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Issue 17 | Hilary Term 2014

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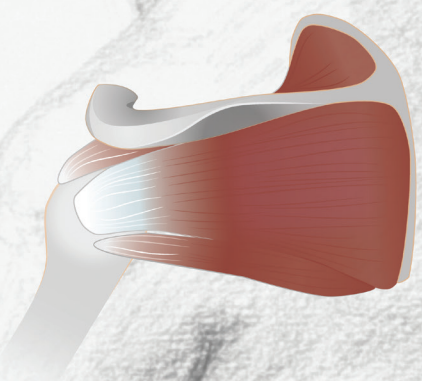

Toxoplasmosis
Can parasites affect our behaviour?

Genetics and Family Planning
What traits will your offspring exhibit?

Obesity and Evolutionary History
Why are some populations more inherently susceptible to obesity?

Frameshifts and Master Regulators
Dr Robert Gilbert on his use of structural biology in understanding RNA functions

cover image by
Maria Kuzma-Kuzniarska
this issue's winner of the SNAPSHOT scientific image competition
page 31

Future Therapies:
Aptamers • Hijacking the injectisome • Cures for HIV?

Prof Chris Ponting on open access publication

How clear are your data presented?

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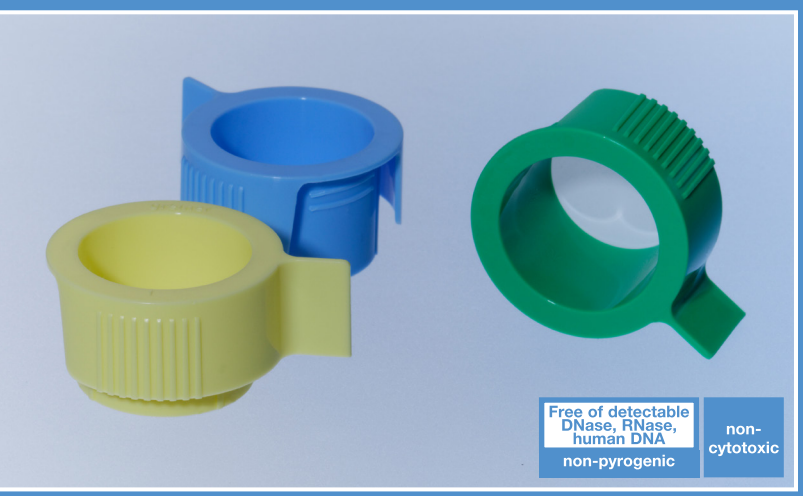

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EDITORIAL

Welcome to the seventeenth issue of *Phenotype*! This issue is brimming with exciting articles contributed by PIs, research staff and students from across the University.

Wonder how RNA has anything to do with shaping our world? A welcome guest, Dr Robert Gilbert from the Division of Structural Biology, reveals to us the nano-scale world of the ribosome and microRNA maturation to demonstrate our understanding of how RNA is controlling biological processes, all by the power of structural biology. We also feature an interview with Prof Kevin Foster from the Department of Zoology, in which he divulges success (and disaster!) in his lab.

Also in this issue, we highlight novel avenues of treatment for human diseases. Thomas Mortimer introduces us to the bacterial injectisome and how we could hijack it as a nano-scale hypodermic syringe, Dr Maria Mogni evaluates the pros and cons of RNA aptamers as alternatives to antibody-based therapeutics, while Susan Graham considers future HIV cures in a collaborative article with Oxbridge Biotech Roundtable.

In our other features, Madeleine Pope makes us shut all our doors and windows tight as she discusses *Invasion of the Body Snatchers* (or rather how a common parasitic infection could affect our behaviour), and Richard Wheeler's colourful comic makes us think about how to illustrate data accurately. And if you wondered why some populations seem to be more protected from obesity, Dr Dyan Sellayah reviews 'chilling' insights from our evolutionary history. Siamak Redhai introduces us to an intercellular postal service and how it is involved from metastasis to mating! Finally, Sarah Dixon questions the ownership of our genomes as she discusses a recent US Supreme Court case.

In our Science and Society section, Dr Jennifer Badger peers into the future of our children as she tells us about genetic screening and prediction technologies. We continue our coverage of science communication and publishing with the case for open access publishing by Prof Chris Ponting, and Clio Korn's report on Oxford's first Science Slam, a hilarious and engaging way of communicating science to the public.

This term, OUBS will be hosting Dr Lukas Tamm, Harrison Distinguished Professor in Molecular Physiology and Biological Physics at the University of Virginia School of Medicine. Find out more about his research from Christoph Treiber, who tells us about the biophysics of lipid bilayers and membrane proteins.

Congratulations to Dr Maria Kuzma-Kuzniarska, the winner of last issue's **SNAPSHOT** competition, who elegantly combined life drawing and computer graphics to portray the rotator cuff of the human shoulder. Further details of her research and unmissable artwork can be found on page 31.

Wake up those neurons by trying your hand at our cryptic molecular biology crossword on page 32 by resident cryptographer *Fish*. And if you figure it all out, don't forget to enter for a chance to win one of the Wiley-Blackwell textbooks reviewed on pages 28-29 of this issue. (If you're still stumped on last issue's crossword, the answers are on page 32!)

If you are interested in science communication, writing and publishing, why not join us on the *Phenotype* team? Contact us at **oubs@bioch.ox.ac.uk**! We are always looking for writers, editors and designers to help us with the next issue. Or why not join the sponsorship team? **Our next issue will have a focus on plant science, so we particularly welcome articles and SNAPSHOT competition images in any way connected to plant science or the environment.**

Finally, thank you to our amazing and creative *Phenotype* team of post-docs and students who put this issue together. This issue is a testament to their hard work and enthusiasm.

Joel Beevers
Editor

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

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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 27 January

Dr Lukas Tamm, *University of Virginia School of Medicine, Charlottesville, Virginia, USA*

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

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OUBS Featured Seminar: Dr Lukas Tamm

by Christoph Treiber

This term, the Oxford University Biochemical Society (OUBS) brings you Dr Lukas Tamm, Director of the Centre for Membrane Biology and Harrison Distinguished Professor in Molecular Physiology and Biological Physics at the University of Virginia School of Medicine.

Oil in the sea: Understanding the biophysics of lipid bilayers

Benjamin Franklin, one of the main contributors to the US Declaration of Independence and the inventor of the lightning rod, bifocal glasses, the urinary catheter and many other important objects, has yet another scientific legacy: he was one of the first scientists working on lipid layers. While sailing across the Atlantic in order to inform the British King George II about the intention of the colonies to gain independence, he noticed that two ships in his fleet were sailing more smoothly than the others. Franklin investigated this phenomenon, and discovered this was due to a lipid layer that had formed around these ships after the cooks had emptied their greasy pots into the sea. This episode, and the contributions of others following this first investigation, eventually led to our current understanding of lipid bilayers, a topic beautifully summarised in the *Biophysical Journal* by this term's OUBS featured seminar speaker, Dr Lukas Tamm (1).

Dr Tamm first became interested in lipid membranes and membrane-associated proteins during his PhD in Basel, Switzerland, where he used nuclear magnetic resonance (NMR) spectroscopy to study the molecular structure of membrane proteins. Membrane proteins usually consist of both hydrophobic and hydrophilic domains, a fact that reduces their solubility in aqueous solutions and hence renders them particularly difficult to be expressed and purified for *in vitro* studies. One way to overcome this problem is to express these proteins in an artificial environment that closely resembles the phospholipid bilayer of cells, the cellular compartment where they are normally located. In 1985, Dr Tamm contributed to the development of artificial bilayers with the development of so-called substrate supported bilayers, which match the properties of cellular membranes remarkably well (2).

Any information that passes between cells has to pass via the membrane. Dr Tamm has been instrumental in studying the proteins that mediate these interactions over the last 30 years and has written more than 120 research papers in this area. Among these publications is work that demonstrates how NMR of the transmembrane (TM) domain of OmpA, an outer membrane protein in *E. coli*, can be used to determine both the molecular structure of the protein in solution, as well as conformational dynamics of certain domains (3). These measurements were made possible by incorporating the TM domain into detergent micelles, thereby making it possible to bring the molecule into solution.

In a more recent study, Dr Tamm and his team have focused on the dynamics of vesicle trafficking in neurons (4). Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are proteins that are thought to mediate the fusion of neurotransmitter-loaded vesicles with the cellular membrane at neuronal synapses, triggering the release of vesicle contents. This process is crucial for the propagation of neuronal signals across synapses. Using NMR and high-resolution interference contrast microscopy in lipid bilayers, Dr. Tamm and his team were able to resolve both the structure (Figure 1) and the dynamic conformational changes of SNARE protein syntaxin-1A, shedding light on a crucial step in neuronal transduction.

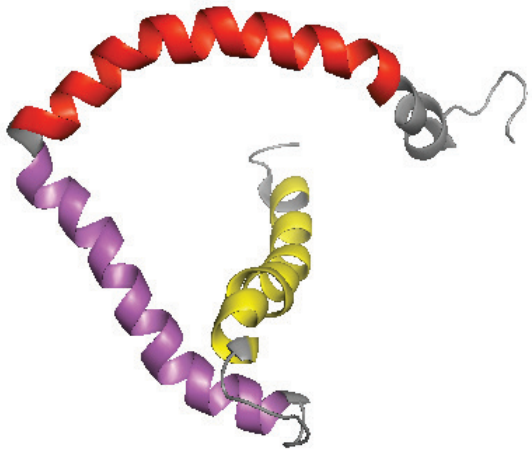

Figure 1: Ensemble representation of 20 structures of syntaxin-1A (residues 180-288) with the lowest violation energies in DPC micelles. RCSB PDB structure 2M8R, coloured to match (4).

Given the difficulty of determining the structure of membrane proteins by X-ray crystallography and the wealth of yet-undetermined membrane protein structures, Dr Tamm's group's use of NMR in this effort is vital. His group's further studies of the functionality of these proteins promises much for our understanding of key processes in health and disease.

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Still an RNA world: Fine-tuning and master-switching gene expression control

by
Dr Robert Gilbert

The 'central dogma' of biology, as defined by Francis Crick, is that the DNA in our genomes gets copied into a complementary sequence of RNA, which acts as a messenger (hence mRNA) from the nucleus to the rest of the cell. This transcriptional process is one of the most important regulations of cell functions. But for many years it has been clear that RNA is not just a messenger, but has all sorts of other functions. Some of these functions are regulatory and some involve the catalysis of key biological processes, such as the way in which the ribosome links amino acids together to build a polypeptide or protein. Thus, unlike DNA, which acts just as information storage, RNA can both carry information and act on it. This has led many to believe that the first kinds of 'life' on Earth – defined by self-replication – were dominated by RNAs, which both coded for and implemented processes. Part of the work in my group concerns processes of biological regulation which show how RNA works in both these aspects – how we still live in an RNA world.

The regulation of the transcription of DNA into RNA in nucleated cells involves protein transcription factors that bind to the DNA and bring with them enzymes such as RNA polymerases. In addition, the tightness with which the DNA is packed together and coiled up affects how effective the transcription factors are and therefore whether genes get expressed. In this article I want to focus on how RNA-based regulation both complicates and undercuts our image of gene expression control at the transcriptional level.

The ribosome translates RNA into proteins by reading three-letter codons made of a four-letter nucleotide code (A, G, C and U). The codons each define either a specific amino acid or a stop signal. This translation involves transfer RNAs (tRNAs), bringing amino acids to the ribosome for polymerisation. tRNAs recognise where they should bind on the mRNA by base-pairing because A (adenine) and U (uracil) bases bind to each other and G (guanine) and C (cytosine) do likewise. So a tRNA bearing a specific three-base sequence will always bind to the same codon on the mRNA. The codons are consecutive and this means that the ribosome reads a continuous strand of mRNA in a particular frame of reference. The fine-tuning aspect of our work concerns how the mRNA itself can cause a frameshift and hence confer a different meaning on the mRNA. We showed that the stability of folded regions, or 'secondary structure', within mRNAs determines the frequency at which ribosomes frameshift, placing a statistical mechanism at the heart of a biological process.

Before we started our own work it was known that two aspects of RNA structure were found together at sites where frameshifts occur – a secondary structure such as a stem-loop or pseudoknot (Figure 1a) and a 'slippery sequence' – that is, a sequence rich in A and U bases which pair more weakly than G and C. What was not known was how these two elements interplayed with the ribosome to make a frameshift happen – and happen at a certain frequency. This matters because there needs to be a specific ratio of the two possible products of the mRNA. A good example of this is the frameshift that occurs with two adjacent HIV-1 genes, *gag* and *pol*, transcribed as a single mRNA transcript. The *gag* gene encodes HIV-1 structural proteins, which build the viral coat, and the immediately downstream but partly overlapping *pol* gene encodes reverse transcriptase (RT) protein. The frameshift enables RT production to occur only approximately 5% of the time so that the right ratio of protein products exists to maintain a balance between the replication of viral genomes and their packaging into progeny viruses.

To determine how this mechanism works we performed structural studies of ribosomes translating a minus one (-1) base frameshifting mRNA (1). We did this work in collaboration with the group of Ian Brierley from the Department of Pathology in Cambridge. Using electron microscopy (EM), we were able to get a snapshot of the ribosome caught trying to unwind the viral RNA pseudoknot (Figure 1b). This showed that a spring-like tension is generated within the intersubunit space of the ribosome as the tRNA tries to keep hold of the mRNA despite the fact that the forward motion of the ribosome into the pseudoknot is blocked. If the tRNA loses its grip on the mRNA, the energy release as it springs back into line causes it to overshoot its previous frame by 1 nucleotide and achieve the -1 frameshift (Figure 1b).

This study is an excellent example of the way in which structural biology can provide a basis for hypotheses about molecular mechanisms. One insight from our work was that the factor determining the frequency of frameshift *in vivo* would be the strength of the mRNA secondary structure. Our work led others to investigate the correlation between pseudoknot structure and frameshift frequency. They showed that the weaker or more flexible an RNA secondary structure, the less it triggered frameshifting. Our work also gave fascinating insights into how tRNAs normally move forwards inside the ribosome, and so help the ribosome move forward in the right frame on the mRNA. It showed that the tRNAs 'stutter', making multiple steps forward over one codon, and some beautiful single-molecule studies from another group showed this in forceful detail. Harry Noller, Carlos Bustamante and Ignacio Tinoco used 'laser tweezers', which are capable of measuring the forces imposed by single molecules, to show the multiple steps that tRNAs take as they move forward (2). They subsequently showed that the ribosome uses two different mechanisms to unwind secondary structures in mRNA: one is based on force, the other on destabilisation of the base pairing at the bottom of the RNA stem (3). These single molecule studies matched insights from our cryo-EM maps, and were interpreted in this light (3).

