PHENOTYPE

Issue 4, Michaelmas 2009

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Reprogramming the nucleus

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CELEBRATING DARWIN
Exploring the life behind the genius

OXFORD UNIVERSITY BIOCHEMICAL SOCIETY

Editorial

Welcome to *Phenotype* Michaelmas 2009! The Oxford University Biochemical Society magazine has a new production team and a new look.

We have some new features, as well as the more familiar ones. *Snapshot* is our research image competition, which we hope will become a regular feature in *Phenotype*. We received some stunning entries from several departments, including Biochemistry, the Dunn School of Pathology and Plant Sciences.

Research Highlights explores recent published biochemistry research in Oxford, from structural studies of membrane transporters to RNA splicing in nematode worms. In the first of our *Features* articles, Dr Lynne Cox gives us a fascinating insight into current ageing research. As part of recent celebrations of the 200th anniversary of Charles Darwin's birth, James Halstead visits an exhibition at the University Museum of Natural History to explore the personality behind the genius.

Four former students give us accounts of their careers after finishing their D.Phil. While one stayed in research, the other three took very different paths, including law and cheesemaking! There's also a double dose of 5' with Professor Mark Sansom and Dr Petros Ligoxygakis from the Biochemistry Department. They tell us about their scientific journeys and impart some invaluable advice.

I hope you enjoy reading the magazine. Visit our website (http://www4.bioch.ox.ac.uk/oubs/phenotype.php) for information about the Hilary 2010 issue!

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We gratefully acknowledge the support of our sponsors:

OUBS Seminars

Michaelmas '09

Monday 26 October

Dr Andrew Carter, University of California, San Francisco

Cytoplasmic dynein: from single molecules to structure

Sponsored by Image Solutions (http://www.imsol.co.uk)

Friday 6 November (4 pm)

Professor Sir John Gurdon DPhil DSc FRS, The Gurdon Institute, University of Cambridge

How do eggs and oocytes reprogram somatic cell nuclei?

Lecture Theatre, Medical Sciences Teaching Centre

The inaugural Sir Richard Gardner Celebratory Lecture

A joint event between OUBS and The Oxford Stem Cell Institute. Followed by a wine reception!

http://www.stemcells.ox.ac.uk/

Monday 16 November

Dr Etienne Schwob, Institut de Génétique Moléculaire de Montpellier, France

Title to be confirmed

The Society for General Microbiology Seminar (http://www.sgm.ac.uk/)

Monday 30 November

Dr Doug Higgs, Weatherall Institute of Molecular Medicine, University of Oxford

Title to be confirmed

Monday 7 December

Dr Tim Humphrey, Gray Institute for Radiation Oncology and Biology, University of Oxford

Break-induced loss of heterozygosity in fission yeast

Monday 14 December

Professor Greg Cook, Department of Microbiology and Immunology, University of Otago, New Zealand

Title to be confirmed

All seminars are held in the New Biochemistry main seminar room from 4 to 5 pm unless otherwise indicated.

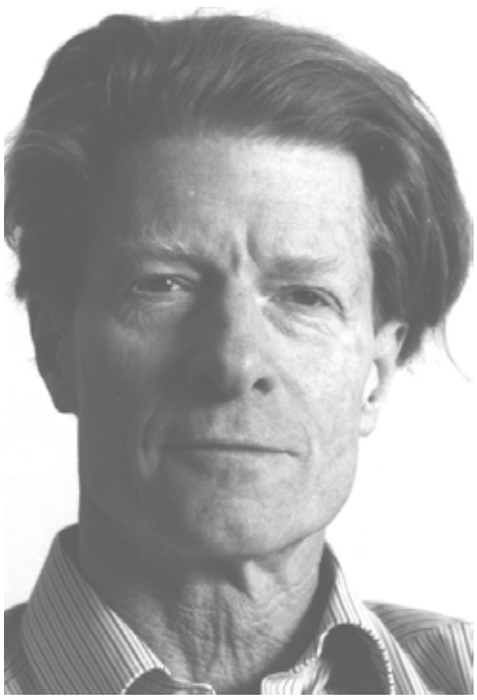
REPROGRAMMING THE NUCLEUS

To mark the inaugural 'Sir Richard Gardner Celebratory Lecture' on 6 November 2009, **William Upcher** revisits the work of Sir John Gurdon, our first speaker.

It's now more than 10 years since Dolly the sheep was born. The first mammal ever to be cloned, Dolly became a symbol of perhaps the most controversial feat of modern biology. The technology behind reproductive cloning was brought clearly into the public eye and sparked fierce debate over the ethics and legality of its potential use.

If claims of successful human cloning by religious cults are to be ignored, the highest mammal cloned to date is the rhesus monkey. The probability of humans being cloned in the foreseeable future remains slight.

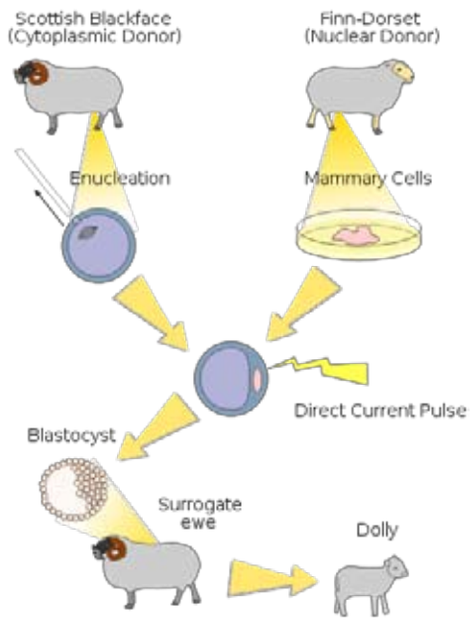
However, the technique underlying reproductive cloning, somatic cell nuclear transfer (SCNT – see diagram), continues to be both contentious and of great scientific importance for stem cell and embryonic development research. It also has potential as an approach to therapeutic cloning - a method for replacing damaged tissues and organs with newly grown cells.



Professor Sir John Gurdon

For most, technology such as this still seems like the realm of science fiction, belonging only to a futuristic world. Indeed, much research is still required to make it a reality, even though it has its inception over half a century ago with the work of a young graduate student at the University of Oxford. At the time, it was the fundamental questions of cellular differentiation that spurred the work of John Gurdon. Previous research had argued for a view of differentiation in which cells discard genes as they specialise, and thereby permanently lose the ability to revert to a totipotent state. In the alternative scenario, cells merely switch genes on and off as needed, and thus can be theoretically directed towards any specialised fate, such as that of a neuron or myocyte. To test this, Gurdon destroyed the nucleus of a *Xenopus* egg with UV light and injected the nucleus from a differentiated adult cell. To the surprise of those in the field, the resulting cell could give rise to every cell type and produce an adult frog genetically identical to the frog from which the donor nucleus was taken. Gurdon's seminal experiments opened up entire fields of research and are still recognised for their importance.

Last month Gurdon was honoured as a co-recipient of the Albert Lasker Basic Medical Research Award. He shared the prize with Shinya Yamanaka, who, building on Gurdon's work, identified the critical factors required for reprogramming differentiated cells. In so doing, Yamanaka was able to convert adult cells to 'induced pluripotent stem cells' simply by adding these factors. Such



Somatic Cell Nuclear Transfer was used to create Dolly

a discovery bypassed the major ethical qualms of obtaining eggs and creating embryos, providing the best hope so far for stem cell-based therapies.

