PHENOTYPE

Issue 7, Michaelmas Term 2010

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Constitutive Lck activity in T cells

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EVOLUTION OF BRAIN DEVELOPMENT

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5' WITH...

Professor Christopher Schofield

BACK TO THE ROOTS

The new Oxford Parkinson's Disease Research Centre

PLUS...

Cancer Stem Cell Theory
Post Traumatic Stress Disorder
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EDITORIAL

First of all, I would like to welcome all new students and staff members to Oxford. I hope you will enjoy your time here. Make the most of it!

To those returning from the summer break: welcome back! Personally, I find Michaelmas term very exciting – each time feels a little bit like being a fresher again.

This issue is the first of three for the new academic year, and with its 32 pages it is larger than ever. We have created more space to accommodate six featured articles and even added a comic to our research highlights.

Human health is the underlying theme of this issue's features, starting off with an introduction to cancer stem cell theory by Shaoyan Liang. Recent advances in sleeping sickness research are described by Richard Wheeler, and Emma Hindley discusses potential drugs for the treatment of Post Traumatic Stress Disorder. On the neuroscience side, we are very grateful to Professor Zoltán Molnár for his in-depth account of the evolution of the brain, and to Stephanie Janezic for introducing the newly opened Parkinson's research centre. Last but not least, Nicola Platt raises our attention to the interesting issue of gender bias in animal experiments.

Furthermore, we have interviewed Professor Chris Schofield, a leading figure in uncovering the biochemical cascade behind the human hypoxic response, and related enzymes involved in epigenetic regulation. Our research highlights section draws your attention to some recent publications from Oxford University and our book reviews might give you some inspiration for home reading.

A brief discussion of science ethics in our science and society section adds some final spice. I hope you will enjoy reading the latest issue!

None of this would have been possible without the people behind *Phenotype*, who have been exceptionally committed and enthusiastic, and are constantly seeking ways to improve the magazine. I would like to thank everyone who has been involved in this and previous issues of *Phenotype*: page editors, distributors, features, regulars and science and society editors, our designers, the fundraising team and of course our contributors.

We are always keen to take new faces on board. Get in touch if you would like to get involved, be it in editing, design, writing for *Phenotype* or fundraising. Or maybe you have an idea that we haven't thought of yet? Even better!

Anna Boleininger
Editor

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

CRACKING COMPLEX I

OUBS Featured Seminar

Daniel Grimes

In November 2010, OUBS is privileged to host Dr Leonid Sazanov of the MRC Mitochondrial Biology Unit, Cambridge. This year in *Nature*, Sazanov's group unveiled beautiful new crystal structures of respiratory complex I, revealing a mechanism that can explain the indirect coupling between electron transport and proton pumping.

We humans derive most of our energy from the food we eat via a process known as oxidative phosphorylation. This involves stripping electrons from metabolites and passing them down a respiratory chain to oxygen. The chain comprises three transmembrane energy-converting enzymes within the mitochondria of which complex I (NADH-quinone oxidoreductase) is the first and most complicated. Electron transfer through these complexes provides the driving force for proton pumping across the mitochondrial membrane, which establishes an electrochemical gradient essential for ATP production by the F_1F_0-ATPsynthase. Whilst the mechanisms at work within complex III and IV, and indeed the ATP synthase itself, have been largely uncovered, the workings of complex I have remained mysterious in the absence of a complete molecular structure.

With a molecular mass of around one million Daltons, the 45 subunit structure of complex I, particularly the membrane-associated section, has long eluded high resolution structural analysis. Information has instead been gathered using an assortment of biophysical techniques coupled with crystal structures of the extra-membrane part. In 1991, an electron microscopy analysis of complex I from *Neurospora crassa* revealed it to be a bipartite structure with intra- and extra-membrane sections forming an L-shape (1). Various studies using electron paramagnetic resonance (EPR) spectroscopy have predicted the physicochemical properties of the redox centres, including the primary electron acceptor, flavin mononucleotide (FMN) and a number of iron-sulphur (Fe-S) clusters. In 2006, Sazanov and Hinchliffe reported the high resolution structure of the hydrophilic extra-membrane domain of complex I from the bacterium *Thermus thermophilus* (2). This structure defined a ~95 Å electron transfer chain with electrons from NADH being passed to FMN and then down a pathway of at least seven Fe-S clusters. It was not clear, however, how electron transfer, almost exclusively within the extra-membrane domain, could be coupled to proton translocation in the membrane-associated part of the complex. The distances seemed too great.

Now, in exciting new research which has graced the cover of *Nature*, Sazanov and colleagues have determined the long-hoped-for crystal structure of the hydrophobic, membrane-associated domain of complex I from *Eschericia coli*, as well as revealing a complete structure from *T. thermophilus* (3). These structures have uncovered an elegant mechanism that might be at work to couple electron transfer to proton pumping. The three largest transmembrane subunits named NuoL, NuoM, and NuoN all resemble Na^+/H^+ antiporter proteins, containing a 'helix-peptide-helix' motif known to be crucial for ion transport. Strikingly, however, NuoL contains an incredibly long α-helix, called helix HL, which extends parallel to the plasma membrane for around 110 Å through complex I. It is thought that conformational changes in the hydrophilic domain driven by electron transfer are transduced to the HL helix, which then tilts helices in the three antiporter-like subunits, changing the conformation of an ionisable residue within the channels and resulting in the translocation of three protons.

In light of these details, Sazanov has called complex I a 'steam engine' which uses the energy of electron transfer to move a 'piston' that in turn drives the opening and closing of three antiporter-like channels. It is helix HL that provides the mechanical link between conformation changes induced by electron transfer and those required for proton translocation. However, the direct driving force of the 'piston' remains to be determined.

This mechanism goes a long way to explaining the transport of three protons per pair of electrons, but the currently agreed-upon number is four. The fourth proton is thought to translocate at the interface of the two main domains in a mechanism that departs from that of the first three. Crucially, some important protein loops close to this interface were not resolved in Sazanov's structure and remained disordered. It might be interesting in the future to obtain crystal structures in which loop conformation has been locked by quinone analogues that inhibit electron transfer.

There are still gaps in our knowledge, particularly in mechanistic details that will have to wait for higher resolution structures. However, the work of Sazanov and his colleagues in the field continue to shed light on the workings of the most elusive of respiratory complexes. These marvellous structures will provide considerable incentive to thrust complex I research forward again.

References:

1. Hofhaus G, *et al.* (1991) Electron microscopic analysis of the peripheral and membrane parts of mitochondrial NADH dehydrogenase (Complex I). *J Mol Biol* 221(3):1027–1043.

2. Sazanov LA & Hinchliffe P (2006) Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. *Science* 311(5766):1430-1436.

3. Efremov RG, *et al.* (2010) The architecture of respiratory complex I. *Nature* 465(7297):441-445.

BLM has early and late functions in homologous recombination repair in mouse embryonic stem cells

Chu WK, Hanada K, Kanaar R and Hickson ID (2010) *Oncogene* 29(33):4705-4714.

DNA damage poses a constant threat to the integrity of our genome and severely increases our risk of developing cancers and ageing faster. Our cells therefore rely on repaiar mechanisms that restore the damaged DNA and maintain genome stability. Double strand breaks, caused by γ-radiation or collapse of a DNA replication fork, can be repaired via the homologous recombination (HR) pathway. This pathway utilises sequences identical to the damaged DNA from either a sister chromatid or a homologous chromosome which thereby act as a template to repair the break.

For a long time the RecQ family helicase BLM, defective in patients with the cancer-predisposing disorder Bloom's syndrome, has been thought to act as an 'anti-recombination' protein in the HR pathway. Individuals who carry mutations that alter or delete the helicase motif of BLM show a loss of heterozygosity and have a greatly increased frequency of sister chromatid exchanges caused by HR. However, despite BLM's link to cancer development and its important role in the HR pathway, the precise functions of BLM are still unknown.

To study the HR mechanism in a mammalian species, Chu *et al*. have generated mouse embryonic stem (ES) cell lines which carry either a *Blm* mutation or a deletion of the *Rad54* gene. *Rad54* encodes a DNA-dependent ATPase proposed to have several 'pro-recombination' functions. The authors also created a double-mutant ES cell line that carries mutations in both the *Blm* and *Rad54* genes to investigate their relationship in the HR pathway.

Chu *et al*. analysed the efficiency of the mutant ES cells to carry out gene targeting, an HR-based genetic technique used to modify endogenous sequences. The authors demonstrated that while the gene targeting efficiency of *Rad54* mutant cells is severely reduced, the *Blm* mutant cells exhibit a hyper-recombination phenotype. This phenotype was completely suppressed in *Blm/Rad54* double mutant cells, which, in combination with previous genetic pathway analysis results in yeast, suggested that BLM mainly functions genetically downstream of Rad54. In addition to this finding, however, Chu *et al*. went on to show that the *Blm* mutation is sufficient to rescue the sensitivity of *Rad54* mutant cells to the DNA crosslinking agent mytomycin C (MMC), suggesting that BLM can also function upstream of Rad54.

This study is the first to demonstrate that BLM has multiple functions in both early and late stages of the HR pathway in a mammalian system and goes on to suggest that the roles of BLM previously established in yeast are likely to be conserved in higher organisms.

Constitutively active Lck kinase in T cells drives antigen receptor signal transduction

Nika K, Soldani C, Salek M, Paster W, Gray A, Etzensperger R, Fugger L, Polzella P, Cerundolo V, Dushek O, Höfer T, Viola A and Acuto O (2010) *Immunity* 32(6):766-777.

T cells are essential mediators of the adaptive immune system, which is responsible for recognising and remembering pathogens that attack our bodies. It is this branch of the immune system that enables us to mount a stronger and faster immune response when we encounter the same pathogen again in the future.

Activation of T cells is induced when cell surface T cell receptors (TCRs) bind to a major histocompatibility complex (MHC) that displays peptides from the invading pathogen. To induce subsequent TCR signalling, intracellular subunits of the TCR are phosphorylated by the tyrosine kinase Lck, a member of the Src family kinases (SFKs). This phosphorylation event initiates a complex signalling cascade that couples the cell surface receptor to the intracellular signalling machinery.

In this study, Nika *et al*. investigated how induction of TCR signaling by Lck is regulated. While traditional views suggest that Lck is activated by peptide-MHC binding to the TCR, the authors had previously demonstrated that activated Lck is present in unstimulated human T cell lines. Here, Nika *et al*. confirmed that this observation holds true *in vivo* by showing that a high proportion of Lck in naive T cells of lymphoid organs in mice is constitutively activated.

The control of Lck activity relies on the equilibrium between phosphorylation and dephosphorylation of an activating tyrosine in its catalytic domain and of an inhibitory tyrosine in the C-terminal domain. Using immunodepletion and quantitative immunoblot experiments, Nika *et al*. identified two distinct forms of activated Lck with different phosphorylation patterns. In addition to the previously known form, which is phosphorylated at the activation site, the study revealed a new type of activated Lck that is phosphorylated at both the activating and inhibitory site. This finding indicates that Lck phosphorylated at the inhibitory site may not solely represent inactive Lck.

The authors then showed that expression of catalytically activated Lck is independent of TCR or co-receptor expression as well as of TCR binding to peptide-MHC. This finding is consistent with a recent Foerster (or fluorescence) resonance energy transfer (FRET) analysis that observed no conformational change of Lck upon TCR ligation. Nika *et al*. suggest that the activated Lck pool needs to be monitored by the HSP90-CDC37 chaperone complex that prevents Lck degradation. Furthermore, when treating immortalized T cells with an SFK inhibitor, the authors observed an exceptionally fast decay of intracellular activated Lck, leading to the hypothesis that Lck kinase activity is also critical to maintain its own activated state, possibly through a trans-autophosphorylation mechanism.

These findings raise new questions, for example about which mechanism prevents the pool of activated Lck from excessively phosphorylating TCRs in unstimulated cells, and imply that a revision of the current TCR signal transduction model will be necessary.

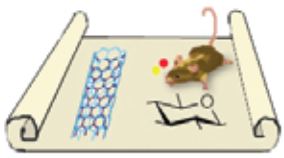
Sugarcoated radiotherapy

Hong, Tobias, Al-Jamal, Ballesteros, Ali-Boucetta, Lozano-Perez, Nellist, Sim, Finucane, Mather, Green, Kostarelos and Davis
Filled and glycosylated carbon nanotubes for in vivo radioemitter localisation and imaging. Nature Materials 2010 9 : 485-490

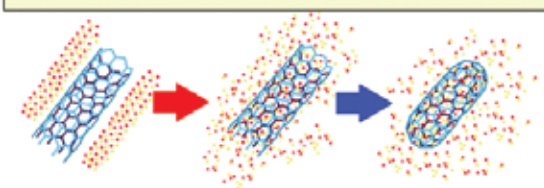
In the unforgiving English summer heat, Oxford chemists try to master localised radiotherapy - used to treat cancer, in new and inventive ways.

Radiotherapy is performed by an ionising source, such as a radio-isotope of iodide. Two things are particularly problematic about iodide use in the body: an iodide transporter steals most of it to the thyroid, and almost all ionising iodide leaves the body within 24h.

Single-walled nanotubes (SWNTs) to the rescue! SWNTs are cylinders made of a carbon hexamer called benzene. The cylinder interior can hold cargo. With this in mind, Oxford chemists used SWNTs, a salt of the radioactive iodide isotope I^{125} and a sugar coating to make a drug delivery vehicle.



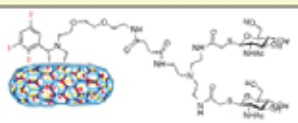
Step 1: Encapsulate the drug
SWNTs, mixed with radioactive sodium iodide salt, are first heated to 900°C to melt the salt. The resulting liquid is densely radioactive and enters the SWNT cylinders by capillary force. As the mixture cools, kinetic energy allows the SWNT's open ends to seal, forming a more stable, united hexameric network where electrons are more delocalised. In the process, SWNTs trap radioactive particles. Any untrapped cargo is removed.



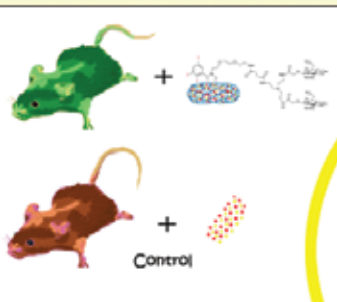
However, carbon nanotube applications face two problems: their influence on cells is the subject of controversy and they mix poorly with water. Therefore Oxford chemists set out to alter the SWNT surface to improve biocompatibility.

Step 2: Tag the SWNT and confirm results
The chemists tag the SWNT with a link that allows them to attach other chemical groups at its end. Both the tag and the cargo are visible using electron microscopy.

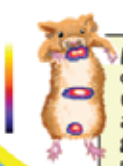
Step 3: Improve biocompatibility and target the tissue
They put sugars onto a branching molecule and sugar-coat the SWNT via the link.