In addition to frameshifting as a complication of gene expression control, more recently, my group has been working on the mechanism by which small RNAs interfere with mRNA translation in order to switch off the expression of certain genes. Some of these microRNAs (miRNAs) act as very important tumour suppressors because they lie upstream of many well-known protein-based mechanisms for gene expression control. Here I will focus on the let-7 family of tumour-suppressor miRNAs (4, 5). Let-7 sequences are first transcribed to primary miRNAs, then processed by a nuclear enzyme called Drosha into shorter pre-miRNAs, which are processed further by a Dicer enzyme in the cytoplasm into mature miRNA duplexes, by a mechanism that relies on measuring their length. A single-strand of the mature miRNA is finally loaded onto an Argonaute protein as part of an RNA-induced silencing complex (RISC).

Gene silencing by let-7 miRNA is regulated by altering the efficiency of its pre-miRNA maturation. The majority of pre-let-7 miRNAs are one nucleotide too short to undergo efficient maturation. But this can be enabled by adding a single uracil (monouridylation). On the other hand, they can be targeted for breakdown by the addition of many uracils (oligouridylation). Both processes are mediated by two terminal-uridylyltransferase enzymes, ZCCHC6 and ZCCHC11. The balance of monouridylation and oligouridylation activities is strongly influenced by another protein called Lin28A, which promotes oligouridylation at the expense of monouridylation. Hence expression of Lin28A can down-regulate let-7 miRNAs, through degradation by the exonuclease enzyme Dis3l2. On the other hand, let-7 miRNAs themselves down-regulate Lin28A production, giving a double-negative feedback control mechanism (Figure 2a). In fact, let-7 miRNAs are involved in the RNA-induced silencing of a host of cancer-causing oncogenes in addition to *LIN28A*, including genes encoding the family of RAS proteins, *MYC*, and *ERBB2*, encoding HER2 protein the target of Herceptin®. In addition, Lin28A promotes the production of the proteins Oct4, Sox2 and Nanog which can be used to re-programme cells to a stem cell state.

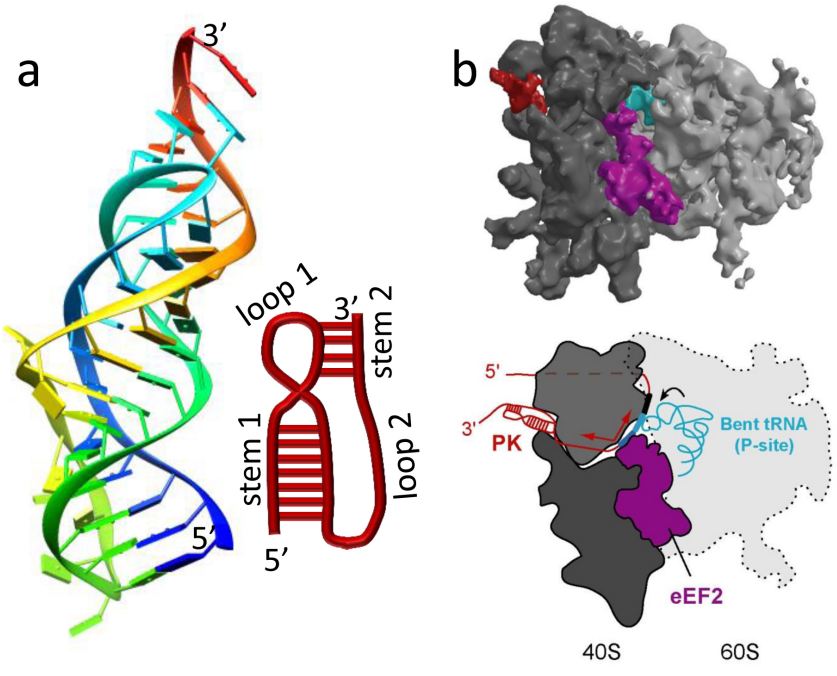

Figure 1: (a) A pseudoknot structure (RCSB PDB entry 2M8K) shown coloured from blue at its 5' end to red at its 3' end. The schematic diagram shows how a typical pseudoknot folds: an RNA stem-loop receives an additional stem through the base-pairing of bases in the loop 1 region. **(b)** The upper panel shows a 3D EM reconstruction of a mammalian ribosome undergoing pseudoknot-triggered -1 frameshifting. The molecular players are, as shown schematically below, the pseudoknot (PK), tRNA in a bent state, and the protein eEF2 (eukaryotic translation elongation factor 2), which promotes tRNA movement. Tension is built up between the tRNA bound to the mRNA and the PK within the mRNA that is blocking the ribosome's forward movement.

Thus, let-7 miRNAs act as master switches because they can suppress the expression of multiple genes involved in cell proliferation, a stem-cell state and cancer. In turn, suppression of let-7 expression promotes 'stemness' and oncogenesis.

Our recent work has shown how ZCCHC6 and ZCCHC11 select uracil over other nucleotides (6) (Figure 2b). Using X-ray crystallography of Cid1, a yeast homologue of ZCCHC11 and ZCCHC6, we have recently identified a critical histidine residue, which specifically selects uracil. Our own mutational studies and those of others have also confirmed that this method of selection is conserved between yeast and human enzymes and is a selectivity filter distinct from that used by the other well-known class of uridylyltransferases, RNA editing enzymes from trypanosomes. Our work on the uridylyltransferases, and on the entire let-7 miRNA system, is a collaboration with Prof Chris Norbury and his group at the Dunn School of Pathology here in Oxford. Cid1 was first properly characterised by this group.

Our current work focusses on seeking a similar understanding of how ZCCHC enzymes, pre let-7 miRNAs and Lin28A come together. It has been clear for a while that targeting the let-7/Lin28A axis of control should be a powerful new approach in drug development, but what was lacking until now was an enzymatic target for the development of inhibitors. Now we have one – or rather two, in ZCCHC6 and ZCCHC11 – and can thoroughly test the viability of redirecting this master switching process for therapeutic ends.

The structural studies of both ribosomal frameshifting and miRNA production control emphasise how we still live, in an important sense, in a world in which RNA is in control. These examples of fine-tuning and master-switching represent good candidates for the development of new therapies and biotechnological tools.

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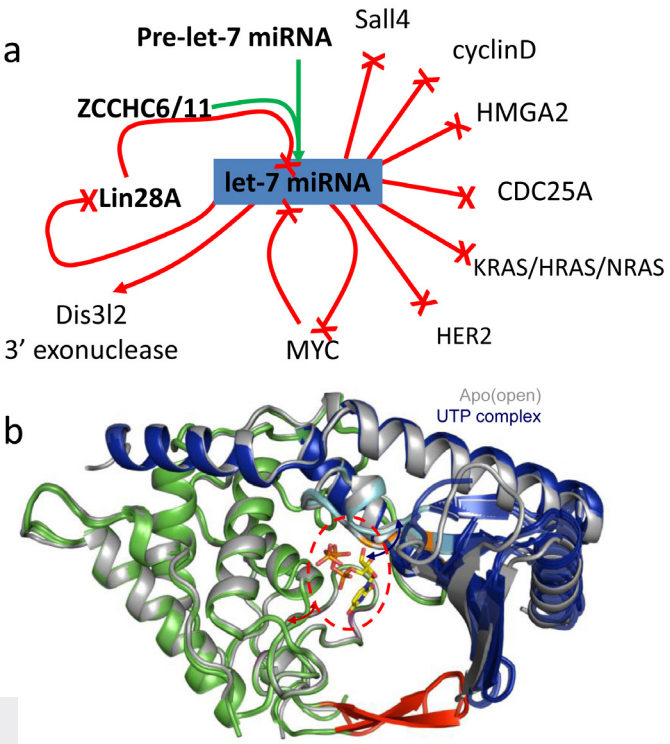

Figure 2: (a) Schematic of interactions in the let-7/Lin28A control axis. Pre-let-7 miRNAs can be matured with help from ZCCHC6/11 but the presence of Lin28A promotes oligouridylation, which switches this off, leading to Dis3l2-mediated breakdown. Lin28A and MYC are both involved in mutually negative regulatory circuits with let-7 miRNAs, which also inhibit expression of the proteins shown (lines ending in crosses), among others. **(b)** X-ray crystal structure of Cid1, the ZCCHC homologue in yeast. The UTP (ringed) is clasped by the formation of a closed state from an empty (Apo) one.

Dr Robert Gilbert is a group leader in the Division of Structural Biology (STRUBI), Nuffield Department of Clinical Medicine.

RESEARCH HIGHLIGHTS

by Freddie Peakman

Loh E, *et al.* (2013) *Nature* 502(7470):237–240.

Temperature triggers immune evasion by *Neisseria meningitidis*

Neisseria meningitidis, commonly known as meningococcus, is an obligate commensal bacterium that colonises the nasopharynx. Under certain conditions it can become pathogenic, resulting in potentially fatal sepsis or meningitis. Loh *et al.* show that meningococcus uses an RNA thermosensor to enhance the expression of genes involved in resisting and evading the immune system in response to increased temperature.

RNA thermosensors, also known as RNA thermometers, are used by bacteria to alter gene expression in response to changes in temperature. They are located around the mRNA ribosome-binding site and form secondary structures, such as hairpin loops, that block the binding of the ribosome at lower temperatures. At higher temperatures the secondary structure melts, allowing the initiation of translation, and thus gene expression.

Loh *et al.* discovered RNA thermosensors in three genes in *N. meningitidis*. Two of the genes encode proteins involved in the biosynthesis of a polysaccharide capsule used to resist the immune system. The third encodes a protein involved in evasion of the complement cascade, which plays a fundamental role in the human innate immune system. What is particularly intriguing about the findings by Loh *et al.* is that the thermosensors they discovered in meningococcus are continuous: expression of the genes increases as the temperature rises. RNA thermosensors found in other pathogens function in an on/off manner: they are 'off' below 37°C when they have no expression, and 'on' above this temperature. These thermosensors ensure that the pathogen does not wastefully express virulence proteins outside the host but can immediately express virulence genes as soon as it is within the host. In contrast, meningococcus is permanently inside the host and always needs expression of its immune evasion genes.

Loh *et al.* propose that the gradual thermosensor mechanism benefits meningococcus during co-infection of the host by another pathogen. Infection triggers the host immune response, causing a rise in temperature and an increase in immune cells and complement proteins, which results in a more hostile environment for the bacteria. Therefore it is advantageous for *N. meningitidis* to increase its defences against the immune system when the temperature increases because this allows it to better survive the enhanced host immune system.

Uphoff S, *et al.* (2013) *Proc Natl Acad Sci USA* 110(20):8063–8068.

Single molecule DNA repair in live bacteria

Both prokaryotes and eukaryotes have extensive DNA repair mechanisms, which are used to maintain genome integrity in the face of damage to DNA. DNA repair is an important area of research; defects in DNA repair pathways are responsible for a number of human diseases and are implicated in the development of cancers. However, our understanding of these pathways *in vivo* is still lacking. Uphoff *et al.* fused a photoactivatable fluorescent protein to the C-termini of DNA polymerase I (Pol I) and DNA ligase. They then used single-molecule fluorescence methods to visualise individual enzymes within individual cells of *Escherichia coli* in real time. These two proteins carry out the last two steps in the base excision repair (BER) pathway, which excises damaged bases, resulting in a short gap in one strand of the DNA; Pol I fills in the gap and DNA ligase ligates the nick left by Pol I.

Being able to visualise individual repair enzymes within a cell provides unprecedented information about DNA repair *in vivo*. Uphoff *et al.* were able to calculate the time taken for individual repair events to take place – 2.1 s for Pol I and 2.5 s for ligase – and to determine a number of other important values, including the copy number and diffusion characteristics of each enzyme.

Interestingly, Uphoff *et al.* showed that only a small proportion (<4%) of Pol I and DNA ligase enzymes are active in the cell under normal conditions. However, under conditions of high DNA damage induced by a change in environmental conditions – for example, following the introduction of a DNA damaging agent – the excess enzymes become vital, allowing the cell to rapidly increase the rate of repair in order to prevent extensive damage. Moreover, the intermediate species in the BER pathway can actually be more toxic to the cell than the damaged bases, and thus it is particularly important that excess Pol I and DNA ligase enzymes are available to ensure a quick increase in the rate of the whole pathway.

Invasion of the body snatchers: Could a common parasite be affecting our behaviour?

by Madeleine Pope

Parasites that live in our brains and affect our behaviour sound more like a concept for the latest zombie movie than a real biological phenomenon. However, this is what some researchers believe *Toxoplasma gondii,* one of the world's most promiscuous parasites, is doing.

Thought to be present in approximately 30% of the world's population, the protozoan *T. gondii* is able to infect almost all warm-blooded animals, including rodents, birds and livestock. It has been a subject of interest for parasitologists since the 1980s, when the first evidence of its ability to manipulate behaviour was published. Since then, *T. gondii* has been widely used as a model for studying the so-called 'manipulation hypothesis', which posits that parasites deliberately alter the behaviour of their hosts in order to promote transmission (1). Recently, *T. gondii* has gained more attention as new evidence hints that it may be able to modulate the behaviour of humans as well. Here we discuss some of the findings from human and rodent studies that suggest this could be the case.

Circle of life

The life cycle of *T. gondii* is a complex one. Whilst it can infect a broad range of hosts, it is only able to sexually reproduce in the cat family. Felines are therefore known as the parasite's 'definitive hosts'. All other susceptible animals are known as 'intermediate' or 'dead-end' hosts and can be any of a huge number of animals. Once an intermediate host acquires *T. gondii*, the parasites form cysts within tissues such as the heart and brain, and reside there for the duration of the animal's life, slowly dividing (Figure 1). When an intermediate host is eaten by a cat, the tissue cysts are broken down, releasing the parasites and enabling them to reproduce sexually in the cat's intestinal epithelium. Their progeny, called oocysts, are excreted in faeces, become infectious and are able to survive for several days. Other animals can then be infected by ingesting soil, water or plant material contaminated with oocysts, thus continuing the cycle (Figure 2).