This November, we are privileged to co-host a talk by Sir John Gurdon himself, marking the first in a series of 'Sir Richard Gardner Celebratory Lectures' held in conjunction with the Oxford Stem Cell Institute (OSCI). This series serves as a tribute to the great support Sir Richard has given the institute as chair of the Royal Society Working Group on Stem Cells and Therapeutic Cloning, in addition to his own pioneering work in the field of stem cell research.

Established last year, the OSCI now stands as a collection of almost 30 research groups working on the many facets and types of stem cells, including haematopoietic, cancer, adult, and embryonic stem cells. Collaborative networks like these will allow the greatest chance of realising the full potential of stem cells. •

Jmjd6 catalyses lysyl-hydroxylation of U2AF65, a protein associated with RNA splicing

Celia J. Webby, Alexander Wolf, Natalia Gromak, Mathias Dreger, Holger Kramer, Benedikt Kessler, Michael L. Nielson, Corinna Schmitz, Danica S. Butler, John R. Yates III, Claire M. Delahunty, Philip Hahn, Andreas Lengeling, Matthias Mann, Nicholas J. Proudfoot, Christopher J. Schofield, and Angelika Böttger. Science 325, 90-93 (2009).

The hypoxic-inducible factor (HIF) system in metazoan cells is activated in response to oxygen limitation. Activity of the HIFα subunits is regulated by oxygen-dependent, post-translational hydroxylation. This study investigated whether the Jumonji domain-6 protein (Jmjd6), a putative homologue of the HIF asparaginyl hydroxylase, is involved in regulating gene expression via post-translational hydroxylation.

Using pull-down assays, Webby *et al.* showed that Jmjd6 interacts with the splicing factor U2 small nuclear ribonucleoprotein auxiliary factor 65-kDa subunit (U2AF65) and cisplatin resistance-associated overexpressed protein (CROP).

Potential Jmjd6-interacting proteins have been previously predicted. A number of these proteins are connected to RNA metabolism, processing and splicing, and approximately 25%, including U2AF6, CROP and LUC7-like2 (LUC7L2), possess Arg-Ser (RS) domains. Thus, the authors investigated whether RS domains are substrates for Jmjd6. Despite the fact that Jmjd6 was recently reported to act as a histone arginine demethylase, Webby *et al.* did not observe Jmjd6-mediated arginine-demethylation of U2AF6, CROP or LUC7L2 RS domains, or histone H4 and H3 fragments. However, Jmjd6-mediated lysine hydroxylation within the U2AF6 and LUC7L2 RS domains was detected. The CROP RS domain peptide did not contain any lysine residues and hence, was not hydroxylated.

LC-MS/MS analysis showed that U2AF65 from HeLa cells was hydroxylated at Lys-15 and Lys-276 at ratios of approximately 1:100 and 1:250 hydroxylated:unhydroxylated, respectively. Levels of U2AF65 hydroxylation were increased following overexpression of Jmjd6.

As U2AF65 has been shown to influence alternative splicing of mRNA, the authors investigated whether Jmjd6 influenced mRNA splicing. Knock-down of Jmjd6 in HeLa cells changed the alternative splicing pattern of the tumour antigen gene MGEA6 and that of a α-tropomyosin minigene construct. However, alternative splicing of the hnRNPA2B1 and TEAD1 genes was unaffected. The data indicated that, in contrast to U2AF65, which acts as a ubiquitous splicing factor, Jmjd6 exhibits selective effects on splicing.

This study suggested that lysyl hydroxylation is the dominant oxidative catalytic activity of Jmjd6. The authors propose that post-translational lysyl hydroxylation may represent a mechanism of regulating alternative splicing.

Regulation of transcription termination in the nematode Caenorhabdtis elegans

Simon Haenni, Helen E. Sharpe, Maria Gravato Nobre, Kerstin Zechner, Cathy Browne, Jonathan Hodgkin, and André Furger. Nucleic Acids Res. 2009 Sep 9 (Epub ahead of print).

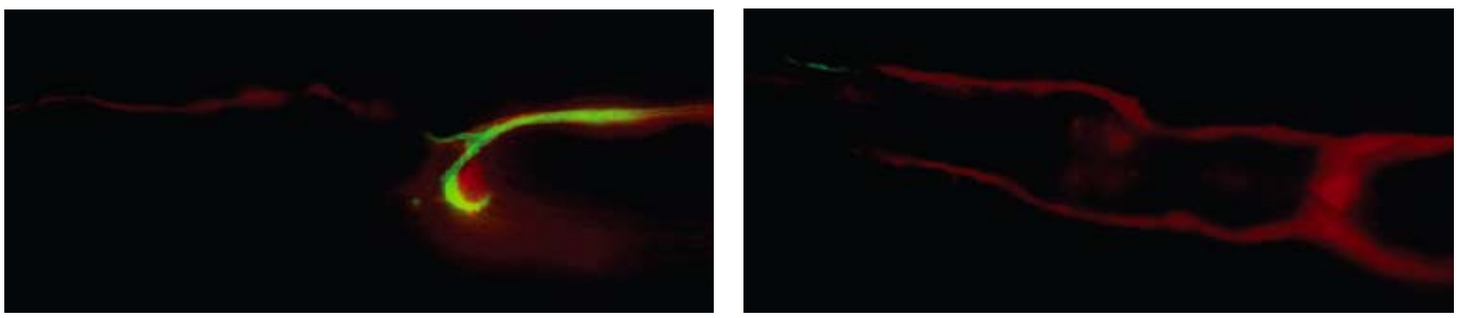
Like some other eukaryotes, *C. elegans* can produce polycistronic pre-mRNAs, similar to bacterial operons. To generate mature mRNAs, polycistronic transcripts must be cleaved and polyadenylated at the 3' end of each gene, and a short leader sequence must be *trans*-spliced at the 5' end of downstream positioned genes.

Using ChIP to identify RNA pol II-bound DNA, Haenni *et al.* showed that pol II terminates ≈1 kB downstream of the 3' poly(A) site of both mono- and polycistronic transcription units. Thus, recognition of the internal poly(A) sites in polycistronic pre-mRNA did not trigger transcription termination.

Analysis of primary transcripts from five different C. elegans operons by RT-PCR indicated that *cis*-splicing of introns in downstream positioned genes occurred before poly(A) cleavage and *trans*-splicing.

To investigate the effect of cis-splicing on transcription termination, Haenni *et al.* used a plasmid designed to express a polycistronic transcript encoding Red Fluorescent Protein (RFP) followed by Green Fluorescent Protein (GFP). They showed that removing the intron within the GFP gene, thus preventing *cis*-splicing of the downstream gene, resulted in decreased GFP expression in *C. elegans* compared with the wild-type plasmid. This indicated that *cis*-splicing of the first intron in downstream genes was important for gene expression.

U1 snRNP specific 70 kDa protein (U1-70K) recognises downstream positioned 5' splice sites and, in retroviruses, this has been shown to prevent the recognition of internal poly(A) sites. The authors showed that siRNA-mediated knockdown of U1-70K expression in *C. elegans* resulted in premature pol II transcription termination. Thus, the authors propose that the association of U1 snRNP with 5' splice sites close to intergenic regions may regulate the recognition of internal poly(A) sites by polymerase associated poly(A) factors, preventing premature termination of polycistronic transcripts.