The drug delivery-vehicle is ready for testing! $Na]^{125}$ radiotherapy enters the bloodstream either as SWNT cargo or unencapsulated, as a control.



Single photon emission computer tomography shows that sugar-coated SWNTs and their cargo are rapidly contained in the lungs. They remain there for a month without causing either cyto-toxicity or fibrosis.



Non-encapsulated iodide, on the other hand, ends up in the thyroid, stomach and bladder. The body gets rid of most of it within a day.

The team managed to deliver an unprecedented high dose of radiotherapy, stably incapsulated and highly localised to the lung. Here, it can act long-term, or alternatively be used to image the tissue non-invasively.

Next the researchers will investigate the sugarcoated SWNT's fate in the body in more detail. They want to explore the delivery vehicle's clearance kinetics and find out how to steer the SWNT elsewhere. Ultimately, they aim to tame the sugar-code, to target any tissue for therapy.

Stay tuned!

by Caroline Dean, in conversation with Ben Davis

EVOLUTION OF BRAIN DEVELOPMENT

Professor Zoltán Molnár

Understanding how the human cerebral cortex (the outer sheet of neuronal tissue of the human brain) has evolved is a fascinating topic for neuroscience, genetics, bioinformatics and comparative biology. We study embryonic brain development in a number of species in order to gain insights into the origins of the mammalian neocortex, which is the newest part of the cerebral cortex in evolutionary terms.

The cerebral cortex is the seat of higher cognitive functions

In Oxford during the 17th century, Thomas Willis proposed that human higher cognitive function originates from the convolutions (folds) of the cerebral cortex rather than from the fluid or other structures in the brain, or other parts of the body. Willis based this on the observation that the convolutions are larger and more numerous in the human cortex than in other species (1). As a practising clinician, Willis was able to follow patients for years and dissect their brains after death, which allowed him to recognize that several cognitive abnormalities were associated with alterations of the cerebral cortex convolutions (Figure 1).

Almost 350 years later, we now know that Willis was right. During the evolution of reptiles, birds and mammals, some brain regions have largely been conserved, whereas the cerebral cortex has undergone a great elaboration since the divergence of these lineages. Moreover, there was a great increase in the total surface area of the cerebral cortex in mammals, which has increased exponentially from lesser shrew (0.8cm^2), rat (6cm^2), cat (83cm^2), to human (2,500cm^2), bottlenosed dolphin (3,745cm^2), and African elephant (6,300cm^2)(2).

Cortical organisation is different in reptiles, birds and mammals

It is not only the size and the shape of the brain that differs in various species. There are also drastic differences in cerebral cortex organisation between reptiles, birds and mammals; deducing how various regions correspond has been difficult. The relative position of the dorsal cortex has now been established based on embryonic gene expression patterns. Whilst the mammalian neocortex contains six layers of dorsal cortical neurons, the equivalent region in reptiles (dorsal cortex) only contains a single cell-rich layer embedded between two fibre-rich layers (Compare N with DC in Figure 2). In birds, there is a non-layered structure in the position of the dorsal cortex. Another distinct feature seen on a cross section of an avian or reptilian brain is the presence of a large ball of cells protruding to the lateral ventricle, called the dorsal ventricular ridge (DVR) or avian hyperpallium (Figure 2B).

In addition to the structural rearrangements, there are major differences in cell numbers between species. The mammalian dorsal cortex contains far more neurons and a greater diversity of neural subtypes than the equivalent brain regions in sauropsids (which includes all existing reptiles and birds). Where did the extra cortical cells come from in the mammalian brain, and what are the major drivers of increased cortical neurogenesis in mammals?

Where are the extra mammalian cortical neurons coming from?

Two major hypotheses exist to explain the increase in mammalian cortical neurons, with both agreeing that accessory sites of neurogenesis would have been required for mammalian cortical evolution. According to Karten's classical equivalent circuit hypothesis, the additional neurons are generated outside the neocortex, then migrate and integrate into the neocortex (4). This hypothesis is based on the presence of similar neurons in the avian and reptilian DVR (Figure 2B), which are believed to establish circuits equivalent to those responsible for sensory pathways in mammalian neocortex. Karten suggests that the DVR in sauropsids has transformed into part of the mammalian multilayered neocortex through the relocation of particular cell groups.

The alternative dorsal cortical germinal zone elaboration hypothesis (5) states that an additional site, containing additional progenitor cells, is present within the mammalian cortical sector of the telencephalic wall (the telencephalon is an embryonic brain structure that develops into regions including

the cerebral cortex) and this is responsible for the production of extra cortical neurons. This hypothesis assumes direct homology between the reptilian dorsal cortex and the mammalian neocortex.

Intermediate progenitors in the developing cortex increase the output from the germinal zone

The cortical ventricular zone (VZ) lies at the most anterior portion of the telencephalon in all vertebrates. The VZ is a proliferative layer of radially oriented neuroepithelial progenitor cells (conventionally called radial glial cells). These progenitor cells go through a series of movements during mitotic cycles, ultimately giving rise to several cell types within the cerebral cortex. In early development of the vertebrate cortex, neuroepithelial cells in the VZ divide symmetrically producing two identical cells, thus increasing the pool of neuroprogenitors. The radial glial progenitors extend through the cortical plate to the pial (outer) surface, and guide migrating neurons to their ultimate destination (6).

In 2004, it was discovered that radial glial progenitor cells in mouse can divide asymmetrically producing a replacement radial glial cell and either a mature cortical neuron that travels to its appropriate layer, or an intermediate progenitor cell (IPC). The IPC then migrates to the zone adjacent to the ventricular zone, the subventricular zone (SVZ)(7,8). Once in the SVZ, the IPCs undergo symmetric division to produce either two identical neurons destined for the same cortical layer or two daughter IPCs that propagate the cycle and amplify cell numbers. Therefore, when compared with the single neuron produced by asymmetric division of radial glial cells (described above), this two-step pattern of neurogenesis is able to increase the output of neurons while maintaining a pool of neuroprogenitors. The regulation of these progenitors could be key in determining brain size.

Substantial species differences exist in the germinal zones of the cerebral cortex

Since there was very little information on the proliferation domains in reptilian and avian brains, we decided to investigate this further. Quantitative comparisons of the germinal zones in reptilian, avian and mammalian dorsal cortex is likely to hold the key to understanding cortical evolution. If the regions and means of neurogenesis differ between birds, reptiles, and mammals it would support the hypothesis that intermediate progenitors in the mammalian SVZ contributed to the expansion of the cerebral cortex. We demonstrated that organized IPCs are not present in reptiles and only present in a specific region (subpallium) of avian brains (9). Thus, the appearance of the SVZ, along with this more prolific, two-step form of neurogenesis, may have facilitated the expansion of the cortical layers in all mammals (5,10,11).

Links with stem cell biology and human developmental disorders

Understanding the origin of the human cortex is not only a fundamental question for developmental, evolutionary and stem cell biology, but also contributes to our understanding of human cortical developmental disorders. Conversely, investigating the disorders that directly affect brain size helps identify factors that control cerebral cortical size.

Human microcephaly (small head) syndromes result from defective neural progenitor proliferation and migration, and can affect the macroscopic appearance of the brain. Baala *et al*. (2007) (12) showed that a homozygous translocation between chromosomes 3p and 10q may be present in individuals with polymicrogyria (characterised by an excessive number of small convolutions of the cerebral cortex, similar to the case shown in Figure 1, right side). This breakpoint on chromosome 3p is close to the SVZ-specific gene *Tbr2*, and quantitative RT-PCR showed that *Tbr2* failed to be transcribed. In mouse, the production of IPCs from radial glial cells involves up-regulation of *Tbr2* and down-regulation of *Pax6*. In *Tbr2* knockout mouse, the interruption of this cascade leads to a reduction in the size of the cortex (13). Other genes that have

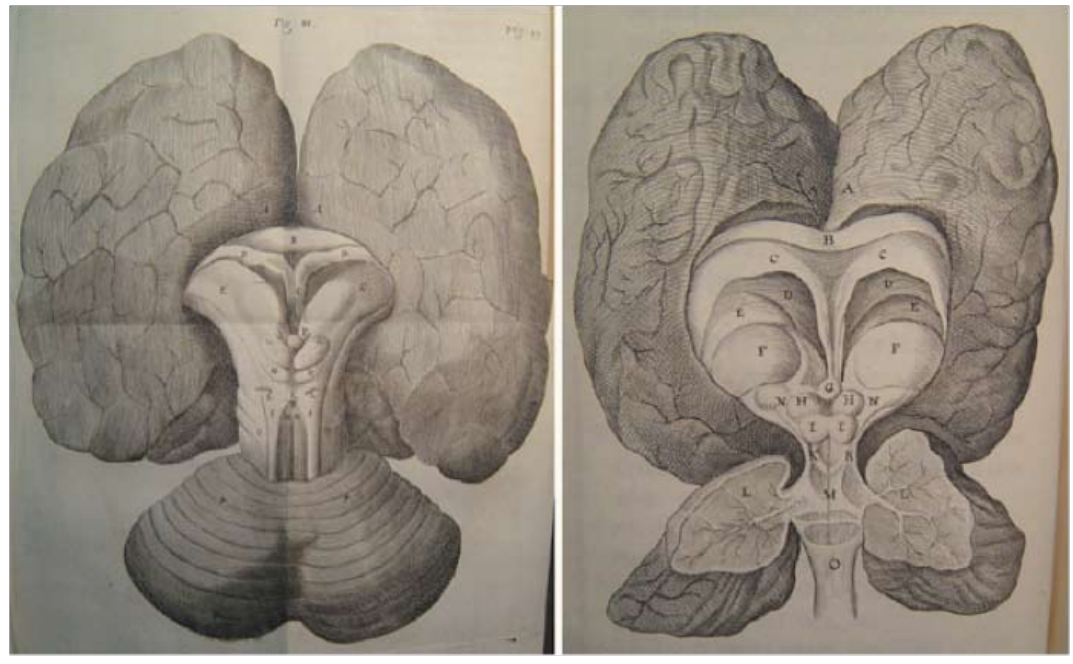


Figure 1. Illustrations from Willis's *Cerebri Anatome* (1664) show a normal brain (left) and a brain from a case of "congenital idiocy" (right). Reproduced with the permission of the library of St John's College, Oxford.

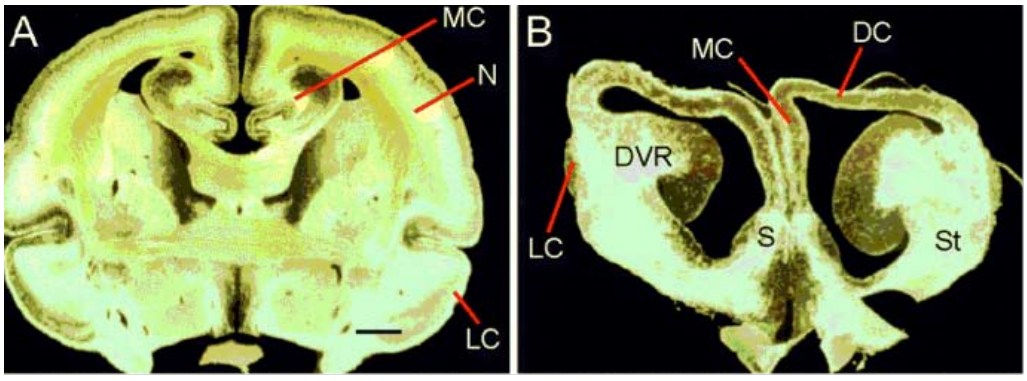


Figure 2. Fibre-stained coronal sections of (A) marsupial (*Dysaurus hallucatus*); (B) turtle (*Pseudemus scripta elegans*) brains viewed under dark-field illumination. Note the thicker dorsal cortex in marsupial compared with turtle (N and DC) and the huge ball-like structure in turtle (dorsal ventricular ridge DVR). N, neocortex; DC, dorsal cortex; ST, striatum; MC, medial cortex; LC, lateral cortex; S, septum (3).

been implicated in microcephaly cases appear to affect the assembling, maintaining and orienting the mitotic spindle in neuronal precursor cells.

Macrocephaly (large head) can occur through mutations of the gene *Pten*, resulting in enlarged cell size, reduced apoptosis and increased proliferation of neural progenitor pools. Interestingly, *Pten* is located close to the translocation breakpoint in polymicrogyria. Further analysis should reveal whether this breakpoint facilitates the overexpression of *Pten*, thus reducing the number of neural progenitors in microcephaly cases. Loss of α-catenin and EphrinA5/EphA7 has been shown to expand the progenitor pool in mice, however their role in human macrocephaly has not yet been established.

Conclusions

In mammalian evolution, the area of the neocortex increases in larger brains. The elaboration of the mammalian brain is a result of the increased neural population facilitated by the emergence and variation of the SVZ. We collaborate with several groups at Oxford (Ponting, Davies, Lu, Robertson, Szele, Monaco, Harrison) to relate the molecular and cellular mechanisms of neurogenesis to changes in cortical organization. Revealing the molecular mechanisms that regulate neural progenitor production and cell migration in different vertebrates, as well as comprehending how they are affected in disease, will allow us to understand how the neocortex evolved.

References:

1. Willis T (1664) *Cerebri Anatome.*

2. Krubitzer L & Kaas J (2005) The evolution of the neocortex in mammals: how is phenotypic diversity generated? *Curr Opin Neurobiol* 15(4):444-53.

3. Molnár Z & Butler AB (2002) The corticostriatal junction: a crucial region for forebrain development and evolution. *Bioessays* 24(6):530-41.

4. Karten HJ (1997) Evolutionary developmental biology meets the brain: the origins of mammalian cortex. *Proc Natl Acad Sci USA* 94(7):2800-4.

5. Cheung AF, *et al.* (2010) The subventricular zone is the developmental milestone of a 6-layered neocortex: comparisons in metatherian and eutherian mammals. *Cereb Cortex* 20(5):1071-81.

6. Rakic P (2009) Evolution of the neocortex: a perspective from developmental biology. *Nat Rev Neurosci* 10(10):724-35.

7. Haubensak W, *et al.* (2004) Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc Natl Acad Sci USA* 101(9):3196-201.

8. Noctor SC, *et al.* (2004) Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci* 7(2):136-44.

9. Cheung AF, *et al.* (2007) Comparative aspects of cortical neurogenesis in vertebrates. *J Anat* 211(2):164-76.

10. Martínez-Cerdeño V, *et al.* (2006) The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. *Cereb Cortex* 16(1):152-61.

11. Molnár Z, *et al.* (2006) Comparative aspects of cerebral cortical development. *Eur J Neurosci* 23(4):921-34.

12. Baala L, *et al.* (2007) Homozygous silencing of T-box transcription factor EOMES leads to microcephaly with polymicrogyria and corpus callosum agenesis. *Nat Genet* 39(4):454-6.

