PHENOTYPE

Issue 10, Michaelmas Term 2011

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DNA replication in archaea
Bionic glasses

Epigenetics and longevity
Professor Jane Mellor discusses treatments for ageing

Human embryonic stem cell research
Political battlefield

Gambling with Parkinson's
Side effects of Parkinson's treatments

5' with...
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Transgenic animals
Changing more than intended?

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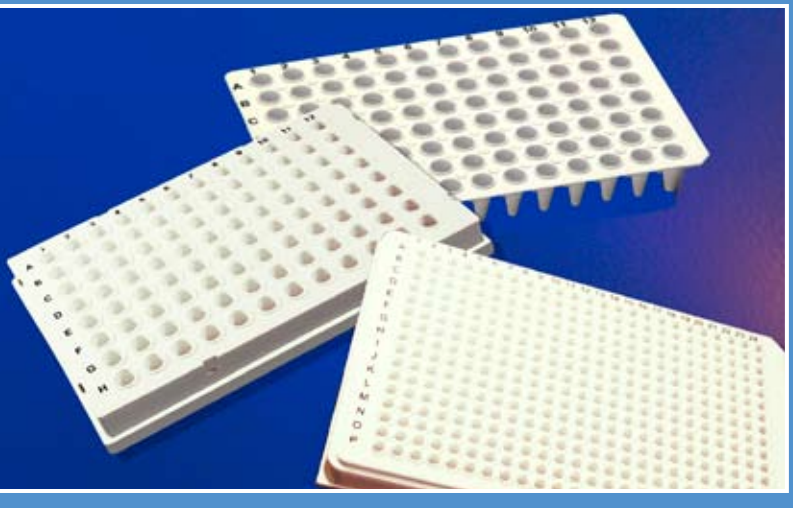

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EDITORIAL

Welcome to the new academic year! It is my privilege and honour to greet you with the 10th issue of *Phenotype*. I wish you all the best throughout the year and would like to thank our talented writers, highly committed editing team, and generous sponsors, especially the Medical Sciences Division Skills Training Fund, who made this issue possible.

As always, this issue includes articles on a diverse range of topics, contributed by students, post-docs and PIs across the University. Professor Jane Mellor discusses the fascinating work underway in her laboratory and others' to understand how cells age, and how this knowledge can be applied to treat age-related diseases. Also concerned with ageing (although apparently not his own; see p30!) is our *5' with* interviewee, Professor Quentin Sattentau, who describes some hair-raising experiences with formaldehyde bombs.

Dr Carinne Piekema introduces us to new research explaining why dopamine agonist treatments for Parkinson's can trigger compulsive behaviours. The focus on dopamine continues in Nicola Platt's exploration of the use of transgenic techniques in neuroscience. She describes the unexpected ways that these techniques can affect the pathways and systems that they are used to study. We also bring you articles addressing the challenges involved in controlling bovine TB, the role of complement in immunity, and the politics surrounding human embryonic stem cell research.

Our Regulars section includes a review of *The Immortal Life of Henrietta Lacks*. This article certainly reminded me of our responsibilities as scientists, not only for ensuring that our experiments are performed ethically and results reported honestly, but also for accurately communicating science to the public. This line of communication failed in the case of the Lacks family who, through genuine lack of scientific understanding, and the failure of scientists and medics to comprehend (or possibly even care about) this lack of understanding, were subjected to unnecessary trauma.

The importance of taking responsibility to promote gender equality in science was recently brought to my attention as Oxford University's Medical Sciences Division faces a cut in funding of £100 million from National Institute for Health Research (NIHR) if it fails to show it is taking sufficient and effective action on gender equality by 2015. The Chief Medical Officer of the Department of Health, Professor Davies has issued an ultimatum to all Medical Schools in the UK, stating that NIHR funding will be cut if they fail to attain an Athena SWAN Silver Award as proof of their commitment to promoting equality. The Athena SWAN Award is given to departments who show that they are taking constructive initiatives to make progress on equality. The key to a successful Athena SWAN application is the support and energy of the department in carrying through its self-assessment process, and in delivering on its action-plan. Every member of a department, from undergraduates to post-docs to tenured academics and technical staff, has a role to play in their department's Athena-SWAN application. Whether you have anecdotes surrounding childcare and flexible work, ideas for how to improve our record on gender equality, or would like to take a leading role as a catalyst in your department, get in touch with the contacts below*.

Moving from responsibilities to rewards, I am excited to announce the new Article of the Year competition. All Features and Science and Society articles published in *Phenotype* this academic year will be entered into the competition, judged by our 2011/12 guest judge, Michelle Grayson, Associate Editor of *Nature Outlooks*. I hope that this serves as inspiration for those of you considering writing for *Phenotype*! If you would like to get involved with any aspect of *Phenotype* production, please do get in touch via oubs@bioch.ox.ac.uk.

Shaoyan Liang

* Eric Liu, Medical Sciences Divisional Representative: eric.liu@hertford.ox.ac.uk; Yuan Yang, Vice-President (Women), Oxford University Student Union: women@ousu.org.

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OUBS SEMINARS

Michaelmas 2011

Monday 10th October
Dr Thomas Clarke, *School of Biological Sciences, University of East Anglia.* "Title tbc"

Monday 17th October
Dr Anton Wutz, *Centre for Stem Cell Research, Cambridge University.* "Gene dosage in mice: From X-inactivation to new tools for genetics"

Monday 24th October
Dr Jenny Gallop, *The Gurdon Institute, Cambridge University.* "Understanding cell shape: Reconstitutions of actin polymerisation at the membrane/cytosol interface"

Monday 31st October
Dr Jason Carroll, *Cancer Research UK - Cambridge Research Institute.* "Understanding estrogen receptor transcription in breast cancer"

Wednesday 2nd November
Prof David Livingston, *Harvard Medical School.* "Title tbc"

Friday 4th November
Sir Richard Gardner Celebratory Lecture 2011 (With Oxford Stem Cell Institute):
Prof Konrad Hochedlinge , *Harvard University*
"Dissecting the mechanisms of cellular reprogramming". Lecture Theatre, Medical Sciences Teaching Centre

Monday 7th November
Prof Francis Barr, *University of Oxford* . "Title tbc"

OUBS FEATURED SEMINAR:
Monday 21st November
Prof Pierre Gönczy, *École polytechnique fédérale de Lausanne.* "Mechanisms of centriole formation"

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

Centrosomes and Cell Division

David Yadin

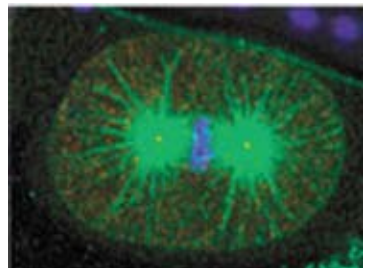
On Monday 21 November, OUBS will host a talk by Professor Pierre Gönczy from the École Polytechnique Fédérale de Lausanne in Switzerland. Dr Gönczy's laboratory examines the roles of centrosomes in eukaryotic cell division.

The mitotic spindle in a dividing eukaryotic cell is important for the correct segregation of chromosomes from the mother cells into the daughter cells. Centrosomes, which comprise a pair of centrioles and the pericentriolar material, organise microtubules into the mitotic spindle during mitosis. Prior to mitosis, centrioles duplicate and then during mitosis move to opposite poles of the cell. The spindle attaches to and aligns the chromosomes along the equator of the dividing cell and subsequently contracts, pulling the chromatids to opposite poles. While centrosomes have been known about for a century, a molecular understanding of their function has only recently started to emerge.

Gönczy's lab has successfully employed the nematode *Caenorhabditis elegans* to study centrosome function. *C. elegans* is a powerful model organism because of established tools for manipulating gene expression and its transparent exoskeleton, which allows direct observation of cell division in live worms. Important genes can be identified by random mutagenesis and screening of worms for particular phenotypes, followed by genetic mapping. RNA interference can then be used to knock down the expression of a particular gene, in order to validate its function. In addition, fusing a gene of interest with a gene encoding a fluorescent protein can allow visualisation of the protein's function in a transgenic worm. Using these approaches, Gönczy's group has identified proteins involved in centriole formation and deduced the pathway of their assembly.

Work by Gönczy's group and others has implicated several proteins in the initial formation of centrioles in *C. elegans*: the coiled-coil proteins SAS-4, SAS-5, SAS-6 and SPD-2 and the kinase ZYG-1 (1). SAS-5 and SAS-6 are recruited by SPD-2, which in turn is dependent on ZYG-1 for localisation to the nascent procentriole. Recently, a collaborative study investigated the structural role of one of these proteins (2). It had long been known that centrioles have nine-fold radial symmetry, but the structural basis of this was unknown. The structure of the SAS-6 homologue Bld12p, from the green alga *Chlamydomonas reinhardtii*, was determined by X-ray crystallography. By combining this result with the dimeric structure of the N-terminal portion of Bld12p, and electron microscopy of the oligomer, a model of the oligomeric structure was built, a ring with nine-fold symmetry. The importance of the oligomerisation of SAS-6 was demonstrated in *C. elegans* embryos by introducing specific mutations into the *sas-6* gene. These mutations perturbed the interface between the SAS-6 monomers and resulted in the inhibition of centriole formation.

Gönczy's group has also investigated the role of centrosomes in the timing of cell division (3). The introduction of a mutation into *C. elegans* that prevented centrosome assembly led to defective timing of the nuclear envelope breakdown of the male and female pronuclei, which usually occurs at the beginning of mitosis. The group showed that centrosomes recruit the Aurora A kinase, which can phosphorylate and thereby activate Cdc25 phosphatases. These phosphatases activate the cyclin-dependent kinase Cdk1/cyclin-B, which drives the cell into mitosis by phosphorylating various targets. Defects in centrosome assembly therefore slow the onset of mitosis by stalling this phosphorylation/phosphatase cascade.



A one-cell-stage *C. elegans* embryo in preparation for division. Alpha-tubulin of the mitotic spindle is seen in green and condensed DNA in blue. Yellow dots show the overlay of alpha-tubulin with the SAS-4 centrosomal protein which is red-labelled (Reprinted by permission from Macmillan Publishers Ltd: Nature Cell Biology, 6: 656-664 (2004)).

Asymmetric cell division is important for the generation of different cell types. During development, the breaking of symmetry is essential for the formation of axes (anterior-posterior, dorsal-ventral, left-right), which give rise to developmental patterning. A number of factors are involved in generation of asymmetry, including centrosomes. One of the key steps is the asymmetric positioning of the spindle. The molecular motor dynein pulls the microtubules to allow correct, asymmetric positioning of the spindle. Gönczy has shown the importance of Gα proteins (one of the components of heterotrimeric G proteins that interact with G-protein coupled receptors) in the generation of pulling forces through interactions with dynein (4). The group has identified a multitude of other factors involved in regulation of asymmetry, including showing that Gβ proteins can regulate Gα-mediated force generation.

This snapshot of Professor Gönczy's research illustrates the diversity and depth of current centrosome research. His talk in November will no doubt provide insights into the latest research in this exciting field.

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MicroRNA Controlled Adenovirus Mediates Anti-Cancer Efficacy without Affecting Endogenous MicroRNA Activity

Cawood R, Wong SL, Di Y, Baban DF, Seymour LW (2011) *PLoS One* 6(1):e16152.

Viral therapeutics provide a novel approach for vaccines, gene therapy and virotherapy, but to date, no universal mechanism has been demonstrated to control all viruses in a tissue-specific manner. MicroRNAs, which are non-coding RNAs that regulate mRNA translation, are required by viruses to exploit host mRNA translation, facilitating viral replication. By designing viral vectors which contain binding sites for tissue-specific microRNAs, a universal control mechanism for viral replication may be achieved, allowing viral replication to be limited to target cell types.

A major caveat in the use of viral therapeutics is the expression of toxic viral proteins. For example, the wild-type Adenovirus type 5 (Ad5WT) strain possesses anti-cancer activity but also causes acute hepatic toxicity in mice. Toxicity is believed to be linked to the expression of the E1A protein in hepatocytes, which activates expression of viral genes leading to hepatocyte death. To circumvent this, the Seymour group previously inserted binding sites for the hepatocyte-specific microRNA *mir122* into an E1A expression cassette (denoted as Ad5mir122), which resulted in the cleavage of E1A mRNA through an RNAi-like mechanism. Here, Cawood *et al*. investigated whether insertion of the *mir122* target sequences leads to unwanted consequences, such as depleting endogenous levels of microRNAs required for cellular processes.

The authors demonstrated that Ad5mir122 treatment significantly down-regulated E1A mRNA and protein levels. They also showed that inclusion of *mir122* binding sites in the Ad5mir122 strain did not affect the strain's anti-cancer activity when compared to Ad5WT in *mir122* negative tumour cells, as well as in a hepatoma xenograft mouse model. Administration of either strain caused no difference in endogenous liver *mir122* levels. In addition, to rule out that *mir122* is sequestered away from its endogenous mRNA targets by direct competition with Ad5mir122 upon treatment, microarray genome-wide mRNA profiling was performed; this revealed that Ad5mir122 administration induced significantly fewer genome-wide mRNA changes in hepatocytes than Ad5WT administration and that no *mir122* target mRNAs were affected by Ad5mir122 treatment.

Together, these results demonstrate that exploiting microRNAs to control selective viral replication does not necessarily affect levels of endogenous microRNA or mRNA targets and opens the door for the use of viral strains, such as Ad5mir122, in virotherapy.

Replication Termination and Chromosome Dimer Resolution in the Archaeon Sulfolobus solfataricus

Duggin IG, Dubarry N, Bell SD (2011) *EMBO J* 30(1):145–153.

Archaea possess a simplified, and presumably ancestral, form of the eukaryotic DNA replication machinery. However, like bacteria they contain circular chromosomes, replication of which produces chromosome dimers, which must be resolved into monomers. The hyperthermophilic archaea, *Sulfolobus solfataricus*, contains a single circular chromosome with three origins of replication. This study aimed to determine how *S. solfataricus* coordinates termination of DNA replication and dimer resolution, mechanisms that were previously unknown in archaea.

First, the authors used 2D gel analysis to search for sites of replication termination, revealing that *S. solfataricus* lacks defined termination sites and that termination occurs via random collision of replication forks. This resembles the eukaryotic process and is unlike the bacterial termination process, which occurs at a specific site on the chromosome (*ter*).