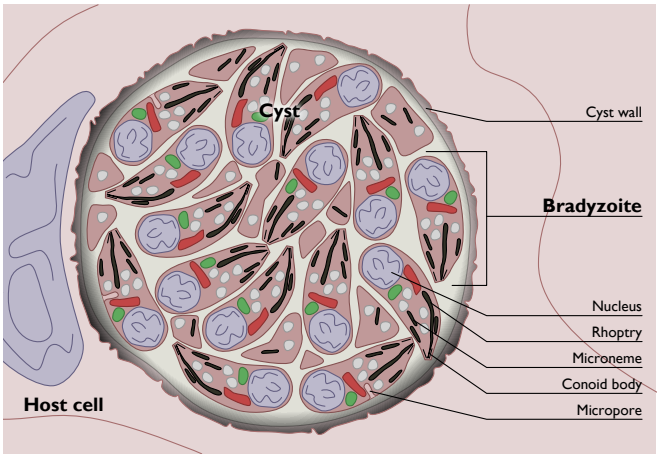

Figure 1: Structure of a *T. gondii* tissue cyst. Schematic of an electron micrograph of a tissue cyst in the brain of a mouse. The cyst is situated in the cytoplasm of a host cell, close to the nucleus. The cyst contains over 100 parasites in their slowly dividing 'bradyzoite' form, and is surrounded by a thin cyst wall. Figure by Richard Wheeler.

Humans fall into the 'dead-end' host category, since we can be infected but are unlikely to be eaten by a cat. For us, ingestion of *T. gondii* can occur by eating undercooked meat that contains tissue cysts, or via handling cat litter or soil containing cat faeces. Additionally, humans can become transplacentally infected, from mother to foetus, if a woman becomes infected for the first time while pregnant (2).

Transplacental infection can have drastic consequences for the developing foetus, including neurological and ocular defects (3), but infections acquired in adulthood have long been considered asymptomatic. However, a growing body of evidence suggests that *T. gondii* infection is associated with a greater risk of developing neurological disorders, such as schizophrenia (2), psychopathology (4), obsessive compulsive disorder, Parkinson's disease, suicidal tendencies and bipolar disorder (5) and, even more strangely, infected individuals are more likely to be involved in traffic accidents (6). But associations are one thing, cause is another; is there any evidence of a direct effect of *T. gondii* on the brain?

Fatal attraction

Most of the current evidence for a direct role of *T. gondii* in altering behaviour comes from rodent studies – rats infected with the parasite are more active, less nervous and have a decreased aversion to cat odour (5). This is thought to be modulated in part by the sexual arousal pathways, and has led to the coining of the term 'fatal feline attraction', which infers that the intermediate host becomes less afraid of cats and thereby increases its chances of

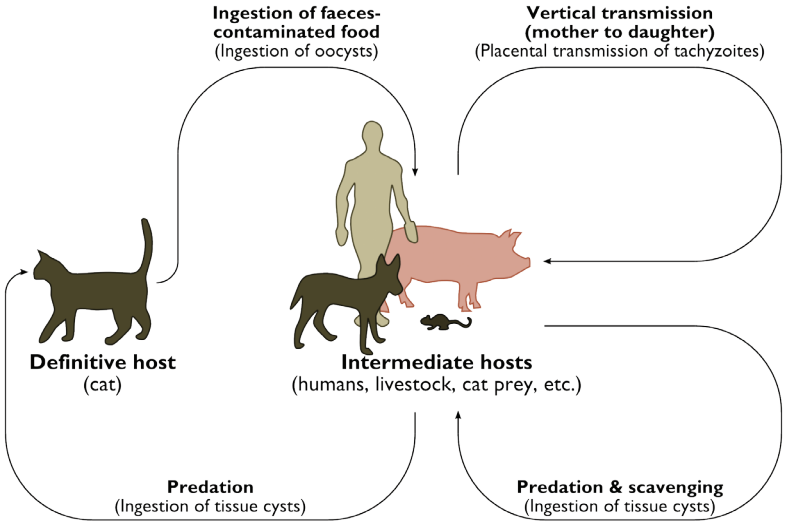

Figure 2: Life cycle and transmission of *T. gondii*. There are a number of ways that *T. gondii* can be transmitted: by ingestion of oocysts, ingestion of tissue cysts, vertical transmission from mother to foetus, and via blood transfusion. Figure by Richard Wheeler.

being eaten, enabling the parasite to complete its life cycle. It is an appealing concept, but some groups argue that neurological symptoms may instead be a secondary pathological effect of the presence of parasites in the brain, perhaps mediated by the immune system (2).

As well as affective and neurological disorders, *T. gondii* infection has been correlated with changes in personality, including increased extroversion and decreased conscientiousness in humans (1). Some groups suggest that these changes could be a human reflection of the behavioural changes seen in rodents. For example, increased activity and delayed reaction times could be likened to reduced predator avoidance. However, it is possible that people with certain personality traits, such as greater risk-taking, are more likely to be infected – for example by being more likely to eat undercooked meat. These same 'risk takers' may also be more likely to be involved in traffic accidents, so association with *T. gondii* infection may be a coincidence.

Towards the future

The game-changer in this strange story will be the publication of evidence directly linking human behavioural changes with *T. gondii* infection. So far, most of what has been reported is correlation. A few studies have made more robust links and, intriguingly, there is some evidence to suggest that symptoms of certain neurological disorders can be improved by treating *T. gondii* infection alone (7). In addition, one interesting study of military personnel analysed serum samples taken up to 11 years prior to the onset of schizophrenia, and found that levels of anti-*T. gondii* antibodies were significantly elevated prior to the onset of illness (8). Despite these developments, we are still a long way from determining whether *T. gondii* has a significant impact on behaviour and mental health. Without systematically infecting people with *T. gondii*, researchers may never be able to tease out a cause and effect relationship. Undoubtedly, if there is sufficient evidence to suggest that targeting *T. gondii* could alleviate some of the symptoms of neurological disorders, further research will prove beneficial to patients. But to suggest that *T. gondii* could significantly increase our risk of developing diseases like schizophrenia is irresponsible. Whilst the field is still in its infancy, researchers must be careful to avoid media scaremongering, which could see many of us believing that our cats really do make us crazy.

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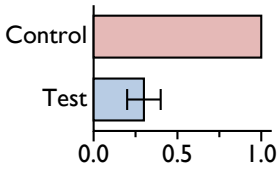
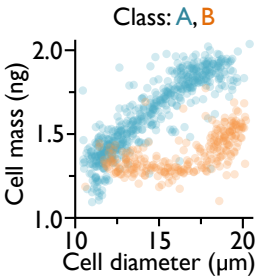
Figuring out Good Figures

If a picture is worth a thousand words, how many words is a scientific figure, chart or graph worth?

The content of figures is actually best thought of in terms of number of numbers...

This is a very simple figure showing just two numbers: that the test is 0.30±0.10 of the control.

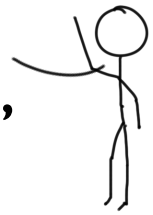




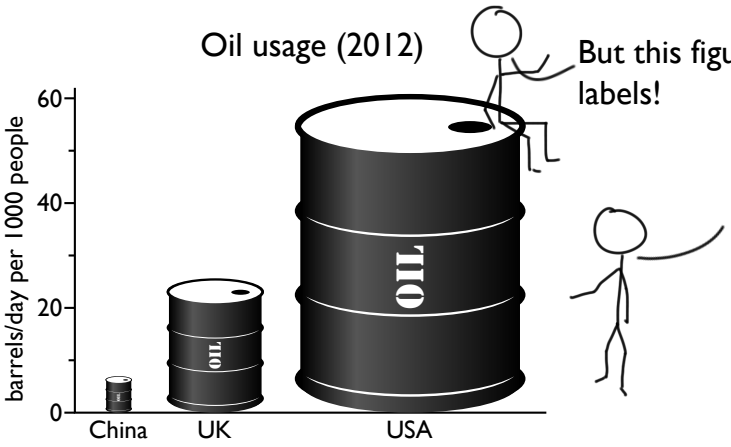
This figure is much more complex, and presents over two thousand numbers simultaneously.

Thinking about figures as a collection of numbers might seem weirdly abstract. Of course, figures aren't all **just** numbers. They often also include pieces of data better presented as images, 3D structures, etc. So why think about them as numbers? It lets you test one important thing: how good is the figure?

> "Graphical excellence begins with telling the truth about the data."
>
> Edward Tufte, 1983



What is telling the truth about data? In short it is making sure the numbers (the actual data) that were used to make the figure are clear. Lying with a graph or figure is just as easy as accidentally misphrasing or deliberately obfuscating text. So what does a lying figure look like?

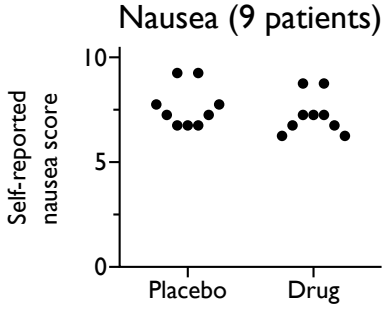


But this figure can't be lying; it's got a clear scale and labels!

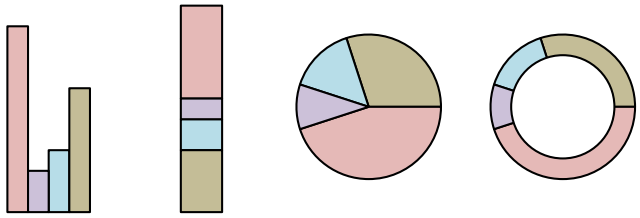
Your brain intuitively interprets the **volume** of the barrels. It looks like the UK uses about an eighth as much oil per day as the USA. Actually the height of the barrel shows the true usage; that the UK uses about half that of the USA. The figure **tricks** you into a 400% error in extracting the data.

It might seem silly, but this figure is lying too. By adding artificial structure to the data (arranging the points as smiley faces) the true values of the data points are hard to see.

Which did you notice first: the sad face, or the lower mean, in the drug-treated group?



If a figure lies to you that certainly doesn't mean the author is trying to mislead you. It is often very hard to present data in the clearest and hardest to misinterpret way.

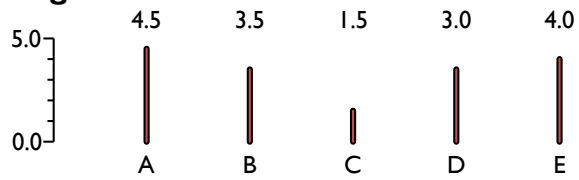


These four charts show exactly the same data, but which is clearest? Is there actually one that is better and easier to read than the rest?

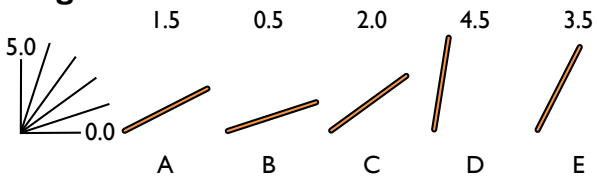
Why not test yourself?

Below are six different data presentation methods, each showing five measurements (labelled A, B, C, D and E) between 0.0 and 5.0. In each set of five measurements there is one deliberate mistake, which differs from the stated value by ±0.5. Can you identify which one it is? The answers are at the bottom of the page.

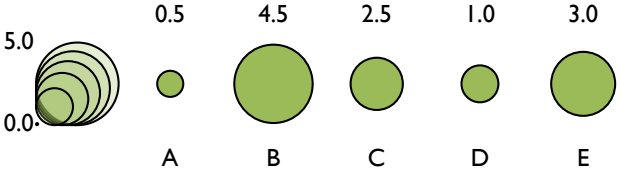
Length



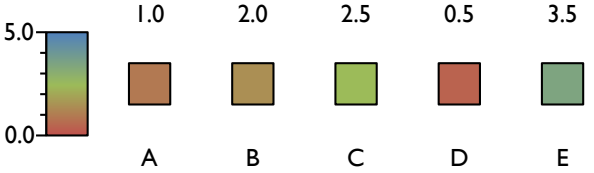
Angle



Area

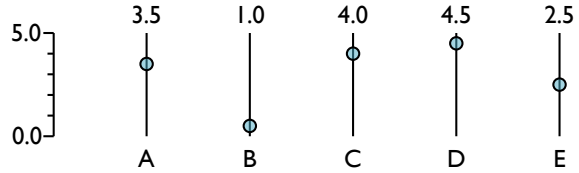


Colour

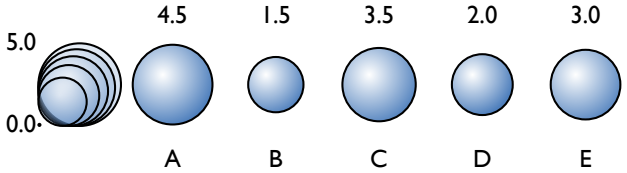


Apologies if you are colour blind, but that's part of the point!

Position



Volume



Most people have a similar order of ability in accurately interpreting data presented in these different ways:

Position is easier than Length is easier than Angle is easier than Area is easier than Volume is easier than Colour

This has important consequences: It means that people typically find pie charts (areas) are harder to read accurately than doughnut charts (angle), and both are harder than bar charts (length). It also means that images (colour brightness), and especially fluorescence colocalisation (colour hue or shade) are very hard to interpret quantitatively by eye.

It is almost impossible to make simple rules for designing the perfect, easy to understand figure for sharing precious scientific data, but with a little thought and energy a lot can be done to help your readers!

Answers: Length: D (actual value 3.5), Angle: B (actual value 1.0), Area: C (actual value 2.0), Colour: B (actual value 1.5), Position: B (actual value 0.5), Volume: D (actual value 2.5).

By Richard Wheeler 2013

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A new perspective on the obesity pandemic

by
Dr Dyan Sellayah

It has been 50 years since Neel proposed the 'thrifty genotype' hypothesis (1). It explained how nutritional abundance and lack of exercise combined with a genetic propensity for energy storage could have led to the obesity pandemic afflicting most industrialised countries today. The thrifty genotype and other hypotheses attempt to reconcile recent lifestyle changes with genetics in an effort to explain the rapidity and extent to which obesity has exerted its devastating influence. However, they cannot explain the variation in obesity prevalence among ethnicities. Why are some indigenous groups such as the Pima Indians of the Americas and Pacific Islanders plagued by excessive obesity rates, while other groups such as the Chinese appear to be obesity-resistant? Surprisingly, the explanation to these mysteries may be found in the journey of human evolution from our origins in Africa through our migration in Asia, Europe, Australia and the Americas. This journey encompassed extreme climates, from the unforgiving heat of the African Savannah to the relentless ice age in Europe and Siberia. Unexpectedly, our ancestral adaptations to cope with differing climates could be at the core of the complexities surrounding the genetic basis for obesity.