GFP (green) and RFP (red) expression in the excretory cell in transgenic *C. elegans:* wild-type with intron (left) and mutant without intron (right)

Ubiquitin ligase ARF-BP1/Mule modulates base excision repair

Jason L. Parsons, Philip S. Tait, David Finch, Irina I. Dianova, Mariola J. Edelmann, Svetlana V. Khoronenkova, Benedikt M. Kessler, Ricky A. Sharma, W. Gillies McKenna, and Grigory L. Dianov. EMBO J 2009 Aug 27 (Epub ahead of print).

Base excision repair (BER) is the predominant cellular pathway involved in the repair of DNA lesions. DNA polymerase β (Polβ) plays a vital role in BER and the regulation of cellular Polβ levels is essential to maintain appropriate levels of DNA repair.

Previously, carboxyl terminus of Hsc70 interacting protein (CHIP) was identified as an E3 ubiquitin ligase involved in polyubiquitylation of Polβ and, consequently, targeting of Polβ for degradation. Wainman *et al.* demonstrated that Mule can also ubiquitylate Polβ in vitro. Using purified Mule, CHIP and Polβ, the authors showed that monoubiquitylation of Polβ by Mule stimulates polyubiquitylation by CHIP in vitro.

Following siRNA-mediated knockdown of Mule in HeLa cells, the amount of ubiquitylated Polβ ($Polβ_{ub}$) was reduced and levels of Polβ were increased. Knockdown of Mule also resulted in an increased rate of repair of hydrogen peroxide-induced DNA lesions, consistent with an increase in Polβ-mediated BER capacity. In contrast, knockdown of the tumour suppressor ARF, known to inhibit the ubiquitylation activity of Mule, resulted in increased $Polβ_{ub}$ and slower rates of DNA repair. Using Polβ-deficient ($Polβ^{-/-}$) mouse embryonic fibroblast, the authors show Mule knockdown in $Polβ^{-/-}$ cells had no effect on DNA repair. However, Mule knockdown in $Polβ^{+/+}$ cells resulted in increased rates of DNA repair. Thus, these results support the conclusion that Mule-dependent effects on DNA repair are mediated via Polβ.

Based on these data, Wainman *et al.* proposed a novel mechanism by which ARF, Mule and CHIP control steady-state levels of cellular Polβ, and hence capacity for BER. In response to DNA damage, ARF was previously shown to be released from the nucleus. Therefore, following DNA damage, cytoplasmic ARF can inhibit Mule-mediated ubiquitylation of Polβ. Polβ is then free to enter the nucleus and participate in BER. Conversely, reduced levels of DNA lesions resulted in a decrease in ARF release. Thus, Mule will monoubiquitylate Polβ, stimulating CHIP-mediated polyubiquitylation and resulting in degradation of $Polβ_{ub}$ and reduced BER capacity. This tuning cycle can be repeated to maintain steady-state levels of BER proteins. However, this model would not allow the system to respond rapidly to dramatic increases in DNA damage, induced by exogenous mutagens. The authors suggested that other mechanisms may control BER in response to severe DNA damage.

Structural analysis of substrate binding by the TatBC component of the twin-arginine protein transport system

Michael J. Tarry, Eva Schäfer, Shuyun Chen, Grant Buchanan, Nicholas P. Greene, Susan M. Lea, Tracy Palmer, Helen R. Sabil, and Ben C. Berks. Proc Natl Acad Sci USA 106, 13284-13289 (2009).

The twin arginine translocation (Tat) system transports fully-folded proteins, with varying size and surface properties, across the cytoplasmic membrane of prokaryotes and the thylakoid membrane of plant chloroplasts. In *E. coli*, the minimal Tat system consists of TatA, believed to constitute the protein translocating channel, and TatBC. TatB and TatC form a multimeric complex which acts as the membrane receptor, recognising the twin arginine motif of Tat substrates.

Tarry *et al.* used single-particle electron microscopy to obtain 3D structures of the TatBC complex, and TatBC bound to two different *E. coli* Tat substrates, SufI and CueO. These images showed that substrate proteins bound to the periphery of the TatBC complex, via the TatC component.

Comparing the size of the TatBC complex in the presence or absence of bound substrate clearly showed that TatBC undergoes a significant structural rearrangement following substrate binding, resulting in a smaller TatBC complex. The scale of this size reduction implied a loss of TatB and TatC subunits from the complex following substrate binding. This is consistent with the current transport model, which proposes that substrate binding to TatBC induces a structural rearrangement, allowing TatA to be recruited to the complex.

Although there are multiple TatC monomers in the TatBC complex, binding of only one or two substrate proteins was observed per TatBC complex. In the latter case, substrates were bound at adjacent sites rather than being randomly distributed on the complex. This led the authors to propose that either distinct TatC protomers in the complex interact with the substrate or there is negative cooperativity of substrate binding.

Based on the estimated mass of the 3D structure, and the distance between bound SufI molecules, it was predicted that the substrate bound TatBC complex consists of 7 copies of TatB and 7 copies of TatC.

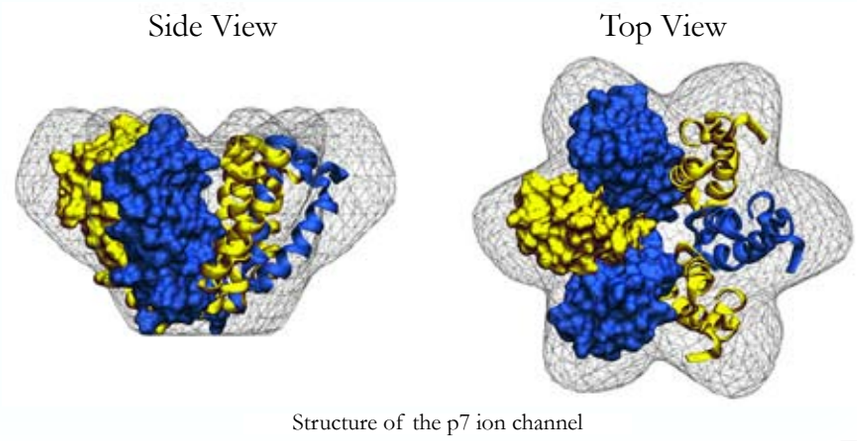
The 3-dimensional structure of a hepatitis C virus p7 ion channel by electron microscopy

Philip Luik, Chee Chew, Jussi Aittoniemi, Jason Chang, Paul Wentworth, Jr, Raymond A. Dwek, Philip C. Biggin, Catherine Vénien-Bryan, and Nicole Zitzmann. Proc Natl Acad Sci USA 106, 12712-12716 (2009).

The hepatitis C virus (HCV) represents a major global health problem, often leading to the development of severe liver disease. The HCV p7 ion channel is critical for the release of infectious virions and hence, has been identified as a potential drug target for the treatment of HCV infection.

Luik *et al.* used single-particle electron microscopy with random conical tilt reconstruction to determine the 3D structure of the HCV p7 ion channel. By chemically cross-linking native p7 monomers, the authors observed hexameric channel complexes. The 3D reconstruction, at ≈16 Å resolution, revealed a flower-shaped structure with six distinct 'petals'. The base of the channel complex is predicted to be 3.2 nm in diameter, broadening to 8.1 nm at the petal tips. Immunoelectron microscopy, using Fab fragments specific for the p7 N or C terminus, showed that both termini of each p7 monomer are located at the wide top of the channel complex. The authors proposed that this wide top is oriented toward the lumen of the endoplasmic reticulum.

