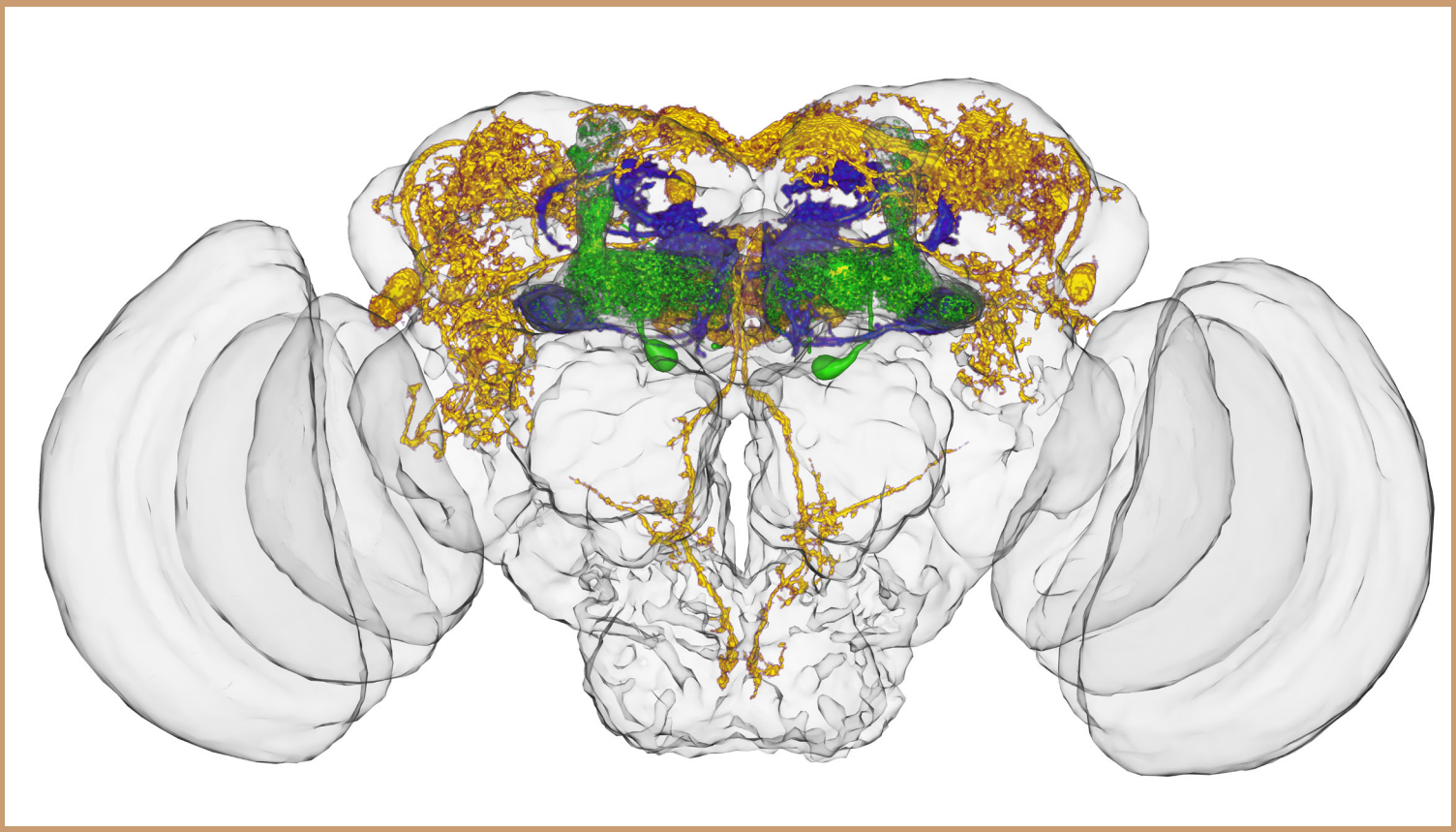
PHENOTYPE

Issue 15 | Trinity Term 2013



Gene regulation
Dr Korneel Hens uses high-resolution techniques to study the molecular basis of homeostatic regulation

HIV
Developing an effective vaccine

Prescription genomes
Treating cancers individually

Conditioned taste aversion
How does the brain remember to avoid sickness-inducing foods?

cover image by
Dr Wolf Huetteroth
this issue´s winner of the SNAPSHOT scientific image competition
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Are pharmaceutical companies 'big bad wolves'?

Antisense transcription

SciBar: Science discussions at the pub

5´ with... Prof Matthew Freeman

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EDITORIAL

Welcome to the fifteenth issue of *Phenotype*! Once again the magazine is packed with varied and exciting articles contributed by PIs, research staff and students from across the University.

Dr Korneel Hens, a new PI at the Centre for Neural Circuits and Behaviour, introduces us to the latest techniques developed to explore gene regulatory elements, and explains how his lab uses a high-resolution microfluidics-based technique to study the molecular basis of homeostatic regulation in *Drosophila melanogaster*. We also feature an interview with Prof Matthew Freeman, the new head of the Sir William Dunn School of Pathology, in which he tells us about his work in developmental regulation and the importance of effective communication in research.

This issue we also highlight treatments for human diseases and problems behind advances in this field. Dr Elizabeth Hartfield reflects on cancer treatments and the necessity of individual diagnoses of cancer genetics, and Rosalind Roberts outlines the complexity of developing an effective HIV vaccine. On the drug development side of research, Chii Fen Hiu presents the media's portrayal of big pharmaceutical companies and discusses how their business model should be taken into account in a critical analysis of their product output.

Other featured articles include an analysis of the ecological impact of eliminating mosquitoes, presented by Dr Maria Mogni, while Dr Gaurav Das explains how we learn and remember to avoid foods that make us sick long after we have consumed them. The oddity of antisense transcription is discussed by Struan Murray, who also presents recent findings in his lab that point to possible effects in gene regulation. Additionally, in graphic format, Richard Wheeler presents the various functions of cilia.

In our Science and Society section, Rupal Mistry takes us down to the pub and tells us about the Oxfordshire branch of the British Science Association, which hosts SciBar, a series of discussions between researchers and the general public. Anna Coenen-Stass takes us on a trip substantially further away and reports on how agricultural and eco-tourism developments in Cambodia have been helping the country move away from its dire political past.

This term, OUBS will be hosting Dr Morgan Beeby. Find out more about his research from Megan Masters, who tells us about his pioneering work using electron cryotomography applied to uncovering the mode of action of the bacterial molecular machinery.

Congratulations to Dr Wolf Huetteroth, the winner of last issue's SNAPSHOT competition, whose fly brain image depicting neurons involved in memory gracefully embellishes our cover. Further details on his work in *Drosophila melanogaster* and on how the image was produced can be found on page 27.

Try your hand at cracking Homarus' cryptic molecular biology crossword on page 28 and you could win one of the Wiley-Blackwell textbooks reviewed this issue. For those of you curious to know the answers to last issue's crossword, the answers are available on the same page.

If you are interested in science journalism, or in any aspect of its production, why not join the team as a writer, editor or designer? Contact us on **oubs@bioch.ox.ac.uk**!

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

Clara Howcroft Ferreira
Department of Physiology, Anatomy and Genetics

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

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TEAM

OUBS SEMINARS

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

Featured Seminar:

Monday 10th of June
Dr Morgan Beeby, *Imperial College London*
"What structural dissection of bacterial flagellar motors *in situ* tell us about structure and function"

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

This issue is supported by:

OUBS Featured Seminar: Dr Morgan Beeby

by Megan Masters

This term, Oxford University Biochemical Society (OUBS) welcomes Dr Morgan Beeby from Imperial College London.

Following a prolific early career in California, Dr Beeby recently braved the British winter to take up a lectureship and the reins of his own lab in the Division of Molecular Biosciences at Imperial College London. His research interests lie in discerning the molecular mechanisms of assembly, function and evolution of the macromolecular machinery of cells. This work has raised the bar in cellular imaging and molecular resolution.

Dr Beeby forged his path as a graduate student at the University of California, Los Angeles, with Dr Todd Yeates. There, he combined bioinformatics and crystallography to investigate self-assembling proteins in Bacteria and Archaea. Dr Beeby was then awarded a prestigious HHMI Scholarship, which took him to the California Institute of Technology, where he undertook postdoctoral training in electron cryotomography with Prof Grant Jensen.

Electron cryotomography is a powerful technique that enables the 3D visualisation of cellular macrostructures to resolutions capable of discerning individual proteins. Moreover, samples are unstained and unfixed, avoiding preparation artefacts and providing novel snapshots of molecules in action in a 'near-native' state. The method involves flash-freezing specimens and imaging them from various angles with an electron microscope. The resulting images can then be collated to determine the 3D structure of cellular machinery analogously to reconstruction by CT/CAT (computerised tomography) scans (1).

As a pioneer of this method, Dr Beeby's work has since unearthed many hidden secrets of microbial life. One such example is his work on the bacterium *Ralstonia eutropha*. This bacterium forms cytoplasmic granules of polyhydroxyalkanoate (PHA), a polyester class of considerable interest as a source of biodegradable plastics (2). Whilst much is known about the biochemistry of PHA production, the cell biology of granule formation and growth remains unclear. Using electron cryotomography, Dr Beeby and colleagues unearthed a new 3D perspective of granule genesis in unperturbed cells, opposing findings from methodologies in which specimen preparation-induced artefacts likely obscure the process (3).

Dr Beeby's recent interests have taken the route of deciphering the diversity of the bacterial flagellum, a micron long, cell-wall-anchored filament that propels the bacterium to and from nutrients and toxins. Despite being a popular nanoscale motor for research, the diversity of flagella across the bacterial kingdom has been largely unexplored. Using electron cryotomography, Dr Beeby and colleagues have shown – in stunning resolution – that the structures of flagellar motors are remarkably different across bacterial species, despite a conserved core (4). Across 11 imaged species, Dr Beeby and co-workers showed novel variations in protein assembly and symmetry (Figure 1). These findings offer insights into the contentious issue of torque generation in bacteria, as well as self-assembly of flagellar protein components. Furthermore, they show how this complex nanomachine has evolved to serve the function of propagating these various forms of bacterial life.

While this work is integral to deciphering basic biological principles and mapping evolutionary variation, it also holds considerable practical applications in sustainable re-utilisation and development of new antibiotics. With the ever-growing problems of waste production and antibiotic resistance, this kind of fundamental research is vital.

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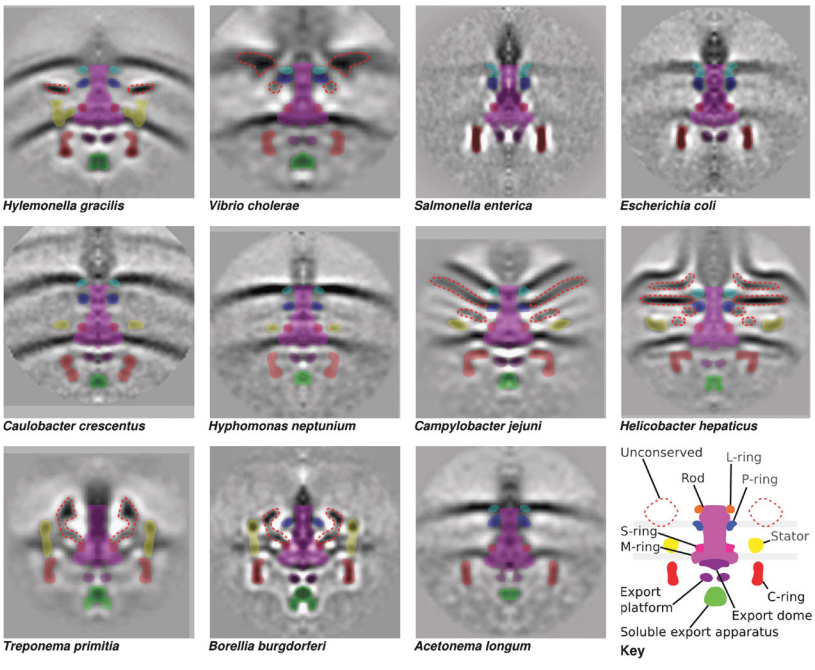


Figure 1: Structural variations of flagella in eleven bacterial species. The conserved core accommodates varying forms of protein assembly and symmetry, directly related to species function. Reproduced with permission (4).

RESEARCH HIGHLIGHTS

by Amy Baxter

DiMattia MA, *et al.* (2013), *Structure* 21(1):133-142.