Next, Duggin *et al*. investigated chromosome dimer resolution. In bacteria, chromosome dimer resolution occurs at a specific site (*dif*), and is mediated by XerCD recombinase. *S. solfataricus* encodes a homologue of XerD, but not XerC. Duggin *et al*. deleted the *xerD* homologue and the resulting strain displayed reduced growth-rates and larger cell volumes, reminiscent of *E. coli* mutants defective in chromosome dimer resolution.

A candidate XerD binding locus was then identified using chromatin immunoprecipitation (ChIP) and was confirmed using electrophoretic mobility-shift assays (EMSAs). The identified binding site, which exhibited sequence similarity to bacterial *dif* sites, was confirmed as a *dif* site using inter- and intramolecular recombination assays. In contrast to bacteria, the *Sulfolobus dif* site was located outside the fork fusion zone, which suggests that in *Sulfolobus* replication termination is spatially and temporally distinct from the dimer resolution process.

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CLAUDE MONET IS FEELING LOW...

BY CAROLINE DAHL
...WHO DOES NOT HAVE STEREOSCOPIC VISION!

EXHIBIT OF BIONIC GLASSES AT THE ROYAL SOCIETY'S SUMMER EXHIBITION AND THE NATURAL HISTORY MUSEUM'S 'SCIENCE UNCOVERED' EVENT 2011, BY S.L. HICKS, I. WILSON AND C. KENNARD, DEPARTMENT OF CLINICAL NEUROLOGY AT THE JOHN RADCLIFFE HOSPITAL

Epigenetics and longevity

by Prof Jane Mellor

Why do some organisms senesce and die, while others exhibit negligible senescence with no loss of fertility over time? My group is using the yeast *Saccharomyces cerevisiae*, the worm *Caenorhabditis elegans*, mammalian cell lines, and mouse models to develop new treatments for age-related conditions and to understand the conserved processes that contribute to ageing. To achieve this, we are collaborating with several research groups and Oxford-based Chronos Therapeutics.

Why do we age?

Only organisms that have a distinction between soma and gametes can age, as material capable of generating new individuals must not age in order for the species to persist. Hydra, for instance, can generate new individuals from any body part, thus appears not to age. This distinction between soma and gamete exists even in single-celled eukaryotes such as *S. cerevisiae*, making yeast an excellent model for animal ageing.

Yeast age over time (1). This is known as chronological lifespan (CLS) and acts as a model for quiescent, non-replicating cells such as neurons and adult stem cells. Yeast also show replicative ageing, or senescence, and are limited in the number of daughter cells they produce, acting as a model for proliferating stem cell ageing. As yeast is single-celled, both forms of ageing must result from the separation of gametes and soma in asymmetrical cell divisions.

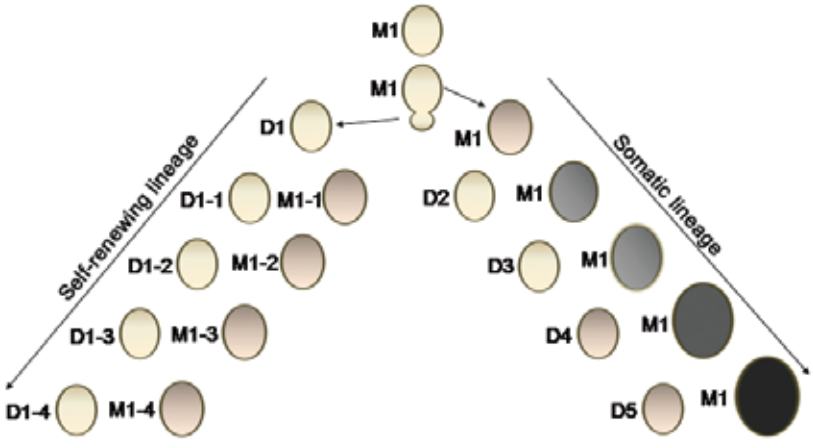


Figure 1. Asymmetric cell division in yeast results in a different fate for daughter (D) and mother (M) cells. Asymmetrical division of a virgin cell (M1) results in two lineages. The virgin daughter cells (D1 to D1-4) constitute a self-renewing germ lineage with infinite replicative potential. The mother cell M1 produces another four daughter cells (D2 to D5), accumulating more damaged protein and organelles at every division, represented by the darker larger cells. As M1 ages, the mechanism for segregating damage to the mother cells gets less efficient and the daughter cells (D4 and D5) also begin to age. Similarly, when D1 divides, the resulting mother cell (M1-1) accumulates damage from her daughter cell (D1-1). The fates of M1-1 to M1-4 and D2 to D5 are not shown for clarity.

A single yeast cell has two phases of life: gamete and mother cell (Figure 1). A gamete may give birth to a daughter gamete, thus becoming a mother cell. Mother cells resemble soma and age, showing senescence rather than quiescence, and a limited capacity to replicate. This is illustrated by the response of mother and daughter cells to glucose starvation, a model for chronological ageing (Figure 2). Mother cells senesce and eventually undergo apoptosis while virgin daughter cells become quiescent, retaining the ability to proliferate once nutrient availability returns. The nutrients released following apoptosis of the mother cells can be utilised by the daughters to aid survival. Non-random segregation of damaged DNA (2) or mis-folded protein (3) into the somatic cells may be used to keep the gametes' lineage 'forever young' (Figure 3).

Asymmetric division between yeast mother and daughter cells establishes a pseudo-programme which results in cell death. A pseudo-programme describes a process by which a secondary, 'unintended' action occurs after the programme has completed its 'intended' task (4). In this case, the intended task is to maintain the stem cell lineage by coordinating cell proliferation with environmental signals such as nutrient availability (Figure 4A). The unintended action is that, in the presence of nutrients, the programme continues to drive cell proliferation despite the fact that mother cells are accumulating damaged DNA, proteins and organelles (Figure 4B). Thus, continued proliferation occurs at the expense of longevity of the mother cells, leading to ageing and death.

Reversing the effects of ageing

Remarkably, this programme is reversible. Lifespan in yeast, flies and mammals can be extended by calorie restriction (CR) and interventions such as rapamycin addition that promote CR by down-regulating pro-growth signalling pathways (5) (Figure 4C). Genetic interventions that mimic CR from birth lead to smaller organisms, suggesting that normal growth is compromised. However, treating middle-aged mice with rapamycin resulted in reversal of the pseudo-programme *after* the growth phase had completed, improving longevity and alleviating age-related conditions in the mice.

These interventions do not target a single 'longevity gene' but promote small changes in the expression of a large number of genes. Both intrinsic and environmental stressors, including ageing, genome instability and

food shortage, induce survival responses to overcome the crisis and extend lifespan. This is illustrated by recent research with mouse models of DNA repair-deficient progeroid syndromes, which cause premature ageing and death. Long-lived mice share similar patterns of gene expression with progeroid mice, both showing suppression of endocrine and energy pathways in response to stress (7). Interestingly, treatment with rapamycin, inducing CR, reduces progeroid symptoms (8).

One paradox in ageing research is that exceptionally long-lived people do not have 'good genes' to explain their longevity. In fact, they have more disease-related genes in their genome than those with normal lifespans, suggesting that something is buffering or cancelling out the negative effects of these harmful genes, allowing them to accumulate. A likely protective candidate is the individual's epigenome. This is consistent with ageing being a result of a reversible pseudo-programme controlling how genes are expressed over a lifetime.

Epigenetics in longevity

The epigenome controls gene expression and DNA repair, thus influencing cell phenotype (9). Three components generally contribute to the epigenome: methylated cytosine residues; histone protein modifications; and RNA molecules. Core histone proteins in the nucleosome, the basic building block of chromatin, are subject to post-translational modifications, including reversible acetylation, methylation, phosphorylation and ubiquitylation at over 100 different sites (10). These modifications guide proteins to specific sites on the chromatin to influence gene expression or DNA repair (11). Non-coding RNA provides an alternative way to guide the modifying enzymes (12).

The enzymes that reversibly acetylate and methylate proteins, DNA and RNA require co-factors that are central intermediates in metabolism or synthesised from essential amino acids and thus are influenced by diet. These cofactors include acetyl coenzyme A (CoA), nicotinamide adenine dinucleotide (NAD^+), S-adenosyl methionine and α-ketoglutarate. Limited periods of extreme CR, used to treat type II diabetics, change energy balances and levels of metabolic intermediates, thus influencing the epigenome and patterns of gene expression. Thus, periodic ritual fasting may reset epigenomes and contribute to better health.

Dietary quality is also likely to influence ageing and behaviour. Newborn rats deprived of maternal care show changes in patterns of DNA methylation linked to anxiety. Anxiety in these rats can be reduced by flooding their brains with nutrients that directly alter the epigenome and patterns of gene expression in the brain. Diet may therefore affect the phenotype by shaping the epigenotype.

Acetylation and ageing

In yeast, reversible protein acetylation is an important component of ageing. The NAD^+-dependent yeast deacetylase sirtuin, Sir2, plays an important role in replicative longevity. Sir2 deacetylates lysine (K) 16 on histone H4 to promote nucleosome stability, particularly at telomeres (13). Age-related decreases in Sir2 levels may thus lead to breakdown in chromatin structure. Interestingly, three mammalian sirtuins also deacetylate H4K16, suggesting that regulated histone deacetylation and nucleosome stability will feature in anti-ageing mechanisms in many eukaryotes. Histone acetylation is also important for nucleosome stability in the wake of elongating DNA and RNA polymerases (14). This requires dynamic acetylation at K56 on histone H3 (H3K56ac) by the Rtt109 lysine acetyltransferase (KAT) using acetyl-CoA as cofactor and the histone chaperone Asf1.

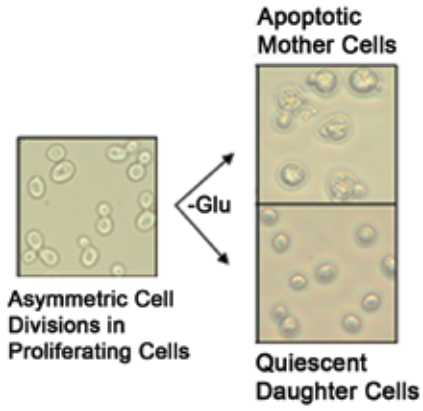


Figure 2. Glucose starved (-Glu) mother and daughter yeast cells form two distinct populations. The daughter cells become quiescent (G0) and are able to survive for extended periods. The aged mother cells become apoptotic.

Sir2 also controls asymmetric cell division and resulting fates of mother and daughter cells (3). Here, Sir2 acts on a chaperone required for the formation of actin cables. Daughter cells are kept young by retrograde transport of damaged proteins along actin cables into the mother cell where they accumulate in aggregates (Figure 2). Extrachromosomal ribosomal DNA circles (ERCs), a toxic product of genome instability in ageing mother cells, are also inherited asymmetrically so that daughters remain ERC free. Sir2 maintains the chromatin structure at the rDNA locus to suppress the formation of ERCs, but not via H4K16 deacetylation.

The co-activator complex SAGA contains a KAT activity (Gcn5) for H3K18. Amino acid substitutions at K18 suggest that acetylation here is detrimental for chronological longevity and that control of KAT activity of the SAGA complex is important during ageing. Acetylation by the Gcn5 KAT promotes growth at the expense of longevity similarly to adenoviral-induced increases in H3K18ac in mammalian cells that promote cell cycling and transformation (15). The integrity of the SAGA complex, and thus its ability to regulate Gcn5-dependent K18ac, is controlled by Spt7. Spt7 is sensitive to oxidation and other stresses in cells. CR reduces loss of Spt7 and improves longevity.

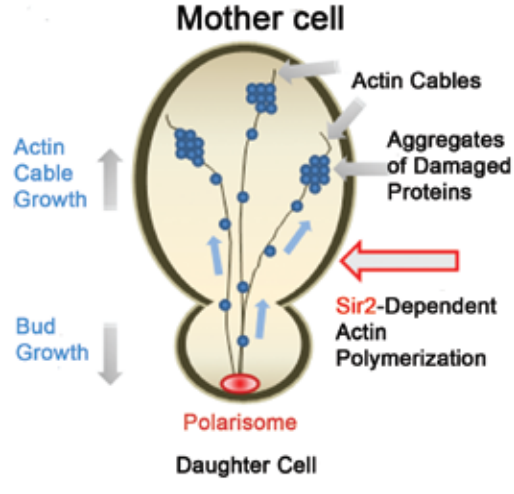
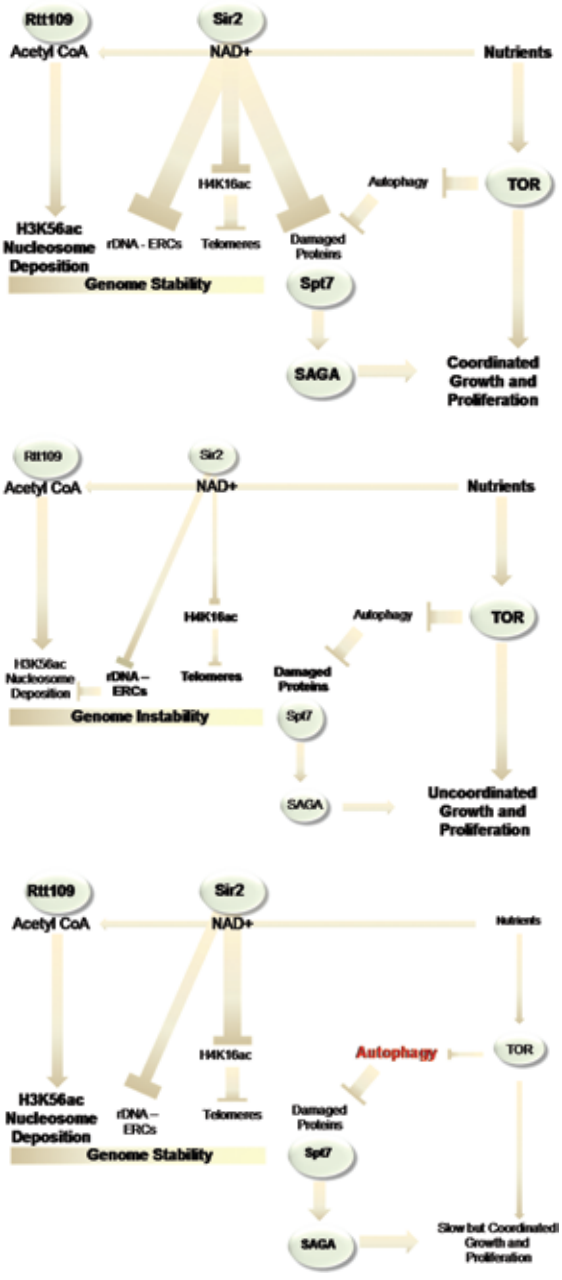


Figure 3. Asymmetric segregation of damaged protein: Sir2-dependent deacetylation of an actin chaperone facilitates the formation of actin cables and movement of damaged and aggregated proteins from the daughter cell into the mother cell.