13. Arnold SJ, *et al.* (2008) The T-box transcription factor Eomes/Tbr2 regulates neurogenesis in the cortical subventricular zone. *Genes Dev* 22(18):2479-84.

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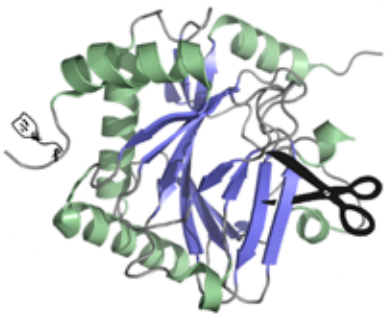

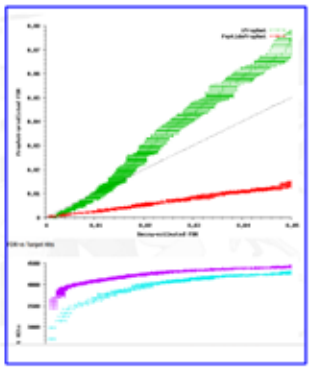

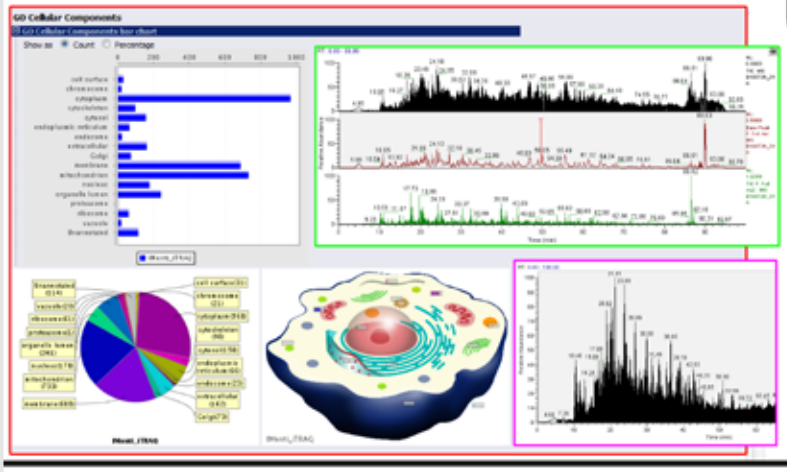

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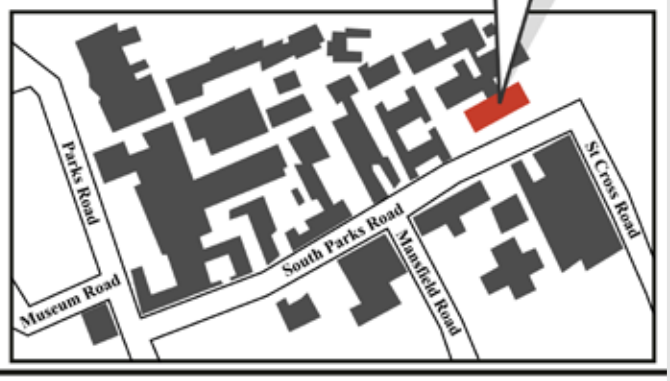

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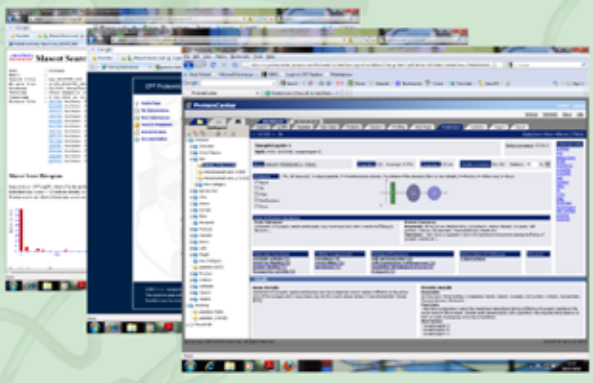


NICK WINCHESTER

Cancer stem cell theory:
Real or an Ideal?

Shaoyan Liang

In recent years, cancer stem cell (CSC) theory has been generating a stir not only within the scientific and medical communities, but across all aspects of the media. It has been heralded by some as a revolution in the understanding of cancer that will transform our approach to combating the disease. Others are more sceptical, and there are concerns that the willingness to jump on the 'CSC theory bandwagon' may divert attention away from other, equally viable, approaches to cancer care. This article will explain what CSC theory is, discuss why some people are so excited about it, and highlight some of the issues that are preventing CSC theory from being universally accepted.

What is Cancer stem cell theory?

Cancer stem cells (CSCs) are a small subset of cancer cells that possess stem cell-like properties – the ability to self-renew via asymmetric division and to produce differentiated progeny. CSC theory suggests that it is this small population of cells within a cancer that are responsible for metastasis and the recurrence of cancers. Hence, specificly targeting CSCs therapeutically might yield great improvements in the treatment of many cancers.

What's new about CSC theory?

Although the existence of CSCs had been hypothesised for many years, it was not until 1997 that strong molecular evidence was published (1). In this study, a subpopulation of immature cells characterised by a specific surface marker phenotype ($CD34^+/CD38^-$) were identified in patients with acute myeloid leukaemia. Following transplantation into immunodeficient non-obese diabetic (NOD)/severe combined immunodeficient (SCID) mice, these immature cells were able to initiate tumour formation in some mice. However, the majority of the leukaemic cells (displaying a $CD34^+/CD38^+$ surface phenotype) were not capable of initiating tumour development. The $CD34^+/CD38^-$ cells were present at less than 1 per 10 000 cells in acute myeloid leukaemia, supporting the theory that the CSCs represent a small subpopulation of tumourigenic cancer cells. In subsequent years, CSCs have also been identified in breast, brain, lung, ovarian, prostrate, gastric and colorectal cancers.

Traditionally, the clonal evolution model has been used to explain cancer growth dynamics. In this model, cancer cells accumulate multiple mutations over time leading to a population of continually diversifying cells. This heterogeneity enables the survival of different clones under varying conditions, allowing growth at metastatic locations, for example, or resistance to chemotherapeutics. CSC theory completely overturns this traditional understanding of cancer, suggesting that only CSCs can promote tumour growth whilst the majority of the cancer cells have limited ability to divide.

While both the clonal evolution and CSC models can explain why not all cancer cells are tumourigenic (as some mutations acquired during clonal evolution would have a negative impact on growth), CSC theory provides the possibility that a distinct group of tumourigenic cells might be identified and targeted therapeutically.

How will CSC theory change clinical management?

If CSC theory is correct, specifically eliminating CSCs will be critical to prevent cancer recurrence. There is increasing evidence that CSCs are more resistant to radiotherapy and standard chemotherapy than other cancer cells, both of which target rapidly growing cells. Therefore, traditional therapeutic regimes risk eliminating only non stem-like cancer cells. Attempts have been made to combine radiation and DNA repair interfering agents, and this approach has shown promising results in mice (2). Other suggested CSC-specific therapeutic targets include the Notch signalling pathway (e.g. γ-secretase inhibitors), Hedgehog signalling pathway, Wnt/beta-catenin pathway, telomerase, and ABC multidrug resistance transporters.

An alternative approach to destroying CSCs is to induce the differentiation of CSCs into non-tumourigenic cells. This approach has shown promising results in malignant brain tumours (gliomas). The addition of BMP4 *in vitro*, which usually acts to induce maturation of neuron precursors into mature astrocytes, reduced the number of tumourigenic glioma cells (3). In addition, BMP4 inhibited tumour growth in mice with transplanted human brain cells.

One complication is that studies identifying CSCs show that they are not necessarily the same in every tumour. Molecular profiling (personalised medicine) of tumours would therefore be required to ascertain the best course of treatment for each patient. Improving our ability to reliably distinguish CSCs from other, non-tumourigenic cancer cells will be key in developing treatment strategies aimed specifically at CSCs. Cell surface proteins are currently the most commonly used markers as cells can be distinguished and sorted by flow cytometry. However, many of these markers have been criticised as unreliable and further research is needed in this area.

Why are some researchers sceptical about the importance of CSCs?

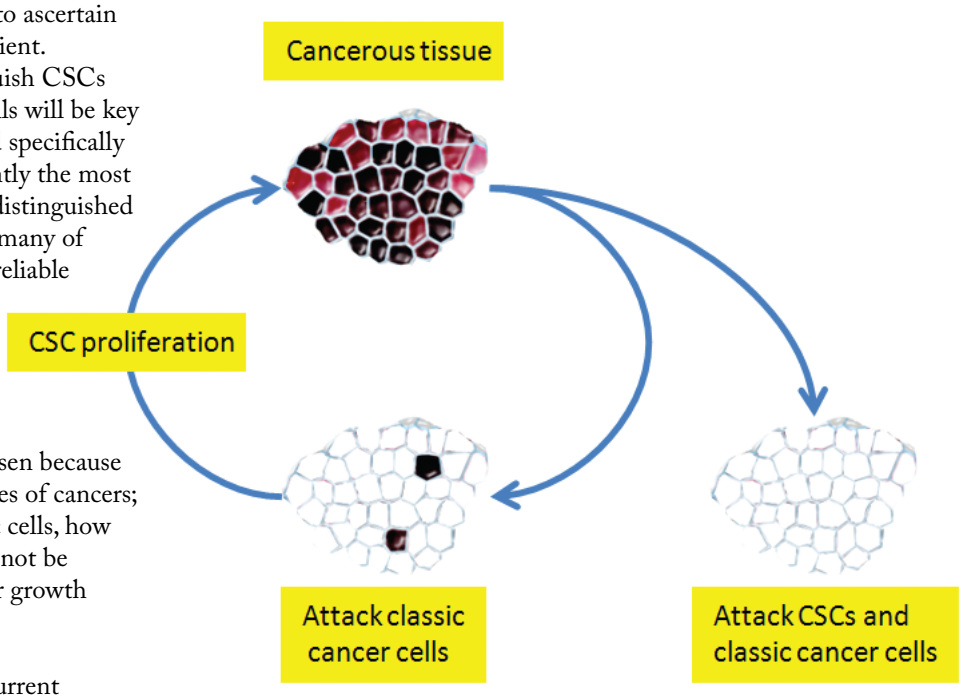
Some scepticism for CSC theory has arisen because CSCs have not been identified in all types of cancers; if CSCs really are the only tumourigenic cells, how is this possible? CSC theory thus might not be the whole story when considering cancer growth dynamics.

Many studies that have contributed to current CSC theory are based on the ability to distinguish tumourigenic CSCs from non-tumourigenic cells. As discussed above, the ability to accurately categorise cells using available markers is questionable. Also hotly debated is the idea that tumourigenic potential is limited to CSCs, rather than a property that any cancer cell may acquire following appropriate mutation.

Also questioned is whether, owing to potential cross-species incompatibility, xenotransplantation in NOD/SCID mice is an appropriate measure of tumour-initiating ability. Although techniques have been developed to improve the validity of rodent models, unidentified factors might still influence the ability of human cancer cells to initiate tumours in the murine microenvironment. It would undoubtedly be unethical to overcome this cross-species incompatibility by injecting human tumour cells into another human. However, in a number of trans-gender organ transplantation cases, recipients developed tumours that not only exhibited the tissue morphology of the transplanted organ, but also the karyotype of the donor indicating that cells within the transplanted organ were able to initiate tumour development (4). Although such observations do not directly support CSCs as tumour initiators, it would be more difficult for a fully differentiated cell to go through all of the de-differentiation steps required to develop into a new tissue type than for a dormant CSC to do so.

Conclusions

Based on current research, the support for CSC theory is convincing but only for some types of cancer. As not all cancers follow the CSC model, the ability to determine when CSC theory is valid is likely to represent a significant step forward in the way we treat cancer.



Specifically eliminating CSCs may be critical in preventing cancer recurrence. Image provided by Caroline Dahl and and Gabriel Villar.

References:

1. Bonnet D & Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine* 3(7):730-7.

2. Bao S, *et al.* (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444(7120):756-60.

3. Stupp R & Hegi ME (2007) Targeting brain-tumor stem cells. *Nature Biotechnology* 25(2):193-4.

4. On the move (2008) *Economist* 387(8583):92-3.

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Advanced TC™: A Novel Cell Culture Surface Improving the Cultivation and Differentiation of Embryonic Stem Cells

Introduction

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation *in vitro*[1,2]. The importance of embryonic stem cells rests in their lack of specialisation. These basic cells are present in the earliest stages of developing embryos and are able to develop into virtually any type of cell and tissue in the body. As such, an understanding of their unique attributes and control can lead to in-depth knowledge about early human development. Furthermore embryonic stem cells can offer a prospective limitless source of cells and tissue due to their self-renewing potential. Based on this feature embryonic stem cells have gained enormous importance over the past decades in medical science. Purposive cell therapeutic approaches for example aim to replace damaged or diseased cells and tissues by embryonic stem cells. But due to limited knowledge in stem cell research with the first human embryonic stem cells being isolated approximately twenty years ago[3] there is still an extensive need for basic scientific investigation to understand the complexity of human diseases as well as stem cell maintenance and differentiation.

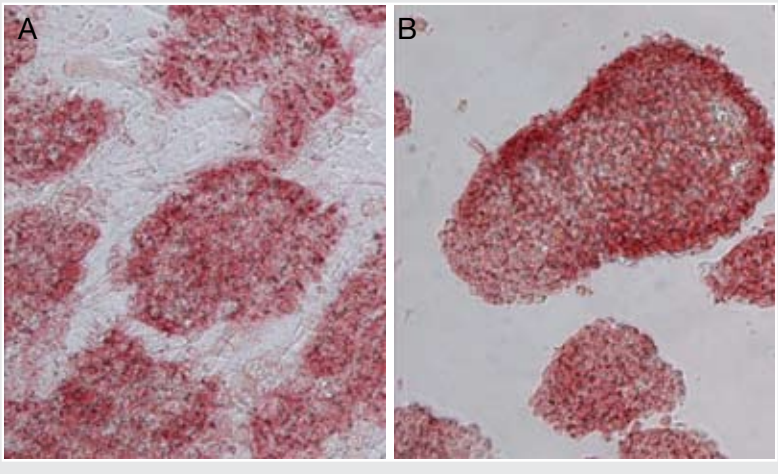

Figure 1: The embryonic stem cell line ES-D3 has been cultivated either on STO-feeder cells (A) or on the novel Advanced TC™ surface (B) in LIF containing stem cell media. 4 days after seeding ES cells in 96 well plates (20,000 cells/well) alkaline phosphatase expression has been determined using the alkaline phosphatase substrate kit (Vector® Red). Pluripotent, undifferentiated stem cells are characterised by a strong red staining.

