PHENOTYPE

Issue 20 | Hilary Term 2015

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Autoantibodies against the central nervous system
A rapidly expanding group of diseases

From bench to bedside in bipolar disorder
The story of the ebselen

Seeing is believing
How intravital microscopy is used to monitor cells inside living animals

Early detection biomarkers
Using mass-spec to detect 50 biomarkers of liver disease

cover image by

Suzan Hammond

this issue´s winner of the **SNAPSHOT** scientific image competition

page 31

Ageing:

Therapeutic interventions · The battle of the sexes

Evolution:

Antibiotic resistance · Catalytic RNAs

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EDITORIAL

Happy New Year and welcome to the twentieth issue of Phenotype! This issue is overflowing with a wide variety of articles written by students, research staff and principle investigators across Oxford and beyond.

Have you ever dreamed that advances in modern medicine might consign age-related disease to the annals of history? Then turn to page 6 where Associate Professor Lynne Cox outlines the biochemistry of senescence. Lynne explains how pharmaceutics that put an organism's cells into 'recycling' mode to remove aggregated proteins, can promote extended lifespan. Continuing the ageing theme, Karolina Chocian laments the incompatibility of the sexes, citing examples where male worms (C. elegans) have been shown to exert a negative influence on the lifespan and survival of their female counterparts (page 20).

Congratulations to Dr Suzan Hammond, the winner of the SNAPSHOT competition, for her beautifully detailed visualisation of the neuromuscular junctions of the transversus abdominis or transverse abdominal muscle. Further details of Suzan's research and career, which has focused on a neurodegenerative disease called spinal muscular atrophy, can be found on page 31. Also on the subject of neuroscience, Dr Anjan Nibber gives in-depth insights into recently discovered auto-antibodies that are responsible for a variety of neurological disorders (page 22). And Olga Kuznetsova shares an inspirational story of the antidepressant drug ebselen, which has been taken from bench to bedside to reduce the toxicity associated with treating bipolar disorder with lithium (Page 10).

On page 30, we interview Dr Bart Cornelissen, a junior group leader in the CRUK/MRC Oxford Institute for Radiation Oncology, who shares his advice for an academic career and tells amazing stories of his adventures in the lab (which include an encounter with a radioactive pig!). Also from the Oxford Institute for Radiation Oncology, Dr Bostjan Markelc takes us on a journey into the fascinating field of intravital microscopy. If you are interested in tracking cells and monitoring their behaviour inside living animals, then page 14 is definitely worth a visit.

We also look at the evolution of antibacterial resistance. On page 15, Dr Alvaro san Millan reveals the dynamics of antibacterial resistance, which hinges on the transfer of plasmids though conjugation, a process that is akin to bacterial sex. Stephanie Oerum puts forth elegant theories for why catalytic RNAs became superseded by protein-only enzymes that catalyse similar reactions (page 12). And rounding up our research articles, Evangelia Tzika discusses the importance of early detection biomarkers in liver disease on page 18.

In our science and society section, Emma Pencheon warns of the need for better education of the public and of practitioners, to promote the responsible use of antibiotics in front line medicine (page 24). Dr Joram van Rheede and Dr Stephen Hicks tell stories of an 'A-Ha' moment in visual impairment, and the development of an innovative pair of electronic glasses that enhance the visual world for the severely sight impaired (page 25). In our Career Highlight, Catarina Vicente explains how she got into publishing as Community Manager at The Node, after leaving academia (page 27).

As if offering all those riveting articles wasn't enough, why not also have a go at the cross-word on the back cover, which is themed around the discovery of DNA! Be sure to send us your answers for a chance to win one of the excellent Wiley-Blackwell textbooks that we have reviewed on pages 28-29.

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Christopher Hillyar
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CONTENT

regulars

science & society

features

TEAM

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RESEARCH HIGHLIGHTS

by Anna Sigurdsson

Lin S, *et al.* (2014) *Nature Neuroscience* 17, 1536-1542. doi: 10.1038/nn.3827

Protein neural correlates of water reward in thirsty *Drosophila*

An animal's internal drive for water manifests as thirst. Whereas water-sated *Drosophila melanogaster* actively avoid water, this paper shows that thirst converts water avoidance into water seeking in naïve flies. Finding water requires foraging behaviour, which is guided by sensory cues in the environment. This article examines how thirst affects nervous system control of water-seeking behaviour, and demonstrates that 'naïve water-seeking', 'learned water-seeking', and 'water learning' behaviour uses independent neural circuitry in the brains of thirsty flies.

Lin *et al.* first determined that flies start approaching water after approximately 6 h of deprivation, and that 90% of flies would approach water after 14 h of deprivation. Thus thirsty flies actively seek water instead of actively avoiding it. At different temperatures drinking was rewarding to thirsty flies via olfactory conditioning. At high temperatures, a more long-lived robust olfactory memory was formed. In contrast, hungry flies trained with dry sugar displayed water-reinforced appetitive memory performance similar to hunger and carbohydrate memory. It was found that water-reinforcement involves the PAM-γ4 neurons on the mushroom body γ lobe, while non-PAM-γ4 neurons in the PAM cluster are required for the reinforcing effects of sugar.

Flies are able to taste water via the osmosensitive ion channel Pickpocket 28 (PPK28), which is expressed in gustatory neurons (on the proboscis). As thirsty flies homozygous for a mutation in *ppk28* (a gene implicated in water taste) displayed defective water learning but naïve water-seeking behaviour, it was concluded that PPK28 – that is, water taste – is required for water learning, but not for naïve water-seeking. Lin *et al.* found that water-seeking relied on a subset of water-responsive dopaminergic neurons that target the mushroom body β′ lobe (the PAM-β′2 neurons). However, these naïve neurons were not essential for learned water seeking.

Lin *et al.* point out that separate mechanisms for food and water learning might allow for more efficient foraging behaviour, and that the individual control of naïve and learned water-seeking might permit flies to seek water using both learned distance cues and reliable signals such as vapour.

Smith, BN *et al.* (2014) *Neuron* 84(2):324-31. doi: 10.1016/j.neuron.2014.09.027

Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS

An important step towards understanding the pathogenesis of human disorders is to identify underlying mutations. Exome sequencing is an efficient approach, but success is limited in late-onset diseases such as familial ALS (FALS), because each individual carries many rare variants. Analysis thus requires DNA from multiple affected family members, and collection of such samples may be particularly difficult in the case of FALS due to the late onset and the rapid progression of the disease.

In order to overcome this limitation, Smith *et al.* suggest exome-wide rare variant analysis as an approach to identify disease genes. They performed an exome-wide variant burden analysis of 363 index cases with FALS and over 13,000 controls in order to identify novel causative genes. The study found that an excess of *TUBA4A* variants in FALS cases. This overrepresentation of the tubulin alpha 4A protein was statistically significant and replicable through analysis of 272 further FALS cases and 5,510 international controls. These results indicated that most *TUBA4A* variants have a deleterious effect. For example, all patients carrying *TUBA4A* mutations had spinal-onset, classical ALS with upper and lower motor neuron signs.

Through functional analysis, Smith *et al.* found that mutant *TUBA4A* can destabilize intraneuronal microtubule networks by incorporating into the microtubules, thus disrupting their dynamics through a dominant-negative mechanism and ultimately damaging their repolymerisation capability. Another interesting finding was that the truncation mutant, $TUBA4A^{W407X}$, is unable to incorporate into neuronal microtubules. Smith *et al.* suggest that this mutation might result in the trapping of tubulin-binding proteins in aggregates or by overburdening the ubiquitin proteasome system.

Apart from identifying *TUBA4A* mutants, this study emphasizes the role of cytoskeletal defects in ALS. Smith *et al.* show how gene-based rare variant analyses may be useful where traditional segregation analysis is unable to identify causal disease genes.

Ageing and potential therapeutic interventions

by
Assoc Prof
Lynne Cox

Ageing is such a gradual everyday process that most of us don't pay any attention to it until we or someone we know is hit by its detrimental aspects. I'm not talking about spotting the odd grey hair or wrinkles round the eyes. The last 9-11 years of most people's lives will be spent with a range of concurrent disease states seriously compromising their quality of life. Cataracts, age-related macular degeneration, non-healing ulcers, cardiovascular disease, pulmonary disease, immune senescence, incontinence, arthritis and dementia. That's only a taster of what may happen: ageing is also the single biggest risk factor for death. So what can biochemistry bring to our understanding of ageing and the possible treatment of age-related diseases?

Dividing cells lose proliferative capacity with age. At each cell division, DNA replication inevitably leads to progressive loss of DNA at the telomeres. This eventually stimulates a DNA damage response (DDR) that results in cells entering replicative senescence - a state of permanent growth arrest (Figure 1). Telomere attrition triggers induction of p53, which in turn induces the cyclin kinase inhibitor $p21^{CDKN1A}$. High p21 levels both institute and reinforce senescence to ensure that cells with damaged DNA do not undergo further proliferation, an outcome that would otherwise predispose to oncogenic transformation. Senescence resulting from gradual erosion of telomeres through multiple rounds of cell division is called replicative senescence. Other types of senescence also occur in response to oncogene activation (oncogene-induced senescence, OIS) and stress (stress-induced premature senescence, SIPS). In all cases, senescent cells exist in a state of chronic DNA damage signalling, with high levels of γ-H2AX, 53BP1 and other damage-associated proteins. Although they initiate a DDR, unlike younger cells, the DDR of senescent cells does not always resolve over time. We are currently testing whether senescent cells are incompetent in some aspect of DNA repair.

In addition to its involvement in ageing, senescence occurs and is important in early mammalian development, specifically in aiding tissue remodelling (1,2). Senescence is therefore a physiological process that we need early in life and serves as a tumour suppressor role later in life. With such crucial functions, senescence must be universally beneficial – or is it?
Senescent cells are phenotypically very different from surrounding non-senescent cells – they increase in size quite markedly, spread flat (on tissue culture dishes) and attach very strongly to their substrate through a multitude of actin stress fibres. They are relatively easy to identify through the production of a pH-specific senescence-associated ß-galactosidase (3), so the simple addition of X-gal under the right conditions yields blue cells (Figure 1). These cells have been shown to occur physiologically in a range of mammalian species, and the incidence of senescent cells rises with age. Removal of senescent cells in mice leads to tissue rejuvenation, suggesting that senescent cells are causative in age-related degeneration (4).

Gene expression patterns change markedly on senescence, probably as a consequence of major alterations to the methylome and histone code. Among the genes activated in senescent cells are those involved in inflammatory signalling including *TNFα*, *IL-6*, *IL-1* and *IL-8*, which contribute to the senescence-associated secretory phenotype (SASP, Figure 1). While these presumably serve a physiological role in developmental tissue remodelling, permitting recognition and clearance by macrophages, the chronic inflammation resulting in later life from accumulation of senescent cells is likely to be detrimental to tissue integrity. Indeed, 'old' macrophages and neutrophils create havoc by destroying the extracellular matrix as they force their way through tissues to get to sites of inflammation (5). This destabilisation of tissue structure is not only directly detrimental to tissue function, but allows metastasis of *in situ* cancerous cells to other sites. Senescent cells further reinforce tissue degradation by secreting a cocktail of enzymes, including collagenase, that break down the extra cellular matrix. An obvious consequence of collagenase secretion is the appearance of wrinkles as we get older. In this sense senescent cells are both beneficial and harmful; they altruistically prevent themselves from proliferating but they lead to tissue degradation and provide a pro-inflammatory and pro-metastatic environment.

So what can we do? Many labs are now pursuing various experimental strategies for evading the detrimental effects of senescence. One is simply to avoid cells becoming senescent in the first place. If telomere shortening is a major cause of replicative senescence then reactivation of telomerase in somatic cells should solve the problem. And it does. Mice with telomerase reactivated in middle age show significant rejuvenation of gut, reproductive organs, muscle and brain (6). So far so good, but telomerase is not normally expressed in somatic cells, and for good reason. Constitutively active telomerase leads to cell immortalisation, a first but necessary step in the development of cancer. Indeed, a subsequent study demonstrated that pre-cancerous lesions became extremely aggressive in mice with reactivated telomerase (7). Therefore, turning telomerase on in a global manner may keep cells young but may kill the organism prematurely through cancer, so this is not a viable strategy for treating age-related disease in the human population.

Instead, we are examining ways of modulating the rate at which cells become senescent without interfering with the cell counting mechanism, or indeed the DDR. A major biochemical pathway in this respect is that mediated by the mTORC1 kinase (Figure 2). Inhibition of signalling in this pathway by rapamycin (a macrolide antibiotic discovered in the soil of Easter Island) leads to decreased cellular proliferation and stimulates autophagy. Cells shift to a 'recycling' mode that allows them to remove aggregated proteins and damaged organelles through autophagy. Remarkably, rapamycin extends mouse lifespan by about 8–10% when fed to them in middle age (8). Even more notably, mice treated with rapamycin have better cognitive function in old age (9). We have been looking closely at the effect of rapamycin and related rapalogues on the cellular proteome during longitudinal cell ageing. It appears to have beneficial effects on the trajectory of cell ageing consistent with the pro-longevity effects seen in mice.

While such agents affect the rate of cell and organismal ageing, the most detrimental effect of senescent cells is likely to be the chronic state of inflammation. Eliminating the senescence-associated secretory phenotype (SASP) may therefore be the strategy of choice to avoid the damaging effects of senescent cells without incurring increased cancer risk. In this field, a different class of drugs, based on resveratrol, shows promise. These act to stimulate sirtuins, a family of protein deacetylases. In mice, activators of this pathway appear to restore 'young' metabolic profiles in muscle (10), and human trials are reported in the popular press (though not to date in the peer-reviewed literature). Since resveratrol is found in chocolate, red wine and other foodstuffs, it is possible that compounds targeting this pathway may enter the anti-ageing market as nutraceuticals rather than having to pass the far stricter regulatory hurdles imposed on pharmaceuticals.

