PHENOTYPE

Issue 26 | Hilary Term 2017

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PACKED WITH POTENTIAL

Stem cells in focus

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EDITORIAL

Welcome to the 26th edition of Phenotype! This term you will notice some changes to the format of the magazine, the most noticeable of which is the integration of the supplement into the main magazine in the form of 'In Focus'. This term we have a selection of articles focusing on various aspects of stem cell research. If you are new to this field, our research infographic by Anne Wolfes on ***page 4*** *provides an excellent introduction.*

Stem cell research is a rapidly growing field and presents great promise for the development of human disease models, which, for the first time, allow researchers to study the early mechanisms of disease in physiologically-relevant cell types that were previously unavailable. This is possible due to the development of induced pluripotent stem cells by the team of Shinya Yamanaka. This technique enables the reprogramming of adult cells, such as skin cells, into stem cells, which retain the genetic information of the donor and can be differentiated into any cell type of the body. On ***page 6****, Adam Phillips provides insight into how this type of system can be used to study neurological diseases, and the challenges that are being encountered along the way.*

In addition to the potential of stem cells for disease modelling, a huge amount of research focuses on the possibility of using them for transplantation. On ***page 12*** *Professor Carolyn Carr tells us about her group's research into whether stem cells can be used to heal the heart after myocardial infarction, and on* ***page 8*** *Kristin Qian looks at the possibility of directly converting skin cells into hematopoietic stem cells for the treatment of leukaemia and lymphomas.*

The field of stem cell research originally arose from the findings of developmental biologists. Their studies identified the potential of cells that are present in the early embryo to develop into any cell type in the adult body. Research has also shown that there are regions of the body where stem cells persist into adulthood, allowing regeneration of certain cell types. Some species, such as the planarian flatworm, possess stem cells that allow them to regenerate large parts of their own body. Claire Hill provides us with a historical account of the discovery of the involvement of neoblast stem cells in this process on ***page 17****.*

In addition to our focus on stem cells, we have a selection of excellent articles covering a range of other fields including DNA editing and cancer biology. On ***page 31****, you will find an interview with Professor Sebastian Nijman, as well as the highlights from some recent publications from his research group on* ***page 3****. If you are interested in sharing your science with the wider community, turn to* ***page 29*** *where Rosemary Wilson tells us about her experience of communicating science to the general public.*

Finally, don't forget to enter our competitions! Check out the winner of our Snapshot Image competition on ***page 32*** *and enter this term's competition for the chance to get your image on our next cover, as well as to win a £50 voucher from Oxford University Press. On the back cover you will find our crossword, which includes some stem cell themed answers; enter this for the chance to win one of the books reviewed on* ***pages 26-27****.*

I hope you enjoy reading this term's edition!

Heather Booth
Editor-in-Chief

If you are interested in getting involved with Phenotype, please contact me at heather.booth@st-annes.ox.ac.uk.

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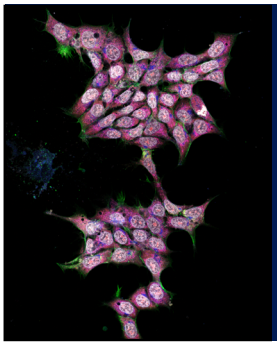


On the cover

Dancing bats

These mammalian haploid cancer cells have fluorescently stained mitochondria, actin cytoskeleton, cytoplasm and nuclei.

Read more on page 32!

EDITORIAL TEAM

RESEARCH HIGHLIGHTS

by
Chandan Seth

Mair B *et al.* (2016) *PLOS Genet* 12(9):e1006279. doi:10.1371/journal.pgen.1006279

Gain- and loss-of-function mutations in the breast cancer gene GATA3 result in differential drug sensitivity

GATA transcription factors are vital regulators that orchestrate normal to abnormal tissue development. Their name derives from the sequence they bind on DNA. Aberrant activity of GATA family members has long been implicated in tumorigenesis and malignancies of the mammary gland. Conventionally, GATA3 regulates differentiation of luminal epithelial cells and cell fate commitment. Its loss forms a molecular switch for cancer cells progressing to a more stem cell-like and invasive phenotype. Interestingly, about 10% of breast cancer samples harbour a mutation in the GATA3 encoding gene, but much remains unexplored and unclear.

The work by Mair *et al.* highlights unique patterns of genetic alterations in GATA3 and establishes the consequent cellular effects. Interestingly, the authors find that GATA3 is analogous to proteins like tumour protein p53 that can have pleiotropic activities depending on the kind of mutation and context. The authors find that a subset of mutations in GATA3 proffer a gain-of-function advantage to the cells, whereas other mutations paradoxically seem to do the opposite. For example, some mutations in the GATA3 gene that lead to an unusual extension of the GATA3 protein, classified as 'GATA3 extension mutants' in breast cancer samples, were shown to lead to a bad prognosis.

The study brings to the fore the impact of GATA3 mutations on patients' prognosis and connects the effect of these mutations to therapeutic vulnerabilities. Mair and colleagues dissect the frameshift mutation pattern in GATA3 and perform a synthetic lethality screen. Strikingly, the authors find that GATA3 extension mutants are inherently sensitive to certain histone methyltransferase inhibitors. For example, UNC0638, an inhibitor of EHMT1 (also known as GLP) and EHMT2 (also called G9A) histone methyltransferases, significantly reduced viability of GATA3 extension mutants in a breast cancer cell line.

Overall, this study links the genetic perturbations in an often mutated and vital gene, GATA3, to possible therapeutic remedies with a putative mechanism of influence.

Smida M *et al.* (2016) *Nat Commun*7:13701. doi:10.1038/ncomms13701

MEK inhibitors block growth of lung tumours with mutations in ataxia–telangiectasia mutated

With the growing incidence of cancer across the globe, much drug discovery has happened, yet most areas of cancer therapeutics remain with an unmet need. The dire need of an efficient therapeutic intervention for cancer paves the way to a more specific protocol such as personalized medicine, which can cater to tumours with a myriad of genetic alterations and contexts. Whilst the conventional drug discovery and clinical trial processes take about a decade to declare chemical moieties as useful drugs, the more recent approach has been to reuse approved drugs to treat stratified groups of cancers.

This study by Smida et al. has accentuated the employability of MEK inhibitors, such as the well-known drug trametinib, for ataxia-telangiectasia mutated (ATM) lung tumours. Smida and colleagues performed an isogenic cell-based pharmacogenetics screen including frequent tumour suppressor genes in lung cancer, such as APC, ATM, PTEN and SMAD4, as well as FDA-approved drugs. The authors observed a synthetic lethal interaction between ATM and MEK; the absence of ATM led to a hyper-sensitised response to MEK inhibitors. Furthermore, this gene-drug interaction was widely observed in patient samples harbouring ATM mutations and correlated with their response to MEK inhibitors, like trametinib.

This work also establishes that restoration of the mutation in ATM can unlink the sensitivity of the cell to MEK inhibitors using the CRISPR/Cas9 system. In addition, the work reveals the compensatory pathways like AKT/mTOR signalling that cooperate to maintain the drug sensitivity in lung cancer cells in a DDR (DNA damage repair) independent manner.

This work propounds ATM as a biomarker for lung cancer tumours that can stratify patients into drug-sensitive and drug-resistant classes, especially for MEK-based therapeutic methods.

STEM C

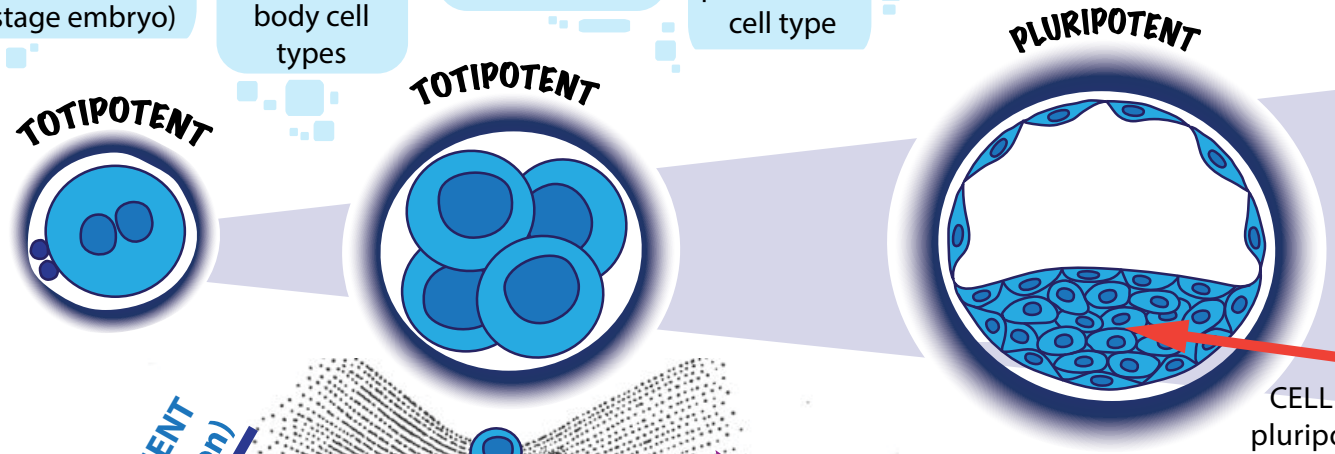
Stem cells (SCs) are unspecialised but can give rise to their own or more differentiated cell types

Totipotent SCs can make any cell type (up until 8-cell stage embryo)

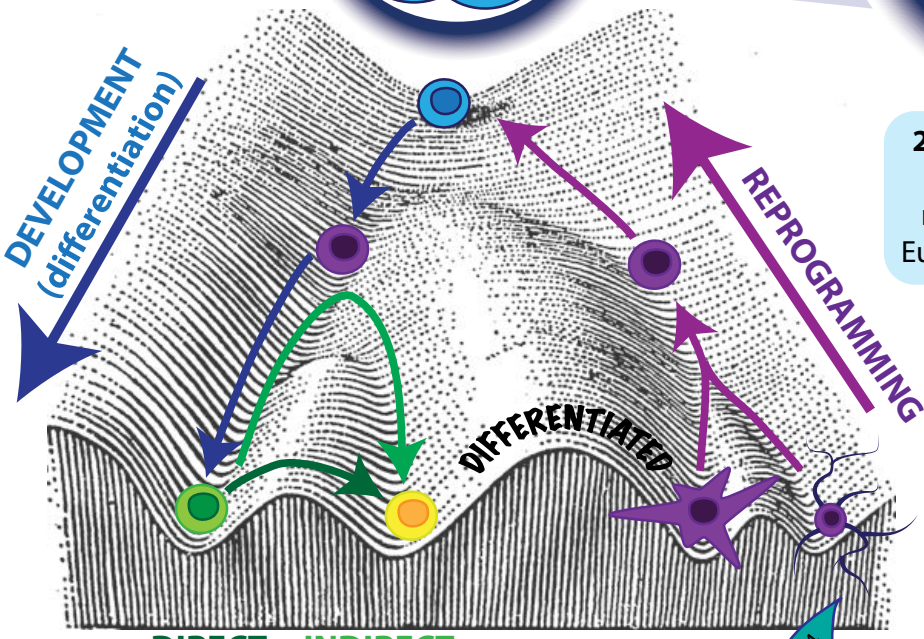
Pluripotent SCs can differentiate into all ≈250 body cell types