Cultivation of Embryonic Stem Cells

In general propagation of cells and tissue *in vitro* can be challenging. *In vivo* cells of a multi-cellular organism are embedded in the three-dimensional structure of the extracellular matrix (ECM) of adjacent cells. In addition to providing structural support, the ECM also comprises a wide range of cellular growth factors and mediates biochemical signals which essentially influence cellular proliferation and survival[4,5].

In addition cultivation of cells *in vitro* mainly refers to a two-dimensional culture on plastic surfaces lacking the vital signals provided by the connective tissue. Stem cells being a very sensitive cellular system with a high risk of spontaneous differentiation under inappropriate culture conditions are therefore usually co-cultured with “feeder” cells derived from mouse or human. These feeder cells provide secreted factors such as leukemia inhibitory factor (LIF), extracellular matrix, and cellular contacts for the maintenance of stem cells in the undifferentiated state without losing their pluripotency. However feeder cells may poses a potential risk of cross-contamination such as passing animal pathogens or retroviruses to human embryonic stem cells hindering clinical application of these cells. Although feeder-free systems[6-7] have been reported in the last couple of years, these approaches required addition of feeder conditioned medium carrying a similar risk of pathogen contamination as well as batch to batch variations. The major aim therefore is to develop chemically defined media and conditions to eliminate the risk of infection from animal components as well as to reduce lot-to-lot variability resulting in consistent and comparable cellular behaviour and facilitating eventual use in clinical applications.

Feeder-free Expansion of Embryonic Stem Cells on the Novel Advanced TC™ Surface

Beside optimal chemical conditions feeder-free cultivated stem cells also require an appropriate physical environment to enable cellular survival, attachment and proliferation. To exclude any risk of cross-contamination and pathogen carryover Greiner Bio-One GmbH has developed the novel Advanced TC™ surface, a non-biological polymer modification mimicking the cellular surrounding to positively influence cell adhesion.

Cultivating embryonic stem cells on the Advanced TC™ surface leads to equivalent results when compared to expansion on feeder layers. Their undifferentiated state can be characterised by high levels of alkaline phosphatase (**Figure 1**) as well as the expression of the surface marker SSEA-1 and the transcription factor Oct-4 (**Figure 2**).

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Differentiation of Embryonic Stem Cells

Aside from the propagation of embryonic stem cells, keeping their pluripotency throughout the cultivation process, the differentiation of these cells is of major importance for their therapeutic application. One of the most promising application fields for such a cell therapy approach are neurological disorders like parkinson, stroke and multiple sclerosis. Under normal circumstances, the nervous system is incapable of healing itself. In the case of disease or injury, patients can be left with impaired motor function, paralysis or other disorders. Stem cells, however, can be used to create new neurological cells and tissue, and in theory could be used to repair damaged cells and restore normal function.

Similar to the general cultivation of stem cells the cellular or physical environment is also critical for the differentiation process of these cells.

Induction of neuronal differentiation of the embryonic stem cell line ES-D3 cultivated on the Advanced TC™ surface by the addition of retinoic acid leads to a high number of neurons and facilitates long term cultivation for more then ten days after initiation of neuronal transition **(Figure 3)**.

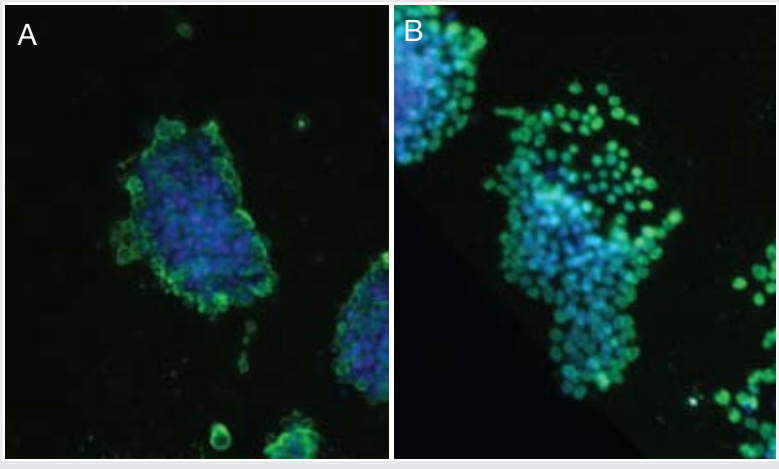

Figure 2: The ES-D3 cell line has been cultivated on the Advanced TC™ surface in LIF containing stem cell media. 4 days after seeding the expression of the surface marker SSEA-1 (A) and the transcription factor Oct-4 (B) has been analysed using a mouse-anti-SSEA-1 or rabbit-anti-Oct-4 antibody detected by the respective Alexa 488 coupled secondary antibody and a DAPI counter stain.

Conclusion

The novel Advanced TC™ cell culture surface improves cell adherence, leading to optimal condition for embryonic stem cell cultivation while excluding any risk of cross-contamination and pathogen spreading possibly caused by feeder cells. Embryonic stem cells remain pluripotent determined by the high expression lever of alkaline phosphatase, the surface marker SSEA-1 and the transcription factor Oct-4.

Beside the positive effect during the general propagation of these cells Advanced TC™ also supports neuronal differentiation by retinoic acid induction and long term cultivation of neuronal cells. In summary these results emphasise the capability of Advanced TC™ as a powerful tool for embryonic stem cell research and encourage new therapeutic approaches.

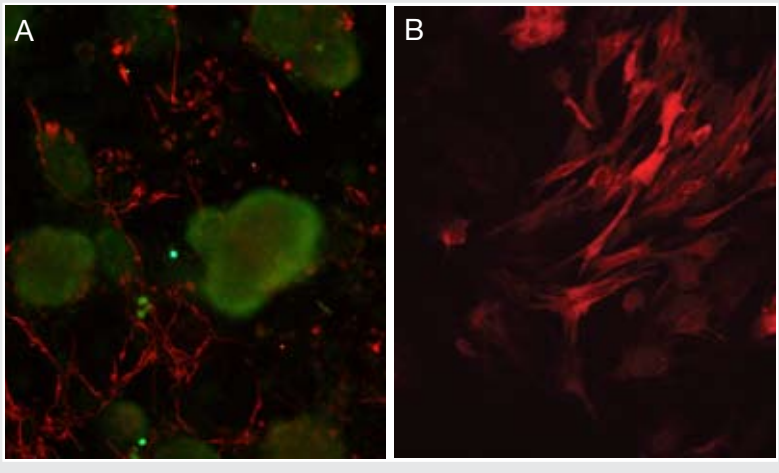

Figure 3: Neuronal differentiation of the embryonic stem cell line ES-D3 cultivated on the Advanced TC™ surface has been induced by the addition of retinoic acid. Expression of the neuronal specific marker ßIII-tubulin (red) and the marker for neuronal precursor cells Nestin (green) was analysed 6 (A) and 10 days (B) after induction of neuronal differentiation. While after 6 days neuronal precursor cells are still detectable, a pure neuronal culture can be reached within 10 days.

References:

[1] Evans M., Kaufman M.; Nature vol. 292; p.154 (1981)
[2] Martin G.,*et al*.; Proc. Natl. Acad. Sci. U.S.A. vol. 78, p. 7634 (1981)
[3] Thomson J.A., *et al*. ; Science vol. 282; p.1145-1147 (1998)
[4] Bacakova, L. *et al*.; Phys. Research vol. 53, p.35-45 (2004)
[5] Zamir, E: *et al*.; J Cell Science. Vol. 114, p. 3583-3590 (2001)
[6] Cowan C.A., *et al*.; New England J Med vol. 350; p.1353-56 (2004)
[7] Lanza R., *et al*.; Handbook of stem cells vol.1., Elsevier p. 437-49 (2004)

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Post Traumatic Stress Disorder:

New approaches to treatment

Emma Hindley

Currently, 5.2 million people in the USA suffer from post traumatic stress disorder (PTSD), and that number is expected to rise hugely over the next few years owing to the large numbers of military personnel returning from Iraq and Afghanistan. The National Institute of Mental Health estimates that about 30% of men and women who spend time in war zones will experience PTSD with varying degrees of severity.

PTSD is triggered by a life-threatening or terrifying event and was first documented in the Victorian age, where it was known as 'railway spine' and associated with crash survivors on the newly-established railway lines. Symptoms of PTSD include flashbacks, nightmares, persistent memories of the event, and sleep problems. Sufferers describe memories of the traumatic event being brought back by inappropriate stimuli – for example, upon hearing a door slam they think they are in Afghanistan under mortar fire. For some, the symptoms will recede over a few months but for others they remain for years, often becoming so debilitating that the patient will not leave their house for fear of exposure to a trauma-reminding stimulus.

Until recently, the only treatment available for PTSD was cognitive therapy – talking over your experiences and problems with a psychiatrist. Although helpful for many, this is time-consuming and expensive. Could there be better ways of dealing with this problem?

PTSD is considered a problem of memory consolidation (the process by which memories are converted from a temporary form into a version that will be stored long-term). The conditioning theory of PTSD, proposed by Terence Keane at the University of Mississippi (1), suggests that as the traumatic memory forms it becomes associated with everything present at the time, instead of just the relevant, dangerous aspects. This indiscriminate association may be linked to the excessive release of stress hormones such as cortisol during a terrifying event. According to this theory, the symptoms of PTSD may be cured if this memory could be disrupted.

Anisomycin is a chemical that inhibits the production of proteins in the brain. It has been repeatedly demonstrated that anisomycin prevents the consolidation of memories, as this process needs protein synthesis in order to occur. In 2006 Hagit Cohen, working at the Ben-Gurion University in Israel, demonstrated that anisomycin could be used to cure a model of PTSD in rats that had been 'traumatised' by the scent of cat urine but only if the drug was given immediately after the event (2). The medicated rats showed much lower levels of anxiety than their unmedicated counterparts, and did not react in a fearful way to cues that had been present at the same time as the cat urine smell. However, as this approach does not work if there is a delay between the traumatic event and treatment, it may not be very useful in human situations.

The effects of stress hormones, such as those responsible for increased fear memories in PTSD, can be blocked with a chemical called propranolol. Propranolol, commonly used to treat high blood pressure or migraines, prevents activation of a type of adrenaline receptor known as a β-adrenergic receptor. This stops the increase in heart rate and blood pressure associated with a stressful event, as well as changing the way in which memories are stored.

A clinical trial led by Alain Brunet at McGill University in Montreal examined whether propranolol is effective after trauma for those who have already developed PTSD (3). In Brunet's study, patients were asked to describe the event leading to their PTSD in order to reawaken the memory, and were then given a dose of either propranolol or placebo. This was conducted as a 'double-blind' experiment, in which neither the subjects nor the researchers knew which patients were taking the active drug. A week later, when the drugs were no longer active, the subjects returned to the lab. They were asked to listen to the recording of their description of their experiences and to imagine how they had felt at the time. Their heart rate and skin conductance (a measure of how much the subjects were sweating) were recorded while they listened. These data allowed the researchers to infer each subject's stress level.

When Brunet and his team analysed the results, they found that the subjects that had been given

propranolol had much lower heart rates and skin conductance while listening to the recordings compared with the placebo group. In fact, many of the propanolol subjects responded at stress levels significantly below the threshold that would be used in diagnosis of PTSD; in effect, they acted as though cured.

> "PTSD is considered a problem of memory consolidation...."

The mechanism of propranolol treatment is not yet fully understood and Brunet's trial lacked sufficient controls to determine whether propranolol acts on memory consolidation. Another possibility is that, by decreasing heart rate and blood pressure, propranolol somehow fools the body into thinking that it is calm. This feeling of calm could over-ride the irrational fear connected with the traumatic event which becomes activated when discussing it. Either way, pharmacological treatment of PTSD may be very successful in the near future, and the US Army is investigating the use of propanolol and anisomycin to treat combat-related stress in its members.

Pharmacological treatment, though, is not without controversy. Penny Coleman, the widow of a Vietnam War veteran suffering from PTSD who committed suicide after returning home, believes that removing the emotional context of a memory removes what makes a person human. "I cannot imagine what aspects of selfhood will have to be excised or paralysed so soldiers will no longer have to be troubled by what they, not to mention we, would otherwise consider morally repugnant," she says (4). "I am afraid that the drug that protects soldiers [from PTSD] will leave an indifference to violence that will make them unrecognisable."

However, propranolol does not remove memories or even the repugnance that a person feels when confronted with a horrible memory; it only removes the fearful response. Brunet tested this by measuring the frowning response made by subjects during playback of their recordings, and did not find a difference between the propranolol group and the controls. Another study, carried out by Merel Kindt at the University of Amsterdam (5), found that after being trained to associate a picture of a spider with a mild electric shock, subjects given propranolol still expected the shock to come when they saw a spider. They just weren't afraid of it.

This research has the potential to transform treatment for PTSD, changing the lives of millions of people. As with all psychiatric drugs, though, pharmacological treatment of PTSD with propranolol must be approached with caution until we have a better understanding of the long-term effects.

References:

1. Keane T, *et al.* (1985) A behavioural formulation of post-traumatic stress disorder in Vietnam veterans. *Behav Therap* 8(1):9–12.

2. Cohen H, *et al.* (2006) Anisomycin, a protein synthesis inhibitor, disrupts traumatic memory consolidation and attenuates posttraumatic stress response in rats. *Biol Psych* 60(7):767-76.

3. Brunet A, *et al.* (2007) Effect of post-retrieval propanolol on psychophysiological responding during subsequent scrip-driven traumatic imagery in post-traumatic stress disorder. *J Psych Res* 42(6):503-6.

4. Coleman, P. Pentagon, Big Pharma: Drug Troops to Numb Them to the Horrors of War. *AlterNet* www.alternet.org/health/72956?comments=view&cID=807687&pID=807673

5. Kindt M, *et al.* (2009) Beyond extinction: erasing human fear responses and preventing the return of fear. *Nature Neuroscience* 12(3):256-8.

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Back to the Roots:

How Oxford Scientists aim to unravel the origins of Parkinson's Disease

Stephanie Janezic

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Neurodegenerative disorders such as Alzheimer's and Parkinson's disease affect about five million people in the US and Europe, and many more worldwide. As life expectancy continues to increase and our population continues to age, neurodegenerative disorders are becoming a great challenge for our society. However, since James Parkinson first described Parkinson's disease in 1817, research has progressed slowly and to date the disease remains incurable. Despite the large impact on society, government funding for research into neurodegeneration lags far behind support for cancer, heart disease and stroke. As a result, research relies heavily on charities such as The Michael J. Fox Foundation in the US and Parkinson's UK, the leading supporter of Parkinson's disease research in the UK.

