PHENOTYPE

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Death Stars

Professor Jonathan Hodgkin FRS and Dr Maria Gravato-Nobre

Winners of the SNAPSHOT Scientific Image Competition ***Page 39***

Women in Science *Pages 20–30*

Prof Judith Amitage, Dr Sylvia McLain, and Prof Elspeth Garman

Sophie Costello, CEO of Costello Medical Consulting

Why men should participate in women's initiatives

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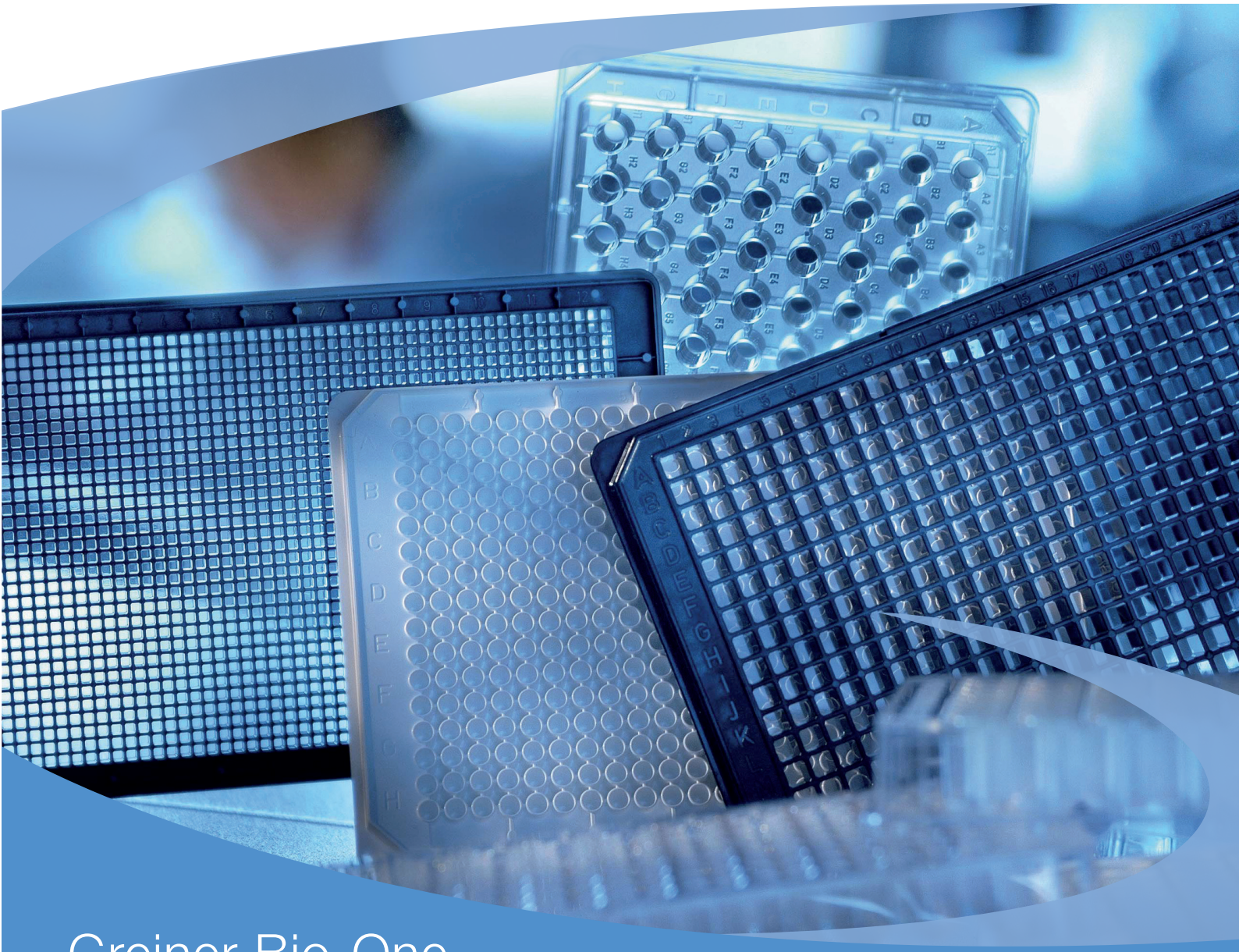

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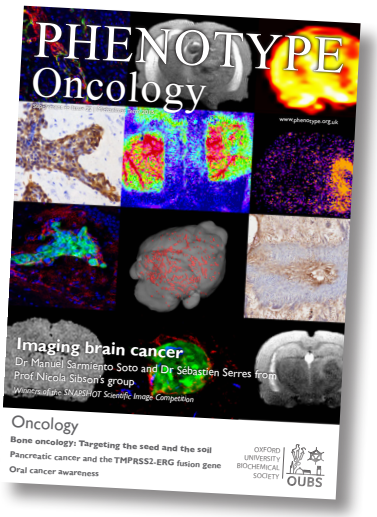
EDITORIAL

Welcome to a very special 22nd instalment of Phenotype! All of our articles have been produced and edited by undergraduates, postgraduates, postdocs, principle investigators and alumna from a range of disciplines throughout the University of Oxford. This term we are delighted to have been able to deliver a focused Women in Science section, as part of our biggest ever issue (40 pages!). I hope you enjoy reading our science magazine as much as we enjoyed producing it.

In this issue, our **Features** *section focuses on… Regulation of cytokine activity in the extracellular matrix: Turn to page 8 where Prof Penny Handford explains how the extracellular matrix is increasingly recognised as a modulator of cell function. On page 12, Natalie Ng contemplates the transcriptional regulation of breast cancer in an article entitled In the Loop. In five mini-features, Dr Suzanne Cole suggests that balance is the key to the innate immune response to Influenza A virus (page 14), Alba Libre discusses the use of histone deacetylase inhibitors for curing HIV/AIDS (page 15), Drew Duglan warns of the health risks of sleep deprivation (Page 16), Dr Burcu Anikirmizitas explains the importance of interactions between histones and DNA methyltransferases (page 17), and Zarina Zainudeen shares novel insights on populations of B cells in the thymus that can be targeted with rituximab, an anti-CD20 antibody (page 18). Then skip to pages 32 to 34, where Dr Joanna Lee writes about drug binding to the transporter SV2, which is found on synaptic vesicles. And, finally, on pages 34 to 35, Leah Taylor Kearney highlights the role of PHD2 in the regulation of HIF, which is a key transcription factor that becomes stabilised under conditions of low oxygen (hypoxia).*

The **Women in Science** *section begins on page 20 with a two-page comic by Dr Natalie Connor-Robson and Dr Óscar Cordero Llana that shows the work that is still needed to bridge the equality gap. Turning to pages 22 to 24, Prof Eslpeth Garman, Dr Sylvia McLain and Prof Judy Armitage kindly provide insight into their careers in academia. In three short focused articles, Brid Cronin explains the great work being done on the Athena SWAN initiative (page 25), Anna Muszkiewucz discusses why men's engagement in women's initiatives could accelerate social change (page 26), and Dr Naveed Akbar gives a historical perspective on women in science. On pages 28 to 29, Sophie Costello, an Oxford graduate and CEO and co-founder of Costello Medical Consulting, gives an inspiring story about starting a healthcare consultancy. And, finally, Dr Ashwag Albukhari highlights the importance of international travel when undertaking doctoral studies, which she describes as 'a dream come true'.*

As always, our **Regulars** *section brings you Research Highlights, a featured seminar, book reviews, an interview with an academic, the winners of the SNAPSHOT scientific image competition and more... In Research Highlights, Dr Myriam Elschami summarises two key papers, one which reports on TARDBP mutations that reduce calcium signalling in motor neurones, and another that highlights a novel biomarker of amyotrophic lateral sclerosis (page 5). This term's Featured Seminar covers the Joel Mandelstam Lecture, which will be given by Prof C. Neil Hunter FRS, Krebs Chair in Biochemistry, University of Sheffield, on Thursday 19th November 2015 (pages 6 to 7). Rebecca Hancock spends 5 min with… Dr Elena Seiradake, who has submitted an impressive 30 X-ray crystallography protein structures to the RSCB Protein Data Bank (page 38). We congratulate Prof Jonathan Hodgkin FRS and Dr Maria João Gravato Nobre on winning this term's SNAPSHOT competition for their 'death star', showing Caenorhabditis elegans (C. elegans) co-aggregating as a result of tail adhesion caused by the bacterium Leucobacter n.sp, Verde1. Further details can be found on page 39. As always, have a go at the crossword on the back cover, which is themed around women in science. Be sure to email your answers to rebecca.hancock@linacre.ox.ac.uk for a chance to win one of the excellent Wiley-Blackwell textbooks reviewed by Drew Duglan, Kate Dunne, Laura Godfrey, and Anna Sigurdsson on pages 36 to 37.*



Finally, we would recommend taking a look at the new themed **Supplement**, *which this issue has a oncology theme! This term the supplement contains articles on oncology from Prof Claire Edwards, Dr Sebastien Sérres, Dr Slav Ovtcharov, Sara Ahrabi and Dr Marzyeh Parvvizi. We would also encourage you to get involved in science communication, writing, and publishing by joining the Phenotype team. We have excellent opportunities to get involved in science writing, editing, designing and advertising. Please get in contact by email to the incubent Editor-in-Chief Becky Hancock at rebecca.hancock@linacre.ox.ac.uk.*

As my final term as Editor-in-Chief I would like to thank all contributors for what has been a highly successful year for Phenotype!

Christopher Hillyar
Editor-in-Chief

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Phenotype is also available to read online via our website: **www.phenotype.org.uk**

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EDITORIAL TEAM

RESEARCH HIGHLIGHTS

by Dr Myriam Elschami

Mutihac R *et al.* (2015) Neurobiol Dis. 75:64-77
doi: 10.1016/j.nbd.2014.12.010

TARDBP pathogenic mutations increase cytoplasmic translocation of TDP-43 and cause reduction of endoplasmic reticulum Ca^{2+} signalling in motor neurons

Amyotrophic lateral sclerosis (ALS) is an adult onset progressive neurodegenerative disorder characterised by the loss of motor neurons. The transactive response DNA binding protein (TDP-43), is a ubiquitously expressed nuclear protein that plays an important role in the complex biology of this multifactorial disease. In ALS, TDP-43 mislocalises into cytoplasmic neuronal inclusions that are the hallmark of the disease. Moreover, mutations in *TARDBP*, the gene encoding TDP-43, account for a significant proportion of familial ALS cases, underlining an integral role of TDP-43 in the cell biology of ALS.
Mutihac *et al.* investigated the pathophysiology of disease-associated mutations in *TARDBP*. By using the full-length genomic locus of *TARDBP* for expression in HEK cells, they developed a novel physiological model that avoids overexpression-induced artefacts. Using confocal microscopy to detect the fluorescently tagged transgenic TDP-43 variants, they could show that mutations in *TARDBP* increase the propensity of the protein to mislocalise to the cytoplasm and sensitise the cells to apoptosis. Furthermore, an increase in the ER-binding protein Bcl-2 (a negative regulator of Ca^{2+} flux from the ER) was noted in cells with TDP-43 mutations.
These findings prompted the researchers to investigate Ca^{2+} flux in cells with mutant TDP-43 using a fluorescent Ca^{2+} binding dye. The authors recorded the release of Ca^{2+} from the ER following pharmacological activation of ER-Ca^{2+} release channels. In accordance with the inhibitory role of Bcl-2 for Ca^{2+} release, cells with mutant TDP-43 displayed a delayed response in store opening and decreased amplitude of Ca^{2+} release. Importantly, these findings were corroborated by measurements with spinal motor neurons from transgenic mice harboring the same *TARDBP* mutations, showing the relevance of these events in a disease-related cell type.
To investigate if the aberrant Ca^{2+} signalling in mutant TDP-43 cells is mediated by an increase in Bcl-2, Mutihac *et al.* used RNA interference to knock down Bcl-2 in the respective cell lines. Intriguingly, they found that for one of the mutations analysed, knock down of Bcl-2 fully restored ER-Ca^{2+} release and partially restored the nuclear localisation of the TDP-43 mutant. Conversely, in the second mutant cell line neither of these parameters were rescued, suggesting that other factors may be involved in disrupting Ca^{2+} signalling in cells with mutant TDP-43. With their study, showing that ALS-associated *TARDBP* mutations lead to aberrant ER-Ca^{2+} signalling, Mutihac *et al.* have contributed novel results that shed light on the complex pathophysiology of ALS.

Lu CH *et al.* (2015) Neurology. 84(22):2247-2257.
doi: 10.1212/WNL.0000000000001642

Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the motor neurons. With a survival time of only 2 to 5 years after diagnosis, there is an urgent need for the development of an easily accessible, reliable biomarker to accelerate diagnosis and improve assessment of prognosis of ALS patients. However, this remains a challenging task due to the clinical heterogeneity of ALS, the variable progression rate and the lack of a recognisable preclinical state. Neurofilaments, the main byproducts of axonal breakdown, are described as universal biomarkers of neurodegeneration. Importantly, they show a distinct increase in the cerebrospinal fluid (CSF) of ALS patients and are robustly detectable.
Lu *et al.* studied the potential of neurofilament light chain (NfL) from CSF, plasma or serum as a prognostic marker for ALS. Using an electrochemiluminscent ELISA, they analysed two separate cohorts of ALS patients and healthy controls in regular intervals over a period of up to 3 years. They found that NfL levels from the three biofluids (CSF, plasma and serum) correlated and reliably discriminated ALS patients from healthy controls. Moreover, they discovered that blood levels of NfL at recruitment were strong indicators of survival and remained stable during follow-up. Lu *et al.* have identified blood-derived NfL as an easily accessible biomarker capable of predicting survival of ALS patients with, due to its stable expression, the potential as a pharmacodynamic measure of therapeutic response.

OUBS FEATURED SEMINAR

Oxford University Biochemical Society brings you

Prof C. Neil Hunter FRS, Krebs Chair in Biochemistry, University of Sheffield

Joel Mandelstam Lecture

Thursday 19th November 2015

Prof C. Neil Hunter was born in 1954 and he is a proud son of Knaresborough, a market town in the Yorkshire Dales. He is the Krebs Professor of Biochemistry in the Department of Molecular Biology and Biotechnology at the University of Sheffield, UK.

Prof Hunter obtained his BSc degree at the University of Leicester in 1975, where the lectures of Prof Hans Kornberg FRS and a final year laboratory project with Prof Peter Henderson inspired a lifelong interest in metabolism and bioenergetics. With a desire to learn about photosynthesis, Prof Hunter moved to Bristol University, where Professors Trevor Griffiths, Owen Jones and Tony Crofts were studying many aspects of this topic in their laboratories, from pigment biosynthesis through to electron transport.

Following this, Prof Hunter studied for his PhD under the guidance of Prof Owen Jones on the subject of bacterial photosynthesis. A sabbatical visit by Prof Robert Niederman in 1978 to the Jones laboratory led to Hunter taking a postdoctoral fellowship with Prof Niederman at Rutgers University, New Jersey. This fellowship, on membrane assembly in bacterial photosynthesis, furthered Prof Hunter's interest in photosynthetic bacteria.

But, why are these organisms worth studying? Purple phototrophic bacteria are the most metabolically versatile organisms on Earth, and display a huge diversity of energy modes and metabolic capabilities. They can grow phototrophically or in darkness by respiration, fermentation, or chemolithotrophy. Organic acids, amino acids, fatty acids, alcohols, carbohydrates, and even C-1 compounds are metabolised by different species of these phototrophs, which are also able to produce hydrogen, fix nitrogen, and use H_2S, S_0, $S_2O_3^{2-}$, H_2, and Fe^{2+} as electron donors. Various species can grow at 57 °C or as low as 0 °C, at pH values as low as 3 or as high as 11, and at high salinities.

Prof Hunter's interests in light-harvesting complexes and membrane assembly developed further at this stage, and the relative simplicity of its photosynthetic apparatus made the purple phototrophic bacterium *Rhodobacter (Rba.) sphaeroides* an attractive model for photosynthesis research. However, research in this area at this time was hampered by a lack of molecular genetic tools. Accordingly, in 1980, Prof Hunter returned to Bristol to learn about molecular biology in the laboratory of Prof Geoff Turner, where transposon Tn5 mutagenesis was developed, so that genes encoding the enzymes for bacteriochlorophyll and carotenoid biosynthesis could be identified, mapped and cloned. Subsequently, Prof Hunter created a toolkit for site-directed mutagenesis of photosynthetic complexes. The rapid development of laser spectroscopy and the emergence of 3D structures of these complexes, plus the ability to genetically engineer their properties created a fertile area of molecular research. The discovery of the genes encoding the enzymes for bacteriochlorophyll biosynthesis in *Rba. sphaeroides* led to the cloning and overexpression of their counterparts in chlorophyll biosynthesis and the provision of recombinant enzymes for a series of papers on their kinetic properties. Nowadays, there is a high level of molecular genetic, biochemical, structural and spectroscopic characterisation for *Rba. sphaeroides*, and this bacterium is an appropriate vehicle for redesign of photosynthesis, for example, by creating hybrid photosystems, and its versatile metabolism forms a basis for future biosynthetic, biomass or biofuel applications.

In 1984, Prof Hunter was appointed to a Lectureship at Imperial College, London, attached to the group of Prof James Barber FRS. He then returned to his native Yorkshire in 1988 to a Senior Lectureship at Sheffield University. In 1996, Prof Hunter was awarded a DSc by Bristol University, and he was elected to the Fellowship of the Royal Society in 2009. He is now the Krebs Professor of Biochemistry at Sheffield, and he continues to apply a combination of molecular genetic, biochemical, structural and spectroscopic approaches to dissect the pathways for bacteriochlorophyll and carotenoid biosynthesis, and to investigate the assembly, structure, function and organisation of bacterial photosynthetic membranes.

The central motivation for Prof Hunter's work is the global importance of photosynthesis as

the ultimate source of oxygen, all food and most energy resources on Earth. Sunlight is a diffuse energy source, so billions of tonnes of chlorophylls have to be synthesised every year on land and in the oceans to collect this energy. Pigmented membranes proliferate within chloroplasts and single bacterial cells, each one housing an extensive network of light-harvesting complexes that form an antenna consisting of many hundreds of pigment molecules. Solar energy harvested by this antenna is channelled towards specialised chlorophyll-protein complexes called reaction centres, initiating a series of electron transfer reactions that capture some of the solar energy prior to its storage in a chemical form that powers the metabolism of the cell.