Multipotent SCs can make multiple but not all cell types

Unipotent SCs can only produce one cell type

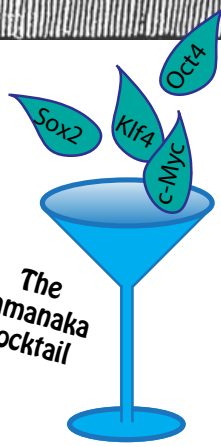


273 SC lines used for research in Europe (2012)

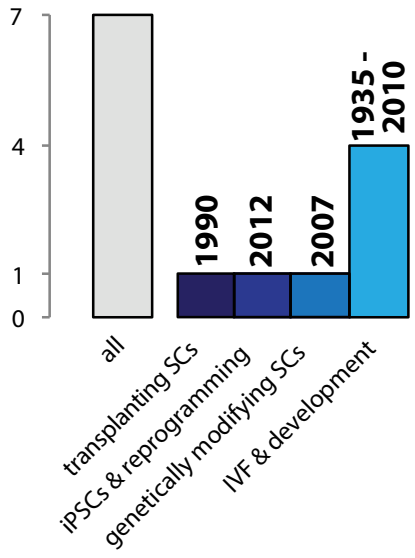


DIRECT REPROGRAMMING **INDIRECT REPROGRAMMING**

Induced pluripotent SCs (iPSCs) can be made from skin cells using four transcription factors, a.k.a. the "Yamanaka cocktail"



STEM CELL NOBEL PRIZES



1868, Germany: Ernst Haeckel first uses the term "stem cells" (SCs) to describe how multicellular organisms derive from a single cell

1958, USA: Leroy Stevens traces fetal teratomas back to pluripotent SCs in 12-day old mouse embryos

1968: Bone marrow transplant between two siblings treats severe com - bined immuno-deficiency

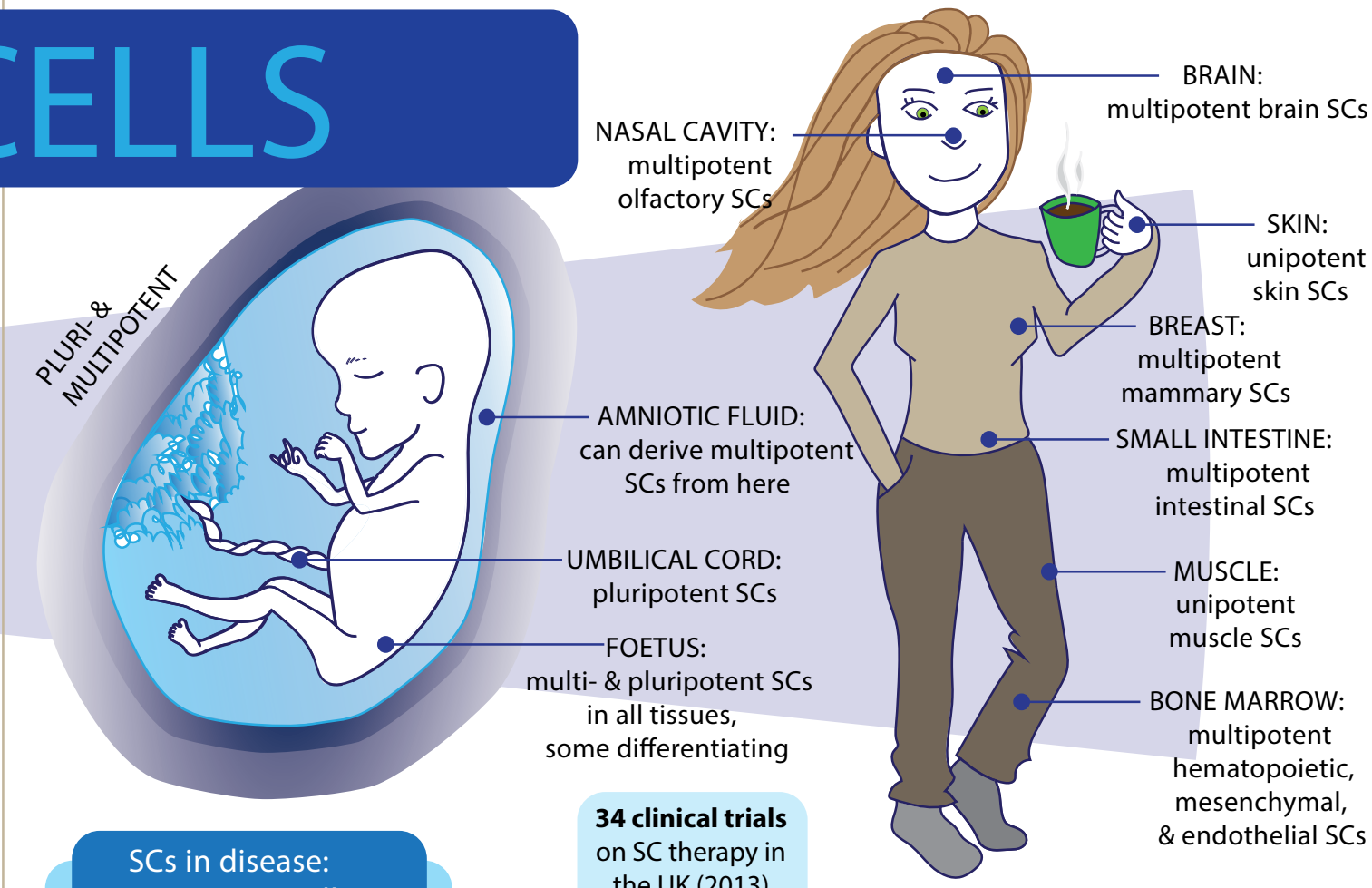
1975, USA: Beatrice Mintz & Karl Illmensee show embryonic SCs can give rise to a whole organism

1981, England: Martin Evans, Matthew Kaufman, and Gail R. Martin isolate embryonic SCs from mouse embryos

1986, USA: Tom Doetschman creates knockout technology using mouse SCs

Created by Anne C Wolfes

CELLS



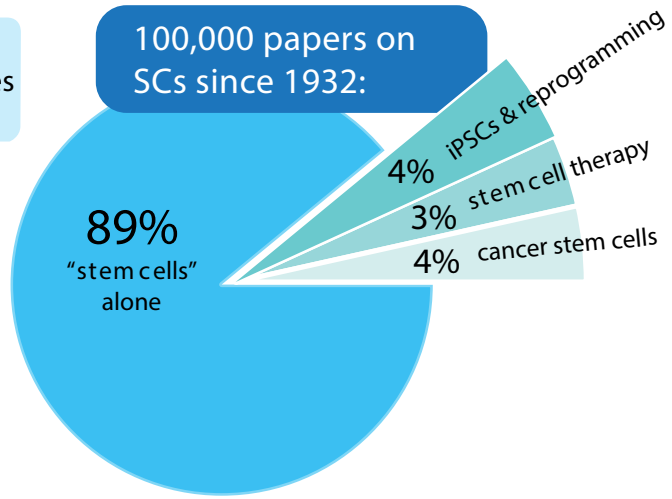
SCs in disease: Cancer stem cells

- Only minor population in tumours, but enhanced resistance to drugs and radiation
- If a tumour includes cancer SCs, these may cause tumour relapse even after therapy

34 clinical trials on SC therapy in the UK (2013)

113 Regenerative medicine companies in the EU: 26 in the UK, 29 in Germany

100,000 papers on SCs since 1932:



Late 90s & 00s: "embryonic SC hype"
Multiple new embryonic SC lines are prepared and shared

Since 2006: "The iPSC Era"
iPSCs widely used, particular focus: personalised medicine

1992: Neural SCs cultured in vitro as "neuro-spheres"

1996, Scotland: Dolly the sheep is born - the first artificial animal clone

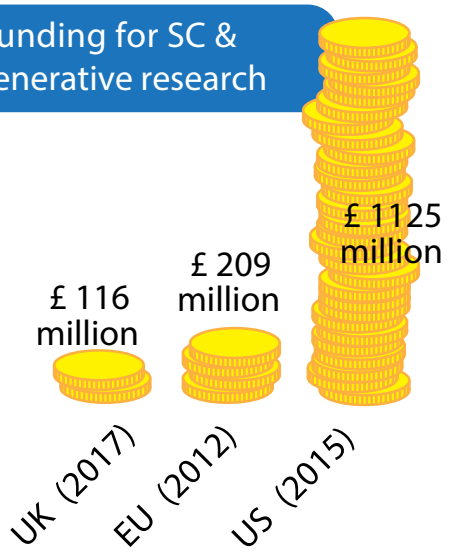
1998, USA: James Thomson & John Gearhart isolate and maintain human embryonic SCs

2006, Japan: Shinya Yamanaka makes iPSCs from adult mouse skin cells (see photo on the left)

2014, USA: Dieter Egli uses iPSCs from diabetic woman to prepare insulin-producing beta cells

2016: CRIPSR-Cas system used extensively to prepare tailor-made iPSCs from patients

Funding for SC & regenerative research



iPSCs - a better model for neurological drug discovery?

by
Adam Phillips

Disorders of the nervous system comprise a wide range of complex diseases and steady progress has been made in providing new therapeutics. On average, a new drug has come to market once every two years since the 1950s. The identification of GPCRs and ion channels as drug targets in the 1950s and the elucidation of neurotransmitter pathways in the 1990s caused surges in drug development, but since then there has been a dearth of ground-breaking therapeutics.

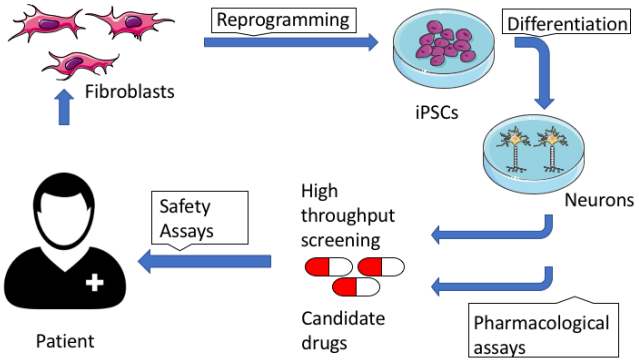


Figure 1. Schematic detailing the concept of iPSC disease modelling.