The estimated mass of the complete p7 channel was ≈90 kDa which, together with the recently published 3D structure of the ≈80 kDa adinopectin trimer, makes this one of the smallest objects to be modelled by single particle image analysis. It is hoped that 3D visualization of the p7 ion channel will aid future efforts to rationally design drugs against this promising therapeutic target.



Structure of the p7 ion channel

PROSPECTS FOR A HEALTHY OLD AGE:

A Biochemical Perspective

How do we age and can we do anything about it? Until recently, these questions have been the domain of evolutionary theoreticians and science fiction writers, but recent research is starting to tackle them head on. Ageing is an intrinsically difficult process to study biochemically as it arises from the complex interplay of genes and environment. Historically, there has been little support for ageing research, perhaps as ageing has been viewed as natural and inevitable – not something that could, or should, be altered. Ageing gracefully is, however, not an option for everyone. With an ageing population, the enormous burden in terms of health costs (an estimated extra £30 bn per annum in care costs in the UK alone by 2030) and reduction in quality of life merits serious research and, if possible, intervention. Thus, from being a 'no-go' area for research, ageing has itself come of age.

Ten years ago, I was involved in organising a conference on cell senescence at Oriel College here in Oxford. At that time, several potential contributors to ageing had been identified, with mitochondrial dysfunction and telomere attrition vying for the top spot. At that meeting, we also discussed progeroid syndromes – single genetic human diseases that mimic some or many aspects of normal human ageing but in a greatly accelerated manner. Werner's Syndrome (WS), caused by loss of a combined helicase/exonuclease, looked a very promising start point as young WS patients were phenotypically very similar to normally aged people. The causative gene for WS had been positionally cloned and molecular studies indicated pleiotropic effects in DNA metabolism. A more severe syndrome, Hutchison-Gilford Progeria Syndrome (HGPS), affected young children, some of whom were dying as young as two years old of apparent old age. In 2008, the keynote speaker at the Oriel Cell Senescence conference was Dr Leslie Gordon, founder of the Progeria Research Foundation and mother of a child with HGPS. She described the unprecedented progress from gene identification in 2003 (a mutant form of lamin A called "progerin"), through to the world's first clinical trials for progeria treatment in 2007, involving farnesyl transferase inhibitor therapy. This trial has now been extended to include statins and bisphosophonates as these improve outcomes in HGPS-mice (see http://www.progeriaresearch.org/clinical_trial.html).

But are these rare progeroid syndromes relevant to normal ageing? What happens in WS patients appears in many ways to phenocopy what happens in normal ageing at the cellular, tissue and organismal levels. Thus, studying WS, especially in isogenic short-lived model organisms, can reveal the importance of DNA replication and recombination in maintaining genome stability and keeping cells 'young' and healthy. Although HGPS appears an outlier in terms of disease severity, lessons from premature stem cell ageing (via Notch signalling) may be widely relevant in developing stem cell strategies to renew damaged or aged tissues. Moreover, it turns out that we all produce progerin, albeit in tiny amounts, and that this aberrant protein accumulates with age, with wide-ranging effects, especially in cardiovascular disease. Therefore, treatments developed to help those few unfortunate children with HGPS may be of major value in therapy for age-associated disease in the normal population.

Incremental improvements in our understanding of ageing and age-related disease are a vital part of research but, what makes the headlines in *Science* and *Nature*, as much as the newspapers, is lifespan extension. Two such 'headline' papers published in July this year demonstrated significant lifespan increases in mammals, the first in mice fed the drug rapamycin in their diet (Harrison *et al.* 2009), and the second describing the effects of caloric restriction in rhesus monkeys (Colman *et al.*, 2009). Rapamycin inhibits the TOR complex, which integrates signals from growth factors, insulin, nutrient status and stress to regulate cellular protein synthesis. Inhibition of this pathway by genetic or pharmacological means was already known to increase lifespan of yeast, worms and flies. Extending this to mice, a model mammalian organism, was hugely significant in the field, particular as the rapamycin was not administered until the mice were well into middle age (apparently equivalent to 60 year old humans).

Necropsy studies suggested that there was no major difference in disease profile in the rapamycin-treated mice, so the drug extended lifespan but did not prevent disease. Notably, the mice were kept under pathogen-free conditions, essential as rapamycin is a potent immuno-

suppressive drug, and the doses used to extend mouse lifespan by 14% in females and 9% in males were much higher than used to prevent rejection in the transplant clinic in human patients. Thus, the rapamycin effect in increasing longevity in mice could not be applied to humans, even without considering other significant side-effects (including increased cancer risk) associated with the drug. What the Harrison study does tell us is that mammalian lifespans are not fixed, that they can be modulated pharmacologically even in later life, and that the TOR pathway is a tantalising target for the development of age-specific modulators. Whether it will be possible to dissect out the longevity effects of TOR inhibition from other less desired effects is a moot point, given the hugely complex feedback mechanisms and the integrative role of the TOR complex in cell signalling and metabolism.

By contrast, adult-onset caloric restriction (CR; a 30% decrease in calorie intake compared with an *ad lib* diet, without malnutrition) increased not only lifespan but also health of the rhesus monkeys. Most notably, there were no reported cases of impaired glucose regulation (e.g. diabetes) in the CR monkeys, and a marked decrease in age-associated muscle wasting (sarcopenia) and white matter atrophy in the brain. The CR monkeys looked younger (top right), with glossier coats and better posture, and despite adverse comments in the *New York Times*, did not appear to be more miserable than their *ad lib*-fed counterparts!

A key aim of much ageing research is the compression of morbidity – the period in later life where quality of life is seriously and adversely affected by age-associated disease and frailty. The results emerging from the Wisconsin monkey study, and related research at the US National Institute on Aging, suggest strongly that compression of morbidity can be achieved with caloric restriction, though the extended lifespan of the CR monkeys means that these experiments, currently in their twentieth year, still have a long time to go before full disease and mortality data can be collected.

So are we nearer to improving the quality of life in older age for the general human population? In my view, drugs like rapamycin are important in highlighting pathways but can never be the whole answer (I would like to be proved wrong, perhaps with the next generation of rapamycin analogues, or rapalogs). Interventions such as caloric restriction do seem promising, though applying such a drastic lifestyle change across human populations appears utterly unrealistic. What we now need to understand are the molecular pathways through which CR acts – the literature is a little unclear on this, but there is some overlap with TOR signalling, and drugs that mimic some of the effects of CR are in development. Perhaps a combined approach of age-specific rapalogs and CR-mimetics, together with sensible lifestyle choices, holds out the best hope for a healthy old age. •

See also:

Cox, LS (2009) Live fast, die young: new lessons in mammalian longevity. *Rejuvenation Research.* 12(4) Epub ahead of print DOI: 10.1089/rej.2009.0894

Cox, LS and Mattison, JA Increasing longevity through caloric restriction or rapamycin feeding in mammals: common mechanisms for common outcomes? *Aging Cell* Aug 12 epub ahead of print DOI 10.1111/j.1474-9726.2009.00509.