A recent example of a large-scale research project funded through a major donation given to Parkinson's UK is the newly founded Oxford Parkinson's Disease Centre (OPDC), led by Dr Richard Wade-Martins of the Department of Physiology, Anatomy and Genetics (1). The OPDC team, comprising of 13 group leaders from Oxford University, was chosen from a shortlist of seven UK research teams to win The Monument Discovery Award. Funded by one of the Sainsbury Family Trusts and with a sum of £5 million over five years, the award is the largest single trust donation that Parkinson's UK has ever received. The charity hopes that the award will result in groundbreaking research that will unravel the origins of Parkinson's disease and eventually lead to a cure.

> "The Monument Discovery Award, which is funded by one of the Sainsbury Family Trusts, is the largest single trust donation that Parkinson's UK has ever received."

Parkinson's patients suffer from motor impairments such as resting tremor, muscle rigidity and slowness of movement, all caused by the death of dopaminergic nerve cells in the midbrain. Additionally, most patients are affected by non-motor symptoms, including anxiety, depression, sleep problems and memory loss. The causes of Parkinson's disease are poorly understood and there is no treatment or cure to prevent neuronal cell death. Disease management is made even more difficult because symptoms only become apparent when approximately 70% of the dopaminergic neurons have been irreversibly lost. Furthermore, current therapies such as treatment with the dopamine precursor L-DOPA can relieve only some of the motor impairments and do not tackle the fundamental causes of the disease. The winning project from Oxford will therefore re-investigate the roots of Parkinson's disease.

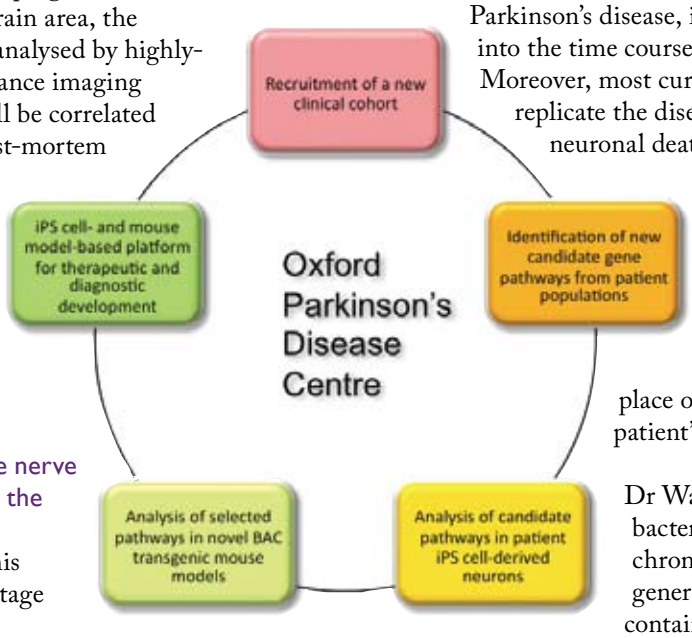
Dr Wade-Martins believes that it is fundamental to understand the early pathological pathways of the disease to allow a diagnosis before symptoms appear, and to develop treatments that will halt the disease progression or even prevent nerve cell loss in the first place. The work of the highly interdisciplinary Oxford team, which combines expertise in clinical neurology and brain imaging with genetics, bioinformatics and stem cell technology, will cover three key areas: the discovery of novel biomarkers and disease-associated genetic variations to enable a diagnosis at early stages of the disease, the generation of new cellular models to investigate the underlying pathological mechanisms and the creation of novel animal models that replicate the disease symptoms and assist in the development of new treatments.

Biomarkers will help to detect the disease before symptoms occur

To date, a definitive diagnosis of Parkinson's disease can only be made post-mortem and clinicians have to rely solely on clinical symptoms when diagnosing the disease. Sensitive and reliable biomarkers that can be translated into objective, simple and inexpensive clinical tests are therefore urgently needed to detect the disease before symptoms occur (2).

To identify such biomarkers, the OPDC team will recruit nearly 2000 Parkinson's patients from the Thames Valley area as well as 300 age-matched healthy controls and 300 close relatives of patients who are 'at-risk' to develop the disease. The study participants will undergo a series of tests that will help detect biological changes that occur in the early

stages of the disease. Blood samples will be used for DNA analysis to identify genetic variations that increase the risk of developing Parkinson's disease. The primarily affected brain area, the *substantia nigra*, will be analysed by highly-sensitive magnetic resonance imaging (MRI) and measures will be correlated with changes seen in post-mortem brains. In addition, researchers will analyse changes in protein and RNA levels in the blood and cerebrospinal fluid to identify peripheral biomarkers.



What happens inside the nerve cells that are lost during the disease?

Dr Wade-Martins and his team want to take advantage of the current revolution in human genetics and stem cell technology to discover the role of the disease-associated genes highlighted by the DNA analysis of the clinical cohort.

Individuals identified to carry disease-associated genetic variations will be asked to donate skin cells, which will be reprogrammed into induced pluripotent stem (iPS) cells. Treatment with specific growth factors directs the iPS cells into a midbrain neuronal fate producing dopaminergic neurons that model the exact patient-specific genetic variations and epigenetic markers. Dr Elizabeth Hartfield, an OPDC Career Development Fellow, explains that these patient-specific neuronal models will enable her to study the cellular disease processes that take place in the patient brain without the need for neuronal samples: "The human brain is inaccessible, and since brain samples can only be obtained post-mortem when the majority of dopaminergic nerve cells have died, our patient-derived iPS cell models will help us to study the early processes of the disease in a cell culture dish." The neuronal models will be analysed for changes in dopamine homeostasis as well as for their vulnerability to toxins which will help to identify how the genetic variations affect dopaminergic neuron function. Dr Hartfield believes that using patient-derived iPS cell models is challenging, with many hurdles yet to overcome, but she is hopeful that the novel models have great potential to unveil new disease mechanisms, serve as new drug screening tools and might even lead to advances in patient-specific neuronal replacement therapies.

Why do we need new animal models for Parkinson's disease research?

A major hurdle for the development of new treatments is the current lack of reliable animal models that accurately recapitulate the disease. Neuronal cell models help to identify chemical and biological responses of neurons in isolation but do not replicate the complex interplay of neuronal processes in the brain. Similarly, while human post-mortem tissue gives crucial information about the end-stage of Parkinson's disease, it does not allow insight into the time course of disease development. Moreover, most current animal models replicate the disease by inducing rapid neuronal death by neurotoxins or transgene overexpression from cDNA-based constructs, and do not reflect the slow process of neuronal cell death that takes place over many years in a patient's brain (3).

Dr Wade Martins uses bacterial artificial chromosomes (BACs) to generate mouse models that contain the whole genomic locus of the desired human transgene. He explains that because the novel BAC mouse models contain all the exons and introns as well as the native promoter and regulatory elements of the transgene, they more accurately reflect the human pathology and help to understand how, when, where and why brain function goes wrong in the early stages of the disease. This knowledge can then be used to identify new therapeutic targets, to develop diagnostic tools and to evaluate the safety and efficacy of novel drugs.

The road to cure Parkinson's disease will not be easy. However, Dr Wade-Martins believes that by combining the diverse areas of expertise of the OPDC team, the consortium will make major advances in uncovering the disease mechanisms, enabling an early diagnosis and ultimately lead to a cure.

References:

1. http://opdc.medsci.ox.ac.uk

2. Graeber M (2009) Biomarkers for Parkinson's disease. *Experimental Neurology* 216(2):249-53.

3. Dawson T, *et al.* (2010) Genetic animal models for Parkinson's disease. *Neuron* 66(5): 646-61.

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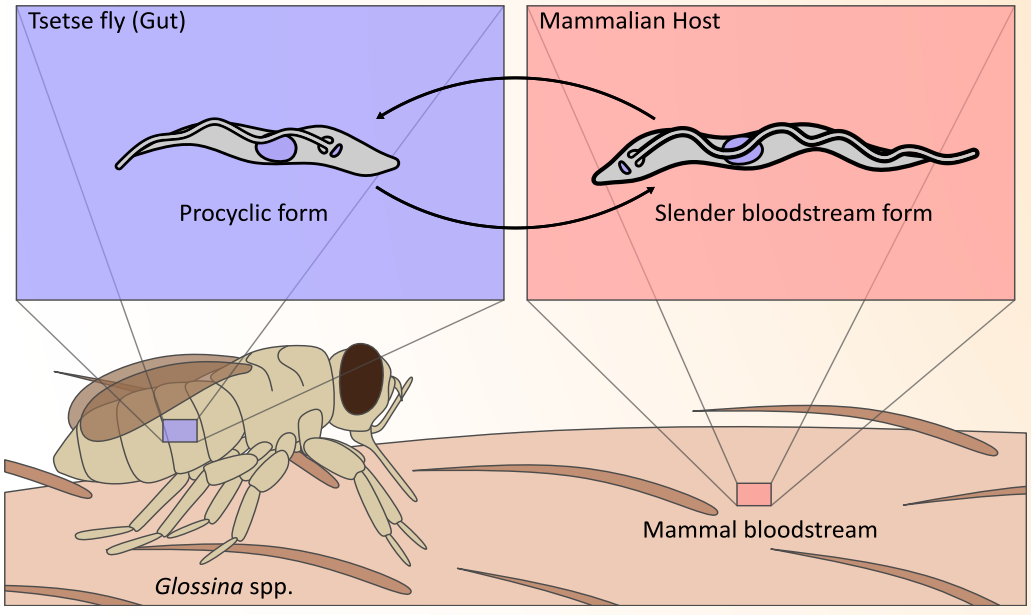
Fighting a Killer: The Trypanolytic Factor

by Richard Wheeler

African trypanosomiasis (more commonly known as sleeping sickness) is a devastating disease, responsible for severe human health and economic problems, and is endemic to sub-Saharan Africa.

Sleeping sickness is caused by *Trypanosoma brucei*, a single cell protozoan parasite carried by the Tsetse fly vector. Treatment is notoriously ineffective as there are few suitable drugs, many of which are outdated and toxic, and vector control faces huge financial and logistical difficulties. Even designing a vaccine has so far proved impossible due to massive antigenic variation – the surface of *T. brucei* is entirely covered by a dense coat of variable surface glycoprotein (VSG) and there is a library of around 1000 VSG variant genes that the parasite can switch between! Fortunately, the human innate immune system can fight back, and the mechanism by which it does this not only has implications for the treatment of sleeping sickness but also for other diseases caused by related parasites; leishmaniasis (caused by *Leishmania* spp.) and Chagas disease (caused by *Trypanosoma cruzi*).



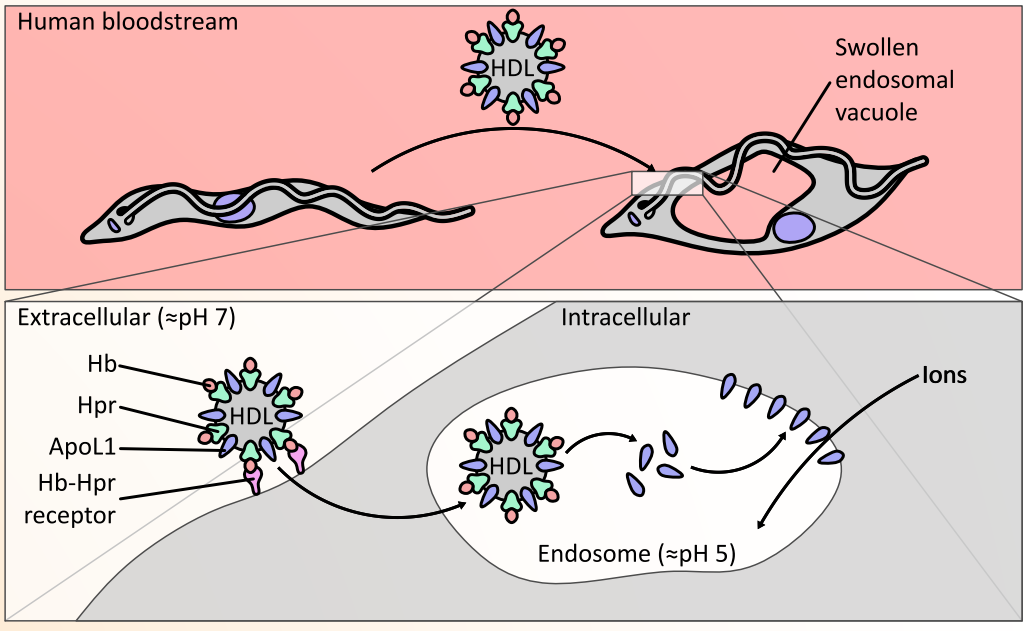
A simple overview of the lifecycle of *T. brucei*. These are the two main proliferative forms of *T. brucei*, the procyclic form in the tsetse fly gut and the bloodstream form in the mammalian bloodstream. *T. brucei* can infect many mammals including humans and cattle.

Trypanosomes are an ancient group of parasites and are locked in a molecular arms race with their host's immune system. This interplay of the immune system and the parasite has lead to a situation in which some *T. brucei* subspecies (*T. brucei rhodesiense* and *T. brucei gambiense*) have evolved tolerance to the human innate immune response while another subspecies (*T. brucei brucei*) cannot infect humans. The complex host/parasite species specificity is not limited to humans; different primates are susceptible to infection by different groups of *Trypanosoma* species and subspecies.

Over 100 years ago, it was discovered that normal human blood causes rapid lysis of *T. b. brucei*, but the actual mechanism of this lysis has only recently been determined. In the 1970s, fractionation of human serum showed that the molecule responsible for causing lysis of *T. b. brucei* is found bound to high density lipoprotein (HDL, 'good cholesterol'), and it was quickly determined that haptoglobin related protein (Hpr), which is found attached to HDL, was required for lytic activity. Unfortunately, discovery of this molecule lead to a wild goose chase; despite several groups searching for how Hpr could cause trypanosome lysis, no one could generate a consistent picture of how this happens. In the early 2000s, a completely different route of investigation identified the actual protein responsible for causing lysis as apolipoprotein L1 (ApoL1), a minor component of HDL. In 1989, a human-serum resistance protein in *T. b. rhodesiense* had been identified as responsible for the human infective phenotype of this subspecies and work in Etienne Pays' group in Belgium demonstrated

that this human-serum resistance protein was bound strongly to ApoL1. ApoL1 even possesses a membrane pore-forming domain, perfect for causing lysis. But how could this protein penetrate the dense VSG coat which protects *T. brucei's* cell membrane?

Trypanosomes are haem and lipid auxotrophs; they lack key metabolic pathways for haem and lipid biosynthesis and therefore rely on their ability to take up these chemicals from the bloodstream. Hpr, which binds haemoglobin and HDL, therefore makes a perfect meal for a trypanosome. An Hpr-haemoglobin receptor was discovered in 2007 and it is this route of HDL uptake which provides the path of attack for ApoL1 and explains the critical role of Hpr in causing trypanosome lysis. It has since been discovered that HDL particles coated in ApoL1 are taken up via receptor-recognition of Hpr and accumulate in the endocytic vesicles of the trypanasome. The low pH within the vesicles allows the pore forming activity of ApoL1 to fill the non-VSG protected membranes of the endosome with anion pores. The resulting osmotic imbalance causes rapid lysis of the trypanosome.