Epilepsy is one such disorder where there has been little improvement on first-generation medications. The first effective anti-epileptic medication was bromide, and since then no anti-epileptic drug has surpassed its efficacy and tolerability (1). Similarly, only symptomatic treatments exist for neurodegenerative diseases such as Parkinson's and Alzheimer's. The lack of effective treatments has seen a 50% reduction in drug development by large pharmaceutical companies since 2010 (2). Animal models have formed the bedrock for researching these conditions. However, in channelopathies such as epilepsy, these models are driven by phenomena rather than pathology. Whilst they are predictive of efficacy in pharmacologically-sensitive patients, they are not useful in studying the disease in refractory patients. Despite most epilepsies occurring in childhood, all established models use adult animals. In neurodegenerative conditions these animal models normally do not develop the same neuropathology or produce the same clinical phenotype despite high amino acid homology of pathogenic proteins (3). Moreover, psychiatric conditions are also extremely challenging to model effectively.

So, is there any hope for novel neurological drug discovery? One cause for optimism is deriving neurons and glia directly from patients' induced pluripotent stem cells (iPSCs). This technology circumnavigates ethical issues and has enabled access to cell types that were once scarcely available from human post-mortem tissue. As these cells are obtained directly from patients, they are more physiologically relevant than previous models as they retain their genetic profile and allow the formation of a 'disease in a dish'. The proliferative nature of these cells makes them amenable to high-throughput screening, allowing a catalogue of compounds to be tested against a range of diseases, including orphan diseases that affect fewer than five in 10,000 people. Novel intellectual property means that this could be commercially exploited as effective market exclusivity can be claimed for seven to ten years, making the technology financially lucrative. This process also allows a quick transition into clinical trials when using a known drug (4).

Despite the apparent benefits of iPSCs in drug discovery, it is challenging to accurately replicate a disease phenotype. 2D iPSC models also pose major limitations, as they rarely produce sufficiently dense networks to create representative epileptic activity. The 2D scaffolds do not produce spontaneous physiologically-representative neuronal-glial activity. Parallel development of functional output of receptive human tissue will also be required to reinforce results obtained from iPSCs.

Possibilities for the future include using stem cells to create 3D organoid models that could also be used to better replicate disease states. Novel protocols have been developed to generate neuron specific regions including forebrain, midbrain and hypothalamic organoids (5). Whilst this technology is still in its nascent stage, it presents an exciting avenue for investigating new therapeutics in a more physiological-representative model. The production of successful neuronal co-cultures with endothelial cells will further improve the viability of the model by providing nutrients and oxygen.

Stem cell technology is still in its early stages for modelling neurological disease but the data obtained, in conjunction with existing models, will provide an exciting avenue for pre-clinical research. This technology has provided new insight into neurological diseases and its continued exploration may reveal new therapeutic opportunities.

Adam Phillips is working in the Cader research group at the Weatherall Institute of Molecular Medicine

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The great therapeutic potential of hematopoietic stem cells

by
Kristin Qian

Hematopoietic stem cells (HSCs) are multipotent self-renewing cells that give rise to all blood cells. They are considered one of the most well understood stem cell types in our body. Nonetheless, there are many unexplored avenues in HSC research; and their clinical significance to treat blood diseases and cancers is tremendous.

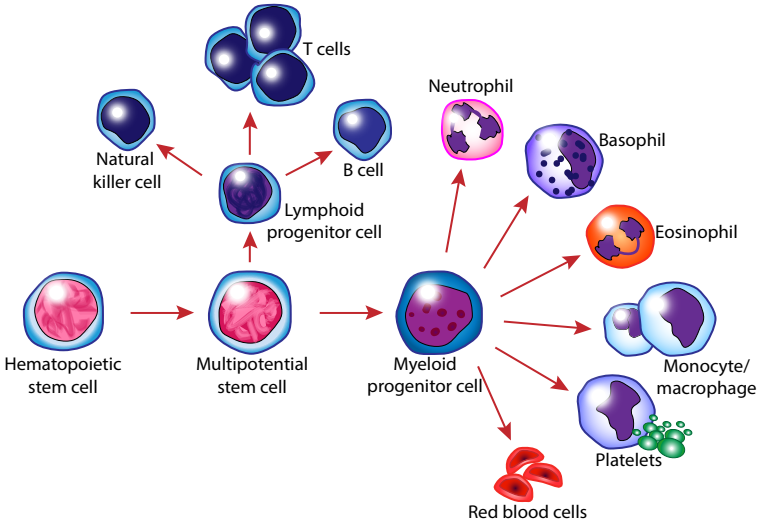

Figure 1. Differentiation of hematopoietic stem cells. Figure by Oleg Sitsel.

Mature blood cells have a short lifespan, so it is up to the HSCs to keep replenishing them. Leukaemia and lymphomas, diseases of white blood cells, typically occur due to either the abnormal growth of undifferentiated precursor cells or to HSCs that have transformed into oncogenic cells. Treatments include high-dose chemotherapy, targeted kinase inhibitors and bone marrow and HSC transplants (1). Although bone marrow and HSC transplantation have been widely established and used in the clinic, there are still many challenges.

Current stem cell therapies include autologous and allogeneic transplants. Autologous transplants involve using the patient's own stem cells, collected in advance and used to replace damaged cells during treatments, such as chemotherapy. Allogeneic transplants are derived from other donors, but are problematic due to potential rejection by the host immune system (2). Another, larger concern is the lack of donor availability and the difficulty of expanding HSCs *in vitro* to replenish blood cells for the patients who need them. Therefore, researchers have been on a pursuit to generate a robust population of HSCs. Ten years ago, adult cells were reprogrammed into stem cells for the first time, using a cocktail of transcription factors. Since then, induced pluripotent stem (iPS) cell technology has made a large impact on the world of developmental biology and regenerative medicine (3).

Kristin Qian is a visiting undergraduate student from Princeton University and is carrying out research in the Zitzmann group in the Department of Biochemistry

This has served as inspiration to many researchers who have been putting effort into trying to convert adult cells into iPS cells, and then differentiate these to become HSCs. It has been reported that mouse fibroblasts can be reprogrammed into hematopoietic progenitors (4, 5). This has kick-started further work to generate HSCs that are fully multipotent, long-lasting and viable for engraftment. In a November 2016 paper, researchers reported the successful reprogramming of mouse skin cells directly into oligopotent blood cell precursors using a combination of transcription factors to redirect the cells' fate. These 'induced hematopoietic progenitors' (iHPs) have the potential to differentiate *in vitro* into several blood cell types and could be maintained *in vivo* for up to four months (6). Although these hematopoietic progenitors are still one level away from HSCs, further studies can be conducted to elucidate additional signals, regulators, or transcription factors that can help to induce the switch to fully functional HSCs. Additionally, these findings show that direct reprogramming using specific factors could be compatible with an *in vivo* environment, supporting long-term renewal of HSCs.

Inducing hematopoietic stem cells from mature fully differentiated cells would be extremely beneficial for patients in need of stem cell transplants and blood transfusions, as well as continued research in disease pathology and drug development. Although there is still much to be done until this is a straightforward process, these recent studies give hope for and reaffirm the great potential of HSCs for use in stem cell based therapies.

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Alternative polyadenylation: the end of the message matters

by
Jessica Hardy

When discussing the cellular changes that promote cancer development, we usually focus on DNA mutations and abnormalities. Indeed, impressive progress has been made in identifying many 'driver' mutations in cancer, from inactivating mutations in tumour suppressor genes such as *P53* (the so-called 'guardian of the genome') to activating mutations in proliferation-driving proto-oncogenes such as *MYC*.

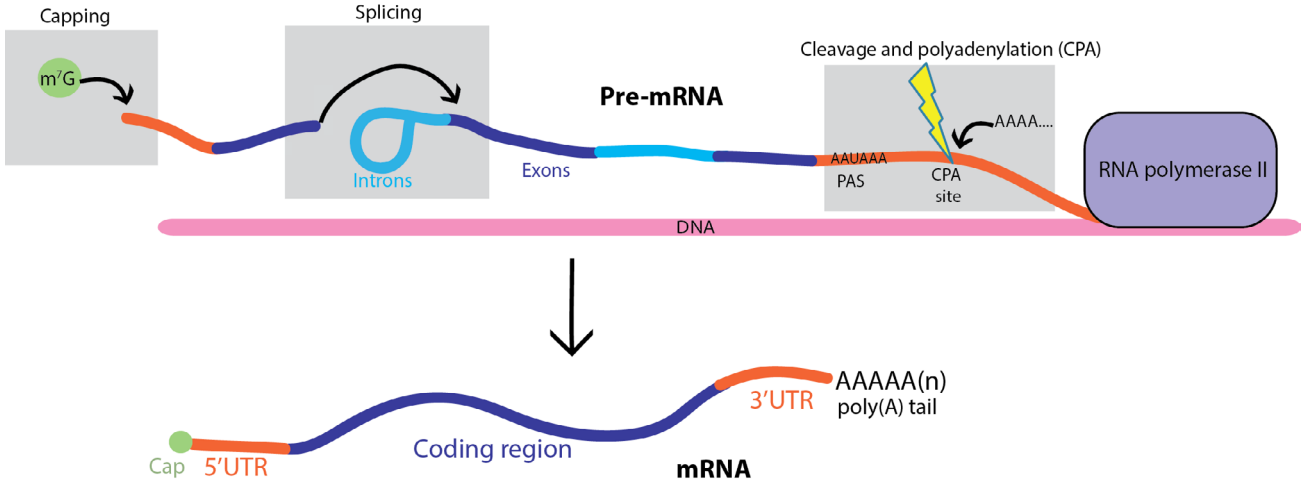

Figure 1. Eukaryotic mRNA processing. Pre-mRNAs are subject to three major co-transcriptional/post-transcriptional processing events. Capping involves the addition of a 7-methylguanosine to the 5' end. Splicing is the stitching together of exons with the removal of intervening introns - here, intron 1 is undergoing co-transcriptional splicing and the formation of the looped lariat intermediate is shown. Cleavage and polyadenylation involve release of the transcript from the polymerase followed by the addition of a poly(A) tail.

However, although such events at the DNA level make a crucial contribution to cancer development, they do not explain everything. It is becoming increasingly apparent that post-transcriptional processes, such as mRNA processing and translation, are key players in regulating gene expression. Accordingly, alterations in these fundamental pathways can help to promote tumourigenic gene expression independently of changes to genomic DNA.

Primary mRNA transcripts, known as pre-mRNAs, must undergo a number of key processing steps to generate a mature mRNA that can be exported from the nucleus and translated. The major processing reactions (Figure 1) are capping and splicing, which will not be discussed further here, and cleavage and polyadenylation.

Cleavage and polyadenylation are closely coupled reactions, and begin with the recognition of a polyadenylation signal (PAS) on the RNA - usually AAUAAA or AUUAAA - during transcription. The PAS and surrounding sequences are recognised by cleavage factors, triggering an enzymatic 'cutting' reaction which releases the transcript from the polymerase and stimulates transcription termination. The mRNA 3' end is then subjected to polyadenylation, which involves the addition of a string of adenosine nucleotides known as the poly(A) tail. This tail protects the transcript from degradation and helps drive its nuclear export and efficient translation.