Colman et al. (2009) Calorie restriction delays disease onset and mortality in Rhesus monkeys. *Science.* 325(5937)pp.201-204

Lynne Cox carried out her PhD at Cambridge University under the supervision of Professor Ron Laskey on "Chromosome replication *in vitro*". She then moved to Dundee to work with Professor Sir David Lane on the role of the tumour suppressor protein p53 in DNA replication and cell cycle control. While in Dundee, she was awarded a Royal Society of Edinburgh Personal Research Fellowship to investigate the replication protein PCNA, discovering the first two examples of PCNA-interacting peptides, or PIPs. She is now a University Lecturer in Biochemistry at the Department of Biochemistry, University of Oxford, and the George Moody Fellow and Tutor in Biochemistry at Oriel College. Her research group studies the molecular basis of ageing, predominantly using Werner's Syndrome as a model of normal ageing. Last year, her group published the first identification of a fly homologue of human WRN, which attracted significant media attention (see http://www.youtube.com/watch?gl=GB&hl=en-GB&v=IGQwz2Wv9FU). She serves on the executive committee of the British Society for Research on Ageing. Her book "Molecular Themes in DNA Replication" is to be published in September by the Royal Society of Chemistry.

5' WITH...

Dr Petros Ligoxygakis and Professor Mark Sansom

When did you realise you wanted to be a scientist?

PL: Probably when I first read a biography of Louis Pasteur when I was 9 or 10. I remember reading it like a detective story. I was struck by how Pasteur, with very simple observations, had seen through the mesh of anecdotal and somewhat metaphysical ways of dealing with infection. Pasteur had built a view of infectious diseases and the host response that still stands today.


Professor Mark Sansom

MS: I think it had been decided for me by a school teacher when I was about 10 years old. I was awarded some random book prize. When I asked for a history book she suggested natural history might be more useful...

If you were not a biochemist, you would be...

PL: I'm not! I'm a geneticist (secret). However, through interactions with people in this Department my eyes have been opened to another way of thinking about biology. If I were not? Probably a music journalist, something I did when I was an undergrad (long story).

MS: Bored. Probably unemployable.

If you are not in the lab you are...

PL: Doing things with my kids, listening to music very late at night.

MS: It depends whom one asks (or who is asking) - most likely either at home or at a conference.

Worst disaster in the lab?

PL: Nothing major yet!

MS: Week two of my D.Phil - spilling my precious enzyme prep down a sink after 20 hours working on it. My supervisor (Louise Johnson) was very understanding... but it was clear that I was not destined to be an experimentalist.

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

PL: Getting my EMBO fellowship to work in Jules Hoffmann's lab. For some time it was like riding the crest of the perfect wave.


Dr Petros Ligoxygakis

MS: There have been two decisive moments, one at the end of my D.Phil and one after about 5 years as a young lecturer. On both occasions, I realized I was ready for a change in research direction.

In your view, what is the importance of luck in research?

PL: Serendipity is always important. However, it is also crucial to realise what happened and grab your chance with both hands. The way one develops that first result is no longer luck.

MS: It is important in two ways. You have to be lucky in: (i) results; (ii) the people who choose to work with you. In both cases the skill is recognizing good fortune early on.

Any memorable findings?

PL: The first screen to identify Toll pathway mutants in fruit fly immunity.

MS: I guess only time will tell. I am convinced most of the things we do are memorable, but then I am biased. The first simulation we ran when a membrane and a protein self assembled correctly was quite fun.

Describe your personality in four words.

PL: Pessimistic nature, optimistic otherwise.

MS: Oh dear...definitely "impatient". Not sure about the other three - maybe you should ask my grad students and postdocs.

One human trait you hate.

PL: Hypocrisy.

MS: Intolerance of differences in others.

Favourite vacation spot.

PL: Anyone that has seen the color of the Mediterranean sea reaching the sandy beaches of southern Crete will have difficulties not to think this is heaven on earth.

MS: Somewhere green and sparsely populated with no wifi.

Best advice you ever received.

PL: Keep your story simple (Jules Hoffmann). I've been trying ever since and it's difficult!

MS: "Have you thought of applying for the job in Oxford?"

Biggest mistake or regret?

PL: Mistakes? Do you have a day or two?

MS: No real regrets, but I am sure I have made many mistakes.

Favourite classical experiment?

PL: Seymour Benzer's genetic screens to identify the circadian rhythm genes and the genes that influence photoreceptor formation in *Drosophila*. There is a fantastic book by Jonathan Weiner called Time Love Memory that could qualify as Benzer's biography, and describes his lab's quest for the genetic control of behavior.

MS: Possibly the first membrane protein X-ray structure. I remember being at a meeting when one of the first high resolution membrane protein diffraction photos was shown and there was spontaneous applause from the audience.

How do you imagine biochemistry research will change in the next twenty years?

PL: More integration with physics and mathematics, especially in the imaging front where super resolution for the detection of single proteins or complexes in real time in living cells will be (hopefully) routine.

MS: I'd like to imagine that we will see theory/computation playing a similar role as in the physical sciences. I think it will also become more 'synthetic' in terms of manipulating our understanding for novel outcomes, whether therapeutic or technological. I also hope there will be further blurring of the physical/biological sciences boundary.

Perspectives on Public Engagement

Dr Penelope Mason, a postdoc in Dr Lynne Cox's lab in the Biochemistry Department, participated in an event for this year's British Science Association festival. She shares her experiences and encourages others to get involved.

The British Science Association (BSA) festival was held on 5 to 9 September 2009 at the University of Surrey campus and around Guildford. One of the events sponsored by the festival is a poster competition called *Perspectives* (http://www.britishscienceassociation.org/web/ScienceinSociety/Perspectives/). The scheme aims to get early career scientists thinking about the wider impact of their research, with a strong component of engaging with the public. This year the remit was specifically how society and research shape each other. Selected from around 85 applicants, 36 'finalists' attended a poster design workshop to design for a non-technical audience. Some of us spent the weekend before the festival in Guildford shopping centre displaying our posters to the general public. As the blurb stated: "See the posters the scientists made about their research. Ask a question. Share your views." The passers-by did exactly that, often surprisingly. One "child prodigy... seemed to know almost as much as some of us about RNA". See Josh's blog at http://www.britishscienceassociation.org/web/ScienceinSociety/Perspectives/_finalists_at_the_British_Science_Festival.htm for more.

Thirty-five finalists, including me, presented posters at the 2009 Festival. We also spent time at other events, including the X-change, where a 'best of the day' review, hosted by the presenter Sue Nelson, was held in the Union Bar (for her blog of this see http://news.bbc.co.uk/1/hi/sci/tech/8240096.stm). Three of us were also interviewed by one of the BSA's media fellows for the Radio 4 programme 'Leading Edge' (10 September). On the third day of the festival, the posters were judged. The prizes up for grabs were £200 for 5 runner-up winners and £750 for the overall winner, as well as an invitation to the festival dinner with the likes of Lord May of Oxford, Simon Singh, Bill Bryson, Adam Hart-Davis and Robert Winston. Also in attendance was Sir Roland Jackson, the Chief Executive of the BSA and an alumnus of the Oxford Biochemistry Department.

Perspectives (and the festival as a whole) was an extremely rewarding event and I would urge anyone eligible to apply next year. I saw a lot of good science, won £200 and got to talk about my work on the radio.
I would say, give it a try!