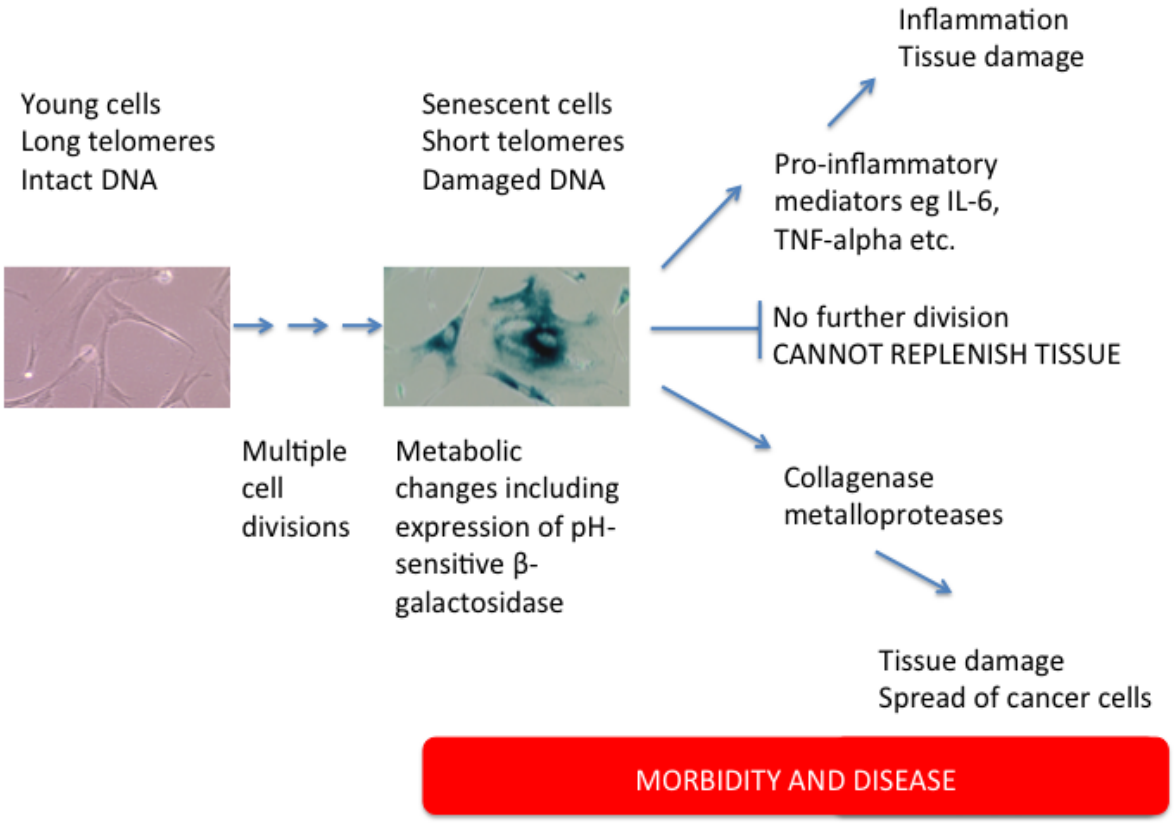

Figure 1: Replicative cellular senescence is a consequence of multiple rounds of cell division. As somatic cells undergo mitotic division, telomeres shorten and cells alter gene expression patterns, resulting in metabolic shifts. Senescent cells lose the ability to divide, entering 'deep' senescence. They also secrete a range of pro-inflammatory cytokines and metalloproteases which result in tissue damage.

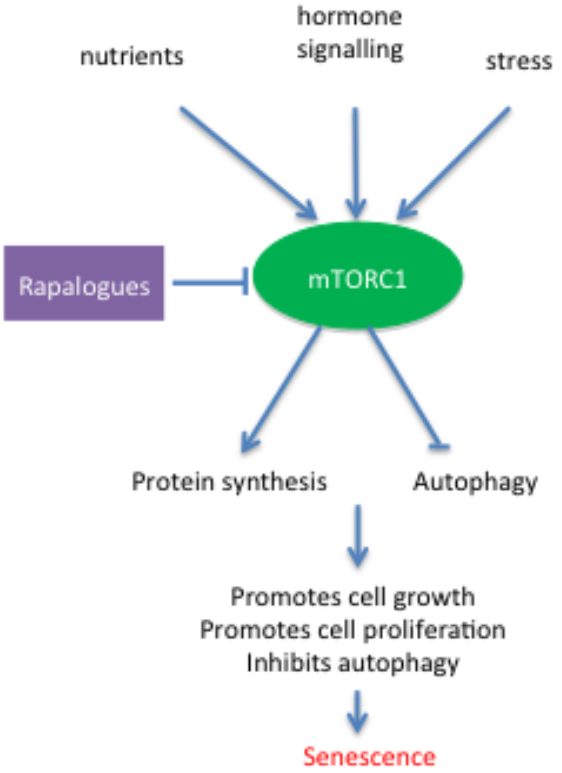

Figure 2: The mTOR pathway regulates cell proliferation and autophagy. Senescence can be modulated by drug treatment. Inhibition of mTORC1 by drugs such as rapamycin increase cellular longevity and organismal lifespan.

Other approaches to ageing analyse the impact of genes that modulate longevity. For many years, my lab has been interested in the human progeroid Werner syndrome, where individuals age much faster than normal. This syndrome results from mutation in a single gene, *WRN*, which encodes a protein with both helicase and exonuclease activities. We have been exploring model systems, including flies and worms, to better understand the action(s) of *WRN* (11). The recent and very exciting DPhil work of Hayley Lees, jointly supervised by Alison Woollard and myself, has demonstrated that *WRN* really is an anti-ageing gene. Hayley has further identified a novel genetic interaction that markedly increases not only lifespan but also healthspan. Because of the genetic tractability of the worm model, she can now pursue these studies to identify components of the biochemical pathways that are important in longevity assurance.

The biochemistry of ageing is at an exciting stage of expansion of primary research, with rapid translation of those findings into the clinic. Whether we choose to pursue the route of developing rapalogues and resveralogues or to target novel biochemical pathways with anti-ageing therapies, as a society it is important we don't lose sight of the aim to alleviate suffering and decrease morbidity. Anti-ageing therapies must therefore be affordable, safe and available to all. Imagine a future where cellular senescence does not lead to inflammation, where cancer risk for older people is as low as in the young, and where vascular ulcers, incontinence, blindness and dementia are historic diseases confined to the annals of the history of medicine.

Assoc Prof Lynne Cox heads the Molecular Basis of Replicative Senescence group, based in New Biochemistry

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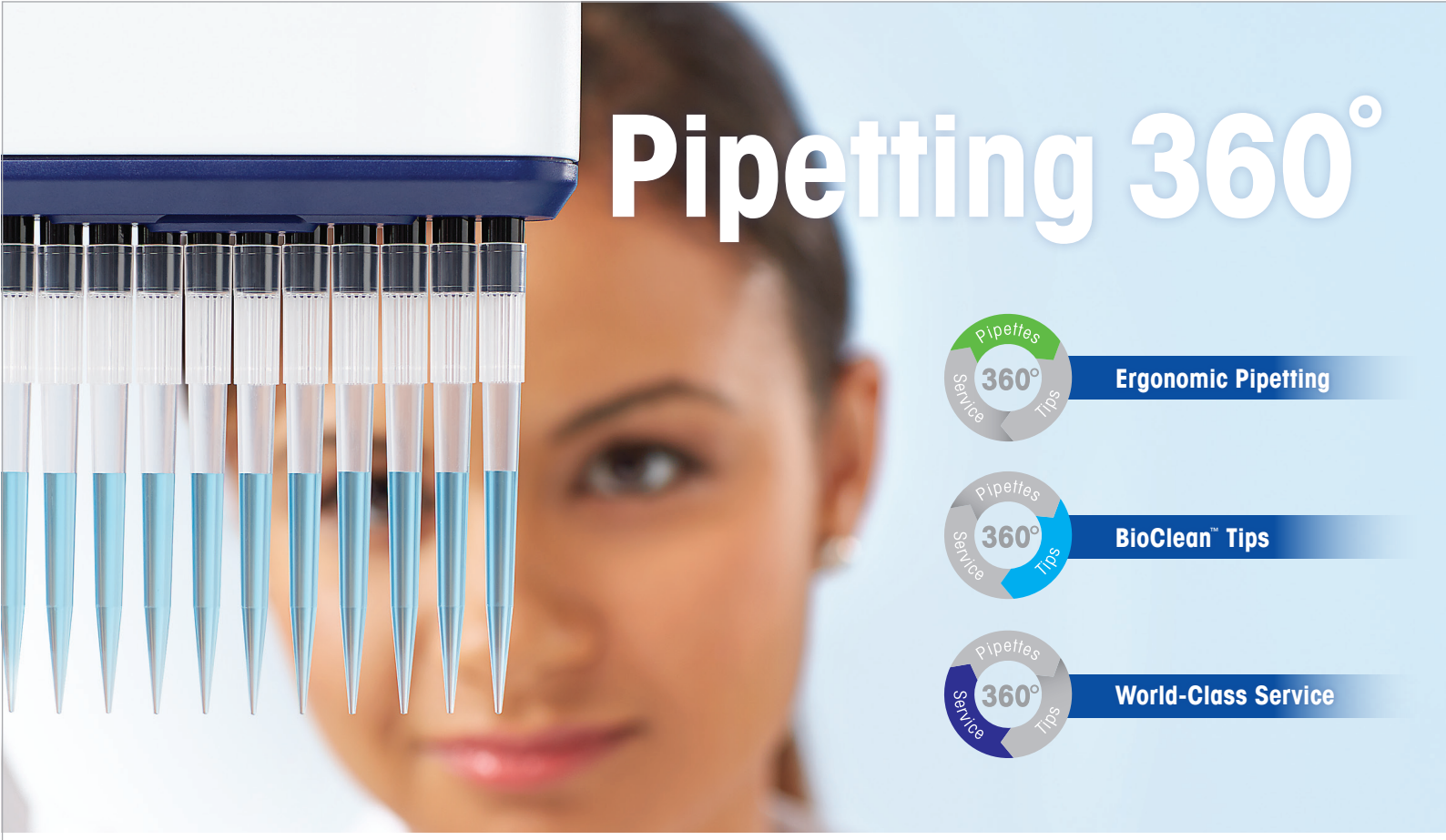

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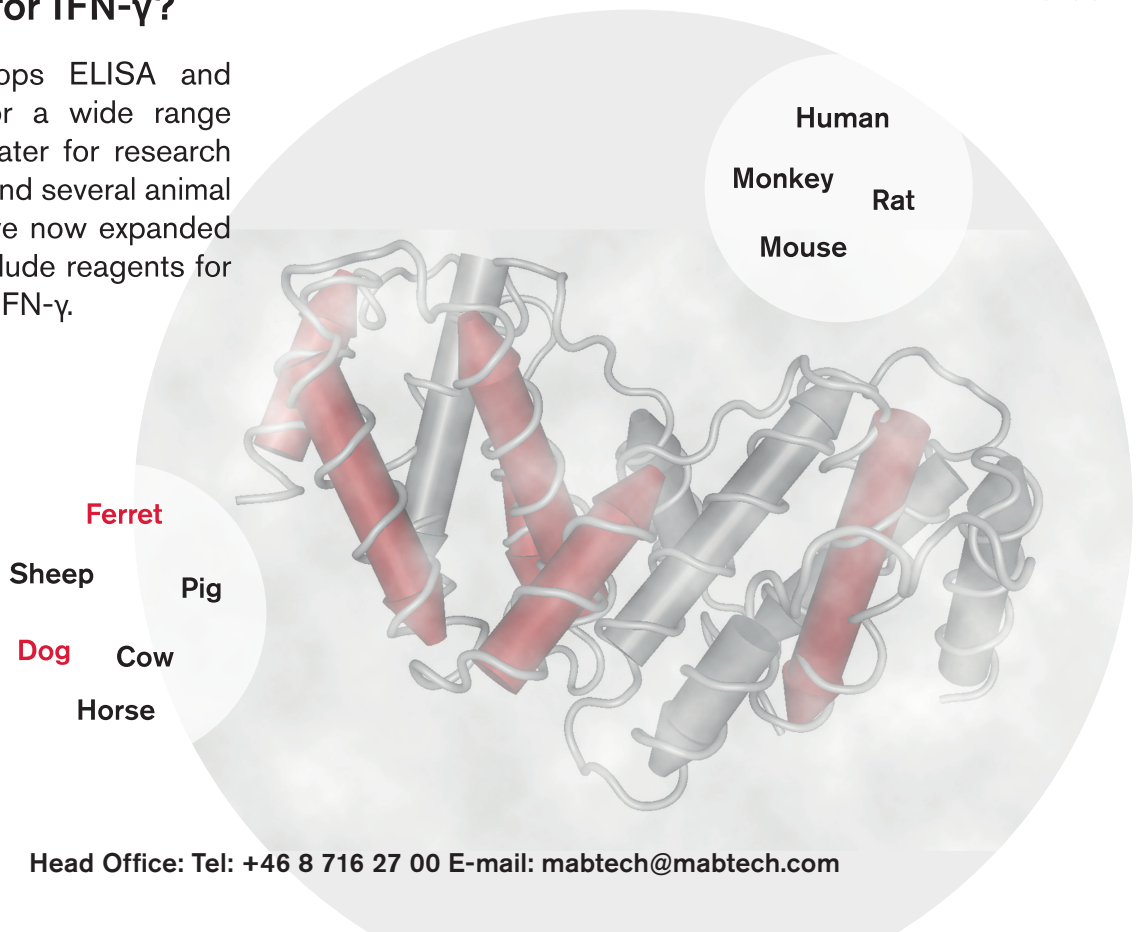

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Bipolar Disorder: The story of Ebselen

by Olga Kuznetsova

To find a new treatment for a disease and to bring it to market has always been a long and challenging endeavour; yet with it comes the potential for astounding improvements in global health - consider the impact of aspirin or penicillin. Historically, new drugs were either derived from traditional, plant-based medicines, or discovered through sheer serendipity. Such approaches were slow and inefficient, and only in recent years have huge leaps in technology and new knowledge about disease mechanisms given drug discovery a significant boost. But even today, drug discovery remains a painfully long and expensive process with low success rates. It is typically only carried out by large companies with access to significant resources: the rich and powerful 'Big Pharma'. The probability of a small laboratory on a shoestring budget developing a drug is close to nil...but that is exactly what Dr Grant Churchill's group at the Department of Pharmacology has achieved. What's more, they focussed their efforts on a complex, neurological condition: bipolar disorder.

Bipolar disorder, commonly known as manic depression, affects up to 3% of the population and has been ranked by the World Health Organisation as the sixth leading cause of disability worldwide (1). It is defined by extreme fluctuations in mood, from periods of depression to periods of elevated mood known as mania. Manic episodes vary in intensity and can feature very severe psychological symptoms such as psychosis, hallucinations and violent behaviour, which can result in admission to a psychiatric hospital. The worst depressive episodes can lead to suicidal thoughts and behaviour. Although treatments do exist, there is an urgent need for new, improved therapies.

The current gold standard treatment for bipolar disorder is lithium, which is known to be effective in more patients with bipolar disorder than other mood stabilisers. It relieves both manic and depressive phases and is the only treatment available that reduces suicidal thoughts and behaviour. So why the need for change? Unfortunately, lithium treatment is risky and plagued with side effects. It is toxic at only twice the therapeutic dose; patients are therefore constantly required to monitor their blood lithium concentration to prevent overdosing. Patients also suffer a variety of other side effects such as tremors, weight gain, and kidney damage. These probably occur because lithium inhibits a large number of processes within the cell, many of which are not specific to bipolar disorder. Ideally, identifying the exact molecular mechanism of bipolar disorder would allow the development of a drug that inhibits one specific target, reducing off-site effects and leading to a much safer treatment.
In 2009, the Churchill group set out to find a drug that inhibits the most likely molecular target implicated in the development of bipolar disorder: an enzyme called inositol monophosphatase (IMPase), which is also inhibited by lithium. There were several ways to approach this, such as screening a large library of previously untested small molecules, modifying the natural substrate of IMPase, or using a computer-based design of 3D ligands and predicting their binding affinity. The Churchill group tackled the problem in a different way: they screened the National Institute of Health Clinical Collection, which contains drugs that have already been through human trials and have previously been shown to be safe. Although these drugs may have failed efficacy tests for other diseases, they could still be proven to be useful for bipolar disorder. The group carried out the screen and identified a molecule called ebselen, which inhibits IMPase at a thousandth of the concentration needed for lithium. Unlike lithium, ebselen binds covalently to IMPase, potentially leading to a longer-lasting inhibition (2).