Through natural selection owing to cyclical episodes of famine and feast, thrifty genes conferred superior energy efficiency upon their bearers (1). Such genes, which allow efficient storage of energy as fat, would be highly advantageous during times of nutritional surplus. However, they become detrimental in the high-calorie and sedentary lifestyle we experience today. Thrifty genes supposedly enabled survival in a nutritionally unreliable world where the intake of a weight-maintaining 2,500 calories per day was, unlike today, by no means guaranteed. The Pima Indians demonstrate staggeringly high obesity rates, and are classically used as a 'case in point' by the proponents of the 'thrifty genotype' hypothesis. Despite its attractive and easily digestible narrative, the conceptual accuracy of the hypothesis has come under attack in recent years. Its main rival, the 'drifty genotype' hypothesis, was proposed by John Speakman (2).

Speakman asserted that, contrary to the 'thrifty genotype' hypothesis, the obesity pandemic is not the result of natural selection for thrifty genes but by the absence of positive selection for genes conferring leanness. According to Speakman, the absence of selection for genes that limit energy storage was caused by the ability of our ancestors to reposition themselves higher in the food chain. By developing sophisticated weaponry, manufacturing tools and cultivating organized social structures, our ancestors were no longer subject to the threat of predation. These distinctly human traits inadvertently rendered predation selection pressures obsolete and endowed us with unfavourable energy-efficient genes. While both theories are plausible, neither can adequately account for the considerable variation in obesity prevalence among ethnicities. In fact, both theories make the assumption that the ancestors of all ethnic groups faced identical selection pressures. Rather, different selection pressures relevant to the climate had profound effects on genes for metabolism.

While the US has the highest rates of obesity, the burden is not shared equally among its inhabitants. Studies show that those of African ancestry are more prone to obesity than those of Caucasian and Chinese ancestries (3). Similar results were found in Britain. To understand how certain ethnicities are more obesity-prone than others, one has to look at how differential exposures to climate in the evolutionary history of modern humans may have impacted on genes for metabolism and energy expenditure.

One such gene encodes for uncoupling protein 1 (UCP1), which is highly expressed in brown adipose tissue (BAT). UCP1 uncouples oxidative phosphorylation from ATP synthesis to liberate energy as heat, a process known as thermogenesis. BAT thermogenesis is activated in response to cold-exposure and shorter days, allowing humans to adapt to cold environments. The heat-producing capability of BAT thermogenesis is metabolically wasteful and enhances energy expenditure. Humans subject to acute cold-exposure exhibit increased BAT thermogenesis and elevated metabolic rate. Not everyone possesses effective BAT thermogenesis, however. Humans without efficient BAT function are obesity-prone while those with enhanced BAT function are obesity-resistant.

Evidence is mounting that those of us whose ancestors were genetically adapted to cold environments possess efficient BAT function while those whose ancestors evolved in hot climates have ineffective BAT capacity and are thus more prone to obesity. Selection for genes with efficient BAT function to cope with colder climates could explain why the majority of people in the US of East Asian ancestry and a large proportion of those of Caucasian ancestry are obesity-resistant. Chinese people are descended from cold-adapted

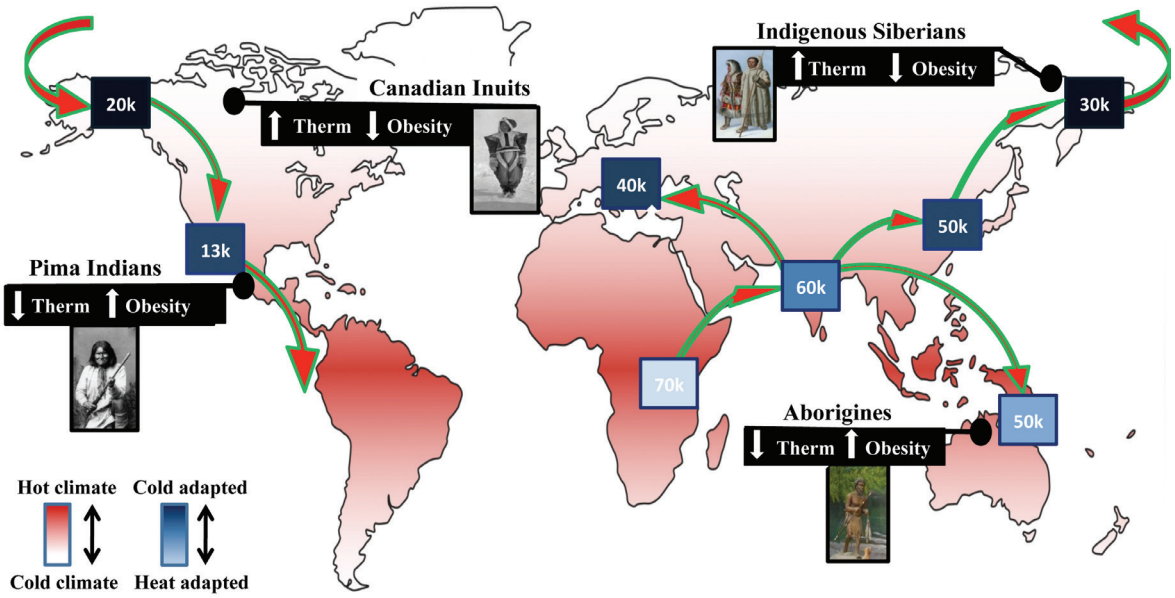

Figure 1: Map portraying the proposed historic human migration out of Africa 70,000 years ago and how it led to differential exposures to climate that subsequently impacted on BAT thermogenesis (Therm), explaining the ethnic variation of obesity today.

'Mongoloids' who flourished in the extreme cold of Mongolia and Siberia during the last ice age. Europeans are also descended from early humans who thrived in ice-age Europe. For both these ethnic groups, potent BAT function would have been imperative.

Conversely, people in the US of African ancestry possess genes which prevent heat stroke and dehydration. While BAT thermogenesis would have been vital for survival in Northeast Asia and Europe, it would have been counterproductive in the tropics. This could explain why people in the US of African and Southeast Asian ancestries are more prone to obesity than their countrymen of Caucasian and East Asian origins. To this end, the lower metabolic rate of Africans has been linked to reduced BAT thermogenesis, while enhanced BAT thermogenesis has been observed in Chinese and Caucasian individuals.

But why are certain Native American groups such as Pima Indians obese? This indigenous group, who inhabit present day Arizona and Mexico, are proposed to be directly descended from cold-adapted Mongoloids who crossed the Bering Land Bridge from Siberia into present day Alaska 20,000 years ago. Evidence suggests that by the time they reached as far south as Arizona and Mexico, they were reacquiring the capacity for heat-adaptation and losing their cold-adapted traits. Interestingly, the reduced metabolic rates and propensity for obesity in Pima Indians have been attributed to lower body temperatures, alluding to a reduced thermogenic capacity (4). Mutations in several genes associated with improved heat-adaptation in Pima Indians are as prevalent as in African populations, indicating that despite their recent descent (<20,000 years) from cold-adapted Mongoloids, exposure to heat had a profound effect on selection. Despite a shared ancestry with the Pima Indians, Canadian Inuits have high metabolic rates and are more obesity-resistant. The Inuits remained in Arctic and Subarctic environments after their ancestors crossed the Bering Land Bridge and retained their adaptations of enhanced BAT thermogenesis. Aboriginal Australians and Pacific Islanders of Indonesia, whose ancestors evolved in tropical climates since they migrated through Southeast Asia around 50,000 years ago, display alarmingly high obesity rates. Low BAT thermogenesis has been implicated in the propensity for obesity in these ethnic groups.

Thus, variation in susceptibility to obesity among ethnicities can be traced to differential exposures to climate of the ancestors of these various populations, beginning with the proposed migration of modern humans out of Africa 70,000 years ago (Figure 1). Migration to northern latitudes would have necessitated the selection of genes for cold-adaptation, such as those that enhanced thermogenesis. Due to their secondary effects on metabolism and energy expenditure, these genes became key players in the genetic predisposition to obesity in today's sedentary and overfed Western population, and explain why the obesity burden is not equally shared across the various ethnicities.

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Raiding the bacterial armoury

by Thomas Mortimer

The hypodermic needle is a key piece of equipment used daily by medical professionals. This highly precise and efficient method of delivering pharmaceuticals is invaluable in everything from vaccination to anaesthesia. However, humans are not the first species to realise the potential of the needle. An abundance of pathogenic bacteria exploit a needle-like type-III secretion system known as the 'injectisome' to pump protein effectors into their host to modify the host's internal environment (1, 2).

The injectisome is found in a rogues' gallery of pathogens including *Yersinia pestis*, the bacterium responsible for the Black Death, and *Salmonella enterica*, a common cause of food poisoning. Our current knowledge suggests a needle-like model (Figure 1). Export begins with the recruitment of effectors associated with a specific chaperone. First, to be threaded through the needle filament they are unfolded by the ATPase. They are then exported in an ordered fashion. The first proteins to be exported include machinery that associates with the needle tip and host cell membrane, subsequently allowing transfer of effectors, powered by ATPase activity and membrane potential.

As our understanding of the injectisome grows, so does our ability to manipulate it. An obvious way to exploit our knowledge is to design specific inhibitors of the system that will, in turn, mitigate the infectivity of the pathogen. This approach has been used effectively to design inhibitors of YscN, a component of the *Yersinia* ATPase. Computationally selected small molecules were tested for their ability to inhibit the ATPase and subsequently demonstrated inhibition of effector secretion in bacterial and mammalian cell culture.

But why not take a more nuanced approach to exploiting the injectisome? Instead of inactivating it, we could recruit it to translocate proteins of our choosing at highly specific locations. Potential applications are limited only by the imagination of the user. This concept has been extensively developed as a means of vaccine delivery. Injecting antigens into the cytosol would allow activation of the major histocompatibility complex class I-restricted antigen pathway. This pathway is a pre-requisite for activation of CD8+ T cells, which are needed to effectively combat intracellular pathogens. Live attenuated *Salmonella enterica* has been created containing a fusion of its effector protein SopE with the *Mycobacterium tuberculosis* protein ESAT-6. This strain secretes ESAT-6 into the cytoplasm of mammalian cells and confers the same degree of protection against tuberculosis as the existing BCG vaccine. The same approach has been used for proof-of-concept malaria and cancer vaccines, both of which show promise but are still far from clinical use. Recent developments have even allowed the entire avoidance of an attenuated pathogen. A *Salmonella* secretion system working in achromosomal bacterial minicells may allow this vaccination approach to be used on children and the immunocompromised. The use of protein delivery has been taken further than vaccines. The *Shigella flexneri* secretion system, for example, has been used to deliver anti-inflammatory cytokines IL-10 and IL-1RA to the sites of wild-type *Shigella* infection, lessening the acute inflammation associated with Shigellosis. This methodology could be used to tackle chronic inflammation brought on by irritable bowel diseases such as Crohn's disease. Combining this delivery of bio-active cytokines/co-stimulatory molecules with transfer of peptide antigens could offer an optimal way of undertaking vaccination.

Hijacking the injectisome is doubtless an approach with extensive dividends. However, our knowledge of how secretion is controlled remains at a stage far too tenuous to allow all possible applications to be realised. With work continuing apace to understand structure and function, we will certainly see significant commercialisation of such concepts in years to come.

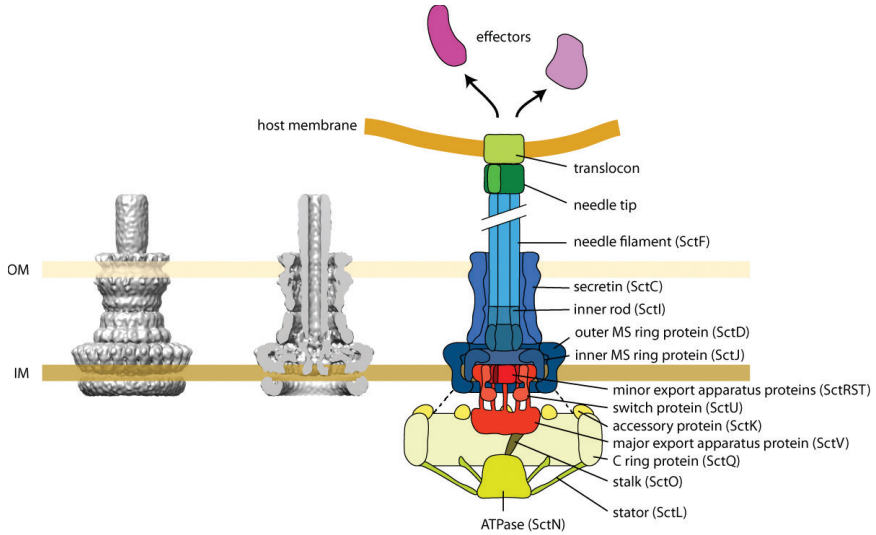

Figure 1: The injectisome is a dynamic multicomponent assembly thought to have a needle-like structure. Figure used with permission from (1).

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Shoot the messenger

Exosomes as novel regulators and biomarkers of metastasis

by Siamak Redhai

Defective cell-to-cell signalling underlies many diseases including diabetes and neurodegeneration, and is one of the hallmarks of cancer. Our current understanding of cell signalling primarily centres on ligand-receptor-mediated interactions. This one-dimensional view of signalling is under review because it is becoming increasingly clear that extracellular vesicles, such as exosomes, can also mediate cellular communication (1).

Exosomes are membrane-bound vesicles formed inside multivesicular bodies (MVBs) and are secreted when MVBs fuse with the plasma membrane (Figure 1). Early studies led scientists to believe that these vesicles were merely 'rubbish bags' used to jettison unwanted transferrin receptors from maturing red blood cells. However, it was shown recently that these vesicles also contain a cocktail of signalling proteins and nucleic acids. Interestingly, these exosomal cargos are functional and can elicit changes in gene expression once transferred to recipient cells (Figure 1). For example, T cell-derived exosomes containing microRNA (miRNA)-335, was shown to curb the translation of *SOX4* mRNA in antigen presenting cells. Therefore, these nano-sized vesicles can deliver an array of information that can simultaneously impact multiple pathways and reprogram cellular processes.

The roles of exosomes in cancer are currently of great interest. A recent study revealed that glioma cells can 'share' oncogenic proteins such as overactive epidermal growth factor receptor VIII via exosome-mediated transfer to induce transformation and anchorage-independent growth in recipient cells (3). Interestingly, melanoma cells use exosomes to reprogram bone marrow cells to form a pre-metastatic niche that subsequently supports metastatic cells. Cancer cells can also use exosomes to communicate with immune cells. For example, cancer-derived exosomes can suppress T cell responses by activating myeloid-derived suppressor cells (3). These studies highlight the diverse roles that exosomes play in malignancy progression.