How to get involved in 2010... The next BSA festival will take place on 14-19 September 2010 at Aston University, Birmingham.
For more information visit:
http://www.britishscienceassociation.org/web/BritishScienceFestival/EventOrganisers/Getinvolved.htm

The Life Of Charles Darwin

(1809-1882)

James Halstead heads to The Oxford University Museum of Natural History to check out the current exhibition dedicated to Darwin.

It is 150 years since the publication of Charles Darwin's seminal 'The Origin of Species' and the biological world is celebrating. Journals have printed special issues in honour of the author and lectures on evolutionary biology have appeared across universities. Sales of the book and those discussing its implications have shot to an all-time high. Now is a better time than ever to find resources to learn about the theory of evolution proposed in 1859 and how it has developed since then. In fact, it is all rather grand, and rightly so you may say. After all, there is no doubt that Darwin's theory of evolution by selection made a profound and lasting contribution to scientific philosophy, despite the ardent opposition it continues to stir, and this is reflected in celebratory exhibits across the country.

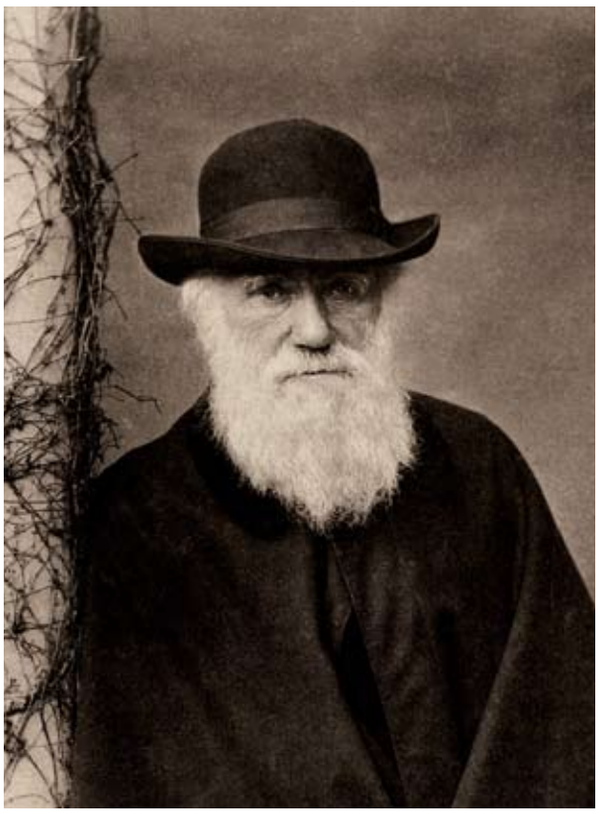
The current Darwin exhibition at the Oxford University Museum of Natural History offers something a little different. While many museums and universities offer excellent introductions to the theory, this exhibit focuses on the man himself and the personality behind the scientist. In 1876, Darwin was approached by a German publisher for an account of "the development of his mind and character" and the naturalist duly obliged. For an hour a day, for the next seven years, Darwin wrote memoirs and collected letters that are sampled in the exhibit.

The first thing that I noticed was Darwin's dry wit. He wrote candidly about how sickened he was by "dull" medical lectures in Edinburgh before quitting to study in Cambridge where his "time was wasted, academically speaking, as completely as it had been in school". He explained his self indulgence as a student and his stubbornness to change, stating that even at the end of his career he refused to "take any pains about my style of writing". There is one particularly funny letter, where he recounted how as a young man with a "smattering of medical knowledge" he was a terrible hypochondriac and had convinced himself that he had fatal heart disease. He was so certain of his imminent death that he refused to go to the doctor in case they forbade him from doing field work. He referred to his own death numerous times in his notes.

For me, one of the most charming facets of Charles Darwin appeared to be his genuine enthusiasm for nature. It was surprising to hear how as a student he never completed a biological science degree, yet as a scientist, he never stopped observing the world around him. In one letter Darwin hilariously recounts how, while relaxing by the banks of the river Cam, he spotted two unusual *Carabidae* (ground beetles) and grabbed one in each hand. Pleased with his catch, he then spotted an even rarer *Panagaeus crux* major beetle underneath some fallen tree bark. Unable to bring himself to lose his previous catches, but loathed to let the *Panagaeus* escape, the young Darwin shoved the beetles in his mouth so that he could carry all three samples back for study. The beetle squirted acid in his throat for the whole journey back to his room.

It was probably this madly inquisitive nature that led him, as a newly ordained clergyman, to board the *HMS Beagle* in 1831 and travel the world as an unpaid naturalist. The journey lasted five years and was catalogued in Darwin's scientific notes and personal thoughts on the voyage.

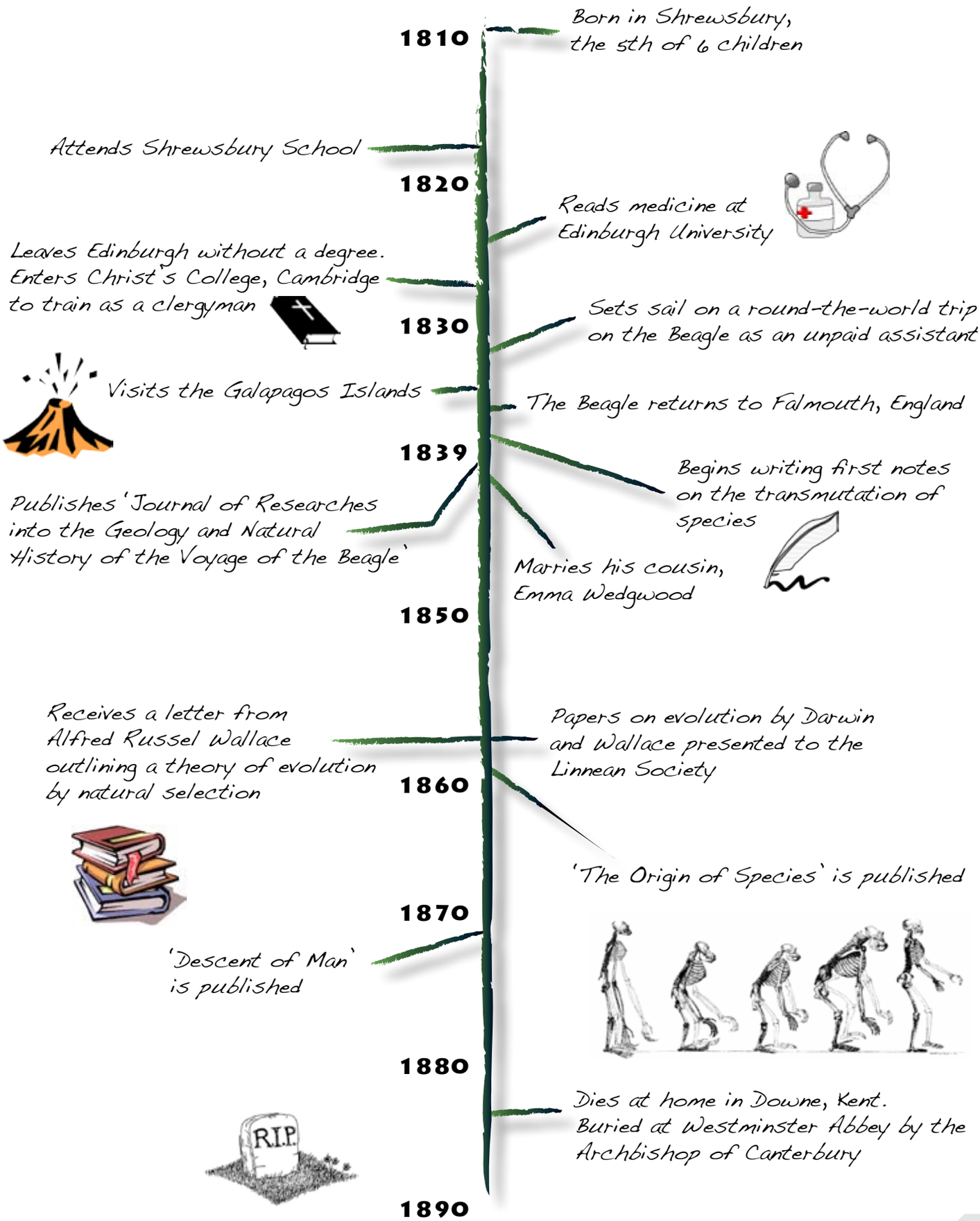
Another fascinating feature of the exhibition is the documentation of the meeting of the British Association for the Advancement of Science in 1860. The meeting took place in the unfinished museum in Oxford and featured many religious and scientific heavyweights of the time, viciously fighting over the theory of selection. This was where Thomas Henry Huxley, the English biologist and supporter of Darwin, was asked by the Bishop of Oxford if "it is through your grandmother or grandfather that you claim your descent from monkeys". In a letter to Darwin, who by then was too ill to attend most public discussions of his work, Huxley described how he