Figure 4



A. Coordination of replicative and chronological longevity with the target of rapamycin (TOR) mediated growth programme in stem cell lineages.

B. The TOR pseudo-programme in somatic cells enhances the accumulation of damaged proteins and genome instability, contributing to ageing and death.

C. Calorie restriction increases autophagy and genome stability by down-regulating the pseudo-programme in soma and improves longevity.

Concluding remarks

In conclusion, cells that change their state, through development, differentiation or ageing, are characterised by persistent epigenetic alterations to their chromatin. An epigenetic state may remain for the life of a cell, surviving cell division and lasting for many generations, but is also changeable in disease states or during ageing. In ageing organisms, high levels of energy intake and poor levels of nutrients, in combination with the pseudo-programme promoting growth, may contribute to senescence and the onset of age-related conditions. The interesting aspect of these conditions is their reversibility, consistent with epigenetic rather than genetic changes.

A number of important questions remain to be addressed. Is there asymmetric, non-random segregation of chromosomes that contributes to fitness of the self-renewing daughter lineage? Are these marked epigenetically? Can we isolate novel small molecules to modulate the enzymes that are implicated in ageing? Exciting times lie ahead.

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Prof Jane Mellor is a University Lecturer and Head of the Laboratory of Cell and Chromosome Biology in the Department of Biochemistry, University of Oxford.

introducing...

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All articles published in *Phenotype* during the 2011/12 academic year will be automatically entered into our competition, to be judged by our editorial team and the Associate Editor of *Nature Outlooks*, Michelle Grayson. The winning article will be announced in the MT12 issue and the author will receive a £50 book voucher.

For further information about submitting articles to *Phenotype* please contact our editorial team at oubs@bioch.ox.ac.uk. Good luck!

Transgenic animals:
Changing more than intended?

By Nicola Platt

The rapid development of genetic and molecular tools over the past decades has propelled the biological sciences forward at an astonishing rate. For many biologists today, it is hard to imagine not being able to fluorescently tag one's favourite protein or label one's transfected cells. One familiar tool is green fluorescent protein (GFP), a protein that absorbs light in the blue-cyan region and emits in the green region. Purified from the jellyfish *Aequoria victoria* and characterised in the 1960s and 70s, it wasn't until it was cloned in the early 1990s that its potential applications in biology were realised. GFP was shown to be easily expressed in systems ranging from yeast to *Drosophila*, and finally mammalian cells.

Since these early expression studies, the applications of GFP and the discoveries resulting from them are incredible. Its utility as a biological tool rests on two properties: it is fluorescent, allowing for non-invasive cell imaging and, unlike many other bioluminescent proteins, it does not require exogenous cofactors (1). Applications have ranged from protein tagging, to investigating protein-protein interactions, to following developmental lineages. Early applications focused on investigating cellular physiology, for example tagging cytoskeletal elements and tracking their movements in living cells (2). Genetic developments have increased the utility of tools like GFP beyond the single cell. In particular, the ability to express such proteins under the control of different promoters has allowed for controlled protein expression in mammalian tissues and whole animals. Thus in recent years, tools like GFP have been regularly used in whole animal research. However, do we really know what we are doing to cells when we introduce such genetic modifications? Do they function normally within a system or whole animal? Are we rigorously investigating new applications of these tools?

Potential GFP pitfalls

GFP is a relatively large protein, composed of 238 amino acid residues with a molecular weight of 27-30 kDa. This is huge in comparison to previously used molecular tags, such as nine residue hemagglutinin (1). Early reports expressed concern that high levels of GFP were toxic to cells and that tagged proteins might no longer be functional. For example, GFP-tagged tubulin expressed in yeast was unable to rescue null mutants (2). However, a huge body of literature has now demonstrated that in the majority of cases, with appropriate expression levels, the presence of GFP is not deleterious to cell function. In addition most GFP-tagged proteins localise and function normally.

The genetic tools used to control GFP expression may also disrupt cell physiology. Gene insertion can disrupt coding and non-coding sequences or the use of an endogenous promoter can disrupt endogenous gene expression levels. For example, a mouse line expressing Cre-recombinase was recently produced using internal ribosome entry site (IRES) technology, designed to minimise disruption of endogenous gene expression (3). Despite this, the mice showed significantly decreased expression of the endogenous protein. These subtle disruptions in cellular physiology, present in many genetically manipulated cells, may have enormous consequences in a whole animal model, particularly in the complex networks of the nervous system.

GFP in neuroscience

Neuroscience is one biological area that benefits particularly strongly from tools such as GFP. A PubMed search for 'GFP' brings up over 20,000 results; a search for both 'GFP' and 'neuron' accounts for over 2,000 of these. The network structure of the nervous system means it can be difficult to identify distinct populations of neurons. It has therefore become commonplace to express tags, such as GFP, under the control of neuron-specific promoters to label different neuronal populations. The ubiquitous use of such tools means that whole animal models are not always rigorously characterised, as evidenced by a recent investigation of striatal neural networks.

The striatum is the input nucleus of the basal ganglia, a group of subcortical nuclei, important for decision-making and action selection. The major striatal projection neuron is the medium spiny neuron (MSN), comprising around 90% of striatal neurons. MSNs can be divided into two populations based on their projection and dopamine receptor expression (expressing either D_1 or D_2 receptors). These two populations play distinct roles in striatal function and disease. However, owing to a lack of clear anatomical structure in the striatum, it has always been difficult to differentiate these cells.

Recently the NIH-funded Gene Expression Nervous System Atlas (GENSAT) project used bacterial artificial chromosome (BAC) technology to produce

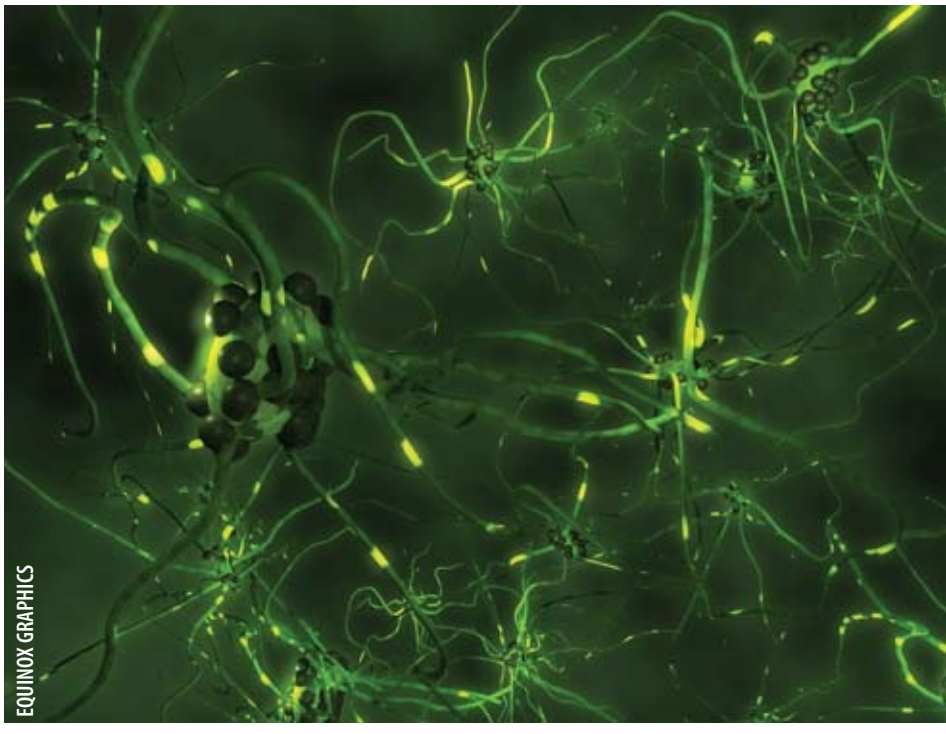
mouse lines expressing enhanced GFP (EGFP) selectively in each population, under the control of D_1 or D_2 receptor promoters (4). These mice have been used in several studies and revealed much about striatal function. The two MSN populations have been found to have distinct projections; distinct physiology, for instance different excitability and synaptic properties (5); and distinct responses to disease or drugs of abuse, for example differential activation of the ERK pathway following cocaine administration (6). However, these mice were never fully characterised, which a recent paper suggests may have been a worrying oversight (7).

Characterisation urges caution

Kramer and coworkers characterised the two mouse lines behaviourally and physiologically (7). Transgenic mice were overtly indistinguishable from wild types. However, the mice expressing EGFP under control of the D_2 receptor promoter (but not D_1) showed locomotor changes suggestive of disrupted striatal dopamine signalling. Further investigation showed a 1.5-fold increase in D_2 receptor mRNA expression, increased D_2 receptor binding, and a corresponding increased behavioural response (decreased locomotion) to D_2 agonists. Electrochemistry showed that dopamine uptake from the extracellular space was also decreased, leading to prolonged dopamine signals. This may underlie their altered locomotor phenotype. Therefore these mice, which have already been used to probe striatal physiology, show significant alterations in this neuronal network with behavioural consequences.

The reason for these disruptions is unclear. Kramer *et al.* rule out the possibility of increased D_2 receptor gene copy, showing the gene has been correctly deleted from the BAC construct. Since D_1-EGFP mice do not show such alterations, striatal expression of EGFP itself is probably not responsible. However, an effect of EGFP expression selectively in this MSN population cannot be definitively excluded. The authors suggest random insertion effects or the presence of a gene of unknown function, *Ttc12*, in the BAC construct, may underlie these effects.

Whilst these changes in neuronal network function are clearly specific to this mouse line, they demonstrate how small changes in gene expression resulting from genetic manipulations can greatly influence the functioning of a system or whole animal. There is no doubt that transgenic animal lines are invaluable tools for a whole range of applications. However, the excitement at their availability can lead to research being carried out before comprehensive characterisation is complete. This example serves as a warning that we do not always know the full ramifications of such genetic modifications, and as researchers we should perhaps exercise greater caution and rigour when using such tools.


EQUINOX GRAPHICS

> “It has therefore become commonplace to express tags, such as GFP, under the control of neuron-specific promoters...”

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From Parkinson's disease to a gambling addiction: treatment side effects

by Dr Carinne Piekema

Ann Klinestiver had never been interested in gambling, but this changed suddenly when she was 45. She began spending hours each day playing the slot machines at the local casino and even stole money from her family to fund her habit. Within a year, Ann lost $200,000, as well as her husband, who divorced her to prevent her from gambling away their life savings. Several years prior to this, Ann had been diagnosed with Parkinson's disease (PD). In order to control her tremors, she had been put on Requip, an agonist that mimics the function of the neurotransmitter dopamine. As the disease progressed, she required larger doses of Requip to control her symptoms and the alteration of her brain chemistry triggered Ann's descent into a gambling addiction.

Neuronal communication and dopamine

Brain cells communicate with each other across the gap between them – the synapse – using chemical messengers called neurotransmitters (Figure 1). An electrical signal, the action potential, travels down the axon of one neuron. When the action potential reaches the end of the axon, it triggers the release of neurotransmitter from the axon into the synapse. The neurotransmitter diffuses across the synapse and binds to receptors on the next neuron (Figure 2). This binding modulates electrical signals in the next neuron, allowing the message to be communicated.

Dopamine is one such neurotransmitter and is known to play a major role in the regulation of movement, as well as motivation and reward. Two key sites containing dopamine neurons are the substantia nigra, giving rise to the nigrostriatal system, and the ventral tegmental area, giving rise to the mesocorticolimbic system (Figure 3). The former is implicated in movement related processes, and the latter is involved in reward processing. The slowness of movement and tremors seen in PD patients are caused by the death of dopamine-producing neurons in the substantia nigra. This is treated with dopamine agonists, which replace lost natural dopamine.

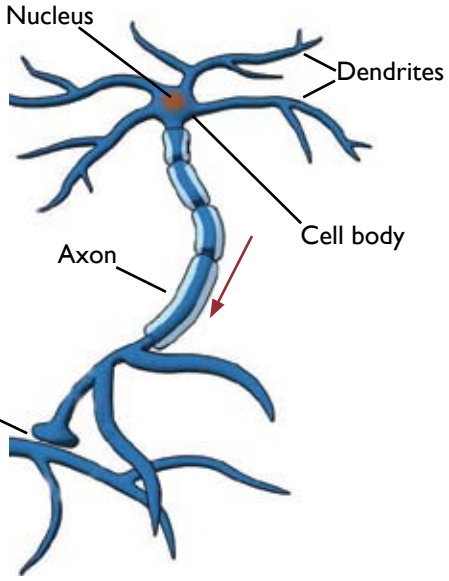

Figure 1. Structure of a neuron and a synapse. The arrow indicates the direction of an action potential (Equinox graphics).

Dopamine agonists and gambling

Dopamine agonists have been used to alleviate motor symptoms in PD for several decades, but over the past decade there have been increasing reports of problematic side effects (1). A growing body of research suggests that dopamine agonists may be linked to pathological gambling, compulsive and impulsive shopping, compulsive eating and hypersexuality. Approximately 15% of PD patients receiving dopamine agonists develop one or more of these Impulse Control Disorders (ICD).

While risk-taking and impulsive behaviours have been extensively documented, the reason why they arise in this particular subgroup is not fully understood. The decision-making process is complex; we weigh up the costs and benefits of our options and calculate the likelihood of positive or negative consequences of our potential choice based on past experiences. Considering this complexity and the influence dopamine agonists could have on any of these processes, the behavioural changes seen are perhaps less surprising.