> “Ebselen inhibits IMPase at a thousandth of the concentration needed for lithium”

While the *in vitro* results for ebselen were very promising, a drug for bipolar disorder is useless unless it can be shown to permeate the brain and cross the blood-brain barrier. To test this, ebselen was injected into mice and their brains extracted. IMPase inhibition was observed in brain homogenates, proving that the drug can cross the blood-brain barrier and perform its function inside brain cells. So far so good, but how to test whether the drug actually stabilises mood? Is there such a thing as a mouse with a mood disorder? In fact, animal models for bipolar disorder do exist, and they mimic both the manic and depressive phases.

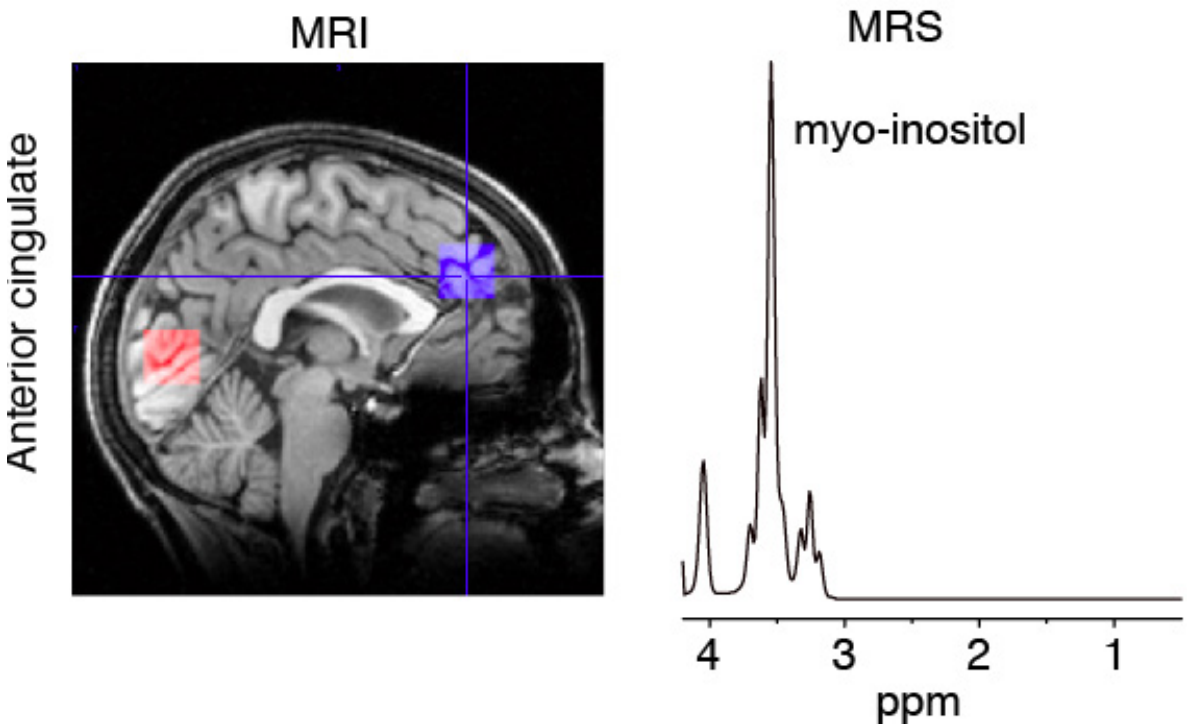

Figure 1: A magnetic resonance imaging (MRI) scan (left) and magnetic resonance spectroscopy (MRS) trace (right) recording the presence of myo-inositol in the human brain. Image courtesy of Uzay Emir and Dr Nisha Singh, Nuffield Department of Clinical Neurosciences, University of Oxford.

To model depression, mice are subjected to a 'forced swim test', in which they swim inside a water-filled cylinder from which they cannot escape. The time that the mouse spends motionless, apart from the small effort required to keep its head above the water, is considered to reflect a depressive and despairing state. Both lithium and ebselen have been shown to increase the amount of time the mouse spends actively moving about, suggesting an anti-depressive action for both drugs. To model the manic phase, mice are put through an 'open field test', where they are allowed to explore an open area. Researchers measure the distance that the mouse explores, and the number of times the mouse stands on its hind legs or 'rears'. Rearing is associated with anxiety and impulsivity, and ebselen reduced the number of times the mice reared. For an even more realistic model of mania, mice were given amphetamine and, again, ebselen and lithium both reduced the hyperactivity resulting from mania.

Unfortunately, animal models do have their limitations, especially when they are expected to model human mental illness. Although ebselen performed well in vitro and in mice, what was lacking were human studies. Before a large-scale clinical trial could take place, a small study using healthy volunteers was run at the Warneford Hospital in Oxford. First, it was necessary to confirm that ebselen permeates the human brain and has an inositol-depleting effect as it does in mice. In the mouse study, this involved extracting the animal's brain. Fortunately, the human subjects did not suffer the same fate, and brain inositol was measured non-invasively using MRI (Figure 1). It was found that inositol levels were reduced, particularly in the anterior cingulate cortex, a part of the brain linked to mood disorders.

What about the effect of ebselen on mood? All the volunteers enrolled in the study were healthy, so it was not possible to assess mood stabilisation. However, the study did analyse emotional processing: the subjects looked at various pictures, and reported the emotions that they perceived. In mood disorders such as bipolar disorder, patients respond to events with a negative bias, and emotions such as happiness and disgust are dampened down during mood swings. In a string of behavioural tests, ebselen repeatedly heightened the subjects' responses to happy or disgusting stimuli, hinting at the promising suggestion that it might do the same in bipolar patients (3).

With an increasing amount of mouse and human data supporting the 'lithium-like' effects of ebselen in models of bipolar disorder, the Churchill group is now looking for funding for a large-scale clinical trial. Although ebselen is not yet proven to be effective for treating bipolar disorder, its success so far shows that even small labs and enterprises can think big and take up the herculean task of drug discovery.

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Olga Kuznetsova is a DPhil student in the Murphy group in the Sir William Dunn School of Pathology

Why were catalytic RNAs replaced by protein-only enzymes?

by Stephanie Oerum

Many scientists may already be familiar with the 'RNA world hypothesis', which states that the origin from which all life descended was an RNA dominated world. Today, biochemical reactions are mainly driven by protein-based catalysis and very few catalytically active RNA components (ribozymes) are known. In fact, only around 15 types of ribozyme have been identified, one of which is the RNase P complex, which consists of a catalytic RNA component with between one and ten proteins depending on the organism (Figure 1) (1).

The RNase P complex is responsible for 5'-end processing of pre-tRNA and was originally thought to be a ribozyme in all species until, in 2008, it was discovered that human mitochondrial RNase P is a protein-only complex composed of three different subunits (Figure 1E) (2). Subsequently, protein-only RNase Ps were identified in all plant sub-cellular compartments, including cytosol, chloroplasts and mitochondria, with homology to one of the three subunits in the human mitochondrial RNase P complex (Figure 1D and 1G). This important finding suggested that a ribozyme and a protein-only enzyme could catalyse the same reaction, and thus provided the scientific community with an opportunity to investigate the potential reasons behind the shift from an RNA-based world to a world where most biochemical catalysts are protein-based.

A number of reasons that catalytic RNAs may have been superseded by protein-only enzymes have been proposed (3) and, with the recent discovery of protein-only RNase Ps, some of these hypotheses have now been tested. Let us explore these in more detail:

Enhanced catalytic efficiency

One hypothesis is that protein-based catalysis may enhance catalytic efficiency, and that such increased efficiency is advantageous in more complex organisms. Biochemical data, however, shows that both the nuclear and organellar protein-only plant RNase Ps (Figure 1D and 1G) have catalytic activities that are one to two orders of magnitude slower (~$1x10^5$ $M^{-1}s^{-1}$) than the bacterial RNA-based RNase P complex (~$4x10^6$ $M^{-1}s^{-1}$) (Figure 1A) (3). This indicates that increased efficiency was not the driving force behind the replacement of the catalytic RNAs with proteins.

Substrate specificity

As the genome expands in size from bacteria to eukaryotes, so does the sequence and structural complexity of the encoded RNA universe. Faced with such an increase in potential substrate complexity, the appearance of protein-only RNase Ps in higher organisms could have been driven by the need for a higher degree of substrate specificity than that achievable by a ribozyme. Although conceivable, this hypothesis is not supported by biochemical data. The simple bacterial RNase P ribozyme (Figure 1A) is only active on pre-tRNAs and RNAs that resemble the structure of pre-tRNAs at the point of cleavage. In the same fashion, the protein-only mitochondrial plant RNase P (Figure 1G) is only active on pre-tRNAs and t-elements with a pre-tRNA-like secondary structure. Despite this apparent similarity, the bacterial RNase P will only process canonical L-shaped bacterial pre-tRNAs (Figure 2), whereas the protein-only plant RNase P will process both cytoplasmic canonical pre-tRNAs and mitochondrial non-

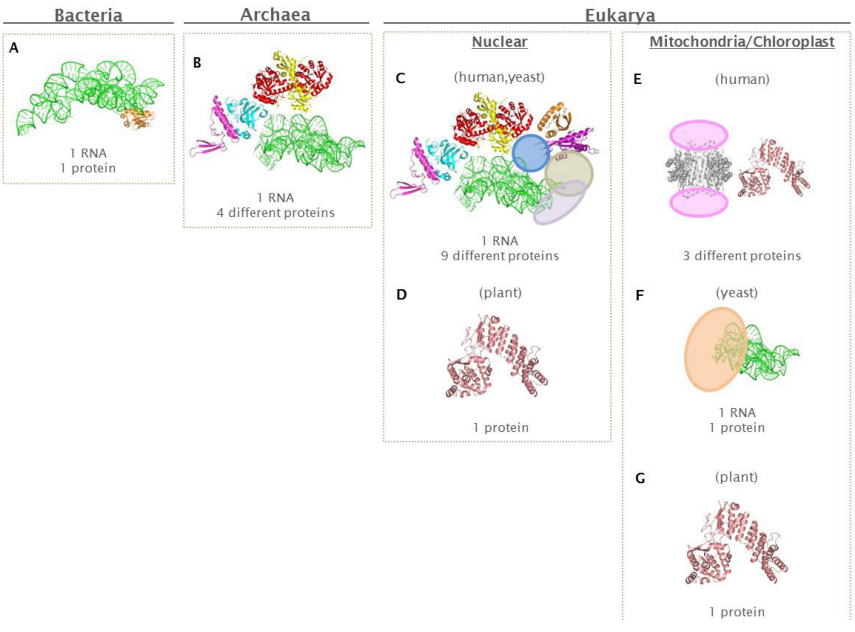

Figure 1: A comparison of the RNase P complex from different domains of life. The RNA component in Archaea and Eukarya is taken from the bacterial RNase P structure. Proteins in Archaea and Eukarya are arbitrarily positioned. One human protein is represented by the plant homologue. Where no crystal structures are known, proteins are shown as coloured circles.

canonical pre-tRNAs that lack various arms found in the canonical structure. This suggests that, if anything, the ribozyme is more specific than the protein-only enzyme. Human nuclear RNase P consists of a catalytic RNA component and nine different proteins (Figure 1C) and may therefore be considered an intermediate between the simple, bacterial RNase P (one RNA and one protein) and the protein-only plant RNase P. This human RNase P complex has been shown to cleave single-stranded RNAs *in vitro* in a relatively sequence-independent manner. In contrast, plant protein-only RNase P had no activity towards the tested RNAs, even in the presence of excess protein (3), further supporting the notion that increased substrate specificity was not a significant driving force behind the shift from RNA to protein catalysis.

> "The discovery of the diversity of RNase Ps offers a fascinating example of 'evolution at work'"

Cross-membrane transport

A third hypothesis suggests that the difficulties of transporting large RNA components across membranes may have been a driving force behind the evolution of protein-only RNase Ps. For example, the human mitochondrial RNase P (Figure 1E), which is encoded in the nucleus, must be imported into mitochondria and is a protein-only complex. Currently, approximately 900 nuclear-encoded proteins have been identified in human mitochondria. In contrast, the presence of nuclear-encoded RNAs in mitochondria is limited to only a few tRNAs. The mechanism of mitochondrial import of large RNA components is currently controversial and unresolved. In yeast, the mitochondrial RNase P complex still holds a catalytic RNA component (Figure 1F) and, interestingly, this RNA is encoded in the mitochondrial genome rather than imported across the mitochondrial membrane from the cytosol. Furthermore, the human mitochondrial genome itself encodes an RNA component, which further suggests that the import of large RNA molecules across membranes presents a potential difficulty for cells.

Conclusion

To date, studies on RNase P complexes from different species have so far not identified singular drivers that might explain the transition from ribozymes to protein-only enzymes. In fact, obvious drivers, such as a need for increased catalytic activity or enhanced substrate specificity, do not seem to have played a role, suggesting that the transition was the result of a combination of several subtle drivers. In addition, it seems that different organisms have acquired different solutions to the same problem. For instance, in humans and plants, where organellar RNase P is a protein-only enzyme, the RNA-to-protein transition may have been driven by the difficulties in transporting larger RNA components across membranes, whereas in yeast this transport issue has been circumvented by encoding the RNA component of mitochondrial RNase P within the mitochondrial genome itself.

The instability of RNA compared to protein could also have been a potential driver of the RNA-to-protein transition in subcellular compartments, as RNA is more sensitive to the higher pH and free radicals in these organelles. Also, as organisms become more complex, the need for molecules to participate in sophisticated regulatory networks increases. Participation in such networks may be easier to achieve with protein-based RNase Ps, which enable a structural diversity that is not available for the RNA-based RNase Ps built from only four building blocks.

Due to the degeneracy of the genetic code, many changes at the RNA level will not necessarily cause a change at the protein level and so will not affect enzymatic activity. However, it is highly probable that changes at the RNA level will alter secondary or tertiary ribozyme structure and so catalytic activity. As such, it is tempting to speculate that the transition from RNA to protein-based catalysis might have been driven by a need to desensitise critical enzymatic activities, like that of RNase P, to genetic mutations. Whatever the drivers, the discovery of the diversity of RNase Ps offers a fascinating example of 'evolution at work' and provides an attractive system to further understand the many forces that help to shape biological molecules.

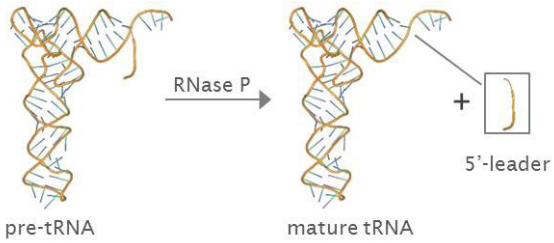

Figure 2: The reaction catalysed by the RNase P complex involves 5'-end processing of pre-tRNA.

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Seeing is believing: Intravital microscopy

by Dr Bostjan Markelc

The desire to see what is happening at the microscopic level has fascinated the scientific community since the beginning. Ever since Robert Hooke published his Micrographia in 1665, there has been a strong desire to see things in greater detail and, if possible, alive. With the advent of modern microscopy techniques such as confocal and multiphoton microscopy, scientists were given a tool which enabled them to see deep into tissues. When combined with fluorescent proteins or fluorophores, this provided an unprecedented level of detail. For seeing things on a microscopic level in living animals, the emerging field of Intravital Microscopy (IVM) uses different imaging windows where a plastic or metal frame is surgically implanted into an animal and then covered with a cover glass; thus providing the means to use the microscope to see the internal workings of living organs and tissues.