Antigenic Switching of Hepatitis B Virus by Alternative Dimerization of the Capsid Protein

Chronic infection with Hepatitis B virus (HBV) is a major worldwide cause of cirrhosis and liver cancer. In this paper, the researchers solve the 3.3 Å structure of HBV e-antigen (HBeAg). Unlike the closely-related HBV core antigen (HBcAg), HBeAg is not assembled into the viral capsid and instead is capable of being secreted by infected cells; it is also a key clinical marker for viral replication, infectivity and disease severity. DiMattia and colleagues describe the structural differences between HBeAg and HBcAg and how these differences may reflect their functions *in vivo*.

The group's analysis showed that HBeAg, like HBcAg, is dimeric, countering previous suggestions that HBeAg was monomeric. However, while HBcAg and HBeAg share a monomeric fold, they dimerise via different methods; while HBcAg dimerises via a disulphide bond, HBeAg possesses an *N*-terminal extension which blocks disulphide bond formation and forces HBeAg to dimerise non-covalently via a different interaction surface. When DiMattia and co-workers analysed the oxidised versus reduced forms of HBeAg they found that when the *N*-terminal extension is disrupted, HBeAg reacquires its ability to form capsids, demonstrating the significance of this bond in the structural properties of each antigen.

Previous studies showed that many HBcAg and HBeAg antibodies are not cross-reactive. Using their structure, DiMattia and colleagues demonstrated that many of the immunodominant HBcAg epitopes do not exist in HBeAg and that the HBeAg structure opens up a large accessible surface that may be targeted by HBeAg-specific antibodies.

Furthermore, it has been noted that the antigens are differentially regulated by the immune system: HBeAg triggers a tolerogenic immune response, while HBcAg is more pro-inflammatory. This has previously been attributed to the differential ability of HBcAg to bind and activate B cells without any T cell help, but the mechanism behind it was unclear. DiMattia and co-workers' observation that the HBeAg structure precludes formation of capsids may explain its inability to activate B cells. The group conclude that the discovery of HBeAg's ability to dimerise, and the significance of disulphide bond formation, will help elucidate the differential immune responses to these antigens.

Papadakis M, *et al.* (2013), *Nature Medicine* 19(3):351-357.

Tsc1 (Hamartin) Confers Neuroprotection Against Ischemia by Inducing Autophagy

Ischaemic stroke, where a loss of blood supply to the brain results in neuronal death, is the major type of stroke seen in clinical settings. However, not all neurons respond equally to the ischaemic cascade. Here, Papadakis and colleagues identify a protection mechanism employed by ischaemia-resistant CA3 hippocampal neurons, which is absent in the ischaemia-sensitive CA1 neurons.

The group began by performing a comparative proteomic analysis of CA1 and CA3 hippocampal regions from rats subjected to forebrain ischaemia. In addition to the activation of the PI3K-Akt pathway, which has already been associated with protection against ischaemic damage, they also identified a single protein, hamartin, which played an important part in many of the networks that underwent ischaemia-induced changes in CA3 neurons. Knocking down hamartin expression *in vitro* rendered neurons more vulnerable to ischaemia-induced cell death, resulting in a 35% increase in death rate. Importantly, the group replicated these results *in vivo*, showing that knock-down of hamartin expression resulted in increased CA3 neuron sensitivity to ischaemia and lower survival rates.

In line with hamartin's role as a suppressor of mTORC1, the group found that mTORC1-associated phosphorylation was significantly increased when hamartin was knocked down in hippocampal neurons. Furthermore, Papadakis and co-workers found that markers of autophagosome formation and accumulation were significantly upregulated in hamartin-depleted neurons, suggesting that while autophagosomes were being formed efficiently, their degradation – a process dependent on mTORC1 – was failing. Taken together, these data suggest that hamartin may mediate neuronal protection against ischaemia via induction of efficient autophagy through mTORC1, highlighting hamartin's potential as a therapeutic target for neuroprotection in stroke.

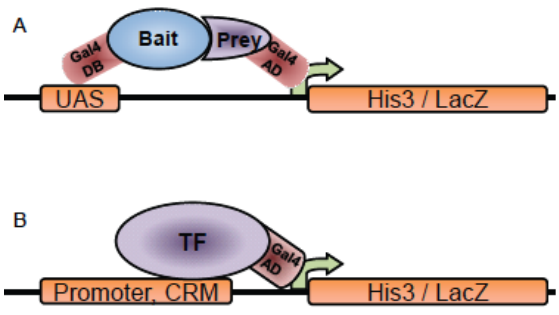
Gene regulatory network mapping in the fruit fly *Drosophila melanogaster*

Drosophila has one of the largest collections of annotated gene regulatory DNA elements. To explore how these elements contribute to gene regulation, we have developed microfluidics and *"in yeasto"* techniques to identify proteins that interact with these DNA elements. My lab now uses these techniques to study the gene regulatory networks governing the transcription of neuropeptides and peptide hormones in response to changes in the interior milieu.

by Dr Korneel Hens

With the advent of next-generation sequencing techniques, genomic sequences for a large variety of organisms are becoming available ever faster. The biggest challenge is currently therefore not the generation, but the functional annotation of this vast quantity of sequence data. The first step to genome annotation is delineating the set of protein-coding genes, and several computational pipelines have been developed for this purpose. Consequently, near complete lists of protein-coding sequences exist for several species. However, although some bacterial species can achieve coding densities of more than 90% of the genome (1), a large portion of the genomic DNA of complex organisms does not code for proteins (e.g. less than 2% of the human genome (2)). Although this non-coding DNA was sometimes referred to as "junk DNA", it has become clear that a significant portion of it has a defined function.

One class of functional non-coding sequences consists of *cis*-acting DNA elements that are responsible for specifying the spatial and temporal expression of genes. The first *cis*-regulatory modules (CRMs) were identified in viruses with the observation that certain viral DNA elements can enhance, in *cis*, the transcription of a nearby host organism gene even when they are located several thousand base pairs away from the promoter (3). Later, endogenous DNA elements were discovered in the fruit fly *Drosophila melanogaster* (4) and the mouse (5) with the same enhancing activities as their viral counterparts.



Development of new technologies has facilitated the discovery of new CRMs. For example, the precise location of transcriptional start sites can be determined using cap analysis of gene expression (CAGE) which helps identifying the promoter driving gene expression (6). Chromatin immunoprecipitation assays coupled to microarray (ChIP-chip) or high-throughput sequencing techniques (ChIP-seq) can be used to map enhancer-associated proteins like the transcriptional activator p300 or histone modifications that are enriched at active enhancers (e.g. histone H3K4 monomethylation), at active promoters (e.g. histone H3K4 trimethylation) or at repressors (histone H3K27 trimethylation) (7, 8). In addition to these experimental innovations, new bioinformatics approaches have been developed which correlate expression data with statistically overrepresented patterns within non-coding DNA (9, 10). Furthermore, comparative genomic-based approaches are used to identify evolutionary conserved stretches of non-coding DNA which have a high propensity to behave as CRMs.

Figure 1: Yeast two-hybrid and one-hybrid techniques. A. The yeast two-hybrid technique is based on the modularity of the yeast GAL4 transcription factor. GAL4 can be split into a functional DNA binding domain (GAL4 DB) and transcriptional activation domain (GAL4 AD). The DB is fused to a bait protein and this fusion construct is used to screen a library of prey proteins which have the AD fused to them. If bait and prey proteins interact, a functional GAL4 is reconstituted, which will bind the upstream activating sequence (UAS) and activate the transcription of a reporter gene such as *HIS3*, allowing the yeast to grow on media lacking histidine, or *LacZ* which can be detected colorimetrically. **B.** The one-hybrid technique is used to test the DNA binding specificity of proteins. A GAL4 AD is fused to the transcription factor to overcome potential intrinsic repressive qualities of the transcription factor. The readout is identical to the yeast two-hybrid assay.

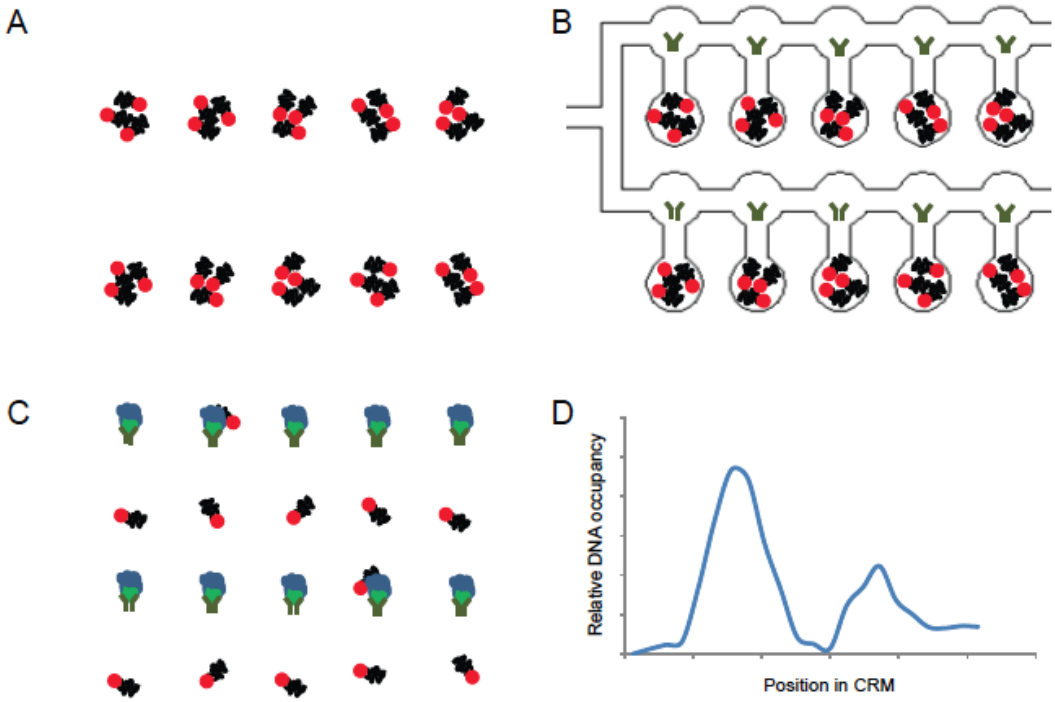


Figure 2: Overview of the MARE technique. A. A CRM is divided in short DNA elements. These DNA elements are fluorescently labelled (Cy5) and spotted on a microscope slide. **B.** Each DNA spot is captured in a chamber of a microfluidics device. The slide surface in the adjacent chamber is coated with an anti-GFP antibody. **C.** Expression templates coding for transcription factor-GFP fusions are translated to protein off-chip and introduced in the microfluidics device. The transcription factor is pulled down by its GFP tag on the anti-GFP antibody. DNA elements bound by the transcription factor are pulled down as well. **D.** The slide is scanned for Cy5 and GFP fluorescence. The Cy5/GFP ratio reflects the relative DNA occupancy for a specific transcription factor.

Drosophila has always excelled as a model organism in the discovery and study of new CRMs, exemplified in the fact that the *even-skipped* stripe 2 enhancer, that drives expression of the *even-skipped* gene in a dorso-ventral band in the *Drosophila* embryo, is one of the best characterised metazoan CRMs. This is due to the availability of a high-quality genome sequence, a convenient large-scale enhancer trapping assay, the modENCODE project with its ChIP-chip and ChIP-seq data and, last but not least, a convenient transgenesis system, illustrated by the recent publication of almost 3,900 transgenic *Drosophila* lines containing CRMs that drive expression in distinct neuronal cell types (11).

Specific short DNA sequences within CRMs act as interaction sites for DNA-binding proteins or transcription factors (TFs) that, together with activating or repressing cofactors, can determine the transcriptional status of a gene by respectively recruiting RNA polymerase II to or blocking it from the promoter. Several techniques have been developed to study the binding of specific TFs to regulatory elements. These so-called TF-centered techniques can be aimed at determining the binding of TFs to selected regulatory elements or binding sites, e.g. electromobility shift assays, or at identifying TF binding sites genome wide, e.g. ChIP-chip or ChIP-seq. However, an increasing challenge in science is to achieve a quantitative understanding of complex biological processes. Such a 'systems biology' approach requires techniques that allow us to uncover the full complement of genes and proteins involved in biological processes, as well as their interactions.

We have developed an automated, yeast one-hybrid (Y1H) technique that can map protein-DNA interactions on a genome-wide level, providing a powerful tool to de-orphanise, in a high-throughput manner, the many functional *Drosophila* promoters and CRMs for which the interacting TFs are still unknown (12). The Y1H technique was developed as a modification of the yeast two-hybrid (Y2H) that is used for detecting protein-protein interactions (Figure 1). To increase the sensitivity of our high-throughput *Drosophila* Y1H system, we have established, to our knowledge, one of the most comprehensive, full-length, fully sequence-verified TF ORF clone collections for a metazoan organism, containing over 90% of predicted *Drosophila* TFs. Since the ORFs were cloned open-ended (without

a stop codon) in the versatile Gateway system, this collection should be of significant value to the *Drosophila* community.