The process of trypanosome lysis by the trypanolytic factor. The trypanolytic factor is a protein, ApoL1, found bound to HDL in the human bloodstream. *T. b. brucei* takes up HDL particles as a means of obtaining haemoglobin (Hb) and lipids from the blood. The ApoL1 cargo leaves the HDL particle and inserts into membranes under low pH in the endosome, this forms ion pores in the membrane. Osmosis causes uncontrolled expansion of the endosome and lysis of the trypanosome.

The toxic function of ApoL1 is not limited to *T. b. brucei*. For example, a rare human case of *T. evansi* infection, normally a livestock pathogen, was linked to loss of both copies of ApoL1 from the patient's genome. Another intriguing discovery was the effectiveness of the trypanolytic factor in reducing the level of infection of a related pathogen, *Leishmania*, in transgenic mice. Unlike *T. brucei, Leishmania* do not live in the bloodstream but are intracellular parasites that live in the phagolysosome of macrophages. It appears that these parasites are phagocytosed by the host macrophage and that during this process ApoL1 from the surrounding serum is also taken up. When reaching the acidic environment of the phagolysosome, ApoL1 causes pore formation in the *Leishmania* cell membrane. It will be fascinating to see what other pathogens are affected by ApoL1.

The billion pound question is of course: "Can this information be used to treat or prevent trypanosomiasis?" In short the answer is yes, but there are many complexities and caveats. ApoL1 is a potent trypanosome killing toxin, however it is only active against the non-human infective *T. b. brucei*. Both human-infective subspecies, *T. b. rhodesiense* and *T. b. gambiense*, are resistant to the effects of ApoL1. Two approaches to overcoming this limitation are being investigated. One research group is considering using a truncated version of ApoL1 which is not neutralised by the human serum resistance-associated protein in *T. b. rhodesiense*. A second approach is to take advantage of resistance which has already evolved in other primates as, for example, the ApoL1 found in one baboon species (*Papio hamadryas*) has lytic activity against *T. b. rhodesiense*. Furthermore, another baboon species (*Papio papio*) is not affected by *T. b. gambiense* and may carry another useful homologue of ApoL1.

Converting a protein (such as modified ApoL1) into a practical therapeutic is not simple, and may prove to be impossible for the time being. However, there are other routes to use these toxins to indirectly benefit human health. Trypanosomiasis is a zoonotic disease; there are large parasite reservoirs in both wild and domesticated animals, particularly cattle livestock. Genetic modification could be used to give livestock innate resistance to trypanosomiasis. This technique has been proven to work in a mouse model, and resistant cattle could be of enormous help to African communities, both economically and in terms of human health. Genetically modifying cattle to give them resistance to *T. b. brucei* would have an immediate positive economic impact. Unfortunately eliminating *T. b. brucei* from cattle would also leave an open niche, and it is possible that human infective *T. brucei* subspecies would move in

> "Trypanosomes ... are locked in a molecular arms race with their host's immune system."

and become prevalent in cattle. This pool of human-infective *T. brucei* subspecies in the livestock population would most likely lead to a large increase in human cases of trypanosomiasis. The alternative, giving cattle resistance to only human infective *T. brucei* subspecies, would give much less economic benefit but would be expected to reduce human cases of trypanosomiasis. As is often the case with a complex epidemiology, choosing the best route of action is not simple.

Research into this trypanolytic factor has also identified other pathways to target in the fight against trypanosomiasis. The Hpr-haemoglobin receptor is an interesting target as a means of driving drug uptake. It has a subtly different specificity from the equivalent human receptors so it could be used as a targeting system for a range of toxins to the endocytic compartments of trypanosomes. Haem uptake is also vital for *T. brucei* and may present another therapeutic target. Genetically modified *T. brucei* lacking the Hpr receptor (responsible for haemoglobin uptake) are not viable *in vivo*, most likely because they are not able to prevent oxidative damage from macrophages. Drugs targeting haemoglobin uptake are therefore also worth pursuing.

Trypanosomiasis has long been regarded as a neglected disease but this situation is slowly changing. Research such as that discussed here is paving the way for the development of new treatments and preventative measures against this deadly tropical disease.

References:

1. Thomson R, *et al.* (2009) Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes. *Proc Natl Acad Sci USA* 106(46):19509-14.

2. Vanhamme L, *et al.* (2003) Apolipoprotein L-I is the trypanosome lytic factor of human serum. *Nature* 422(6927):83-7.

3. Vanhollebeke B, *et al.* (2008) A haptoglobin-hemoglobin receptor conveys innate immunity to *Trypanosoma brucei* in humans. *Science* 320(5876): 677-81.

4. Wheeler RJ (2010) The trypanolytic factor-mechanism, impacts and applications. *Trends Parasitol* Available at: http://www.ncbi.nlm.nih.gov/pubmed/20646962.

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SEXISM IN RESEARCH?

Nicola Platt

Gender bias in the sciences is a widely discussed issue. Whilst the number of female science students increases year on year, and blatant sexism is no longer prominent, at least in the West, issues surrounding maternity and childcare still lead to a significant earnings gap between male and female scientists. In response, many campaigns exist to promote the careers of young female researchers and encourage the return of young mothers to science. However, intriguing research suggests that sex discrimination in the life sciences stretches further, beyond the researchers themselves into the science they practise.

Almost 20 years ago, researchers highlighted the fact that across the life sciences there is a huge bias towards the use of male animals as model organisms. In 1992, a survey of 100 neuroscience papers found that almost half did not specify the sex of the animals used, but the large majority of those that did specify sex used only males (1). Two decades on, it appears that little has changed. Recent research found a sex bias in almost all areas of biological research, with neuroscience being the worst offender. For every neuroscience study that used female animals, 5.5 studies used only male animals (2). A study published in 2005 (3), which investigated publications on animal experiments in one neuroscience journal, found that over 75% of the published studies used male animals alone, and that this sex-based bias had remained constant during the preceding 10 years.

So, what is the impact of this discrepancy? If a neurochemical pathway functions a certain way in a male ferret, should it not be identical in a female? If a drug has a certain pharmacology in male rats, will it not be the same in females? Human data suggests that this is not necessarily the case. For example, women respond differently to anaesthetic delivery and tend to experience more adverse drug side effects than men (4). These differences are not surprising given the fundamental differences in physiology between the sexes. Recent research suggests that the difference between an X and Y chromosome may lead to differences in the expression of hundreds of genes. At a systems level, sex-specific hormones underlie a myriad of gender differences in the brain and the rest of the body. Given these broad ranging biological differences, can we justify not investing in basic research into the physiology of half the world's population?

From an epidemiological perspective, the study of male animals also seems counterintuitive. The choice of male animals may be appropriate for, say, the study of Parkinson's Disease, where the prevalence can be 2-3 times higher in males than females (5), but for many neurological and psychological disorders the opposite is true. In women over 95 years, the cumulative risk of suffering from Alzheimer's Disease is 0.22, compared to just 0.09 for men of the same age (6), and major depression is twice as common in women as it is in men (7). Therefore, a continued focus on male animal models of such diseases may mean that the pathophysiology of women is neglected.

Why are male animal models favoured? The major reason cited is the minimisation of variability. The oestrous cycles of female animals increase physiological variation and therefore more animals are needed to reach statistical significance. Therefore, the use of females conflicts with the reduction principle of the 3Rs of animal research, since fewer male animals may be required. Ultimately though, if the sole use of male animals produces results relevant for only half the human population, long term animal use may be reduced by addressing the gender imbalance now and therefore reducing the need for future experiments. Gender bias in many areas of the life sciences is being actively tackled in order to improve women's healthcare, including a drive to increase recruitment of women to clinical trials. However, if the basic research underlying new treatments shows such a gender imbalance in its animal subjects, it is possible that some trials may already be destined for failure. Given that personalised medicine is becoming a realistic target for the future, gender targeted medicine may be an appropriate starting point.

References:

1. Berkely KJ (1992) Vive le Difference! *Trends Neurosci* 15(9):331-2.

2. Zucker I & Beery AK (2010) Males still dominate animal studies. *Nature* 465(7299):690.

3. Mogil JS & Chanda ML (2005) The case for the inclusion of female subjects in basic science studies of pain. *Pain* 117(1-2):1-5.

4. http://www.worldallergy.org/professional/allergic_diseases_center/anaesthetic_agents/

5. Van Den Eeden SK, *et al.* (2003) Incidence of Parkinson's Disease: Variation by Age, Gender, and Race/Ethnicity. *Am J Epidemiol* 157(11):1015-1022.

6. Andersen K *et al.* (1999) Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence Research Group. *Neurology* 53(9):1992-7.

7. Kuehner C (2003) Gender differences in unipolar depression: an update of epidemiological findings and possible explanations. *Acta Psychiatr Scand* 108(3):163-74.

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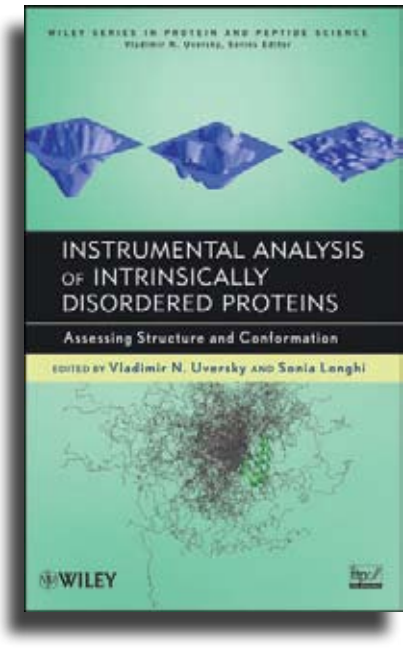
Instrumental Analysis of Intrinsically Disordered Proteins: Assessing Structure and Conformation. By Vladimir Uversky, Sonia Longhi (Reviewed by Leopold Kong)
Published May 2010 by Wiley-Blackwell, 760 pages, £100.50

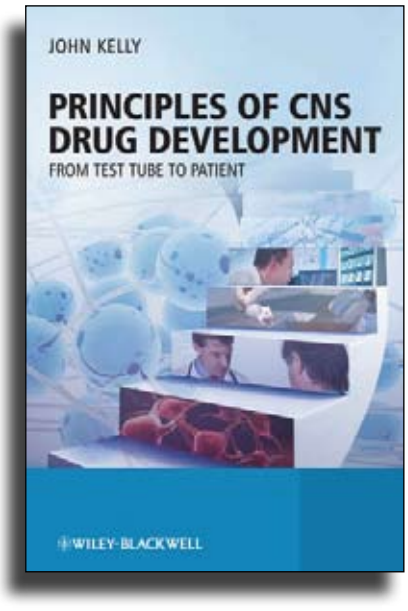


This book provides an overview of classic macromolecular chemistry techniques that have been modified to address the specific problems associated with proteins that sample multiple conformations instead of folding into one native shape. The role of this "intrinsic disorder" in enhancing the binding promiscuity, the size of the interaction surfaces, and the specificity of both structural proteins and enzymes can be advantageous in numerous functional contexts but is offset by decreased expression levels and sensitivity to degradation. Researchers, therefore, need to know not only how to analyse these proteins but also how to isolate these potentially fragile molecules. This text summarises all these aspects in a comprehensive way and is a suitable introduction for biochemists and systems biologists interested in looking at proteins from this perspective.

As the title suggests, most of the chapters are organised around techniques associated with particular instruments. Following a brief introduction to the complex relationship between disordered proteins and many protein degradation apparatuses, the book quickly delves into methods of probing these proteins in live cells with nuclear magnetic resonance (NMR). Innovations in other spectroscopic and single-molecule techniques such as circular dichroism and Foerster (or fluorescence) resonance energy transfer (FRET) that analyze the extent of the different conformations these proteins sample are then discussed. The text also covers methods ranging from small-angle x-ray scattering to more traditional denaturation and mass spectrometry that explore the size and stability of proteins. All of these approaches are introduced with solid background material and backed up with examples using the techniques. Some of the chapters are written by leading experts in the field while most other chapters are contributed by scientists who have worked on disordered proteins for at least 10 years. The absence of comparison of techniques and justification of methodological details, however, makes the text less useful as a practical guide and is unfortunate, given the emphasis on instrumentation.

Furthermore, while the theories of specific approaches are thoroughly described in the book, there is little emphasis on the theory of protein folding and its relation to intrinsically disordered proteins. This would have led to discussion of computational modelling of folding, which has seen enormous progress in the past 15 years. Its application to intrinsically unstable proteins is obvious.

Overall, this book is a good introductory text for the various techniques and approaches that are used in its field. However, supplemental reading, particularly on computational methods, would be needed for a complete understanding.



Principles of CNS Drug Development: From Test Tube to Patient. By John Kelly (Reviewed by Vicki Patterson)
Published January 2010 by Wiley-Blackwell, 324 pages, £75.00

When presented with this book to review my first reaction was relief – it is not a large book, allowing easy handling and reading. However, I was soon daunted by the lack of colour evident when flicking through before starting to read. All the pages consist of black text on white paper, interspersed with diagrams, graphs and tables in varying shades of grey. While initially off-putting, this proved helpful later as it prevents the eye from straying to figures, allowing one to concentrate on the text. And it needs concentration.

While well written, the text is difficult to dip in and out of; chapters require reading as a complete entity to gain the full benefit of the information contained within. While this may lessen the usefulness as a reference book, it makes the text more engaging and able to hold the reader's attention. Each chapter ends with a list of references, making further research into the topic more accessible.

Taking the reader on a journey from the serendipitous discovery of the first CNS drugs to the targeted drug development approaches currently underway, "Principles of CNS Drug

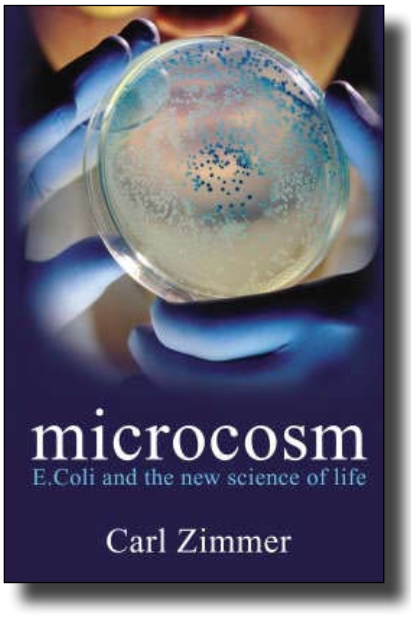
Development" offers in depth information on the etiology of debilitating CNS disorders, the drugs currently available and the techniques being employed to improve existing therapies.