had "sharpened up [his] beak and claws in readiness" for the debate.

This exhibition is small and conservative, and easily enjoyed in 30 minutes. It is refreshing to learn more about the character of the Darwin and not to let the grandeur of his achievements overshadow his eccentric and exciting personality.

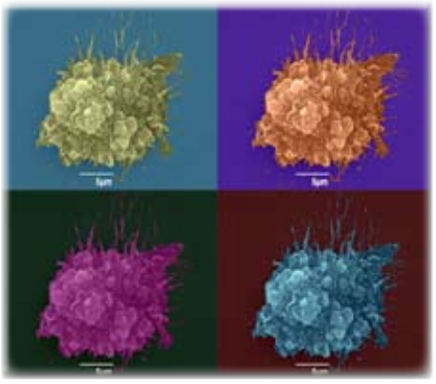
The exhibtion ends on 31 December 2009 •



SNAPSHOT

Snapshot is *Phenotype's* new research image competition. We received pictures of very high quality and are grateful to everyone who submitted an entry.

The winners of *Snapshot* Michaelmas 2009 were Dr Kate Gartlan and Dr Rebecca Russell from Dr Quentin Sattentau's group in the Sir William Dunn School of Pathology. Their prize was a bottle of champagne and a copy of 'The Oxford Biochemistry Department 1920-2006' by Dr Margery Ord.



The science behind the winning picture...

The striking image on the cover is an artistically rendered scanning electron micrograph of a human macrophage. It was captured as part of research into the transmission of human immunodeficiency virus-1 (HIV-1) from macrophages to T cells.

HIV is the causative agent of acquired immunodeficiency syndrome (AIDS), a major global health problem. There is currently no cure for HIV infection. A significant problem in treating HIV is the persistence of the virus. Recent work suggests that HIV can 'hide' in macrophages, which act as reservoirs of virions. The primary role of macrophages is to engulf pathogens and dying or infected cells. In an HIV-infected individual, macrophages can become infected if they engulf T cells containing the virus. Macrophages, in turn, perpetuate the spread of the virus within the host by transmitting virus particles to other T cells through the transient formation of virological synapses.

The work behind this image aims to investigate the mechanisms by which macrophages become infected through T cell engulfment. It has potential therapeutic applications, as inhibition of T cell engulfment might aid successful elimination of the virus from patients. The competition winners co-cultured macrophages, isolated from human peripheral blood, with autologous T cells infected with CCR5-coreceptor-tropic HIV-1. After incubating for two hours, the cells were fixed and then imaged by scanning electron microscopy (SEM). The winning image shows a macrophage not in contact with a T cell, allowing its surface complexity to be observed in spectacular detail. In order to highlight the important features of the macrophage, it was pseudocoloured and the protrusions were traced manually. The result was a very impressive picture – particularly so, as this was Dr Gartlan's first attempt at colouring an SEM image.

Dr Rebecca Russell & Dr Kate Garland with their prizes

The winners would like to acknowledge the help of Dr Fedde Groot with sample preparation and Dr Michael Shaw for his 'terrific microscopy skills'. •

Snapshot Hilary 2010: how to enter...

Do you have an image from, or inspired by your research? Why not enter it for *Snapshot*?

We are now accepting entries for pictures to be featured in the Hilary 2010 *Phenotype*.
The prize for this competition will be announced soon.
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 20 November 2009

Alumni Adventures

Examining the varied careers and lives of D.Phil students after graduate school

Maria G ('Pita') Enriquez Harris studied biochemistry at Oxford from 1984 to 1988, followed by a D.Phil at the Dunn School of Pathology. After a couple of brief postdocs in Oxford, Pita left science to set up a local IT company, The Oxford Knowledge Company. In 2004, a ski accident left her recuperating with a broken leg, with an opportunity to alter the trajectory of her career. Writing as MG Harris, Pita began to pen a series of children's adventure novels called *The Joshua Files*.

The book deal that followed with Scholastic Children's Books UK was the largest deal of the year for a debut children's author. The first title of the series, 'Invisible City', was 2008's fastest-selling debut children's novel in the UK.

The Joshua Files tells the story of an Oxford teenager who discovers a family secret linking him to the ancient Mayan prophecy of global catastrophe in 2012. Pita describes it as "a blend of mystery, mayhem, myth and teenage angst".

In her new life as an author Pita has appeared at the Oxford, Edinburgh and Hay literary festivals – an enlightening change from the scientific conference circuit!

MG Harris at the official launch of the second book in her series, 'Ice Shock', March 2009

Jemima Cordle cuts the curd as a cheesemaker

After completing a D.Phil in the Biochemistry Department in 2005, **Jemima Cordle** *tells us how she became a cheesemaker*:

In many respects my life in research was perfect; I love to investigate things and to discover. But something was missing and I needed inspiration. Two years ago, I visited the Slow Cheese festival in Italy. While waiting for the plane, I found myself standing next to Jamie Montgomery, the country's leading artisan cheddar maker, who talked of the need for scientists in the world of farmhouse cheesemaking. At the Festival, I marvelled over the fact that the huge diversity of cheeses could all be created from the same raw material – milk. The more I considered this, the more interested I became.

On returning to England, I arranged a visit to a dairy and, after a week, I was hooked. One month later, I bought a small cheesemaking business in the Somerset village of Westcombe. Since then, I have been making and selling up to half a tonne of Duckett's Caerphilly every week while simultaneously teaching myself milk biochemistry and microbiology. It has been a deeply satisfying experience and I have no regrets. The two-year anniversary of my move has just been marked with a return to the Slow Cheese festival. This time, Duckett's Caerphilly was on sale!

Former OUBS president **James Wilding** *tells us how his career has progressed since leaving the Biochemistry Department in 2003*:

After completing my D.Phil, I initially stuck with research and went to Paris for a two year postdoc. I had enormous fun but, as my funding ended, I decided to hang up the pipettes and joined HLBBshaw as a trainee patent attorney.