The 3D structures of light-harvesting and reaction centre complexes have revealed the internal arrangements of chlorophyll-protein complexes that foster efficient solar energy harvesting and charge separation. Prof Hunter used atomic force microscopy (AFM) to show how the supramolecular organisation of light harvesting and reaction centre complexes creates extensive membrane networks for light harvesting and energy trapping. Nanoscale maps of these membrane networks, together with the 3D structures of reaction centre and antenna complexes determined by X-ray crystallography, allowed computation of atomic-level models of whole membrane assemblies (Figure 1 A and B) that predict energy transfer and trapping behaviour and identify desirable design motifs for artificial photosynthetic systems. In separate work, new surface chemistries and nanoscale patterning methods have been developed for surface fabrication of artificial light-harvesting and charge separating arrays on gold, silicon and other substrates (Figure 1 C). This approach is being extended to include *de novo* designed proteins called maquettes, the brainchild of Prof Hunter's collaborator Prof Leslie Dutton FRS. Prof Hunter believes that assemblies of native and man-designed proteins will advance our understanding of natural energy-converting systems, and will guide the design and production of devices for capturing and storing solar energy. One day, next-generation biohybrid structures could incorporate principles familiar in biology such as self-organisation, self-repair, redundancy and tolerance of failure.

The Hunter research team seeks to answer many other scientific questions in this field. For instance, how are cofactors delivered to photosystem apoproteins, and how are chlorophyll-protein complexes folded and assembled within the membrane bilayer? Can we test our understanding of these processes by engineering new combinations of pigments and proteins *in vivo*? Can native and non-native pigments be used, along with naturally evolved or artificially designed polypeptide sequences, to create new organisms for harvesting and trapping solar energy? Rapid advances in genomics and metagenomics, structural biology, spectroscopy, and computation are coming together to create new opportunities in photosynthesis research that will enable the construction of proteins and organisms with new capabilities for biological light capture and energy storage.

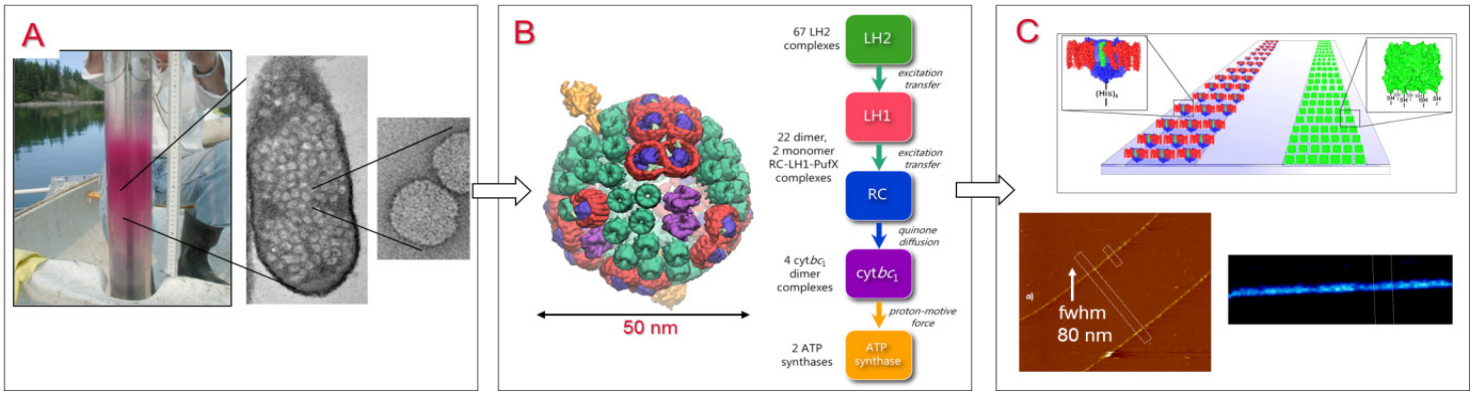

Figure 1: (A) Photosynthetic bacteria grow in stratified layers; the cells contain hundreds of membrane vesicles that convert light to adenosine triphosphate (ATP). (B) 1.9 million atom vesicle model derived from X-ray crystallography of the complexes and atomic force microscopy (AFM) of the membranes, comprising 67 LH2 complexes (green), 11 LH1-RC-PufX dimers & 2 RC-LH1-PufX monomers (blue/red), 4 cytbc_1 dimers (magenta), and 2 ATP synthases (orange). The diagram depicts the interconversion of harvested solar energy, eventually forming ATP. (C) (top) Concept for nanolithography to create surface-immobilised arrays of LH2 complexes (green) and dimeric RC-LH1-PufX complexes (red/blue); (bottom) atomic force microscopy of a surface-fabrocated LH2 nanoline and fluorescence (inset) of an 80 nm wide LH2 line. Nanofabrication allows new energy transfer geometries to be explored that are inaccessible through genetic or biochemical methods.

Regulation of cytokine activity in the extracellular matrix

by
Prof Penny Handford

For many years the extracellular matrix (ECM) of animals was thought to be an inert insoluble polymer comprising proteins and glycosaminoglycans that gave tensile strength and resilience to tissues. While undoubtedly imparting biophysical properties to connective tissues such as bone, tendon and ligament, the ECM is increasingly recognised as a modulator of cell function through specific binding of growth factors and integrins. My group has been studying the fibrillin/LTBP family of ECM proteins and the mechanisms by which they bind and regulate growth factor activity. The identification of human fibrillin-1 (*FBN1*) gene mutations in Marfan syndrome (MFS) as well as in the acromelic dysplasias and related stiff skin syndrome (SSS), which have strikingly different phenotypes to MFS, shows the variable functions performed by this extracellular matrix protein in connective tissues. The existence of such heterogeneity in patients suggests that understanding the complex molecular mechanisms operating in these diseases will lead to new insights into matrix and growth factor regulation and the development of novel therapies.

The fibrillins and functional insights from genetic disorders

There are three fibrillins (fibrillin-1, fibrillin-2 and fibrillin-3) and four LTBPs (latent transforming growth factor beta binding proteins) in humans, all of which share a related domain architecture (1). Fibrillin-1 is a 350 kDa calcium-binding protein that is a major component of 10–12 nm microfibrils in the ECM. These microfibrils may occur independently as networks, or form the periphery of the elastic fibre, where they act as a scaffold for the assembly and cross-linking of elastin. Sequence analysis of simple (*Cnidaria*) and complex (human) species demonstrates striking conservation of fibrillin domain organisation, suggesting spatial arrangement is important for microfibril assembly. Furthermore, rotary shadowing electron microscopy (EM) has demonstrated similar beaded structures for extracted microfibrils from jellyfish and human tissue. The vast majority of heritable FBN1 mutations result in classical Marfan syndrome (MFS), an autosomal dominant common connective tissue characterised by skeletal, ocular, cardiovascular and joint defects. More recently, identification of a subset of FBN1 mutations in patients with acromelic dysplasias (2), which have clinical features almost the opposite of MFS (short stature, joint stiffness and skin thickening) as a result of excessive ECM production, has revealed new tissue-specific roles for microfibrils (Figure 1). Intriguingly, although the phenotypes associated with the various fibrillinopathies are quite different, they all appear to lead to TGFβ dysregulation, where too much active TGFβ is present in tissues.

The major factor in MFS that results in increased TGFβ activation and signalling is thought to be due to the reduced ability of microfibrils to bind the LTBP-TGFβ complex. The LTBPs bind specifically to the N-terminal domains of fibrillin via their C-terminal region. In the ECM, the small latent complex of TGFβ (bound to its latency-associated peptide, or LAP) is anchored by covalent attachment of LAP to specific domains within LTBP-1, -3 and -4. In recombinant studies, fibrillin also interacts directly at distinct sites with BMP and GDF growth factors, but the contribution to the MFS phenotype of disrupting these interactions is less clear-cut than in the case of TGFβ. Thus, the assembled form of fibrillin, i.e. the 10–12 nm microfibril, acts as a major reservoir or docking station for latent cytokines, as well as performing a structural role within connective tissues (Figure 1). Various FBN1 mouse models, expressing lower levels or a defective form of fibrillin-1, exhibit developmental defects associated with TGFβ dysregulation (defective lung septation), and cardiovascular and skeletal manifestations which are similar to those seen in MFS (aortic dilatation and dissection and overgrowth of the long bones). The phenotypic features seen in these mice can be rescued to a large extent by infusion of an anti-TGFβ antibody in the perinatal period, or by administration of Losartan, a licensed drug known to inhibit TGFβ signalling. This suggests that a loss of microfibrils leads to too much TGFβ activation (3, 4). Further strong evidence for the fibrillin/MFS/TGFβ link comes from another genetic disorder, Loeys-Dietz syndrome, which is caused by mutations in TGFβ receptors (TGFBR) I and II and has a similar, but not identical, phenotype to MFS.

Whilst the fibrillin/MFS/TGFβ story was developing, the genetic basis for a distinct set of connective tissue disorders, the acromelic dysplasias and the related stiff skin syndrome (SSS), was reported in separate studies (5, 6). The autosomal dominant forms of these diseases were also found to be attributable to FBN1 mutations, however unlike MFS, the majority were found to be missense mutations affecting two protein domains of fibrillin only. My laboratory has been investigating the effect of these mutations on domain structure, fibrillin biosynthesis and microfibril assembly utilising a variety of molecular and cellular techniques to unravel the very different mechanisms operating in these diseases.

Fibrillin-1 structure

We have been able to solve the high resolution structures of the major domain types in fibrillin-1 including calcium-binding epidermal growth factor-like (cbEGF), latent transforming growth factor beta binding protein-like (TB), hybrid, fibrillin unique N-terminus (FUN) and EGF domains, utilising either NMR or crystallographic techniques (7, 8) (Figure 2). We were also able to study

the calcium-binding properties of the cbEGF domain and observed a range of affinities associated with this domain, which were dependent upon domain context. These data have allowed us to model the native structure of fibrillin-1 and suggest ways in which the microfibril may be assembled. Our structural data, combined with antibody mapping data of microfibrils and EM data suggest a staggered model for assembly (Figure 2).

MFS missense mutations predominantly affect domain structure

The most common types of MFS missense mutation are those affecting cbEGF domains, specifically conserved cysteine residues important for stabilising the domain fold, and those affecting calcium-binding consensus residues important for maintaining calcium binding and imparting rigidity to domain/domain interfaces. These destabilising mutations were found to have a variety of effects on recombinant fibrillin structure, biosynthesis and secretion, suggesting that a reduction in the quantity and/or quality of microfibrils would result in the ECM (9). These data, together with the observation that ~25% of MFS mutations result in premature termination codons and haploinsufficiency, suggest that a quantitative loss of microfibrils in the ECM results in MFS. The proposed explanation for the observed TGFβ dysregulation is either there are not sufficient binding sites for the LTBP/TGFβ complex, and/or the mechanical properties of a fibrillin microfibril-deficient matrix are not sufficient to allow force-mediated activation of latent TGFβ.

Many acromelic dysplasia/stiff skin syndrome mutations are also structural

The majority of mutations associated with these disorders are highly restricted to two specific domains within fibrillin-1 (TB4 and TB5). TB domains are the second most common domain type in fibrillin (seven in total) and, like EGF domains, are disulphide-rich. They have a globular fold with a hydrophobic core and in most cases pack against a C-terminal cbEGF domain to enhance calcium-binding affinity and create a rigid domain/domain interface. In addition, TB4 contains a functional integrin-binding site, while TB5 is postulated to contain a heparin-binding site. Examination of the types of missense mutations that give rise to the acromelic dysplasias and related disorders, rather than MFS, perhaps surprisingly does not give an obvious clue to the molecular mechanisms underlying these very different diseases. An example is the case of residue C1719, which is involved in stabilising the TB5 domain fold via a conserved disulphide linkage. Substitution of this cysteine residue by tryptophan (C1719W) gives rise to acromelic dysplasia whilst substitution by tyrosine (C1719Y) causes Marfan syndrome! Similarly, in domain TB4 C1564S gives rise to SSS, while C1564Y gives rise to MFS.

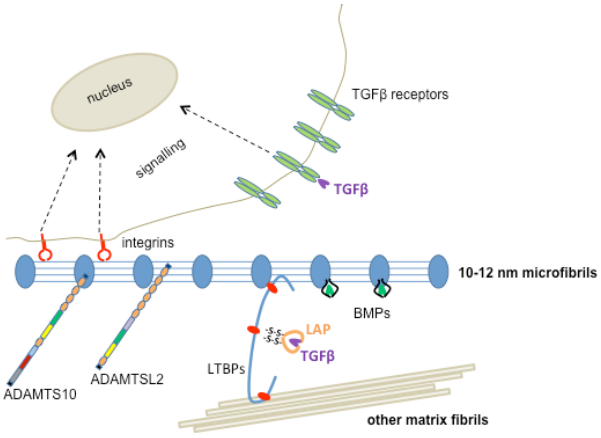

Figure 1: Fibrillin microfibrils are involved in multiple cell-matrix interactions and affect signalling either directly through interactions with cell-surface proteins such as integrins, or indirectly through the regulation of growth factors such as BMPs and TGFβ in the ECM. Interactions with other proteins, such as members of the ADAMTS(L) family of proteins, also affect the regulation of growth factors through unknown mechanisms.

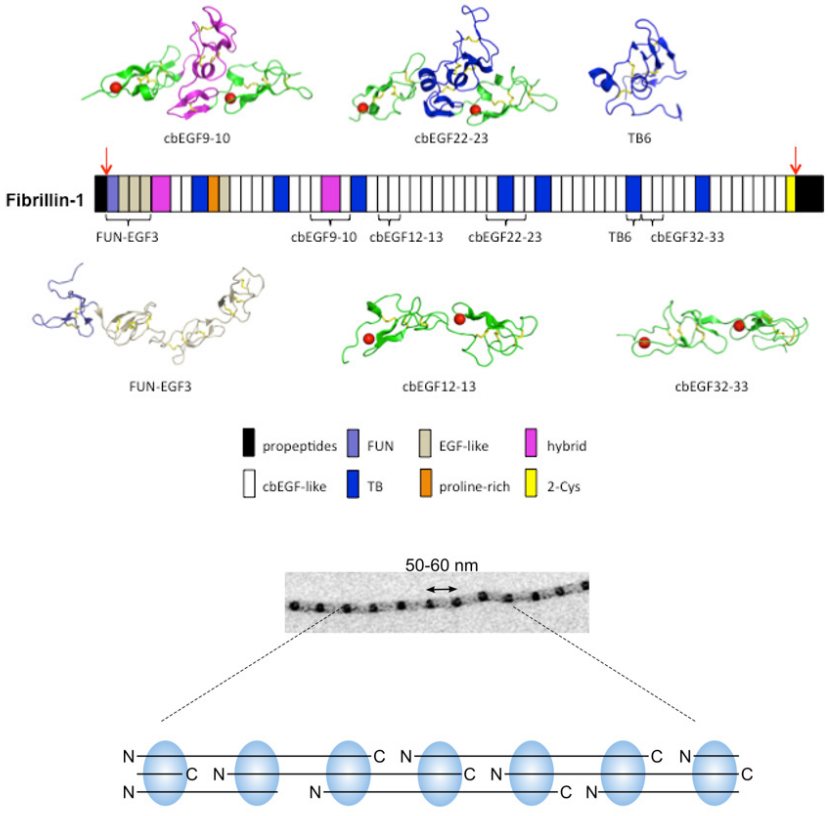

Figure 2: Structures have been determined for most fibrillin-1 domains including the cbEGF, TB, hybrid and fibrillin unique N-terminal (FUN) domain. When viewed by rotary shadowing electron microscopy, isolated microfibrils appear as beaded filaments with a 50 - 60 nm periodicity. Our high resolution structural data support a model of microfibril organisation in which individual fibrillin monomers have a near-linear conformation spanning at least 2 interbead distances.

Development of new assays for microfibril assembly

In order to address this puzzle, Dr Sacha Jensen in my laboratory set out to design a new assay to look at the incorporation of mutant fibrillins into microfibrils, alongside assessing their secretion profiles. He designed a GFP-fibrillin-1 fusion construct based on information obtained from the NMR structure of the FUN-EGF3 N-terminal fragment of fibrillin-1 (determined by David Yadin and Christina Redfield), and used a Gly rich region as the insertion site so as not to perturb the FUN fold or microfibril assembly (10). Using transient transfected HEK293T cells, expressing the GFP-fibrillin-1, co-cultured with human fibroblasts which produce endogenous fibrillin and are able to assemble microfibrils, he was able to track incorporation of the normal fusion protein into microfibrils (Figure 3). What happened when he assayed GFP-fibrillins with various disease-causing mutations in his co-culture assay? The GFP-fibrillins containing acromelic dysplasia (TB5) or related SSS mutations (TB4) showed clear evidence of secretion from cells and incorporation into microfibrils, while MFS variants were not present in the extracellular medium, and did not therefore incorporate into microfibrils (Figure 3).

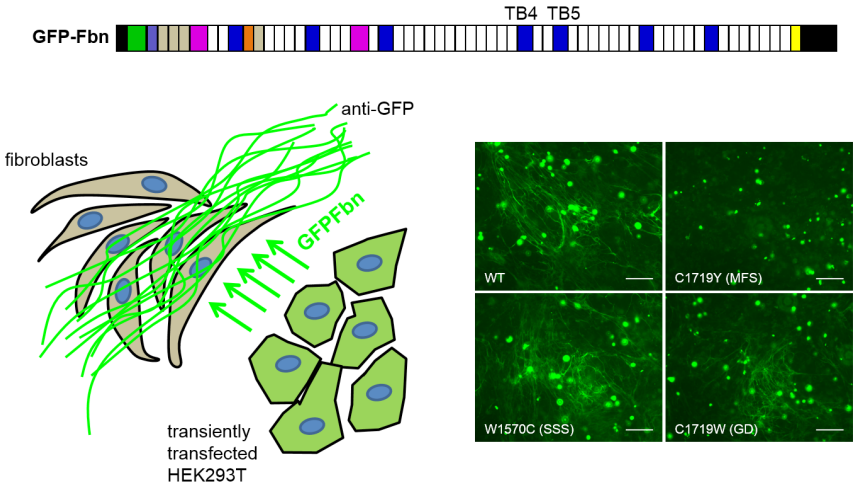

Figure 3: To track the incorporation of fibrillin-1 into the extracellular matrix, a GFP-tagged version of fibrillin-1 was created in which the GFP sequence was placed after the N-terminal propeptide. Co-culture assays combining human dermal fibroblasts with transiently transfected HEK293T cells expressing GFP-Fibrillin-1 variants were used to assess the incorporation of fibrillin-1 variants. TB domain mutants causing MFS resulted in a lack of matrix incorporation while mutants that caused stiff skin syndrome or acromelic dysplasias incorporated like the wild type protein.