Cleavage and polyadenylation (CPA) is a fundamental step in the gene expression pathway, essential for defining the end of the message and ultimately ensuring its successful translation. Despite this, it has become clear that the location of the CPA site within a gene, and thus the resulting 3' end sequence preceding the poly(A) tail, is not always predictable and invariable. Rather, most genes have at least 2 different CPA sites that can be used – a phenomenon known as alternative polyadenylation (APA) (Figure 2). Individual examples of APA were first identified in the 1980s, and more recently advances in 3'end-biased RNA sequencing have revealed that over 70% of human genes have at least 2 CPA sites (1).

APA research has attracted much recent attention, with two major questions being the focus of investigation. The first question relates to how RNA-binding proteins and other factors interact to influence the choice of one CPA site over another. The second, which will be discussed further here, is how APA influences gene expression and physiology, particularly in disease states.

Most commonly, APA sites are found within the 3' untranslated region (3'UTR) of a gene. Whilst in these cases the use of alternative sites does not alter the encoded protein, the change in 3'UTR length alone

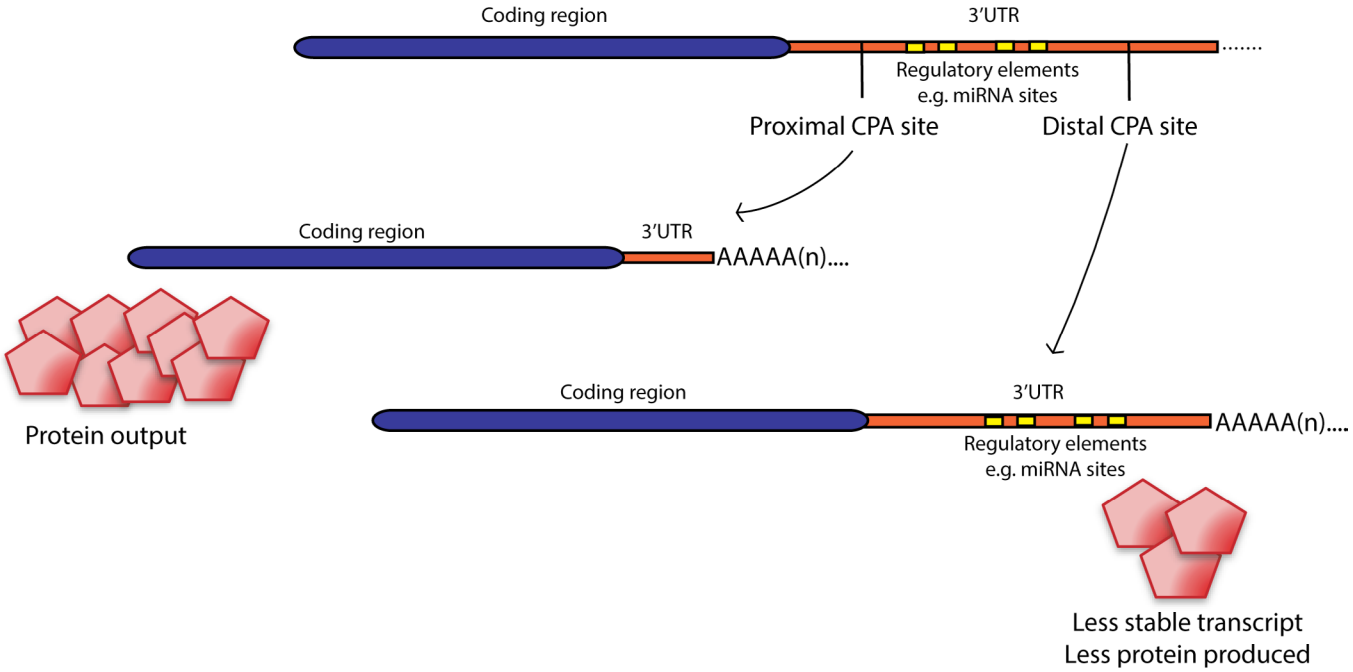

Figure 2. Alternative polyadenylation (APA). An illustration of the most common form of APA, UTR-APA, where alternative CPA sites are found in the 3'UTR (orange). Choice of the proximal CPA site may exclude positive or negative regulatory elements within the 3'UTR, influencing aspects of transcript fate and often protein output. Here, loss of miRNA sites (yellow) when the proximal CPA site is used leads to down-regulation of protein expression.

can have dramatic consequences on protein output. 3'UTRs contain sequences that regulate many aspects of mRNA dynamics, including stability, localisation and translational efficiency. For example, binding of microRNAs (miRNAs) to target sites in the 3'UTR can lead to transcript degradation or translational inhibition. Shortening of 3'UTRs through the choice of more proximal CPA sites can allow 'escape' from certain regulatory elements, potentially leading to up-regulation or down-regulation of protein expression without changes in transcription rate (Figure 2).

Strikingly, the transition from cellular quiescence into proliferation, and further into cancer development, tends to involve a progressive and widespread 3'UTR shortening through APA (2, 3). This observation has been made both in cell culture and when comparing colorectal cancer cells to healthy bowel cells within individual patients, and has been associated with poor prognosis. This raises some important questions – in particular, do APA-induced changes in expression of specific genes contribute to the cancer phenotype?

A major example illustrating the potentially critical impact of APA in cancer is 3'UTR shortening of the cyclin D1 (*CCND1*) transcript in some mantle cell lymphoma (MCL) cases. MCL characteristically exhibits CCND1 overexpression, which results from an activating chromosomal translocation and drives increased cell proliferation. Despite this shared genetic trait, an analysis of MCL samples revealed the extent of CCND1 overexpression to be highly variable. It was found that in tumours with the highest levels of *CCND1* expression, the *CCND1* transcripts had very short 3'UTRs and were more stable due to loss of destabilising sequences (4). This shortening often arose from a point mutation generating a new, premature CPA site, leading to early CPA and exclusion of most of the 3'UTR. The 'short *CCND1* 3'UTR' patients had the lowest survival rate as a result of the higher CCND1 expression, illustrating the potential for altered APA to heavily influence cancer biology.

Whilst in this example the APA event results from a genomic point mutation, the cancer-associated increased selection of existing proximal CPA sites in *CCND1* and other oncogenes (e.g. *IMP-1*, *CCND2*) can enhance tumourigenic transformation through similar mechanisms (3). In a recent study, recurrent shifts towards the use of a proximal CPA site were found in the *NRAS* and *c-JUN* oncogenes in a cohort of triple-negative breast tumours. This 3'UTR shortening increased *NRAS* and *c-JUN* expression, partly due to escape from negative regulation mediated by the RNA-binding protein PUMILIO, and was associated with increased tumour invasiveness (5).

APA has also been implicated in the pathology of several other diseases, ranging from immunodeficiency syndromes to neurological disorders. One well-studied example is the genetic muscle disease myotonic dystrophy (MD). In MD, DNA microsatellite expansions lead to production of RNAs that sequester and impair the function of the muscleblind-like (MBNL) RNA-binding protein. RNA sequencing in cells from MD patients revealed that MBNL sequestration caused pervasive changes in APA, involving both 3'UTR shortening and lengthening events (6). Furthermore, many affected genes showed changes in expression that would be expected to contribute to key pathological features of the disease – namely, impaired protein

synthesis and enhanced protein catabolism – suggesting a potential causative role for APA in MD.

In summary, APA research is uncovering many interesting examples of the biological impacts of changes to the 3'UTR length in normal physiology and disease. Alongside continued identification of these gene-specific events, further efforts will aim to improve our knowledge of how the choice of CPA site is regulated genome-wide, and how this regulation changes in disease states. This may highlight targets for therapeutic intervention, allowing pathological APA patterns to be reversed, as well as improving our fundamental understanding of the complex world of gene expression.

Jessica Hardy is a DPhil student the Norbury research group at the Sir William Dunn School of Pathology

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Can stem cells mend a broken heart? A controversial question

by
Carolyn Carr

The human heart contains around two to three billion cardiac muscle cells supported by smooth muscle and endothelial cells, fibroblasts, mast cells and other immune cells. During a myocardial infarction (MI), a coronary artery is blocked and blood supply to part of the heart is cut off, leading to cell death and the formation of a fibrotic scar.

Clinical practice aims to re-open the blocked artery as quickly as possible, and restore delivery of oxygen and substrates to the damaged area, but in the majority of cases this does not prevent cell death. Indeed, reperfusion brings its own problems as the sudden influx of blood can induce calcium overload, mitochondrial collapse, rupture of capillaries and the sarcolemma, and an influx of inflammatory cells (1). Over time, the heart remodels as the resulting fibrotic scar is not able to contract and therefore, the surviving muscle hypertrophies to maintain cardiac output. However, the increased workload on the surviving cells cannot be maintained *ad-infinitum* and the heart may begin to fail. Pharmacological attempts to reduce infarct size have had limited success and current therapies, such as angiotensin-converting enzyme (ACE) inhibitors and β-blockers, are aimed at maintaining viability of the surviving muscle for as long as possible (1). Improvements in treatment have led to a reduction in short-term mortality, however there are currently 2.3 million people living with coronary heart disease in the UK.

