and-off ever since!

Favourite conference location?
Cold Spring Harbor is hard to beat: always top science, the best auditorium in the world for 350 people and a relaxing environment. If not, then Keystone Colorado in the snow with afternoon skiing is pretty nice.

Best advice you ever received?
Chase after the unexpected. When you can't explain a result, yet you are sure you did the experiment right, keep an open mind and guide your thoughts away from what you think you understand towards new directions.

Any regrets?
I am sorry I gave up being a tutor at Oxford. Though tutorial teaching at Oxford has its frustrations, it is still the most rewarding way I know to interact with the next generation of scientists. Not being a tutor breaks your connections with college life.

Favourite classical experiment?
The discovery that mammalian genes are discontinuous and that gene exons can be shuffled by splicing to make lots of different proteins.

In your opinion, what makes a good scientist?
Three critical ingredients: hard work, hard thought and especially, good luck.

How do you imagine biological research will change in the next twenty years?
It has already changed way beyond my expectations as a starting graduate student in the mid '70s. I feel that nowadays there is far too much massive data set collecting. I hope that in the future there will be a return to doing more focused experiments that unravel detailed mechanisms that can then be extrapolated to the general, rather than starting out with a bewildering and often unintelligible 'omic analysis.

Biotechnology and Innovation

Dr Peter Dean

Dr Peter Dean, the founder of Cambio and several other companies, gives an entertaining account of the ups and downs of his career in biotechnology.

As a scientist in modern Britain, one gripe is that work done by British scientists gets neither proper reward nor appreciation; my experiences may help to explain some of the shortcomings of the system. One problem of the 1950s and '60s was that commercialisation (and therefore innovation) was a dirty word. Starting a company was not an obvious science route.

As a young researcher, I was working to confirm a report that a steroid sex hormone was present in pomegranates. We found sex hormones - although they were non-steroid, they could account for the idea of pomegranate as a contraceptive (don't try this at home!). We also kept warm that winter with homemade pomegranate wine - which is rather good! My students got thoroughly involved in steroids after this and after getting an assay for testosterone going, our graduate student started to measure everyone in the lab; numbers for male students were 600 to 1200 units; tired professors with two kids were 350 and yours truly only 250. When asked why I made so little, apparently I was heard to say, I don't make much but I use it very economically! We beavered away for ten years measuring, amongst other things, cortisol in humans and horses. This turned out to be important in measuring stress not only in the mentally ill, but also in internationally-travelling racehorses.

After ten years of making antibodies to steroid hormones (enabling us to measure these hormones in blood), I was visiting the laboratories of Ayerst, Harrison and McKenna in Montreal. Romano Deghenghi and I went for a farewell dinner on the last day just as a big snowstorm started. He casually asked me if I knew any good way to measure glycated haemoglobin (HbA1c), adding, "If you want to get us to fund your lab forever, invent us a test, make a name for yourself but whatever you do, get back to me". The plane skidded down the runway (the last to take off before the big freeze-up) and I started doodling.

"One problem of the 1950s and '60s was that commercialisation (and therefore innovation) was a dirty word."

I knew that boron was used in buffers to assist sugar separation; it was not too hard to immobilise boron and design a column-based test. Most red blood cells have a

A biotechnological journey from sex hormones in pomegranates to equine cortisol and glucose monitoring for diabetics.

half-life of about 120 days. Every time we eat sugar, the aldehyde form of glucose reacts with proteins - including haemoglobin (Hb). Since this cannot escape from the RBC, it rather usefully records all the insults of high sugar snacking. Take diabetic blood and measure HbA1c: a badly controlled diabetic will have up to 20% HbA1c whereas a normal person has 5% or less. The old method was to electrophorese the proteins and look for the 'fast' Hb. My student Saroj had just been diagnosed as diabetic, so we tested her with the new method and she proved negative. About to chuck our method, someone suggested, "Maybe she's not diabetic?", and he was right (having beta-thalassaemia, she had fast Hb). Like DNA fingerprinting, the first case proved someone innocent, which made us feel that we might be on to a good thing. The test is now a world standard and it's British!