Exosomes are also considered to be potential biomarkers for various cancers, since their encapsulated contents are protected from extracellular degradation and reflect the molecular constituents of their cells of origin. Khan *et al.* highlighted that exosomes containing the protein survivin were highly enriched in patients suffering from prostate cancer and correlated with an elevated relapse rate after chemotherapy (4). Recently, biotechnology companies have begun to develop novel technologies to take advantage of exosome-based biomarkers. In 2010, Caris Life Sciences launched Carisome®, which was designed to analyse prostate-derived exosomal markers in blood samples. Although these approaches are still in their infancy, they potentially represent a novel method for early cancer detection. However, a real obstacle in the exosome field has been the lack of an *in vivo* genetic system to dissect out exosome biogenesis and function.

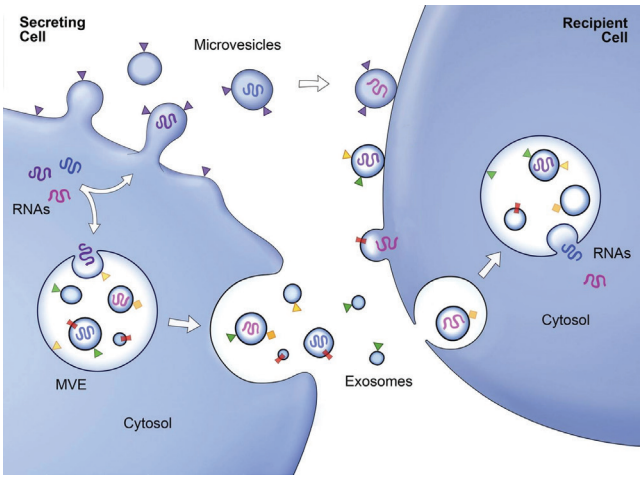

Figure 1: Exosomes are secreted and can reprogram recipient cells. Modified from (1).

Our group studies the cellular and molecular aspects of the *Drosophila* accessory gland (AG), a secretory organ related to the mammalian prostate, containing a subset of cells that secrete exosomes into the glandular lumen. We have shown that male AG-derived exosomes are transferred to females during mating, and are involved in reprogramming the post-mating behaviour of females (5), suggesting that exosomes can potentially signal between individuals. Using this system, we hope to unpick the elusive regulatory mechanisms that govern exosome biogenesis. Understanding these mechanisms will, in turn, allow us to selectively regulate the secretion of exosomes in cancer cells and thus help to block their progression.

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Aptamers: The future therapeutics?

by
Dr Maria Mogni

Nucleic acids not only hybridise to one another based on complementary sequences, but can also create complex 3D shapes that act as scaffolds, supporting the formation of protein complexes. Recently a series of technological advancements allowed the development of *in vitro* evolutionary methods for the discovery of additional, non-biological oligonucleotides that can bind to protein targets. When synthesis of degenerate oligonucleotides and PCR were combined with the ability to partition oligonucleotides on the basis of their binding or catalytic activities, *in vitro* selection of functional nucleic acids – **S**ystematic **E**volution of **L**igands by **Ex**ponential Enrichment (SELEX) – was born. Nucleic acid ligands generated using SELEX are called aptamers. Similar to antibodies, aptamers can be engineered to bind to multiple, distinct targets. In a typical SELEX experiment, a random sequence oligonucleotide library is synthesised that covers 20-100 residues in length, where each nucleotide is flanked by constant sequences essential for enzymatic manipulation.

Since the invention of SELEX, researchers have been able to identify high affinity aptamers that target a large cross-section of protein families, including cytokines, proteases, kinases, cell-surface receptors and cell-adhesion molecules (Figure 1). Although traditional SELEX methods require a soluble, pure form of the target, novel methods have been developed that target cell-surface proteins and proteins in the human plasma. Structurally-constrained peptides and protein domains can also be used to create aptamers targetting a specific protein domain or site. Aptamers with properties more suitable for systemic administration are currently being developed, mainly against targets in the bloodstream, such as thrombin, factor IXa and von Willebrand factor, or targets on cell surfaces, such as the epidermal growth factor receptor (EGFR). In the future it may be possible to use aptamers against intracellular targets, using technology such as exosomes to transport the molecules across cell membranes, either by themselves or in combination with other drugs. Furthermore, aptamers are being engineered to act as agonists or antagonists. How the structural, chemical and pharmacokinetic characteristics of these and other aptamers influence their eventual clinical utility is the focus of this article.

For therapeutic applications, aptamers are in competition with small molecules and antibodies. However, one advantage of aptamers is that they typically have longer half-lives. For example, aptamers targetting vascular endothelial growth factor (VEGF) were successful in the treatment of wet macular degeneration, since they had long half-lives in the ocular compartment (1).

Unlike conventional antibody generation, no organisms are required for the *in vitro* selection of oligonucleotides. This freedom from cellular biochemistry offers a massive advantage in manipulating the process of directed evolution. While the targets of antisense oligonucleotides and silencing RNAs (siRNAs) are intracellular, aptamer therapeutics can be developed for intracellular, extracellular and cell-surface targets. Aptamers are smaller than antibodies, allowing more efficient entry into biological compartments. Furthermore, they can be reversibly denatured, so purification is more straightforward than for antibodies. In addition, it has been possible to use chemically modified nucleotides in SELEX experiments. These approaches use DNA or RNA polymerases that can accept appropriately-modified nucleotide triphosphates as substrates. The presence of these modified nucleotides stabilises oligonucleotides against nuclease-mediated degradation, and imparts greater affinities to selected aptamers.

Several aptamers that are selected to bind to a specific protein have been shown to function as agonists. For example, aptamers isolated against the extracellular domain of the protein human epidermal growth factor receptor 3 (hEGFR-3) can promote its oligomerisation (2). Moreover, a DNA aptamer has been shown to enhance the binding activity of an isoleucyl tRNA synthetase (3).

Although the selection of aptamers for therapeutic applications is relatively straightforward, the adaptation of aptamers for *in vivo* use has required extensive research. Relevant features of protein therapeutics can be extrapolated on the basis of characteristics of known, circulating proteins. However, there are few circulating nucleic acids from which similar comparisons can be drawn. Moreover, even though aptamers are chemicals, they are large by the standards of traditional, non-protein drugs and do not readily cross biological barriers such as cell membranes. It follows that many of

the general rules that are typically applied by medicinal chemists during drug development do not apply to aptamers. In addition, most targets for therapeutic aptamers are either in solution in the blood plasma or displayed on the surface of cells that are accessible from the blood plasma, such as on the surface of the vasculature. Aptamers in this medium are subject to nuclease-mediated degradation by serum nucleases, renal filtration, and uptake by the liver and other tissues such as the spleen.

Particular attention has been paid to avoiding renal filtration of aptamers. Since most aptamers have a molecular mass of 5-15 kDa, below the 30-50 kDa cutoff for renal glomerulus filtration, they are susceptible to renal filtration regardless of how resistant they are to nuclease-mediated degradation. However, aptamers that are conjugated to polymers in this size range show a significant reduction in renal filtration rates. The most widely used polymer for avoiding exclusion by renal filtration is high molecular mass polyethylene glycol (PEG) (4). Indeed, unconjugated aptamers are cleared from the mouse circulatory system with a half-life of 5-10 minutes, while 40 kDa PEG-aptamer conjugates can have circulating half-lives as long as one day. Cholesterol conjugation has been reported as an alternative strategy to decrease renal filtration rates; however, this seems to be less effective than PEG conjugation. Aptamers are particularly amenable to modifications since they lack many of the functional groups commonly present in proteins. Therefore a single functional group can be introduced site-specifically and used as a unique site for conjugation of other molecules to the aptamer without disrupting structure or function.

As with other therapeutics, aptamer toxicity can manifest itself through on-target or off-target mechanisms. Off-target effects of oligonucleotides have been extensively studied and include anti-coagulation, complement activation, and innate immune stimulation. Furthermore, although antibodies to synthetic oligonucleotides have not been generally observed, antibodies to some oligonucleotide conjugation partners such as PEG have been reported. Innate immune activation occurs as a consequence of the activation of Toll-like receptors that respond to single or double-stranded RNA, or unmethylated CG motifs in DNA. Moreover, anticoagulation has been reported in primates after administration of oligonucleotides. It is thought that this is a consequence of low-affinity interactions between the oligonucleotide and protein components of the clotting cascade. In addition, complement activation has been attributed to the interaction of oligonucleotides with complement factor H.

Several aptamers have undergone clinical trials (5-7). However, to increase the number and range of aptamers the selection process must be automated.

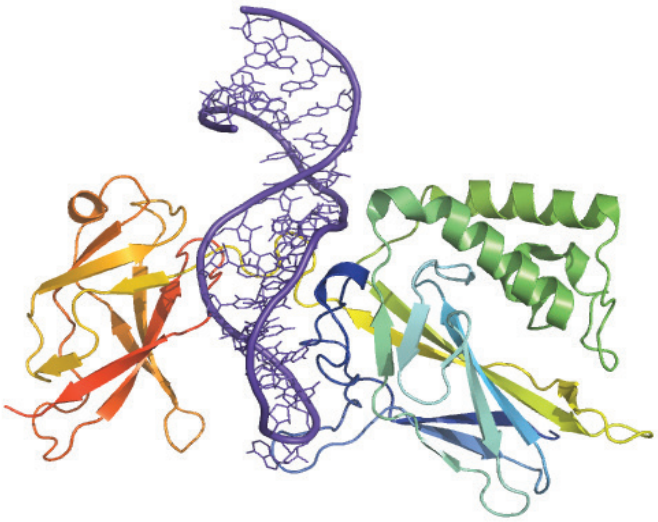

Figure 1: Crystal structure of an RNA aptamer (purple) complexed with mouse NF-κ-B p50 subunit (rainbow). Only one monomer is shown from RCSB PDB entry 100A.

Techniques based on affinity-capture onto magnetic beads are being developed. Nonetheless, special effort is still being focused on the engineering of novel interactions, and on the creation of aptamers that bind to the cell surface, allowing them to persist in the vicinity of a specific cell or tissue type. Finally, phototoxic aptamers for the targeted therapy of specific cancer cells are being developed. The wide-ranging potential of this technology holds great promise for the future.

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HIV cures: What do they mean?

by Susan Graham

To date, one infant and possibly three adults have been cured of HIV (1-3). Many other patients' immune systems can control the virus (4, 5). Do these cases give hope to the other 34 million people living with HIV (6)?

Potential HIV cures can be divided into two main categories. Sterilising cures completely remove the virus, with no possibility of recurrence apart from re-infection, whereas functional cures mean the patient remains *infected* but not *affected*. Here, we analyse the cases of reported cures and compare them with reports of 'elite controllers' and 'long-term non-progressors': those whose own immune systems can keep HIV at bay.

HIV mechanics

HIV (human immunodeficiency virus) is an RNA-based retrovirus, which is further sub-classified into HIV-1 and HIV-2. The virus infects the cells of the immune system, thus attacking its attacker. Once inside the cell, HIV converts its RNA into DNA through an error-prone process known as reverse transcription. Mutations acquired during reverse transcription may be beneficial to the virus, e.g. if the mutation provides resistance either to the drugs the patient is taking or to the patient's own immune system. The DNA is then transported to the nucleus where it is integrated into the human genome, converting the host cell into a factory for more HIV viruses.

The immune cells specifically targeted by HIV are $CD4^+$ 'helper' T cells. These cells reside in the blood stream and aid in the recognition of viruses via the CD4 glycoprotein and either CCR5 or CXCR4. HIV persists in these cells and subsequently forms reservoirs in $CD4^+$ 'memory' T cells, which 'remember' previous infections and are quickly re-activated upon re-infection. Reservoirs are also formed in long-lived cells such as dendritic cells, macrophages and cells of the central nervous system. These reservoirs allow the virus to 're-awaken' from latency upon breaks in therapy.

Possible hope: The Berlin Patient

The Berlin Patient was first reported in 2009 by Dr Gero Hütter and is the most widely accepted case of HIV being cured in an individual. A male patient infected with HIV for 10 years was diagnosed with acute myeloid leukaemia. HAART (highly active antiretroviral therapy) had been effective in preventing any AIDS (acquired immunodeficiency syndrome)-related illnesses until that point. After initial chemotherapy failed to treat the leukaemia, an allogenic stem cell transplant (derived from a human donor) was performed. The donor cells were homozygous for the *CCR5-Δ32* allele, a mutation known to provide some protection against HIV. After a relapse of leukaemia, the patient received a second transplant from the same donor. Incredibly, 20 months after HAART had been discontinued, no viral RNA or DNA could be detected in the body (1). For the first time in medical history, the patient was then cured not only of leukaemia but also of HIV. This case inspired a new wave of enthusiasm for possible HIV cures. The mutated CCR5-Δ32 receptor became a candidate for gene therapy, providing hope for an end to this disease (7).

Since that first report, a similar study of two men receiving bone marrow transplants for treatment of cancer and HIV has been published. Each patient was heterozygous for the *CCR5-Δ32* mutation but received bone marrow transplants from donors with 'wild type' CCR5 receptors, i.e. lacking any beneficial mutation. After treatment, there was a substantial reduction in the peripheral blood reservoir of HIV (3), providing another indication that bone marrow transplants could be used to eliminate viral reservoirs.

Early treatment may have cured an infant

In March 2013, it was announced that a newborn baby in Mississippi, USA, had been cured of HIV. Although there are adequate therapies that prevent mother to child infection, 1% of babies born to HIV-positive mothers are still infected with HIV (8). In this case, the infant tested positive for HIV DNA at 30 hours. Immediate antiretroviral therapy (ART) was given and continued for 18 months. At 26 months, standard clinical assays did not detect any virus or antibodies against HIV in the infant's system (2), indicating that the infant was able to suppress the disease. This was the first reported functional cure of an infant born with HIV.

Such intense combined therapy is unlikely to be effective for the majority of adult patients with HIV, as most patients are diagnosed long after the virus has formed its reservoirs and can continuously re-populate its host. In terms of fundamental research, however, this case does suggest that the outset of infection may be a weak point in HIV's attack. The apparent curing of the Mississippi-born baby sparked a prompt response from researchers to begin a clinical trial testing the efficacy of early treatment in delivering a cure for babies born with HIV.

Elite controllers and long-term non-progressors

A small subset of individuals infected with HIV do not progress to AIDS even in the absence of treatment. These individuals are termed 'elite controllers' as their own immune system is able to maintain virus levels under 50 copies per ml (the detection limit of current assays) and their $CD4^+$ T cell counts remain stable.