Valerie Voon and colleagues investigated the causes of increased risky decisions in PD patients (2). Patients were tested on a risk-based task, both whilst on and off dopamine agonist medication, in order to probe the aspects of choice behaviour that might be affected. They used two versions of a gambling task where participants choose between a probabilistic 'risky' option and a certain 'safe' option. In the 'gains' version, participants were given a choice between a certain small reward and the risky option that could lead to either a high monetary reward or to no gain at all. In the 'loss' version, they chose between a certain small loss of money and the risky option that could either lead to no loss or a large loss of money.

The researchers observed that PD patients with ICD took riskier gambles in the 'gains' task compared to PD patients without ICD. Nevertheless, the patients with ICD were still sensitive to the relative level of the risk and chose to gamble less when gambles were

more risky and also adjusted their behaviour on the trials directly after a loss.

Voon *et al.* collected functional neuroimaging data from the patients while they were performing these tasks. They found that PD patients with ICD showed significantly less activity in orbitofrontal and anterior cingulate cortices compared to those without ICD (Figure 3). These regions are known to be involved in the evaluation of risks and the value of options, and a decrease in activity may result in a tendency to behave more riskily.

Deep brain stimulation and gambling

But if these patients are able to learn from their experience, why do they continue to make riskier decisions? Researchers at the University of Oxford, led by Professor Robert Rogers, are working to understand gambling behaviour. Typical behavioural patterns of gamblers, both social and pathological, include a tendency to continue gambling to recover sustained losses. Rogers and his group investigated this 'loss-chasing' behaviour in a game where participants had to make decisions to either sustain certain losses or gamble in order to recover the loss. Each participant started off with a pot of £20,000. At the beginning of each round, a certain amount (£10, £20, £40, £80 or £160) was subtracted from the pot.

Participants could choose to 'quit' the round and lose this amount, or 'play' the round. Playing could result in either recovering the money or doubling the loss. If they won, the round ended. If they lost, they could either quit or play again, the latter with the chance of recovering the double loss or doubling the already doubled loss. The maximum loss per round was £640. To make the game worthwhile, the researchers promised a prize of £70 to the participant who had the largest pot left at the end of the experiment.

With functional magnetic resonance imaging, the researchers showed that loss-chasing behaviour activated regions in the brain that are associated with expectancy of positive outcomes. This type of activation is consistent with personal accounts from gamblers who say they keep gambling because they feel a win is imminent. Other regions of the brain involved were the same as those activated when we feel extremely hungry. It is thought that a dysfunction in these regions may result in pathological gambling (3).

Rogers and colleagues have also started to focus attention on PD and ICD. In *Experimental Neurology*, they report that ICD may also be linked to another effective treatment of PD, deep brain stimulation of the subthalamic nucleus (STN DBS) (4, 5). Together with a group in Düsseldorf, Germany, Rogers and colleagues tested 22 PD patients with the 'loss-chasing' task, both on and off

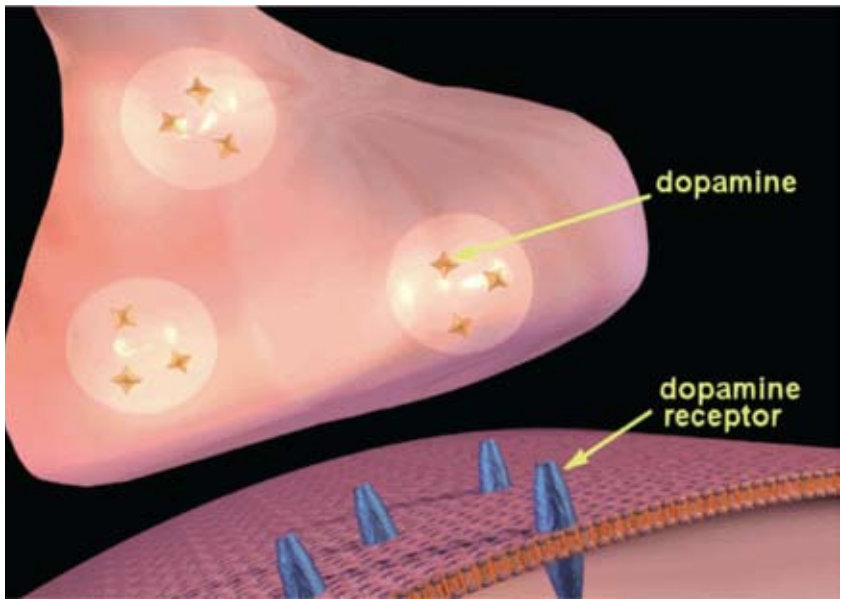

Figure 2. Dopamine is released from the presynaptic terminal into the synaptic cleft, where it binds the dopamine receptors of the adjacent cell (www.morphonix.com).

STN DBS, and found that their gambles were riskier while undergoing treatment. This suggests that STN DBS might change the way in which some patients evaluate loss and that treatment with STN DBS may exacerbate or even cause ICD in patients with an underlying vulnerability to develop addictions. As STN DBS is currently used as a replacement treatment for dopamine agonists in some PD patients who have developed ICD, this may mean re-evaluating treatment protocols in ICD patients (4).

Potential Causes

Why do PD treatments have such unwanted side effects in a significant number of patients? PD has long been thought of as primarily a motor disease. The nigrostriatal dopamine system, originating in the substantia nigra, has been considered to be

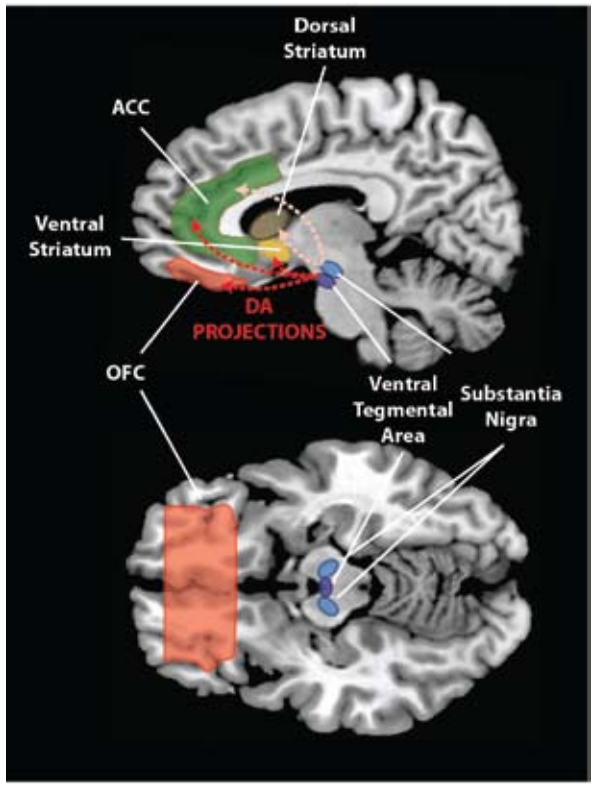

Figure 3. Location of the dopamine producing regions involved in decision-making (ACC = anterior cingulate cortex, OFC = orbitofrontal cortex). Arrows indicate the two major dopamine pathways (red = mesocorticolimbic dopamine system, pink = nigrostriatal dopamine system) (courtesy of Dr Peter Rudebeck).

primarily involved in the selection and control of voluntary movement. Those studying the other dopamine system, the mesocorticolimbic dopamine system originating in the ventral tegmental area, have focused mainly on its role in motivational and reward processing (Figure 3). These two strands of dopamine research have proceeded largely in parallel, due to the idea that these two dopamine systems are functionally distinct (6).

However, the changes in decision-making in subgroups of PD patients documented by Voon, Rogers and others demonstrates that attempting to treat PD simply by re-equilibrating the loss of nigrostriatal dopamine may have several drawbacks. Firstly, given that PD primarily affects the nigrostriatal dopamine system while sparing mesocorticolimbic dopamine, dopamine agonists or stimulation might in fact impair the functionality of the latter system in certain patients, leading to maladaptive decision-making. PD patients who develop ICD as a result of their treatment may have underlying genetic neurobiological vulnerabilities that exacerbate this (7).

Secondly, several lines of basic research suggest that the two dopamine systems interact both anatomically and functionally. Both systems send projections to parts of the frontal lobes as well as the striatum (8), and the cell bodies in both systems can sometimes be found in the same midbrain structure (6). There is also increasing evidence that interactions between ventral striatum – innervated by the mesolimbic dopamine system – and dorsolateral striatum – innervated primarily by the nigrostriatal dopamine system – underlie the development of habitual addictive behaviours, and that these interactions are mediated by dopamine transmission (9).

But possibly the most compelling reason for why the strong distinction between 'motor' and 'motivational' dopamine systems may be regarded as artificial is seen in every-day life. Sometimes we choose to walk to the fridge because we are hungry, scratch our back to relieve an itch, or exercise to stay fit. In a study published in the *Journal of Neuroscience*, Pietro Mazzoni and co-workers showed that one of the key 'motor' symptoms of PD – namely bradykinesia or slow movement – might arise not because patients could not make the movements, but because they were less motivated to expend energy on movement (10). This indicates that nigrostriatal dopamine activity plays a role in enabling voluntary movement, not just through modulations of motor pathways in the basal ganglia, but also by participating through calculations of the cost/benefit value of a response. Thus, an important class of voluntary movements frequently affected in PD rely on dopamine and are motivationally driven.

These studies are gradually revealing the mechanisms behind pathological gambling and other compulsive behaviours caused by different PD treatments and in doing so, they aid our understanding of the role of dopamine in risk-taking behaviour. Until we understand the precise mechanisms more fully, it is important for clinicians to remember that interactions between treatment and underlying vulnerabilities can lead to highly undesirable side effects.

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The politics of human embryonic stem cell research

Since the first human embryonic stem cell (hESC) lines were isolated in 1998, our perception of hESCs has changed drastically. This breakthrough could potentially combat a wide spectrum of diseases, but equally it has raised a number of ethical concerns and controversies. In the United States in particular, hESC research has become a highly political issue, on which politicians' opinions are paid intensive attention by voters.

by Shaoyan Liang

An introduction to hESCs

hESCs are derived from the inner cell mass (ICM) of a blastocyst, which is formed five days after fertilisation. The harvested cells are cultured on a layer of mouse feeder cells to keep them undifferentiated while propagating, thereby generating a continuous cell line. The process is not efficient and cell lines are not always formed. The embryos used to make hESC lines are unused from *in vitro* fertilisation (IVF) procedures, donated for this use by the parents. These embryos would otherwise be discarded. Compared to stem cells from adult humans, hESCs have the advantages of easier isolation, shorter cell cycle, and fewer mutations due to less sunlight (UV radiation) and toxin exposure.

hESCs are pluripotent, meaning they can develop into all types of cells, tissues, and eventually organs in the human body. Theoretically they could provide replacement tissue for treating injuries and various degenerative genetic diseases such as Parkinson's, Alzheimer's, and Type I diabetes. However, further research is required to define the correct cellular programming and *in vitro* culturing environment needed to produce a particular type of tissue.

The transcription factors NANOG and OCT-4, expressed in many stem cells including hESCs, inhibit differentiation. hESCs and other human undifferentiated cells also express cell surface markers called Stage Specific Embryonic Antigens-3 and -4. A more prominent character of hESC is that they can form a teratoma, an encapsulated tumour with tissue or organ components resembling normal derivatives of all three germ layers, in immunocompromised mice. This demonstrates that hESCs are potentially cancerous.

Objections to hESC work

Harvesting hESCs as described above inevitably results in the destruction of the blastocyst. An alternative technique utilising a single ICM cell and leaving the blastocyst intact was first reported in 2006, but this has not become the routine method of hESC production. The standard technique raises the issue of whether it is ethical to destroy a human embryo to conduct biomedical research. The ethical considerations fall into two broad categories.

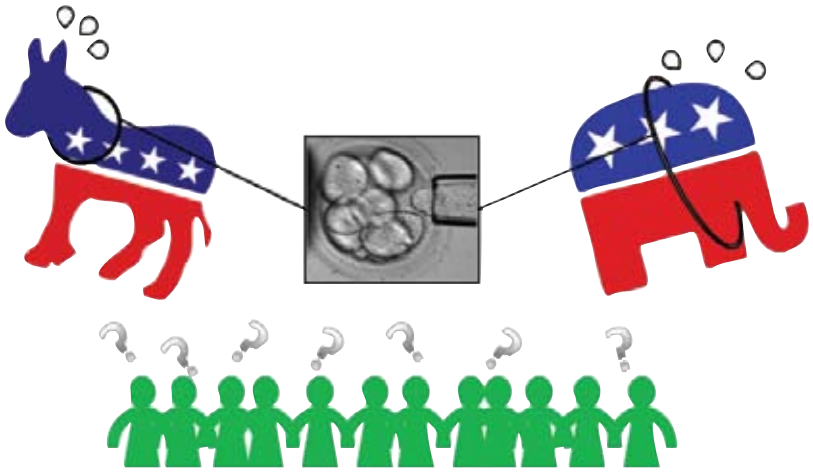
Firstly, we should consider whether the embryo has rights which can be infringed upon. Pro-life advocators believe that the embryos generated in IVF have the potential for life, thus they deserve respect and should not be used as a natural resource for exploitation. Some of these groups suggest that the embryos could be adopted, with the biological parents' consent, by couples that have fertility difficulties. The embryo is treated as in conventional IVF but implanted into the adoptive mother's uterus. Since unused embryos are frozen between IVF and implantation, the resulting children are referred to as 'snowflake babies'.

The second area of consideration is the existence of alternative sources of stem cells. Stem cells can be derived from sources such as umbilical cord, amniotic fluid, and adult bone marrow, the isolation of which does not require the destruction of embryos. In 2007, two independent research groups in the US and Japan successfully reprogrammed human skin cells to a hESC-like state using modified viral shuttles. These induced pluripotent stem cells (iPSC) should result in lower clinical immunogenicity compared to ESC-derived transplantation, because the source of iPSCs are the patients' own somatic cells rather than a donor. However, it was recently demonstrated that mouse iPSCs could still lead to an immune response and rejection in the transplantation recipients, while ESCs were not rejected and formed teratomas in mice within the same strain. Therefore, further work is required before iPSCs can realistically replace ESCs.