An early PCR machine

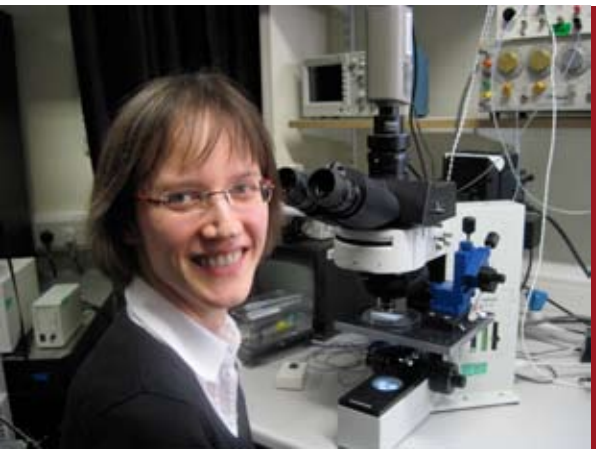
Our test evolved because someone asked the right question. On 'Tomorrow's World', Pete McCann publicised a visibly pink test tube to show a patient was diabetic. The Canadians applied for a patent on our behalf then promptly decided they were no longer interested, but they did give us the rights. My dear old University declined the offer to support the application and I sold the licence to Amicon in Massachusetts for a princely 50p (plus royalties); Letters patent were granted and they started to make kits. Then the big boys got involved; W. R. Grace bought out Amicon and someone started to look at their intellectual property portfolio. The upshot was a call from New York and me on the first Concorde flight to NY (they offered to pay!). The lawyer was more interested in lunch until he discovered I had sent letters accusing competitors of trying to break the patent, and had copies to prove it. This led to the companies in question all paying indecent amounts of damages, but I was more incensed that our work had been copied by unscrupulous competitors claiming to be first even though the patent came out three years before the published work. Adding insult to injury, the invention is still never claimed as British, which it most certainly was. In those days universities were not interested in defending the intellectual property rights of their staff. It is happily somewhat different today.

After a number of years I was invited to move to Cambridge to help set up a new company devoted to innovation in biotechnology. 55 projects and 1500 scientists left me unable to do any science myself, so I moved to start a company (Cambio) with Mike Gronow and Bill McRae. We started by trading in restriction enzymes, and as luck would have it one of our scientific advisors was a certain Martin Evans. His opening remark to me was, "Are you interested in making a PCR machine?" Clearly someone up there loves me. We started another new company (Genesys Instruments) almost immediately. Martin's design was based on the exact opposite of available models. Some of these were based on large water tanks: cycling was achieved by pumping water through the tube-holding block. Other types used (occasionally explosive) Peltier chips. Our machine used a cooking lamp (quartz halogen), a thin black copper plate with tube holder and a computer fan for cooling (by forcing air under the copper plate). It was furnished with real-time graphics, a BBC computer and a flying probe to measure the in-tube temperature. We called it the Intelligent Heating Block; happily the original now rests in the London Science Museum.

Apart from experiencing patent abuse, the company did very well (I think we sold about 2000 machines). However, it soon became clear that a better Taq polymerase was needed, and it was Smithies and Evans who made sure Cambio knew what was best. Their reward came when the Nobel Prizes were announced a few years later. Everyone else profited too - in an early sales offer, one received a bottle of champagne with every 1000 units (not unpopular!) Cambio has grown steadily since then, but truthfully, it was the quality and specificity of advice from our local scientists that made it a success story. This just goes to show what can happen when you read the right subject. The most important thing in my view was that it should be fun, and if it makes a few pennies, that's extra.

Peter Dean read chemistry at Jesus College, Oxford (1959) and went on to his D.Phil under Michael Whitehouse in the Department of Biochemistry. From there he went to Harvard and under Nobel Laureate Konrad Bloch, worked on squalene epoxide cyclases. After Harvard, he settled in Liverpool where he worked on affinity chromatography. He founded P&S Biochemicals and Medesign. In Cambridge, he started Cambio and Genesys Instruments with the now Nobel Laureate Martin Evans. The next 20 years saw Dean and his friends transfer technology from universities to industry. He was awarded a DSc in 1984 for his work in Biochemistry.
In his spare time he raises money for charity. In 2003, he moved to Stow in an attempt to retire with his friend Judy. Golf has been replaced by painting. He married Judy in 2006. He has failed to retire from Cambio!