Another group of individuals maintain stable $CD4^+$ T cell counts but have replicating virus in their system; these are termed 'long-term non-progressors' (4). Early combinational antiretroviral therapy (cART) for long-term non-progressors may be sufficient to allow their immune systems to control HIV (5).

The genetics and/or environment that allow these patients to control HIV without the need for drugs, and in effect be functionally cured, may inform the treatment of patients whose immune systems alone cannot control the virus.

The future for HIV cures

These few reports of individuals being cured provide hope for millions living with HIV and new directions for future research. However, despite these encouraging developments, HIV continues to pose an extremely complicated challenge to researchers attempting to develop a cure.
The immune system alone cannot clear HIV from the body because the virus specifically attacks the immune system and also mutates in order to evade it. Additionally, the high mutation rate is one reason for the failure of traditional vaccine approaches. Anti-viral drugs do not cure patients because any lapse in treatment allows the virus to multiply from pre-established reservoirs.

Unconventional approaches are where we may see real progress in the fight against HIV. Research into the inherent resistance seen in the immune systems of 'elite controllers' and 'long-term non-progressors' could lead to an artificial therapy that mimics these natural mutations.

If a cure is developed by one of the many groups working towards this goal, the process of testing it in clinical trials may be slow. The latest update from the WHO claims that patients receiving ART can have a normal life expectancy (9); for these people, the risk associated with trying new strategies, with a view to a cure, may seem unnecessary. For those like the Berlin Patient, however, where their overall medical condition is more critical, the risk-to-reward ratio may be tipped in favour of more experimental therapies. By testing these strategies, a cure for the other 34 million infected people may be revealed.

Also published online at http://www.oxbridgebiotech.com/review/science-basics/hiv-cures-mean/.

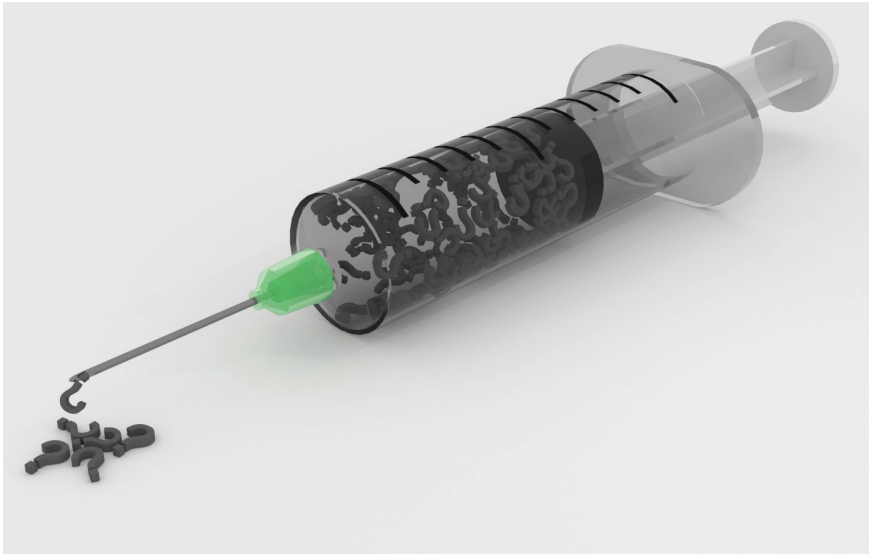

Illustration by Richard Wheeler

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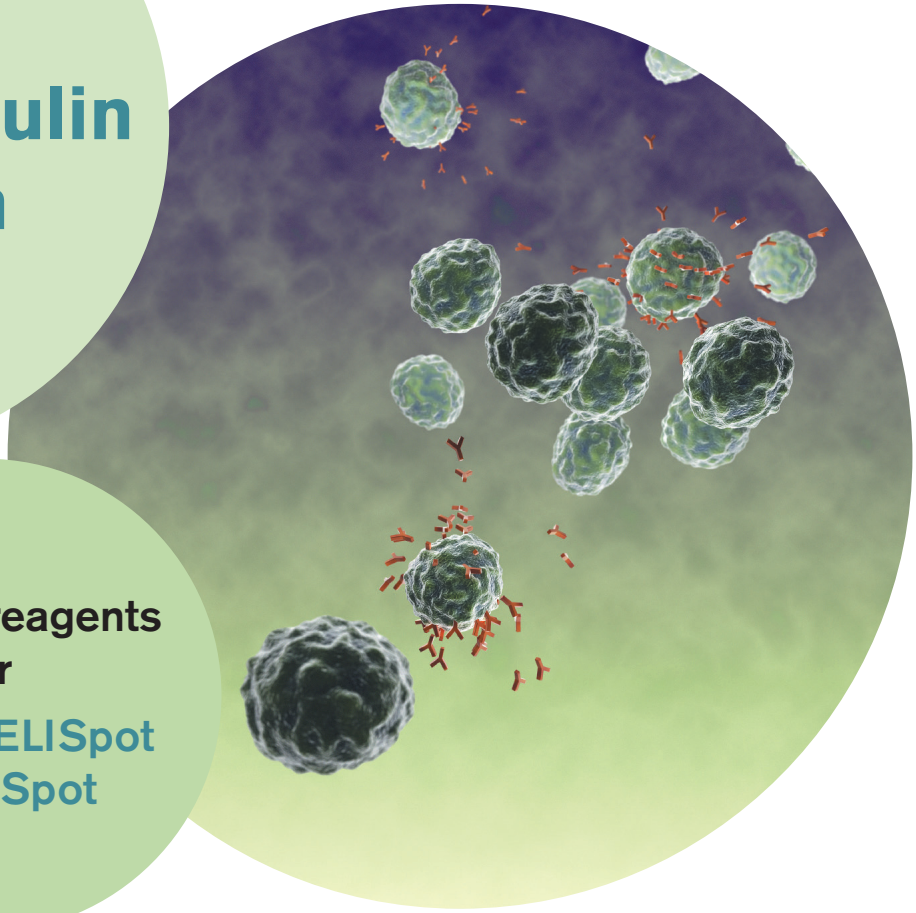
Immunoglobulin detection



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Who owns your genome?

In May 2013 Angelina Jolie revealed that she had undergone a preventative double mastectomy, owing to her familial risk of breast cancer. Shortly after this announcement the focus of the media turned to the company that had, for the past 18 years, held a monopoly in the US in providing the genetic test to determine predisposition to certain cancers. Less than a month after the publication of the Hollywood A-lister's moving story in the *New York Times* (1), the US Supreme Court seemed to put to bed the long-standing controversy over the legal right to patent genetic material when it overruled patents held by Myriad Genetics, Inc. ('Myriad') on the two genes associated with hereditary forms of breast and ovarian cancer, *BRCA1* and *BRCA2*.

by Sarah Dixon

In 1995 and 1996, as soon as they identified a correlation between mutations in the *BRCA* genes and a high predisposition to cancer, researchers at the Salt Lake City-based biotechnology company patented the gene sequences (2-3). This cemented their exclusive right to develop diagnostic tests, and their ability to set the going rate for their service. Consequently, they are now the only company in the US that offers targeted analysis of the two genes, costing patients $3,100 each. This is extortionate considering that, with the advent of next-generation sequencing technology, the cost of whole-genome sequencing is rapidly decreasing and currently stands at a little over $5,500 (Figure 1).

Recently, objections have arisen to Myriad's inflated margins, amounting to $57 million in profits, as the research was originally funded by public sources. The patents held by Myriad have inhibited further research on the *BRCA* genes and have left Myriad's consumers, who are at high risk of developing cancer, with only one gene sequencing service provider and no possibility of a second opinion. The patent system exists to protect and monetise the intellectual property rights of inventors, but Myriad created neither the *BRCA* genes, nor the PCR or Sanger sequencing technology implemented in their patented BRAC*Analysis*®. Their only claim is to the sequence of the primers used in their diagnostic tests and the reference sequence of the *BRCA* genes. But should anybody have the right to patent a gene, or part of it, when the sequence is found in nature? In June 2013, a case against Myriad was brought to the US Supreme Court by the Association for Molecular Pathology with a view to answering this question. During the proceedings, Justice Clarence Thomas ruled that "separating [a] gene from its surrounding genetic material is not an act of invention" (4), partially invalidating the *BRCA* patents. However, laboratory-created cDNA, being not technically a "product of nature", was ruled patentable.

In these first months following the Myriad ruling, its effect on research, therapeutic agents and biotechnological innovation, both in the US and worldwide, remains to be seen. While introducing competition to the *BRCA* screening market represents a victory for women's health to some, the wider implications are still very much in question. The future of patent applications for other naturally occurring biomolecules and gene-derived products, including antibodies and hormones, is under threat. However, perhaps the biggest risk is that investors will be dissuaded from backing innovative research into genetic testing due to a lack of confidence in the value of intellectual property rights in this area.

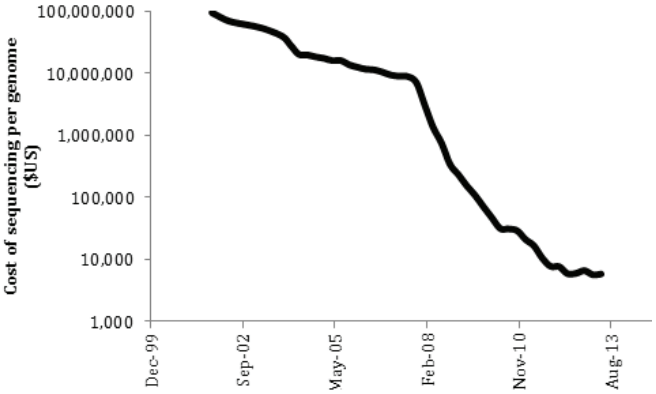

Figure 1: The cost of whole-genome sequencing has plummeted since next-generation sequencing replaced Sanger sequencing in 2008 (5).

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Will genetic screening and selection guide future generations?

by
Dr Jennifer Badger

In this post-genomic era, we are becoming more aware of our own genetics. With the advent of affordable genetic profiling we are but a cheek swab away from a wealth of information about our own DNA. Several companies now offer prospective parents a chance to peer into the future and predict attributes of their future child, based upon their own genetics.

The company 23andMe have recently patented a technology allowing prospective parents to select a sperm or egg donor based upon the donor's genetic profile (1). Inspired by their 'Family Inheritance Calculator' tool, infertile couples can now select a donor carrying desirable traits, based on predictions generated when the donor's genetic profile is theoretically crossed with their own. Of course, this service is also open to prospective parents who are simply curious to know how their child would look and which personality traits they may inherit. You can discover the probability of your child having a particular eye or hair colour, sporting ability or lactose intolerance, to name just a few traits. Some of you may be thinking that this sounds very much like a 'Designer Baby' service, but 23andMe have stated that they do not anticipate that their tool will be utilised by fertility clinics for embryo selection. 23andMe are only interested in offering excited parents the chance to see how their child might be, statistically speaking. In addition, they offer those seeking *in vitro* fertilisation (IVF) the choice of egg and sperm donors based upon these predictions.

23andMe were previously offering customers a full genetic test that screens for single nucleotide polymorphisms in genes that increase your risk for specific diseases, such as Parkinson's disease or breast cancer. This information would inform you on how likely you are to be affected by the disease and the likelihood of transmission to your children. The $99 saliva swab makes this technology relatively affordable and easily available to the curious. However, in a recent turn of events, the US Food and Drug Administration (FDA) ordered 23andMe to stop their heavy marketing campaigns for the kits, culminating in a complete change of service from 23andMe. This results from failing to provide the FDA with evidence supporting the accuracy of the data they provide to customers. 23andMe believe that we all have the right to access our own genetic information, but we must also be able to trust its accuracy. To be told that you have a genetic mutation is potentially life-changing. In the UK, information on one's genetic profile is not provided without genetic counselling. This is to help people come to terms with the results and to make carefully considered and well-informed decisions, if they so choose. Does 23andMe need to consider more fully the implications of the data they are sending to customers? Indeed, in efforts to comply with the FDA's requests, 23andMe now only sell their kits for ancestry reports and provision of raw genetic data with no interpretation – this includes the removal of their predictive traits tool.

Aside from the political and ethical issues surrounding 23andMe, the actual technology behind their campaign is very exciting, if used responsibly. A large number of diseases that have a devastating effect on a person's quality of life are heritable. People with a condition caused by a genetic mutation stand a great chance of passing this to their children. What if this technology could eliminate the chance of their child being afflicted by the same condition?

An American company called GenePeeks proposes to offer clients a 'baby check' service (2). Prospective parents looking to conceive using a sperm donor are advised which donors to choose in order to avoid their child inheriting a recessive disorder. This is achieved by theoretically crossing maternal DNA with many potential donors and screening for approximately 600 genetic diseases. However, a simpler solution may be to remove from the archives any donors carrying alleles for recessive diseases, negating the need for such selection. Do sperm banks not have a moral responsibility to ensure their donors are 'safe'? Indeed, sperm banks do screen for the most common genetic diseases, but perhaps the emergence of companies such as GenePeeks implies a demand for more vigorous testing. At $2,000 however, these tests are not something that many can afford, especially when coupled with IVF costs.

The concept of selection against disease is not novel and those not wishing to use sperm or egg donors do have another choice. Pre-implantation genetic diagnosis (PGD) is a service offered by fertility clinics to parents who are known carriers of genetic mutations that result in familial disease (3). As few as two cells can be removed from the early embryo to screen for a familial mutation. Screening

embryos in this manner does not compromise the health of the embryo and can improve the chances of successful pregnancy; up to 50% of embryos produced for IVF do not have the correct number of chromosomes and consequently miscarry, or fail to implant. Interestingly, if the inherited disease is sex-linked, PGD also allows parents to carry out gender selection legally. For example, female children are unaffected if they carry a copy of the mutated gene that leads to recessive X-linked Duchenne muscular dystrophy, while male children would develop the disease.

The ability to remove heritable diseases from our gene pool might be the key to achieving a healthier population with increased longevity. But not everyone knows they are a carrier until the onset of disease, generally much later in life, after they have had children. However, although screening may be viewed as desirable to reduce the risk of disease, others may argue that every child is special and deserves a chance of life, regardless of their genetic make-up. Embryos may be discarded needlessly, given that not all genetic mutations have complete penetrance and disease severity may vary with environmental factors.