US policies and public opinion

In 1995, the US Congress passed the Dickey Amendment prohibiting the Department of Health and Human Services from granting federal funding to research involving direct destruction of human embryos of any source. However, there was no clear guidance on whether federal money could fund research on hESCs derived from privately funded routes. Although President Clinton decided to allow this, the decision was not enacted before his tenure ended.

President George W. Bush was informed of the need for new legal guidelines on federal funding of hESC



"...the hESC issue became a political defaming tool with the basic scientific facts often misunderstood and misrepresented to the public."

research in late January 2001. As the media picked up on the issue, months of consultation with both pro- and con-hESC advocates ensued. Bush announced his final decision in August 2001 using a primetime speech, a timeslot usually used by presidents in their capacity as Commander in Chief discussing military issues, in an effort to garner public support. He concluded that the prohibition on federal funding of any direct embryo destruction would remain, but private funding for the creation of new cell lines was still allowed. He conceded that taxpayer money could fund research on hESC lines created before his announcement, but not any that would follow. Federal money was now applicable to over 70 hESC lines derived in the United States, India, Israel, Singapore, Sweden and South Korea.

However, the decision was publically opposed by both conservatives and liberals. The former considered all research on hESCs immoral. The liberals, at the time becoming increasingly influential, argued that federal money should be available to fund research on new hESC lines in order to broaden the number of lines available to facilitate potential therapies. Over the next decade, the hESC issue became a political defaming tool with the basic scientific facts often misunderstood and misrepresented to the public. In the 2004 presidential election, Democrat candidate John Kerry called the Bush policy a "ban" on hESC funding, although Bush allowed federal funding on the existing hESC lines. Kerry's running mate John Edwards declared that if there had been no Bush hESC policy, handicapped people would have got up out of their wheelchairs and walked again.

Many Republican politicians switched sides as the media increasingly criticised the Bush policy. The House and Senate passed a bill in 2006 to increase the number of federally funded hESC lines. However, this was vetoed by President Bush with the support of families that had adopted 'snowflake babies' the next day. After gaining a majority in both the House and the Senate, the Democrats launched another attempt to reverse the Bush policy in 2007, but it was vetoed again.

In March 2009, President Obama repealed the Bush hESC policy with an Executive Order and two days later, signed an act including the Dickey Amendment. This effectively means a return to the 1995 restrictions preventing federal funding of hESC production, but explicitly allows federal funding of research using any hESC line, regardless of when and how it is created. Despite the total National Institutes of Health (NIH) budget increasing by less than 3% in Obama's first year, federal funding on hESC research through the NIH was increased from 88 to 120 million US dollars from fiscal year 2008 to 2009.

Informing public opinion

The hESC issue is not the first scientific controversy and it will not be the last. This debate serves as a reminder that public and political opinion can change with little basis in fact, yet heavily influence the agency funding agendas that we as scientists rely on. It is our responsibility as scientists to establish effective communication channels with the government, lawmakers, and the general public, in order to assist decision-making based on scientific facts. By keeping aware of the political arena and maintaining that communication, the scientific community can ensure that science does not fall victim to political demagoguery.

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Bovine tuberculosis and badgers:
to cull or cure?

Deemed "the most pressing animal health problem in the UK today" by the Government (1), bovine tuberculosis (bTB) costs the taxpayer tens of millions of pounds each year in compensating farmers and research (2). How this is spent is a contentious source of public and scientific debate. The crux of the problem lies in how to control the predominant reservoir host: whether to cull or vaccinate badgers.

by Peter Ibbetson

Bovine Tuberculosis

Mycobacterium bovis, the causative agent of bTB, is a pathogenic mycobacterium with a broad host range, able to infect all mammals and birds. Tuberculosis as a result of *M. bovis* infection has a complicated epidemiological pattern due to transmission of the infection within and between cattle and wildlife populations. Infection of humans in the UK is negligible as milk pasteurisation effectively eliminates transmission (3). Nevertheless, *M. bovis* remains a pathogen of high economic importance. Driven by political, animal welfare and potential public health agendas, current government policy entails the culling of infected cattle and quarantine of afflicted farms.

Epidemiology

The principal mode of transmission between hosts is inhalation of aerosols containing live bacilli. A wide body of evidence supports the suggestion that badgers are the main reservoir host of bTB in the UK. It has, for instance, been observed that hitherto disease-free cattle populations succumb to bTB in areas of medium to high badger density, and that the incidence of bTB is higher in badgers than in other potential reservoir hosts. The application of modern diagnostic methods such as DNA fingerprinting has proven that the same *M. bovis* strains are present in cattle and badgers. Direct evidence of transmission between badgers and cattle in their natural habitat is lacking, but experimental studies have demonstrated that cattle can be infected with bTB through contact with infected badgers (3).

Cattle may become infected by inhaling bacilli surviving on badger faeces during investigative behaviour, or by consuming contaminated grasses. Once in the rumen, fermentation generates aerosols containing these bacilli, which are expelled by belching. However, a large proportion of these contaminated gases can enter the lungs *en route* to expulsion, providing an alternative transmission route (4). This path is particularly significant as substantially fewer bacilli are needed to establish infection via the pulmonary route than the alimentary route.

Culling proves ineffectual

Prof John Kerbs' influential 1996 review of the link between bTB and badgers stated that despite compelling evidence for this association, control policy was hindered by the lack of scientific information on the effectiveness of culling (5). In response, the Randomised Badger Culling Trial (RBCT) was established to investigate culling effects. Proactive culling over four to seven years in 100 km^2 zones saw a 23.2% decrease in bTB incidence within the culling area. However, the 2 km ring of land surrounding culling zones saw a 24.5% increase in cases compared to 'survey only' regions. It is thought that culling disrupts the badger's social structure, causing survivors to roam more widely and come into contact with more susceptible animals (6).

Monitoring bTB incidence five years post-culling (Table 1) demonstrated only short term beneficial effects within proactive culling areas. Benefits were

Table 1: Estimated effects of culling on TB incidence in cattle within trial areas and in the adjoining ≤ 2 km of land. 95% confidence intervals are in brackets. Source: Jenkins *et al.*, 2010

	Inside 100km^2 proactively culled trial areas	Adjoining lands ≤ 2km outside culled trial areas (not culled)
During-trial period		
1st to 2nd cull	-3.5% (-30.6% to 34.1%)	43.1% (-5.6% to 116.8%)
2nd to 3rd cull	-12.8% (-36.6% to 20.1%)	22.8% (-16.9% to 81.7%)
3rd to 4th	-39.4% (-57.6% to -13.4%)	17.8% (-23.4% to 81.1%)
After 4th cull to end	-31.5% (-46.8%to -11.9%)	14.7% (-13.8% to 52.6%)
Entire during-trial period	-23.2% (-32.7% to -12.4%)	24.5% (-0.6% to 56.0%)
Post-trial period		
Months 1-6	-52.7% (-71.8% to -20.8%)	-17.5%(-51.2% to 39.5%)
Months 7-12	-41.1% (-64.0% to -3.8%)	-26.9% (-60.0% to 33.5%)
Months 13-18	-49.4% (-67.9% to -20.4%)	-19.5% (-51.9% to 34.8%)
Months 19-24	-27.8% (-52.4% to 9.4%)	37.9% (-15.5% to 125.2%)
Months 25-30	-35.0% (-59.5% to 4.3%)	14.1% (-33.5% to 95.5%)
Months 31-36	9.9% (-36.7% to 90.7%)	-2.1% (-55.2% to 113.8%)
Months 37-42	Insufficient breakdowns to calculate estimates	Insufficient breakdowns to calculate estimates
Entire post-trial period	-37.6% (-48.4% to -24.6%)	-5.6% (-31.4% to 30.0%)
During- and post-trial periods combined	-28.7% (-35.8% to -20.8%)	11.7% (-12.9% to 43.2%)

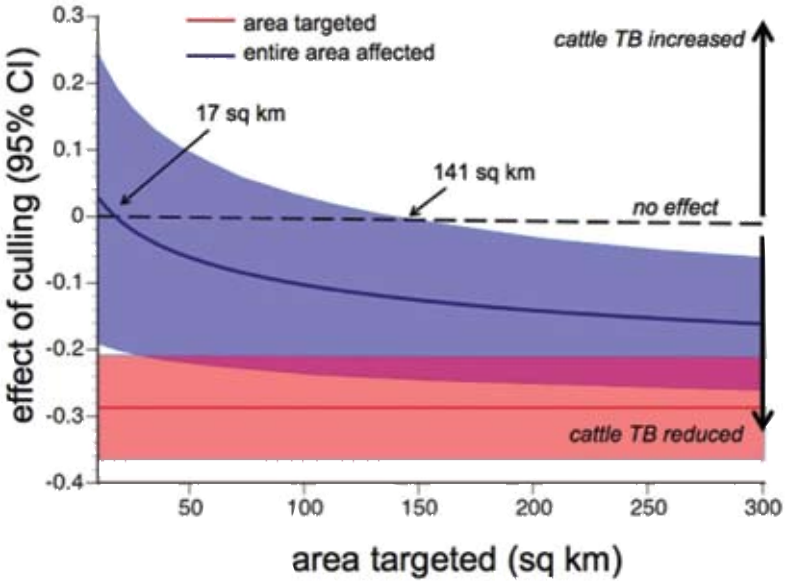
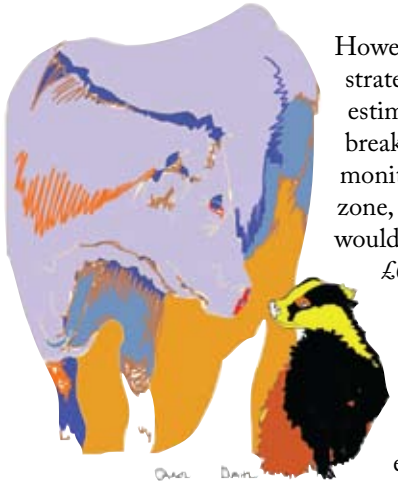


Figure 1: Estimating the effects of culling in areas of different size, within the proactive culling zone alone (red) and within both the culling zone and 2 km^2 surrounding ring (blue), showing 95% confidence intervals. Source: Jenkins *et al.*, 2010.

no longer present four years after the last culling trial. For the 2 km ring outside this region, culling was found to reduce bTB incidence for the 18 months post-trial, but this reduction was never significant. Variable results have been found in other culling trials. In Ireland, a five year culling trial resulted in bTB incidence dropping by between 51% and 68%. Geographical barriers reducing badger immigration may account for this difference compared with the RBCT trial (7).

Recent work has considered increasing the culling zone. Assuming the cull zone is circular, as the region of proactive culling increases, its size relative to the 2 km surrounding ring also increases. Thus the result of culling becomes dependent on the effects within the culled area rather than on the surrounding 2 km ring of land. Jenkins *et al.*, using RBCT's findings, found that culling must be carried out over a minimum area of 141 km^2 to give a statistically significant net benefit (Figure 1).

"Cattle may become infected by inhaling bacilli surviving on badger faeces..."



However, the same group showed this strategy to be economically unfeasible. They estimated that each herd experiencing TB breakdown costs £27,000 in treatment and monitoring. Using an idealised 150 km^2 cull zone, 22.6 of a predicted 187 breakdowns would be prevented over 7.5 years, saving £610,200.

However, conducting five annual culls over 150 km^2 would cost significantly more than this, regardless of the method used. Cage trapping is estimated to cost £3,800 per km^2 per year, totalling £2.14 million; snaring or gassing £2,400 per km^2 per year, totalling £1.35 million; and farmer-led culling, excluding training farmers or coordinating their efforts, costs £1,000 per km^2 per year, totalling approximately £562,500. Unsurprisingly, Jenkins *et al.* concluded that "on the basis of cost-effectiveness, badger culling is unlikely to contribute to the control of bTB in Britain" (7). Similar work focusing on financial outcomes when using various transmission models for bTB found that all scenarios led to a net loss (6).

The Public View

'To cull or not' continues to be hotly debated in scientific and political communities, as well as within the public domain. The National Farmers Union (NFU) welcomed the Agriculture Minister's proposal in September 2010 to license farmers in England to shoot badgers on their land as "a major step forward". In November 2010, Wilshire NFU renewed calls for a badger cull (1). Such a stance is understandable from those whose principal concern is their own cattle and livelihood. In contrast, the general public is clearly anti-cull, influenced by the iconic image of the badger as a positive feature of the British countryside. Furthermore, badgers are protected in the UK by their own act of Parliament (Protection of Badgers Act, 1992). Demonstrative of the difficulties inherent within any cull, the Welsh Assembly was recently forced to spend over £57,000 in a protracted legal battle to defend its proposed five year annual badger cull throughout North Pembrokeshire, following an appeal by the Badger Trust (1).

Given the lack of scientific evidence and the controversy surrounding culling, it is important to consider alternative control methods. The licensing in March 2010 of an injectable BCG badger vaccine (BadgerBCG) based on attenuated *M. bovis* is a major step forward. This may be considered an effective use of £11 million public funds invested by Defra since 1999 on vaccine research and development. Both laboratory and field studies have been completed and demonstrate a significant reduction in badger blood tests positive for bTB (8). BCG-based vaccines can now contribute to control of bTB, but questions remain, especially related to vaccine delivery, programme implementation and the durability of protection.

The future of bTB vaccines

Vaccination presents a more humane approach to controlling badger bTB than widespread culling. However, in its present form, there are practical difficulties in delivery and high programme implementation costs. Nonetheless, vaccines can be effective and oral vaccines are preferred as the next step in widespread control.

Successful control of wildlife rabies in North America and Europe highlights the potential of oral vaccination. Success in this instance hinged on an efficacious vaccine; a delivery formulation resistant

to destruction by the gut; and species-specific bait. Such challenges seem surmountable for bTB vaccines. Research at University College Dublin demonstrated that orally applied BCG encapsulated in a lipid matrix resulted in a lessening in both the number and severity of gross lesions, fewer sites of infection, and reduced bacterial load in the lungs of vaccinated badgers, compared to a control group (9).

If *M. bovis* is to be controlled more effectively, the importance of developing a vaccine suitable for broad application cannot be understated. Furthermore, by understanding how to control bTB we may learn how to intervene with other diseases of public concern that have a wildlife reservoir.