The learning curve as a trainee has been very challenging. I started the job with the technical expertise but knowing little about law. Becoming familiar with legal concepts and how they are applied is a large part of the training. The job requires a logical mind and an ability to express arguments well. The arguments can seem surreal, for example when asserting that a range continuous between 1 and 10 does not actually include the values 1 and 10, but for me that is part of the fun. Indeed, I found generating new ideas and arguing about them the most enjoyable aspect of research!

A senior colleague at HLBBshaw, Richard Bizley, who read biochemistry at Christ Church, has been involved in many high profile cases, including the Harvard oncomouse technology, PCR, IL-2, Protein-C and β-IFN, and humanized antibodies, demonstrating just how exciting the job can be.

Another former OUBS president, **John Knowland**, held the position in 1968/9. At the time, he was completing a D.Phil on "The initiation of synthesis of different types of RNA in early amphibian development" in Dr John Gurdon's lab in the Department of Zoology. John then spent a period at the MRC Laboratory of Molecular Biology in Cambridge, working on hormone-controlled gene expression in *Xenopus* in John Gurdon's lab.

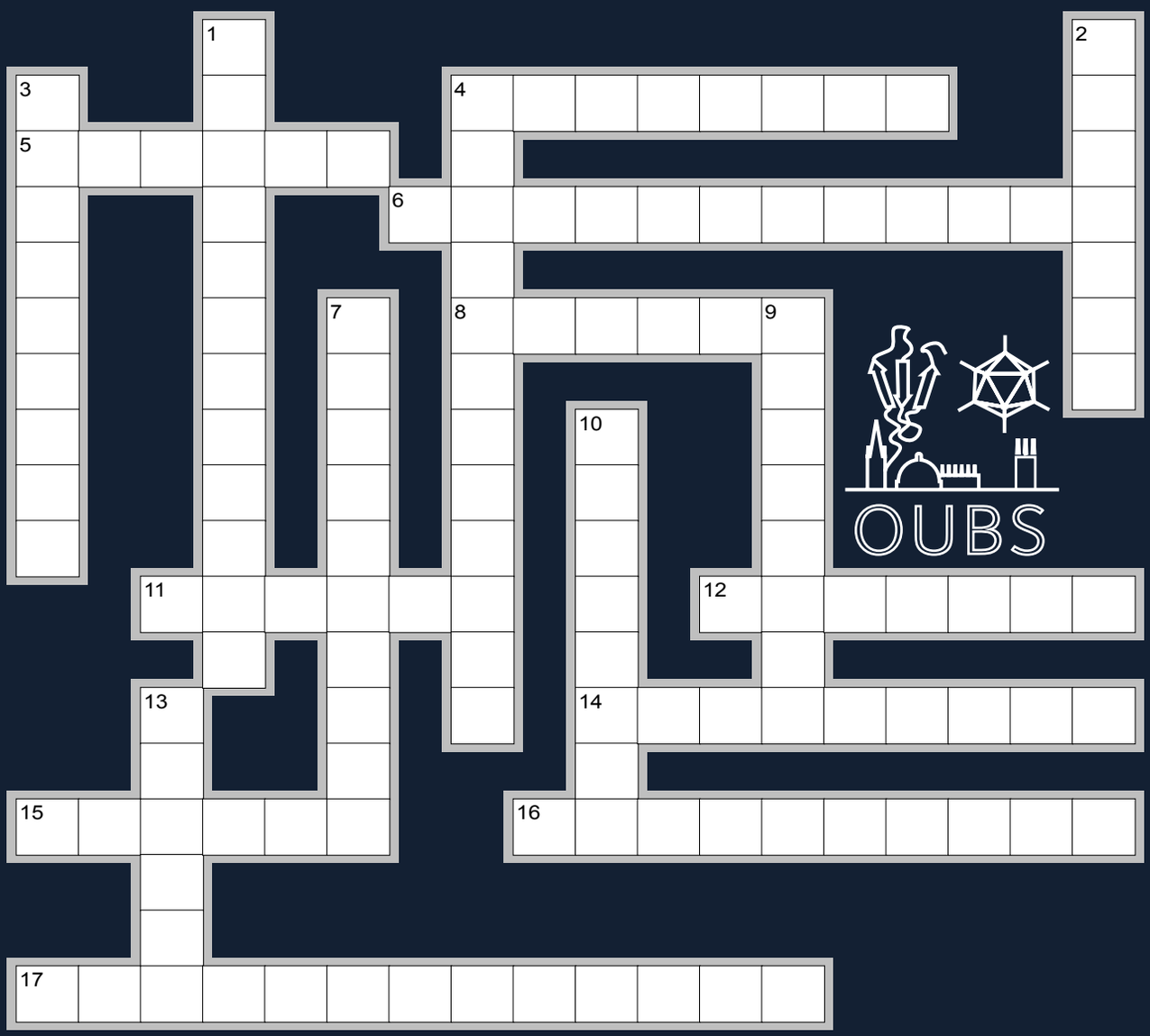
In 1976, John returned to Oxford as a Lecturer in Biochemistry and Fellow of Pembroke, where he remained until 2001. During his long career, John tells us that "perhaps the most fun thing that I did was to investigate the DNA-damaging effects of chemicals in sunscreens when exposed to sunlight". He observed that some of these chemicals, including esters of dimethylaminobenzoic acid and dibenzoylmethanes, can generate carbon-centred free radicals following exposure to UV-radiation. These free radicals are capable of causing DNA damage and hence this work has implications for the use of these types of chemicals in sunscreens. Unsurprisingly, these findings caused quite a media stir!

In 2001, John left the Biochemistry Department but stayed in Oxford as the Bursar of Brasenose until his retirement on 30 September, 2009.

CROSSWORD

Try your wits against this term's *Phenotype* crossword!
Some of the answers can be found in the articles in this issue of *Phenotype*. Others are related to subjects in the magazine.
Send us your answers by Friday 20 November 2009. All correct entries received by this date will be entered into the prize draw.
The winner will receive a £10 book voucher.

You can e-mail us the answers (oubs@bioch.ox.ac.uk) or leave a paper copy in a sealed envelope in the OUBS pigeonhole at the New Biochemistry reception.



Across

4. Microscope that uses a small pinhole to filter out out-of-focus light (7)
5. Author of *The Joshua Files* (6)
6. Type of electron microscope (12)
8. Name of the first cloned dog (6)
11. Type of $CD4^+$ T cell (6)
12. Alternative name for a beetle (7)
14. Drug that can extend lifespan (8)
15. Ship on which Charles Darwin sailed during his voyage that led to his formulation of the theory of evolution by natural selection (6)
16. Type of cheese (10)
17. Description of mRNA transcribed from genes in an operon (13)

Down

1. Competition for young scientists run by the British Science Association (12)
2. Professor Mark Sansom's D.Phil supervisor (7)
3. Discovered Green Fluorescent Protein (Nobel Prize 2008) (9)
4. Oxford college of which Sir Richard Gardner is a fellow (6,6)
7. Cellular reservoir of HIV-1 (10)
9. Co-recipient of 2009 Albert Lasker Basic Medical Research Award (8)
10. Inherited condition that results in premature ageing (8)
13. Chemical used for negative staining of samples for electron microscopy (6)
_____acetate