This was not due to cell type-specific differences, since fibroblasts as well as HEK293T cells transfected with mutant fibrillin constructs showed similar secretion profiles (11). Therefore the simple explanation for the different phenotypes associated with TB4 and TB5 mutations is that MFS arises by functional haploinsufficiency with retention of the mutant fibrillin-1 in the endoplasmic reticulum (and therefore a quantitative loss of microfibrils in the ECM). In contrast, SSS and the acromelic dysplasias are caused not by a deficiency of fibrillin and microfibrils in the ECM, but by altered microfibril interactions with other cell-matrix components. Given the presence of an integrin-binding site in TB4 and a heparin-binding site in TB5, we hypothesise that altered microfibril/cell interactions underlie the acromelic dysplasia/SSS phenotypes. Mouse data support this idea since a fibrillin TB4 mouse model with an RGE mutation in the integrin-binding motif has been created, and recapitulates aspects of the SSS phenotype, directly linking defective integrin-binding in TB4 with excessive matrix production (12).

Questions remaining

These, and other studies, suggest that we need to look closely at the properties of a fibrillin deficient matrix to examine what happens to the latency of TGFβ. Dr Ian Robertson (with Christina Redfield) is looking closely at the structures of the fibrillin/LTBP interaction domains, to see if this binding event might modulate TGFβ activation (the small latent complex is bound adjacent to this site). In parallel he is developing a TGFβ reporter assay to test his structural predictions. Dr Sacha Jensen is creating various CRISPR knockouts in fibroblast cells to allow us to design ECM with different deficiencies in the fibrillins and LTBPs. A long term goal is to create fibroblast cells expressing homozygous fibrillin/LTBP variants to examine how the cell response may change in the absence of a normal fibrillin signal. Could this lead to an upregulation in the TGFβ production by cells? The next few years should prove to be exciting times in ECM research, with new fundamental mechanistic insight into matrix regulation as well as understanding pathogenesis of connective tissue disorders.

*Prof Penny Handford is a University Lecturer in the Department of Biochemistry.**The work presented is funded by Arthritis Research UK and the Medical Research Council.*

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In the loop: Transcriptional regulation in breast cancer

by
Natalie Ng

The expression of many genes is precisely regulated in response to environmental cues via epigenetic control of transcription. Irregular upregulation of oncogene activity is a hallmark of breast cancer, which has the second highest mortality rate of UK females (24%). Evidence has emerged demonstrating that chromatin 'loops', areas in the genome that form a looping structure, are involved in long-range interactions. This article aims to provide insight into the involvement of chromatin loops in transcriptional regulation with specific examples of chromatin looping events and implications for breast cancer subtypes and resistance to therapy.

Normal cellular growth and function require precise gene expression mechanisms. Transcription, the first step in gene expression, is the process of copying DNA into RNA. Transcription in eukaryotic cells involves three RNA polymerases (I, II and III). RNA polymerase II (Pol II) binds to the core promoter located upstream of the transcription start site (TSS) to initiate transcription. *Cis*-acting regulatory sequences downstream of the core promoter, such as enhancers and insulators, increase and repress transcription respectively. Regulatory proteins called transcription factors (TFs) bind to regulatory DNA sequences on the gene to activate or, less commonly, repress gene expression. The eukaryotic genome is highly organised, with DNA being packaged with proteins called histones into a supercoiled three-dimensional structure known as chromatin. The structural properties of chromatin selectively restrict the access of TFs and polymerases to the DNA sequences where they bind. The action of chromatin remodelling complexes is required to 'open' the chromatin (the open form of chromatin is referred to as euchromatin), allowing TFs and Pol II to access the DNA. For instance, biochemical modification of histones which affect their positive charge, e.g. acetylation of lysine residues can disrupt the electrostatic charges between histones and DNA, thereby creating a less compact chromatin structure and 'opening' regions of DNA, allowing transcriptional machinery access. Thus, precise epigenetic mechanisms allow eukaryotes to fine tune transcriptional activity in response to intra- and extra-cellular signals.

Control of gene expression can occur locally, on a given gene's promoter, or over long genomic distances between promoters and enhancers. An enhancer can be thousands of base pairs away from its corresponding promoter, which raises the question: how do distal regulatory elements achieve specificity and cope with unwanted influence from other genes? Techniques developed to investigate chromosome conformation at high resolution reveal that chromatin loops juxtapose important *cis*-acting genetic sequences in long-range interactions, bringing the sequence into close proximity in 3D space. Recent data published by the ENCODE consortium suggested that long-range chromatin interactions are commonly observed for gene transcription regulation (1). Mechanistic details of how these loop-structures are directed are not completely understood. There is growing evidence that a protein complex called cohesin stabilises chromatin loops in long-range chromosomal interactions in addition to its well-described function in sister chromatid separation during mitosis. Cohesin is a multi-subunit, ring-shaped heterodimer comprised of two structural maintenance of chromosomes (SMC) proteins, Smc1 and Smc3, and two non-SMC proteins, Scc1 and Scc3. Cohesin associates with both CCCTC-binding factor (CTCF), which is a zinc-finger DNA-binding protein and transcriptional regulator, and the mediator complex, which is a central component in the pre-initiation complex (PIC), helping to regulate Pol II activity (Figure 1). Depletion of cohesin is linked to disruption of long-range promoter-enhancer interactions in thymocytes, in which the distortion of the chromatin architecture of the T cell receptor α locus affects regulatory elements over 80 kilobases (kb) (2). Furthermore, Schmidt and colleagues identified genomic sites that share binding of cohesin with the oestrogen receptor α (ERα), a nuclear transcription factor that is a major therapeutic target in ER-positive breast cancer patients (3). These examples demonstrate the association between cohesin and gene expression, however the causal relationship and molecular mechanisms remain to be understood. Breast cancers express various surface receptors such as the oestrogen receptor (ER), human epidermal

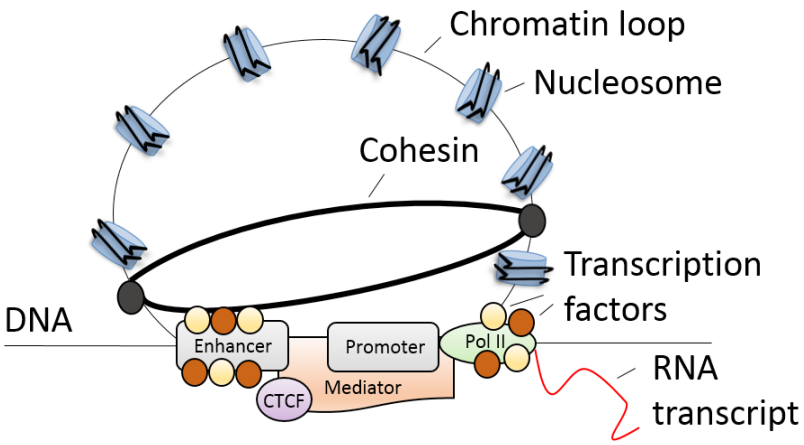

Figure 1: Cohesin has an important role in transcriptional regulation, stabilising chromatin loop formation in enhancer and promoter interactions. Mediator and CTCF associate at the promoter allowing the binding of transcription factors and transcription to take place.

growth factor 2 receptor (HER2) or progesterone receptor (PR). The expression level of each of these receptors conveys prognostic information and may be used to directly target such receptors with drugs known as antagonists, which bind to and inactivate receptor signalling. Some ERα-positive breast cancers do not respond to tamoxifen, an ER antagonist, while, interestingly, some tamoxifen-resistant ERα-positive breast cancers show vast regression with oestrogen (Figure 2). These obscurities are puzzling and imply that we do not understand enough about the effects of targeting these receptors in cancer. It is tempting to speculate that chromatin rearrangement might have an impact on breast cancer therapy. Defects in chromatin organisation are emerging as hallmarks of this disease. Indeed, long range chromosomal interactions in breast cancer cells were first observed in the distal binding site of ERα and the promoter of the *TFF1* gene. Genomic technologies such as Chromatin Interaction Analysis with Paired-End Tagging (CHIA-PET) have allowed the analysis of chromatin loops bound by ERα, highlighting genes that are misregulated and may lead to cancer pathogenesis.

It has been observed that depletion of the cohesin subunit Smc3 or the mediator subunit Med12 rapidly decreased the expression of *ERS1*, the gene that encodes ERα. This demonstrates that the co-localisation of Smc3 and Med12 on *ESR1* are required for Pol II occupancy, also facilitating long-range chromosomal interactions with enhancer sites (4). These observations indicate that *ERS1* is transcriptionally-dependent on the cohesin-mediator complex. Therefore finding ways to control ERα-chromatin looping may provide a treatment for oestrogen-dependent breast cancer. Observations from numerous studies imply that ERα is unlikely to act alone in mediating long-range chromatin looping and will require the addition of several augmentative protein factors in the maintenance of long-range interactions. For example Forkhead Box A1 (FOXA1) is an early factor in mediating ERα chromatin loops by initial recruitment to the receptor and co-localising with half the ERα binding sites in the breast cancer genome (5).

The *ErbB2* gene, which encodes the HER2 receptor, is overexpressed in 15% of breast cancers. The *ErbB2* amplicon on chromosome 17 comprises a core cluster of genes surrounding the *ErbB2* locus which are co-amplified and contribute to the growth of HER2-positive breast cancer cells. Therapies that target the HER2 receptor have greatly improved the outcome of HER2 positive breast cancer patients. Trastuzumab (Herceptin) is a monoclonal antibody that binds and blocks HER2 receptor signalling, which results in reduced growth in breast cancer. However, acquired resistance to HER2-targeted therapy often circumvents effective treatment.

The mechanisms of transcriptional regulation and amplification of the *ErbB2* amplicon remain elusive. A study by Mungamuri *et al.* highlights that epigenetic chromatin modifications enhance *ErbB2* gene overexpression (6). This, together with the association of cohesin in long-range chromosomal interactions in ER-positive breast cancers, suggests that transcriptional activation may operate in a similar fashion in different breast cancer subtypes. It will be interesting to study the role of epigenetics in the pathogenesis of HER2-positive breast cancers.

In summary, the topology of the chromosome in the nucleus can affect the expression of a given gene, acting as an additional regulatory layer. The role of cohesin as a stabiliser of chromatin loops is emerging as a pivotal contributor to the pathogenesis of breast cancer. Discovering and characterising the transcriptional behaviour of certain breast cancer subtypes may provide an insight into how cells respond to targeted therapies, and will begin to answer the perplexing question of how resistance arises, with the ultimate aim of improving the survival of breast cancer patients.

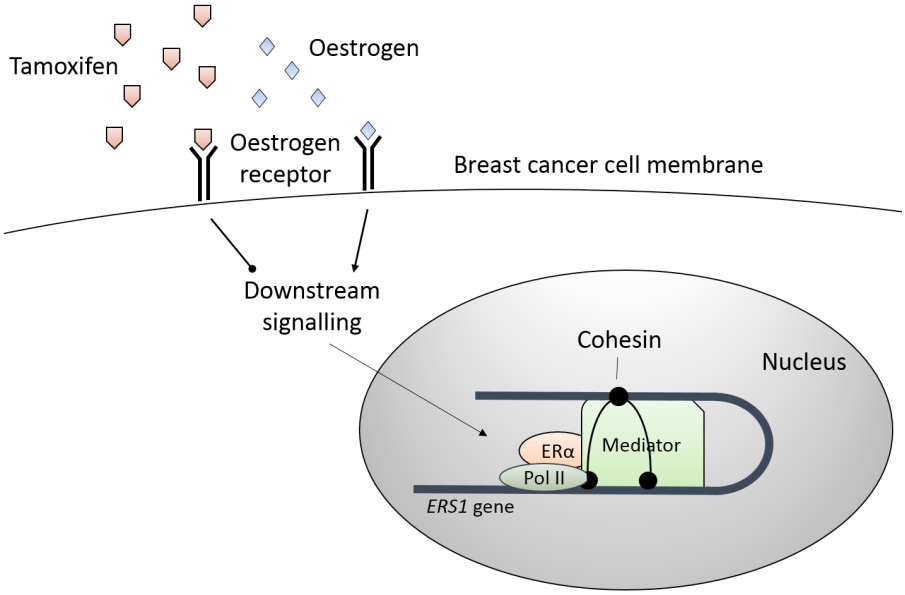

Figure 2: ERα-bound chromatin loops require the cohesin-mediator complex for transcription of the ERS1 gene. Tamoxifen competes with oestrogen for the oestrogen receptor on the breast cancer cell membrane, thereby disrupting downstream signalling and ERS1 gene activity.

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Balance is the key: The innate immune response to Influenza A viruses

by
Dr Suzanne Cole

Influenza A viruses (IAV) remain a constant global healthcare threat that can cause severe disease in healthy people without warning. This is largely because the host fails to generate long-term protective immunity as a result of frequent mutations in the viral genome. This is due to viral RNA polymerase, unlike the human DNA coding system, lacking a proofreading ability. The rapid mutation of IAV was demonstrated particularly well in the last flu season where the predominant H3N2 virus began mutating after vaccine preparation had begun. The resulting vaccine offered little or even no protection against the newly mutated strain. As a result, the ensuing disease may be more severe.

Disease severity upon IAV infection is determined by a multitude of factors including pre-existing illnesses, access to healthcare, pregnancy, prior vaccinations, and cross-protective immunity generated by previous exposure to different IAV strains. However, it is the innate immune system that is at the forefront of the response to IAV infection. The magnitude and effectiveness of the innate immune response play a large role in dictating the outcome of infection. IAV is first detected in the respiratory tract via respiratory epithelial cells, lung-resident dendritic cells (DCs), and macrophages that sense viral components. These sensing mechanisms are pattern recognition receptors; primarily retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), Toll-like receptors (TLRs) and nucleotide oligomerisation domain (NOD)-like receptors (NLRs). Viral sensing by these receptors will trigger the production of type I interferons (IFN) and pro-inflammatory cytokines.

> “The level of cytokine production can partly be dictated by the genetic makeup of IAV”

Type-I IFNs are the key antiviral cytokines. It has become apparent that excessive levels of type-I IFNs can lead to immune pathology in response to highly pathogenic IAV strains (1). This occurs as IAV-induced type-I IFNs mediate the production of pro-inflammatory cytokines and chemokines that recruit inflammatory cells to lungs. The subsequent cellular immune response comprises primarily of myeloid cells, of which monocytes are the most numerous, attracted in response to the chemokine MCP-1. Once monocytes are recruited to lungs, they can differentiate into macrophages and DCs. Macrophages phagocytose infected or dying cells while DCs process viral antigens and present them to T cells in lymph nodes, in order to prime the adaptive immune response. While blocking monocyte recruitment to lungs reduces the T cell response and delays viral clearance (2), excessive early monocyte recruitment also leads to severe immune pathology and more severe disease (3).

This process highlights a crucial balance where the same mediators can cause either beneficial or pathogenic effects depending on the magnitude of the response. The level of IFNs or cytokine production can partly be dictated by the genetic makeup of IAV strains. As IAV is a single-stranded RNA virus comprising only 8 segments, the role of each individual segment is starting to become clear, and adds depth to our knowledge of why certain strains are more pathogenic than others. The NS1 protein, for example, enables the virus to disable the host's type-I IFN defence system, and mutation of NS1 was shown to contribute to the increased virulence of avian H5N1 IAV strains.

The outcome of an individual to IAV infection is therefore likely to be determined by a fine balance of the types of innate immune mediators, such as IFNs, produced early in infection. This may differ in response to different IAV strains, and may furthermore affect individuals adversely depending on their health status prior to infection.

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HDAC inhibitors for curing HIV/AIDS

by Alba Libre

Today, an estimated 35 million people are infected with HIV worldwide (1), and according to the World Health Organisation, 'HIV/AIDS is one of the world's most important public health challenges' (1). Research during the past three decades has led to significant advances in treating HIV with drugs that block the production of further HIV particles by inhibiting viral replication. Antiretroviral therapy (ART) as it is called has been a turning point in the history of HIV/AIDS and is the current gold standard for HIV treatment. However, a prominent drawback of ART is its inability to target the latent HIV reservoir.

The latent HIV reservoir consists of a pool of non-replicating or 'latent' HIV particles that is established in a particular type of immune cell called the resting memory CD4+ T cell. In contrast to 'active' replicating HIV particles, the lack of viral replication inside resting memory CD4+ T cells means that latent HIV particles cannot be cleared by ART.

The latent HIV reservoir can have dire consequences for HIV-infected patients, because it maintains the HIV infection by converting to actively replicating form of the virus, so that when a course of ART ceases high levels of replicating HIV particles rapidly reappear. Thus, although ART is effective at blocking further HIV replication, it cannot completely eradicate the latent copies of the HIV genome, and this makes curing HIV/AIDS with ART an impossible task.

Our immune system has evolved an effective mechanism that enables it to clear a wide range of viral infections. Although we may suffer for a little while, our immune system is usually equipped to fight and overcome such infections as the common cold, influenza, viral gastroenteritis, etc. The successful immune-mediated clearance of these viral infections relies on the recognition and targeting of virally-infected cells by the immune system. However, the lack of viral replication inside resting memory CD4+ T cells means that these cells are not recognised as virally-infected by the immune system. This raises the question, is it possible to activate latent copies of the HIV genome and thus make the resting memory CD4+ T cells detactable by the immune system?

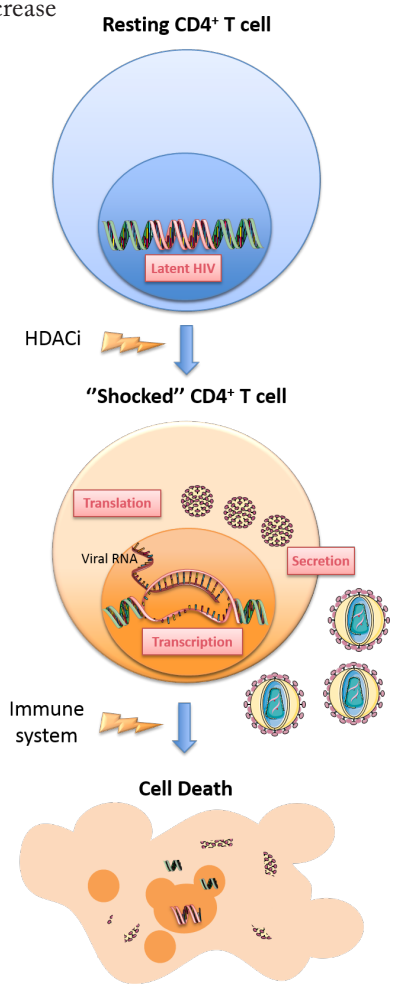
To answer this question, researchers are turning to histone deacetylase (HDAC) inhibitors, which block the removal of acetyl groups from histones. Histones are positively charged proteins that become closely associated with copies of the HIV genome, which, like human DNA, happens to be negatively charged. And just like human DNA, the association of postively charged histones with the negatively charged HIV genome results in a condensed chromatin structure that does not allow replication. The removal of negatively charged acetyl groups from histones makes this association even stronger and, therefore, by reducing the strength of the association of histones with the viral genome, HDAC inhibitors can be used to decondense the HIV genome and activate viral replication.