Over the last 15 years, the use of stem cell therapy to restore viable cardiomyocytes and rescue cardiac function has been a rapidly expanding topic of research. Until relatively recently, it was thought that the reason the damaged heart was not able to restore its function was that myocytes were unable to proliferate. However, in 2001 the group of Piero Anversa reported two interesting findings, namely the presence of dividing myocytes around the infarct region of a human failing heart and that myocytes, coronary arterioles, and capillaries with a Y chromosome could be identified in hearts from male patients who had received a transplant from a female donor (2). They also reported that injection of GFP-labelled bone marrow cells into the border-zone of an

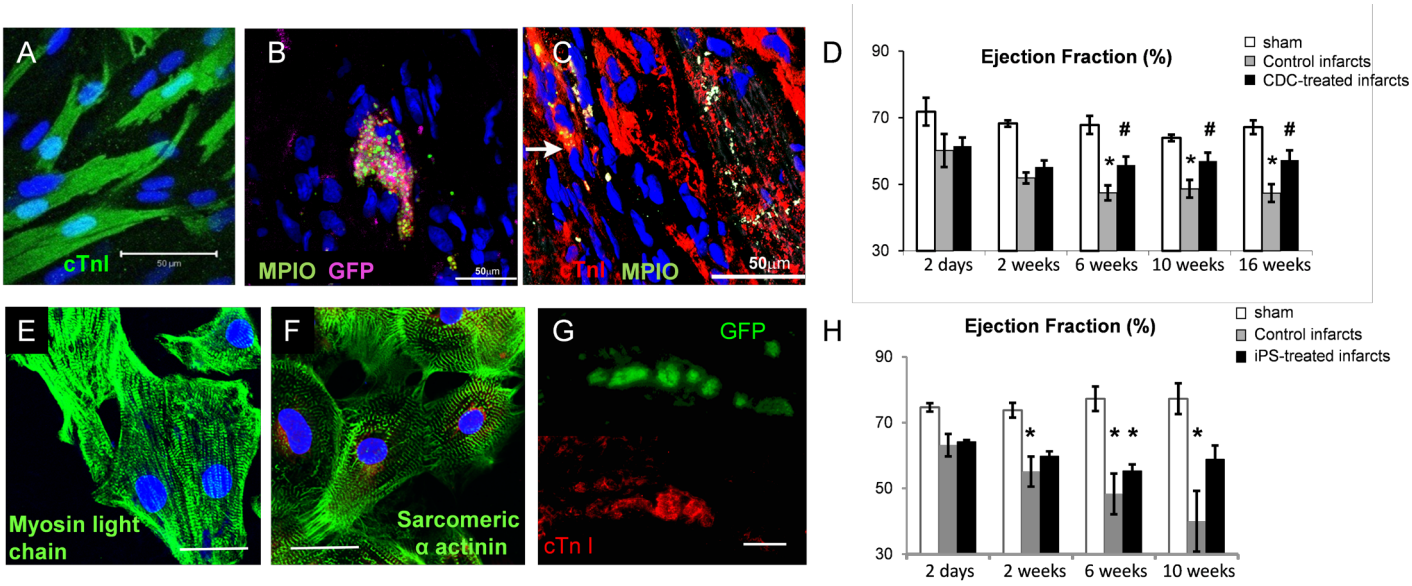

Figure 1. *In vivo* testing of cardiosphere-derived cells and induced pluripotent stem cell-derived cardiomyocytes in the infarcted rat heart. Cardiosphere-derived cells (CDCs) can be differentiated *in vitro* to express cardiac markers (A) but do not beat. When injected into the infarcted rat heart, GFP+ CDCs, labelled with green fluorescent iron oxide particles (MPIO) could be identified in the myocardium (B) and on rare occasions MPIO-labelled cells were found which expressed cardiac troponin I (C). CDC-treated hearts (n = 7) had a significantly higher ejection fraction than control infarcted hearts (n = 7) at 6 weeks after infarction and this improvement was maintained out to 16 weeks (D). Human induced pluripotent stem cells (iPSCs) can be differentiated to beating cardiomyocytes which show sarcomeric expression of contractile proteins (E, F). When partly differentiated GFP+ iPSCs were injected into infarcted immuno-compromised rat hearts, GFP+ iPSCs could be detected within the myocardium and expressed cardiac troponin I (G). iPSC-treated hearts (n= 4) did not show the same decline in function after MI as control infarcted hearts (n = 5) (H).
Scale bar = 50 μm; * $p < 0.05$ vs sham, # $p < 0.05$ vs control.

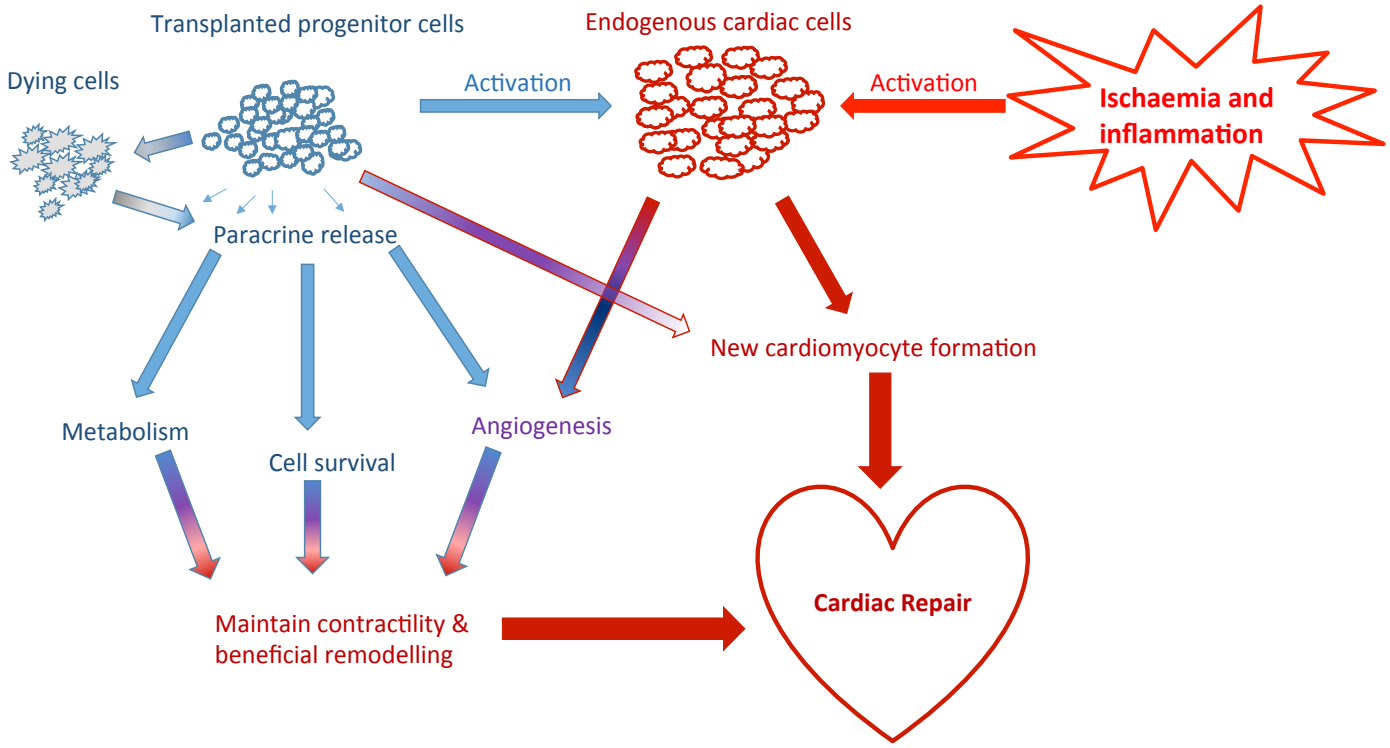

Figure 2. Mechanism of action of stem cell therapy post-MI. The transplanted cells and their progeny are activated by the local inflamed and ischaemic milieu. The transplanted cells can exercise beneficial effects on the heart directly by differentiation or indirectly by the secretion of paracrine factors. Similarly, the transplanted cells may recruit and activate endogenous cells from the heart or from elsewhere within the body, which may differentiate or induce further paracrine signalling. In addition, the death of the transplanted cells may modulate the inflammatory environment (taken from (5)).

infarcted mouse heart induced the formation of new myocytes and blood vessels within the scar.

Our first foray into cardiac cell therapy was to investigate whether bone marrow stromal cells (BMSCs), expanded *in vitro* from bone marrow isolate, could improve function in the infarcted rat heart (4). We used high field magnetic resonance imaging (MRI) to measure cardiac function and labelled the BMSCs with iron oxide particles so that we could track them *in vivo* by the hypo intensities they caused in the MR image. We found that the cells were retained in the infarcted heart, with an increased number of cells remaining in the more damaged hearts, but we did not find any differentiated donor cells or any improvement in cardiac function.

Bone marrow cell therapy has the advantage that sufficient autologous cells can be obtained with relative ease, however these cells are not of cardiac origin. In 2003, three groups reported the existence of cardiac progenitor cells resident in the heart, identified by the surface markers c-kit or Sca1 and the cardiac transcription factor Isl1. These progenitor cells were shown to be clonogenic and capable of differentiating into the three main cardiac lineages (5). Subsequently, in 2011, we showed that cardiac progenitors could also be expanded from cardiac biopsies as a heterogeneous population, via the formation of cardiospheres (6). More recently, Paul Riley and Nicola Smart found that cells from the epicardium could be activated to repair damaged heart muscle (7). We tested the potential of cardiosphere-derived cells (CDCs) from neonatal rats in the infarcted rat heart, again using iron oxide labelling to track the cells (6). We found that donor cells were retained in the heart for 16 weeks and that some cells had differentiated into cardiomyocytes or formed new blood vessels. The capillary density was increased and, more importantly, by six weeks after MI the CDC-treated hearts had significantly better function than the untreated hearts (Figure 1). Despite this positive outcome, the number of differentiated donor cells remaining in the heart after 16 weeks was very small. CDCs and c-kit+ cells have now been tested in the clinic and have shown some improvement, albeit small (5).

It is generally agreed that the beneficial action of administered stem cells, either from the bone marrow or the heart, is predominantly via the release of paracrine factors which induce new blood vessel formation, reduce cell death and may activate resident cardiac progenitor cells (5) (Figure 2). Indeed, stem cells from other sources, such as adipose tissue and cord blood, have also been shown to give a comparable benefit to that seen with cardiac progenitors, at least in animal models. This begs the question of why we saw no improvement in function in our study using BMSCs (4). One answer may come

from a comparison between two clinical trials using bone marrow cells, the REPAIR and ASTAMI studies (3). Although very similar in design, REPAIR showed a positive result whereas ASTAMI did not. Further investigation by the REPAIR team, suggested that the conditions used to isolate the cells differed, which affected the efficacy of the cells, so that apparently minor differences in isolation and culture may have a profound effect on the end result.

Having shown that CDCs from neonatal heart can improve cardiac function after MI, we have been investigating whether the cells are affected by age or disease and how to maintain their efficacy through expansion *in vitro*, in order to generate sufficient cells for therapy. We have found that the number of CDCs that can be isolated from the heart decreases with age but is not affected by the mild cardiac impairment found in the MDX mouse (a model of muscular dystrophy) nor by long term consumption of a high fat diet (8). We have also found that expansion under hypoxia maintains these cells in a more stem cell-like state and increases the release of the angiogenic cytokine, VEGF (8). More recently, we have found that we can produce a more homogeneous progenitor population that has a promising resistance to serum starvation. We have also attempted to overcome the issue of low cell retention by applying CDCs to the heart in a supportive porous collagen scaffold, although to date this is proving less successful than we had hoped.