Lately, there has been an expansion of different chamber models, all specially adapted for an organ or tissue of interest (Figure 1A). A cranial imaging window enables imaging of the brain, a mammary imaging window is adapted for imaging of breast cancer, an abdominal imaging window enables imaging of internal organs, and a dorsal skin-fold chamber enables imaging of the vasculature in the skin and different tumours (1). What is especially attractive with IVM is that it allows repetitive imaging of the same animal for several days, providing insight into the development and behaviour of cells, tissues, organs and tumours.

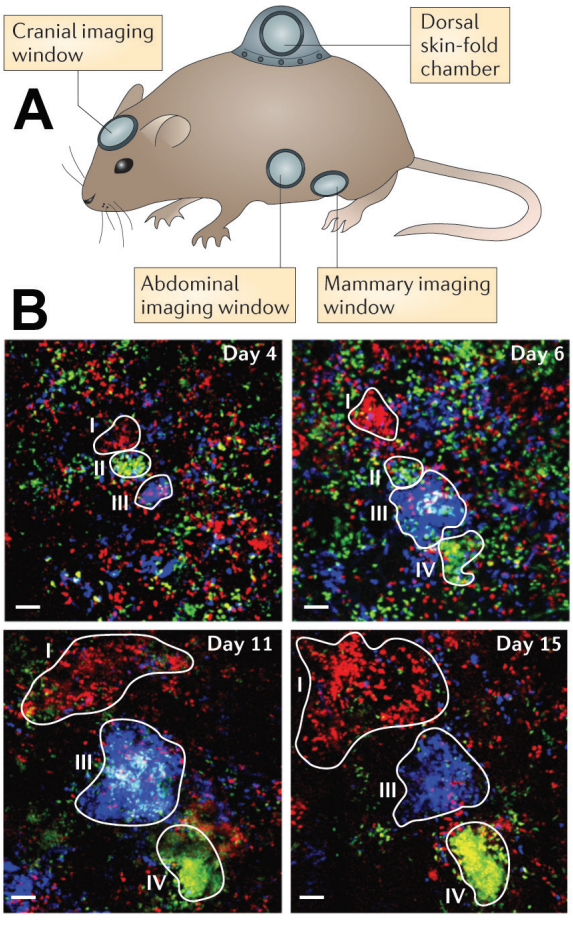

Figure 1: A) Position of imaging windows on a mouse B) A series of intravital images of a growing mammary carcinoma after induction of the Confetti label randomizer. Only four Confetti-labelled cells were able to form small clones (white outline, regions I–IV). Scale bars represent 50 μm. Part A is reproduced with permission from (1) and B from

IVM has already shown its value in providing information on several hallmarks of cancer. For example, IVM has revealed that it is not only the tumour cells which invade healthy tissue around the primary tumour, that are motile and responsible for metastasis; but the cells in the primary tumour are also highly motile and able to invade blood vessels (1). Furthermore, by following single cells in pre-micrometastasis in the liver, researchers were able to show that the motility of cancer cells is crucial for the establishment of a micrometastasis and as soon as it is formed, the cells stop moving (1).

IVM has also been used to delineate the abnormalities of tumour vasculature. By injecting different fluorescent markers intravenously, several aspects of the blood flow in tumours can be observed. This includes the leakiness of tumour blood vessels as well as irregular blood flow or, in some cases, even an absence of blood flow. All of which can provide important information on the distribution of different drugs in the tumour (1).

Determining the clonality of different populations has also been possible with IVM. By using cancer cells carrying a special genetic construct called the Confetti construct, which fluorescently labels each cell differently, it has been shown that only some cells within a growing tumour are able to proliferate (Figure 1B). A similar approach has also revealed intestinal crypt homeostasis at a single-stem-cell level (2).

Thus, if your interest lies in tracking single cells and their behaviour in their natural living environment, or in the development of a growing tumour mass, or even in how neurons communicate in the brain and you want to see this happening with your own eyes, then IVM might just give you what you are looking for.

Dr Bostjan Markelc is a post-doc in the Mechanisms of Metastasis

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New pieces of the plasmid puzzle

by
Dr Alvaro
San Millan

Antibiotics are indispensable for the treatment of bacterial infections and have played a pivotal role in the development of modern medicine. However, since the introduction of antibiotics in clinical practice, the development of antibiotic resistance amongst bacteria has dramatically increased. Antibiotic resistant bacteria cause diseases which are difficult to treat, raising mortality rates and the economic costs associated with infection. Antibiotic resistance is therefore considered a major threat to human health in developed countries (1, 2).

Bacteria can acquire antibiotic resistance through plasmid acquisition. Plasmids are circular DNA molecules that are not part of the bacterial chromosome. They control their own replication and, most importantly, they can be horizontally transferred between bacteria in a process known as conjugation. Plasmids carry genes that help bacteria to adapt to new niches and stresses, playing a key role in bacterial evolution. In recent decades they have become especially relevant as vehicles for the spread of antibiotic resistance (3). Plasmids can encode multiple resistance genes, so can therefore supply multi-resistance in a single step, jeopardising the effectiveness of a spectrum of antibiotics.

The downside of plasmid carriage for the bacterial host is the associated physiological cost (Figure 1). In the absence of antibiotics, a bacterium carrying a plasmid that confers antibiotic resistance has a 'fitness' disadvantage compared to a plasmid-free bacterium. Bacteria can also lose plasmids during cell division, in a process known as segregational loss (Figure 1). Previous studies have predicted that, given these factors, the only way for plasmids to survive in bacterial populations is to spread by conjugation. However, analysis has shown that almost half of the plasmids in nature lack the genetic tools required for conjugation (4).

This puzzling finding raises a question: how do plasmids survive in the absence of conjugation? We have addressed this question using experimental evolution, mathematical modelling and whole genome sequencing. We found that a costly, unstable non-conjugative plasmid conferring antibiotic resistance could be stabilised in populations of the pathogenic bacterium *Pseudomonas aeruginosa* by a combination of compensatory adaptation and positive selection. Bacteria adapt to the plasmid via compensatory mutations, recovering the cost associated with plasmid carriage (Figure 1). However, this alone is not sufficient to maintain the plasmid. Positive selection, mediated by antibiotic exposure, is necessary to increase the frequency of plasmid-bearing bacteria and offset the effects of segregational loss. Crucially, we found that feedback occurs between these two processes. Positive selection acts to increase the effect of compensatory adaptation by enlarging the population of plasmid-bearing bacteria, thus tipping the balance in favour of new adaptive mutations in the population. Compensatory adaptation, in turn, enhances the effect of positive selection, by slowing the rate at which plasmids are lost between episodes of positive selection.

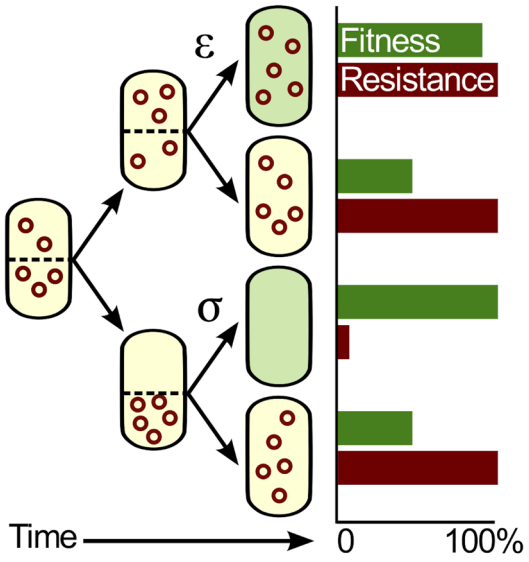

Figure 1: The level of antibiotic resistance and relative fitness of different bacteria evolving from a parental clone carrying a plasmid that confers antibiotic resistance. The stability of the plasmid in a bacterial population depends on: (i) the rate of loss of the plasmid during cell division (**ε**) and (ii) the difference in fitness between plasmid-bearing and plasmid-free bacteria, which determines the competitive disadvantage of plasmid-bearing bacteria. Compensatory mutations appear at a constant rate (**ε**) and alleviate the cost of plasmid carriage, stabilising plasmids in the population. Figure by Rafael Peña-Miller. Modified from (5).

Our work therefore indicates that antibiotic use plays a key role in stabilising plasmid-mediated resistance genes in pathogen populations. This reinforces the importance of minimising the use of antibiotics in order to control antibiotic resistance.

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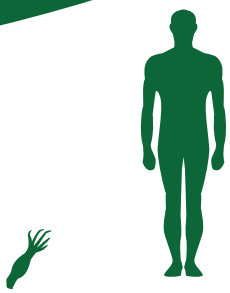
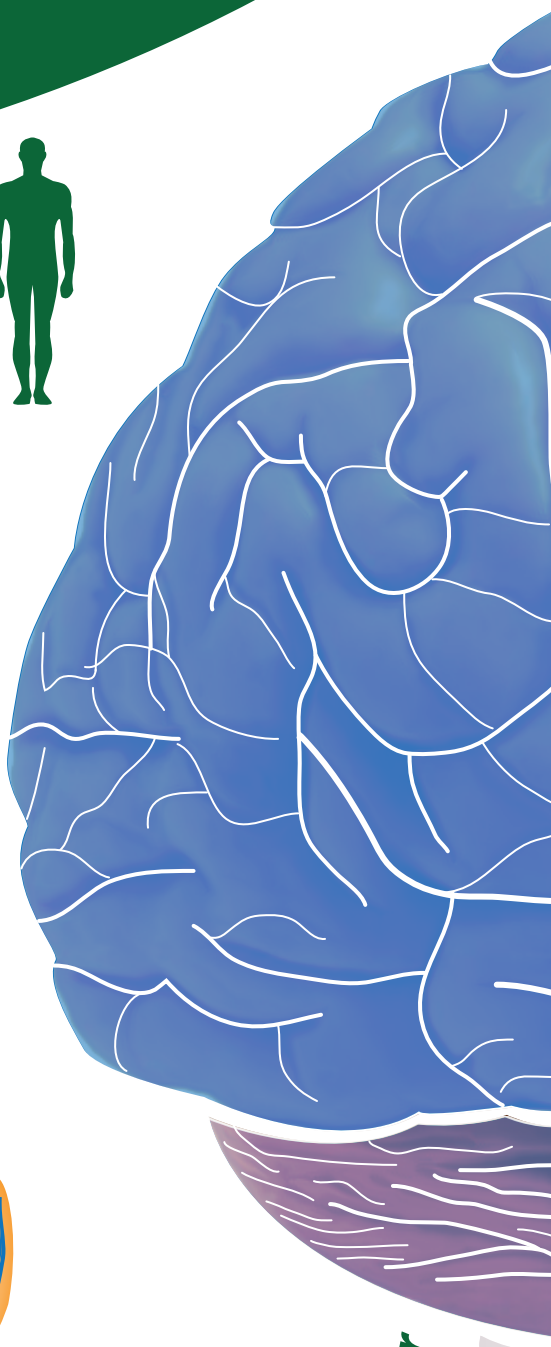
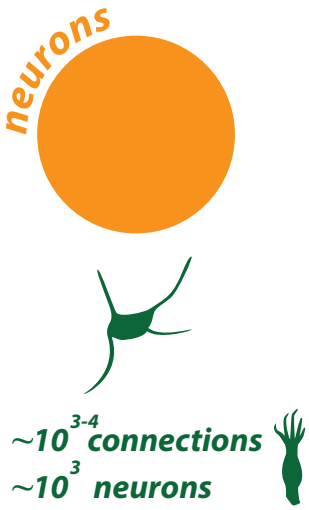
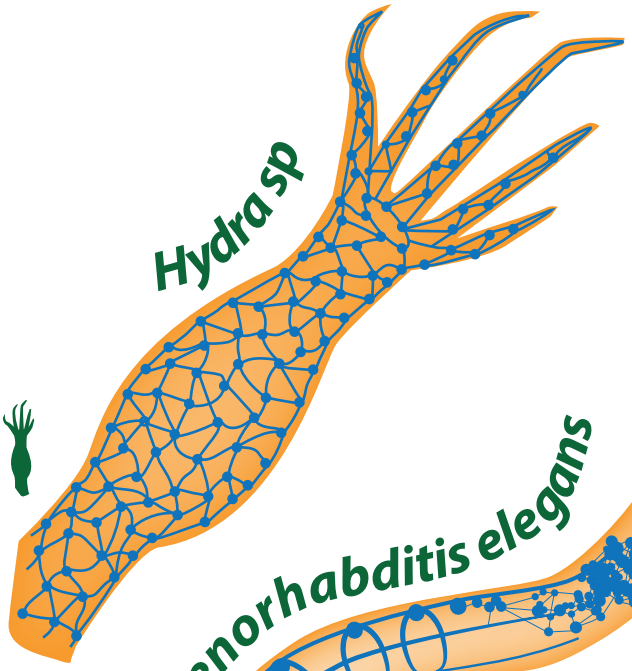
More than neurons...