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A Complementary Discussion:

The roles of complement and properdin in immunity

By Alexandra East

Each person possesses an innate and an adaptive immune system, each with cellular and humoral aspects. Innate immunity is the more primitive in evolutionary terms and responds to the presence of pathogens in a non-specific manner. In contrast, adaptive immunity develops responses to pathogens as the body encounters them, ensuring that the response to infection is much more rapid upon subsequent exposures to a pathogen, such as a certain kind of bacterium. Cellular immunity in the context of innate immunity refers to the actions of leucocytes, commonly known as white blood cells, which function to capture microbes, foreign particles and cellular debris. However, this article will focus on humoral immunity, which supports the action of the cells of the immune system, and includes complement.

The complement system

Complement is in many ways an abstract concept that acts to link innate and adaptive immunity in an effective manner. It consists of a carefully regulated cascade of enzymes, protein complexes and receptors that acts as a rapid and efficient immune surveillance system. This cascade has distinct effects on both healthy and altered host cells, like cancer cells, as well as foreign intruders, such as bacteria entering through an open wound. It acts to eliminate cellular debris and infectious microbes, to orchestrate immune responses, and to send danger signals. This enables discriminatory recognition of cells which need to be removed, whether they are foreign or dying cells of the body, followed by an appropriate response by the body.

It is thought that complement acts as one of the first lines of defence against microbes, after physical barriers like the skin. It rapidly recognises, tags and eliminates pathogens to give the adaptive immune system time to become fully activated. The response of complement to healthy, dying or foreign cells is tuned via regulatory mechanisms. Complement has also been shown to be involved in various aspects of body function including tissue regeneration and lipid metabolism. Although it is sometimes described as a series of linear cascades for the sake of simplicity, it is in fact a tightly connected network, linked to a number of other systems in the body.

Activating complement

Complement is activated in three different ways (Figure 1). The classical pathway is stimulated by the recognition of antigen-antibody complexes on the surface of foreign cells by a component known as C1q. A series of proteases are then activated which eventually cause cleavage of a molecule called C3, and initiate amplification and downstream effector functions. The lectin pathway is similar to the classical pathway, with a few minor differences in the initial stages. However, both the lectin and classical pathways lead to the central cleavage of C3 and the formation of its active fragments, C3a and C3b.

The alternative pathway is slightly different to these two paths. In its native form, C3 is inert and does not bind to many ligands. However a small fraction of the C3 molecules are hydrolysed in blood plasma, exposing new binding sites. These are then bound by factors in the blood and a molecule is generated that activates C3. C3b is produced at a significant rate by this C3 cleavage, which is considered spontaneous as it does not require the presence of a pathogen. The spontaneously activated C3 is always available in the plasma, able to bind to pathogen surfaces, thus keeping complement alert. The alternative pathway also includes an initiation mechanism involving properdin.

Properdin

Properdin was discovered in 1954 by Dr Pillemer at the Institute of Pathology, Case Western Reserve University, USA. It is a 53 kDa positively charged protein comprising six globular domains. It associates with itself to form dimers, trimers or tetramers. Synthesis of properdin occurs in macrophages, T cells and neutrophils. In neutrophils specifically, properdin is stored in granules and released upon microbial stimulation of white blood cells. Individuals with properdin deficiencies are susceptible to fulminant meningococcal disease.

It is believed that properdin in the blood acts to stabilise the convertase enzymes that cause the cleavage of C3 into its active components. Properdin can also bind to target cells and microbes, thus providing a platform for convertase assembly and function, and promoting phagocytosis (engulfing of pathogens by cells of the immune system). This pathway may serve in the identification and clearance of undesirable cells. Properdin may direct complement activation more selectively by recognising and binding only to specific targets. It has been shown to bind early apoptotic T cells, but not resting or activated T cells, and to then promote their phagocytosis by macrophages and dendritic cells. There are currently two theories about how this is achieved. Properdin may bind apoptotic T

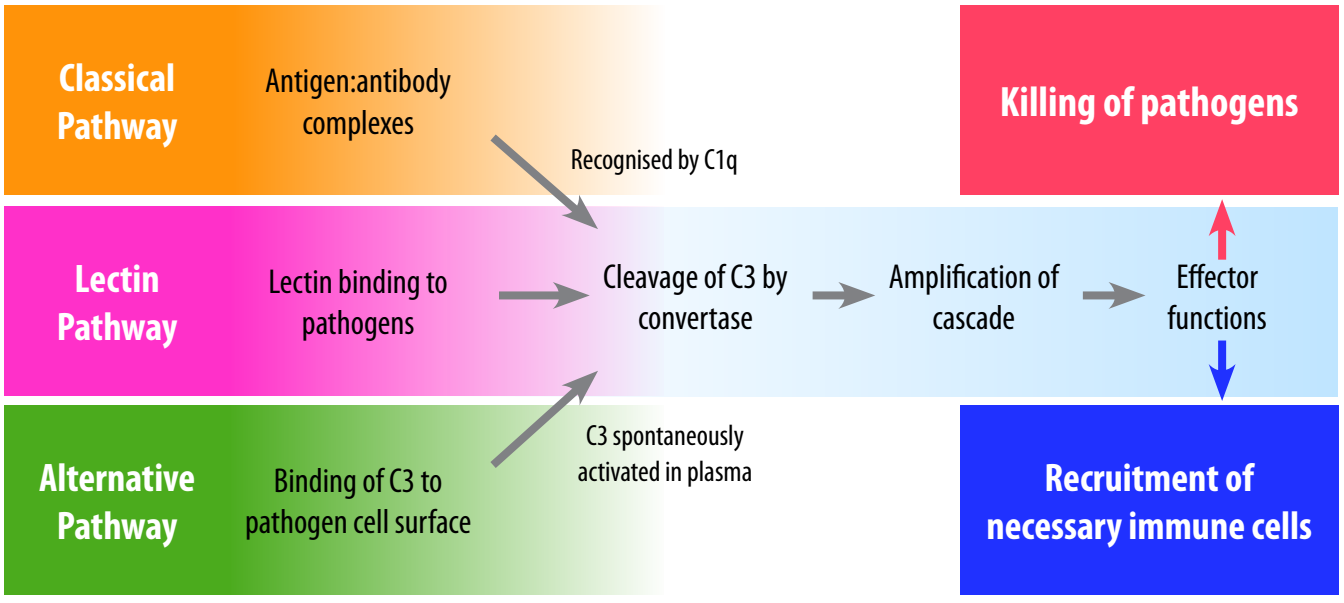

Figure 1. Activation of complement via the classical, lectin and alternative pathways.

cells and then activate *in situ* complement activation. Alternatively, it may bind to apoptotic T cells and then mediate contact with phagocytes, the details of which still need to be elucidated.

Properdin thus interacts with other aspects of immunity beyond complement. For example, activated T cells secrete soluble molecules that induce the release of properdin from neutrophils. These effects together allow swift clearance of early apoptotic cells, which is necessary to avoid the harmful inflammatory and autoimmune reactions that would otherwise occur. There is now discussion as to whether properdin could be used as a therapeutic target, since C3 convertase plays critical roles in exacerbation of injury. However more work needs to be done in this area before such treatments are possible.

Immunity interconnectivity

The cellular component of innate immunity is often considered in isolation. However, the influence of humoral complement stresses the interconnectivity of the immune system. The recognition of pathogens by complement, the coating of these particles with marker molecules that single them out for destruction (opsonisation) and the lysis functions of complement are fundamental aspects of innate immunity. In this respect, complement influences immunity very strongly, and we would be highly unlikely to survive if all complement components were removed from the plasma. The classical pathway appears to play a key role in facilitating the exposure of lymphocytes to antigens that activate them.

The humoral components of both immune systems are also intertwined, as complement can affect B cell development. Complement-inhibitory proteins have been implicated in the direct modulation of these cells, which present antigens to the adaptive immune system and assist immunological memory by producing antibodies, thereby representing the humoral arm of adaptive immunity. The decreased priming of $CD8^+$ and $CD4^+$ cells in C3-deficient mice suggests that complement also plays a role in T cell adaptive immunity.

These and other examples show that complement has a strong influence over both cellular and humoral aspects of immunity and extensive research continues to take place in this field, along with examination of the way in which complement mediates between innate and adaptive immunity.

In conclusion, it is clear that the complement cascade is an important part of immunity, both innate and adaptive. Regulation and varying methods of activation mean that complement is activated when required and that deficiencies in one pathway can be compensated for by the others. However, the role of complement, and especially of properdin, still requires further study since pertinent detailed information is a relatively recent development in this field. More information will allow therapeutic methods relating to complement to be used for the treatment of conditions such as hereditary angioedema, which is a deficiency of the C1 inhibitor that usually controls the complement cascade.

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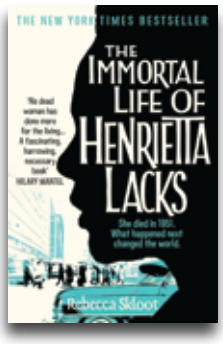
BOOK REVIEWS



The Immortal Life of Henrietta Lacks

Rebecca Skloot

MacMillon (hardcover), 2010, 352 pages, £18.99; Pan (paperback), 2010, 384 pages, £7.99

(Reviewed by Dr Blanka Sengerová)

The Immortal Life of Henrietta Lacks is a biographical work exploring the abruptly shortened life of Henrietta Lacks, the woman who produced the HeLa cell line, interspersed with the story of the cells themselves. These cells arguably took on a life of their own, contributing to countless medical and biological discoveries over the years since Lacks' death.

Skloot writes in a captivating style, drawing both novice and specialist readers into the story. The author describes her fascination with HeLa, the cells she learnt about at school, which had been obtained from a certain Helen Lane (a false name to preserve the donor's anonymity). To her dismay, little was known of the woman who had donated the cells, and it became Skloot's personal mission to discover and tell her story. This she achieves admirably in a book that has been 10 years in the making.

The HeLa cell line originates from a poor young black woman who died of an aggressive cervical cancer in 1951; cells from her biopsy became the first ones to be successfully cultured *in vitro*. This was conducted without the knowledge of Lacks or her family, in an era when informed consent was not legally required. Since then, the cells have been used in many areas of science, from research into the polio vaccine in the 1950s to the effects of toxic substances and radiation on cells. Sadly, despite the amount of money that HeLa-based research generated, Lacks' surviving family remained poor and unable to afford healthcare, unaware for many years that Henrietta's cells were still 'alive' in many labs across the world. In her book, Skloot travels on a journey of discovery as she acquires the trust of Lacks' surviving daughter in order to learn the story of her mother's life. The inclusion of many poignant details constantly reminds us that this was a real person, not just a set of code-letters. I thoroughly recommend this well-researched book as very readable for scientists and the general public alike.

The Immune Response to Infection

Edited by Stephan H. E. Kaufmann, Barry T. Rouse and David L. Sacks.

Wiley-Blackwell, 2010, 666 pages, £120.65

(Reviewed by Amy Baxter)

The Immune Response to Infection brings together world experts in immunology, virology, vaccinology and pathology (including three of Oxford University's own) to discuss our current understanding of the innate and adaptive immune responses to infection.

An unusual feature of this textbook is its structure, which is broken into sections to address different aspects of the topic. The first section looks at the immune response *in situ* and provides a general overview of host defences. It includes chapters on 'sexy' themes such as mucosal immunology as well as further sections on both innate and adaptive immunity. The second section looks at the immune system in the context of pathogens, including viral, parasitic, bacterial and fungal organisms. The pathology of specific infections, such as those caused by African trypanosomes and *Helicobacter pylori*, are used to demonstrate the complex relationship between pathogens and their hosts. The third section investigates how pathogens have evolved to evade the host immune system and what happens when the immune system itselfmalfunctions. The final section describes current and in-the-pipeline attempts at immune intervention, particularly with respect to vaccines in the context of the four major killers, HIV/ AIDS, TB, malaria and influenza. This structure makes the textbook easy to dip in and out of, allowing the reader to locate areas of interest.

A further positive is the attempt of the authors to keep the text as up-to-date as possible, referencing primary literature and novel findings of the last 5-10 years rather than reviews. This may help keep the textbook relevant for longer. The one criticism of this book is the lack of colour illustrations. There are numerous diagrams accompanying the text, however (with the exception of the colour plates) these are in black and white. In some cases, diagrams have clearly been produced with this in mind; however, this is not always true, the result being that many diagrams are unclear and difficult to interpret. All in all, this textbook is a very valuable resource for anyone with an interest in the immune response to infection who would like to explore the broader context of their field.

Science communication: a journalist's perspective

Science Communication Conference 2011 keynote speaker and ex-editor at the *Guardian*, Tim Radford, explains how news reporting has changed over the years and why newspapers still have a place in the online world.

Dr Blanka Sengerová

Postdoctoral researcher in the DNA Damage and Repair lab at the Weatherall Institute of Molecular Medicine.

"People don't buy newspapers for science, the science stories are incidental," says Tim Radford, a journalist with decades of experience. Radford addressed delegates at the Science Communication Conference, taking them on a tour of his weaving career, discussing investigative journalism in the 1960s and the current state of play.

Radford described himself as both up-to-date and out-of-touch; whilst meticulously aware of the world around him, he admitted to never having blogged and never receiving an 'e-invite.' Early on, working for the *New Zealand Herald*, there was no such thing as a press officer- if you wanted a crime story, you went to the local police station. It was not possible to e-mail for information on a story. Instead, you went to the scene, with a pencil and paper in hand, and a tuppence for the phone box to ring the news desk once you had the story.

Radford argued that, like today, the newspapers back then, above all, had to make money. Science stories didn't sell papers and the science correspondent - if there was one - was regarded as rather a bore. Radford described the 1960s as heaven to be a journalist: there were the Beatles, the Vietnam War, the Cuban Missile crisis, the Moon landings and the Kennedy assassination. But some stories were missing. Radford suggests that while cosmology used to be a bit of a joke, the Big Bang theory now explained where the Universe came from, suddenly giving the field a much more credible position. Elsewhere, the theory of plate tectonics was proposed when geophysicists noticed how South America and South Africa fitted together like a jigsaw. Slowly, journalists including Radford started noticing how thrilling stories about science actually were, with the *Guardian* soon catching on and starting a weekly science supplement.