Perhaps the most promising source of new cardiomyocytes, and the least controversial (if you exclude the ethical issues around embryonic stem cells), are pluripotent stem cells. Early work testing cardiomyocytes derived from human embryonic stem cells (ESC) in mouse models of MI showed promise, but was hindered by limited engraftment to form a beating syncytium, possibly due to the difference in heart rate between man and mouse. More recently, Murry *et al* have injected one billion human ESC-derived cardiomyocytes into a macaque heart following MI and have shown large areas of functional new myocytes (5). Of course, ESC-derived cardiomyocytes are, by nature, not autologous, but induced pluripotent stem cells (iPSCs) can also be differentiated to form beating cardiomyocytes and may be equally effective. We tested differentiating human iPSCs in an immunocompromised rat model. We injected cells that were part-way through the differentiation protocol (after completion of the differentiation protocol but before the cells started beating) and showed that donor cells were retained and differentiated to form cardiomyocytes and smooth muscle cells, albeit again at a very low level (9). Although we were limited by low numbers of animals, we showed that the treated hearts did not decline in function post-MI to the same extent as the untreated hearts (Figure 1). *In vitro*, iPSC-derived cardiomyocytes resemble foetal heart cells rather than mature myocytes. This should not form an obstacle for their use in cell therapy, indeed it may well be an advantage as less mature cells may be more likely to survive in the ischaemic scar for long enough to engraft and form new muscle. However, we and others are working towards generating more mature iPS cardiomyocytes for use as *in vitro* models of human heart cells.

So, can stem cells mend a broken heart? Pluripotent stem cell-derived cardiomyocytes can generate new cardiac muscle, however at present the cost of generating autologous iPSCs and differentiating them in sufficient numbers is prohibitive. Time will tell whether this becomes clinically viable. Adult stem cells can act as paracrine factories, capable of delivering beneficial cytokines over several days to induce angiogenesis and reduce cell death, and can activate the formation of new myocytes. Whether these form from resident cardiac progenitors, division of existing myocytes or even recruitment of bone-marrow derived progenitors is still a matter of hot debate.

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The story of the very first life

by
Siu Shing
Wong

A natural response to the cell theory postulate that all cells arise from pre-existing cells is to ask where the first cell came from. To answer this question, scientists hypothesised a simple Darwinian chemical system, a protocell, as the ancestor of all living things. Unlike the complex machinery of cells that we are used to, a protocell had only rudimentary genetic material and a membrane. Thus, its survival was at the mercy of the hostile primordial environment. In this article, we will consider how these components might cooperate synergistically to form a protocell.

The RNA world hypothesis is composed of two major ideas: that RNA was the first genetic material and it carried out protein-like functions (1). However, synthesising ribonucleotides involves numerous steps, complicated even in laboratory conditions (2), not to mention the fact that RNA is unstable. Therefore, perhaps an alternative genetic polymer, more chemically simple and stable, might have predated RNA. However, it should also be capable of Watson-Crick base pairing with itself and RNA so as to conserve base sequence information during the transition to RNA.

A suitable candidate for this genetic ancestor would be Threose Nucleic Acid (TNA) in which threose can be chemically synthesised simply by dimerisation of glyceraldehyde (3). It was demonstrated that TNA can form structures that bind thrombin and evolve in vitro (4). However, TNA can be further simplified by replacing threose with glycol as the backbone (3). The resulting polymer is Glycol Nucleic Acid (GNA), which can form a stable duplex with RNA but not with TNA. Although many alternatives have been intensively studied, the missing link of evolution from primitive genetic polymer to RNA still remains elusive. Despite this mystery, we can anticipate that the functions of the primordial polymer must have been conserved and passed to RNA so that sequence information remained intact.

Now we have an idea of what the alternative genetic polymer predating RNA might be. What might have been the first feature of the polymer? One reasonable answer is self-replication. Otherwise, it would have been wiped out by the invisible hands of Darwinian evolution. Of course, self-replication is no easy task. Nucleotides can polymerise to form short stretches of random sequence on clay surfaces, due to hydrophobic base-stacking interactions. However, the length of the randomers formed is only around 20-40 nucleotides, whereas the length of artificial ribozyme ligase is around 170-220 nucleotides (1). How can such a short randomer perform self-replication so similarly to a long ribozyme ligase? Perhaps small functional randomers cooperate as an autocatalytic set to facilitate the duplication of themselves (1) (Figure 1a).

However, the first problem with this idea is that a multicomponent ribozyme formed by individual randomers is unlikely to have the control to ensure that there is well-balanced abundance of the cohort. In other words, the replication is futile and does not result in the complete catalytic set (Figure 1b). Therefore, evolution to a one-piece ribozyme may be more favourable because this ensures that each component duplicates exactly once per cycle. The first ribozyme synthesised artificially was a ligase, not a polymerase (1, 3). If the first natural ribozyme was also a ligase, this could potentially explain the formation of a one-piece ribozyme from its autocatalytic set of short randomers (Figure 1c). Moreover, ligases can offer an additional competitive edge through diversifying the functions of ribozyme by random modular ligation. In fact, such a phenomenon is known to occur when DNA duplication and insertion generates a new protein by combining different domains.

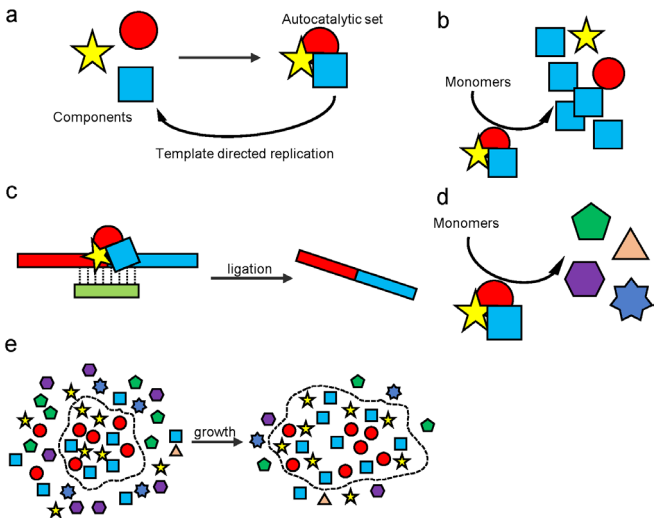

Figure 1. (a) Autocatalytic set catalyzes the formation of the set members. All three members must be present for replication. (b) Unbalanced replication results in the over-abundance of a particular component. The number of effective descendants is not maximized. (c) Autocatalytic set may be a ligase. Ligation may join two set members together. Gradually an autocatalytic set will become a single stranded ribozyme. (d) Replication of other non-self sequences is not helpful for the autocatalytic set to dominate. (e) Membrane ensures the replication of only the polymer within, thus excluding other sequences.

The second problem is altruism towards non-self sequences (1) because ribozyme might not have evolved an advanced discrimination mechanism to exclude other randomers (Figure 1d). Such benevolence might jeopardise functional ribozyme generation in a pool of randomers. This problem could have been overcome by

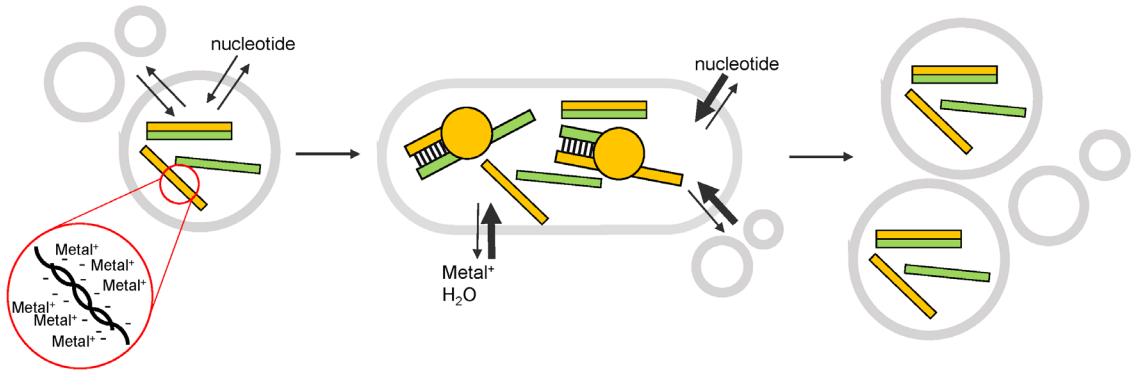

Figure 2. Replication of impermeable genetic polymers drives the entry of water into the protocell by osmosis, thus the growth of the protocell. This results in the instability of the membrane so that the protocell divides into two daughter protocells.

the involvement of membranes which encapsulate and prioritise the duplication of the members within the physical barrier (5) (Figure 1e). We would anticipate that the protocell membrane had a similar level of simplicity as its nucleotides. It should be made of permeable amphiphilic molecules because protocells lacked delicate membrane transporters so they could only obtain nutrients by passive diffusion (6).

We understand that when the critical micelle concentration is reached, fatty acids will assemble. What determines assembly into a membrane instead of a micelle? Perhaps, it could be pH. A neutral fatty acid can be imagined as cylindrical because the electron distribution is approximately homogeneous. When it is deprotonated, its shape is more cone-like because the electron distribution is skewed towards the ionic head. When environmental pH is larger than the pKa of the fatty acid, it will deprotonate, causing the majority of fatty acids to adopt a cone shape. Thus, the surface curvature increases, forcing the fatty acids to form a micelle (6). Using the same logic, when environmental pH is less than the pKa, the fatty acids become protonated and neutral, leading to the formation of lipid aggregates (6). When pH is similar to pKa, the populations of fatty acids with cone and cylinder shapes will be approximately equal, and will be more likely to form a bilayer. Besides, the fatty acids will partially ionise and pseudodimerise by forming hydrogen bonds which stabilise the bilayer structure, resembling a two-tailed phospholipid (6). Fatty acids are constantly leaving and incorporating into the vesicle, so an equilibrium exists. Assuming that the primordial environment had a continuous supply of fatty acids, the fatty acid will either self-assemble or incorporate into existing vesicles.

A tempting hypothesis coupling nucleotide replication and membrane formation has been proposed, which renders fatty acid incorporation an active process. Fatty acid membranes are generally impermeable to replicable genetic polymers, whereas they are permeable to monomers (5). The replication of the polymer spawns more polymers inside the protocell, demanding that more monomers enter the protocell (5). These factors raise the osmotic pressure inside the protocell, promoting water entry (5). To accommodate the volume increase, active membrane growth is promoted (5). It shifts the equilibrium towards fatty acid incorporation (5) (Figure 2). Therefore, nucleic acid-containing vesicles have a selective advantage over other vesicles. The ability of ribozymes to replicate also allows exclusion of other randomers. This relationship is mutualistic and synergistic.

The growing cell would eventually become unstable and divide. It was also found that the vesicle grows filamentously because the increase in surface area is faster than that of volume (6, 7). By light agitation, many descendant vesicles form (5, 6). The process would not be possible without the involvement of physical force inside hydrothermal vents (7). The convection created inside the vent might separate the protocells (7). Besides, it may also help to concentrate fatty acids so that they can start self-assembly into vesicles and provide a fluctuating temperature for separation of a duplex genetic polymer (7). It would eventually become a competition in which the cells that can grow and divide efficiently will predominate. One of the participants in this competition is likely to be the common ancestor of all life on planet Earth.

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The mystery of planarian regeneration

by
Claire Hill

Planarians are flatworms of the order *Tricladida*, often referred to as 'the masters of regeneration'. Remarkably, Thomas Hunt Morgan determined that a planarian fragment as small as only 1/297th the size of the original organism could regenerate into a complete and fully functioning one (1).