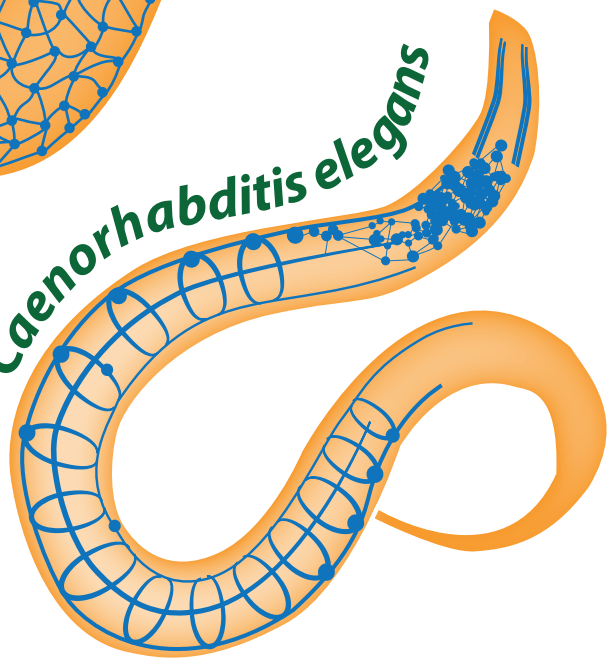
Number of neural cells

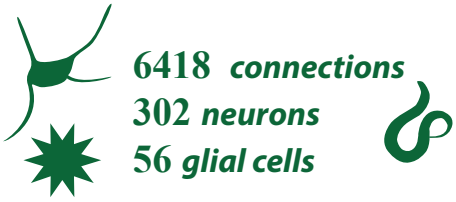


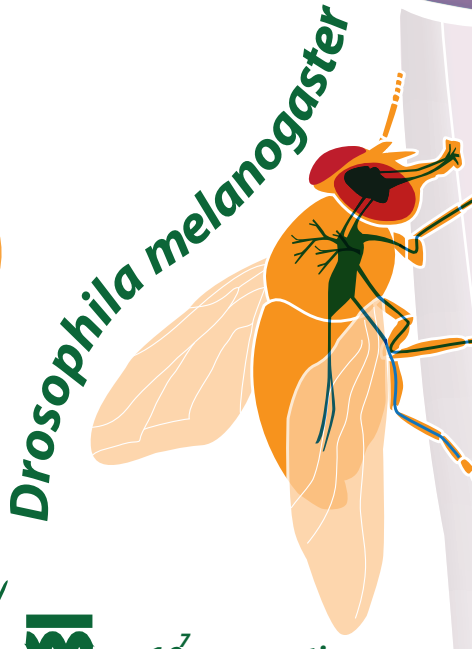


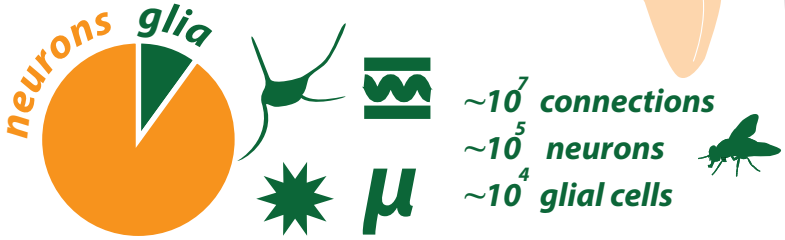


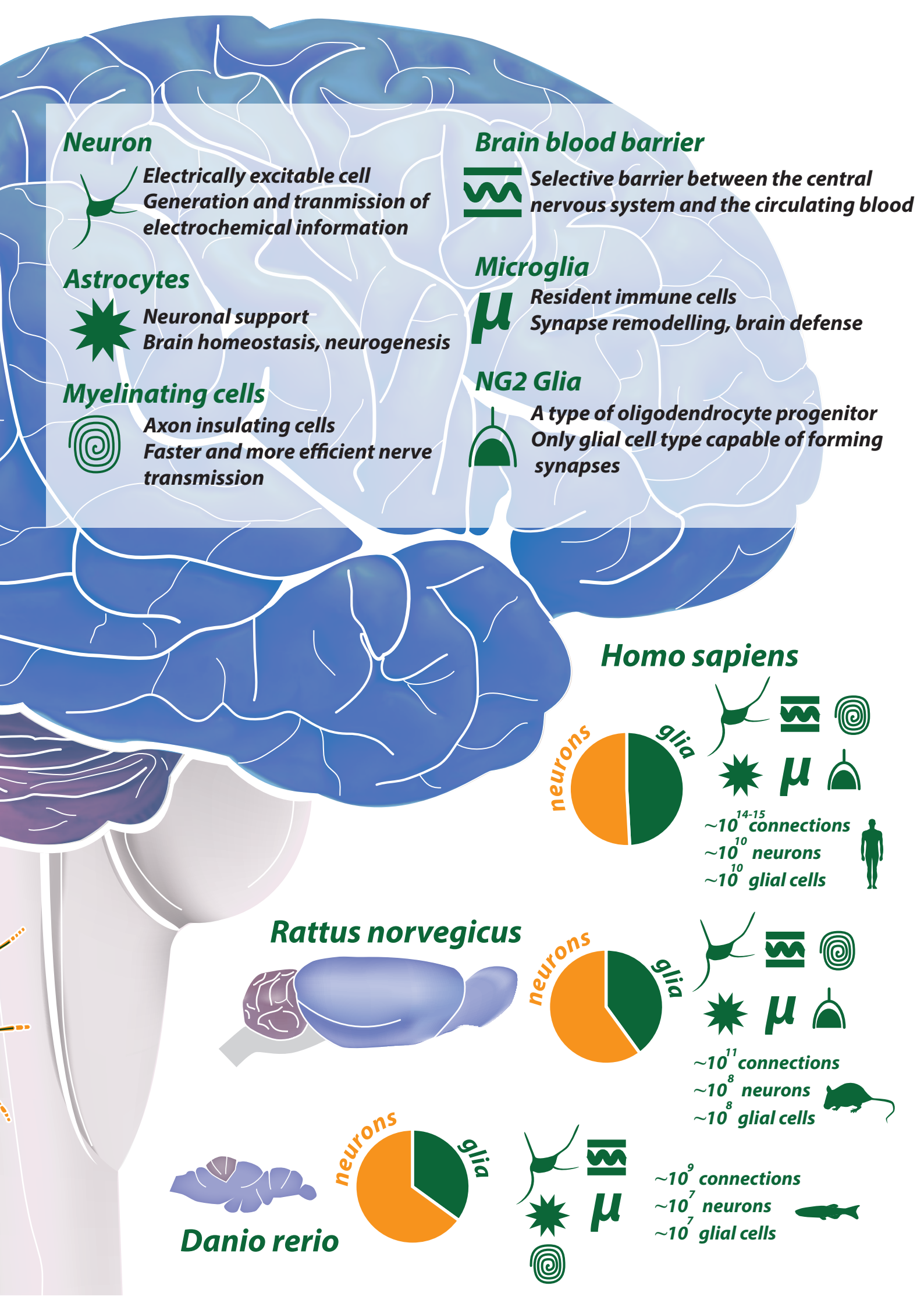











Neuron
Electrically excitable cell
Generation and tranmission of electrochemical information
Astrocytes
Neuronal support
Brain homeostasis, neurogenesis
Myelinating cells
Axon insulating cells
Faster and more efficient nerve transmission
Brain blood barrier
Selective barrier between the central nervous system and the circulating blood
Microglia
Resident immune cells
Synapse remodelling, brain defense
NG2 Glia
A type of oligodendrocyte progenitor
Only glial cell type capable of forming synapses
Homo sapiens
neurons
glia
~10^14-15 connections
~10^10 neurons
~10^10 glial cells
Rattus norvegicus
neurons
glia
~10^11 connections
~10^8 neurons
~10^8 glial cells
Danio rerio
neurons
glia
~10^9 connections
~10^7 neurons
~10^7 glial cells

Early detection of liver fibrosis biomarkers

by Evangelia Tzika

Hepatic fibrosis is the overgrowth of connective tissue, or scar tissue, within the liver as a reaction to inflammation. Inflammatory cytokines activate specialised macrophages called Kupffer cells, which in turn activate the hepatic stellate cells; liver fibroblasts that lay down the scar tissue. During this process, excess collagen and other matrix constituents can fill the spaces between liver cells, disrupting blood flow and normal liver function. As a consequence, extensive scar tissue accumulation can progress through fibrosis to an advanced stage known as cirrhosis (Figure 1), and subsequently to liver cancer (hepatocellular carcinoma).

Hepatic fibrosis is triggered by many of the known chronic liver diseases, including chronic viral hepatitis B and C, alcoholic liver disease, and non-alcoholic fatty liver disease. In the early stages of the disease the patient is typically asymptomatic; liver fibrosis can therefore be considered a silent attack. Cirrhosis may take over a decade to develop in a chronic alcoholic, and more than two decades to develop in a patient infected with hepatitis C virus. Detection of the initial stages of hepatic fibrosis is therefore vital, as early intervention can delay or even prevent the development of cirrhosis.

The diagnosis of liver disease through biopsy is currently considered the reference standard in the clinic. The most significant drawback to this method is its invasive nature. A liver biopsy is painful and may require an overnight hospital stay at a cost of over £2,000 to the National Health Service. An additional disadvantage of biopsy is the potential for sampling error, which arises due to the fact that fibrosis is not homogenous.

Liver biopsies represent only 1/50,000th of the total liver mass, meaning that a biopsy result may be unrepresentative, and could lead to an inaccurate diagnosis. A number of non-invasive immunoassays requiring blood plasma or serum are currently available to clinicians. FibroTest, one of the earlier assays, is based on an algorithm of serum marker levels combined with age and the gender of the patient. Similar immunoassays are FibroMeter and the Enhanced Liver Fibrosis (ELF) test. Although such immunoassays are used in the clinic, there can be a significant overlap between the results for neighboring stages of fibrosis. Therefore, there is a need to develop a more reliable non-invasive assay to help diagnose the individual stages of hepatic fibrosis.

Prof Nicole Zitzmann's group at the Oxford Glycobiology Institute aims to produce a non-invasive, rapid, and inexpensive method for the diagnosis of liver fibrosis. The group uses proteomics techniques to identify and quantify novel liver fibrosis biomarkers. Group member Dr. Bevin Gangadharan has identified novel liver fibrosis biomarkers by comparing serum proteins from patients diagnosed with a range of fibrosis stages by liver biopsy. The technique used to discover these serum protein biomarkers was two dimensional gel electrophoresis (2-DE). In 2-DE, serum proteins are separated according to their charge (pH) in the first dimension and their molecular weight in the second dimension, resulting in a two dimensional array of features on a gel. This separation was performed for serum samples taken from patients with different stages of fibrosis, which allowed the team to identify protein features which increased or decreased in intensity across the stages. In an initial study over a wide pH range (pH 3-10), several protein features were detected which changed in expression according to fibrosis stage. The proteins were identified by mass spectrometry and could be used as potential biomarkers for liver fibrosis (1, 2).

To identify additional biomarkers, the team developed novel proteomics methods (Figure 2). 2-DE of blood serum was performed in the pH range 3-5.6, which is outside of the range of separation of highly abundant proteins such as albumin, transferrin and immunoglobulins. This

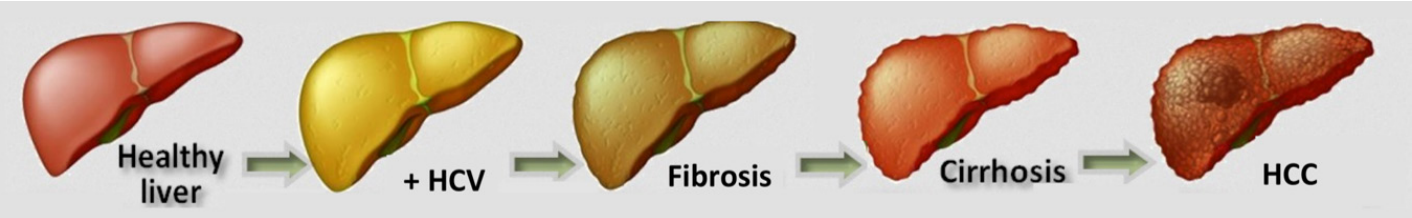


Figure 1: Stages of Liver Fibrosis. HCV, Hepatitis C Virus; HCC, Hepatocellular Carcinoma.

was the first time serum had been separated using this pH range. This approach allowed four times more protein to be loaded onto the 2-DE gels and therefore enhanced the detection of low abundance proteins. Comparison with 2-DE gels of plasma from individuals with and without liver fibrosis led to the identification of novel biomarkers. Furthermore, Western blot analysis of plasma samples from individuals at different fibrosis stages showed that the novel biomarkers are very promising in comparison to those currently used in the clinic; the novel biomarkers may be more effective at distinguishing between the different stages of fibrosis (3, 4).

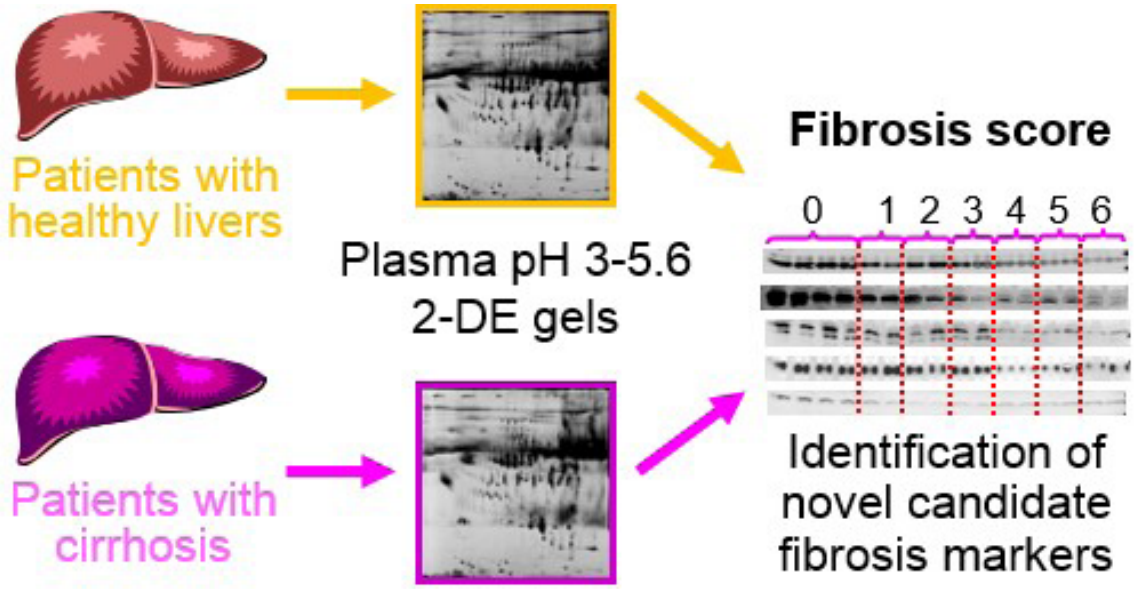


Figure 2: Identification of novel liver fibrosis biomarkers. Plasma samples from individuals with healthy livers and patients with liver cirrhosis were separated by 2-DE gels using a narrow pH 3-5.6 range. Gel features which changed in intensity were identified by mass spectrometry and later confirmed by Western blotting. Figure adapted from (3).

The future plan of Prof Zitzmann's team is to detect and quantify biomarkers using mass spectrometry. With this approach, results with high sensitivity can be achieved with the detection and quantitation of low abundance proteins. Quantitative mass spectrometry provides significant advantages over immuno-based techniques for the diagnosis of liver fibrosis, as it is robust, fast, sensitive, and antibody-free. Antibodies utilised in immunoassays are known to vary in binding specificity, which may give rise to false positives. Antibodies are also unable to detect biomarkers if protein degradation compromises the integrity of the binding epitope. However, mass spectrometry overcomes these problems, since detection is based on filtering pre-selected tryptic peptides and analysing their unique fragments. As a result, it does not matter if a biomarker is degraded as long as the selected peptide is intact. Additionally, the cost of antibodies is generally higher than the peptide standards used in quantitative mass spectrometry. Mass spectrometry is able to detect and quantify more than 50 biomarkers within a single 30-minute analysis, while commercially available liver fibrosis immunoassays may only be used to detect a maximum of five biomarkers.

Mass spectrometry based approaches, such as the assay being developed by Prof Zitzmann's team, have the potential to be used not just for the staging of liver fibrosis, but also for the diagnosis of a wide variety of diseases in the future. Together with high throughput techniques that are already established, this could herald a new era of rapid and non-invasive disease diagnosis.

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Men! Can't live with them, can't live without them…

by Karolina Chocian

Men! Can't live with them, can't live without them…This flippant phrase tends to be the inevitable verdict over a couple of glasses of wine with my female friends. We often feel exasperated about how little men understand us; sometimes it seems like the two sexes are separate species! And yet, something drives us together. Genetics? Perhaps. Surprisingly similar conclusions were recently drawn in lab meeting with Prof Alison Woollard where we discussed several articles on the effect of the opposing sex on health and lifespan in lower model organisms like *C. elegans* and *D. melanogaster*. Are we all in the middle of the 'battle of the sexes'?