But does this approach actually work? And, does it make HIV visible to the immune system?

Archin *et al.* tested resting memory CD4+ T cells from 16 HIV-infected patients with the HDAC inhibitor vorinostat (2). The amount of detectable HIV RNA increased significantly in 11 samples (69%) after treatment with the drug. Similar results have been seen with the HDAC inhibitor romidepsin (3). This novel therapeutic strategy has become known as "shock and kill" and, in theory, has the potential to be the very first cure for HIV/AIDS (4). However, there are several scientific and ethical concerns that must be considered. Firstly, to date, no study has shown a reduction in the latent HIV reservoir following increased HIV replication. Secondly, HDAC inhbitors may also increase the non-specific transcription of human genes, the consequences of which has not yet been explored. Thirdly, HDAC inhibitors have been shown to affect T cell function (5) and, alarmingly, increase susceptibility of uninfected CD4+ T cells to HIV infection (6).

Since the 1980s, when HIV was first identified, the progress made on treating HIV/AIDS has been outstanding. However, much rigorous research is still needed before HDAC inhibitors become a reality in HIV treatment. There is still much work to be done, but new approaches using HDAC inhibitors provide hope that a cure may be in sight.

Figure 1: Latent HIV reservoirs in resting memory CD4+ T cells can be activated by the addition of HDACis. Active viral replication and production of HIV particles occur in these 'shocked' cells, making them more susceptible to immune recognition and, potentially, killing. Image credit, Lisette Krabbendam.



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Have a sleep-in. It could save your job as well as your health

by Drew Duglan

It is midnight on the luminescent digital clock of your personal devices. "I just need to edit this paper, I can always catch up on sleep over the weekend". These are the excuses we give ourselves as today rolls into tomorrow and we're still soaked in blue electronic light, even as we climb under the duvet.

It's no wonder that we're getting less sleep than ever before. Indeed, recent reports from the UK Sleep Council state that a third of the population are subsisting on only 5 to 6 hours of sleep per night. This finding is mirrored by the Centre for Disease Control and Prevention (CDC) in the US. This permanent sleep deprivation almost seems to be an inevitable product of our modern society, where competition for employment is greater, funding in several sectors has diminished, and commute times are longer. Add to this the huge technological advances in consumer electronics, and we have the perfect excuse for continuing to work when we're at home, away on vacation or right before bed. This pattern of working harder and resting less has now embedded itself as a meme in our everyday world, where phrases such as 'just sleep faster' or 'I can sleep when I'm dead' are aimed at motivating us to professional success, even if it's at the expense of our long-term health.

> "poor sleep quality was associated with shrinkage and atrophy of key regions of the cortex"

Lack of sleep is associated with a number of detrimental conditions, such as chronic inflammation, depression, and obesity, as well as increasing our overall risk for developing type 2 diabetes and cardiovascular disease. What's even more worrying is that recent evidence now suggests poor sleep patterns could be linked with permanent decreases in brain volume. A study recently conducted by Dr Claire Sexton and colleagues at the Oxford Centre for Functional Magnetic Resonance Imaging (MRIf) assessed sleep quality in nearly 150 adults aged between 20 and 84 years of age. MRIf brain scans of these individuals demonstrated that over 3 to 5 years, poor sleep quality was associated with shrinkage and atrophy of key regions of the cortex (1). These regions are involved in a whole host of functions ranging from reasoning and problem-solving, to memory and perception, as well as actions like movement and speech. Although the authors of this study responsibly acknowledge that poor sleep quality may not have directly caused the changes in brain volume, the findings are in line with other recent work linking sleep disturbance in adolescents to poorer academic performance in schools (2).

It is tempting, and yet troubling, to believe that an irregular sleep pattern beginning in our critical teenage years could initiate a number of structural changes in the brain that may ultimately determine our levels of cognition, and thus our social and occupational abilities further down the road. While much more research into this area is required, it is already becoming clear that sleep is a key component of our lives that is often overlooked. A large part of this could stem from our scientific struggle to unravel the complexities of sleep and the role it plays in maintaining our overall health and well-being. But perhaps the other, much larger part can be attributed to this notion of sleep as lost time, better spent performing tasks with more tangible outcomes.

The strange irony in all of this is that our dedication to working late into the night could well be triggering a biological cascade that makes us far less capable at our jobs over the long-term. Of course, in a world of e-commerce, endless social media platforms and TV on-demand, work is far from being the only distraction. Hopefully innovative studies like these will help to provoke a gradual shift in attitudes, where we instead see sleep as a crucial ingredient for performance and improvement, whether that is in our working lives, in recreational activities, or in the formation and support of meaningful relationships.

Drew Duglan is a final year DPhil student in Cardiovascular Medicine in Professor Barbara Casadei's group

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Interactions of DNA methyltransferases with histone tails

DNA methylation is a key epigenetic mechanism with important roles in cellular events (cell differentiation and X-chromosome inactivation) and also pathological events (carcinogenesis). In mammals, DNA is typically methylated on cytosines in the context of CpG dinucleotides by enzymes called DNA methyltransferases (DNMTs). DNMTs work alone or in combination with other DNMTs. However, the exact mechanism by which the enzymes locate at specific parts of the genome is still not known in detail.

by Dr Burcu Anilkirmizitas

DNA methylation patterns vary between cell cycle phase, cell type, chromosomal location and are determined by the sequence of the gene to be methylated. Research in the Brockdorff lab is aimed at understanding the molecular details of the mechanism by which DNMTs target histones. We use biophysical and structural biology methods to directly answer two main questions: "Do DNMTs recognise specific histone modifications directly?" and "How do the intra- and inter-molecular interactions of DNMTs affect their function and targeting?"

The two main types of DNMTs are DNMT3 and DNMT1. DNMT1 is mainly responsible for the maintenance of DNA methylation, whereas DNMT3s establish *de novo* methylation. There are two different catalytically active DNMT3s, DNMT3A and DNMT3B. Various isoforms of these enzymes methylate CpGs that have not been previously methylated. DNMT3L is the third and last protein that belongs to the DNMT3 family. DNMT3L is not catalytically active but enhances DNMT3A and DNMT3B activity.

Lysine methylation on histone tails is one of the most widely studied epigenetic modifications. Histones are proteins that assemble into octamers around which DNA is coiled to create the nucleosome. So-called 'histone tails' protrude from the nucleosome and are subject to many different kinds of modifications. Previous studies have shown that DNMT3A recognises histone H3 at its tail region when it is not methylated on lysine-4 (1).

The ATRX-DNMT3-DNMT3L (ADD) domain of DNMT3A is responsible for its ability to recognise histone tails, and the same may be true of DNMT3B. There are, however, reasons to believe that DNMT3A and DNMT3B might also recognise specific histone H3 lysine methylation marks. Firstly, this is because both proteins contain a domain (the PWWP domain) that can recognise H3 lysine methylation marks. PWWP domains belong to the Tudor domain family of proteins that contain aromatic cages which provide binding pockets for methylated lysines. Secondly, both proteins are found in association with modifications such as H3K9me3 and H3K36me3. H3K9me3 has a repressive role on the genome whereas H3K36me3 is found in gene bodies of actively transcribed genes. (2, 3).

A recent study showed that the catalytic domain of DNMT3A is blocked when the ADD domain is in its default conformation, rendering the protein inactive (4). However, DMNT3A can become active again when it encounters an unmethylated H3 histone tail on a nucleosome, through the binding of the ADD domain to the histone tail (Figure 1).

Both DNMT3A and DNMT3B oligomerise *in vitro*. The multimeric species formed by both proteins can range from dimers to octamers. DNA methylation occurs in a processive manner with DNMT3 molecules arranging themselves as multimers on DNA. However, whether oligomerisation occurs *in vivo* is not yet known.

Mutations of DNMT3A and DNMT3B are widely detected in different types of cancer such as acute myeloid leukemia (AML) and lymphomas. Consequently, information about DNMT3A and DNMT3B at the atomic level is essential for tackling these diseases, as it will provide the means for designing drugs that target these proteins. Needless to say, we may also gain further mechanistic insight of the important role these enzymes play in normal cellular events and pathological ones, such as carcinogenesis.

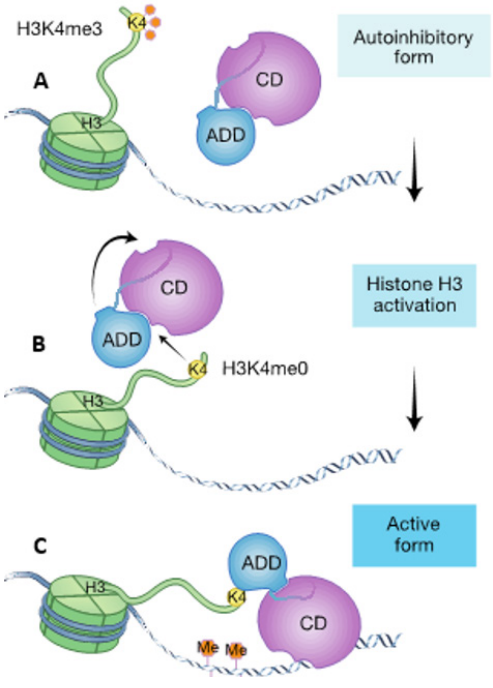

Figure 1: The prevailing model for the autoinhibitory mechanism of DNMT3A as it encounters its nucleosome substrate. (A) Dnmt3A in auto-inhibitory conformation, (B) ADD domain of Dnmt3A recognises unmethylated H3 tail, and (C) the active form of the enzyme docks onto DNA via its catalytic domain and DNA methylation is initiated. Adapted with permission from (4).

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Burcu Anilkirmizitas is a Postdoctoral Research Associate in the Brockdorff Lab at the Department of Biochemistry

Career Insights: Medical Writing

by Dr Elizabeth Hartfield

As an aspiring scientist, I was always certain that I would spend my career in the laboratory, working on cutting-edge research and helping to contribute to healthcare breakthroughs. After 4 years of postdoctoral research, I was running out of steam and, surprisingly, I found myself more interested in reading and writing papers than in working at the bench. I started writing for *Phenotype* and progressed to the position of Section Editor. I realized that I preferred writing about science to doing it!

When I first heard about medical communications, it was an unknown and mysterious field to me. I went to a networking event run by the University and met with writers from over 20 different companies. I was keen to learn more about medical communications, so I enrolled in a 2-day workshop in which we learned what it was like to work in this industry directly from those involved. This convinced me that I would be well suited to a career in medical writing and, more importantly, that I would enjoy it. I wrote to Oxford PharmaGenesis to enquire about opportunities and was sent a writing test to complete at home. I was then invited for an interview, where I met team leaders from several groups. After a second interview, I was very pleased to be offered the position of Trainee Medical Writer.

It was a difficult decision to leave scientific research, but since joining Oxford PharmaGenesis I have not looked back! I have been involved in many different projects in different disease areas. The work is varied and it is definitely not a case of writing research manuscripts all day. We work on a wide range of materials, including slide decks, training and patient education materials, product monographs, systematic reviews, regulatory material and more. I still feel that I am involved in helping patients by enabling high-quality clinical research to be published in peer-reviewed journals. Furthermore, we are involved in developing evidence-based policy initiatives with an aim of improving global health. This requires political as well as scientific

and economic insight, and therefore involves a holistic approach to HealthScience. We must take responsibility for what we write and follow the International Society for Medical Publication Professionals (ISMPP) Good Publication Practice Guidelines to ensure that ethical practices are followed when reporting medical research sponsored by pharmaceutical companies.

I was surprised at how easy the transition from bench scientist to writer has been; I am very well supported by my team and learn new skills every day. The skills that you have already acquired in the laboratory can be applied to writing: attention to detail, literature research and critical evaluation of data are all important. Learning about a variety of diseases and exciting new products, as well as knowing that our work makes a difference, definitely keeps me interested and motivated.

If you are looking for a career in which you are involved in cutting-edge science without having to do the practical work, I would highly recommend medical writing. Oxford PharmaGenesis offers a supportive training environment in which you learn on the job and are part of the team from day one.

Dr Elizabeth Hartfield is a Medical Writer at Oxford PharmaGenesis

A career as an editor

Another role that enables you to use your scientific knowledge is that of an editor. This is a very varied role, with involvement in the editing, design, typesetting and printing stages of projects. At Oxford PharmaGenesis, editors add polish to the content and enhance the appearance of a wide variety of publication types, and work across many different therapy areas. If you have a passion for science, an aptitude for editing, a keen eye for detail and an interest in design, this may be the role for you!

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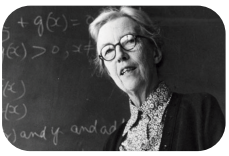

Mary L. Cartwright


Stephanie Kwolek


Virginia Apgar


Rosalind Franklin

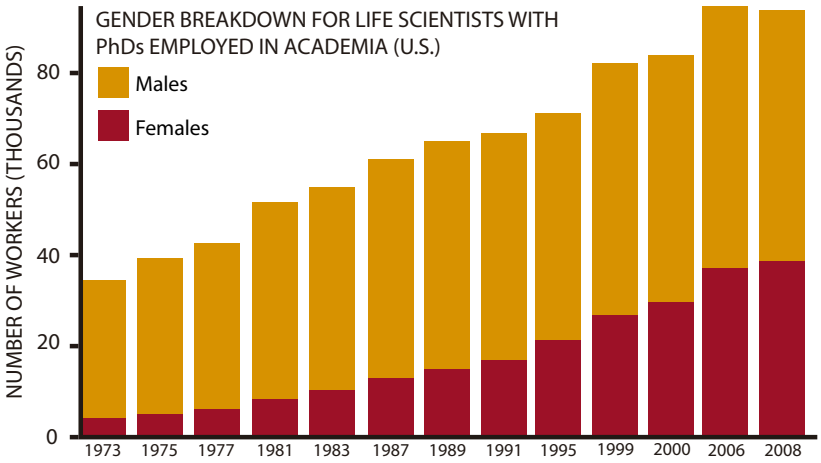

Barbara Hohn


Angela Vincent


Susan Lindquist

Bridging the Gap

Gender inequality in the number of doctorate graduates in academia and their income disparity.



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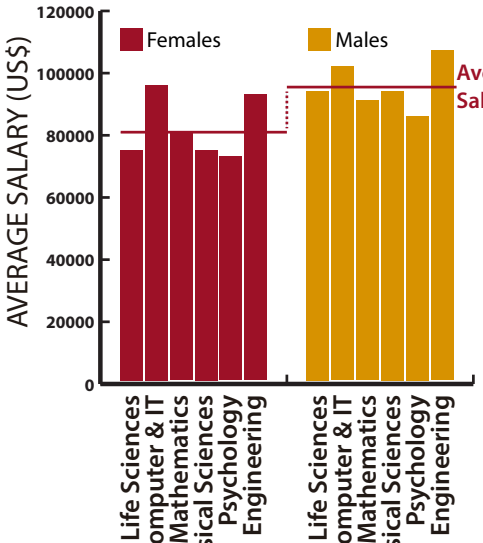

Studies demonstrate that improvements are being made to address the gender bias associated with hiring and funding women, though more still needs to be done to prevent women from being discouraged by careers in Science, Technology, Engineering and Medicine (STEM).


Sonia Kovalevsky


Sally Ride

Issues identified women in a survey:

Prove it again: having to repeatedly prove themselves as their successes were discounted and expertise questioned.

Isolation: Lack of mentors and role models as well as lack of other females at a similar career stage.

The tightrope: being seen as too feminine to be competent or too masculine to be likeable.

The maternal wall: upon the decision to have a child, the commitment to their job was questioned and opportunities began to disappear.