However, science still remained an elitist pursuit. Thatcher was quoted as saying that those interested in hearing about science should expect to pay for their indulgence, like opera-goers. Science, and opera, were not for the masses, only a select few. So even though science had become an exciting conversation topic, writers did not have a passion for communicating science per se, but were more concerned about getting stories in the paper and avoiding the 'spike' (referring to a sharp metal object on the editor's desk, where unused articles were disposed of!). Unlike today, when many science editors and science journalists have at least a first degree in science, if not a PhD or Masters, Radford was not too worried about having a limited science background. Science and journalism, he said, are not so different since they both start from the position of constructive ignorance.

Discussing online newspapers, Radford expressed some concern. When print was the major news medium, people tended to read the whole newspaper, cover-to-cover, including the science articles. This meant readers were exposed to and learnt about science just by reading the news. This is not necessarily true with online news, where readers cherry-pick articles and can easily ignore those that don't immediately peak their interest. Radford was particularly concerned that 16-24 year olds avoid being told things they don't explicitly look for.

Why is this important? Why should young people be informed about things they don't have an immediate interest in? Radford proposed a scenario where the government, in money-saving mode, starts cutting research budgets. If unaware of the importance of scientific research, the public may be unconcerned and think this an easy way to economise. Yet scientific research is important, and as long as the public are informed and can be convinced of this, they will step up to defend it, even in today's fast-moving, vivid and entertaining world.

To conclude, Radford argued that in a democracy, people (including scientists) have an obligation to tell others what they do. On the contrary, there is no obligation to listen. This means that scientists inevitably have to work hard to make themselves heard and to remind us why, particularly today, public engagement and science communication are a key part of a scientist's remit.

Bang Goes the Theory - LIVE!

Bang goes the theory is the BBC's flagship science television programme, 'putting science and technology to the test'. It airs on BBC1 in a primetime evening slot and, in my opinion, does a great job of engaging the general public, particularly the youth, in science. The programme aims to make science 'fascinating – and fun', whether explaining the principles behind everyday objects or exploring some unconventional applications of scientific theories.

Nicola Platt

3rd year D.Phil student in Dr Stephanie Cragg's laboratory in the Department of Physiology, Anatomy and Genetics.

As a public service, the BBC has an obligation to 'inform, educate and entertain'. In accordance with these core values, BBC Learning runs campaigns aiming to inspire individuals and communities, in a number of academic and recreational areas. One such campaign involves taking *Bang goes the theory* live to a series of roadshows around the country this summer. As part of this initiative, *Bang goes the theory* live was present at the Coventry Godiva festival, a 3-day long family-friendly music festival on the 2-3 of July, and I was lucky enough to be in a team of volunteers helping out.

Alongside the television programme, the roadshow was a fantastic chance to engage the public with science in a fun and entertaining way. All four of the presenters of the show were there; from the hardcore scientist Dr Yan Wong, who has a PhD in computational and mathematical modelling of self-incompatibility systems in plants and was a lecturer in evolutionary biology before joining the show, to the not-so-hardcore scientist Dallas Campbell, an actor and presenter. The presenters performed 15-minute shows every hour to capacity audiences of over 500 people, covering topics from climate change to the chemical composition of glue. Who would have guessed that glue made from rhubarb and custard sweets could support the weight of a fully grown man?!

Between shows there was plenty to keep everyone entertained, such as displays of the crazy contraptions built by the show's resident engineer, Jem Stansfield. These gadgets included square-wheeled skateboards, a microwave ray gun and a fire extinguisher-powered go-kart. For the more biologically minded, there were interactive displays showing the length of DNA in our cells (I think I was as surprised as the children by this!). In addition, at the Society of Biology stall, you could rearrange Velcro body parts, or watch white flowers turn pink, blue or orange by adding food colouring to the water in which they stood.

There was also the chance to participate in some real science. In conjunction with Northumbria University, Dr Yan is conducting an experiment to investigate what we project about ourselves through our movements, and so the public were welcomed to 'Dr Yan's dance lab'! All visitors to the Bang tent over the age of 18 were encouraged to take part. Participants filled in a questionnaire about their age, sexuality, health and personality. They then danced in a booth for about a minute. Motion sensors captured their every move and were used to generate an avatar, which was projected live to the whole festival as they danced! The neutral-shaped avatars will be put online and the public asked to rate different aspects of the movements. Scientists at Northumbria will then look for any relationships between the questionnaire responses and reactions to the dances. Having participated in a number of physiology and psychology experiments over the years, this definitely rates (for me) as the most fun, although possibly the most embarrassing as well!

One of the most striking things about the roadshow was the diverse audience it attracted, which was great for providing a balanced population sample for Dr Yan's dance experiment. Since the show was part of a music festival, many families came who would never normally have visited a museum or science fair, making it a fantastic opportunity to inspire children who are not normally encouraged to think about science outside of the classroom, as well as to engage and educate their parents.

I had a brilliant weekend at the roadshow. Within the narrow scope of academic research it can be easy to forget why you were first excited by science, and this weekend completely renewed my love for it. I remembered how much fun it is to carry out weird and wacky experiments, and how satisfying it is to puzzle out why they work. It was a great encouragement to me to engage the public further in our work – to show that the science we do is not only valuable but also comprehensible and fun! For me it was also an excellent revision of some long-forgotten fundamental principles: midway through explaining why a beach ball floats in a column of air I realised I had no idea why it wasn't flying out of the door! *Bang goes the theory* live broadened my horizons scientifically and socially as well as being a great opportunity to help inspire the next generation of scientists. Overall I'd recommend a visit to anyone, especially if you have kids, or if you're a kid at heart!

I´m a scientist, get me out of here

"If you discovered an option for immortality, would you use it?" This was just one of the many thought-provoking questions raised by school pupils for real-life scientists, as part of the Wellcome Trust-funded *I'm a Scientist, get me out of here!* initiative.

Dr Blanka Sengerová

Postdoctoral researcher in the DNA Damage and Repair lab at the Weatherall Institute of Molecular Medicine.

How do you engage one of the most challenging audiences and encourage them to take an interest in science, leading them to become scientifically literate engaged citizens in due course? *I'm a Scientist, get me out of here!* may be an answer, as panellists Shane McCraken and Sophia Collins of communication service company Gallomanor, and Dan Hannard, a Leeds-based physics teacher, aimed to convince us as they introduced the concept during the Science Communication Conference, held in London last May.

Firstly, an excited participating pupil demonstrated how video-links are used in the *I'm a Scientist…* competition. Scientists, ranging from graduate students through to professors, are linked up with classes of school students, ranging in age and ability, from top set 18 year olds taking their final exams, to Year 7 students with little experience of science. The groups interact online, with students able to ask the scientist any question they like. This is a new experience for the students as in most lessons they are the ones asked questions, not the ones to set them. Scientists do not have to travel anywhere as they answer questions from their own computer screen, in their own time. Additionally, there are live chats, where scientists and students are scheduled to be online at the same time and conversations can occur in real time.

The panel gave workshop participants a taste of a live chat session by linking up to five past scientist participants, one in the Antarctic, another in the jungle and yet another in an industrial research lab. Members of the audience could ask not only about the experience of taking part in *I'm a Scientist…*, but also questions about life in the lab. It felt chaotic, but cleverly brought across the excitement of how the communication format feels to participating students. "Why is the sky blue?" and "What's it like to type in gloves?" were among the questions raised.

When questions are answered, both in live chats and in longer question formats, the students decide which scientists stay in the competition and which leave. Some 'grown-ups' may scorn at this reality TV style of decision-making, but it is very familiar for children who have grown up with *Big Brother* and *The X Factor*. As it is entirely up to the pupils to decide who stays and who goes, the process is empowering and engaging.

Dan Hannard, who has been involved with the project since its inception, gave a very enthusiastic address. He described science communicators as the mouthpiece of science and teachers as the receiver, arguing that sometimes there is a slight disconnect between the two. *I'm a Scientist…*, in his eyes, is a great way of communicating science directly to the pupils, without teachers as mediators, resulting in engaged and informed pupils, but not at the expense of dumbing down the science. It wasn't just the usual suspects that were coming up with questions; in the online environment, the usually quiet and subdued students at the back of the class were suddenly posing well argued questions, giving a voice to those who at other times may feel too intimidated to speak. Importantly, the project worked with all abilities, from those aiming at Oxbridge university places to those doing work based qualifications.

Early on, the issue was raised as to whether the students would focus on superficial qualities when voting out scientists – is he good-looking, does she come from a poor family, does their project sound cool just because they're based at a space station – but, in Hannard's experience, the pupils cut through these surface qualities very effectively. They concentrated instead on whether the scientist was a good speaker and explained his or her science clearly, and how significant the scientific contribution was.

What an exciting way of getting school kids interested in science – it has certainly made me want to take part as a scientist!

Write for *Phenotype*?

The deadline for article submissions is 25 November 2011 • We accept articles on any aspect of biological sciences research, books or science education • Articles can be either 650 or 1300 words. If interested, please get in touch: oubs@bioch.ox.ac.uk.

Work for *Phenotype*?

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

Lizzie Burns, science artisan

Interviewed by Caroline Dahl

It was in 1996, the first year of her DPhil at Oxford, that Dr Elizabeth Burns had a stroke of genius. Dr Burns, or 'Lizzie' to the children that she has taught at Cheltenham Science Festival for the past seven years, realised that molecular biology did not have to be ugly. One could portray subcellular mechanisms in oil colours and thus bring stained, dead specimens back to life.

Caroline Dahl

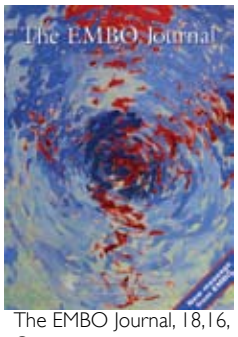
Third year DPhil student in Prof Mark Sansom's laboratory in the Biochemistry Department.

A few years earlier, she, like many of us, had made the difficult choice between the Arts and the Sciences, and had opted to squeeze art into her weekends. It was the logical thing to do, as it fit much more easily than science into her spare time. So when she first realised that her two favourite topics did not have to be mutually exclusive, it was a revelation.

Within a year she had an art exhibition in the top floor of the old Biochemistry building that drew in 150 people. She also wrote a play and, when she eventually handed in her DPhil thesis, each chapter opened with a print of a relevant oil painting. When her supervisor spotted an advertisement in the *EMBO Journal* asking for artistic scientists to contribute cover art, he knew who to forward it to. Lizzie painted four covers for *EMBO*, which at the time was housed by the Oxford University Press. She finished off in style with an OUP exhibition where her talent was spotted and she was commissioned to do a book cover, another few science magazine covers and a mouse pad in the shape of a kidney.

Amongst well-received exhibitions and happy nephrologists, Lizzie built up her artistic confidence and her portfolio. When her three-year postdoctoral fellowship came to an end, she scavenged the internet to find work for herself as a science artisan. As Lizzie told the OUBS at a talk that she gave in May this year, such positions are scarce. There was the well-established Wellcome Trust's 'Sci/Art' fund, but the art critics involved were not convinced by Lizzie's impressionistic works.

Art meets science: a Lizzie Burns painting based on imaging the body (courtesy of Dr Elizabeth Burns).



The EMBO Journal, 18,16, ©, 1999

The EMBO Journal, 17,22, ©, 1998

Lizzie's work has been featured on the covers of a number of journals.

Instead, Lizzie invented her own future. She spotted a clause in the UK Medical Research Council's (MRC) mission statement that emphasised the need for them to support public awareness of science. Although there was no official MRC grant for artistry, Lizzie read the statement as an open invitation. She wrote up a proposal, landing a succession of grants across five years that took her and her camera to over 20 laboratories scattered across the UK. There she would peer at fellow researchers through an inch-thick camera lens, capturing the every-day stubbornness of hundreds of scientists who collectively choose to squeeze art into their weekends for the greater good. Her MRC work culminated in a British Council invitation to travel to India to exhibit an MRC art collection and teach girls there about the prevention of malaria.

This experience sparked Lizzie's interest in engaging with people, and she realised that she wanted to work more closely with the public than the messenger-at-a-distance role that she had adopted for the MRC. Consequently, she set up workshops and started taking her work to festivals. At the Cheltenham Science Festival she encouraged children to express their ideas of subcellular structures in fluorescent clay. Pictures of brightly coloured organelles in the hands of proud five-year-old owners with rows of UV-blue beaming little round teeth bear witness to

her travels. Back in the city, at a University College of London hospital, Lizzie arranged for cancer patients to paint and draw. She summed up the patient feedback as simply: "You need to express yourself in a sterile, clinical environment". This observation might seem to contradict her days as a wet lab academic where sterility was a virtue, but Lizzie is quick to correct this assumption. "To me, the lab was always a very creative space. You went in there to figure something out; you had to be innovative." This might have been particularly true in Lizzie's lab; that of Professor Edwin Southern, of Southern blot fame, who went on to develop pulsed field electrophoresis and DNA microarray technology. Today, royalties from these DNA chips fund a programme where scientific equipment is shipped to schools to aid and promote natural science education, demonstrating the shared interests of Lizzie and her former PI.

While public engagement might leave you with a warm and fuzzy feeling inside, it does not necessarily feed you. In the light of this, since her time as an MRC artist in residence, Lizzie has done several commissions, including brightening up the interior of the University of Brighton and the GlaxoSmithKline Imaging Centre. The Wellcome Trust eventually came around to her way of thinking and invited her to participate in an event at TATE Modern where neuroscientists talked about creativity from a neuroscience perspective. Lizzie encouraged participants to stick colourful clay onto large windows with full view of the London skyline. Despite Lizzie's encouragement, none of the neuroscientist speakers dared to approach the clay, whereas the patterns created by audience participants curled up and down the glass facade.

Lizzie had more luck with some other neuroscientists, following a few glasses of wine at the 2007 British Neuroscience Association dinner, where she challenged teams of competitive neuroscientists to create neuroscience-themed art installations. They were assessed based on their sense of humour and visual appeal. A couple of homunculi appeared that battled it out to achieve the largest erection, only limited by the persistence length of clay, but the winning artwork was 'Escape from the Morris water maze'. It featured all the tools needed for a rodent's escape: eyes, a hippocampus, a cerebellum and some motor neurons. In addition there were a couple of seahorses, allowed on artistic licence, and also a contemplative mouse, looking on.