The Y1H system described above can be used to map interactions between TFs and CRMs but lacks the resolution to identify individual binding sites within a CRM. To overcome this limitation, we have developed a microfluidics-based technique called MARE (MITOMI-based Analysis of Regulatory Elements) (Figure 2). MARE allows the relatively straight-forward analysis of multiple TFs across a large panel of individual DNA sequences on one chip while they are simultaneously providing a quantitative read-out for the observed interactions. Coupled to the high-throughput Y1H system, this pipeline therefore uniquely enables us to identify TFs binding to an uncharacterised CRM, and subsequently locate the specific binding site for each of these TFs within this element.

We are currently using these techniques to study the molecular underpinnings of homeostatic regulation. Homeostasis is the result of regulatory mechanisms that maintain a constant environment within and around living cells, while faced with changes in external environmental conditions, with regard to pH, salt concentration, temperature, and nutrient levels. This regulation requires accurate sensing of changes in the interior milieu, conveying this information to synthesis sites of bioactive effector molecules such as hormones and neurotransmitters, which in turn trigger a response in their target organs to counteract the effects of the environmental change.

A good example of how changes in the interior milieu lead to changes in the expression of neuropeptides is the *Drosophila* insulin-like peptide (*dilp*) gene family. *Dilp* expression is modulated in response to changes in nutritional status and fertility. Insulin is a peptide hormone that has been extensively studied for its regulatory role in carbohydrate and fat metabolism in mammalian organisms. Additionally, failure to produce insulin or resistance to insulin leads to diabetes mellitus which is a rising global hazard: about 10.3% of men and 9.6% of women aged 25 years and over have diabetes in the European region and the number of adults with diabetes has more than doubled over nearly three decades.

The insulin/IGF-1 signalling pathway is evolutionary conserved between mammals and *Drosophila* both at the structural level and at the functional level. In addition, the elevated haemolymph carbohydrate levels in larvae lacking insulin-producing cells are reminiscent of the accumulation of carbohydrates in the blood seen in human diabetes mellitus. This observation, combined with the fact that the fruit fly has proven to be extremely useful to study gene regulation underscores the use of *Drosophila* as a model organism for studying metabolic diseases such as diabetes. Our aim is therefore to uncover how changes in the nutritional status and fecundity are sensed by the brain, how this information is integrated and translated to changes in the regulatory state of the *Drosophila* insulin-like peptides, and thus how *Drosophila* can serve as a model for diabetes.

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HIV vaccine developments

by Rosalind Roberts

Since its emergence in the early 1980s, AIDS has developed into a worldwide pandemic. It has devastated many developing countries, where life expectancies have fallen dramatically as a result. A vaccine holds the greatest promise for slowing the pandemic, but will it be possible to design an effective vaccine against HIV, a virus well adapted to function under the radar of the immune system?

In 1984 a team of researchers led by Dr Robert Gallo at the National Institute for Cancer, Maryland, identified the human immunodeficiency virus (HIV) as the causative agent of acquired immunodeficiency syndrome (AIDS). A decade later, an assembly of HIV experts in Italy discussed strategies for HIV vaccine development. The outcome was the founding of the International AIDS Vaccine Initiative (IAVI), the first consortium with a primary focus to advocate collaboration across industry, academia and government, with the goal of developing robust vaccines.

Almost 20 years later, we now have a fuller understanding of the virus and its mechanism of infection, but no vaccine against HIV. HIV preferentially infects $CD4^+$ T cells, gaining entry via interaction of the viral envelope proteins, gp120 and gp41, with the CD4 receptor and one of its co-receptors, CCR5 or CXCR4 (Figure 1). Once inside the host cell, the virus integrates its DNA into the host genome, where it can lie latent for many years. In contrast, the process of HIV replication kills the host cell, and AIDS develops when T cell titre drops so low that infections can no longer be successfully fought off by the immune system.

Highly active anti-retroviral therapy (HAART), a combination of drugs that target different stages in the HIV life cycle, has been successful in maintaining a life expectancy for Western HIV positive individuals of only ten years lower than the population average. HAART has also shown effectiveness as a prophylactic treatment against HIV. However, despite its success, HAART is not a satisfactory alternative to a vaccine as it requires strict adherence to a complicated treatment regimen, and has unpleasant side effects. In addition, HIV infection is primarily a problem in developing countries; the World Health Organization currently estimates that of the 34 million people infected with HIV worldwide, 70% are in sub-Saharan Africa. Access to HAART in developing countries is limited, with only 56% coverage in this region, compared to approximately 90% in Western Europe. In its place, preventative strategies, such as encouraging the use of condoms and voluntary male circumcision (shown to prevent transmission by up to 60%), are used to limited effect. The development of a vaccine that could be administered once and provide lasting immunity would prevent the spread of the virus, and reduce the cost of treatment and awareness programmes around the world.

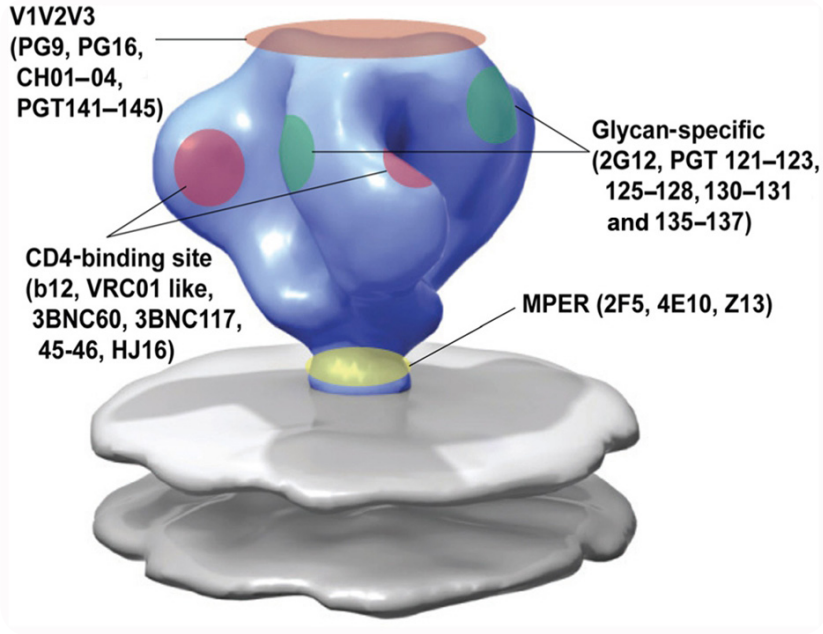

Figure 1: The HIV-1 envelope (Env) with epitope specificity of known broadly neutralising antibodies indicated. Reproduced with permission (1).

The RV144 vaccine trial in Thailand, the results of which were published in 2009 (2), was a milestone for the HIV vaccine community. Since 1987 there have been 187 separate vaccine trials, none of which showed much effect on preventing the acquisition of HIV. The RV144 vaccine consisted of two previously trialled candidates: ALVAC-HIV, a canarypox virus vector carrying genes encoding envelope proteins and enzymes required for viral assembly; and AIDSVAX B/E, consisting of recombinant gp120 protein. The vaccine was administered to Thai adults at heterosexual risk of HIV infection. The treatment group showed a 31% decrease in acquisition of the virus, representing the most

promising results to date. However, those who went on to be infected did not have decreased viral load or increased CD4+ T cell counts.

Understanding the immune system's response to vaccination is the next step to design a more efficacious vaccine. Currently, there are no identified markers for a productive response to a vaccine, particularly as the immune system is unable to surmount an effective response under normal infection conditions. This is vital to enable rational design for future improvements.

A recent study suggests that an immune response against components of the variable viral envelope loops 1 (V1) and 2 (V2) is key to the efficacy of the RV144 vaccine (3). Patients who responded to the vaccine produced IgG antibodies against the V1/V2 loop, which is an important region for mediating the interaction with the CD4 receptor. A follow-up study identified key mutations in this loop which correlated with a vaccine-induced immune response (4). However, these mutations are only found in certain strains of the virus, which may explain the relatively low efficacy of RV144.

Many avenues of research are being pursued to build on the results of the RV144 trial, bringing the reality of a preventative vaccine closer. An ideal vaccine would be able to protect against multiple strains of the virus. This would require a broad humoral (antibody) response, with antibodies targeting epitopes in the envelope proteins on the surface of the virion. However, HIV is very adept at evading recognition by antibodies; the surface proteins are heavily glycosylated, effectively creating a glycan shield which hides neutralising epitopes. There is also a great amount of inter-strain variability, and the flexibility of the surface proteins means that conformational masking may occur.

The identification of broadly neutralising antibodies (bnAbs) in the sera of HIV patients (5, 6) has given hope that the development of a vaccine capable of providing protection against diverse strains is achievable. Some bnAbs are capable of neutralising up to 80% of the >150 strains of HIV tested. BnAbs are highly unusual in their sequence and structure – the acquisition of mutations during maturation of the B cell is five- to ten-fold higher than that of the average human memory B cell receptor. It may therefore be difficult to induce these in naive individuals after one administration of a vaccine, as they are usually only found in patients after many years of infection. Structural biologists are studying the conformations of these antibodies in detail to understand which region of the viral envelope they target, and thus which regions are vulnerable to neutralisation by antibodies. It may then be possible to reverse engineer immunogens capable of inducing productive responses.

Alternatively, it may be possible to circumvent the requirement for production of heavily mutated antibodies working on the hypothesis that HIV exploits a gap in the B cell repertoire. This limitation of the B cell response could be overcome by relaxing B cell tolerance checkpoints simultaneously with immunisation. As bnAbs were only recently identified, there is much work to be done to understand how they are produced and how best to enhance their production in vaccinated individuals.

Vaccine research often focuses on the humoral response, but cellular immunity is also a worthwhile target for a vaccine. Individuals that have been exposed to the virus many times, but remain uninfected (known as exposed seronegatives) display cellular immune responses against HIV, but not antibody responses. It is hypothesised that cellular immunity could be easier to induce and key in controlling virus replication. Activation of both $CD4^+$ and $CD8^+$ T cells would be necessary to ensure a fully productive response. It is likely that a combination of humoral and cellular immunity will provide the best protection.

The IAVI remains optimistic that a vaccine can provide a lasting solution to the HIV epidemic, and aims to have a vaccine inducing broadly neutralising antibodies in clinical trials by 2015, as well as a repertoire of vector-based vaccine candidates. It seems likely that, similarly to HAART, a vaccine against HIV will require a combinatorial approach. A similar approach in research will be required to get there.

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Rosalind Roberts is a 2nd year DPhil student in Dr Richard Wade-Martins' laboratory in the Department of Physiology, Anatomy and Genetics.

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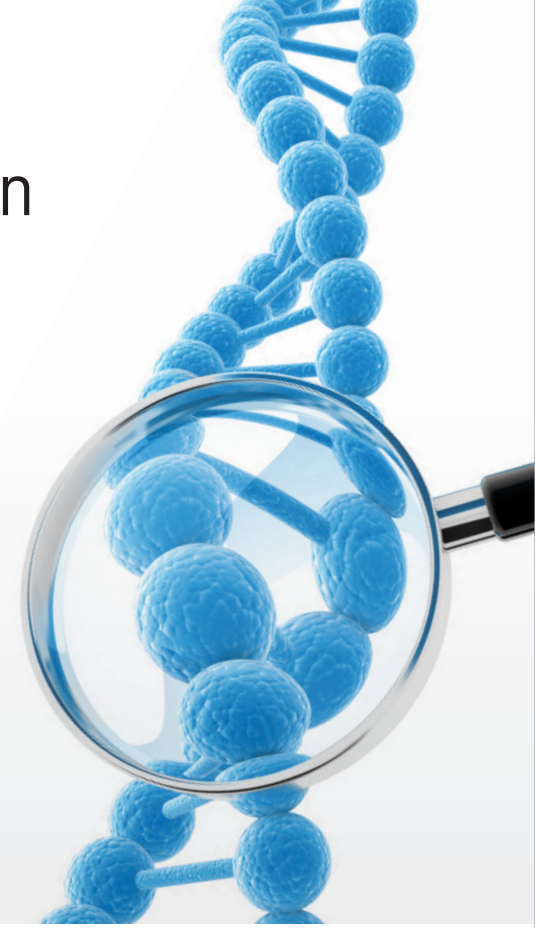