*Adapted from Williams et al., 2015

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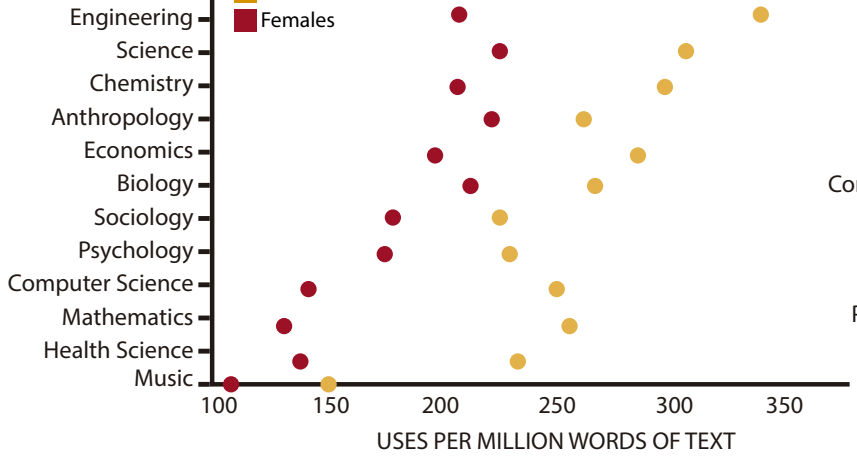
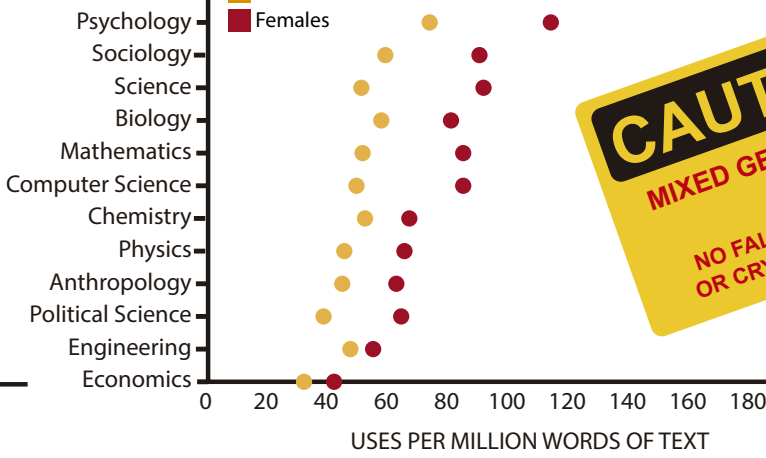
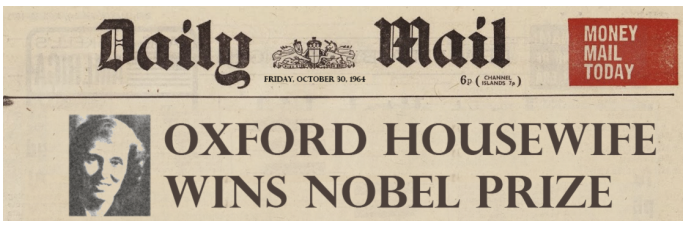
Ada Yonath


Nancy Roman






Dorothy Hodgkin



Gendered Language

A surprising number of words have a gender split which may reinforce particular stereotypes for the role of women in socie

* Student reviews of lecturers compiled by Ben Schmidt

** Data from ratemyprofessor.com

IENCE

r Cordero Llana

1 in 5 countries
as reached gender parity
5-55% of researchers are women)


Barbara McClintock


Louise Richardson


Magdalena Götz


Ida Noddack


Elsie Widdowson

0% of the
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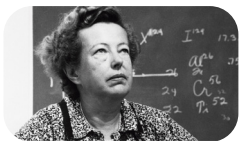
Maria Goeppert - Mayer


May-Britt Moser


Hypatia


Linda B. Buck


Rachel Carson


Elizabeth Blackburn


Gertrude B. Elion


Athene Donald


Gerty Cori


Jocelyn Bell Burnell


Dian Fossey


Mary Leakey


Shirley A. Jackson


Lise Meitner


Irène Joliot-Curie


Linda Partridge


Pallas Athena

"*It would probably also be beneficial to find one or two male biologists to work with (or at least obtain internal peer review from, but better yet as active co-authors), in order to serve as a possible check against interpretations that may sometimes be drifting too far away from empirical evidence into ideologically biased assumptions*"

PLOS ONE Reviewer

***www.nature.com/sexist-review-causes-twitter-storm-1.17457**

17%
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Marie Curie

47%
Early Post-Docs

"*Let me tell you about my trouble with girls… three things happen when they are in the lab… You fall in love with them, they fall in love with you and, when you criticise them, they cry*"

Sir Tim Hunt


Carol W. Greider


Sally Davies


Winifred Watkins


Pascale Cossart


Rosalyn Sussman -Yalow

The leaky pipeline
Gradual loss of women through the science career pathway
* Female:male ratio at a given stage
** UK specific data 2010 (*She Report*)
*** Across all sciences

46%
PhD Students


Joan A. Steitz

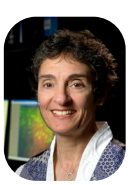

Bonnie Bassler


Sheina Marshall


Michal Schwartz


Ann Bishop


Kay Davies


Carla J. Shatz

TION
ENDER LAB!
LLING IN LOVE
YING PERMITED

Spotlight: Prof Elspeth Garman

I am 7 and I do my first experiment. My mother returns from the nearest town 20 miles away with a tube which produces red stripes on the outside of white toothpaste. I am very curious and ask if the stripes are already on the paste inside or if they appear as the paste comes out of the end. I get a non-committal 'adult' reply, so after dark I dissect the tube with scissors to find out. I make a big mess and get into trouble.

I am 11, I have failed the 11+ and I am at a Church of England Convent boarding school (St. Hilda's, sadly closed in 1995) in Whitby, Yorkshire. I am bottom in the exams for most subjects, with only Algebra left to go: the 'B' stream beckons. Two 'friends' lock me in the school library for 3 hours as a joke. All I have for entertainment is my Algebra book, and it occurs to me that I could read through it. I come top in the exam with 87% and thus cling to my A stream status and acquire a competitive urge.

I am 18 and have just failed the entrance exams to read Natural Sciences at Newnham College, Cambridge. I leave 6th Form to teach Maths and Science for 9 months in a large girls' secondary school in Manzini, Swaziland. I cover every subject on the curriculum except Zulu. I discover I am born to teach. This experience changes my life and eventually results in the amazing gift of a Swazi foster daughter, the orphaned child of one of my pupils.

I am 20 and in my second year at Durham University reading Physics. I work and play (row and sing) hard and love it. I go as a summer student for 15 weeks to CERN in Geneva and join the team determining the magnetic moment of the muon. I realise that not many women go into nuclear physics research, but decide I will be one of them. I go to Oxford to do a DPhil in low energy nuclear physics. I am trained to use the workshop (lathe, milling machines, etc) which has stood me in excellent stead ever since.

I am 24 and rowing for Osiris with OUWBC, having started a women's club at my College, Linacre. During my DPhil and the seven subsequent years as a Research Officer and College tutor in the Nuclear Physics Department, I tutor Physics at seven different Colleges. Having married my landlord, Dr John Barnett, I want to stay in Oxford, but Nuclear Physics research funding is starting to dry up.

I am 33 and while tutoring Physics at Somerville I am approached by Louise Johnson from the Laboratory of Molecular Biophysics (LMB), who asks 'What next?' I don't really want to think about this since I now have a two year old daughter and am prime carer for my 81 year old mother-in-law, who lives with us. However, she persists, and six weeks later I am working in LMB 66% of the time looking after their new X-ray generator and electronic detector and have changed fields to protein crystallography/structural biology.

After 12 years of looking after the LMB X-ray equipment, and with a second daughter (now 8) at home, I begin my own research group. I am lucky enough to work with a continuous stream of great graduate students, and together we make well recognised contributions to methods development for structural biology. I travel the world teaching, demonstrating and lecturing, and make many friends. In 2009 I am seconded for 5 years to the MPLS Division EPSRC funded Doctoral Training Centre as Director of first the Life Sciences Interface and then the Systems Biology Programmes: a wonderful interdisciplinary experience which resulted in four outstanding graduate students joining my group.

I have not regretted moving research fields for a second. Macromolecular crystallography requires intellectual contributions from many different fields of science. Despite now having a Senior Rail Pass, I am still learning and I feel fortunate to work with the next generation of inquisitive and enthusiastic young scientists.

For a BBC Radio 4 'Life Scientific' interview with Prof Elspeth Garman please see:

http://www.bbc.co.uk/programmes/b04kbjhg

Spotlight: Dr Sylvia McLain

@girlinterruptin

Water is ubiquitous to life, yet an understanding of how water contributes to biological structure and function is largely unknown. The overarching theme in my research group within the Department of Biochemistry is to understand how biological molecules work on the atomic scale when in solution, and how water contributes to biological function.

I came to Oxford, and my current position as an EPSRC Career Acceleration Fellow, via a somewhat circuitous route. It took me longer than usual to finish my undergraduate degree in Zoology (Tennessee, US), because while I was studying I had the opportunity to work in some unique scientific areas. I spent two years working as a fisheries technician for the Great Smoky Mountains National Park (Tennessee/North Carolina) and as a technician doing ecological river/stream surveys. I also worked in a variety of research laboratories within the Zoology Department, including a spider lab that I hoped might cure my arachnophobia (it didn't!).

After graduating, I worked for a few years as a white water rafting guide, and then subsequently for four years as a technician in an evolutionary genetics laboratory, investigating multiple paternity in snakes using microsatellite markers. The lab was transitioning from more traditional research into genetics, and I was responsible for leading this transition. It was a fun job for me, as I learned many new techniques and got to be a part of the beginning of a new research direction for an established laboratory.

However, I left my technician job in order to return to university to become a qualified secondary school teacher, and received an MSc in Education in 1999. As part of my training I taught physical science and biology in a US high school, but I also took a few courses in the Chemistry Department so that I could teach chemistry. I discovered that I really liked chemistry and was able to get a place as a graduate student in the department. As I didn't have all of the appropriate qualifications at the time, I spent the summer before entering my graduate studies learning undergraduate chemistry so that I could pass the examination to get into the program at the University of Tennessee. Thankfully, I did.

My PhD research focused on understanding the structure of superacids, and I also synthesized silver fluoride compounds with unique magnetic properties in highly reactive solvents – anhydrous HF and F_2. In order to do this, I had to design and build a gas/vacuum line to handle these compounds, which was challenging given their chemical properties. Part of my PhD research was also performed at Argonne National Laboratory (near Chicago, US), where I was lucky enough to be funded by them for the latter half of my research.

After completing my PhD, I was awarded a National Science Foundation International Postdoctoral Fellowship to work at the ISIS Facility, a world-leading centre for research in the physical and life sciences at the STFC Rutherford Appleton Laboratory near Oxford (UK), to investigate the role of hydration in the structure of simple biomolecules – amino acids and di-peptides. After three years at ISIS, I was awarded a fellowship to work at the newly built SNS neutron facility at Oak Ridge National Laboratory (ORNL) in the US. There I began investigating sugars and lipids in solution, with the aim of understanding the role of water in helping determine the conformation of these molecules. After a year and half at ORNL, I quit my job to immigrate back to the UK to get married. After returning to the UK, I was awarded a Wellcome Trust Value In People Fellowship through King's College London's Pharmacy Department, where I began work on membrane and peptide structure. I moved to Oxford in 2011 to form my own research group, thanks to an EPSRC Career Acceleration Fellowship.

I have been very lucky in being able to obtain funding to do research for most of my career, but I have definitely not been successful every time I applied for it – you just have to keep at it and try to stay as positive as you can.

Sylvia McLain is a biophysicist at the Department of Biochemisrtry. Sylvia regularly writes about science, science policy and philosophy for The Guardian (http://www.theguardian.com/profile/sylvia-mclain). You can also follow Sylvia on Twitter at @girlinterruptin.

Spotlight: Prof Judith Armitage FRS

My career in 650 words? I can probably write it in much less, as I've made few decisions and moved rarely. I decided I wanted to be a bacteriologist at school thanks to a charismatic biology teacher. I attended a small girl's grammar school in Yorkshire with limited ambition for its students, but this teacher encouraged me to think more ambitiously. The school only sent pupils to universities in Yorkshire or, at a push, Lancashire, but this was 1969 and I wanted somewhere different. I applied to UCL not because it was a leading university for biological sciences (which it was and is), but because it was in the centre of London and close to all the excitement.

At the end of a socially and academically stimulating first degree, I decided I liked research – formulating a hypothesis and testing it, not knowing what might happen. I applied for a PhD at Sussex and Oxford, but when I was offered a place to continue at UCL I chose that. I loved my PhD; I worked in a small group (two grad students and a technician) under David G. Smith and Robin Rowbury, and was allowed to develop my own project. There were ups and downs – realising there was a link between growth and migration in the bacterium I was interested in was a high point; someone throwing away three months' worth of lipopolysaccharide samples painstakingly isolated using boiling phenol was a low point. I wrote six papers during my PhD and when I finished was offered a UCL postdoctoral fellowship to continue for three more years. During this time I became increasingly interested in how bacteria swim. Part way through my fellowship Mike Evans' group moved to the next lab. They used photosynthetic bacteria to study the bioenergetics of photosynthesis. One bacterium fascinated me, *Rhodobacter sphaeroides*. It could grow aerobically, in which case light was toxic, or photosynthetically, when oxygen was toxic. A 'Friday night' experiment showed me that it swam towards light and away from oxygen when growing photosynthetically but vice versa if growing aerobically. That was the start of my next 30+ years of research, leading me to the analysis of sensory signalling in bacteria and their control of the activity of the flagellar motor.

With some preliminary data, after a short period with Bob Macnab at Yale, I applied for a Lister Institute Fellowship. This was 1982 and Lister was the first funding agency to offer five year grants that paid a salary and consumables. Four of us got grants in that first round, including the now Prof Sir Alec Jeffreys. I was now totally free to develop my own research direction. I applied for two permanent positions during this time. At my interview in London I was asked by the Dean "how do you combine housework with science?" My interview at Oxford for a University Lectureship (UL) in Microbiology, associated with St. Hilda's, however was rigorous and stimulating and involved no mention of housework. I expected Oxford to be a stepping stone to somewhere else, but I am still here! Where do you go to from Oxford?

I spent 10 years at St. Hilda's before resigning over their then unwillingness to become a mixed sex college, and discovered the 'market' for a female scientist UL. I moved to Merton where I have been ever since, first as a UL and from 2006 as a Professorial Fellow.

So, I have stayed in the same place and focussed on the same question. Why have I been reasonably successful? I think because I have approached the question with every tool available and always assumed that if someone else can do something, so can I. My research has been interdisciplinary from the beginning, allowing me to gain insights that would not have been possible by sticking to one approach. I have collaborated with physicists, engineers, mathematicians, structural biologists and statisticians to look at the problem from many different angles.

At 16 I decided to be a bacteriologist, at 64 I am still a bacteriologist. Maybe that makes me narrow minded, but I think it makes me unbelievably lucky.

Athena SWAN: Equality for all

by Brid Cronin

The Athena SWAN Charter was established in 2005 as a national scheme to encourage and recognise commitment to combatting the underrepresentation of women in science, technology, engineering, maths and medicine (STEMM) in higher education and research. In May 2015, the charter was extended to recognise work undertaken in other academic disciplines (arts, humanities, social sciences, business and law). Athena SWAN is based upon ten principles, with the core understanding that academia cannot reach its full potential unless it can benefit from the talents of all.

Going through the Athena SWAN application process gives the University and its departments the space to reflect upon, and celebrate, current organisational and cultural practices that promote gender equality, as well as identify areas for improvement. As part of the process, Departments are asked to reflect upon:

- The representation of women at all levels
- The progression of students into academia
- The support offered at key career stages
- The working environment for all staff

Receiving an Athena SWAN award does not mean that a department is perfect, but rather recognises a commitment to engaging with staff and students to improve their environment and the support available for their career progression and leadership aspirations. All of the departments in the Medical Sciences and Mathematical, Physical and Life Sciences Divisions hold an Athena SWAN award and have strategies in place that respond to their own particular issues. Departments have introduced a range of actions to improve practice in the areas of:

- Recruitment and selection
- Induction
- Career development
- Meeting the needs of carers
- Instilling a transparent and friendly workplace

Although this activity has primarily focused on addressing the disadvantages faced by women, Athena SWAN has offered a valuable framework for introducing cultural changes that create a better, more inclusive working environment for all.

At Oxford, there are ongoing Athena SWAN activities at all levels of the University. A central Athena SWAN and Gender Advisory Group established an institutional Bronze action plan in consultation with staff and students. In the last couple of years this has resulted in:

- New procedures for recruitment of Statutory Professors
- Agreeing gender equality targets as a public statement of our commitment to increasing the proportion of women at senior levels
- Signing up to the United Nation's HeForShe campaign to promote men's engagement with gender equality initiatives
- Introducing the Vice Chancellor's Diversity Fund, to provide resources for innovative projects that will promote gender equality
- Establishing the Returning Carer's Fund, to support academic staff in re-establishing their research career following a career break for Carers

Similar advisory groups oversee activity at a Divisional level and implement appropriate Divisional actions. For example, in 2014 the Medical Sciences Division established a scheme of peer group mentoring to support researchers. Divisional groups also support departments during the charter application process, provide networking and information sharing opportunities, and give advice on best practice.

Each department has an Athena SWAN self-assessment team comprised of individuals from all of the constituent staff and student groups in the department. Care is taken to ensure gender balance on the team as well as a representative mix of roles and seniority. The team analyses data and consults with staff and students through online surveys and focus groups, and then creates an action plan to respond to the issues identified.

As an example action, the Department of Biochemistry identified the transition from Postdoctoral researcher to Research Fellow as an attrition point for women. In response, in addition to the support they provide for researchers, they plan to establish clear and open procedures for Fellowship support. They will circulate advertisements inviting potential fellows twice a year and invite a short-list for interview by a selection panel.

Further reading

Equality Change Unit (ECU): ECU's Equality Charters – Athena SWAN Charter. Available at: www.ecu.ac.uk/equality-charters/athena-swan/

University of Oxford Equality and Diversity Unit: Athena SWAN at the University of Oxford. Available at: www.admin.ox.ac.uk/eop/gender/athenaswan/

University of Oxford Medical Sciences Division: About us – Athena SWAN. Available at: www.medsci.ox.ac.uk/about/athena-swan

University of Oxford Mathematical, Physical, and Life Sciences Division: Equality and Diversity – Athena SWAN. Available at: www.mpls.ox.ac.uk/equality-and-diversity/athena-swan

Brid Cronin is the Athena Swan Advisor and Facilitator at the University of Oxford

Men's engagement in women's initiatives could accelerate social change

by Anna Muszkiewicz

The gender equality movement has come a long way. At Oxford, a number of initiatives supporting and promoting women have been established. These include vibrant student societies such as Oxford Females in Engineering, Science, and Technology (OxFEST); Oxford Women in Computer Science; Oxford Women in Engineering Group; and Oxford Women in Business. Despite these laudable efforts, we still face big challenges ahead. A recent United Nations report predicts the gender pay gap will not close completely for another 70 years (1). I believe part of the problem is that men are too often not encouraged to join gender equality initiatives and addressing this issue could accelerate the pace of social change.

A common misconception is that gender inequality only affects women, while in fact both women and men are disadvantaged by the gender roles assigned to them by society. The unconscious bias tests designed by Harvard University's Project Implicit (2) demonstrate that over 75% of test responders associate women with family and men with careers. Such societal bias leads to irrational prejudices, and carries very real consequences for both genders. For instance, in the UK, women are paid on average 20% less than men for the same number of hours of work (3). On the other hand, men are often perceived as 'less-important parents' due to the association of women with caregiver roles. Furthermore, men also face disparities in mental health (they are three to four times more likely than women to commit suicide (4)). Despite the fact that men are clearly disadvantaged by gender inequality, they tend not to participate in gender equality initiatives; a typical event organized by the student societies mentioned above predominantly attracts women attendees.

It is, however, unsurprising that over 90% of the attendees at gender equality events are women. After all, while women comprise almost 50% of the workforce, only 8% of the engineering workforce in the UK are women (5), 6% of executive directorship positions are occupied by women (6), and 3% of tech start-ups are owned by women (7). Studies show that women need more structural support (such as mentoring, encouragement to apply for promotions, etc.) than men, especially in traditionally male-dominated fields such as sciences or engineering (8). This is the central reason why initiatives tackling gender inequality have explicitly targeted women. Unfortunately, this acts as a double-edged sword, as the focus on promoting and supporting women results in men feeling excluded. Indeed, the very names of the societies mentioned above may imply exclusivity towards women.