Both in the wild and in isolated lab strains, the nematode worm *C. elegans* exists predominantly in hermaphrodite form, producing sperm early in development and switching to egg production as an adult (Figure 1). Males, which by virtue of being male only produce sperm, can arise via a non-disjunction event during meiosis, and constitute less than 1% of the population. The number of males increases in difficult conditions such as high temperature or stress. When males mate with hermaphrodites, an exchange of genetic information occurs, increasing genetic variation in the population. This gives the entire species a greater chance of survival in harsh conditions. Thus, worms show that when times get tough men can actually be quite useful (in ensuring the survival of the species of course).

But what about the other extreme? The hypothesis that female worms "can't live with men" has been tested in ageing experiments, where an artificial ratio of hermaphrodite and male animals is created. In the case of *C. elegans*, a population of worms solely made up of hermaphrodites, have an average lifespan of about 15 days, with the majority of wild-type hermaphrodites dying within three weeks. Interestingly, if male worms are introduced into this artificial population, the average lifespan of the hermaphrodites decreases drastically. However, the amount of offspring they produce increases, because male sperm can outcompete the hermaphrodite sperm.

Hermaphrodites tend to die prematurely (shortly after egg laying) if co-mixed with their male counterparts, and ageing phenotypes also occur prematurely. Co-mixed hermaphrodites may also shrink and appear less healthy when compared to worms of the same age that have never been exposed to males. The magnitude of the aging effect directly correlates with the number of males the hermaphrodites are exposed to (the male to hermaphrodite ratio). The more males on the plate, the faster the hermaphrodites age.

Scientists initially thought this effect was due to the physiological stress of mating. Indeed, genetically modified, spermless hermaphrodites co-mixed with mutant males, which lacked gonads (and therefore the ability to produce seminal fluid and sperm for successful mating), did not cause the hermaphrodites to shrink or their lifespan to shorten, even though some 'safe sex' occurred. On the other hand, sterile hermaphrodites suffered from reduced lifespan when exposed to fertile males. Therefore, it was neither the production of progeny nor successful fertilisation that was to blame for the aging of hermaphrodites (1,2). Therefore, it seems that the early death of the hermaphrodite is actually the result of an 'arms race' between the sexes: the competing physiological demands of the longevity of the individual (female animals) and survival of the species (through male-mediated genetic diversification). In the worm world, hermaphrodite worms that mate with male worms may die soon after laying eggs. Male worms also prefer to mate with hermaphrodites that have not been fertilised by another male - 'virgin' worms seem to be more attractive. This increases the male's reproductive success as it eliminates the chances of the male being the second one to mate with a particular hermaphrodite and therefore having a lesser chance at producing offspring.

> "Are the two sexes simply bad for each other?"

But that is not the end of the story. Have you ever walked into a sports changing room, and known straight away that males have recently been there? Well, it turns out that worms can sense males in their absence too! In one cleverly designed experiment,

it was noted that the lifespan of hermaphrodites were shortened after being kept on dishes that were initially primed with males (i.e. the males were put onto the dishes for 24 hours and then removed). It turned out that some kind of signal secreted by fertile male worms was enough to cause shortening of the hermaphrodites' lifespan. To sense the male signals, hermaphrodites developed a response network consisting of (amongst others) the insulin signaling peptide ins-11 and che-13 protein (responsible for the development of sensory cilia in sensory neurons). Depletion of those two genes in hermaphrodite worms makes them non-responsive to the signals from the opposite sex and can ameliorate male-induced short-lifespan phenotypes (3).

Does this suggest that girls should really stop smelling their boyfriend's T-shirts? And that men really are bad for women? Not quite. Fortunately, our species is more complicated than *C. elegans*; and so far a single factor has not been proven to affect lifespan of either of the sexes (or the attractiveness, despite what some advertising companies would like you to believe). Furthermore, there are even more lessons to be learned about the effect of the other sex on the lifespan from a different model organism, the fruit fly (*D. melanogaster*).

It has been previously shown that exposure to food-based odorants can partially reverse the lifespan extension caused by dietary restriction and that loss of olfactory function can promote longevity and alter fat metabolism. The question, then, is whether the smell of the opposite sex can also alter lifespan in the fruit fly. Considering the previous results from studies in *C. elegans*, it shouldn't come as a surprise that smell does indeed have an effect on lifespan in the fruit fly. Similar studies have shown that female flies exposed to other females that are genetically modified to produce male pheromones have reduced life expectancy. However, males pay an even higher price than females. Male flies exposed to purified female pheromone or other males that have been engineered to produce female pheromones, had a significantly reduced lifespan. It was then shown that these effects were mediated by taste perception through gustatory neurons near to the fly mouthparts (4).

Considering the continuous adaptation and co-evolution of the sexual behaviors of the species, these kind of responses are perhaps not that surprising. In some recent experiments, however, it has been shown that both male and female mice had higher stress levels when handled by male scientists in comparison to female scientists. Importantly, it was firmly established that this was not due to the harshness of the handling technique, as the results were replicated simply by subjecting the mice to the male scientist's sweaty t-shirt. However, the levels of stress experienced by the mice was not increased when they were exposed to a female scientist or her sweaty T-shirt (5).

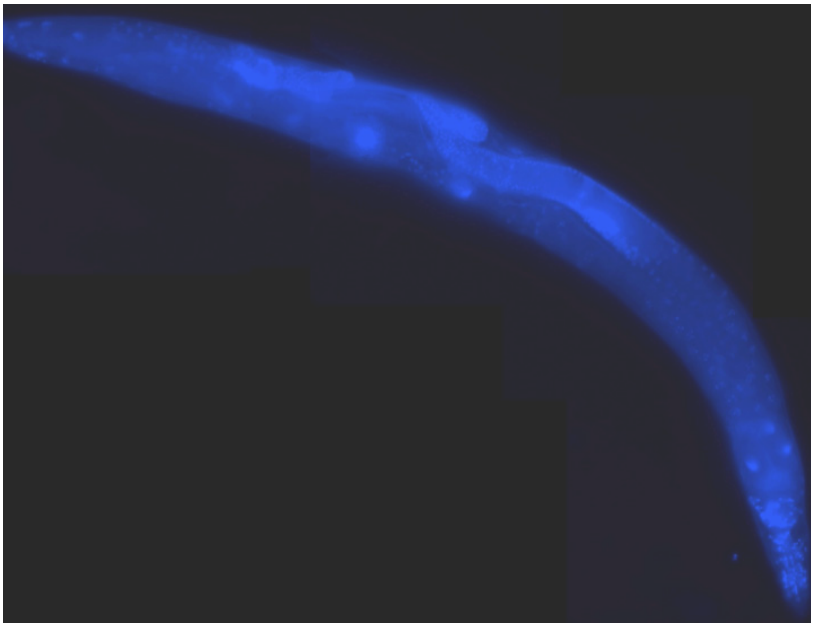

Figure 1: DAPI-stained C. elegans hermaphrodite. Region of dense, blue nuclei highlights the gonads, from which secreted signals are partially responsible for establishing a healthy lifespan.

To sum up with a question: are the two sexes simply bad for each other? Or are we entangled in a never-ending conflict between longevity of the individual (female animals) and survival of the species (through male-mediated genetic diversification)? Whilst a difficult question to answer, it is clear that even amoungst humans, the people we spend most time with do definitely affect our environment, and therefore our biology. For example, statistics show that married men live longer than single ones (6).

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Karolina Chocian is a DPhill student in the labs of Prof Alison Woollard and Prof Jane Mellor, in the Department of Biochemistry

Autoantibodies in CNS disorders

by Dr. Anjan Nibber

Over the past 15 years, autoantibodies directed against extracellular epitopes of cell-surface proteins expressed in the brain and spinal cord have been identified in a number of neurological disorders, such as limbic encephalitis, epilepsy and various forms of encephalopathy. These disorders comprise a rapidly-expanding group of central nervous system (CNS) diseases, and the identification of the antigenic target is important in understanding the relevance and pathogenic potential of these antibodies.

Neurological conditions with an autoimmune etiology were first recognised in the peripheral nervous system. Diseases of the neuromuscular junction, such as myasthenia gravis and Lambert-Eaton myasthenic syndrome, are autoantibody-mediated diseases that have formed the paradigm of autoimmune neurological diseases. Antibodies against the acetylcholine receptor and voltage-gated calcium channels have been identified in these diseases, and both *in vitro* and *in vivo* experiments have shown them to be pathogenic, by interfering with proper neuromuscular transmission (1).

A more recent discovery was of antibodies that target extracellular epitopes of receptors, ion channels and ion channel-associated proteins expressed in the CNS. Antibodies to these cell surface-expressed proteins have been identified, namely the NMDA, AMPA, GABA and glycine receptors and proteins that are part of the voltage gated potassium channel (VGKC) complex (1,2), with all these proteins having important roles in synaptic transmission. By using cell-based assays, it is possible to detect antibodies against conformationally-expressed neuronal surface proteins in patient sera and CSF, which can aid in the diagnosis of these diseases (Figure 1).

While optimal treatment strategies still need to be explored, clinical studies have shown that many patients treated with immunomodulatory regimens show neurological improvements (2). Serum samples often show that antibody levels correlate well with clinical severity, suggesting that these antibodies are pathogenic and contribute to the development and progression of disease. While some antibodies (e.g. NMDAR antibodies) are commonly found in CSF and are able to access brain parenchyma, the details of how other antibodies (e.g. VGKC-complex antibodies) gain access to the brain remains unknown. Prodromal infections have been reported in some patients with antibody-mediated encephalitis, and infection may compromise the integrity of the blood-brain barrier, allowing antibodies to gain access to the brain. Indeed, region-specific permeabilisation of the blood-brain barrier may explain the development of neurological symptoms associated with a select area of the brain, despite these proteins being ubiquitously expressed. In vitro and in vivo experiments have demonstrated the pathogenic capabilities of these antibodies, once they have accessed the CNS. Many of these antibodies have been shown to down-regulate (internalise) the autoantigen from the cell surface or activate the complement cascade, leading to neuronal death. Documentation of a direct modulatory effect of autoantibodies has been less commonly observed.

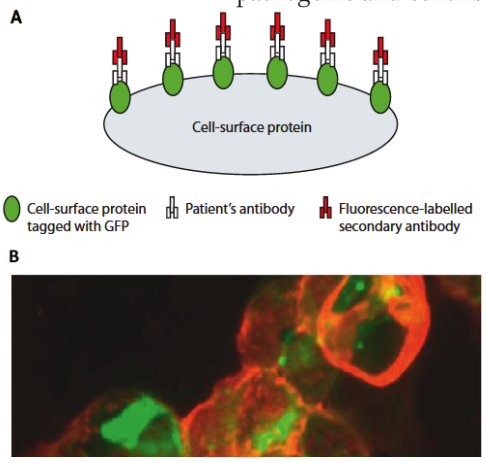

Figure 1: A) Schematic of the cell-based assay protocol. Human embryonic kidney cells are transfected with GFP-tagged cDNAs encoding the protein of interest and then incubated with the patient's sera or CSF. Binding of the patient's antibodies is detected with a fluorescence-labelled secondary antibody. **B) Representative image of a cell-based assay.** The binding of patient IgG (red) to cells transfected with the protein of interest (green) is shown. Adapted from (1).

What triggers the development of these autoantibodies is an area of great interest. Some patients have tumors, e.g. ovarian teratomas, thymomas or small-cell lung cancers, which express CNS autoantigens and may stimulate the production of these antibodies. However, there are a number of non-paraneoplastic cases, and although a prodromal infection may be involved, the etiology in these patients remains unclear.

While these disorders are relatively rare, autoimmune-mediated CNS diseases are a very exciting and interesting newly-defined group of diseases. The identification of known and novel antigen targets may be vital to our understanding of the generation of autoantibodies and optimisation of treatment strategies.

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Dr Anjan Nibber recently completed her doctorate in Dr Bethan Lang's laboratory in the Nuffield Department of Clinical Neurosciences

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An A-ha moment in visual impairment

"I feel like I have walked into an A-ha music video", commented one of our participants. "You look like the holograms in Star Wars", said another. The slightly unusual representation of the visual scene on the displays of the smart glasses (worn by one of our volunteers in Figure 1a) certainly inspired some imaginative feedback. But when Ms O, one of our legally blind volunteers, told us that we had "turned from smudges into people" for the first time, we knew that our approach might make a real difference for visually impaired individuals.

by
Dr Joram van Rheede and Dr Stephen L Hicks

We are the Oxford Smart Specs Research Group, and we are developing a set of electronic glasses that aim to improve the vision of severely sight-impaired people. While their impact can be impressive, what the glasses do is based on a simple principle that can be implemented with relatively low cost components. Most sight-impaired people, even those who meet the criteria for legal blindness, have some remaining vision. However, the very detailed visual world around us can be too complex for them to interpret. The glasses are therefore designed to simplify the visual scene, and boost its most relevant elements. While intelligent computer vision approaches could be used in the future to determine the elements of the visual scene that are most important to the wearer, the glasses currently use proximity as a marker of relevance. The glasses identify the elements of the visual scene that are nearest by combining information from a depth camera (a structured light sensor, such as the one found in the popular Kinect) and a regular RGB camera. The glasses then display parts of the visual scene that are nearby in a brighter, contrast-enhanced fashion, while discarding the potentially distracting background (see Figure 1b for an example of what this looks like).

The glasses were initially conceived to enable visually impaired people to navigate in unfamiliar environments independently – loss of independence being one of the main factors affecting quality of life in visual impairment. The first version of these 'residual vision glasses' therefore focused solely on representing the distance of objects as brightness on the displays, such that brighter objects on the displays corresponded to objects closer to the wearer. We hypothesised that this approach would enable even people with only rudimentary light perception to spot mobility hazards. After a number of trials with people with varying levels of residual vision, we realised that this approach was only beneficial to people on the lowest end of the visual impairment spectrum. The next stage of the project has focused on adding layers of high-contrast detail to objects in the foreground using image processing strategies that are selectively applied to the parts of the visual scene that are nearby. This has resulted in a form of vision that may be more reminiscent of an A-ha video than our normal visual experience. Yet since the day we "turned from smudges into people" in the eyes of Ms O, another of our participants has been able to pick out her favourite cheeses in the Oxford Covered Market by sight, and others have seen their guide dogs walk around for the first time.

Assessing changes in visual mobility performance

Testing improvements in visual ability in the context of the Smart Specs Project presents its own challenge. Measures of visual acuity, visual field size or contrast sensitivity are not appropriate, since the glasses do not technically improve the visual status of the wearer. Instead, we require measures that could probe improvements in visual performance relevant to everyday life. In order to allow us to closely monitor changes in performance, however, our tasks need to result in quantitative, objective and repeatable outcomes.