With the structure of the book outlined in the preface, the subject is compartmentalised into sensible segments. Each chapter addresses a new segment, with the initial chapters concentrating on the background of the CNS disorders and the history and development of presently available drugs. The discussion is heavily patient-orientated, with detailed description of symptoms and their classification into diagnosable categories as well as the cost to the individual and society as a whole. Five categories of CNS disorders are chosen to exemplify the stories of drug development. However, I would have preferred to find all the information on one disorder collected together. While splitting it according to the type of information may make it easier to find required information quickly, it somewhat interrupted the flow of the reading.

The latter part of the book is focused on the development strategies utilised in pharmacology. The processes of clinical trials are outlined, with particular attention paid to the design of such trials and discussion of the suitability of different parameters of the trial, for example the end-point as well as the analysis of the data generated, and how to extrapolate these data to the human condition. There is also good detail on the use and generation of animal models, and how to choose the most appropriate model for the study in question. The efficacy of the drug is also considered, in discussing the so-called pharmacokinetics (how the drug behaves in the body). The adsorption, distribution and metabolism of the drug is discussed, and a whole chapter is dedicated to the safety considerations and how they are controlled for.

Overall, "Principles of CNS Drug Development" provides an accessible starting point for the understanding of the drug development process.

Microcosm: E. coli and the new science of life. By Carl Zimmer
(Reviewed by Elizabeth Anscombe)
Published July 2008 by William Heinemann Ltd., 256 pages, £20.00



Many of us who work with *E. coli* might use the bacterium as a tool on a daily basis without really knowing too much about its inner workings. Carl Zimmer, the author of "Microcosm: *E. coli* and the new science of life" believes that this bacterium deserves far more attention than this. After all, the placid workhorse of the lab, obediently expressing our proteins, is only one facet of *E. coli* biology. I found it fascinating to learn a little more about the secret life that *E. coli* leads in our large intestine, how it survives as a free-living bacterium in soil, and what genetic differences allow this normally harmless gut bacterium to become lethal in some strains.

This book provides a rapid romp through *E. coli* biology, covering such diverse areas as systems biology, biotechnology and genomics. It also describes some intriguing features of *E. coli* behaviour, such as their capacity to engage in chemical warfare by producing compounds called colicins, their ability to form biofilms and other complicated communities, and adaptations to allow them to survive a journey through stomach acid.

This book would be a good read for a bit of background knowledge on *E. coli*, without going into too much depth. It is written for a general audience, so readers with a knowledge of biochemistry may find some sections a bit basic, but the style is gripping enough to keep most readers engrossed. Of particular interest to those involved in biochemistry and genetics, the second chapter nicely describes the importance of *E. coli* in many classic experiments during the birth of molecular biology.

Zimmer employs an engaging mixture of experimental evidence, anecdotes and metaphor to guide the reader through many facets of *E. coli* biology, explaining why this bacterium has become the organism of choice in so many labs. As well as being interesting in its own right as part of our gut flora and as a pathogen, our detailed and growing understanding of *E. coli* means that it can also provide us with insights about fundamental questions regarding human biology.

In The Neutral Zone:

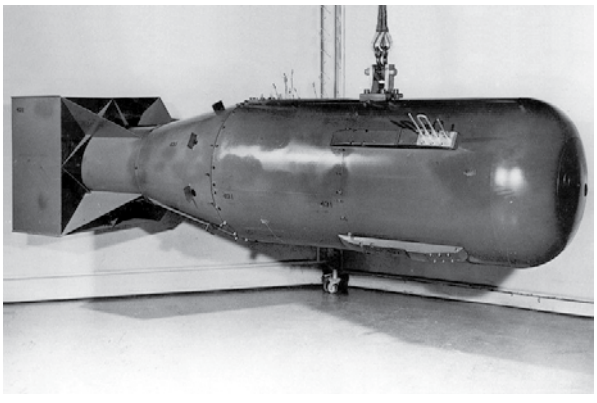
is there an ethical dimension to science?

by Dr Carinne Piekema

"I promise to work for a better world, where science and technology are used in socially responsible ways. I will not use my education for any purpose intended to harm human beings or the environment. Throughout my career, I will consider the ethical implications of my work before I take action. While the demands placed upon me may be great, I sign this declaration because I recognize that individual responsibility is the first step on the path to peace."

The above is the text of a 'Hippocratic Oath for Scientists' proposed by the physicist and Nobel Peace Prize laureate Sir Joseph Rotblat in Science in 1999 (1). Rotblat, a British physicist who grew up in Poland and moved to England just before the Second World War, was originally part of the Manhattan Project that created the atom bomb. When in 1944 it became obvious that the Germans were themselves not capable of building such a device, Rotblat was the only member of the group – which included many of the finest physicists of the 20th century such as Niels Bohr and Richard Feynman – who decided he no longer wanted to be part of the creation of such a powerful weapon of mass destruction. Throughout the rest of his life, he advocated that scientists had the responsibility of protecting the environment and humankind and should be main players in the quest for world peace.

Rotblat did not see science as amoral; he felt that scientists had a clear responsibility to the public and if the results of certain lines of research could lead to adverse effects for humanity and the environment, scientists should decide against conducting those experiments. But many have argued that, while the misuse of scientific knowledge can certainly have dangerous consequences, the scientific process itself is, and has to be, ultimately neutral.



A replica of the bomb dropped over Hiroshima, Japan on August 6th, 1945

Arguably the strongest advocate for such a position is Lewis Wolpert, Emeritus Professor in Cell and Developmental Biology at University College London. In 2000, in a published exchange of letters (2) between Wolpert and the environmentalist and founder of The Ecologist, Edward Goldsmith, he argued that "it is the very nature of science that it is not possible to predict what will be discovered, or how these discoveries could be applied", and suggests that one should not confuse knowledge gained by scientific research with the (technical) application that follows. While he also argues that "scientists have neither the right nor the skill to make ethical decisions about the application of their work", he does suggest that scientists have a responsibility to provide the public with the tools to make informed decisions about the application of science: they should explain their research to the public, explore possible consequences – positive and negative – of their scientific results, and make sure that the research is trustworthy (3). This then would allow for informed debates amongst policy makers and the general public such that society as a whole would be involved in decisions as to how scientific progress is applied.

The debate about the ethical responsibilities of scientists came to the fore again in May this year when the controversial American scientist Dr Craig Venter announced that his team of researchers at the J. Craig Venter Institute had been able to create what he referred to as 'synthetic life'. This was achieved by replicating DNA from a bacterial cell artificially and replacing the original DNA of the cell with the artificial DNA (4). With many scientists arguing that rebooting a living cell with synthetic DNA is not equal to the creation of new life, even the terminology surrounding these research findings is a subject of discussion. While the technical advancements of this study are largely undisputed, it has been the ethical questions surrounding this research that have caught the headlines. Some of the more controversial reactions to Venter's research came from Professor Julian Savulescu of the Uehiro Centre for Practical Ethics, who is quoted in the Times Online saying that "Venter is creaking open the most profound door in humanity's history, potentially peeking into its destiny", and even goes on to say that "he [Craig Venter] is going towards the role of a God: creating artificial life that could never have existed naturally … the risks are also unparalleled. These could be used in the future to make the most powerful bioweapons imaginable." David King, director of the Human Genetics Alert watchdog also accused Venter of playing God: "what is really dangerous is these scientists' ambitions for total and unrestrained control over nature." Although these statements seem extreme and guided by emotion rather than reason, they do illustrate an important issue: that there is a discrepancy in understanding of scientific research between scientists and lay-audiences – even highly educated ones – that needs to be addressed.

This research has also grabbed the attention of environmentalist groups who are calling for a moratorium on the release of any synthetic life forms into the environment. They argue that artificial life forms may threaten existing wild life and could lead to hastened extinction, resulting in diminished biodiversity. At the UN Convention on Biological Diversity held in Kenya at the end of May this year, the

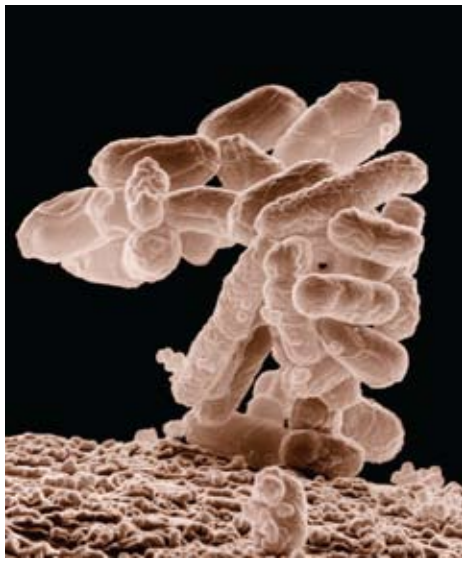
Action Group on Erosion, Technology and Concentration (ETC Group) - an international civil society organisation that researches the impact of new technologies on marginalised peoples and is based in Ottawa, Canada - helped formulate a 'de facto moratorium' on synthetic biology, which calls for a total ban on any experiment where artificial life forms are released into the wild (5). Environment ministers of the 193 member countries of the Biological Convention will meet in Japan later this year to decide whether to adopt such a moratorium.

Browsing through the popular media coverage, it might seem as if Rotblat would not have approved of Craig Venter's experiments; however, as Venter himself has pointed out, the ability to create synthetic life allows us to start understanding the function of the fundamental components of life. This may ultimately lead to cures for genetic diseases, such as cystic fibrosis and Alzheimer's disease by replacing damaged DNA with synthetic DNA that does not have mutations. The environment may benefit significantly too. Synthetic cells may be used to develop synthetic fuels that could address our need for fossil fuels and the large amount of carbon emission in our atmosphere. Such technologies therefore have huge potential for business; BP and Exxon Mobil are already large funders of Dr. Venter's research.

To a young scientist, it might seem quite straightforward to sign up to Rotblat's proposed oath, but what would it practically mean to adhere to this code? How do you decide where your personal responsibilities lie? Are you always aware the possible effects of your research? These are particularly difficult questions to answer, especially in a scientific world where high profile publications are ever more important as a token for research money.

As Wolpert argues, scientific discovery is not necessarily predictable, and the suggestion that controversial results can be avoided by not carrying out such experiments seems naïve. Furthermore, while some findings may be used in harmful ways, often their benefits far outweigh their risks. For example, 14% of the world's electricity needs (30% in the EU alone) are provided for by nuclear power (6,7). Thus, despite its major image problem, this positive use of nuclear fission is of vital importance in today's society.

While the argument for the scientific process being neutral seems well founded, it is harder to conceive exactly how the application of science should happen democratically. There are only a few scientific minds in the current UK parliament. The crucial question is whether we would really want to leave decisions about the application of synthetic life or genetically modified crops up to politicians or groups of activists. Many comments on Venter's study came from philosophers, and they could potentially play a pivotal role in democratising the application of scientific progress. In the current era, where major scientific discoveries follow each other in rapid succession, there is a need for ethicists to guide scientists and policy makers on how to use controversial results responsibly. This need is beginning to be widely acknowledged and has already led to the founding of numerous centres dealing with the ethical side of scientific discovery worldwide. Scientists could, or maybe even should, play a major role in this process by helping ethicists and policy makers understand the science behind discovery.



While it may seem that the media increasingly represents scientific advances as lead to increasing a threats to our existence, such fears are by no means new. An image of the 'mad scientist', tinkering with the natural world, can be seen as far back as Aristophanes' comedy *The Clouds*. Mary Shelley's famous 19th century novel *Frankenstein* expressed the uneasy feelings many people might have about science. However, one has to bear in mind that controversial advances have always led to criticism and ethical questions, but equally, controversial advances in science as well as literature, art and society, has made our society the way it is today.

References:

1. Rotblat JA (1999) Hippocratic Oath for Scientists. *Science* 286(5444):1475.

2. Wolpert L & Goldsmith E (2000) Letter exchange: Is science neutral? *The Ecologist* 30 No. 3 (May).

3. Wolpert L (1999) Is science dangerous? *Nature* 398(6725):281-2.

4. Gibson DG, *et al.* (2010) Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome. *Science* 329(5987): 52-56.

5. Etc Group (2010) Synthia is Alive … and Breeding: Panacea or Pandora's Box? http://www.etcgroup.org/en/node/5142.

6. International Energy Agency (2009) Key world energy statistics. http://www.iea.org/publications/free_new_Desc.asp?PUBS_ID=1199.

7. European Commission (2009) Eurostat. http://epp.eurostat.ec.europa.eu/portal/page/portal/product_details/publication?p_product_code=KS-SF-09-055.

Dr Carinne Piekema is an alumna of the Neuroscience Department, University of Oxford who is now studying an MSc in Science Media Production at Imperial College London.

School's in for Summer!

by Dr Mark Roberts

Public engagement in science can take on many forms, including communicating the work we do in an attempt to inspire the next generation of biochemists. For many of us, the pressures of applying for higher education, with course choice and getting that all important personal statement right, may seem like a very distant memory. Every year, you can't help reading in the papers how terrible it is that world class establishments such as Oxford University do not take enough students from the state sector, despite state school pupils making up the majority of Oxford's UK undergraduate students. One major issue with this type of media coverage is that people who would make good students do not even think of applying to top institutions. There are also countless stereotypes about the people attending Oxford University and many unhelpful myths surrounding student life that result in able students choosing not to apply.

So how does Oxford try to change these views and encourage the widest range of students to apply? Well, Oxford runs over 1,500 access events a year, working with students and teachers from schools that do not routinely send students to Oxford. These events include summer schools, where students come and experience what it's like to be at Oxford. Such summer schools have been run for the past thirteen years, funded by the Sutton Trust. During that time, thousands of summer school students from non-privileged backgrounds have subsequently attended Oxford and other prestigious universities. The Biochemistry department ran a Sutton Trust summer school for the first time last year, hosting 30 students who took part in a varied programme.

This year, thanks to a donation from the Helsington Foundation, the university has been able to expand the summer school programme, building on the success of the Sutton Trust summer schools. 500 students will come to Oxford in July on the UNIQ programme, 30 of whom are on the Biochemistry UNIQ summer school.

Students are accepted to the summer school from state-funded schools with preference given to students from schools which do not have a history of sending students to Oxford. Transport to Oxford and a week living in an Oxford college is provided free of charge to the students. A number of colleges participate in the programme so students stay in mixed groups, giving them a taste of the varied academic environment in an Oxford college. There is also an organised social programme covering everything from rock climbing to salsa dancing!