That said, there is a clear benefit to having women-only events. Women are more inclined to discuss certain topics openly in an exclusively women audience, especially when they feel that the issue affects their gender disproportionately. Speaking from personal experience, workshops on topics such as assertiveness and leadership take a very different tone with a women-only audience, facilitating openness and getting to the heart of difficult questions. However, such events may be inadvertently seen as 'preaching to the choir', since the attendees are often all too aware of the problems associated with gender inequality.

There is no doubt that large-scale social change is taking place, as evidenced by the decreasing gender gap in terms of representation and pay in Science, Technology, Engineering and Medicine (STEM) over the last 50 years (see the Women in Science comic on pages 20–21 of this issue). However, if we are to close the gender inequality gap more quickly, the debate over equality must engage the elephant in the room – men!

Gender equality, and diversity among communities, is beneficial for everyone (9). With women being historically underrepresented in STEM, women-only initiatives still serve a valid purpose. However, to accelerate the pace of social change, men should be more actively encouraged to contribute to gender equality initiatives. Shifting the emphasis from 'supporting women' to 'promoting gender equality' amongst men and women is vital in achieving this goal. If we engage the entire population, it will be much easier to bring about the social change that will benefit us all.

Anna Muszkiewucz is 3rd year DPhil student in the Department of Computer Science

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Historical perspective of women in science

by Dr Naveed Akbar

The involvement of women in science has long been of significant interest to academics, historians and, more recently, grant funding agencies. Women have contributed to the advancement of scientific knowledge throughout history and therefore a conscious effort has been made by historians to further understand the vital role women have played in some of the most important discoveries.

One of the major figureheads of success for women in science is Marie Curie. Celebrated first and foremost for her major discoveries in radioactivity, she is also accredited as the first woman to receive a Nobel Prize and the only woman to win the prize twice (1). Whilst celebrating the success of Marie Curie, however, we should also acknowledge and raise awareness of less well-known women who have played significant roles in scientific progress, and seek to discover their contributions to advancements that are all too often solely attributed to men.

> "we should also acknowledge and raise awareness of less well-known women who have played significant roles in scientific progress"

The descriptions of women in science that exist from before the 20th century are often associated with male scientists. Women frequently undertook associate or assistant roles with their spouses or fathers, which upon the man's death resulted in them taking over teaching and research duties. Daughters of educated scholars were taught practical techniques and often made significant contributions, principally in astronomy and mathematics. The best-described historic examples are Hypatia, a Greek mathematician and astronomer who followed in her father's footsteps and taught at the University of Alexandria, and Jane Colden Farquhar, an American botanist described as the "first botanist of her sex in [her] country" (2).

During the 19th century the number of women scientists increased, albeit usually in partnership with their husbands — this included Marie Pasteur, assistant and co-worker to Louis Pasteur who is famous for the technique of pasteurisation (3). However, at this time the formal education and training of women in the sciences remained scarce. This is clear, for example, from the fact that women were not allowed to matriculate or graduate from Oxford University until 1920. The revocation of this rule at Oxford and other universities therefore marked a significant advancement in the education of women.

On the other hand, it is important not to assess the involvement of women in science solely in terms of university entry and numbers of primary publications? Women were conducting scientific investigations but the extent to which they were involved remains under-appreciated. This, in part, is because quantifiable measurements do not paint a full picture of the diverse and vital role that women frequently played behind the scenes. The ability to track the involvement of women in science historically through publications is further complicated by three principal factors: (i) women often changed their names after marriage; (ii) women were not accredited with the full extent of their contributions to scientific investigations and were overshadowed by their male co-workers; and (iii) women often adopted male pseudonyms for publication.

In the 21st century, women remain under-represented in senior scientific roles. The factors that contribute to the absence of women in professional roles have been widely discussed and include childcare responsibilities, poor future career prospects, prior restrictions on university entry, and marital status (4). The principal integration of women into science is being encouraged by educational establishments and remains a core driver for the development of ideas and achievements. We must continue with this endeavour.

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Starting a healthcare consultancy

by Sophie Costello

On completing my Chemistry degree at Wadham College in 2000 I had no idea what to do next. I did know that a life in the lab was not for me. Soon after dipping my toe into the world of accountancy the realisation hit that although bench science and I were not suited, the utilisation of my scientific training was key to a happy working life. I now feel incredibly fortunate that, as one of the co-founders of Costello Medical, I have achieved that aim working in an area that during my degree I had no idea even existed.

Founding Costello Medical

Following a brief encounter with the world of finance, fate played a hand and I saw an advertisement for a new Master's course at the University of Cambridge that combined training in science and business. Through my studies I was exposed to the field of evidence-based medicine, which is the rigorous critique and review of clinical data to inform decision making (such as the most appropriate place of a drug within the treatment pathway), and I began work for a consultancy specialising in this for the pharmaceutical industry. Then in 2008 my brother experienced a very serious illness and I left my job to focus on my family. Reviewing and analysing clinical data can at times be a little abstract, but during those difficult months I witnessed first-hand the true impact of medicine. When the time came to think about returning to work I was doubly certain that I wanted to work in the healthcare field. But also, I felt brave enough to take a risk and truly follow my career ambitions. Fear of a wrong turn in my career paled into insignificance compared to seeing my loved ones suffer.

And so Costello Medical was born. My husband (also an Oxford graduate but in Classics) and I co-founded it in November 2008 and ran the business from a box room in our house. My job was to deliver the scientific work and my husband's was to figure out how to run a company! We started with one small project for a mid-sized pharmaceutical company and set a limit of how much of our own savings we were willing to invest before giving up the dream. But one small project turned into another, and then another and soon we could see that there was a genuine opportunity to build something.

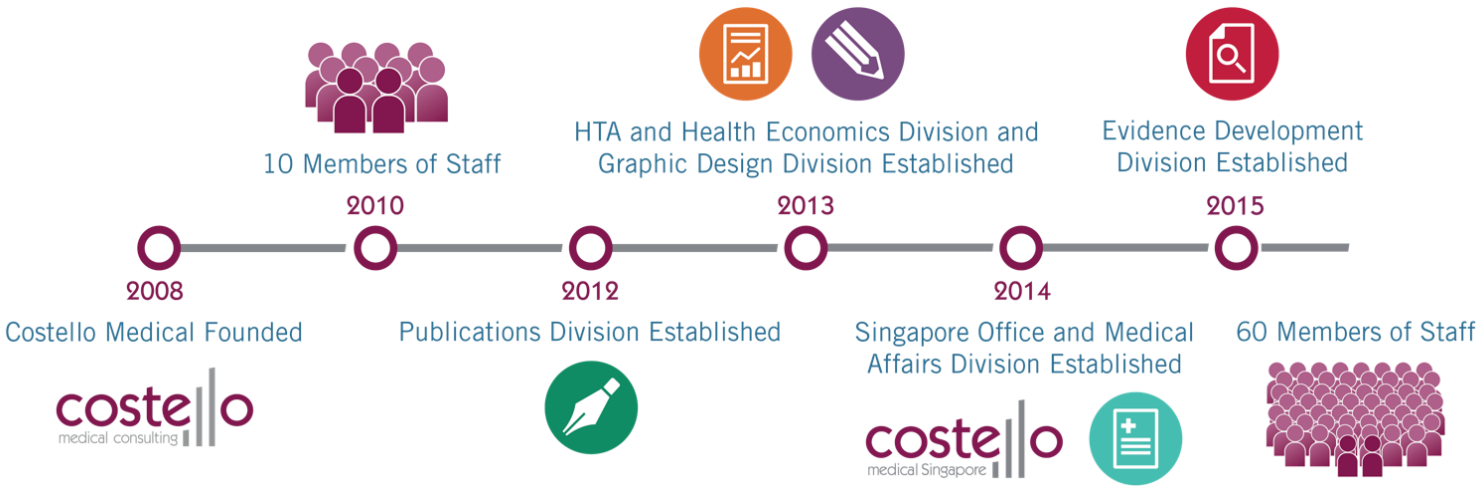
What do we do?

Today what we do can be summarised as the provision of scientific support to the healthcare industry to analyse, interpret and communicate clinical and health economic data.

But what does that actually mean? A good example of what we do is the advisory boards that are delivered by our Medical Affairs Division. Advisory boards are meetings where pharmaceutical companies seek guidance on their drug's data from the top 10 physicians on the disease of interest. At Costello Medical we help our clients develop presentations for these meetings and formulate questions that need to be answered. Typical questions include "which patients are most suited to treatment with this drug?" and "what additional analyses are needed to understand how this drug should best be used?" We also summarise the outcomes of these meetings and provide guidance on the next steps.

The Publications Division develops abstracts, posters, manuscripts and press releases that communicate data to the research, clinician and patient communities, and works closely with the authors to generate scientifically robust and compelling publications. Our Evidence Development Division reviews scientific literature to help clients answer specific questions. Reviews can vary from targeted literature reviews of the current landscape to rigorous systematic reviews and meta-analyses to identify, quantify and compare the efficacy and safety of different treatments. Systematic reviews frequently feed into the work done by our Health Technology Assessment and Health Economics (HTA and HE) Division. This team develops the submissions and economic models needed for agencies such as the National Institute for Health and Care Excellence (NICE), who assess the clinical and cost effectiveness of health technologies and ensure that all NHS patients have equitable access to the best treatments. We do many other projects in addition to these examples – but at the heart of each is the need to have a thorough understanding of scientific data and the ability to analyse and communicate findings in the context of the management of a particular disease.

Our ongoing aim is to develop the unparalleled quality of our work, complemented by unprecedented customer service. The projects we work on are not only complex but also time-critical, and our clients trust us to deliver unfailingly thorough, accurate and professionally presented work that is enhanced by novel



insights and creative input.

Growing the company

It was always our intention to grow our company, rather than use it as a vehicle for me to freelance. Six months after its foundation we realised we had enough work to expand. We posted a job with the Cambridge and Oxford Careers Services, never really expecting that anyone would apply given we had no office and no track record! But to our amazement some people did, and among the applications was an outstanding CV from Jeanette Kusel, a Cambridge Natural Sciences graduate. Jeanette joined us in October 2009 as the first member of staff and has been with us ever since. Jeanette is now Head of our HTA and HE Division. Our model of employing people with relatively little work experience, but outstanding scientific credentials, has been the foundation for the last 7 years. Our team has been growing steadily and we now number over 60.

One of the best parts of developing our company, and the factor that keeps us cheerful even when other things are going wrong (which they frequently are!), is the fantastic team we are lucky enough to work with. Retaining our staff is a key aim and we do this by providing them with limitless opportunities, and where possible a career path aligned with their ambitions and interests. Our third ever employee, Craig Brooks-Rooney, expressed an interest in living abroad. After exploring opportunities in different regions Craig established our Singapore office in April last year. We do our best to give the team a high level of autonomy so that they can take genuine pride in first creating and then representing Costello Medical.

A key challenge as we have grown is maintaining the quality of what we provide as well as the friendly and innovative small company feel. Laura Hamerslag, who joined us in the scientific team, developed a deep interest in how this is done and last year transitioned to Head of Operations. A key aspect of her role is to ensure we remain true to our founding values as we grow in size.

Could it be for you?

The vast majority of the team came directly from their undergraduate or postgraduate degrees and almost all state the desire to use their scientific skills outside of the lab as a key reason for joining. If that resonates with you, then a career in healthcare consulting could be what you are looking for. The work is particularly suited to those who would thrive combining scientific understanding with a passion for delivering customer service in a commercial environment, and who can adapt to the fast-paced nature of the work and exhilarating but demanding variety of the project content.

Could starting a company be for you? There is no doubt that it is hard work, and there have been some very tough times for us as we have grown Costello Medical. But it has been a fantastic experience and one that (most days!) we would not change. The feeling of creating something has been second to none, and we aim to replicate this for our senior team through our divisional structure, which essentially simulates mini companies. For me, learning to take risks in my professional life was one of the main positives to come out of the challenges faced by my family. I would urge anyone with an idea for a new venture, and the passion to live that venture as you get it up and running, to go for it.

For more information on Costello Medical please visit www.costellomedical.com

Sophie Costello is co-founder and CEO of Costello Medical and has been providing scientific support to the healthcare sector for thirteen years. In her role as CEO, Sophie provides strategic guidance and scientific direction to the company. She is responsible for overseeing the senior management team and ensuring that everyone at Costello Medical is working in line with the core values of exceptional scientific quality and commitment to outstanding customer service. Sophie holds a first class Master's degree in Chemistry from the University of Oxford and a Masters in Bioscience Enterprise from the University of Cambridge.

A DPhil journey; a dream come true

by Ashwag Albukhari

Studying abroad can be a critical step and a major turning point in the life of any young academic. Students are able to engage and share their experiences with like minded academics, attend and present work at national and international conferences and form collaborations with world-renowned researchers whilst also experiencing the thrills of a new culture.

I graduated from the Biochemistry department at King Abdulaziz University in Jeddah with a first-class honours degree. After the completion of my Masters degree I was appointed as teaching assistant in the Biochemistry department. Here I taught lab techniques to undergraduate students and also got involved in research and scientific committees. Additionally, I was also involved in establishing the Experimental Biochemistry Unit at King Fahd Medical Research Center (KFMRC). This experience helped me to develop my academic career and acquire research methodologies skills. My ambition, desire to learn, and interest in being more involved in the scientific community led me to the next phase of my life: A DPhil at the Weatherall Institute of Molecular Medicine at the University of Oxford. My DPhil focused on studying the response of a molecular subtype of breast cancer known as triple negative breast cancer (TNBC) to a particular targeted therapy, and investigating the underlying molecular mechanisms of their primary and acquired resistance to that therapy.

This was a huge turning point in my career and was a period of great professional and personal growth. Being a student at one of the top universities in the world was a great achievement and had a tremendous impact on my personality. Of course, studying abroad does not come without its challenges. The journey from being a girl who lived her entire life with her family to becoming an independent woman with my own responsibilities in a completely different culture was at times very difficult. During my time as a student at Oxford, I not only had to focus on producing high quality research and scientific articles, but also learn how to best accomplish this whilst ensuring that I make the most of this amazing opportunity to study in this historical city.

Best poster award at the Saudi International Conference, University of Edinburgh

I can happily say that my time as a student in Oxford was one of the most enjoyable, fruitful and successful periods of my life. My DPhil research resulted in two first-author papers (currently under submission), a chapter in a recently published book in cancer research and another paper from a joint project with my colleagues. I believe that building a network with people from different institutes is a critical step towards success, and I had the opportunity to develop my networking and communication skills throughout the 5 years by attending scientific meetings and conferences. During my DPhil, I had the opportunity to present my work at different international and national conferences such as the NCRI conference and Breakthrough Breast Cancer Triple Negative Conference. As a result, I have been recognised as a distinguished Saudi student in the UK by HRH Prince Mohammad bin Nawaf, the Royal Ambassador of Saudi Arabia to the UK, and my abstracts have been selected as distinguished abstracts in different conferences.

Saudi ladies awarded as distinguished Saudi students in the UK by HRH Prince Mohammad bin Nawaf, the Royal Ambassador of Saudi Arabia to the UK.

However, it was with a heavy heart that I had to say goodbye to the city of dreaming spires. As they say, when one door closes another opens. After the completion of my DPhil, I returned to my home university and became Assistant Professor of Biochemistry at King Abdulaziz University in Saudi Arabia. As a faculty member, I have had the pleasure of being able to share my knowledge, and journey, to a future cohort of young scientists (both undergraduate and postgraduate students). In addition, I have established my own research team with the hopes of carrying forward the research from my DPhil project. Whilst this new role has and will continue to present new challenges and responsibilities, I firmly believe that my experiences abroad have prepared me for the challenges ahead and I look forward to pushing back the frontiers of research in this new environment.

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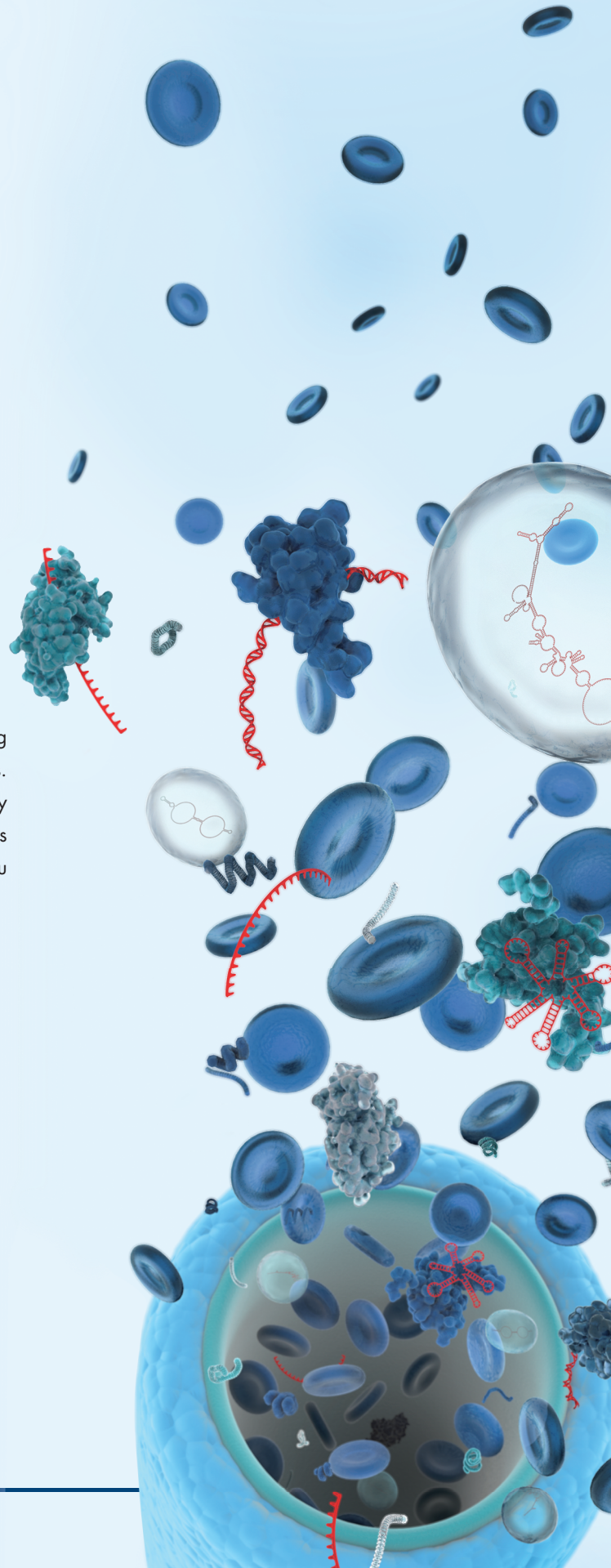

Sample to Insight

Drug-binding to SV2A

by
Joanna Lee

The Major Facilitator Superfamily (MFS) transporter Synaptic Vesicle 2A (SV2A) is a protein found in the membrane of synaptic vesicles and is the target for the successful anti-epileptic drug (AED) Levetiracetam (LEV). LEV is a second generation AED with a novel mode of action and the only one known to act by interacting with SV2A. In order to investigate the structural basis for this interaction, a model of SV2A was constructed and theoretically tested using Molecular Dynamic (MD) simulations with the aim to better understand drug-target interactions. As a member of the MFS family, SV2A is thought to be accessible to either the synaptic vesicle or the cytoplasm. Hence, there are two potential conformations to which LEV binds. Investigation of both conformations revealed a putative binding site for LEV in the central cavity of the transmembrane domain of the protein.