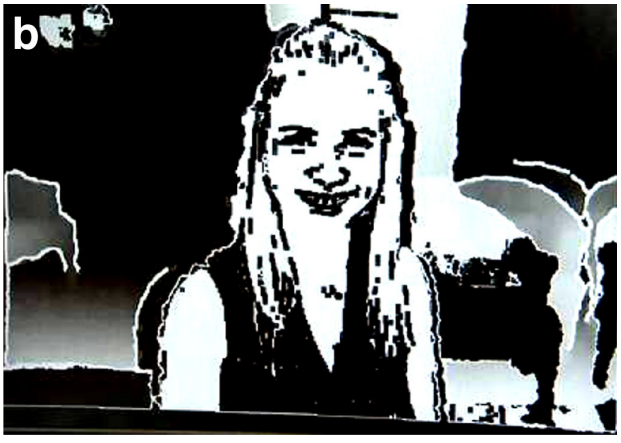

Figure 1. (A) A visually impaired participant wearing our experimental prototype during a trial in Oxford's Covered Market. (B) Nearby elements of the visual scene (in this case, a face) are represented in high contrast, while elements in the background are de-emphasised

The focus of our testing has been on assessing independent mobility in unfamiliar environments. We have tested for improvements in mobility by monitoring how visually impaired volunteers were able to navigate an obstacle course. Such mobility trials have frequently been scored simply using a stopwatch and a tally sheet; the stopwatch to estimate walking speed from obstacle course completion time, and the tally sheet to mark the number of collisions. However, this only provides a rough estimate of walking speed, and only registers actual collisions with obstacles while ignoring near misses or hesitations. Moreover, these aggregate measures are only meaningful when all participants follow roughly the same path.

We were interested in more fine-grained measures of visual awareness of the environment, which could also be used in tasks allowing participants to explore their environment freely. We therefore used a tracking system to continuously log the position of our participants in the room, allowing us to trace their paths and monitor their speed as shown in Figure 2. Initially, the tracking system consisted of overhead cameras, and a heroic research assistant who spent a few long days going through the footage, tracking participants with a mouse. For a later set of experiments, it was decided that a motion capture system was a more humane approach.

The tracking allowed us to probe the dynamics of visual mobility performance. We were able to quantify hesitations through investigating fluctuations in walking speed, giving us a measure of visual confidence. Moreover, not only could we quantify collisions with obstacles, but we could also measure how close participants came to colliding with an obstacle even if they managed to avoid it in the end. This resulted in a 'detection distance', a measure of obstacle awareness. Both these measures could differentiate between visually impaired participants and sighted participants, with low hesitation and large detection distances characteristic of normally sighted volunteers.

In trials with the smart glasses, we saw a dramatic drop in the number of collisions for those participants that were normally unable to avoid obstacles. Moreover, the detection distance increased for those people who previously had many near misses with obstacles. On the other hand, we found that wearing the glasses led to slower walking speeds and increased hesitation. We think these results are very encouraging, and hope that the negative effects on walking speed and hesitation will disappear with training.

A vision for the future

The next step for the project is investigating whether enhancing nearby parts of the visual scene is effective in assisting with everyday tasks, such as objects and face recognition. But more importantly, we want to assess whether the glasses can make a real difference in everyday life. The Smart Specs Project recently won a Google Impact Challenge Award that will allow us to investigate exactly that. In 2015, we will be able to produce a large number of prototypes to loan to participants for up to a month. User feedback will tell us to what extent the glasses are used, and what situations they are most and least useful for. Moreover, running mobility trials and other lab tests, both at the beginning and the end of such a 'loan' period, should indicate the extent to which individual performances improve when using the glasses.

Lastly, there is the challenge of turning our rather unfashionable prototype into something that people can wear on the streets without standing out, and the exciting step of starting the company that will aim to bring the glasses to market for no more than the price of a smartphone. Ray-ban, watch your back…

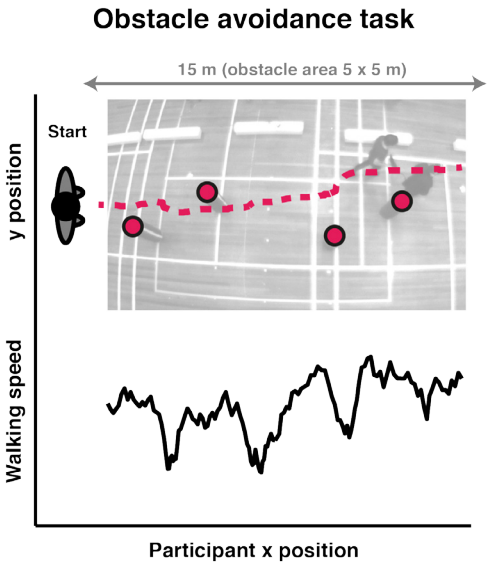

Figure 2: Monitoring of participant position over time during an obstacle avoidance task allowed us to examine the paths they took (top) as well as any variations in walking speed (bottom).

More information can be found at:
http://www.eye.ox.ac.uk/research/oxford-smart-specs-research-group
http://www.smart-specs.com/

A first person account from one of our participants:
http://hannah-thompson.blogspot.co.uk/2014/06/smart-glasses-phase-two-adding-detail.html

Dr Joram van Rheede is a Postdoctoral Research Associate in the Oxford Smart Specs Research Group of Dr Stephen L Hicks

Antibiotics: Why Complacency can Kill

by Emma Pencheon

The threat posed to human health by pathogenic bacteria has gained much attention in recent years. Back in 1928, infection-related mortality was curbed following the serendipitous discovery of penicillin. For the first time, clinicians were well equipped to tackle bacterial infections. However, concerns quickly arose due to the emergence of drug-resistant strains (1). Decades later these fears have been realised to a frightening degree.

Antibiotic resistance occurs when bacteria evade the action of drugs at the molecular level (2). Treating bacterial infections can introduce a Darwinian selective pressure that enables bacteria capable of withstanding the effects of antibiotics to thrive. It is essential that clinicians choose the most effective drugs for a specific pathogen, and that patients complete the treatment course to prevent recurrent infection.

> “If we don’t take the necessary steps now, we will face a very different medical landscape”

Careless handling of antibiotics supports the emergence of drug resistance. So why are we not treating antibiotics with greater respect? A 2013 Europe-wide survey indicated that 41% of UK citizens believed antibiotics kill viruses (3). This highlights the need for better communication from the medical establishment regarding the action of antibiotics. Improved education may alleviate pressure on GPs from anxious patients wanting prescription antibiotics for non-bacterial illnesses. This will in turn reduce the exposure of pathogenic bacteria (which may be present in the patient at levels that are not clinically overt) to antibiotics, thus minimising conditions that drive the selection of drug resistant strains.

Antimicrobial resistance is increasingly under the political spotlight. It has become a public health priority in the UK, being the focus of Chief Medical Officer Dame Sally Davies’ first annual report (4). This year, Public Health England (PHE) published a five-year antimicrobial strategy to mitigate the growing threat of resistant organisms (5). This is a global collaborative challenge, but progress is being made: the WHO published a 2014 global report on antimicrobial surveillance (6) and more recently David Cameron announced the launch of a major international review on antimicrobial use (7).

The joint goals of these initiatives can be nicely summarized by the strategic aims of the PHE five-year plan. The first is to better educate practitioners and members of the public about antimicrobial resistance. This includes highlighting the issue in the healthcare curriculum and raising public awareness through events such as the “European Antibiotic Awareness Day”. The second aim is to safeguard existing treatments, both by preventing infections and ensuring responsible use of antibiotics. The third aim of the PHE strategy is to stimulate the development of novel therapies and diagnostics.

New drugs are not a standalone solution: without better stewardship they represent a short-term fix at best. In future, genomic technologies may allow personalized prescriptions. Improved point-of-care diagnostics will help clinicians make informed decisions, an area of development supported by the 2014 Longitude Prize from innovation charity Nesta (8).

No longer can we deny the threat of antibiotic resistance. The mounting evidence and political momentum behind the issue makes inaction unacceptable. If we don’t take the necessary steps now, we will face a very different medical landscape and a return to the days when common infections come with a death warrant. What will make a difference is if we all take responsibility, in whatever capacity, to handle antibiotics with respect.

Emma Pencheon is a third year medical student at Somerville College.

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Career Insights: Publishing

Catarina Vicente, Community Manager of the Node, shares her experiences after leaving academia.

Why did you leave academia?

My experience of academia was mostly positive. I was part of a well-funded lab led by a supportive and enthusiastic supervisor, my experiments mostly worked, and with a Wellcome Trust studentship I even had a generous stipend. However, when I started applying for postdoc positions during the final year of my PhD, I quickly realised that the last thing I wanted to do was research.

Deciding to leave research was not easy. Academia is structured in such a way that becoming a group leader is the obvious career path, and all your mentors are passionate and successful researchers. Consequently, no one ever tells you that only 10% of PhD students will ultimately become tenure-track faculty and hence you aren't the exception in wanting to leave the bench.

> “Leaving the world of academia can open the door to a new and exciting career”

How did you get into your current position?

I finished my PhD more quickly than expected, and the main catalyst for this was a job advert that I simply could not ignore. The job was advertised by The Company of Biologists, a not-for-profit publishing company, looking for someone to fill the position of Community Manager for the Node. The Node (thenode.biologists.com) is a community blog for developmental biologists, run by the journal Development, where researchers write and discuss topics of interest to those in their field. While anyone can post, a manager is needed to maintain the blog, assist its users and spread the word. It is a well-established blog, with over 5,000 readers every month, and I contributed to it when I was a student. This role really appealed to me and it required a skill set that I had already developed in my free time, such as blogging and social media skills. In addition, I had some interest and experience in building science communities; for example, I had organised the first Oxford fly retreat.

What does your job entail?

My daily routine is quite varied. Typical activities include commissioning pieces for the Node, writing articles and helping others to do so, managing our social media accounts, keeping an eye on science news and papers, and filming and editing movies. I also travel a lot, attend several conferences a year, and give talks at various institutes about social media for scientists and careers in science communication/publishing. The variety of tasks is one of the positive aspects of this position, while the short-term nature of many of the projects gives me a strong sense of achievement. I really enjoy the way that my work allows me to interact with scientists in many different ways, and through this I've gained a broader view of science. In addition, I apply the skills and knowledge that I acquired during my PhD every day, which makes my career choice feel like a natural progression from research.

Working in the office of a publishing company is different from working in the lab and I particularly miss the camaraderie of the latter. In the 'real world' my work is more intense and most of my colleagues have family responsibilities. However, one of the benefits of leaving academia for me has been a better work/life balance.

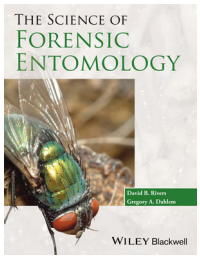
Can you share any advice for those pursuing a career outside academia?

Think about alternatives early. Give yourself time to try different projects and activities outside the lab. This will give you the opportunity to discover what you really enjoy doing, while boosting your CV and improving your job prospects. Most importantly, don't let the idea of leaving research get you down. Leaving the world of academia can open the door to a new and exciting career, and like me you may thoroughly enjoy it.

Affiliation:

Development, The Company of Biologists. Catarina was previously a DPhil student in the Raff Lab at the Dunn School.

BOOK REVIEW



The Science of Forensic Entomology

David B. Rivers and Gregory A. Dahlem

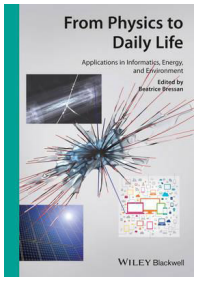
ISBN: 978-1-119-94037-1, Wiley-Blackwell (2014)
Paper, 400 pages, £37.50

Reviewed by Anna Sigurdsson

The Science of Forensic Entomology is an introductory textbook that explores the fundamental concepts and principles that underlie the forensic science of insects in civil and criminal matters. The book is primarily aimed at students, and there is a strong emphasis on pedagogy and easy learning. Each chapter provides an overview and a list of key concepts, which are discussed in detail. At the end of the chapter, these concepts are reviewed and summarised, and there are also questions to reinforce learning. The language is light-hearted and references are made to popular cultural phenomena such as Sherlock Holmes, CBS' show *CSI: Crime Scene Investigation*, and the animated cartoon *Pinky and the Brain*; making the reading very entertaining.

The 18 chapters of this book can be read in any order. Chapters 1-4 consist of introductions to forensic science, the history of forensic entomology, the role of insects in legal investigations, and entomology. Here, we learn that the very first case in forensic entomology took place in 1235, where the Chinese Death Investigator Sung Tz'u solved a murder involving blowflies which were attracted to the murder weapon. Chapters 5-8 focus on necrophagous flies in the families *Calliphoridae* (blow flies) and *Sarcophagidae* (flesh flies), which are most important as evidence in cases of suspicious death or homicides. Different fly species are attracted to a body at different stages of decomposition! The most important species for determining the amount of time between death and post-mortem, are species that use a corpse for reproduction. However, this picture is complicated by insects, such as parasitoid *Nasonia vitripennis* (pteromalid wasp), that can destroy the developing larvae that might otherwise indicate the length of the post-mortem interval. Chapters 9-13 discuss more applied matters, e.g. the biology of post-mortem decay, insect activity influencing bloodstain evidence, and more. Chapters 14-18 take a broader view of forensic entomology, with discussions of archaeoentomology and, interestingly, of how insects can pose threats to humans (by being toxic), and can even threaten national security when used as biotic terrorist tools.

Overall, *The Science of Forensic Entomology* is highly informative, pedagogical, and fun to read. It provides a great introduction to the field of forensic entomology as well as providing the reader with some fascinating general knowledge.



From Physics to Daily Life

Edited by Beatrice Bressan

ISBN: 978-3-527-33261-8, Wiley Blackwell (2014)
Hardback, 386 pages, £65

Reviewed by Kristijan Jovanoski

From Physics to Daily Life explores how technologies originating from fundamental physics have been successfully applied to biomedical research and healthcare. In particular, it details how many of these technologies can trace their origins back to CERN (The European Organization for Nuclear Research), home of many ground-breaking discoveries and fundamental inventions in particle physics. Each chapter has been drafted by a relevant expert, while Bressan has edited the entire book to ensure a coherent and logical structure.

Although the title, "*From Physics to Daily Life*", suggests a book describing physics in everyday life, readers expecting the biomedical equivalents of special relativity in GPS might be surprised to find that one third of Bressan's book focuses on knowledge management and technology transfer instead. These parts of the book read as though they are from a business, economics or human resources textbook and, as such, may have a greater appeal for policy makers, investors, planners and technology companies rather than for members of the research community.

Nonetheless, the rest of the book strives to highlight how knowledge and technologies from physics have incredibly made their way into fields as varied as proton therapy, the 'omics' sciences and public health surveillance. It manages to do so without introducing any equations, making it much easier for readers without a background in physics or mathematics to follow. Although undefined terminology can be found on occasion throughout the book, this does not detract from Bressan's impressive portrayal of an incredible array of topics concerning various sciences and humanities, resulting in a book with interesting content for a wide audience.

Whilst this book may not be what the title initially suggests, it remains a very useful point of reference for readers investigating how physics has affected their particular biomedical research area. It provides an adequate amount of history for each technology described and clearly presents the future directions in research and development. This is a recommended read for students aspiring to lead a biotech start-up or work in areas such as science policy and commercialisation.

Cell and Molecular Biology and Imaging of Stem Cells

Edited by Heide Schatten

ISBN: 978-1-118-28410-0, Wiley Blackwell (2014)
Hardback, 304 pages, £100

Reviewed by Monika Krecsmarik

Cell and Molecular Biology and Imaging of Stem Cells presents a summary of recent developments in stem cell research, with an emphasis on its potential for biomedical and therapeutic applications. It is an interesting read for students, researchers, and clinicians who work in this rapidly evolving field. The book also contains some fascinating new results in veterinary stem cell research, making it equally valuable for veterinary students and professionals. However, the title suggests a much broader content than the book presents and reading the book requires prior knowledge in both the technical and academic aspects of stem cell biology. Nonetheless, each chapter does contain a helpful graphical presentation of each working model, making it more accessible for those less familiar with the subject.