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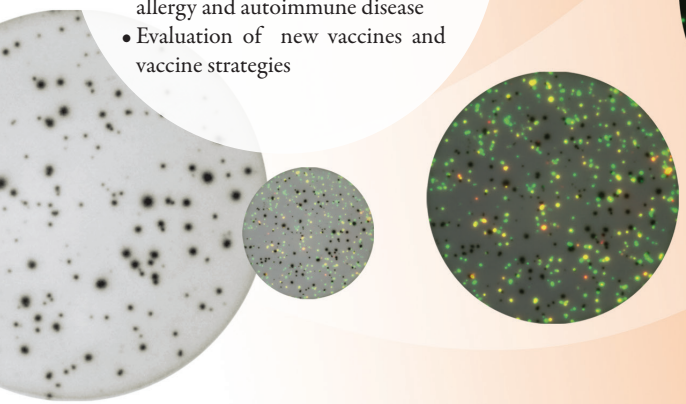

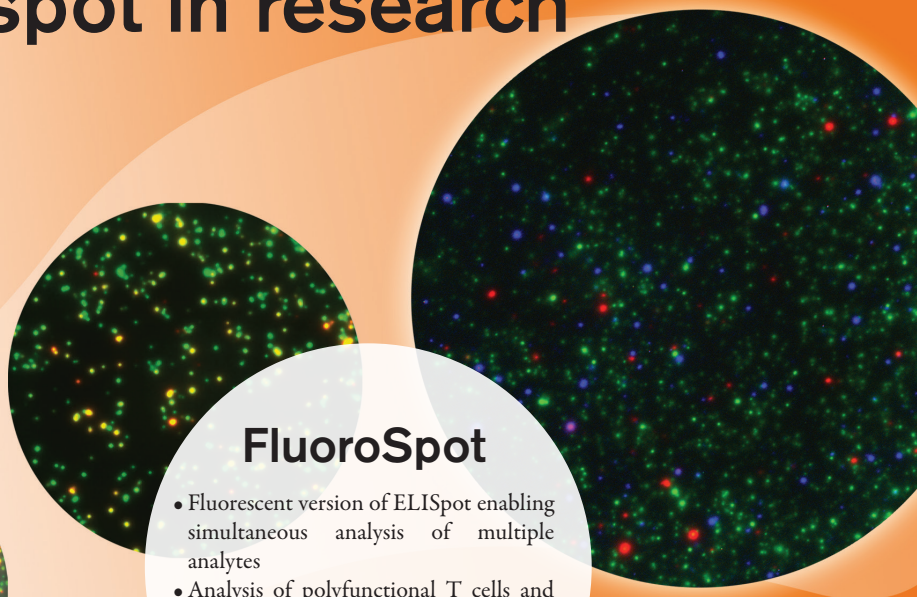

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Prescription genomes

Cancer arises in the body as a result of accumulating somatic mutations in a rogue group of cells, or tumour. Each tumour genome is unique and distinguishable from the normal genome of an individual. Traditional treatments have adopted a 'one-size-fits-all' approach, with surgery, chemotherapy and radiotherapy being prescribed across the board. However, with the increasing understanding of the different types of cancer and their aetiologies, treatments can now be tailored to the individual, based on the types of somatic mutation(s) their cancer harbours. Such mutation-specific therapies will target specific cancer cells uniquely, maximising the efficacy of treatments and increasing survival rates.

by Dr Elizabeth Hartfield

Colorectal cancer treatment is a good example of how personalised therapy can increase survival rates. This cancer is the third most common form of cancer diagnosed in the UK and accounts for 13% of all cancer cases. The standard treatment regimen for this cancer is chemotherapy, yet recent findings identified that subtypes vary in their response: tumours with high thymidylate synthase expression and microsatellite instabilities respond poorly, whereas patients that are homozygous for the *UGT1A1* gene respond well to some types of chemotherapy but experience severe haemolytic side effects with others. Furthermore, almost 50% of colorectal cancers carry a mutation in the *KRAS* gene and are thus resistant to anti-angiogenesis agents, a major class of chemotherapeutics. With the aid of genomic analysis, the post-treatment survival time for colorectal cancer patients has doubled between 1990 and 2006 (1).

Herceptin™ (also known as trastuzumab) is a highly effective monoclonal antibody treatment against early breast cancers that overexpress the HER2 receptor (Figure 1). *HER2* genetic screening has now been implemented as part of the diagnostic routine. An eight-year US-based study of over 6,000 women with breast cancer found that 90% of patients were screened and of these, 77% received Herceptin™ as the appropriate treatment for their disease (2). This demonstrates that the genetic analysis of individual patients is crucial for accurate and predictive therapeutic strategies, and that money is not being wasted in administering expensive drugs to patients with tumours that will not respond.

Genomic mutations are important when considering cancer prediction as well as treatments. Mutations in the genes *BRCA1* and *BRCA2*, although only responsible for around 5% of breast cancer cases, confer a higher risk for breast and ovarian cancers in women. These genes are inherited in an autosomal dominant fashion, and therefore 50% of children born to a mutation carrier will inherit the mutated copy. Identifying which mutation is carried can help directly inform treatment choice, as *BRCA1* mutation carriers are unresponsive to Herceptin™, whereas *BRCA2* carriers respond well.

Next-generation sequencing has been evolving since the mid 1990s and has enabled low-cost, high-throughput processing of samples. Thousands of sequencing reactions can be produced in parallel from the genome and aligned to give a read from a single reaction tube. The whole genome can be mapped in under a day for less than £1,000. NICE (the National Institute for Health and Clinical Excellence) has recommended that genetic screening for breast cancer and the drug, Herceptin™, be made available on the NHS in the UK at a cost of £17 million per year. This is expected to help around 2,000 women annually. Despite this large cost, savings will be made in shorter treatment times and longer periods of good health due to the correct diagnosis and quick administration of the appropriate treatment.

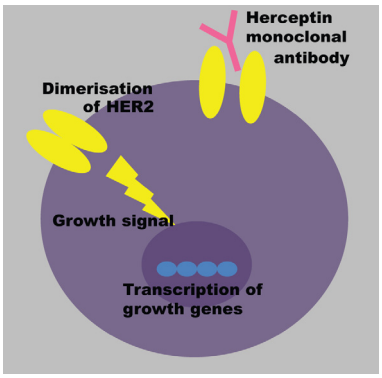

Figure 1: Mechanism of action of Herceptin™.
Herceptin™ binds to the extracellular portion of the HER2 protein, preventing dimerisation and repressing the rapid cell growth and proliferation that is associated with tumour formation.

By using data produced from genomic analyses of cancers, we will be able to prescribe targeted, and thereby the most effective, treatments to date. What remains to be seen is whether the NHS will be able to adapt and cope in the post-genomic era of cancer diagnosis.

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Dr Elizabeth Hartfield is a Career Development Fellow in Dr Richard Wade-Martins' laboratory, Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics.

Blame it on the sushi! Conditioned taste aversion as a model for learning and memory

by
Dr Gaurav Das

My graduate advisor once recounted to me how one night, in a downtown Seattle sushi bar, he had a raw quail egg on top of a dollop of salmon roe. Hours later, seized by violent cramps, he lay clutching his stomach on the bathroom floor. Needless to say, he would not touch anything remotely eggy or runny for years to come.

We have an elaborate system for detecting and rejecting toxic food and often perceive them as bitter tasting or foul smelling. It is when toxins evade detection by taste or smell and upon ingestion make us sick, that a robust aversive learning mechanism kicks in. This makes us avoid such malaise-inducing food in the future. The memory of the toxin-induced sickness is even stronger when the food is question is novel or exotic: thus one is less likely to assign blame to the regular morning muesli than to the once-in-a-blue-moon sushi. This phenomenon is known as conditioned taste aversion (CTA).

In the early 1950s Dr John Garcia observed that irradiated rats avoided drinking from provided water bottles in the radiation chambers. Curious, he exposed rats that had exclusive access to either water or sweet saccharin solution, to gamma radiation. Two days later, these rats had to choose between water and saccharin solution to drink.

Now, rats normally have a high preference for sweet taste. True to that trend, rats that had water during radiation exposure preferred the saccharin solution. But, remarkably, rats that had saccharin solution during irradiation now showed a marked aversion to it (1)! This aversion to saccharin was still evident a month after conditioning. Dr John Garcia went on to show that other nausea-causing cues, such as toxic lithium chloride injected after saccharin consumption, also formed CTA in rats. Subsequent research on CTA showed that similar aversions are formed in other species. For John Garcia's contributions to the study of CTA, the phenomenon also came to be known as the 'Garcia effect'.

In Pavlov's classical conditioning experiment, meat (unconditioned stimulus, US) elicits salivation (unconditioned reaction, UR) in dogs. When a neutral stimulus, like the ring of a bell (conditioned stimulus, CS) is paired repeatedly with the presentation of meat, the dogs subsequently salivate merely upon the bell ring. CTA closely parallels Pavlovian conditioning: gamma radiation (US) causes sickness (UR) in rats. After the saccharin solution (CS) has been paired once with radiation exposure, presentation of the CS lead to its prompt rejection. Interestingly, for Pavlovian association to form, the CS and US must be almost perfectly contiguous, while in CTA a large time lag between the CS and US is tolerated – sickness can hit hours after ingestion, but a strong association is still formed between these two events.

This has important implications for the neural and molecular basis of CTA. In Pavlovian conditioning, specific neurons represent the coincidence of US and CS. Studies on the anatomical/neural circuit basis of CTA also point to critical brain regions. But how do neurons in these regions encode the delayed association? How is the neural representation of the novel foods held in waiting in a neuronal circuit for the subsequent sickness cue? Ultimately, an understanding of the neural basis of CTA would help build a neurobiological theory of our likes, dislikes, attractions and aversions.

Eat glorious raw fish on subtle fat rice
Chase it with bad karma sake, drunk
See sunrise from paddy field throwing-up
Is glorious fish to blame?

Haiku, origin unknown

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Dr Gaurav Das is a postdoctoral research assistant in Prof Waddell's group at the Centre for Neural Circuits and Behaviour.

Mosquitoes: a necessary evil?

For some people mosquitoes constitute one of the most gruesome aspects of nature, spreading a variety of diseases. They are responsible for infecting 247 million people with malaria and causing around one million deaths every year (1). Mosquitoes are also pests, infecting plantation crops such as tea, or asphyxiating caribou in Alaska by forming dense groups. Hence, many wish mosquitoes were wiped off the surface of the Earth. Nevertheless, since they have been around for over 100 million years and have co-evolved with many species, there is concern that eradicating a mosquito species could leave a predator without a prey, or a plant without a pollinator. Scientists, however, believe that the ecological scar left by eradicating mosquitoes would heal quickly and other organisms would fill the vacant niche.

by Dr Maria Mogni

Ecologically, the Arctic tundra would probably suffer the most from eliminating mosquitoes. From Canada to Russia, there is a short period during the year in which mosquito species such as *Aedes impiger* and *Aedes nigripes* are abundant, forming thick clouds. Their disappearance was estimated to result in more than a 50% decrease of migratory birds that establish their nests in the tundra, due to the lack of food source. However, a recent study revealed that Arctic mosquitoes are not highly abundant in bird stomach samples and that midges are a more important food source. In contrast, since mosquitoes suck up about 300 ml of blood a day from each animal in a caribou herd, they influence the herd's path, which moves into the wind to escape the swarm (1). If the path were to change, consequences could include trampling other areas, nutrient transportation and impact on wolves' feeding.

Around the world, the absence of mosquito larvae would force hundreds of species of fish, such as the mosquitofish, *Gambusia affinis*, to change their diet. This would be problematic as they have a highly adapted feeding behaviour, thus affecting the food chain. Additionally, numerous species of insects, spiders, salamanders, lizards, frogs and birds would lose a primary food source, which could however potentially be replaced by other insects emerging in its place. Some insectivores, like bats, may not miss mosquitoes at all, as they constitute less than 2% of their gut contents (1).

In aquatic ecosystems, mosquito larvae form a considerable biomass, feeding on decaying leaves, organic detritus and microorganisms (Figure 1). The impact of their elimination would depend on the body of water in question. Tight-knit communities in 25-100 ml pools inside pitcher plants on the East Coast of North America, for example, are highly dependent on mosquito larvae. When other insects drown in the water, the midges chew up their carcasses and the larvae feed on the waste products, producing nutrients available for the plant. As larvae feed, they also keep the number of dominant species of protozoa down (1). Thus, eliminating mosquitoes might affect plant growth and the local aquatic ecosystem.

Without mosquitoes, plant species like cacao would lose a group of pollinators. Indeed, adult mosquitoes depend on nectar for energy, as females of only some species require a blood meal to obtain the proteins necessary to lay eggs. However, pollination by mosquitoes is not crucial for crops that humans depend on.

One important ecological role mosquitoes play is as a vector, constituting a major route of disease transmission: they are efficient at sucking blood from one individual and injecting saliva into another, transferring parasites and/or viruses. Eradicating mosquitoes would save millions of lives. Several techniques like RNAi-based insecticides, improved chemicals, mosquito traps and genetically engineered mosquitoes are in development for this purpose. These efforts aim to diminish the detrimental effect on human health and consequent societal burden. However, the relief obtained using these techniques might only be temporary as one insect vector species can be easily overtaken by another.