However, the most important part of the course is giving the students an idea of what academic life is like at Oxford – and for those on the Biochemistry UNIQ summer school - what it is like to be a biochemist at Oxford. During their time in Oxford, they attend lectures in the department and, using the material from these and the library, prepare for a tutorial. For many, this is the first time they will have been taught in small tutorial groups, providing a unique insight into the method of teaching that is at the heart of the Oxford system. Those on the Sutton Trust programme last year found the tutorial experience the most rewarding and enjoyed getting to tackle a new topic in depth. This year the Biochemistry UNIQ summer school students learned

about DNA structure and anti-viral drug development in tutorials. Biochemistry is an experimental science so the students also get to do a wide mix of lab work during the course. These range from using affinity chromatography to purify tagged proteins, through purifying and analysing their own DNA by PCR, to examining different tissues tagged with fluorescent proteins in *C. elegans* worms. Qiagen provides the materials for these practicals. For these students, it is a first-hand opportunity to use a micropipette and run a gel! Students also get experience with the computational side of biochemistry, doing a drug docking experiment devised by the laboratory of Structural Bioinformatics and Computational Biochemistry. The students are also exposed to modern structural biology through a visit to the Diamond Light Source and the departments solid state NMR facility, which are both at the Rutherford Appleton Laboratory at Harwell, just south of Oxford. Furthermore, these practicals give students an opportunity to see the instruments used in techniques discussed in the summer school lectures.

> "The aim of this Summer School is to inspire students to apply to university to study biochemistry and hopefully help debunk a number of myths that they may have about applying to Oxford"

On their final day, the students get advice about the UK university admissions process (UCAS), on writing a personal statement, as well as information about Oxford's admissions process, including discussions about the infamous and often misunderstood interview. This is designed to help make the admissions process clear and transparent and to reduce the pressure around applying for university in general and Oxford in particular. Overall, the programme hopes to inspire all the biochemists of the future and encourage students from all backgrounds to seriously consider applying to Oxford University.

For more information visit http://www.ox.ac.uk/admissions/undergraduate_courses/working_with_schools_and_colleges/uniq/new_summer_school.html

Dr Mark Roberts is a Postdoctoral Researcher in the laboratory of Professor Judith Armitage, Department of Biochemistry, University of Oxford.

5' WITH... Professor Christopher Schofield

This issue's 5' with features Chris Schofield, Professor of organic chemistry in the Department of Chemistry. The Schofield lab works on different biological problems applying chemical principles and techniques. Current areas of interest include how human cells respond to variations in oxygen supply, how complex antibiotics are made by biosynthesis in a few steps, and how small molecules regulate transcription in humans. Professor Schofield is a fellow of Hertford college.

When did you realise that you wanted to be a scientist?

I remember sitting in a tutorial in Manchester, given by Bob Ramage who was a very enthusiastic teacher, and thinking that the life of a scientist was very attractive. As a child I was also interested by scientific stories, including that of Alexander Fleming, who was presented as a 'hero' alongside the more conventional warrior heroes. Coming from a moderately religious background I was also motivated to find out if there were other ways of thinking about the universe.

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

I really don't know – it's rather difficult to think of doing anything else! My mother was a primary school teacher and my father a merchant seaman/River Pilot. The latter was not an option for me as I suffer from travel sickness, so teaching would be one option. I do enjoy working with young children, in part because they are developing so rapidly.

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

At work, teaching, reading/writing, or a victim of the 'bureaucracy oncogene'. At home taxi-driving children or, more enjoyably, sporting activities, usually in a cheerleading role with children, occupy most of my time.

Worst disaster in the lab?

Thankfully, we have never had a serious injury in our laboratory, and it is always a relief to find the group safe and sound after being away. In the 'old days' fires and minor explosions were commonplace, but safety and terrorism legislation mean they are now rare. I do sometimes wonder if the rules mean some adventurous young people are less attracted to chemistry. As a graduate student in Jack Baldwin's laboratory, we were allowed a very high degree of independence (with significant help from senior postdoctoral workers), that was extremely important in my scientific development, but did occasionally result in accidents. One of my students did cause a gas-leak alarm that made the local newspaper when he accidentally released a thiol compound that smelt like household gas, but much more potently so.

What has been the most important moment of your career so far?

Getting married.

Any memorable findings?

Over the last decade or so our group has had a highly productive collaboration with Peter Ratcliffe's and Chris Pugh's group on the role of oxygenase enzymes in the regulation of gene expression by oxygen in humans and other animals. This has been a wonderful experience because it has enabled us to correlate detailed biochemical with physiological analyses within the framework of a project that is of considerable medicinal interest.

Best advice you ever received.

Don't be afraid to start work on apparently difficult long-term projects at an early stage of your career. Do what interests you.

Any regrets?

The only thing I regret about my job is that I don't spend more time outdoors.

Favourite classical experiment?

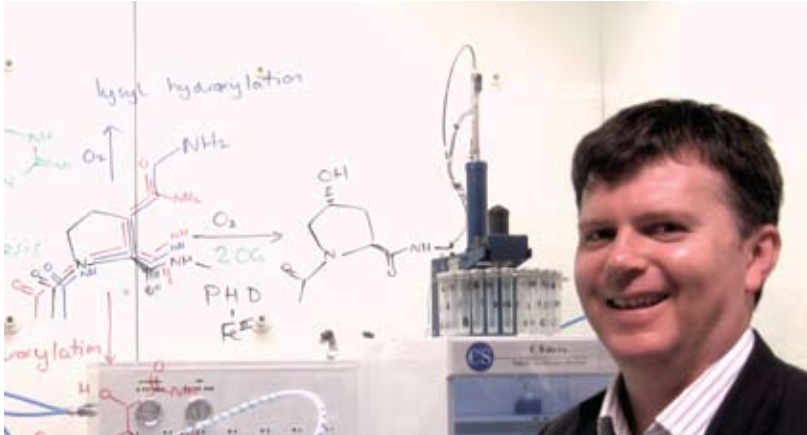
Without doubt the most important development relative to my career has been the advances in genome sequencing – these have opened up biology to quantitative chemical analyses.

In your opinion, what makes a good scientist?

There is room for many types of people with very different styles in science today, but the two essential requirements are motivation and luck.

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

Arguably the two most important basic questions for 'molecular biology' are the origins of cell-based life and a chemical understanding of how the brain works. It would be interesting to see major efforts to address these questions, not least because the answers may be of some medical use. I think it is likely that in ageing populations such as in the UK there will be enormous pressure from society to find treatments for brain/dementia related diseases. However, I'm not sure that our current system sufficiently incentivises research relating to difficult diseases such as dementia.



SNAPSHOT Research Image Competition

We are very pleased to announce that this issue's winner of the *Snapshot* scientific image competition is Anna Franz, a PhD student in the lab of Professor Jordan Raff at the Sir William Dunn School of Pathology.

She submitted an amazing phase contrast image of sperm flagella from a mutant fruit fly, featured on the front cover of this issue.

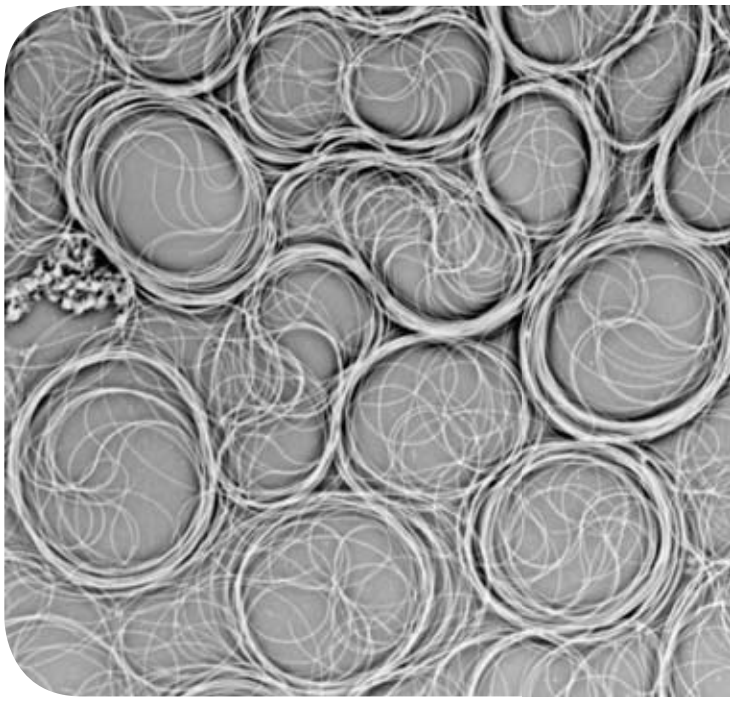
In recognition of her contribution, she will receive a £50 book voucher, kindly provided by our sponsor Oxford University Press.

We hope she will enjoy her reading!

OXFORD UNIVERSITY PRESS

Centrosomes are the major microtubule organising centres in animal cells. They comprise a pair of centrioles surrounded by an amorphous pericentriolar matrix. Centrosomes play an important role in cell polarity and cell division, while centrioles form the basal bodies of the cilia and flagella that are important for sensory function and cell motility. The main goal of research in Professor Raff's lab is to understand how centrosomes and centrioles function at the molecular level. The lab uses the powerful genetic, biochemical and cell biological tools available in the fruit fly Drosophila to tackle these questions.

Anna joined the lab of Professor Raff as a PhD student in 2006, and her work has since focused on unravelling the molecular mechanisms of centriole duplication. In Drosophila, five proteins are required for this process. A few years ago, a screen performed in Professor Raff's lab revealed novel genes involved in centriole duplication in fly tissue culture cells. Anna's work centres on one of them, called DCP110. As part of her thesis work, Anna has created a mutant fly that lacks the DCP110 gene, and she is now investigating the consequences of the mutation on the fruit flies' phenotype.



Her stunning cover image shows the large flagella of sperm cells from a DCP110 mutant fruit fly, using phase contrast optics. The image was taken as part of an experiment designed to determine whether sperm motility is impaired in DCP110 mutant flies, as might be expected from the role that centrioles play in the formation of flagellar basal bodies.

Anna began working with Professor Raff when his lab was based at the Gurdon Institute in Cambridge; she joined the lab's move to Oxford in 2009. Anna is thrilled by the opportunities that microscopy offers for making the invisible visible. When asked about her motivation for studying centrioles in Drosophila, she said "I am just fascinated by watching these very basic cellular processes as they happen in real time in a living organism".

Snapshot Hilary 2011: 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 2011. 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 Friday 10 December 2010.

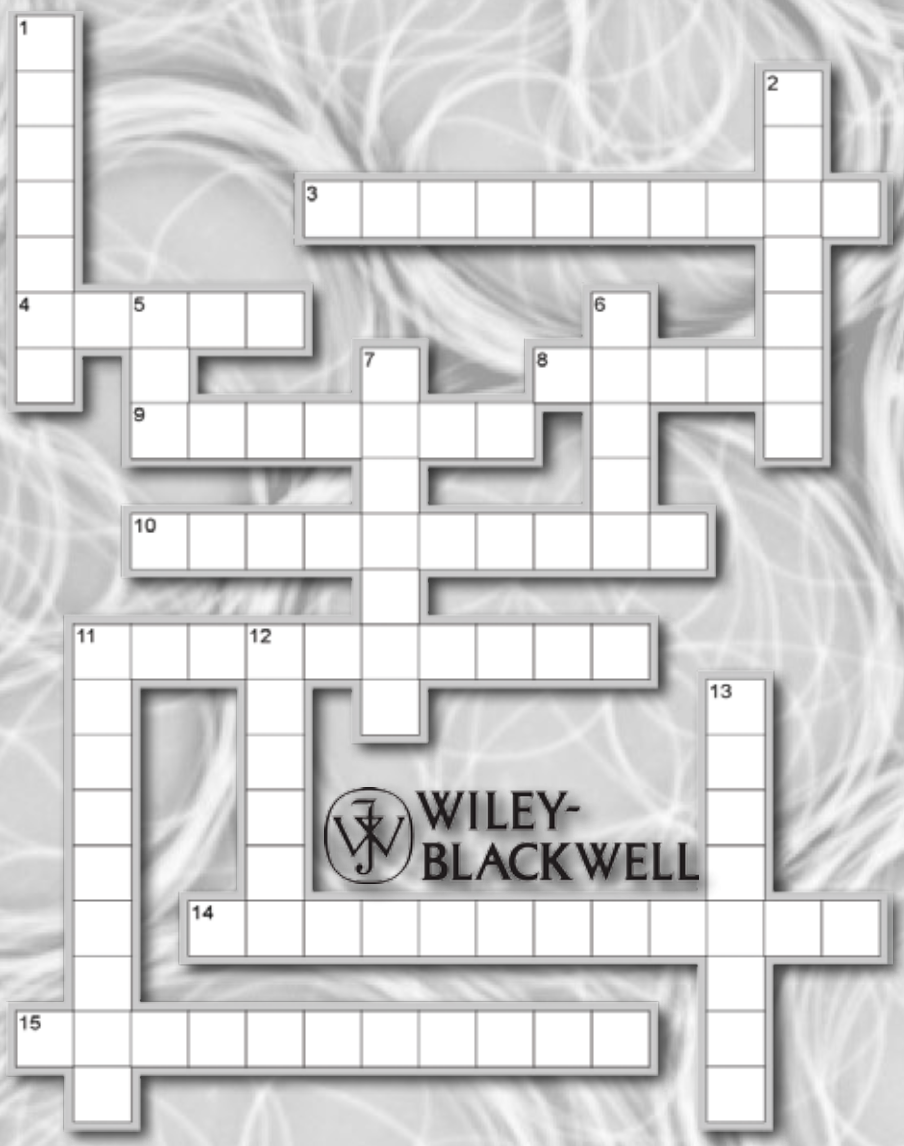
CROSSWORD

Test your knowledge of Neuroscience with this term's *Phenotype* crossword!

Enter the competition by sending your answers to oubs@bioch.ox.ac.uk or leave a paper copy in a sealed envelope in the OUBS pigeonhole at the New Biochemistry reception. Entries received by 10 December 2010 will be entered into the prize draw.

The winner will receive their choice of two Wiley-Blackwell textbooks reviewed in this issue.

Congratulations to Martin Krehenbrink from the Department of Biochemistry, who won the Trinity '10 crossword competition.



Across

3. The decrease is response of a receptor following a continuous steady stimulus
4. Receptor for the delta ligand
8. Nobel Prize winner who claimed his experiment to demonstrate the existence of neurotransmitters came to him in a dream
9. The site of communication between two neurons
10. The process by which neurotransmitter vesicles are released
11. Most common neurodegenerative disease
14. Brain region that controls hunger and body temperature among other functions
15. Area of brain involved in memory and navigation, named after the Greek for 'seahorse'

Down

1. Type of glial cell that insulates neurons to speed up conduction
2. Dorsalizing agent released from Spemann's organizer in the developing embryo
5. Non-invasive technique used to excite neurons in the brain
6. Silver-based stain for visualising entire neurons, named after its Nobel Prize-winning inventor
7. Sea slug used for the study of synaptic potentiation
11. Pain due to a stimulus that would usually not cause pain
12. Researcher who, along with Hodgkin, first modelled conduction of electrical impulses along the axon
13. Neurotransmitter whose levels are increased by nearly all drugs of abuse

OUBS