SV2A and epilepsy

Epilepsy is one of the most debilitating neurological disorders. It is characterized by a spectrum of seizures that take both chronic and acute forms. Furthermore, almost a third of patients with epilepsy can currently not be treated. A recently developed AED that has had much success is LEV. Though its mode of action is distinct from other AEDs, it is not well understood at the molecular level and binding to its target SV2A remains incompletely resolved. Elucidating the exact mode of binding would however be a prerequisite to further define the drugs' mode of action (1).

MFS structure and function

One of the most striking properties of MFS transporter proteins is their highly conserved fold. There are currently 44 MFS structures in the Protein Data Bank (PDB) and all have the same 12 transmembrane helix fold, arranged into two domains with pseudo two-fold symmetry. This arrangement is key to the function of MFS proteins. The 'secondary' active transport mechanism operates via a cycle of conformational changes such that the central cavity between the two domains is open either to the extracellular space or the cytoplasm (Figure 1).

MFS proteins are found in all organisms, and though the most commonly researched are the sugar transporters, their substrates are incredibly diverse. Often the substrate is co-transported with an ion that moves in either the same or opposite direction across the membrane bilayer. This ion is thought to be the driving force for transport as the movement of ions is with the electrochemical gradient, allowing uphill transport of substrate. The transporters are specific to their substrate and the structural basis for this specificity appears to be in the central cavity, which is the substrate binding site embedded within the two transmembrane domains. This implies that the common 12 helix fold is crucial for these proteins to transport their substrates across the membrane, rather than specific interactions of the substrate with amino acid side chains within the central cavity.

Despite this common fold, MFS proteins have low sequence identity – as little as 11% between solved X-ray crystal structures. The result of this is that MFS transporters fall into the 'twilight zone' where proteins of larger folds can share apparently little homology and yet maintain the same secondary structure, namely 12 transmembrane helices in the case of MFS. While this is an interesting feature, it complicates the structure prediction of novel MFS proteins from existing MFS crystal structures. When two sequences are diverse, the confidence of a paired alignment for structure predictability is low.

Predicting structure from sequence

One method used to aid the construction of a model for SV2A was to use evolutionary conservation in similar sequences. Protein structure prediction from amino acid sequences is possible when there is high homology between target protein and a protein with a solved X-ray crystal structure so that a simple paired alignment can be used to generate a 3D model of the target. However, this is not the case with SV2A, the sequence being too distant to other MFS proteins. Thus, the resulting alignment contains too many ambiguous regions which do not accurately predict structure.

A multiple sequence alignment of proteins with high homology to SV2A provides more confidence in the alignment – the more amino acid sites that are the same between sequences the easier it is to align less conserved sites. This is important because advances in genome sequencing provide hundreds of sequences for related proteins and so it is essential to have confidence in the alignment. From these alignments, patterns of amino acids can be identified across the family that may be structurally or functionally important. This was first done for Class A G-protein coupled receptors for which Ballesteros and Weinstein (2) used amino acid conservation to predict the location of equivalent residues, which were subsequently confirmed by X-ray crystal structures.

Conservation of amino acid properties such as hydrophobicity (Figures 2A and 2B) was used to help predict the location of helices in the sequence.

Subsequently, conservation of polar residues was used to predict the helix most likely facing the central, solvent-accessible cavity (Figure 2C). This information refined the

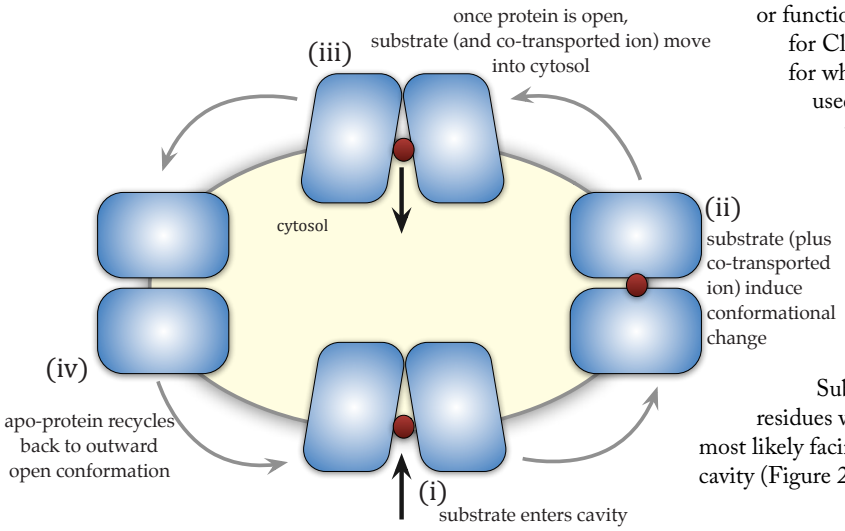

Figure 1: Alternating access mechanisms of MFS transporters. When the direction of transport is into the cycle, the substrate enters the central cavity between the two domains (i) and instigates a change in conformation to an occluded (ii) and then inward (iii) facing state. This facilitates release of substrate into the cytoplasm and the empty transporter (iv) transitions back to the outward open conformation.

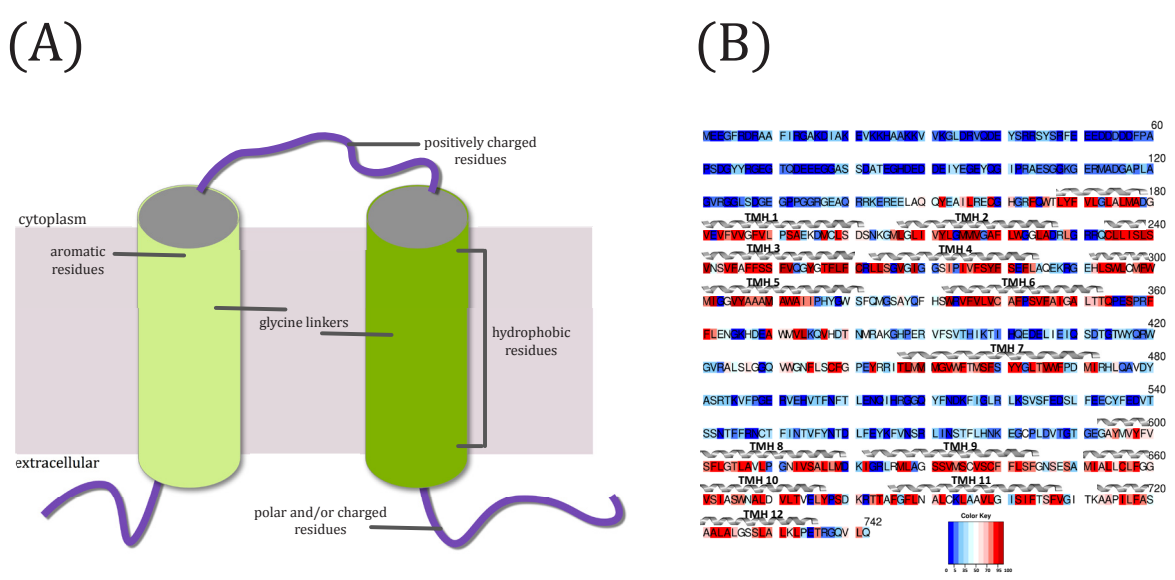

Figure 2: (A) The conservation of amino acids according to chemical properties can be used to predict secondary and tertiary packing of transmembrane helices. (B) The conservation of hydrophobic residues in the multiple sequence alignment of SV2A-like proteins can be used to predict helix position. The heat map is coloured blue-white-red to represent 0–100 % conservation. (C) The MFS fold shows a central cavity between the two domains. The cavity is solvent-accessible in both the cytosolic and extracellular open conformation. Hence, the helices in proximity to the solvent-exposed cavity have conserved polar residues. (D) The LEV-homologue binding cavity, depicting the presence of three polar or charged residues in the cavity (N667, D670, K694). All residues (C297, W300, Y462, N667, D670, K694) affect LEV-homologue binding (5, 6). Panels (B) and (D) are adapted under creative common licence from (6).

the alignment of SV2A to X-ray crystal structures with luminal and extracellular facing MFS transporters and thus generates potential models that could be explored further with regard to drug-target interactions.

Using MD simulations to investigate LEV binding

After constructing the luminal and extracellular conformations of SV2A, a homologue of LEV was docked into the respective central cavity. The cavity is mostly hydrophobic, with five conserved polar or charged residues, which is in keeping with the cavity composition of other MFS transporters that are known to transport sugars, for example six conserved polar or charged residues in the case of FucP (3, 4). Of the conserved polar or charged residues situated in the central cavity of SV2A, some are in the LEV-homologue binding site (N667, D670 and K694) (Figure 2D). MD simulations of the LEV-homologue bound SV2A system with SV2A embedded in a POPC-lipid bilayer showed that these residues are crucial for the drug-protein interaction. Two of these residues were previously identified as important for the LEV-homologue binding to SV2A (5), but D670 was newly identified and further confirmed by site-directed mutagenesis and binding studies (6). Sequence analysis techniques as well as MD simulations have helped to further elucidate the drug binding site in SV2A, the target for the anti-epileptic drug LEV. Specific interactions of the LEV homologue and amino acid residues were identified within SV2A conformational models providing insight into key LEV-SV2A interactions on the molecular level.

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The path most travelled

by
Leah
Taylor
Kearney

Oxygen is essential to all aerobic life. In animals, the cellular response to a lack of oxygen (hypoxia) is mediated by the transcription factor hypoxia-inducible factor (HIF). HIF up-regulates multiple genes such as endothelial growth factor and erythropoietin, which help the body cope with low oxygen conditions. HIF levels and activity are regulated by four enzymes: prolyl hydroxylase domain enzymes 1-3 (PHD1-3) and factor-inhibiting HIF (FIH). PHD1-3 catalyse the hydroxylation of specific prolyl residues in HIF, marking it for degradation by the proteasome. FIH catalyses the hydroxylation of an asparaginyl residue in HIF, inhibiting its transcriptional activity. Collectively, the HIF hydroxylase enzymes reduce cellular HIF concentrations as the oxygen supply increases. PHD2, however, stands out from the crowd as kinetic studies show that it reacts more slowly with oxygen than FIH and related oxygenases. Somehow PHD2 restricts its reaction with oxygen, and we believe that this is connected to its oxygen-sensing role (1,2). What, however, do we mean by 'sense'? How does PHD2 control its reaction with oxygen, and therefore, what is the molecular basis for cellular oxygen sensing?

When PHD2 is examined in detail, its motifs and structural quirks show controlled and highly organised inefficiency. It is these structural features in which we have become immersed. We hope to dissect PHD2, understanding exactly what makes it tick. One notable feature is a loop region connecting beta sheets 2 and 3 of PHD2 (β2β3 loop). Interest in this region was sparked by the sequence alignment of the three PHD enzymes, which showed that the β2β3 loop has the greatest variability across the catalytic domains of the three PHDs. This loop has been linked to substrate recognition (5) and may be involved in guiding oxygen into the active site. We hope that detailed kinetic analysis of targeted variants of PHD2 will help us to understand the role of this important loop in PHD2 catalysis, particularly with respect to oxygen.

However, kinetic data can only tell us so much, with other questions remaining unanswered. How does oxygen make its way into the active site? How would changing the chemical characteristics of the loop affect its path, and how, above all, do we prove it? This is where we throw the book away and wheel out the physical chemistry kit-bag. To 'see' what happens to oxygen once taken up by the enzyme, we have to monitor its transition from the bulk solvent through PHD2 itself. The experimental techniques to investigate this vary from fluorescence to Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry. Another very insightful tool at our disposal is molecular modelling, which allows us to expose PHD2 to a series of computer-generated conditions and monitor its subsequent behaviour. Once a certain physical characteristic has been 'observed', it is then time to experimentally prove it. The easiest way to do this is to monitor physical changes in the enzyme upon encountering oxygen.

One such avenue involves intrinsic tryptophan fluorescence quenching. Tryptophan is an amino acid with a chromophoric side chain. That is, its side chain has an electronic transition responsible for a given spectral band. Phenylalanine and tyrosine are also considered chromophores, but tryptophan has the highest quantum yield. As such, tryptophan fluorescence spectra can be used to monitor structural and local environmental changes within a protein. Fortunately, oxygen is a known fluorescence quencher and PHD2 only has four tryptophans, two of which are located both at the pocket and the active site. This enables us to monitor the transition of oxygen through PHD2 to the active site. We can achieve that by producing a variant of PHD2, removing the two external tryptophans, and leaving only those that monitor oxygen. We can then observe the changes in fluorescence upon the introduction of oxygen. This way, we hope to establish an experimental basis for the suggested oxygen pathway, not simply inferring it from crystallography or modelling outputs.

One series of data, however, does not establish a convincing argument. Uncovering the oxygen pathway to the active site via fluorescence is one thing, but the question still remains: is something restricting oxygen's access to the active site of PHD2, resulting in slower kinetics? If oxygen is indeed being restricted we need to be able to show evidence of this. Our chosen experimental avenue is a little adventurous; it involves molecular oxygen excitation using high-energy laser pulses. This is an old technique to probe an enzyme's structure, which has been used to elucidate key residues in enzymatic activity (6). The idea is simple: excite molecular oxygen from its ground triplet state to its highly reactive singlet state. This excited oxygen molecule can then covalently modify an amino acid, but only one that would otherwise come into contact with an oxygen molecule. Once the modification has been confirmed by mass spectrometry, the amino acid of interest must be identified. Trypsin, a serine protease, is commonly used to digest proteins into peptides which can be analysed using matrix-assisted laser desorption ionization mass spectrometry time of flight (MALDI-TOF). By analysing both modified and unmodified protein via this technique, the oxidised amino acid can be identified, inching us closer to elucidating the specific details of the

Figure 1: Overlay of the crystal structures of PHD2 with (3) and without (4) substrate in grey and blue respectively. The pink region denotes the β2β3 loop region of interest locking the substrate in place. The C-terminal oxygen-dependent domain of HIF (CODD) substrate is depicted in black.

pathway.

The final part of the experimental trilogy will likely involve NMR spectroscopy. ^{15}N NMR is the chemist's bread and butter, using the labelled nitrogen of the peptide backbone (and the select few suitable side chains) to monitor protein structural changes as a function of environmental factors. Local structural changes within the protein can be observed using other NMR active nuclei such as ^{19}F, ^{31}P and ^{169}Tm. As none of the essential amino acids contain these elements, unnatural, isotopically labelled amino acids must be introduced into the structure of a protein. In the case of PHD2, the labelled amino acids would be introduced in, and around the proposed oxygen pathway. Changes in NMR spectra of the labelled amino acids would then be monitored upon the introduction of oxygen, perhaps allowing further insight into the oxygen pathway. NMR is further down the line. That, however, is a vision for the future. Understanding how PHD2 senses molecular oxygen could reveal the nuts and bolts of a cell's oxygen sensing mechanism from an enzymatic perspective and may allow us to glimpse into what remains a remarkably unexplored pathway.

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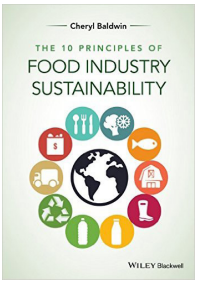
Leah Taylor Kearney is studying MSc(Res) in Chemical Biology in the Department of Chemistry. Leah wishes to thank her supervisor, Dr Emily Flashman, for her unwaivering support and the opportunity to work on such a rewarding project.

BOOK REVIEW



The 10 principles of food industry sustainability

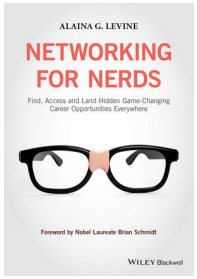
Cheryl J Baldwin

IISBN: 978-1-118-44773-4, Wiley-Blackwell (2015)
224 pages: Paper, £55.00 / eBook, £49.99

Reviewed by Drew Duglan

Consideration for the longevity and optimisation of our food supply has never been so important. This well-written book provides an excellent insight into the many issues surrounding food sustainability. Dr. Baldwin has amalgamated information from leading researchers, professionals in the nutrition sector and commercial directives to produce detailed sections outlining each principle covering the full spectrum. Topics discussed range from agricultural methods and animal welfare to processing and distribution, as well as a discussion of far-reaching social implications. Each chapter has a precise structure, making it easy to find material relating to the topic in question. Graphs and charts, where appropriate, also aid interpretation. The book would be ideal for (but not restricted to) either new suppliers, or for existing companies and local authorities looking to improve current practices. It provides advice on how to implement, monitor and assess certain approaches relating to the principles, while also recognising the level of investment necessary to fulfil each sustainability goal. Moreover, each chapter explores company initiatives as real world case studies, also touching on exciting new innovations such as interactive edible packaging. Particularly salient is the author's discussion of the unsustainable nature of current agricultural methods, detailing the desertification and loss in biodiversity that result, inevitably damaging ecosystems and exacerbating climate change. Additionally, the book provides a fresh perspective on the energetic demands at each step of the supply chain, especially in the generation of highly refined products, such as high-fructose corn syrup.

The book would have benefited from a deeper examination of the latest permaculture efforts being developed internationally, particularly the holistic approaches advocated by the Savory Institute. Nevertheless, the book effectively conveys the challenges surrounding food sustainability we face as a culture, covering all aspects relevant to producers, manufacturers and consumers. The reader is left with a lasting message on the security and equity of our food supply, and encouraged to support the most important aspects of future food production – the seeking of local smaller-scale producers, the empowerment of women in farm labour, the minimisation of food wastage and an improvement in our dietary behaviours.