It is worth noting that the technology used by 23andMe and GenePeeks does not take into account mitochondrial DNA (mtDNA) mutations. Approximately one in 3,500 people in the UK are at risk of developing a disease from mutated mtDNA (4). mtDNA is inherited maternally and, until recently, the only way to avoid passing on diseases through mtDNA was by using an egg donor. Discussions are currently underway in the USA concerning the use of a technology that replaces the nucleus of a healthy donor egg with the nucleus of a maternal egg, or the pro-nuclei from a fertilised egg (5). By this procedure, a child would inherit the chromosomal DNA from its mother, but the mtDNA of the egg donor. This would mean that the child will have genetic information from three parents; the father (sperm donor, chromosomal DNA), the egg donor (mtDNA) and the mother (chromosomal DNA) (Figure 1).

Our understanding of genetics and inheritable diseases is constantly expanding and it is evident that technologies such as those discussed here will become increasingly more common as they progress and become more accessible. The opportunity to remove rare and debilitating diseases from our gene pool is something few would ignore if they were aware of the option. There are also ethical issues associated with these techniques that require technology owners to be sensitive, exert adequate control over their use and ensure that their data is accurate and secure.

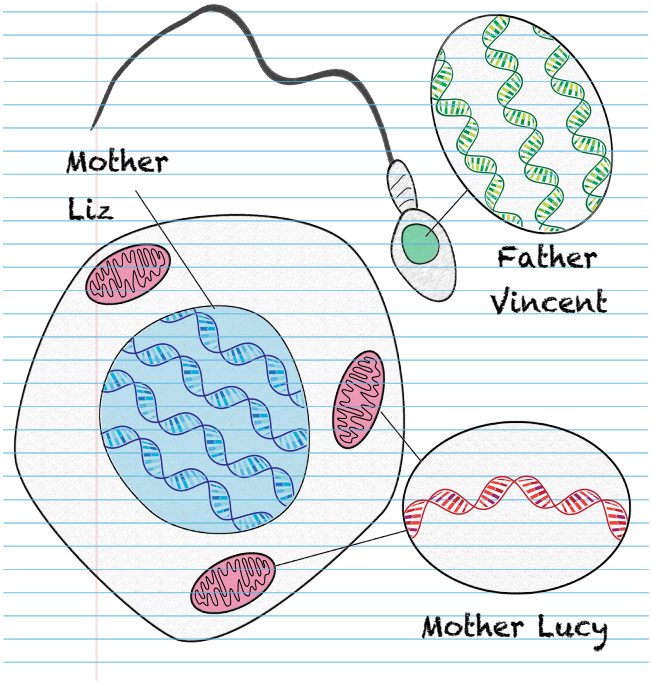

Figure 1: Three parents. The nucleus is removed from a donor egg (supplied by Mother Lucy) so that it only carries the mitochondrial DNA. The nucleus from the egg of the other mother (Mother Liz) and the sperm from the father (Father Vincent) then supply the chromosomal DNA. Figure by Óscar Cordero Llana.

As for 23andMe, it remains to be seen whether they will resume their full genetic service to clients. In the meantime, I'd better think of something else to buy my partner for Valentine's Day.

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by Clio Korn

So you think you can slam?

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Ten minutes to talk.
The clock starts…**NOW!**

This is the setting for a Science Slam – a communication contest for scientists. The trick is not just to translate complex ideas and academic jargon into a story for a lay audience, but also to entertain them in the process. Slammers must make their science scintillate, sparkle, surprise, and inspire.

Science Slams began in Germany, spread across Europe and recently hopped the channel into the UK. Oxford's first Slam took place in the Old Fire Station's black box theatre. The atmosphere was part talent show, part public lecture. The event's organiser – a joke-cracking German neurobiologist who is himself an experienced Slammer – bounded onto the stage, accompanied by dramatic music and lighting effects, as if about to host an episode of *The X Factor.* Six contestants – all biologists pursuing PhDs or postdocs – waited nervously in the wings. One by one, they took the stage and, for ten minutes, pitched their research to the audience. They pulled out geeky jokes, colourful diagrams, quirky props, and theatrical tricks to keep us all entertained and laughing, or gripping the edge of our seats as the drama of an experiment unfolded before our eyes.

With the aid of a 'clap-o-meter', the audience narrowed the competition to two front-runners: Cedric Tan and Sally Le Page. Tan, a zoologist and dancer who has made several award-winning dance videos about his PhD research, presented a skit entitled *Chicken Sex: Variety Is the Spice of Life*. Accompanied by a four-piece band, Tan and his ensemble danced, sang, and recited their way through an experiment on whether bringing along a male relative as a wingman is a successful courting strategy for chickens. The skit opened with a bearded scientist explaining the experiment in rhyming verse. Enter the experimental subjects: two brother chickens, rapping about the night of love awaiting them, and a hen, appropriately adorned with a white feather boa. The brothers' courtship of the hen is interrupted by the arrival of a foreigner – Tan, playing the role of a Chinese cock that the scientists introduced to their flock of British chickens. The foreign male woos the hen with dance moves rather than vocal skills, and, as it turns out, she prefers quickstepping to rapping. The audience was delighted by the skit – although the Q&A afterwards was necessary to clarify just what the experiment was all about.

However, it was Le Page who took first prize. Her presentation *You Can Run But You Can't Hide: A Story About Ladybirds* was as entertaining as Tan's but managed to pack in a good deal more scientific detail, thus achieving the dual goals of a Science Slam: to both educate and entertain. Le Page is an accomplished science communicator: she maintains a website of blog posts, photos, and videos that all aim to convey her enthusiasm for science and the natural world. That experience shone through in the Slam. The performance was funny, exciting, and informative, with a soundtrack perfectly timed with the slides and narration. From an experiment that would strike most as tedious and dull – an attempt to model competition between native and invasive species of ladybirds in a lab petri dish – Le Page wove a dramatic story of a *Hunger Games*-style fight for survival in an arena (the petri dish) where competitors (the native ladybird, the invasive ladybird, and aphids) battle for their lives while being avidly watched by an omnipresent audience (the scientists). Le Page certainly had me on the edge of my seat, and she managed to educate me about the relative merits of different ladybird survival strategies – fight versus flight, run versus hide – so engagingly that I barely noticed just how much I was learning.

An extended version of this article was originally published on the *Bang!* blog, accessed at http://www.bangscience.org/2013/10/oxford-science-slam/.

Clio Korn is a 3rd year Neuroscience DPhil student jointly in the Walton Group in Experimental Psychology and the Tunbridge Group in Psychiatry.

Open access: Time for change

by Prof Chris Ponting

On occasion, I have navigated a scientific article through its difficult writing, submission and editorial processes only to realise on its publication date that I am disbarred from reading my own words in their final published form. On other occasions, scientists from around the world have asked me to source articles their own libraries cannot afford. A biochemist friend suffering from Chronic Fatigue Syndrome cannot benefit from articles detailing the latest advances in treatments, despite most of this work being funded by taxpayers or charities. Meanwhile, publishers of scientific and medical journals enjoy large annual profits. Such has been the state of scientific publishing.

We have now reached a knife-edge moment in publishing where access to scientific knowledge will either be wrested from profit-making publishing houses, or remain restricted to those who can pay. To tip the balance, the UK Research Councils now require their funded, peer-reviewed published research articles and conference proceedings to be made freely available within six months of publication, although the effectiveness of this is up to individual researchers (1).

For many in science, open access publishing will only be achieved when findings are freely available immediately upon publication, and when any reader has the right to make use of these results whilst noting full author attribution. The investigators and their institutions will inevitably bear the financial cost. Nevertheless, this cost would be lower than it is now if all papers were published by not-for-profit organisations. Recently, three prominent funders, the Howard Hughes Medical Institute in the USA, the Max Planck Society in Germany and the Wellcome Trust in the UK, jointly launched a new journal, *eLife*, that promotes enhanced open access (2). *eLife* promises to challenge the 'CNS' journals (*Cell*, *Nature* and *Science*) for the very best science worldwide. It takes advantage of the expertise of active scientists – all of whom are paid – at every level of the journal, to rapidly publish impactful science. Currently, the median time for decision after full peer review is only 27 days.

The new journals aim not just to reform the business of scientific publishing, but also to change how scientific output and impact are assessed. Publication metrics, such as journal impact factors, are known to distort the scientific process and scientific careers by placing too great an emphasis on where the results are published, as opposed to the research itself (3, 4). They are often used as a crude measure of scientific quality by those who have insufficient time or background to appreciate fully the arguments being made. Frequently, these metrics are produced non-transparently with considerable limitations. To begin to turn the tide, a group of editors and publishers recently recommended together that "journal-based metrics, such as Journal Impact Factors, [not be used] as a surrogate measure of the quality of individual research articles, to assess an individual scientist's contributions, or in hiring, promotion, or funding decisions" (5). Instead, they argue, a greater emphasis should be placed on each individual article's scientific content.

Until recently, we have paid over-the-odds to publish our findings, then given away our rights to our science, only to pay again in order to read this science. We are a conservative bunch and so might cling to the old publishing order. Yet, if I might hazard a guess, the root-and-branch reforms coming to scientific publishing will soon forever change how we produce, read and assess our science.

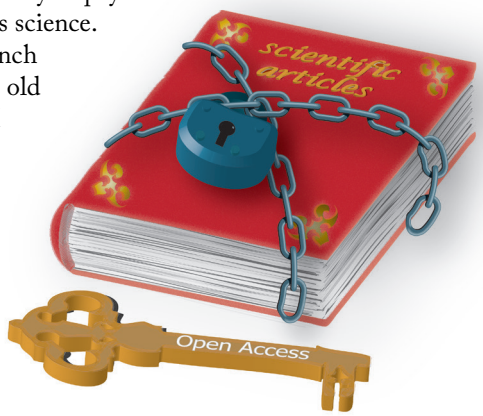

Illustration by Maria Kuzma-Kuzniarska

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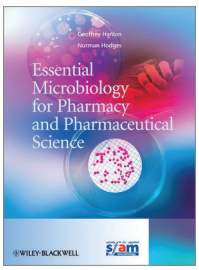
Chris Ponting is Professor of Genomics in the MRC Functional Genomics Unit at the University of Oxford.
Disclosure: he is a paid senior editor at eLife.

BOOK REVIEW



Essential Microbiology for Pharmacy and Pharmaceutical Science

Geoffrey Hanlon and Norman Hodges

ISBN: 978-0-470-66534-3, Wiley-Blackwell (2013)

Paperback, 234 pages, £27.50

Reviewed by Mariam Ayub

Essential Microbiology for Pharmacy and Pharmaceutical Science is aimed at undergraduates studying for a pharmacy-related degree. Written by two pharmacists, each with over 30 years' experience in teaching, research and publishing in pharmaceutical microbiology, the book covers the elements of microbiology required by practising pharmacists and pharmaceutical scientists. It also covers all microbiological components of the Royal Pharmaceutical Society's indicative syllabus that is at the heart of every pharmacy degree in the UK.

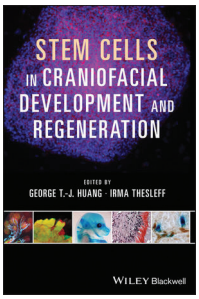
The book is neatly structured into three parts, each of which is logically divided into shorter chapters. All chapters begin with a 'key facts' box and a glance back at the history of theories relating to the chapter. Part I of the book emphasises the fundamental differences between prokaryotic cells and human cells, focussing on the characteristics of microorganisms such as bacteria, viruses, fungi and protozoa. This section also touches upon the importance of safety when handling or growing these microorganisms in order to avoid the risk of infection.

Part II of the book is concerned with the practical aspects of infection and immunity. The initial chapters cover why we as scientists are interested in microorganisms and their potential to harm or infect us. This section highlights the importance of the treatment of infectious disease and provides detailed information regarding the use of antibiotics for this purpose. Part II ends with a review of the key factors that need to be considered when determining which antibiotic is most suitable for a particular infection and includes discussions on toxicity, dosage and susceptibility to resistance development.

Finally, Part III details the manufacture of medicines, covering the processes of producing and preserving microbiologically sterile medicines and antibiotics.

All of the chapters provide material in an easy-to-digest format by using flow diagrams, tables, images and additional summary boxes. The book also contains an extensive self-assessment section that includes multiple-choice, short-answer and essay-style examination questions, which can be used to test one's knowledge.

Overall, this book provides an essential guide for undergraduates studying microbiology modules during degree courses in pharmacy and the pharmaceutical sciences. Although the textbook targets students within this field, I would highly recommend it to other readers as a teaching aid, as it provides comprehensive coverage of the world of infectious diseases.



Stem Cells, Craniofacial Development and Regeneration

Edited by George T.-J. Huang and Irma Thesleff

ISBN: 978-1-118-27923-6, Wiley-Blackwell (2013)

Hardback, 584 pages, £100

Reviewed by Anna Sigurdsson

The purpose of *Stem Cells, Craniofacial Development and Regeneration* is to summarise the current knowledge and research in the field of craniofacial tissue development and regulation, a goal that the book satisfies. The reader will benefit from having some prior knowledge of the topic, although the clear structure of the book will help even an unfamiliar student. The book is logically divided into three parts. Each chapter begins with a helpful introduction, followed by topical sub-chapters and a conclusion that also points towards future research questions.

The first part of the book consists of nine chapters that summarise the current knowledge in the field, from the very first stages of craniofacial development to a more specialised discussion on the developmental mechanisms of particular parts of the head. This includes the development and regeneration of the complex shapes of organs, craniofacial intramembranous bone, temporomandibular joints, craniofacial muscles, the tongue, and the teeth.

The second part of the book expands to the niche functions of stem cells, including embryonic and induced pluripotent stem cells as well as stem cell-based renewal and regeneration of tissues. Postnatal cells and their potential application for craniofacial tissue regeneration are discussed. There is also a focus on tissue-specific stem cells; for example, the importance of satellite cells - the tissue stem cells of skeletal muscles - in the process of muscle regeneration after an injury. The activation, proliferation and differentiation of satellite cells are determined by the local microenvironment as well as the stiffness of the neighbouring tissues and the soluble factors that surround them. A better understanding of this process will improve skeletal muscle engineering, enabling treatment of a wide range of muscle defects and diseases.

The final part of the book covers ongoing research in the bioengineering of craniofacial tissue, including bone, muscle, dental tissues and teeth. The use of growth factors, stem cells and scaffolds is crucial to engineered tissue regeneration technologies, some of which are already being studied in clinical trials – for example, the use of 'bioactive' scaffolds with different tensions/textures that can 'nudge' stem cells in a certain developmental direction. Huang and Thesleff note that tissue regeneration and regenerative medicine will undoubtedly become mainstream medical practice and, judging from the content of this book, the future of the field will be very interesting and exciting.