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Figure 1: Mosquito larvae form a considerable biomass in water pools. Reproduced with permission (1).

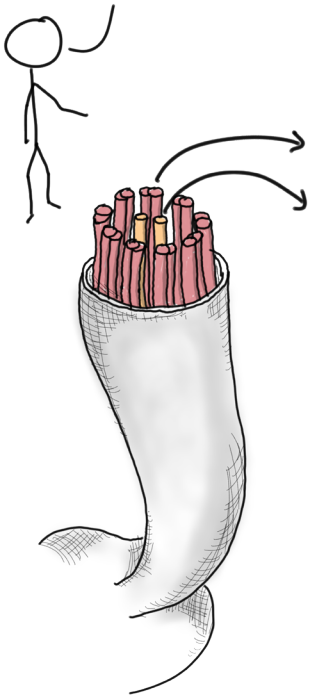
Dr Maria Mogni is a DPhil alumna from the Sir William Dunn School of Pathology.

Cilia

Cilia are probably the most multifunctional cell structure.

A huge number of eukaryotic cells have cilia and the last universal ancestor of *all* eukaryotes had one.

Sometimes cilia are called flagella, but they share the same core structure made of microtubules.

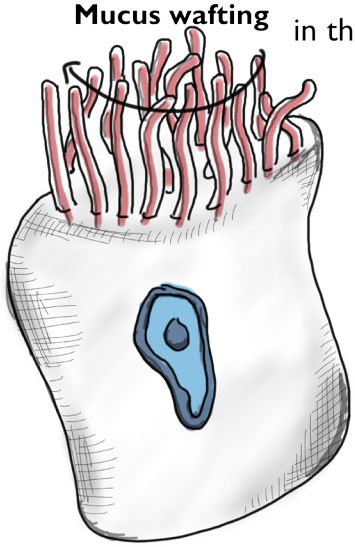


Outer microtubule doublets - in all cilia

Microtubule central pair - only in cilia that move

Cilia and flagella often move cells, or move things in the cell's surroundings:

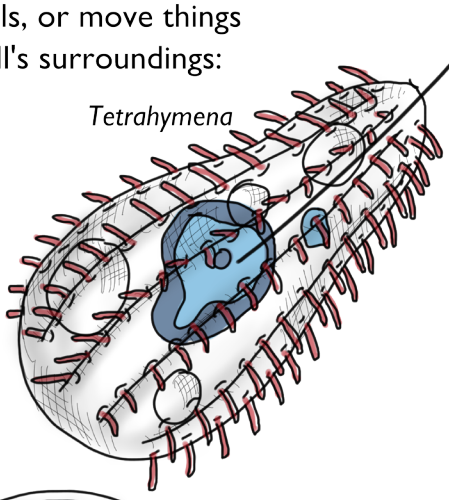
Mucus wafting

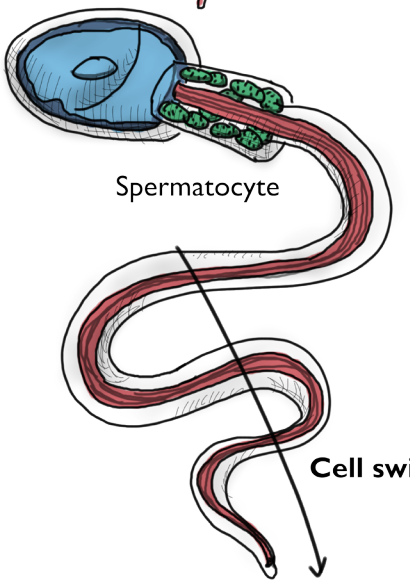


Ciliated epithelial cell

Cell swimming

Tetrahymena



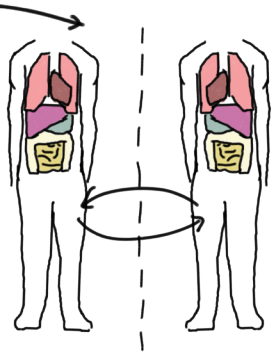


Spermatocyte

Cell swimming

Movement is just the most obvious of many ciliary functions though; genetic diseases which affect their formation can cause severe developmental problems.

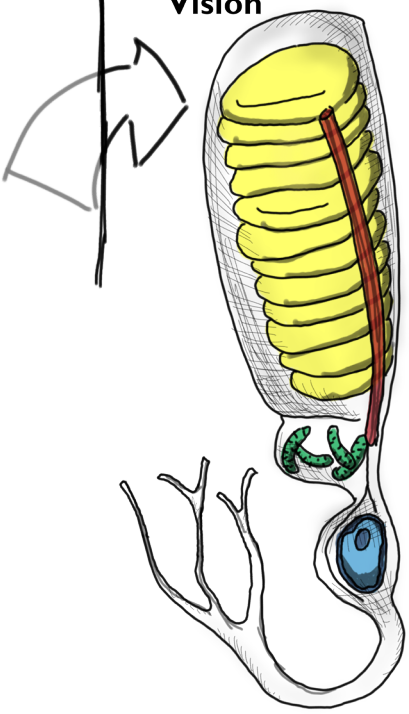
One mild (but interesting!) phenotype is *situs inversus* where the position of a person's organs are mirrored left to right. About ¼ of cases are caused by primary ciliary dyskinesia where there are defects in the movement of cilia.



Movement may play a role in some cilium-mediated development, but cilia can also have a direct sensory role...

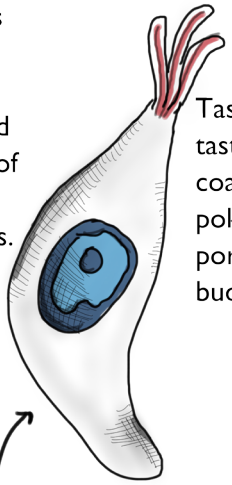
Four of our five senses rely on cilia which have dedicated sensory functions:

Vision



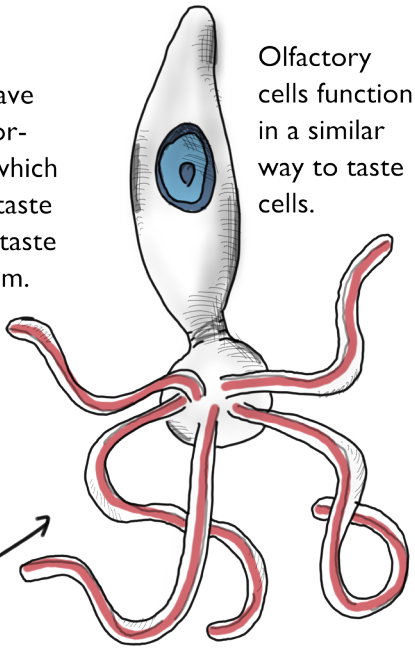
Rod (and cone) cells in the retina are mostly made up of one huge cilium filled with stacked layers of membrane coated with photoreceptors.

Taste

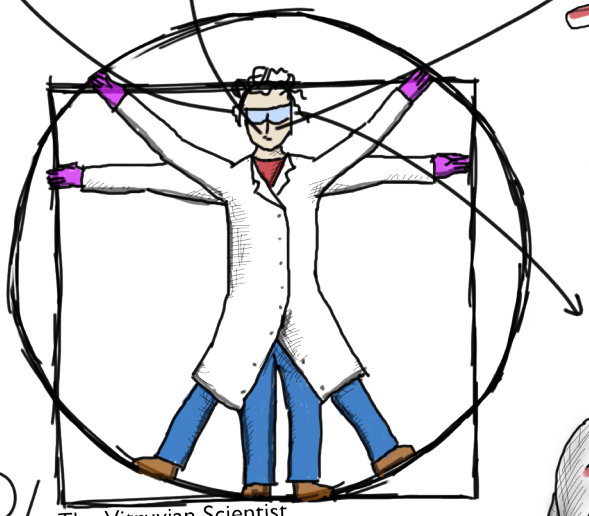


Taste cells have taste receptor-coated cilia which poke out of taste pores in the taste bud epithelium.

Smell



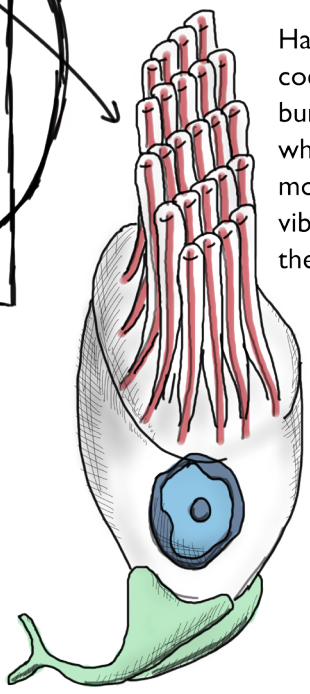
Olfactory cells function in a similar way to taste cells.



The Vitruvian Scientist

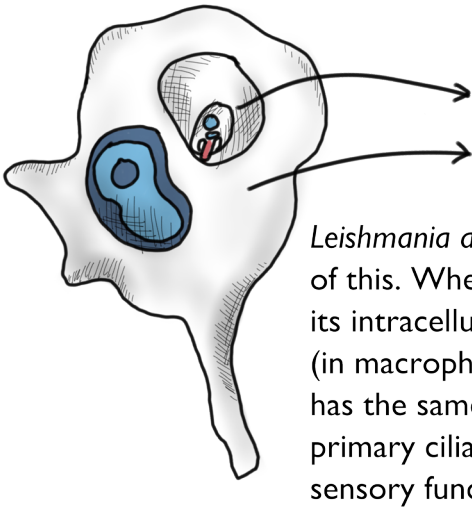
These aren't the only examples of sensory cilia. New examples keep emerging, like the primary cilium found on many human cells... so how many cilia have as-yet unknown sensory functions?

Hearing



Hair cells in the cochlea have a bundle of stereocilia which detect movement caused by vibrations (sound) in the cochlear fluid.

(These are actually more like actin-based microvilli, but require a cilium for their development and function)



Leishmania are a great example of this. When the parasite is in its intracellular life cycle stage (in macrophages) its flagellum has the same structure as primary cilia. Could it have a sensory function too?

While so much is known about cilia it seems our understanding has only just scratched the surface. It's 'cilly' to underestimate this organelle!

By Richard Wheeler 2013

Confusing our view of a gene: when transcription goes backwards

by Struan Murray

In yeast, most genes experience non-coding transcription of their antisense strand. Recent research suggests this 'gene symmetry' might have important regulatory consequences, but is this the case in mammals?

The human genome project left everyone underwhelmed by the lack of genes inside our cells, and the great swathes of 'junk' DNA found in their place. Despite such vast gene deserts, most of the genome seems to get transcribed anyway, albeit at often low levels, leading to the curious term 'RNA polymerase promiscuity' – the idea that transcription in genomes may be a messy, unselective process, biological noise of no real consequence, or an artefact of genome-wide experiments. Current research in my lab suggests low-level non-coding transcription could have dramatic and functional genomic effects.

Recently, a curious phenomenon has arisen from the observation that many genes in eukaryotes are transcribed on their antisense strand – a class of non-coding transcription in which transcripts are made from the terminator to the promoter (1). The proposal that this might be a common feature of genes has been met, rather predictably, with both controversy and scepticism, due to the difficulty in measuring antisense transcription. Most genome-wide experiments designed to measure transcription do not directly measure transcription, but rather measure the transcripts. This distinction is not always important; however, for antisense transcription the distinction is crucial as antisense transcripts are often rapidly degraded: the moment you go looking for them, they are already gone.

The recent development of an RNA-seq based method, in which elongating RNA polymerase is immunoprecipitated and the nascent RNA purified, allows a more accurate determination of the actual transcription happening on both DNA strands (2). In budding yeast, this technique has demonstrated that antisense transcription is abundant within the genome, on the same order of magnitude as conventional, coding transcription. Moreover, the terminators of genes exhibiting antisense transcription display features normally associated with the promoter, and vice-versa – a surprising gene symmetry. This suggests that both ends of a gene might be subject to extensive regulation, tuning the levels of sense and antisense transcription (3).

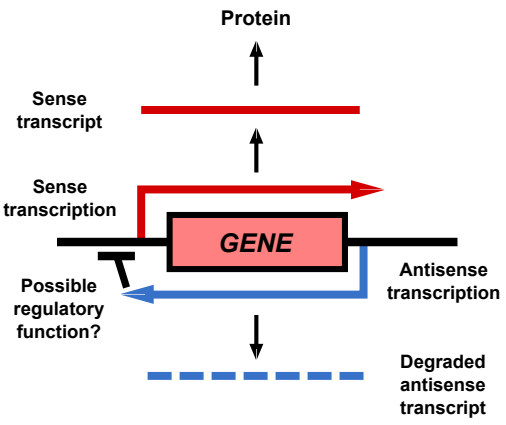


Figure 1: Illustration of a sense transcript which leads to production of a protein and the antisense transcript which may possibly have a regulatory function.