The book is a compilation of twelve chapters, each written as short reviews by experts in their respective areas of research. The chapters are well written, easy to follow and very informative. They have a similar structure, beginning with a short introduction which is followed by the main text, a conclusion and bibliography. The first three chapters describe the use of multipotent perinatal stem cells as candidates for cell therapy and the use of biomaterials and ultrasound in stem cell biology. Later chapters explain how mitochondrial metabolism and cell differentiation are linked and how cell division is altered in the case of cancer stem cells. There are also chapters dedicated to ovarian, hair follicle and intestinal stem cells and their potential use in regenerative medicine for orthopedic lesions and skin diseases.

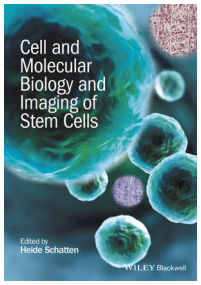
Overall, this book is a valuable tool to keep updated with the rapid advances in stem cell research, including the maintenance of the stem cell microenvironment and stem cell-based therapies. However, the lack of coherent links between chapters, and the inclusion of an introductory chapter, makes it difficult to consider this book more than a collection of reviews.

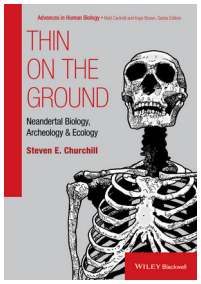
Thin on the ground: Neanderthal Biology, Archeology and Ecology

Steven E. Churchill

ISBN: 978-1-118-59087-4, Wiley Blackwell (2014)
Hardback, 472 pages, £100

Reviewed by Karolina Chocain





It is an exciting time for research into human evolution. In this book the author attempts to bring recent research on Neanderthals together, despite acknowledging that, in a field as fast moving as this (especially with the sequencing of ancient DNA), his views might be out of date by the time of publication. Churchill also assumes his readers have previous knowledge of *Homo sapiens* evolution, human anatomy and the history of Pleistocene Europe.

Homo neanderthalensis or 'Neanderthals' lived during a difficult era in Europe, inhabiting the continent approximately 500,000 years before *H. sapiens* arrived from Africa. Neanderthals survived the waxing and waning of glacier episodes in Europe and therefore must have been well adapted to climatic and ecological variations. Despite this, they lived 'thin on the ground' in small communities of low population densities. Considering the demographic growth of modern humans and their adaptive success, the interesting question is "what kept Neanderthal numbers in check?" Churchill tries to answer this in his publication.

A noteworthy point for discussion is the thermodynamic approach to understanding Neanderthal biology. The estimation of the caloric demand for the average Neanderthal to move camp, hunt, engage in everyday activities and reproduce requires a significant amount of approximation, and therefore the final figures are largely guesswork. Fortunately, the author recognises this and acknowledges it as only one of many possible ways to look at Neanderthal biology and ecology.

There are many astonishing facts that can be learnt from this book and if, like me, you are interested in evolutionary genetics, this is a very good read. From a perspective of scientific methods, however, the virtues of the calorimetric approach to Neanderthal biology requires further explanation. It would, perhaps, be useful to first get to know the topic better by reading additional publications. Nevertheless, this book is written using a clear and accessible language, and would also be useful to the reader who possessed no prior knowledge of Neanderthal Biology.

5' with ... Bart Cornelissen, PhD

Bart Cornelissen studied at the Universities of Hasselt and Ghent in Belgium and remained in Ghent to complete his PhD on the topic of radiolabelled growth factor proteins. After spending some time in Toronto and at Oxford as a postdoctoral fellow and lab manager, Bart became a career development fellow, and has been junior group leader in the Department of Oncology in Oxford since early 2012. His group focuses on the development of radiolabelled compounds for medical imaging in oncology with PET (positron emission tomography) and SPECT (single photon emission computed tomography).

How did you get into your field of research?
Significant discoveries. After those many late nights in the lab, you might be the only person in the world that knows about that new finding, the only one that knows the answer to that particular question. It is a great feeling. A good scientist is someone who is passionate and fearless about asking questions and finding answers to them.

How do you think the field will progress over the next decade?
In nuclear medicine imaging, there is already a shift from 'classical' gamma camera imaging to PET imaging. In the future, increasing combinations of techniques will be used to combine the ability of PET to image molecular processes with the advantages of MRI imaging, such as high spatial resolution. As a result, medical imaging as a whole, and functional imaging in particular, will gradually become more integrated into standard clinical practice.

What are you most proud of in your career so far?
In my first year at Oxford, we designed a method that enabled non-invasive visualisation and quantification of DNA double-strand breaks in a tumour in a living organism. It was a good example of being at the right place at the right time. I was able to apply specific techniques I had learned, before testing the ideas of my then supervisor.

Do you have any amusing stories from the lab?
In the first year of my PhD, colleagues were doing imaging studies with pigs. One day, one of them escaped, and we had to chase a radioactive racing piglet down the lab. We only caught it after half an hour - that was not a good day!

What do you think makes a 'good' scientist?
There are actually a lot of different ways to be a good scientist - it depends how that 'goodness' is measured. Having an open mind and being creative are some good traits. And never say something won't work until you've tried it.

When you are not in the lab, you are..?
With my wife, our two-year-old son and our two dogs, out and about in the countryside around Oxford.

What do you like most about being a research scientist?
The thing I personally like most is the ability to come up with an idea or concept that can then be worked on from the design stage to chemical synthesis, over *in vitro* to *in vivo* applications, and sometimes all the way to patient studies.

Do you have a scientific hero?
It may be a cliché, but as a boy I was fascinated by Einstein and the science-fiction writer Asimov. I also really enjoy watching anything by David Attenborough.

What advice would you give to students looking to pursue research as a career?
Don't plan your career too much. Make choices based on what you enjoy doing. Work hard. Take opportunities as they present themselves. Also, the grass is not necessarily greener on the other side.

Dr Bart Cornelissen is a junior group leader in the Oxford Institute for Radiation Oncology

Write for *Phenotype*?

- The deadline for article submissions is Friday of 8th week, 13 March 2015
- We accept articles on any aspect of biological sciences research, books or science education
- For next issue we particularly welcome articles related to plant science or the environment
- Articles can be either 650 or 1300 words

If interested, please get in touch: christopher.hillyar@jesus.ox.ac.uk.

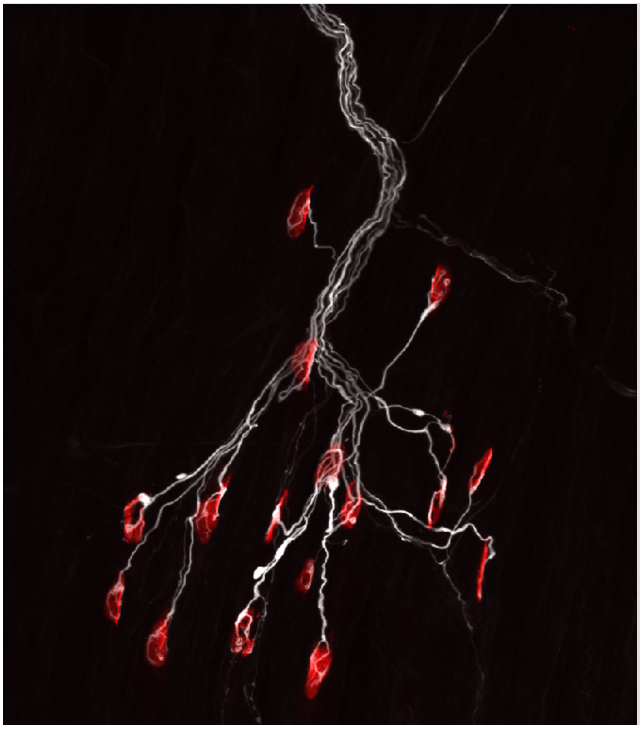
Work for *Phenotype*?

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

SNAPSHOT Research Image Competition

This issue's winner is...

Dr Suzan Hammond



Suzan received her degree from Northwestern University in 2010. Since then, Suzan has been working as a post-doctoral researcher in Professor Matthew Wood's laboratory. She has also held a Somerville College Fulford junior research fellowship for two years. Currently, Suzan is involved in translational research for spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD).

The winning image of the SNAPSHOT competition shows neuromuscular junctions (NMJs) of the transversus abdominis or transverse abdominal (TVA) muscle. The axon and the end terminals of motor neurons (white) were stained using antibodies against neurofilament and synaptic vesicles. Muscle endplates (red) were stained with alpha-bungarotoxin, a 74 amino acid peptide isolated from Bungaris multicinctus venom which binds to the acetylecholine receptor at the neuromuscular junction. This work was performed to study the development of NMJs in a mouse model of spinal muscular atrophy following various forms of treatment. The TVA muscle was harvested at early post-natal development, which accounts for the less defined structure of the endplates compared to that of mature endplates.

Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality due to motor neuron degeneration, with an incidence of one in 10,000. SMA arises from loss-of-function mutations in the survival motor neuron 1 (SMN1) gene. SMN1 protein is ubiquitously expressed and is indispensable for motor neurons. The nearly identical gene SMN2 produces transcripts in which exon 7 is preferentially excluded (~90%), generating only small amounts of functional SMN2 protein. Restoring sufficient levels of SMN protein may lead to the restoration of function of motor neurons in patients affected by SMA. Increasing the level of SMN protein centrally and peripherally by forcing SMN2 exon 7 inclusion with a systemically delivered compound is an ideal approach to therapy and, therefore, a high priority for clinical research on the topic.

Both spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD) are treatable with splice switching oligonucleotides that have the effect of correcting such pathogenic genetic mutations. Novel peptides and antisense oligonucleotides, which effectively act as gene therapeutics, have been developed for systemic biodistribution. Antisense oligonucleotides have been used safely in clinical trials; however success has been hampered by poor distribution toward important tissues such as skeletal muscle, heart and, in the case of SMA, central nervous system. Through a productive collaboration with Professor Michael Gait's group at the MRC Laboratory of Molecular Biology, University of Cambridge, Suzan seeked to improve these therapies using highly potent peptides, which can be directly conjugated to oligonucleotides to enhance their systemic biodistribution. These peptide-oligonucleotides have had great success in the pre-clinical studies for DMD. Suzan's most recent work focuses on the passage of these peptide-oligonucleotides across the blood brain barrier to treat SMA.

When asked about her time in Oxford Suzan remarked, "Moving to the UK to work at the University of Oxford has been a wonderful experience. I am particularly proud to work in the department of physiology anatomy and genetics, which puts emphasis on the support of women in science."

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SNAPSHOT
Research Image Competition

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

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT**?
We are now accepting entries for pictures to be featured on the cover of *Phenotype* Trinity 2015.

To enter, send images to christopher.hillyar@jesus.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.
The deadline for the competition is 16 March 2015.

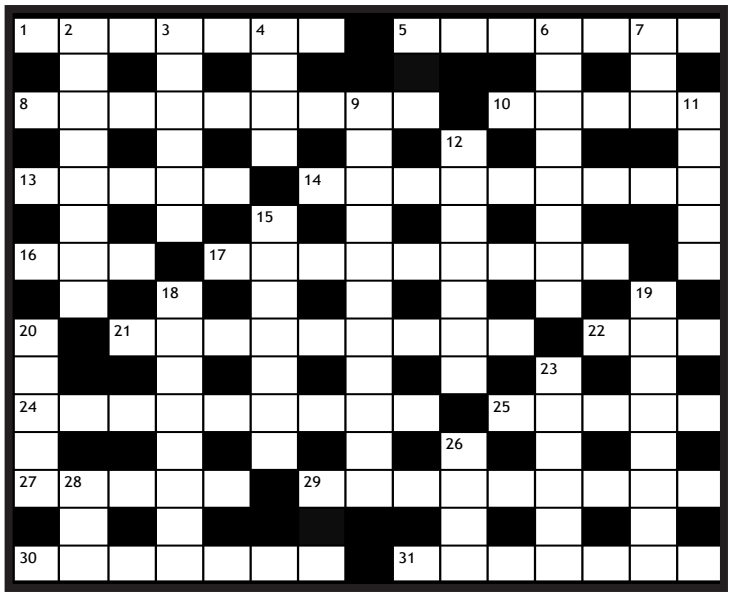
PHENOTYPE crossword

Check out the answers to last issue's crossword at the bottom of the page. Fish challenges Phenotype readers to this latest cryptic crossword on the theme of evolution. Can you crack it?

Enter this term's competition by sending your answers to christopher.hillyar@jesus.ox.ac.uk. Entries received before the 16 March 2015 will be entered into the prize draw.

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

WILEY-BLACKWELL



ACROSS

1. *31's partner asks about your old pit (7)*
5. *Copper takes about three seconds when following princess in debate (7)*
8. *A way to cut through attitudes regarding criteria (9)*
10. *"It increases blood flow?" - good man swallows a number (5)*
13. *He's a pain in the neck (5)*
14. *A component of the 9 is caught in a wave of excrement (9)*
16. *see 22*
17. *Taking sides? (9)*
21. *Is cheeky to support French cats (9)*
22,16 *Cryopreservative is witty and cool (3,3)*
24. *Guardians dispatched Ernie the golfer (9)*
25. *It's not common to contaminate (5)*
27. *Part of a flower in mountainous country changes polarity (5)*
29. *Crushed 16 is used by the Spanish back so he can stretch (9)*
30. *Protozoa have no right to love in Italy, or love in modern England? (7)*
31. *In 9, A commercial with european number (8)*

DOWN

2. Chop and trim yttria in event of three parts (3-5)
3. Principality enclosed by a stream on a coast (6)
4. It's straight and tidy (4)
6. Young baseballer to pitch holding second innings lead while on base (8)
7. Students back a star (3)
9. Round and round? (6,5)
11. Article about a case (5)
12. Put in position where politicians lie? (7)
15. He's the sort to put about his triumphs (7)
18. Scatter a distortion and cut the bollocks! (8)
19. She was, to be honest, the seed (8)
20. Foundations at the heart of the 9? (5)
23. He reports happenings (6)
26. 18 horse wearing back support and headdress (4)
28. Steer is beheaded by tree (3)

Congratulations to Daniel Gbra from Corpus Christi College who won the Michaelmas Term 2014 crossword competition.

Answers to the crossword from Issue 19 - Michaelmas '*14*

Across 1. candied, 5. species, 9. ava, 10. spoiler, 11. flosser, 12. inlet, 13. uteri, 14. anoint, 17. hamper, 18. fossil, 21. deseed, 25. dorks, 26. tetra, 27. upslope, 28. reroute, 29. nay, 30. fittest, 31. ovaries

Down 1. cuspid, 2. neoplasm, 3. islet, 4. darwin, 5. safari, 6. evolution, 7. instep, 8. survival, 15. of/the, 16. headstone, 17. handcuff, 19. sastrugi, 20. preset, 22. silent, 23. embryo, 24. navels, 26. terra

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