Essential Microbiology (2nd Edition)

Stuart Hogg

ISBN: 978-1-119-97890-9, Wiley-Blackwell (2013)
Paperback, 526 pages, £34.95

Reviewed by Jessica Beevers

Essential Microbiology is an introductory microbiology textbook aimed at those for whom "the study of microbiology will be a brief encounter, forming only a minor part of their course content". In contrast to a typical full-size hardback microbiology textbook, *Essential Microbiology* presents a compact, readable, and – importantly – inexpensive introduction to the world of microbes.

The tone is more conversational than might be found in most textbooks. Unfortunately, in the early chapters it is easy to feel overwhelmed by the quantity of information presented – particularly when the principles of optics, organic chemistry, biochemistry, and cellular biology are introduced *en masse*. Furthermore, the level of detail included was not always necessary to understand the rest of the book and may be confusing rather than helpful.

Having summarised these fundamental topics, Hogg moves on to present general microbiology in reasonable bite-sized chunks using a more engaging style. Topics are covered in enough detail to make the text useful while not losing the reader's interest. The book works well either read from cover to cover, or as reference material in chapter blocks according to a structured microbiology module. Topics are easy to find and chapters are well laid out with section headings that direct attention to the appropriate places. Explanatory textboxes appear throughout to describe unfamiliar terms, to introduce points of discussion, or to complement the text with interesting facts. The final sections of the book are easily the most interesting for a general reader – as is common with texts of this nature – discussing the roles of microorganisms in the environment, in human disease, and in industrial exploitation and food production.

The text is also accompanied by an Instructor Companion Website that students and instructors alike would find valuable, with PowerPoint versions of all figures from the book, short answer format quizzes for each chapter, and additional online resources. All could be put to good use both inside the lecture theatre and out.

One of the greatest strengths of this particular text is its ability to whet the appetite, provoking questions such as: what are further examples of viroid diseases in plants? Do taxonomic distinctions really mean anything in the microbial world? Can microbes realistically be used for industrial purposes without disturbing ecosystems as much as man-made processes already do?

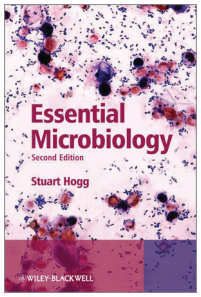
Essential Microbiology would be best recommended to those who already have a solid foundation in the life sciences and require an introduction to microbiology. It would especially suit those transitioning into a medical field.

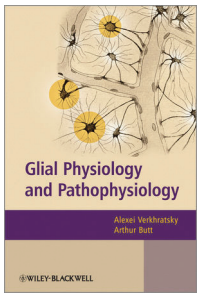
Glial Physiology and Pathophysiology

Edited by Alexey Verkhrastsky and Arthur M. Butt

ISBN: 978-0-470-97853-5, Wiley Blackwell (2013)
Paperback, 560 pages, £50

Reviewed by Óscar Cordero Llana





When we consider the human brain, we usually think in neuronal terms. How many neurons do we have? Do elephants have more neurons than us? Why do we lose specific neuronal populations in a particular neurodegenerative disease? But we tend to forget that neurons are not the only cell type in our nervous system and that human intelligence would not be possible with a brain composed exclusively of neurons. *Glial Physiology and Pathophysiology* is a fascinating book focusing upon the other neural cells: the forgotten glia.

This book is a beautiful demonstration of how every aspect in the life of a neuron and our brain is dependent upon glial cells. Glial cells are involved in brain development, maturation and ageing. They are required for nerve transmission and synaptic plasticity. Moreover, they police and protect our brain against damage and rapidly react to any change in brain homeostasis. All types of glia are systematically described in this work, from their developmental and evolutionary origins to their biochemical and functional properties. Even the most hardened neuroscientist will discover new facts and perhaps even a new type of glia, like the mysterious NG2 cells, which are exclusive to mammals and are the only cell type, besides neurons, that is capable of establishing synapses with each other.

Written from a historical perspective, *Glial Physiology and Pathophysiology* also incorporates current developments in the field, citing original publications for the reader to look up if desired. The authors conclude with the implications of glial pathology for neurological diseases. This aims to reinforce the importance of glial cells for brain physiology but it is perhaps the weakest section. Complex diseases such as Alzheimer's and Parkinson's appear oversimplified and recent findings linking glia and neurodegenerative mechanisms are omitted.

Nevertheless, this is a stimulating book full of fascinating facts and a great read for any initiated neuroscientist.

5' with... Prof Kevin Foster

Kevin Foster studied at the University of Cambridge before researching the social interactions of wasps during his PhD at the University of Sheffield. After spending some time in Berlin, Helsinki and Harvard, he joined Oxford as Professor of Evolutionary Biology in 2010. His group currently focusses on the evolution of social interactions of bacteria.

Interviewed by Stuart Thomas

If you are not in the lab you are…
In the pub. I should really be somewhere else I expect.

If you weren't a scientist, you would be…
An economist. The idea of trying to understand society through mathematical principles really appeals, although it is not clear how often it actually works.

What was your first disaster in the lab?
My worst moment was not closing an autoclave properly at Harvard. The damn thing filled a room with steam and the fire brigade was called. It took ages to sort out because I had been sterilising cultures of a pathogen. And the entire building was evacuated late on a Friday afternoon so everyone had to wait outside angrily rather than go home!

What has been the most memorable finding of your career so far?
During my PhD, we studied worker policing in wasps, which is the idea that the workers in insect societies act as a police force and prevent rebellious workers from reproducing. We predicted that workers would remove eggs of other workers, but not eggs of the queen. When I introduced eggs into the colony in a comb, it only took about fifteen minutes for the workers to strip out the worker eggs, leaving behind neat rows of queen eggs. It felt great to see worker policing in effect.

What is the best advice you have ever received?
Realising that when things are going wrong, or not going the way you expected, is when you are learning the most. Indeed, some of my best work has come out of experiments behaving strangely and ultimately revealing biology that was new and exciting.

If you were starting your career again, are there things you would do differently?
I don't think so. There are many ways to go through a career in science. Mine is characterised, like many, by becoming an expert in one field, in my case social evolution, and then applying this to a different field, microbiology. This works, although it is never all that planned.

Do you have a favourite classical experiment?
The bobtail squid houses symbiotic bacteria on its front that generate light and are thought to help the squid not cast a shadow as it swims around in moonlight. This means that it cannot be detected by predators or prey. A very simple experiment involves shining light on the back of the squid at varying intensities and recording the light produced by the bacteria from below. The really nice thing is that the two light sources closely correlate. The squid apparently detects light on its back and then signals to the bacteria, which vary the light they produce accordingly. This is not only a great example of a cooperative interaction between bacteria and a host, it is also pretty close to the evolution of the cloaking devices that they have in sci-fi films.

How do you imagine biological research will change over the next twenty years?
In my own field of microbiology, there will no doubt be a better fusion of the information that one can get from growing cells in a lab and that which one can get from assessments of DNA and RNA in environmental samples. At present, we are very good at seeing which bacteria are in a particular environment but not what they are doing there. I am hopeful that this will change.

Write for *Phenotype*?

- The deadline for article submissions is Friday of 8th week, 14 March 2014
- We accept articles on any aspect of biological sciences research, books or science education
- For next issue we particularly welcome articles related to plant science or the environment
- Articles can be either 650 or 1300 words

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

Work for *Phenotype*?

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

SNAPSHOT Research Image Competition

This issue's winner is...

Dr Maria Kuzma-Kuzniarska

Dr Maria Kuzma-Kuzniarska is a postdoctoral research assistant in the Hulley group in the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS).

The winning image is a merge of a pencil drawing, prepared during a life drawing session at Fusion Arts, Oxford, and vector graphics created using Adobe Illustrator. It shows the **rotator cuff**: a group of muscles and tendons that stabilize the shoulder joint.

Maria studied for her Bachelor's and Master's of Science in Biotechnology at the University of Gdansk in Poland, followed by a PhD in Biological and Biomedical Sciences at the University of Liverpool. She also worked at the Mario Negri Institute for Pharmacological Research in Italy as an early-stage researcher, and further as a researcher at the Université Pierre-et-Marie-Curie in Paris. She came to the University of Oxford in October 2012 to work as a post-doctoral researcher in Dr Philippa Hulley's group at NDORMS. Dr Hulley's group focuses on understanding disease pathology in skeletal cells and on devising effective regenerative strategies.

In Dr Hulley's group Maria investigates the mechanobiology of the human tendon. She is particularly interested in the molecular mechanisms involved in mechanotransduction and the structure-function relationship of the bone-tendon interface. Tendons act as force transmitters between muscle and bone. They are made up of bundles of collagen fibers, with cells (tenocytes) aligned in rows. Under physiological conditions, tendons are exposed to considerable mechanical loads. Nevertheless, the mechanism by which tenocytes sense the mechanical load has not been well studied.

Rotator cuff injuries are common and cause significant shoulder disability. A better understanding of tenocyte biology will facilitate the development of improved treatments for rotator cuff injuries in the future.

Currently, Maria's research focus is on the role of intercellular channels, called gap junctions. Gap junctions play an important role in intercellular communication, and hence influence a variety of cellular activities and responses, including the response to mechanical stimulation. In order to study gap junction-mediated communication in human tendon cells, she utilises fluorescence recovery after photobleaching (FRAP), a non-invasive technique that allows continuous monitoring of intercellular communication. Using FRAP, Maria has demonstrated that human tenocytes form functional gap junctions in both monolayer and three-dimensional cultures. Additionally, she has shown that it is also possible to experimentally manipulate gap junctional communication in tendon cells.

Maria is a biologist and self-declared artist interested in exploring the complexity and beauty of living organisms. In particular, she is fascinated by the inherent beauty of the human body and the constant changes it undergoes. In addition to her work in Dr Hulley's group, Maria is also a freelance biomedical illustrator and figurative artist as well as a volunteer graphic designer for Sense About Science – a London based charity – since 2012. You can find more of her scientific illustrations at http://mybioscience.org/.

A manuscript by Maria and her colleagues, highlighting the use of FRAP as a tool to quantify the function of gap junctions in human tendon cells has been accepted for publication in the next available issue of the Journal of Biomedical Optics.

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* Trinity 2014.
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.
The theme for our next issue is Plant Science, so any images that you can link to plants or the environment are especially welcome.
The deadline for the competition is 14 March 2014.

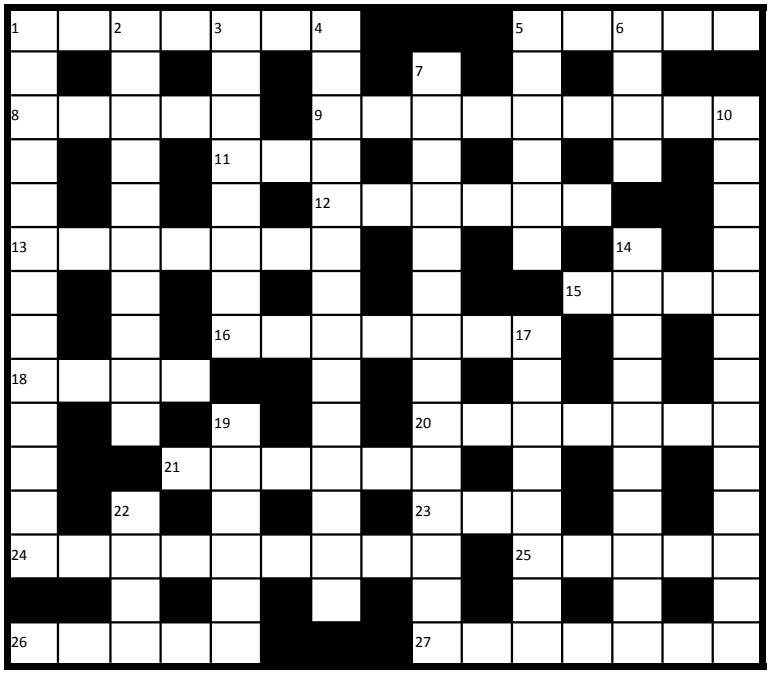
PHENOTYPE crossword

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 28 March 2014 will be entered into the prize draw.

Our resident cryptographer, *Fish*, challenges *Phenotype* readers to crack this cryptic crossword on the theme of molecular biology.

The winner can choose one of the four books reviewed in this issue, generously provided by Wiley-Blackwell.

WILEY-BLACKWELL



Across

1 To pass with it is painful? (7)
5 One of the 1 down left whale in a muddle (5)
8 One of the 1 down said to regret the drill (5)
9 Two letters (in Greek) about party for new photoreceptor (9)
11 Appeal vocally for a small French coin (3)
12 Odd chaps stress the classes (6)
13 Inject sera, endotoxin in back, since it's closest (7)
15 'e's no longer carried? (4)
16 Backing solidifies around the Air Force as it fires on ground troops (7)
18 It's heartless, fearsome, divine (4)
20 Gas-fed one is just 15 (7)
21 Lunatic murderer reverses over Oxbridge graduate (6)
23 Note: sing for your supper (3)
24 She ends tryst with one short manservant who's swallowed foodstuff (9)
25 One of the 1 down makes dunderhead look back with love at the Right (5)
26 One of the 1 down got its groove back by taking molybdenum (5)
27 Core students' collective has no right to filthy lucre (7)

Down

1 What Wolsey does with documents describing inflammation? (8,5)
2 Research facility of short dog speech (10)
3 Sailor talks of those who 18 for Asian primates(8)
4 School of linguists (or crystallographers?) who analyse racist slurs and tut (14)
5 Twitching is a tedious task at first (6)
6 see 22
7 One of the 1 down added later - type of mutation (4,2,8)
10 Offhand, it's unlikely to happen? (3,2,3,5)
14 20's soft spot for the dancing of Nate and of Nell (10)
17 Return works of revolutionary who refuses to use aluminium on occasion (8)
19 Bacon is more dangerous (6)
22,6 Hothead with weapons keeps the French safe (8)

Congratulations to Gabriel Aughey from DPAG who won the Michaelmas '13 crossword competition.

Answers to the crossword from issue 16 | Michaelmas '13
Across: 1 phenolphthalein; 8 titration; 9 bijou; 10 unsound; 11 prayer; 14 rotor; 16 identical; 19 alkaloids; 20 ascus; 23 capsid; 26 operons; 28 uvula; 29 phenomena; 30 bromophenol blue
Down: 1 pith; 2 extinct; 3 litmus; 4 hyoid; 5 amber; 6 enjoy; 7 neutralise; 11 pons; 12 acids; 13 breadcrumb; 15 opals; 17 Dad; 18 void; 21 congeal; 22 lean to; 24 Pluto; 25 imago; 26 ochre; 27 base