This raises the question of what antisense transcription is actually doing. Budding yeast have no RNAi machinery, and antisense transcripts are quickly degraded, confounding things further – why bother making them if they are going to get broken down immediately? A possible answer could be that the act of transcription, rather than the transcript itself, might be exerting regulatory effects. Antisense transcription tends to proceed right into the gene promoter, and it is hard to envisage this not having certain consequences. Indeed, current research in our lab suggests that antisense transcription brings about changes both at the gene promoter and across the gene itself. Antisense transcription appears to actually reset the epigenetic state of the gene – continually replacing its histones with fresh ones, altering the pattern of histone modification. It seems to be capable of bringing about such changes even at the sorts of low levels some might consider 'noise'.

In mammals, the sheer size of the genome has shown to be a major hindrance in measuring nascent low-level transcription. Only very abundant transcription events are sampled, namely coding transcription. It still remains unclear how prevalent non-coding antisense transcription in mammals might be. Our findings in yeast demonstrate that most genes (~75%) experience antisense transcription, and that it has unique effects on gene behaviour. Further sequencing experiments will be required to assess whether such gene symmetry is also prevalent in mammals, and whether it has similar effects.

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Struan Murray is a final year DPhil student in Prof Jane Mellor's laboratory in the Department of Biochemistry.

A pie, a pint and some quantum physics

Rupal Mistry

1st year Biochemistry DPhil student working in the Ligoxygakis group.

Established in 1831, the British Science Association was founded to encourage individuals to engage with science, engineering and technology. Science is an open-ended field full of potential, continuously evolving and endlessly churning out new discoveries. Many assume that to be involved with the latest findings and cutting-edge research you must be a formally trained scientist. SciBar was introduced to show that this is not the case, and that people from all walks of life can access science over a pint in their local pub.

SciBar was founded in 2009 with the main goal of making science more accessible, urging people of all ages to voice their opinions and ask questions. The typical audience at SciBar varies depending on the speaker, and events are open to all, from professors to graduate students to interested locals. With such a mixed group of spectators, it is a great way to meet people and understand science from another perspective. Each month the Port Mahon Pub in Oxford hosts the Oxfordshire branch of the British Science Association's SciBar, where a range of speakers, such as Prof Peter Atkins and Prof Colin Blakemore, have discussed topics ranging from detox diets to the possibility of the world's end in 2012 (1).

At a recent SciBar meeting, Dr Joey O'Gorman asked "Where is the art in science?" (2). Being passionate about both art and science, he discussed the dilemma he faced due to the lack of collaboration between the two, explaining that neither subject really understands the other, yet they could both benefit from cooperation. To quell the ongoing antagonism between the arts and science, each party must highlight the concept of their field to the other. Recognising this, Dr O'Gorman currently researches and lectures in MA Art and Science programmes. During his talk, he rationalised that although science has infinite possibilities, day-to-day science is limited by facts and structured protocols. However, the liberal nature of art means that nothing is ever concrete and the interpretation of art depends on the individual's perception. Regardless of the distinct differences between the two disciplines, science depends on art to communicate complex ideas without the jargon. Recently, creativity and innovation in science are starting to be promoted. Likewise, much of art requires science, from mixing paints over pottery to the composition of materials. Cooperation between the two is mutually beneficial.

Figure 1: The British Science Association promotes science since 1831.

In addition to SciBar, the Oxfordshire branch of the British Science Association has also launched SciBox, a sophisticated blog for those thirsty for science news, fascinating stories and opinions on all things science. Written by the Oxfordshire committee members, SciBox is an easy and exciting way to keep on top of the latest scoops.

SciBar receives its funds primarily from the British Science Association, but also collaborates with Oxford University Press, which provides three speakers a year. The academic excellence of the University of Oxford means that SciBar is spoilt for choice from a pool of scientists.

Figure 2: The Port Mahon Pub on St. Clement's Street hosts the monthly SciBar.

As a registered charity, the Oxfordshire branch of the British Science Association is solely run by dedicated volunteers. You can support it without donating any money, by shopping online through *www.easyfundraising.org.uk*. If you are interested in taking part, the team is always looking for volunteers. You can dedicate as little or as much of your time as you want to assist in the organisation of events, setting up the venue, designing posters, updating the website and liaising with speakers.

SciBar runs every third Thursday of the month at 6.30 p.m. and admission is free. You are guaranteed a fascinating discussion about science over a pie and a pint at the Port Mahon Pub. Science could not get simpler!

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Chii Fen Hiu

1st year DPhil student in the Department of Experimental Psychology.

The 'Big Bad Pharma' wolf: is it as bad as the media say?

Nowadays, most public connotations of 'Big Pharma' have become synonymous with 'Bad Pharma'. Undoubtedly, the pharmaceutical industry is historically laden with scandals of questionable ethics, dishonesty and the promotion of bad science. Due to the prominent role in the health care system, this industry is under constant scrutiny and criticism, and it has become a trend in the media not only to criticise 'Big Pharma', but to demonise it. Is this cynicism called for, and is it constructive for the industry and the welfare of its consumers, the patients?

As a profit-driven industry, one of many concerns is that pharmaceutical companies do not have the incentive to produce drugs that provide cures, but rather deliver treatments for chronic symptoms. The media is outspoken on this issue, pointing out that the most profitable drugs on the market don't ever cure you.

Drug	Company	Sales (£m)	Indication
Nexium	AstraZeneca Pharmaceuticals	3,743	A proton pump inhibitor used to treat symptoms of gastro-oesophageal reflux disease and other conditions involving excessive stomach acid.
Abilify	Otsuka Pharmaceutical Co.	3,719	A partial dopamine agonist (atypical antipsychotic) with antidepressant properties used in the treatment of schizophrenia, bipolar disorder and clinical depression.
Crestor	AstraZeneca Pharmaceuticals	3,162	A statin used to treat high levels of cholesterol and to prevent cardiovascular diseases.
Advair Diskus	GlaxoSmithKline	3,065	A combination a corticosteroid and beta-2 adrenergic receptor agonist used in the management of asthma and chronic obstructive pulmonary disease.
Cymbalta	Eli Lilly & Co.	2,969	A serotonin-norepinephrine re-uptake inhibitor used to treat symptoms of major depressive disorder and generalised anxiety disorder.

Table 1: The top five pharmaceutical drugs by U.S. retail sales for 2012 and their indications (1).

The most profitable drugs do seem to be the ones that require repeated and prolonged dosing, and that treat symptoms without providing an outright cure (table 1). But does this point towards foul play within the pharma industry? Is it deliberately churning out blockbuster medicines that treat symptoms rather than the cause?

At first glance, this may seem likely. However, upon further reflection, high profit probability may be a necessary prerequisite for prescription drugs. In essence, drugs that must be continuously and frequently consumed will naturally bring in more money; a drug that could provide an outright cure wouldn't have the same sustainability in driving profits. As such, using the profitability of drugs as a measure of good conscience is a flawed and pre-determined construction from the start. Nevertheless, as John LaMattina, former Pfizer President of R&D, admits in his book *Devalued and distrusted*, big pharmaceutical companies cannot address an obvious unmet medical need for new antibiotics because the "commercial return on a new antibiotic would pale in comparison to a new treatment in areas like cancer or Alzheimer's disease" (2). LaMattina attributes this to the fact that antibiotics are generally used acutely, not chronically, making them unattractive for a big company. A likely extrapolation from this would be that 'Big Pharma' prioritises research towards moneymaking prescription drugs and treatments over one-off, outright cures. However, before pointing a damning finger, an in-depth analysis into the type of research that 'Big Pharma' invests in is first required. Similarly to the criticisms for being profit-driven, the pharmaceutical industry has also been condemned for investing excessively on marketing and promotion, even more than on research (3). Moreover, a 2012 report in the *British Medical Journal* suggested that "current incentives reward companies for developing large numbers of new drugs with few clinical advantages over existing ones" (4). So, investing a lot of resources in new, ground-breaking medications is less profitable than creating products that only differ marginally from drugs already on the market.

Indeed, it does seem that an astonishing amount of money is needed to bring a new drug to market. According to an article on Forbes.com, the average drug developed by a major pharmaceutical company costs at least £2.7 billion, with AstraZeneca spending £8 billion on research for every new drug approved (table 2) – as much as their top-selling drug made in annual sales (5).

Company	No. of drugs approved	R&D spending per drug (£m)
AstraZeneca	5	7,827
GlaxoSmithKline	10	5,424
Sanofi	8	5,250
Roche Holding AG	11	5,180
Pfizer Inc.	14	5,129

Table 2: The top five pharmaceutical drugs by U.S. retail sales for 2012 and their indications (1).

With figures like these, can 'Big Pharma' be blamed for investing in less risky ventures? A quick comparison of tables 1 and 2 shows that the average R&D expense is considerably higher than the annual sales profit from top grossing drugs. 'Big Pharma', like any other industry, are cogs in a capitalist system, and the business of inventing new drugs seems like a pretty unsustainable one. We might like 'Big Pharma' to act as saviours of the 21st century. However, in reality, not only do they have a responsibility towards the well-being of mankind, they are also liable to shareholders, employees and the financial security of the company. So while as consumers we condemn them for over-pricing or over-marketing drugs, as businesses it would be irresponsible not to maximise profits where they can.

Today, the pharmaceutical industry is widely blamed for creating a culture of drugged-up, over-prescribed denizens, and was even accused of inventing new diseases. An article by Martha Rosenberg viciously attacked the pharma industry, compiling a list of eight allegedly invented diseases, including fibromyalgia and adult autism (6). In the case of autism, where academic longitudinal studies have shown poor outcomes for many adults, regarding it as a "created" disease is an insult to its sufferers and to the wider mental health community. The case of fibromyalgia, an umbrella diagnosis of widespread muscle pain with fatigue and poor sleep, might be more complex. The FDA requires a drug to be indicated to treat a disease, not just their symptoms. So while it could be that fibromyalgia was invented in order to create demand for a product, it is also possible that 'Big Pharma' made up a condition so that it could sell a drug that only alleviates symptoms, as the cause of fibromyalgia remains largely unknown.

Big pharmaceutical companies have done many inexcusable things: suppression of unfavourable research findings; intimidation of doctors and researchers; dishonest marketing and more. But for all their faults, being profit-driven isn't one of them. They are placed within a broken system, the same way we all are. They are no saints, but importantly, they make no claims to be: they aren't non-profit organisations or charities but large, revenue-maximising, multinational conglomerates. These companies have undoubtedly earned their reputation as wolves. However, society seems to have thrust upon them the role of our gentle grandmother, expecting them to provide care and support as a matter of duty, and so we are vexed and appalled when we get bitten. It's not that there's nothing wrong here. There are a thousand things wrong with a system in which the major incentive to save lives and cure diseases is money. And so we should continue to be critical, always mindful of marketing gimmicks and never stop calling for open access to data from drug studies and clinical trials. However, it shouldn't stop there. Short of overhauling the current economic system within which healthcare operates, it is not enough for us to only focus our attention on the pharmaceutical industry. We also need to hold doctors responsible; it is their duty and obligation to patients not to be drug-mongers for the pharmaceutical industry and patients must be able to trust their advice and recommendations – these drugs cannot be over-prescribed if doctors do not pander to the will and whims of the pharmaceutical industry. The onus is also on us, as patients and as consumers, to be well informed and diligent when it comes to our own health.

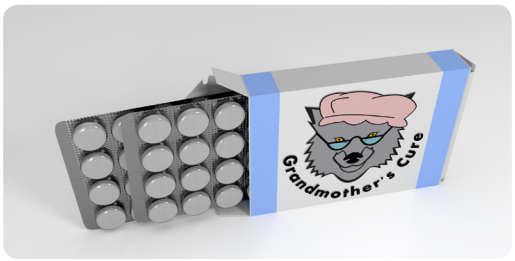

Figure 1: Illustration by Richard Wheeler.

As an industry, 'Big Pharma' faces a moral conundrum: a conflict of interest between their own survival and their basic obligation towards their fellow man. In the long run, the sustainability of a profit model within healthcare is at best questionable. For now, rather than spreading vicious depictions of 'Big Pharma', the media should be a source of education and impartial information. So, while we should always keep a critical eye on 'Big Pharma', we should also save some scepticism for what is written about them. Ultimately consumer decisions must be information driven and evidence based, neither falling prey to marketing ploys nor to sensationalist media propaganda.