The principles of planarian regeneration have been explored for over two centuries; nonetheless, many aspects of the process remain a mystery. Peter Simon Pallas was the first to describe planarian regeneration in 1766. Later, in 1814, Dalyell characterised planarians as "immortal under the edge of the knife". With this statement, interest was sparked: could planarians hold the key to eternal youth?

It was not until 1897 that the underlying mechanisms behind planarian regeneration began to be revealed. In that year, Harriet Randolph coined the term 'neoblasts' to describe a population of small, rounded cells with large nuclei, a thin cytoplasm and high ribosomal RNA content; these cells are what make planarian regeneration possible.

Neoblast cells make up approximately 20-35% of the total cell count (2) and are the only proliferating cell type, giving rise to all other types. They are localised within planarian tissue, in all areas of the body, except above the eyes and in the pharynx. These cells migrate to sites of wound and organ formation, where they accumulate to form a regeneration blastema. They then differentiate and integrate into the surrounding tissue, replacing missing, damaged, or aged cells (3).

Investigations carried out by Wolff and Doubousm in 1948 demonstrated that X-ray irradiation of planarians reduced neoblast populations and consequently prevented regeneration (3). Then, in 1989, Baguñà and colleagues showed that regenerative abilities could be restored to X-irradiated planarians upon the injection of purified neoblasts (4). This confirmed that these cells were responsible for wound repair and regeneration and held stem cell-like functions. Neoblast number has been associated with animal length and volume, with larger planarians exhibiting decreased neoblast density and lower regenerative abilities (3). Neoblasts play a role in planarian homeostasis, growth and repair. It is likely that the pathways involved in these functions are conserved and, subsequently, similarities between planarian and mammalian stem cells activity have been investigated.

The potential of planarian models to improve our understanding of mammalian disease and aging is only beginning to be appreciated. Stem cell therapies are showing great potential in the field of regenerative medicine; however, these therapies are currently restricted due to concerns about safety, effective delivery and integration. Moreover, studying neoblast function in planarians has also improved our understanding of neoblast-like cells in other flatworms, such as the parasitic *Schistosoma mansoni* and *Fasciola hepatica* (Figure 1), thus, aiding the identification of novel drug targets for the treatment of animal and human infections worldwide.

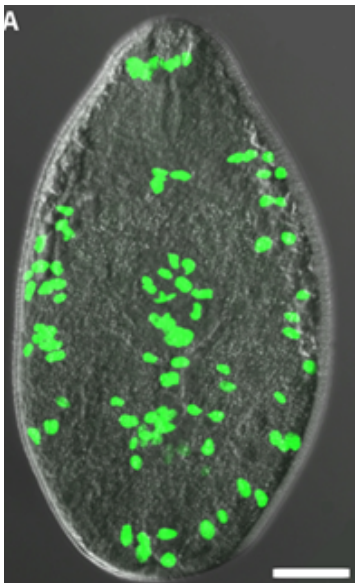

Figure 1. Green fluorescence shows the incorporation of 5-ethynyl-2-deoxyuridine (EdU) in proliferating cells with neoblast-like morphology in the parasitic flatworm Fasciola hepatica (5).

In summary, our understanding of planarian regenerative abilities can provide a valuable insight into the structure and function of stem cells and the mechanism of tissue repair. This will be of major relevance in potentiating regenerative medicine-based applications.

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Light sheet microscopy: a new age of faster volumetric imaging

by
Sonia Muliyil

The advent of new imaging techniques that aid the capture of in vivo events in real-time has revolutionised biology. However, the quest to improve our knowledge and technical expertise in microscopy-based imaging continues. Many such explorations have involved biologists, physicists and chemists alike. Developments have been mainly directed towards improving resolution, reducing phototoxicity or maintaining optimal imaging conditions that can capture various natural phenomena within a living cell or an intact organism.

Some of these scientific advances have been widely recognised, as is the case with super-resolution microscopy techniques like Photoactivation Localisation Microscopy (PALM), Stochastic Optical Reconstruction Microscopy (STORM) and Stimulated Emission Depletion Microscopy (STED), for which the Nobel Prize in Chemistry (2014) was collectively awarded. While some of these techniques attempt to break the current limit of resolution, others have made imaging faster, and the rest have attempted to capture more volumetric or 3D information from specimens. There are very few techniques that can boast combining all of these features into one.

One of the most common problems faced by fluorescence microscopy since its infancy has been to eliminate out-of-focus light from samples. A conventional widefield or standard fluorescence microscope illuminates a sample almost in its entirety and collects the reflected light from all planes, thus producing a blurry image of the sample. There are two ways to circumvent this problem: one possibility is to minimise the capture of out-of-focus light; the second being to eliminate this information post-acquisition. While a conventional confocal microscope utilises the principle of a pinhole to eliminate out-of-focus light coming from the specimen, it can often contribute to phototoxicity and bleaching owing to deep tissue laser penetration, especially in cases of live imaging. Using a two-photon laser helps to reduce the amount of phototoxicity, since the fluorescence is generated only in a small volume, owing to a non-linear interaction between light and matter. However, both of these techniques lack sufficient depth penetration in heterogeneous samples, fail to bridge the gap between lateral and axial resolution, and are associated with slow temporal imaging speeds. In order to find better solutions to these problems, a group of scientists looked back to a discovery made way back in 1903. At this time, R. A. Zsigmondy developed the ultramicrosope as a new illumination scheme which allowed the visualisation of particles close to the size of the wavelength of light. This method of detection was based on light scattering rather than light reflection, a discovery which was later awarded the Nobel Prize in 1925.

Taking this technique a few more steps forward, Huisken et al subsequently developed a means of imaging whereby optical sectioning was achieved by illuminating samples with a thin sheet of light along an axis orthogonal to the detection axis (1). This technique, termed as Single Plane Illumination Microscopy (SPIM), used a cylindrical lens to generate a sheet of light which would selectively illuminate only a single plane and not the entire depth of the specimen, unlike a conventional confocal microscope. The emitted fluorescence is imaged from above and below the sample with optimal conditions, resulting in almost no out of plane excitation or emission, thus achieving fast and efficient optical sectioning. Additionally, only the plane in view is imaged and thus subject to bleaching, not the entire depth of the sample. Furthermore, this technique scores highly in aspects of spatial resolution. It is known that a confocal microscope is intrinsically dependent on the same Numerical Aperture (NA) of the objective for both lateral and axial resolution, with the former being inversely proportional to NA and the latter inversely proportional to NA2. However in the case of SPIM, the lateral and axial resolution can be uncoupled, since the former depends on the NA of the light-collecting

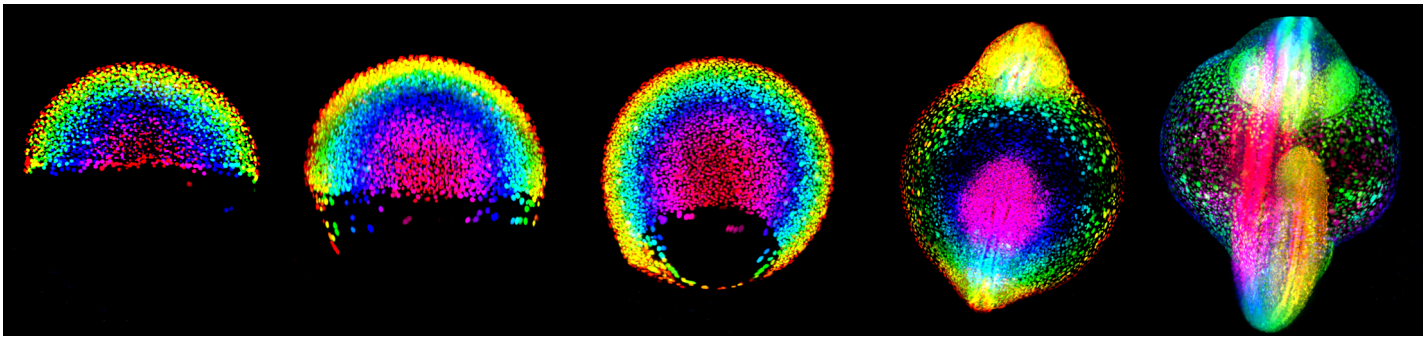

Figure 1. Developing zebrafish embryo from 5 - 18 hours of development post fertilization (hpf) showing the formation of an embryo from a ball of cells. Four views of the nuclei labelled embryo were acquired and reconstructed at each time-point. Color code indicates depth, enabling the visualization of various parts of the embryo. Image acquired on a home-built 4-lens Selective Plane Illumination Microscopy (SPIM) setup. Courtesy: Gopi Shah, CRUK, Cambridge.

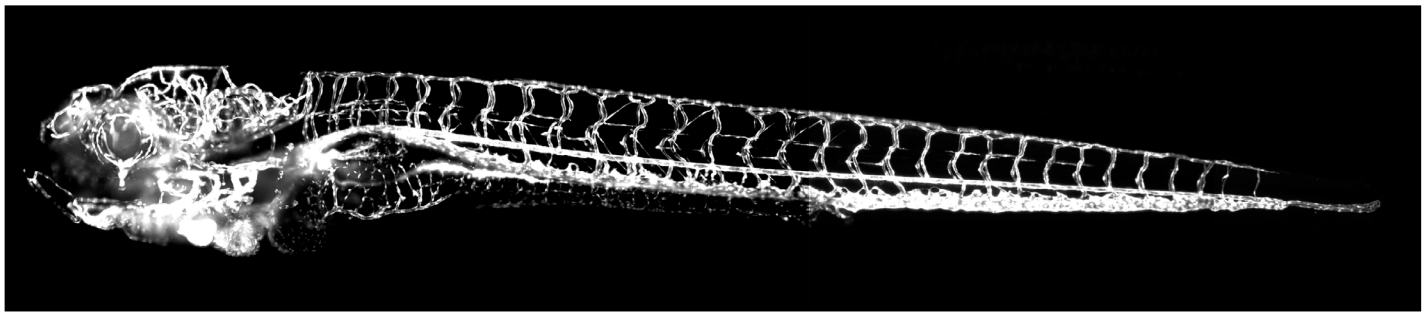

Figure 2. Entire vascular network of 6 days post fertilization (dpf) live zebrafish larva is shown. Image stacks were acquired in 4 tiles to cover the entire length of the larva. Image acquired on a home-built 4-lens Selective Plane Illumination Microscopy (SPIM) setup. Courtesy: Vikas Trivedi, Department of Genetics, University of Cambridge.

objective lens while the latter depends on the NA of the sample-illuminating objective lens. Flexibility to adjust NA with different objective lenses has helped to achieve a better resolved image. This is especially important when imaging at low magnifications (low NA optics) where one can now maintain a more isotropic resolution (close to same value) in all dimensions, a feature ideal for volumetric imaging. Together, all of the above characteristics are incredibly valuable for imaging live organisms. For example, the first ever SPIM could image a Drosophila embryo at a depth of 500 microns with a resolution of 600 nm in both lateral and axial directions (1). This was almost unimaginable for a standard confocal microscope. These figures have only improved with fine tuning of the optics involved.