Dr Elizabeth Burns left science a decade ago, yet has more publications to her name than most of us, and has probably landed more covers. Her work has been featured in media such as the *New York Times*, *The Guardian*, BBC Radio 4 and *The Times*. You would be forgiven for aiming for less upon abandoning your chosen career. Ultimately, her dad's down-to-earth words echo her bold and entrepreneurial career choices: "Just try doing something you really enjoy".

A classic and contemporary example of art inspired by science

Dr Burns is not alone in her endeavours to make science prettier. In the 1850s, curious people flocked around courageous adventurers that had returned from faraway, exotic countries with dead creatures of all sizes. Around the same time, the submarine was developed, opening up a chance for underwater adventures and new niches for natural science exhibitions. One museum curator commissioned glass replicas of sea anemones from an Austrian family of glass makers, the Blaschkas. These sea anemones were so exquisite in detail that father and son Blaschka became famous and swapped making glass eyes, a profitable industry at the time, for making marine animals, calling themselves "Natural History artisans". Blaschka's glass creatures can be seen in several museums today, including the London Design Museum, and the collection includes jellyfishes, diatoms and dead men's fingers. See http://designmuseum.org/design for examples.

The collaboration between sisters and professors Helen and Kate Storey is a more recent example of science applied to art. Helen designed and managed her own fashion brand, worn by the likes of Madonna and Cher, whereas Kate was a developmental biologist. In 1997, the Wellcome Trust commissioned them to create garments that represent the first thousand hours of human development. Today 27 fashion items are on tour, showcasing items such as the "the uterus" (see figure) and "closing neural tube dress". You can follow the exhibition at www.primitive-streak.org.

Courtesy of Caroline Dahl

5'WITH... Professor Quentin Sattentau

Interviewed by Amy Baxter

Quentin Sattentau moved his laboratory from Imperial College to the Dunn School of Pathology in 2003. Here he has continued his work on HIV-1 biology and vaccine immunology, with a focus on cell-to-cell HIV-1 infection and the design of novel vaccine adjuvants.

When did you first decide you wanted to be a scientist?

I was geeky from a young age! I was about 14 when I first became interested in microbes; I remember looking at pond water through a microscope and being both disgusted and intrigued. At school, I was fairly useless at science, except for Biology, and my careers advisor said (incorrectly!) I couldn't apply for medicine because I didn't have Latin O-level. So, I applied for a medical microbiology degree at Bristol. Three weeks in, I found out that I was actually on a straight microbiology course as my course had been cancelled and they hadn't told me! It was mostly pretty dull, but in my 3rd year I did a research project on the herpes simplex virus and loved it. There was also an element of rebellion – both my parents were artists so the house was always full of clay, which did my head in.

If you are not in the lab you are…

In the kitchen. I do most of the cooking at home. Or building a bicycle or gardening or doing any kind of DIY; painting, plastering, plumbing…

If you were not a scientist, you would be…

A landscape gardener. I love making massive changes to gardens, putting in hills and walls, digging holes. I like being outside, so maybe I should have been a botanist or oceanographer.

What was your worst disaster in the lab?

It wasn't a disaster but it could have been! For my first post-doc, the Category III containment lab was a port-a-cabin on top of a venereal diseases clinic. There was no fumigation system, so to fumigate I had to set off a formaldehyde bomb and run out, then when the lab was fumigated I had to run back in holding my breath and turn the hood on to extract the fumes. I'd get really dizzy and by the time I got out my eyes would be stinging. I could easily have tripped over, breathed in a lung-full of formaldehyde and been unconscious in seconds. I don't think that would be allowed anymore…

What's the best advice you've ever received?

To be generous in science. Don't try to claim things and keep them to yourself. You move the field along much more by sharing ideas and reagents.

If you were starting your career again, are there things you would do differently?

No is the boring answer! It's been a roundabout journey, but I'd do it all over again.

Do you have a favourite classical experiment?

I was a post-doc in Richard Axel's lab at Columbia at the same time as Linda Buck. While I was there, she cloned the olfactory receptor family, the work that won her and Richard the Nobel Prize. I remember her excitedly showing me a gel she had run – the Nobel gel!

She hypothesised that there would be one olfactory receptor with multiple isoforms which would bind all the different odorants. It was a very elegant experiment; she made a subtractive RNA library from rat olfactory epithelium and used degenerate primers to clone out the receptor family. She was looking for one band that, when cut with restriction enzymes, would give lots of different bands, showing multiple isoforms. And that was exactly what the gel showed - beautiful.

In your opinion, what makes a good scientist?

It's critical to be really interested in science, curious, but also persistent and thick-skinned, else you'll be easily defeated by academic life. You also need to be a good communicator to get your ideas out into the scientific community.

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

I hope we'll start to move away from the "–omics" and mass-screenings and back towards curiosity-driven creative research. I also hope we'll move back from the relentless focus on translational research and be able to research how things work just because we want to know. Apart from being useful, science is part of our cultural heritage and scientific knowledge is a large part of what brought us out of the dark ages. I suspect we will see new vaccines and begin to understand more about how the brain works, especially in terms of ageing. It's an important problem as I see all these people ageing around me. Luckily it's not happening to me.

SNAPSHOT Research Image Competition

We are pleased to announce that this issue's winner of the *Snapshot* scientific image competition is Dr Helen Farr, a postdoctoral researcher in Prof Stephen Bell's group at the Dunn School of Pathology.

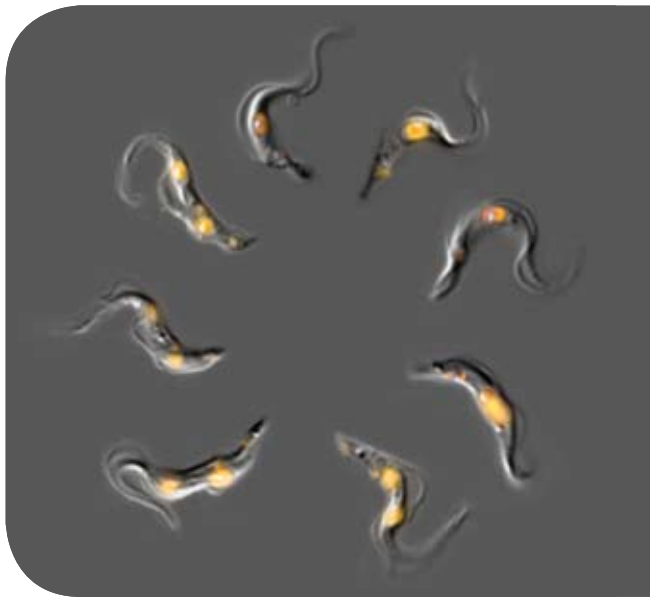
She created our winning cover shot from fluorescent microscopy images of propidium iodide-stained *Trypanosoma brucei* cells undergoing cell division. Both the nuclear DNA and the mitochondrial DNA (the smaller dots) can be seen at all stages of division.

In recognition of her contribution, she will receive a £50 book voucher kindly provided by our sponsor Oxford University Press.

We hope she will enjoy her reading!

OXFORD UNIVERSITY PRESS

Trypanosoma brucei is well known as the causative agent of human African trypanosomiasis, or sleeping sickness. However, it is also scientifically interesting owing to its evolutionary lineage as an early branching eukaryote. As such, *T. brucei* shares characteristics with both eukaryotes and archaea. In particular, its DNA replication machinery contains features from both kingdoms. For example, like other eukaryotes, *T. brucei* utilises a six subunit MCM helicase to unwind DNA prior to replication, but also contains a homologue of the archaeal DNA replication initiation factor Orc1/Cdc6.



Prof Stephen Bell's group studies DNA replication and transcription, using archaeal models to understand related but more complex processes in eukaryotes. *T. brucei* thus bridges the gap between the relatively simplistic mechanisms of archaea and those of eukaryotes.

Helen's research focuses on identifying the origin of replication (*ori*) sites within the *T. brucei* genome. A number of candidate sites have already been identified using ChIP-chip and she plans to confirm their identity using Marker Frequency Analysis (MFA) on cells isolated by fluorescence-activated cell sorting (FACS). While establishing the FACS assay, she encountered problems with cell fixation. A routine check led to the image gracing the front cover of this issue of *Phenotype*.

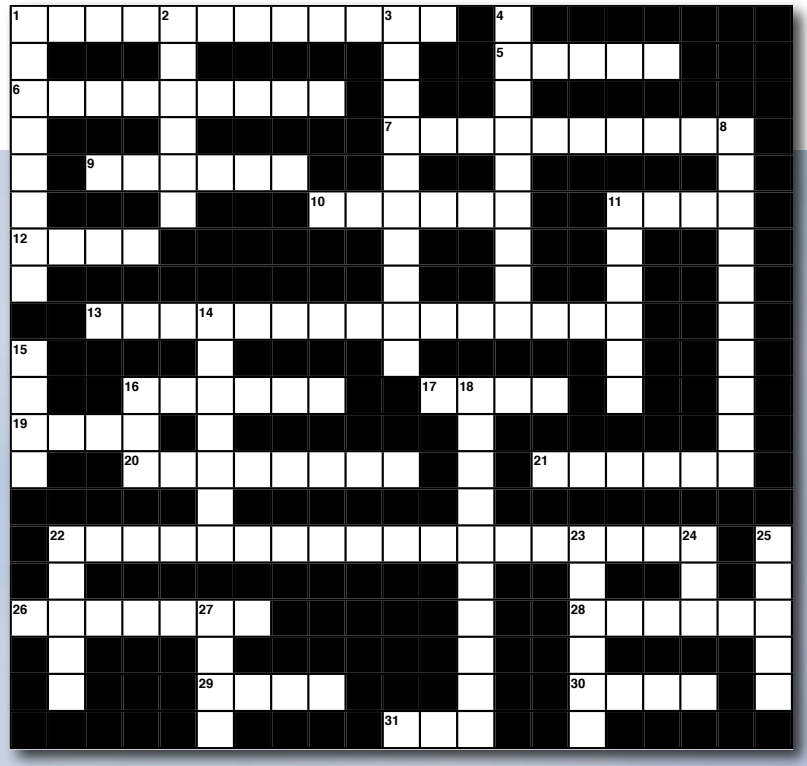
To determine whether her *T. brucei* cells were fixed properly, Helen stained the cells with propidium iodide, which binds to DNA, and subsequently visualised them using fluorescence microscopy. As shown in the cover image, all stages of the *T. brucei* life cycle can be seen. The larger red structures in the cells are chromosomal DNA, while the smaller dots are mitochondrial DNA. In *T. brucei*, DNA from a single mitochondrion is tightly clustered, forming a structure called the kinetoplast. As seen in the image, the kinetoplast is the first to replicate and divide, followed by replication and division of the nuclear DNA. Simultaneously, the kinetoplasts separate in preparation for cytokinesis.

Beyond the fundamental research goals for investigating DNA replication in *T. brucei*, its similarities to archaea and thus differences to both its human host and tsetse fly vector, may also present targets for future therapeutic agents designed to combat sleeping sickness.

Snapshot Hilary 2012: how to enter...

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 in Phenotype Hilary 2012. To enter, send pictures to oubs@bioch.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images. There is no limit to the number of entries per person. The deadline for the competition is 25 November 2011.

crossword



***Phenotype* invites you to explore the weird and wonderful world of *Drosophila melanogaster* gene names, which are often inspired by the phenotype caused by mutation or deletion of the gene.**

Consider yourself a fly expert? Take the challenge below!

Enter the competition by sending your answers to oubs@bioch.ox.ac.uk or leave a paper copy in a sealed envelope in the OUBS pigeonhole at the New Biochemistry reception. Entries received by 25 November 2011 will be entered into the prize draw.

The winner will receive their choice of two books reviewed in this issue.

Congratulations to Roger Brideau, a retired microbiologist from the Dunn School (1979) who won the Trinity '11 crossword competition.

Across

1 Gene required for normal tissue organisation in the epidermis and central nervous system. Indistinct frankfurter? (5, 7)
5 Adult mutants are unusually hirsute. (5)
6 Females mutant for this gene produce spindle-shaped eggs without dorsal appendages. Encodes a protein involved in RNA silencing. (9)
7 Mutant embryos exhibit nerve bundles that are thicker than normal, much like tangled, matted ropes of hair. (10)
9 Example of a gap gene. May also be useful in wet weather. (6)
10, 28 These poor flies have no external genitalia. (3, 3, 6)
11 Interacts with Gustavus, forming a Swedish king. (4)
12, 16 Work together to trigger apoptosis. Death personified. (4, 6)
13 See 26 across.
16 See 12 across.
17 See 15 down.
19, 30 Named for the apical/basal movements observed in the Golgi bodies of mutants, reminiscent of a hideously kitschy decorative light. (4, 4)
20 Mutants cannot fly. (8)
21 Mutant males court but do not mate with females. (6)
22 Zinc finger transcription factor controlling segment polarity. Not to be confused with an unreliable method of contraception. (7, 11)
26, 14d, 13 Child-bearing women will oppose pretty much anything - even *Drosophila* morphogens. (7, 7, 15)
28 See 10 across.
29 Gene abbreviation/acronym. Brother of 31 with entrails limited? (4)
30 See 19 across.
31 (_) Skipped. Strange mutant phenotype seen. (3)

Down

1 Cadherin protein regulating tissue planar polarity. May also be useful in a game of croquet, though only if cooperative. (8)
2 Flies mutant for this gene have learning impairments. Hilariously stupid root vegetable. (6)
3 Protein required for retinal development. Takashi's extra-time winner. (6, 4)
4 Mutants are particularly sensitive to alcohol. (5, 4)
8 Gene required for cuticular development. (10)
11 Mutants of this gene have malformed legs. Named after a similarly disfigured Roman god. (6)
14 See 26 across.
15, 17a Regulates RNA splicing. Partial loss of function causes females to lay much shorter eggs. (4, 4)
16 Dorsal closure and cuticle differentiation defective. Not cooked. (3)
18 Mutant flies are uncoordinated, as if they have had one too many. (10)
22 Mutants have red and white eyes. (5)
23 Mutant embryos form a faint ball of cuticle resembling a small stone. (6)
24 Sister of 29d and 31d. Cry cry cry. (3)
25 Colocalises with 1d at proximal/distal cell boundaries. Probably nothing to do with the Hand of God. (5)
27 Abbreviation for gene involved in axon guidance, named after distinguishing feature of Milton Keynes. (4)

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