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

Cambodia – A Story of Survival

Anna Coenen-Stass

Master student in Prof Matthew Wood's laboratory, Department for Physiology,

Within four years (1975-1979), the communistic Khmer Rouge regime killed almost a third of Cambodia's population through systematic execution, forced labour, disease and starvation. Mass graves throughout the country hold about 1.3 million bodies (1). The larger cities in the country, such as Phnom Penh, Siem Reap and Battambang, were evacuated completely, the deportees being forced to work in the fields for more than 16 hours a day, seven days a week. 30 years later, Cambodia remains one of Southeast Asia's poorest countries and still faces major human right issues. Yet Cambodia's inhabitants started a courageous battle to improve the agricultural-based economy and to attract tourists to their country, which has so much history and natural beauty to offer.

Officially, Cambodia's past seems to be buried, with only a few lines in history schoolbooks referring to the Khmer Rouge years. However, when I went to Cambodia in January of this year, I visited Tuol Sleng, a former high school in the heart of Phnom Penh that served as one of the main re-education centres, and realised that the Cambodians remember the suffering with brutal clarity. Of the more than 12,000 Cambodians that were brought here by the Khmer Rouge regime, only seven survived (2). I met two of them in the courtyard of the building complex, where they sold books recounting their memories of the years of torture and famine. When I learnt that the government still comprises several ex-Khmer Rouge, such as the Prime Minister Hun Sen, it became clear why there are so few pages about the genocide in schoolbooks. Considering these circumstances, it is not surprising that the tribunals against the Khmer Rouge leaders are only slowly progressing (3).

Pol Pot, the leader of the movement, went to school in Phnom Penh before studying radio electronics in Paris. After joining a communistic cell, he served as the Prime Minister of Democratic Kampuchea. Keeping Pol Pot's upbringing in mind, it seems ironic that under the Khmer Rouge regime, everybody that spoke French, wore glasses, had 'soft hands', or had worked for the former government was seen as a traitor and imprisoned. After months of torture, starvation and false accusations, prisoners were taken to the killing fields. In these fields, loudspeakers blared songs about revolution to cover up the screams and noise of farming tools smashing the skulls of the so-called enemies of Angkor (4). Almost 10,000 bodies, including newborns and children, were found at Choeung Ek, located 17 km south of Phnom Penh. An incredibly dusty tuk-tuk ride through the smog of the city outskirts took me to the mass grave, a former orchard, where hundreds of dragonflies were swarming over an idyllic pond. During the rainy season, however, bones and rags still emerge to the surface: a reminder of its horrible past.

Figure 1: Rice and crop fields adjacent to the killing fields.

The Khmer Rouge regime evacuated cities and separated families to send them to labour camps, where they were expected to fulfil impossible quotas of rice: an average of one ton of rice per hectare of land was harvested prior to the Khmer Rouge era, whereas three tons were then expected during the regime. Rice and corn were traded for weapons with China. Famine, diseases and unbearable work conditions resulted in a massive death toll. Nowadays agriculture remains the mainstay of Cambodia's economy (Figure 1). Land rights are still one of the most critical issues. Since 1998, more than 100,000 Cambodians were evicted from their homes because the corrupt government had sold around 60% of the arable land to private companies (4). However, since 2001, with the help of non-governmental organisations (NGOs), Cambodian organisations have been created to fight against land grabs and to teach farmers about their rights.

The leading national NGO is the Cambodian League for the Promotion and Defence of Human Rights (LICADHO), founded in 1992. Financially supported by the Canadian International Development Agency (CIDA) it initiated projects to boost the capacity of local farmers by teaching

them new farming techniques and providing better crop seeds. Furthermore, Cambodians themselves have developed more efficient farming strategies, e.g. planting rice in single shoots instead of batches of 10, as was traditional practice. This technique yields a twofold increase in rice production, which in turn avoids famine and allows farmers to invest the profit in houses with foundation, means of transportation and education for their children (5).

Lately, some of Southeast Asia's least explored terrains, such as the Koh Kong Province, are becoming accessible through roads and bridges. Influenced by Khmer Rouge presence until the 1990s, these provinces used to be marginalised by a total lack of infrastructure, and tourism is still in its infancy. The Koh Kong region comprises the wild rain forest of the Cardamom Mountains, home to many of Asia's most endangered species. The area is of untouched beauty, including large regions of mangrove forest and a 12-island archipelago in the Gulf of Thailand, with pristine sand beaches and crystal-clear waters.

Community-based ecotourism projects have recently been initiated in the Koh Kong region. These projects aim to employ local Cambodians, providing them with an income so they can send their children to school and have access to basic health care. One of the pilot projects was started by the NGO Wildlife Alliance in Chi Phat in 2008. Previously, illegal hunting, logging and clearing for farms resulted in the exploitation of the rain forest in an unsustainable manner. So far, the project is proceeding well, with a tenfold increase in visitors over the last four years, and a total profit of almost 100,000 USD in 2012 – given that the average family in this area only earns 1.50 USD a day this is an impressive number. Tourists are hosted by families in the village and offered trekking tours through the National Park, led by local guides. This allows the inhabitants to use their knowledge of the territory and visitors to catch a glimpse of Cambodia's wildest beauty, thereby encouraging conservation of the environment (6).

The kingdom of Cambodia still struggles for independence from its bigger neighbours, which have been trying to squeeze profits from the country for centuries. Additionally, the corrupt Cambodian government has made many fraudulent deals over the centuries leading to massive economic disadvantages for the population. Most strikingly, the company that charges tourist 20 USD per day to visit the ancient temples of Angkor is run by the Vietnamese, and the National Angkor museum is owned by the Thais. Cambodia's past is not an easy one. The country has fought many battles and there are more on the way to reaching political stability and economic independence. Nevertheless Cambodia has survived. I was deeply impressed with the achievements of the country and the nature of the people living there. Through experiences of hardship beyond belief, they seem to have preserved something of irreplaceable value: the joy of living.

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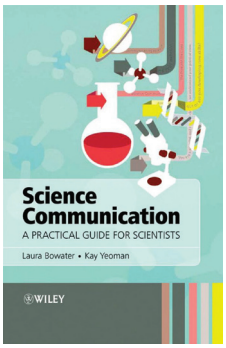
BOOK REVIEW



Science Communication: A Practical Guide for Scientists

Laura Bowater and Kay Yeoman

ISBN: 978-1-1199-9312-4, Wiley Blackwell (2012), Paperback, 384 pages, £24.95

Reviewed by Stuart Thomas

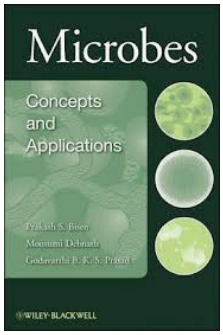
Science Communication is written, in the authors' own words, by "two scientists who really enjoy and feel personally enriched by communicating science to the public". This is evident from the text, which feels more of a passion project than any other science textbook I have read. Perhaps this is because it is not a traditional textbook, but rather a collection of ideas, theories, and examples of effective scientific communication.

Science Communication consists largely of case studies and anecdotes. An unusually high number of these are from Norfolk as both authors are based at the University of East Anglia. These case studies are very useful, detailing how people have organised science communication events and what they have learned in the process. They also provide readers with ideas for events they can themselves hold. Examples include "How do you make chocolate in a slang-free zone?", "Conker tree science", and "Science in a suitcase". A particular case study on World Sight Day 2009 is mentioned throughout the book, focusing on different aspects of the event: organisation, method of teaching, and impact.

Some readers may – as I did – have an immediate prejudice against such words as 'involvement', 'impact' or 'engagement'. However, the authors identify the differences and importance of each of these terms. There is a detailed introduction to the history of science communication and its current status. We are now, according to Bowater and Yeoman, in the third stage of communication, characterised by engaging the public with scientists and encouraging each side to ask questions. This is in response to the previous phases of communication: 'let the public teach themselves' and 'tell the public what they should know', neither of which has been successful.

My one criticism is that all pictures are bound together in the centre of the book. These pictures are linked to articles in the text and include numerous photographs of events and exhibits. These are however badly labelled and consequently serve little purpose, unless a reader constantly flicks back and forth while reading, and are useless if the book is being used as a reference.

Science Communication is a fascinating book, full of interesting ideas and anecdotes. The advice it provides for a scientist interested in conveying their work to others, or a layman wanting to bring scientists and the public together, is smart, engaging, and essential.



Microbes: Concepts and Applications

Prakash S. Bisen, Mousumi Debnath, and Godavarthi B.K.S. Prasad

ISBN: 978-0-470-90594-4, Wiley-Blackwell (2012), Hardcover 724 pages, £133

Reviewed by Tania Hasan

Microbes: Concepts and Applications gives an up-to-date account of current knowledge regarding the roles and applications of microbes in myriad areas. Microbes play an essential role in the biogeochemical cycles of key elements and not only benefit our health, as commensal microflora break down indigestible food and assist our immune system, but also our economy, as we have learned to profitably exploit microbes and their products.

Throughout the book, the impact of technological advances on our knowledge of microbes is discussed. Genome sequencing has revolutionised the classification of microbes, which previously relied on imprecise morphological factors. Furthermore, shotgun sequencing of the 'gut microbiome' (combined genomes of all the microbes in the gut) can highlight differences between normal and pathogenic states, providing insights into the role gut microflora can play in disease.

A recurrent refrain throughout the book is that there is still much to learn. Future applications discussed include the development of novel renewable energy sources through photosynthetic systems or through the use of microbes that can metabolise renewable waste materials. The potential use of bacteria to deliver gene therapy is also presented, expanding on their proven efficacy in drug synthesis and delivery.
The authors make many insightful observations during the course of the book. A particularly interesting point discussed is the limitations of clinical trials: although the same disease can present diverse pathologies, and different treatments can have different effects, researchers still look at an average effect of a therapy on all patients with the disease.

What struck me most from this book was firstly our ignorance in this area, with only approximately 0.01% of microbes having been cultivated and characterised, and secondly the breadth of applications microbial technologies provide – for example the widespread use of biosensors in lab protocols such as ELISA, or in the fermentation and pharmaceutical industries. Though the darker side of microbes in disease and biowarfare is mentioned, implicit in the text is the belief that the benefits of working with microbes outweigh the risks, contrary to the public image of microbes as something to be feared and avoided.

Ultimately, I found this text insightful, interesting and informative; this book is definitely a must-read for any budding researchers looking for a fascinating but poorly exploited area to work in!

Extremophiles: Sustainable Resources and Biotechnological Implications

Edited by Om V. Singh

ISBN: 978-1-118-10300-5, Wiley-Blackwell (2013), Hardcover, 456 pages, £100.00

Reviewed by Hua Wang

*Extremophile*s is an anthology of research reviews organised into 16 chapters. The book is therefore a valuable resource for anyone interested in the applications of extremophiles to the emerging field of biotechnology.

Singh recruited 40 experts from around the globe to contribute to the book. The chapters are very well organised, ranging from an introductory chapter on the molecular evolution of extremophiles, through resourceful chapters covering laboratory methodologies, to ones outlining the limitations of, and strategies used in, isolating and cultivating extremophiles. The book dedicates more than half of its content to covering the significance of extremophiles in biotechnology. A major benefit of this book, if you are a researcher, is that each chapter contains convenient references to relevant patents and research articles.

What I enjoyed most while reading this book were the chapters highlighting the applications that can potentially be derived from a better understanding of extremophiles. Chapter 6, for example, covers possible biotechnological uses of cold-adapted enzymes in the food and pharmaceutical industries, while Chapter 9 describes the applications of extremophiles in biofuel production. Particularly interesting is Chapter 15, which covers the therapeutic implications of radiation-protective extremolytes (natural compounds produced by extremophiles) that could be used in sunscreen, and the biotechnological implications of extremolytes and extremozymes for medical and commercial use.

Unfortunately, although complete and authoritative, this book lacks colourful figures or diagrams which I would have liked to see in order to better understand the mysterious realm of extremophiles. Such images would appeal to readers, and would make the book more accessible to a wider audience.