SNAPSHOT



We are delighted to announce that Dr Leah Herrgen, a post-doctoral fellow from Dr Colin Akerman's lab in the Department of Pharmacology, is the winner of the Trinity Term *Snapshot* competition.

Her amazing cover image was taken with a confocal laser scanning microscope and shows cellular proliferation in the developing frog brain. In recognition of her contribution, she was awarded £50 of books from our sponsor, Oxford University Press.

We hope she will enjoy her reading!

OXFORD UNIVERSITY PRESS

The animal nervous tissue develops by a process called neurogenesis wherein bursts of proliferation generate billions of cells. These cells must be coordinated into specialised domains which are coordinated into the complex working circuitry of the adult brain. Dr Colin Akerman's lab is pioneering the use of a frog species, *Xenopus laevis*, to discover how this remarkable organisation is achieved. Traditionally, neuroscientists have analysed short-lived mammalian brain slices. Since *Xenopus* has an external, transparent embryo, developing neurons can be easily observed while leaving the embryo intact, making *Xenopus* an ideal system to study neurogenesis in a whole organism.

The striking cover image contributed by Dr Herrgen, a post-doctoral fellow in Dr Akerman's lab, shows cell division patterns in the brain of a developing *Xenopus* embryo. Six days before this photo was taken, Dr Herrgen injected the embryo with a nucleotide analog, 5-bromo-2-deoxyuridine labelled with a green fluorescent dye, which was taken up by actively dividing cells; thus, the green dots show a snapshot of cellular proliferation at a single moment in time. Propidium iodide was used to stain all neural cell bodies red for contrast. This type of experiment can be done at various time points during neurogenesis to monitor the spatial distribution of cellular proliferation in the developing brain as a function of time.

Other hallmarks of neurogenesis are oscillating waves of calcium ions that course through the developing nervous system. As Dr Herrgen explains, many studies have examined the effects of oscillatory calcium within a single cell. But given that these waves run through the entire developing nervous system, they may function to convey important spatial and temporal information between cells. Could rhythmic calcium waves be involved in neuronal coordination? Dr Herrgen and the Akerman lab are poised to investigate this exciting question.

Dr Herrgen completed her PhD in Dresden, Germany, where she worked on zebrafish, and subsequently arrived in Dr Ackerman's lab in October 2009 on a Blaschko visiting fellowship. When asked what attracted her to study neurogenesis in *Xenopus*, Dr Herrgen was refreshingly candid - "the sheer beauty of it," she said.

Snapshot Trinity 2010: how to enter...

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 in Phenotype Michaelmas 2010. To enter, send pictures 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 Friday 18 June 2010.