Networking for Nerds: Find, Access and Land Hidden Game-Changing Career Opportunities Everywhere

Alaina G. Levine, Nobel Laureate Brian Schmidt

ISBN: 978-1-118-66358-5, Wiley Blackwell (2015)
Paperback, 248 pages, £20.50

Reviewed by Kate Dunne

Networking for Nerds is, as the title suggests, a guide to networking for those in Science, Technology, Engineering, and Mathematics (STEM) fields. The author, Alaina G. Levine, promises to furnish us with the techniques that will help gain access to the 'Hidden Platter of Opportunities™'. Ever heard of an advertised position that was magically filled within a week? That job/post-doc/grant was actually only ever available via the 'Hidden Platter of Opportunities™', and good networking is our ticket to the buffet.

The author begins by debunking some typical networking myths; it's not about cringey forced encounters and cocky car-salesman types. Instead, networking is about a mutually beneficial maximisation of opportunities. By networking we ensure we are aware of as many opportunities as possible, we can create our own hidden niche opportunities, and become more than just a name on a CV. Chapters cover various aspects of networking, including establishing a brand and reputation, culturing and maintaining your network, determining the right opportunities for you, dos and don'ts of event networking, and managing your social media amongst others. The tone is chatty and entertaining, the advice given is sound, and the author clearly seeks to empower the reader. One small qualm is that the author draws on personal anecdotes to illustrate her points. Nothing wrong with this in itself, but it does become a little tiresome because of the sheer number contained within the book. Although much of the advice is not very STEM-specific, arguably this is simply because good networking technique is the same in STEM as anywhere else.

Competition in STEM is at an all-time high, with hundreds of CVs, all equally perfect and glittering, submitted for a single position. Networking has never been more worthwhile and *Networking for Nerds* is well worth a read, both as an excellent introductory guide and as an exercise to bring your personal networking and career goals into focus.

Principles of Stem Cell Biology and Cancer: Future Applications and Therapeutics

Edited by Tarik Regad, Thomas J. Sayers and Robert C. Rees

ISBN: 978-1-118-67062-0, Wiley Blackwell (2015)
Hardback, 362 pages, £90

Reviewed by Laura Godfrey

Stem cells and their use in scientific research are becoming an increasingly popular field in today's scientific community. As scientists push the boundaries of stem cell research, information on how stem cells contribute to disease and their use in therapeutics is becoming increasingly available. For cancer researchers who use stem cells in their work, such as myself, this book is a must-read.

This book comprises 16 chapters split into two parts. Part I 'Stem Cells' focuses on the basic characterisation and the development of stem cells, as well as the essential process of stem cell differentiation. One area of focus in Part I of the book is haematopoietic stem cells (HSCs) – that is, cells that give rise to an incredibly large number of terminally differentiated red and white blood cells. Part II 'Cancer Stem Cells' centres upon the role of stem cells in a wide variety of malignant tumours, from breast to liver cancer. Each chapter deals with a different type of cancer, highlighting the importance of stem cells for a broad spectrum of different cancer types. Part II also discusses the use of stem cells for therapy of many of these cancer types. At the beginning of every chapter, there is a background section, followed by the main body of the chapter and a conclusion section. This structure allows for easy understanding and provides the reader with a take-home message with each and every chapter.

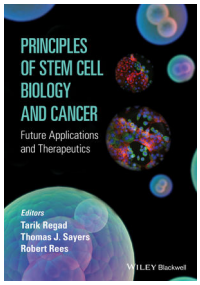
At first glance, the contents of this book could appear daunting to a scientist new to the stem cell field. However, on closer inspection, the reader will find that the chapters are well set out. With detailed background information and clear conclusions, this book is suitable for readers without prior knowledge of the subject. I recommend this book for both a biologist requiring a well-defined reference book, and also an inquisitive novice simply wanting to find out more!

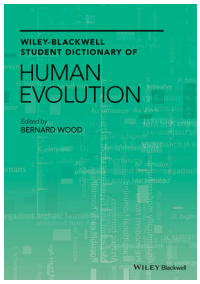
Wiley Blackwell Student Dictionary of Human Evolution

Bernard Wood

ISBN: 978-1-4051-5506-9, Wiley Blackwell (2015)
Hardback, 472 pages, £62.50

Reviewed by Anna Sigurdsson





This A to Z dictionary, with its 5000 entries, provides comprehensive coverage of scientific terms and concepts related to the study of human evolution. Every entry has been written with a student user in mind, and the variety of concepts explained reflects the rapidly increasing complexity of the field.

Not too long ago, students of human evolution could do well by understanding the general evolutionary principles, being familiar with the fairly sparse fossil records and knowing a few analytical methods. However, the field has moved on, with the exponential growth of fossil records, technology, and a much deeper understanding of genetics, evolutionary history and modern human variation. In addition, other related fields such as chemistry, earth sciences, and physics have moved forward. Due to this increasing interdisciplinarity, contemporary students of human evolution need a more detailed understanding of a much wider range of disciplines and methods. This book is the first reference source that provides information on topics as diverse, and yet as interrelated as the sagittal crest, satellite imagery, seasonality, sedimentary rock, sexual selection, social learning, and stable isotope biogeochemistry.

The entries of this dictionary vary in length from a few lines to a couple of pages, depending on the complexity and importance of the term. However, what all the entries have in common is that they clearly explain both the meaning and the significance of the terms to the study of human evolution and to each other. As a former student of human evolution, this is definitely a book that I would have liked to have access to, and it is safe to say that this book will provide valuable support to future students of human evolution.

5' with ... Dr Elena Seiradake

Dr Elena Seiradake completed her PhD at EMBL, Grenoble, in 2006 under the supervision of Dr Stephen Cusack. She moved to Oxford in 2008 after being awarded a Marie-Curie Intra-European Fellowship to work under Professor Yvonne Jones at the Nuffield Department of Medicine. In 2014, she established her independent lab in the Department of Biochemistry, where her research focusses on understanding the structure and function of cell surface receptors responsible for the development of the brain, blood vessels and cancer. Elena is involved in many scientific outreach initiatives and has submitted an impressive 30 X-ray crystallography protein structures to the RSCB Protein Data Bank.

How did you first become interested in cell surface receptors?
I have always been interested in philosophy and what makes us think the way we do. Thoughts are generated in the brain by biological processes that are still poorly understood. During my PhD research I came across neural cell guidance receptors, which are the molecules that control how the brain develops. I realised that working in this field would allow me to contribute to understanding how the brain works.

If you were not a scientist, you would be…?
A professional ballerina.

What do you like most about your field of research?
Until recently, understanding how the brain works seemed impossible given its complexity. With the advent of powerful techniques (such as advanced crystallography, electron microscopy, super-resolution microscopy, mass spectrometry, optogenetics, computer simulation, to name but a few) biologists are now able to tackle questions of ever-increasing complexity. For the first time in history, understanding how our brain works and why we see the world the way we do may be within our reach. I think this is very exciting.

What are you most proud of in your career so far?
Having worked with amazing scientists like Stephen Cusack (EMBL Grenoble outstation), Yvonne Jones (Division of Structural Biology, Oxford) and Ruediger Klein (MPI of Neurobiology). I am proud of my work in these groups. For example, our results have contributed to a new drug designed to help fight fungal disease and provided some of the basic knowledge required for the design of new treatments for cancer and brain-related diseases.

What has been the biggest challenge you have faced during your career?
The biggest challenge was at the end of my postdoc. I didn't know how to go about obtaining a PI position. Fortunately, the Department of Biochemistry offered me lab space and supported my grant applications. I am incredibly grateful to the people who advised me and helped with this.

What advice would you give to students looking to follow in your footsteps?
I think the most important thing for someone working in science is to be enthusiastic. I think the more you love your work the better you will be at it. Therefore, I would not blindly follow 'fashions' or work on 'hot topics' just because other people say that is what you should do. I would try to find what you personally find the most fascinating and work on that.

Who has inspired you during your career?
The list is long, but at the top of it is my PhD supervisor Stephen Cusack. He is a wonderful supervisor and working in his lab was what really kindled my interest in science. Beyond being a world-leading scientist in his field, Stephen is incredibly knowledgeable and takes a keen interest in the world and people around him. His advice is always excellent.

Where do you see the field of cell guidance going in the next 5 years?
Many of the molecules that guide cells and control brain development have now been discovered, but it is still unclear how they work together to give rise to complex tissues. I think that using advanced experimental techniques together with computer simulations will produce a much more detailed understanding of how cell guidance works and what goes wrong in associated diseases.

Write for Phenotype?

- The deadline for article submissions is Friday of 8th week, 4th December 2015
- We accept articles on any aspect of biological sciences research, books or science education
- For next issue we particularly welcome articles related to plant science or the environment
- Articles can be either 650 or 1300 words

If interested, please get in touch: rebecca.hancock@linacre.ox.ac.uk

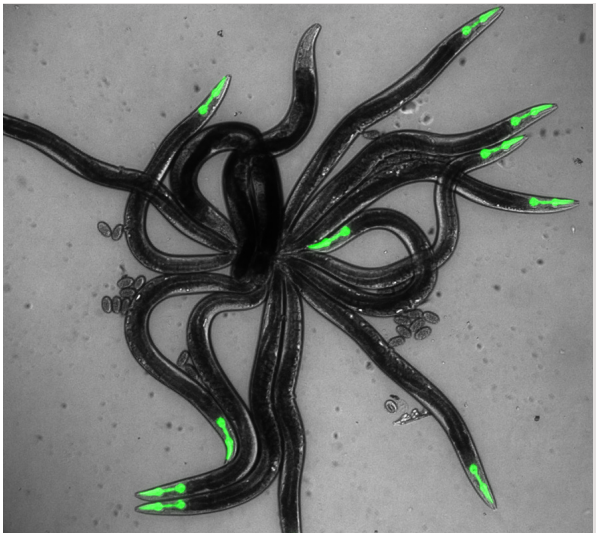
Work for Phenotype?

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

SNAPSHOT Research Image Competition

This issue's winners are...

Prof Jonathan Hodgkin and Dr Maria João Gravato Nobre



The winning image by Prof Johnathan Hodgkin and Dr Maria João Gravato-Nobre shows nematodes *Caenorhabditis elegans* (*C. elegans*), with pharyngeal GFP, and Pristionchus pacificus co-aggregating as a result of tail adhesion caused by the bacterium *Leucobacter n.* sp, Verde1. Worms trapped in these aggregates (worm-stars) will be subsequently killed and digested by the bacteria.

Prof Jonathan Hodgkin

Prof Jonathan Hodgkin FRS holds the Genetics Chair in the Department of Biochemistry, is the Associate Head of Department, Head of the Laboratory of Cell Biology, Development and Genetics, and a Fellow of Keble College at the University of Oxford.

C. elegans provide an excellent model for studying a variety of biological processes. Research in the Hodgkin laboratory has been directed at understanding how *C. elegans* can detect and defend against pathogenic or toxic bacteria. Using microarray analysis, the group was among the first to report that nematode immune responses to infection result in the production of candidate antibacterial factors. While investigating bacterial infections, Prof Hodgkin became interested in dissecting the complex nematode cuticle and surface coat. These outer layers are important as they determine sensitivity to many pathogens and drugs, and impact on many biological properties such as morphogenesis, biofilm formation, locomotion and male mating recognition.

Prof Hodgkin is also a pioneer in making use of *C. elegans* for investigations into the genetics of sex determination and development in *C. elegans*. Through extensive genetic analysis he has discovered a set of major regulatory genes that govern sexual differentiation. His results show how these genes are organised into a control hierarchy that connects sex chromosome dosage to events such as yolk protein synthesis. Prof Hodgkin was also a pioneer in making use of *C. elegans* for investigations into the genetics of innate immunity and its response to bacterial infections.

Prof Hodgkin has made other contributions to nematode genetics, including genome structure, cell lineage control, informational suppression and germ-line immortality and telomere function. He has been the Curator of *C. elegans* Genetic Map and Nomenclature. To mark his outstanding research contribution to genetics, Prof Hodgkin was the recipient of the 2011 Genetics Society Medal.

Dr Maria João Gravato Nobre

Dr João obtained her Diploma in Agronomy from the University of Lisbon in 1984 and her MSc in Nematology from the University of the Algarve in 1999. In 1996 she obtained a PhD from the International Association for Cryptologic Research and the University of Nottingham. She held subsequent positions as a Lecturer at the University of the Algarve and a Research Scientist at Zeneca Agrochemicals. Since 2000 she has been a Research Associate working with Prof Jonathan Hodgkin at the University of Oxford. In 2011, Dr João became a Stipendiary Lecturer at Hertford College teaching Molecular Cell Biology, Developmental Biology, and Genetics to undergraduate students.

Dr João's research focuses on the genetics of innate immunity in the nematode *C. elegans*. Many human pathogens are capable of infecting *C. elegans*, causing morbidity and mortality. In particular, Dr João has been studying *Microbacterium nematophilum* (*M. nematophilum*), a bacterium that adheres to the glycocalyx, inducing an inflammatory response. Through genetic screens of bacterially unswollen mutants she has identified over 21 significant loci. Among these are genes encoding glycosyltransferases, galactosyltransferases, acyltransferases and nucleotide sugar transporters. All of these genes affect the nematode surface and the adherence of both *M. nematophilum* and *Yersinia pseudotuberculosis*. Dr João is also currently investigating nematode immune effectors that are induced upon chronic intestinal colonisation.

The important work being done in the Hodgkin laboratory will continue to increase our understanding of nematode biology and innate immunity, providing insights into ways to augment our own immune response to bacterial infection.

OXFORD UNIVERSITY PRESS

SNAPSHOT
Research Image Competition

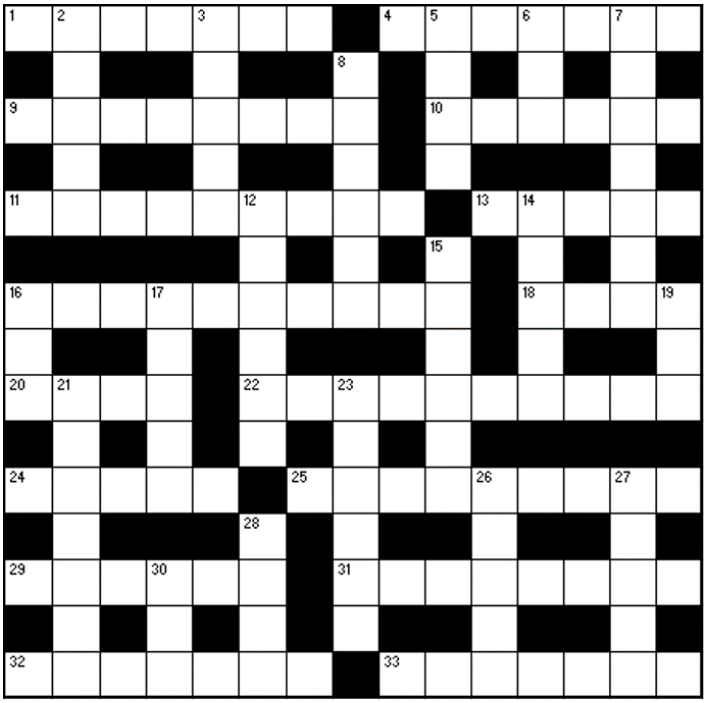
Win a £50 book voucher kindly provided by Oxford University Press!

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT**?
We are now accepting entries for pictures to be featured on the cover of the Hilary 2016 issue of *Phenotype*.

To enter, send images to rebecca.hancock@linacre.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

PHENOTYPE crossword

Fish challenges you to this latest cryptic crossword on the theme of women in science. Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to rebecca.hancock@linacre.ox.ac.uk. Entries received before the 7th December 2015 will be entered into a prize draw to win one of the four books reviewed in this issue.



The winner of the cross-word competition will recieve their choice of one of the books reviewed on pages 36 to 37, kindly provided by

WILEY-BLACKWELL

ACROSS

1. It's crystallising that turning 30 should be of note to family (7)
4. Performing operations without anaesthestic? (7)
9. See 13
10. Ribosomes readthrough commonly on a thrombin mRNA (6)
11. Modify blank curb so it's the same as 33 (9)
13,9. Block Amber from including heartless SOBs in French agreement on HIV (5-8)
16. 17 is disheartened after elements 95, 77, 58 and 44 each turn into elements 88 and 84 (5,5)
18. See 20
20, 18. First computation: 0 + 50 + 1 (8)
22. 1251: New king takes to crushing leading "controlling elements" (10)
24. Selenium interferes with long-term memory backup of the brain's positioning system (5)
25. How do we smell? Lke half a fantail fish with yucky centre (5,4)
29. D'you excise sex chromosome from genomic material using the CRISPR-Cas9 system? (6)
31,32. Venial lord shuns development - even embryonic development! (8-7)
33. Germaine writes about identification of telomerase (7)

DOWN

2. Bird at the college window (5)
3. Kim Kardashian initially about to manage hip-hop style (5)
5. Gemstone forms layers on two axes (4)
6. See 16
7. Complete on "1 vs. 100" - if you have the guts? (7)
8. Reflect that note from Roger ends with gold (6)
12. Enhanced/developed several firsts, but endemic chauvanism annulled my endeavour; . . . (6)
14. . . . give everything to reverse it . . . (5)
15. . . . and put male-backing in the trash can, and finally, in the rearview 8 (6)
16,6. Reverse order for 10 moles in liquid state (6)
17. Say goodnight to Ms. Adler? (5)
21. Every second flare's extensively oxidised (as is this sherry)? (7)
23. Buyer confuses cretin by using left rather than right (6)
26. Alcohol is swallowed in corridor (5)
27. Bell tolling at the Church of Fish as I enter (5)
28. It's just beautiful (4)
30. Carrier is upended, utters cartoonish expression of frustration (3)

Answers to the crossword from Issue 21 - Trinity '15. **Across** 1. Staphylococco, 8. Inasnap, 9. Biofilm, 11. Occultyl, 12. Opened, 14. Hulk, 15. Sistership, 18. Earthiness, 21. Upto, 22. Emboli, 25. Bitintwo, 26. Bacilli, 27. Aprotic, 28. Streptococcus. **Down** 1. Spirochaete, 2. Avascular, 3. Handle, 4. Lapilli, 5. Oligomers, 6. Coffers, 7. Lam, 10. Lee, 13. Diplococcus, 15. Spirillae, 16. She, 17. Heptatitic, 19. Tropist, 20. Stifado, 22. Vibrio, 24. Moa, 26. Bed