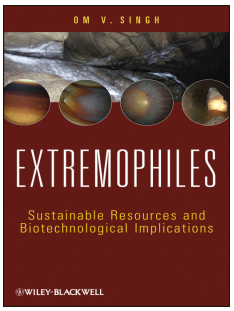
Ultimately, *Extremophiles* is a very helpful book to have in your library if you do not want to wade through multiple books and research articles regarding these extreme and largely unexplored microorganisms. If you are intrigued and curious about the creatures that can survive in extreme conditions – temperature, salinity, pressure, radiation, lack of nutrition and osmotic barriers, to name just a few – and you want to learn from scientific experts in the field, then I highly recommend *Extremophiles!*

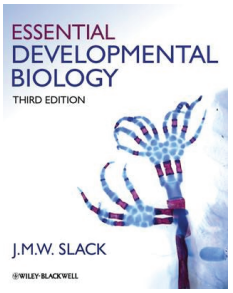
Essential Developmental Biology (3rd edition)

Jonathon M.W. Slack

ISBN: 978-0-470-92351-1, Wiley-Blackwell (2012), Paperback, 448 pages, £39.95

Reviewed by by Jennifer de Beyer





Producing an undergraduate level text for a field as fast-paced and diverse as developmental biology is fraught with challenges. There is a high level of complexity, often overwhelming detail and a vast new vocabulary: problems that are often poorly dealt with. Jonathon Slack's *Essential Developmental Biology* neatly sidesteps these challenges in this clear, concise and easy to follow text.

From the first, this textbook is obviously written by an educator who has test-run the layout on students. Information is split into four overarching sections that differ greatly from other similar texts. *Groundwork* introduces the vocabulary and general concepts of developmental biology and explains some of the techniques used in its investigation. Unlike in similar texts, information is not presented as a series of classical experiments. Instead, classical experiments are mentioned throughout in separate boxes where appropriate. This clearly delineates between the details of the science within the main text and these interesting asides with their rich historical detail.

Major Model Organisms presents each of the 'big six' in its own chapter. Great care is taken to highlight the benefits and challenges of each of these most popular models, and to keep the details of development of each separate from one another. This clarity and differentiation is the greatest strength of this book. *Organogenesis* focuses on organ development in vertebrates, covering each of the major physiological systems, while *Growth, Regeneration and Evolution* dives into the latest findings and the hottest areas of current research. Both sections continue the strong background and clear distinction between species that characterise the earlier sections. The detail of the latter sections also makes this text appropriate as a springboard for early postgraduate students.

Rich illustration is appropriately used throughout, including photographs of models, histology slides and clear cartoons. I was particularly delighted by a series of grinning zebrafish embryo cartoons used to introduce basic genetic concepts. Each chapter concludes with a bullet-point list of key concepts and extensive suggestions for further reading. While a basic knowledge of cell biology or biochemistry is assumed, the appendix provides details of molecules and processes mentioned, filling in any gaps in the reader's knowledge.

Essential Developmental Biology is a well-planned, beautifully produced text that I wholeheartedly recommend for undergraduates, early postgraduates and the curious layperson.

5' with... Prof Matthew Freeman

Prof Matthew Freeman studied for a BA in Biochemistry at Oxford University and then went on to obtain a PhD at Imperial College, London. After a stint in the States, he started his own lab studying rhomboid proteases and development in Drosophila in the Laboratory for Molecular Biology (LMB), Cambridge. In January, he took over from Prof Herman Waldmann as the head of the Sir William Dunn School of Pathology.

Interviewed by Dr Maria Mogni

If you were not a scientist you would be...

Involved in either journalism or politics. During the first three years of my undergraduate studies at Oxford I used to edit for the *Cherwell* newspaper, where I enjoyed writing and reporting on different people. I also enjoy being politically engaged.

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

Spending time with my family and my children. I have also always enjoyed sailing, but it has become more and more difficult to do it since starting a lab and a family.

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

My research took off when I used *Drosophila melanogaster* as a model organism to investigate genes undergoing developmental regulation in the eye. Among the interesting-looking genes that resulted from my screening was one named *argos*. I showed that it encoded a secreted protein with an epidermal growth factor (EGF) motif, and that this protein was involved in regulating the recruitment of cells in the developing eye. Loss of function of *argos* was found to lead to excess recruitment of photoreceptor cells. Later studies showed that Argos acts as a secreted inhibitor of EGF receptor (EGFR) signalling and therefore blocks cell fate determination. Argos was the first such inhibitor of any receptor tyrosine kinase to be discovered. Subsequent work from different groups showed that it works by dimerising with the active ligand of the EGFR, thereby rendering it unable to stimulate the receptor.

Do you have a favourite classical experiment?

My favourite experiment was performed by the Nobel Prize winners Christiane Nüsslein-Volhard and Eric Wieschaus from the European Molecular Biology Laboratory in Germany in the mid-1970s. They aimed to identify genes involved in the development of *Drosophila melanogaster* embryos. At that point, little was known about the genetic and molecular mechanisms by which multicellular organisms develop from single cells to morphologically complex forms during embryogenesis. Nüsslein-Volhard and Wieschaus identified genes involved in embryonic development by chemically generating random mutations in fruit flies and screening for mutations that affected genes involved in the development of the embryo. They took advantage of the segmented form of *Drosophila* larvae to investigate the logic of genes controlling development.

In your opinion, what makes a good scientist?

An ability to cope with failure, an ambition to address important questions, imagination, single–mindedness, and finally the ability to keep in mind the bigger picture whilst staying focused on experiments.

What advice would you give to a young scientist in your field?

Find a subject that interests you, where you can ask fundamental questions. Also, it is very important that you personally engage with your lab supervisor and the people in the lab - the atmosphere in the lab is a key factor when you choose where to do a research project.

What advice would you give on how to cope with experimental failures?

Look for help immediately. Start by talking to lab members and people around you. If necessary, talk to people from outside the department. The people that succeed in science are the people who know how to take advantage of their surroundings.

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

I envisage that research will become focused on the medical implications of science - unexpected discoveries in basic science and fundamental biology will be used to the advantage of human health.

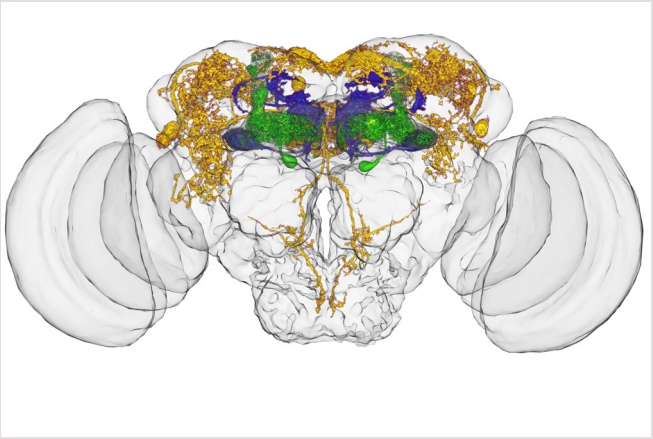
SNAPSHOT Research Image Competition

This issue´s winner is...

Dr Wolf Huetteroth

Dr Wolf Huetteroth is a postdoctoral research assistant in Prof Scott Waddell's group at the Centre for Neural Circuits and Behaviour, and a research fellow at Wolfson College.

Wolf's image of three different types of neurons in a fruit fly brain was produced merging confocal scanning microscope data in AMIRA®, a 3D rendering software.



Research in the Waddell group focuses on exploring neural networks involved in learning and memory in the fruit fly *Drosophila melanogaster*. The group combines behavioural tests with functional imaging using newly developed microscopy technology.

Wolf's image of a fruit fly brain shows three types of neurons involved in learning and memory: the green neurons are crucial for memory consolidation; the others integrate hunger motivation (mediated by Neuropeptide F, yellow neurons) with memory processes (via dopamine, blue neurons).

Obtaining this image involved confocal laser scanning microscopy of three fly brains, each expressing green fluorescent protein (GFP). The three images were then merged using the 3D analysis tool, AMIRA®. This technique allows imaging interior structures and subsequent 3D reconstruction of topologically complex objects. Confocal laser scanning is the only type of imaging that allows neural network analysis with sufficient spatial resolution in all dimensions. Although it takes longer than traditional widefield microscopy, the beautiful and detailed images are worth the wait.

Neurons are embedded into circuits of astonishing complexity in which each cell is influenced by and influences many others through thousands of input and output connections. Owing to their lower brain complexity (a fruit fly has just 100,000 neurons compared to the 75 million neurons in mice!) and technical advantages, it is common to use insects to study behaviours such as associative learning.

A main focus of Wolf's research on insect brains is to understand how internal motivational states like hunger tweak learning and memory. Instrumental for fly memory are the so-called mushroom bodies, dense hubs of dendrite and axon terminals, glia cells, and Kenyon cells, which are located in the centre of the fly brain. They are densely innervated by the green neurons and receive input from the blue neurons depicted in the image. The mushroom bodies constitute the regions where the fly forms associations and stores memory, one reason why they are sometimes compared to the hippocampus in vertebrates.

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SNAPSHOT
Research Image Competition

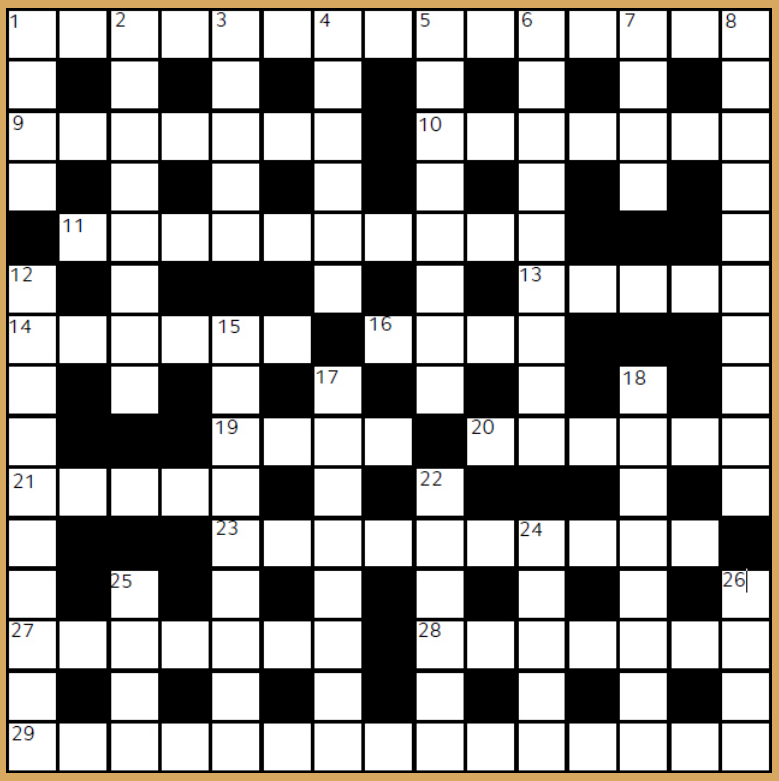
Win a £50 book voucher kindly provided by Oxford University Press!

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 *Phenotype* MT 2013. To enter, send images to oubs@bioch.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images. There is no limit to the number of entries per person.
The deadline for the competition is 14 June 2013.

PHENOTYPE crossword

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

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



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

WILEY-BLACKWELL

Across

1 see 1D
9 He felt tension as serum mixed (7)
10 Manly specimen putting small item into carrier (7)
11 Viagra, perhaps, gone to waste in Bananaman (10)
13 Sheep, somewhere, hold races (5)
14 Cooker attacked insular 10 (6)
16 Fool thrombosis (4)
19 Dimwit returns for groundsheet (4)
20 Similarly hot and crowded (6)
21 Wait for the sound of cats' feet (5)
23 7 about a marten, with no going back inside (10)
27 Fabric of crumbling minaret (7)
28 Fly on the wall makes one duck (7)
29 Flower of addled papacy: "O, profilin!" (10,5)

Down

1, 1A 10 – it may be I sold his/her genome (4,15)
2 Write copy in breaking wave (8)
3 Put up or stood up (5)
4 Herb puts aroma above anger (6)
5 Once more cope with adding new haft (8)
6 Jesus may accept Jews, even, as true fungi (9)
7 Rational, as in "an elf's an elf" (4)
8 Turf over a pair of small referees in a membrane channel (6,4)
12 Posh choir shudders under piano with a capital P (10)
15 Blank page inserted mistakenly after line (9)
17 100 nJ thrust onto flower by a species of 1D (8)
18 Insect to express amazement about collapsing wall (4,4)
22 Small 10 reported to shorten joy (6)
24 Sum of publicity by provincial politicians (3,2)
25 Sounds like a terrible container (4)
26 Whip or swat a fly (4)

Congratulations to Charlotte Dodson from the Department of Chemistry who won the Hilary '13 crossword competition.

Answers to the crossword from HT 2013:
Across: 1. ingot; 4. patens; 9. varnish; 10. teed off; 12. rebut; 13. strappado; 14. ells; 15. low-pitched; 17. chinchilla; 19. ante; 21. marsh frog; 23. azide; 24. admiral; 25. somatic; 26. nassau; 27. drams.
Down: 1. inverted comma; 2. garibaldi 3. thirty; 5. anthropologist; 6. eyespot; 7. stoma; 8. physcomitrella; 11. flood defences; 16. henrietta; 18. cohorts; 20. warmed; 22. roman.