CROSSWORD

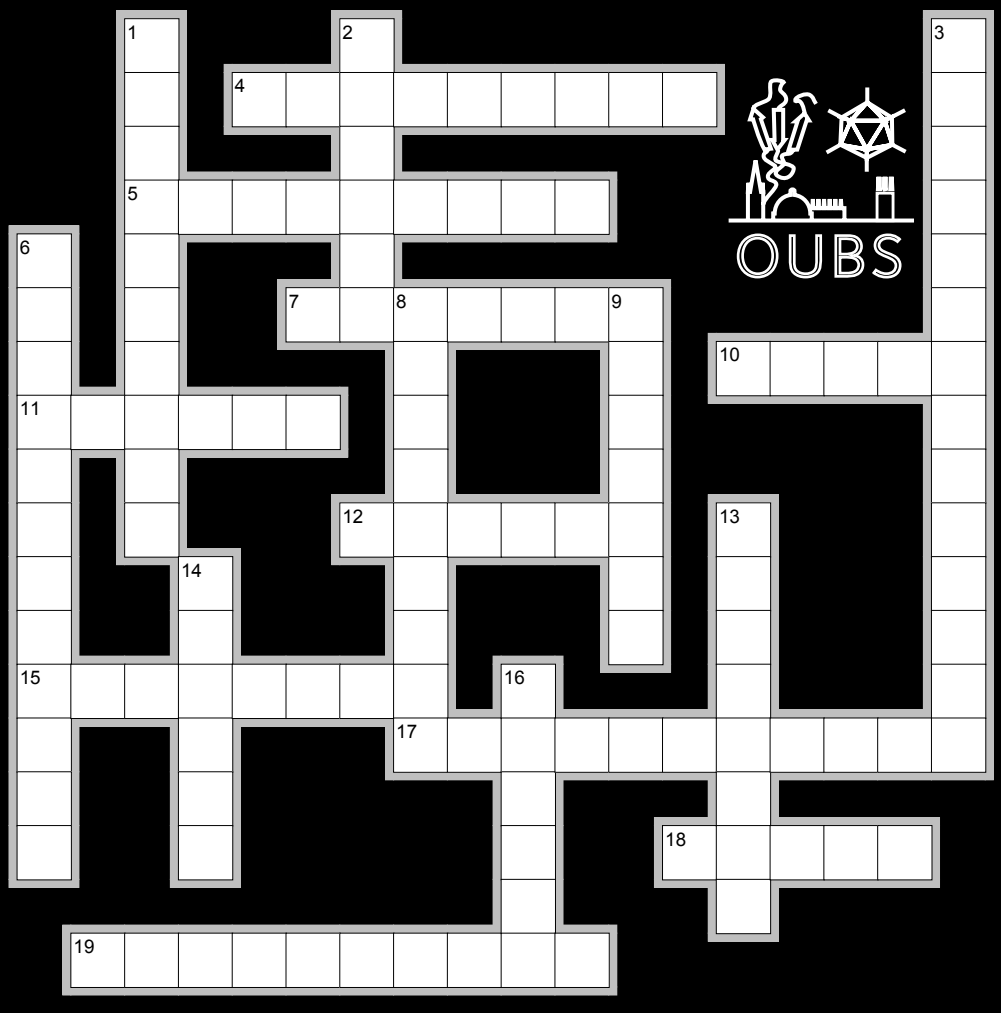
Test your knowledge and win a copy of one of the two textbooks reviewed in this issue (winner's choice).

From Watson and Crick to Celera Genomics, this term's Phenotype crossword is all about historic milestones in biochemistry and molecular biology.

You can either e-mail the 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.

All correct entries received by Friday 18 June 2010 will be included in the prize draw.

Congratulations to Alexander Deng from St John's College, the winner of the Hilary '10 crossword competition.



Across

4. Current Chief Executive of the Biochemical Society.
5. Type of phosphorylation shown to be coupled to electron transport in the electron transport chain by Albert Lehninger.
7. Surname of the chemist who first developed the pH meter.
10. Name of the first cloned mammal.
11. Michaelis-___ is the name of the equation, formulated in 1913, which describes the dependence of the rate of an enzyme-catalysed reaction involving a single substrate on substrate and enzyme concentration.
12. Protein that acts as a biological catalyst.
15. Decade in the 20th century when biochemists first started using radioisotopes.
17. Genus of the first bacterial genome to be completely sequenced.
18. Surname of the scientist who first enunciated the central dogma: "DNA is transcribed into RNA, which is translated into polypeptides."
19. The organism used by George Beadle and Edward Tatum to show that one gene produces one enzyme, which earned them a Nobel Prize in 1958.

Down

1. Type of antibody for which production was developed in 1975 by Cesar Milsein, Georges Kohler, and Niels Kai Jerne.
2. The first enzyme to be crystallised.
3. Metabolic process that includes the Calvin-Benson cycle, first formulated in 1954-1956 and involves fixation of carbon dioxide.
6. The first biological event shown in 1897 to be a chemical process.
8. Laboratory in Cambridge where Watson and Crick worked.
9. Surname of the scientist who first proposed the term "biochemistry".
13. Surname of the scientist who invented DNA blots.
14. Nationality of the inventor of the Petri dish.
16. Founder of Celera Genomics.