All microscopy-based detection systems suffer from additional inherent problems that revolve around the issues of light scattering and absorption by the samples being imaged. This can be overcome to some extent by capturing images of the sample from multiple angles and then combining them post-detection into a 3D view. Again, plane illumination microscopy gives the flexibility of using multiple angles of excitation as well as detection, thus building a more holistic picture of the specimen in question. However, despite the good penetration of SPIM, the original setup required rotation of samples (especially those that are thick) in order to gather entire volumetric information. These requirements gave birth to multi-directional SPIM (mSPIM) and SPIM with four objectives (four lens SPIM). These setups use multiple objectives for both illumination and detection, thus bypassing the need for sample rotation. With the power of such a setup, an entire fly embryo could be imaged in half a minute (2,3). Interestingly, in some cases, four orthogonal views can be obtained if the four objectives are alternately used for both illumination and detection. However, as with any other complex setup, these designs also require careful monitoring of optical alignment. Another significant advance from the original setup has been the advent of Lattice light sheet microscopy. This modified version of the original technique uses a structured light sheet to excite fluorescence in successive planes of a specimen in one go (4). The Lattice method can capture 200 to 1000 planes per second: two orders of magnitude faster than the Spinning Disk confocal, and yet achieves reduced phototoxicity. In fact this improvement, brought about by Nobel laureate Eric Betzig, is believed to be more impactful than his original discovery of PALM. Light sheet microscopy has now become a widely-used technique for microscopists across different fields of research. One very interesting use of this technique has been in imaging whole rodents from head to toe, following tissue clearing. A method termed 3D Imaging of Solvent Cleared Organs (3DISCO) has become popular in this regard (5). This technique renders tissues completely transparent post-fixation while causing an overall shrinkage of its size (up to 65%). Combining this method of fixation with light sheet imaging has allowed one to capture thick volumes in vivo at a resolution of a few microns within a span of minutes. This revolutionary imaging technique has enabled one to capture stunning and highly informative images of individual neuronal projections, as well as incorporation of stem cells inside a living anaesthetised rodent. At a subcellular level, collective efforts combining Lattice light sheet microscopy with multi-colour labelling has painted a highly resolved and dynamic picture of the rapidly changing nature of organelles within a cell, including the mitochondria and endoplasmic reticulum (6, 7). Even though light sheet microscopy has previously been used for single cell resolution imaging, exploration of its applications for single molecule super-resolution imaging has only just begun.

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Would you upgrade your DNA?

by Sofia D'Abrantes

Imagine a world in which we could eliminate all genetic disease. Today, genome-editing technologies could make this dream a reality. In this article, we will explore how our DNA, along with other aspects, determines our health. Additionally, we discuss how current research into DNA editing can be used for therapeutic applications, highlighting what the future of medicine holds for us.

The days when DNA tests were taken just to resolve paternity issues are long gone. Nowadays, we often read headlines such as "DNA-editing could fix 'broken genes' in the brain", "Jurassic Park in real life" or "US start-up matches wines to your DNA". For a fee of £79 and just a 4-6 week wait, we can even use DNA to uncover the history of our ancestors.

The concept of DNA

DNA is a molecule that contains the genetic information used in development, reproduction, growth and functioning of all known living organisms, including humans. Almost every cell in our bodies has the same DNA. DNA is composed of four different chemical bases or units: adenine (A), cytosine (C), thymine (T) and guanine (G). The order, or sequence, of these bases determines every characteristic of an organism, from eye colour, to risk of developing diseases such as cancer.

The Human Genome Project (HGP) was an international project which aimed to determine this sequence in the human genome and to improve the technology, cost and quality of DNA sequencing (1). With its success came a myriad of advances in the field of biochemistry and medicine, but it also unveiled many questions. Since then, the promise of personalised medicine and cure of human disease has revolutionised healthcare and is changing the way we live. Although the project led to a draft of the human genome, allowing us to 'read' our DNA; our ability to 'write' DNA sequences in cells is limited to a small number of short sequences, restricting our capability to understand and manipulate biological systems.

"The promise of personalised medicine and cure of human disease has revolutionised healthcare."

DNA and health

Different people have unique DNA sequences, and depending on their order and stability, it makes them more or less likely to develop illnesses. With the sequence generated from the HGP, the consequent challenge was to identify the sequences that increase the risk of diseases such as diabetes and cancer, and then develop new treatments. Nowadays, your individual DNA sequence can be compared to a reference human genome to detect DNA base changes, known as mutations. Most of the DNA changes represent places where individuals commonly differ. These variations can be linked to a physical feature, personality traits, your body's response to certain medications, or risk of diseases.

Certain inherited genetic disorders are known to be caused by changes in your DNA. For example, 5-10% of all cancers are caused by mutations inherited from our parents (2). It has been shown that women who carry mutations in BRCA1 or BRCA2 genes have significantly higher risk of developing breast and ovarian cancer (3). Companies such as Myriad Genetics have started offering tests for patients to uncover their predisposition to diseases including breast cancer and cystic fibrosis. Others, such as 23andMe, provide an at-home, saliva-based sampling service which reports on over 100 genetic related health conditions and traits.

Genetic changes can arise at any point during an individual's life, and these are called acquired mutations. These are the most common cause of many diseases such as cancer and diabetes (4). Unlike inherited genetic disorders, the causes of these diseases are complex and highly varied. This is because multiple combinations of DNA mutations, in addition to environmental exposures and lifestyle factors, can lead to a health condition. UV radiation, smoking, pollution, diet, and other factors can all trigger mutations that result in disease. It is estimated that over 40% of cancer cases could be prevented by lifestyle changes, including eating a healthy diet and not smoking (5). Smoking tobacco is linked to at least 17 classes of cancer (6). A recent study showed that annually people who smoke a pack of 20 a day for one year generate 6 mutations per liver cell, 18 per bladder cell, 97 per larynx cell, 150 per lung cell, and 39 per pharynx cell (6). Every DNA mutation has the potential to alter cell function and behaviour, potentially rendering it cancerous. Some smokers never develop cancer despite acquiring thousands of mutations; however, higher levels of smoking increase the number of mutations accumulated, therefore elevating the risk of developing cancer.

Genome editing

Until recently, it was thought that whilst it may be possible to change environmental and lifestyle risk factors, genetic risk factors cannot be altered. However, a breakthrough discovery this year might just change that. Scientists have discovered a new way to edit

Figure 1. DNA editing using 'molecular scissors'. An example of this technique is CRISPR/Cas9, which has revolutionised the future of medicine.

DNA and 'fix' mutations to cure previously incurable diseases (and possibly even extend human lifespan) (7). Previously, scientists were unable to make changes to DNA in heart, liver, brain and eye tissues. This is because most cells in these tissues do not divide, making it harder to change mutated DNA. Using a technique called HITI (homology-independent targeted integration, based on the famous CRISPR/Cas9 gene editing technology), researchers have been able to alter DNA in the eyes of rats born with a genetic disease called retinitis pigmentosa. The study demonstrated improved visual responses, and could pave the way for a treatment of this disease in humans (7). This illness affects about one in 4,000 people in the UK (7), and improvements in this type of technology could pave the way for treatments of previously incurable diseases.

Gene editing technologies such as HITI and CRISPR/Cas9 have allowed the precise and easy manipulation of DNA in our cells. These can be thought of as a pair of molecular scissors (Figure 1) which cut mutated, harmful DNA, removing it from cells and preventing these mutations from being passed on to their offspring. Another recent genome editing study has showed that CRISPR/Cas9 could provide a cure for a type of muscular dystrophy (FSHD), a genetic disorder which weakens the muscles of the body over time (8). For FSHD, the way genome editing could help is simple – remove the DNA which produces a toxic protein (8).

Recent studies such as the ones described above have raised the possibility of generating offspring that carry either no risk or a reduced risk of some genetic diseases. However, genome editing is highly controversial, even for medical purposes. It raises concerns about 'designer babies' and 'playing God'. Last year, in a world first, Chinese scientists caused a stir after announcing they had used genome editing in human embryos (9). While their main goal was to cure a disorder known as β-thalassaemia, some people argued that such changes could risk altering the physical traits of future generations.

Nonetheless, genome editing is the only weapon we have against faulty DNA, so it is important to have serious conversations about how to handle this double-edged sword. Some scientists believe people will become comfortable with such technologies, in a similar way to how society has become accepting of the use of in vitro fertilization to help infertile people conceive. If it can be used accurately and safely, human genome editing for the eradication of incurable diseases will no longer just be a possibility, but the future of medicine.

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Classic kit: HPLC

by Sandra Ionescu

Separation of substances from a mixture has held a key role in scientific advancement since the 'beginning' of chemistry. In fact, one Dutch word for chemistry, scheikunde, means 'the art of separation'. One of the most widely practiced separation methods in use today is high performance liquid chromatography (HPLC).

The use of HPLC as a preparative and/or analytical method spans a variety of fields such as biomedical research as well as the cosmetics, energy, food, and environmental industries. The method uniquely enables both the separation and identification of femtomolar amounts of compounds from complex matrices in the research setting and large-scale purification and isolation of synthetic industrial products (1, 2).

The schematic of an HPLC instrument typically includes a solvent reservoir, pumps, a sample injector, a column, and a detector. The injector brings the analyte(s) into the solvent (mobile) phase which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column, which is packed with an adsorbent material (the solid phase). The time at which a specific analyte elutes from the column, the retention time, varies depending on the interaction between the stationary phase, the molecules being analysed, and the solvent(s) used for the mobile phase. Analytes that have the least amount of interaction with the solid phase or the most amount of interaction with the mobile phase will exit the column faster. The retention time measured under particular conditions can be used to identify and separate the analyte. The main types of liquid chromatography (LC) are preparative methods, which focus on productivity, and analytical methods, characterised by increased selectivity. The selectivity of the column can be tuned using different types of HPLC, including hydrophobic interaction, ion-exchange, affinity, and size-exclusion chromatography. The composition of the mobile phase is kept constant or varied depending on the type of HPLC used (1).

The Russian-Italian botanist Mikhail Tsvet was the first to adopt the term 'chromatography' and is considered to have invented the adsorption chromatography technique in 1906 when he reported the separation of plant pigments into a series of coloured bands on a calcium carbonate column. The flow rate of traditional LC columns relied on gravity, and separations could take anywhere from hours to days to complete (1). Around the time that LC was evolving, gas chromatography dominated the analytical field driven by the needs of the petrochemical industry. However, gas chromatography was too harsh for the thermally unstable biological compounds that were of interest to the life sciences. Demand also grew for the efficient separation of polar and charged compound mixtures in the pharmaceutical industry and for purity control of industrial chemical

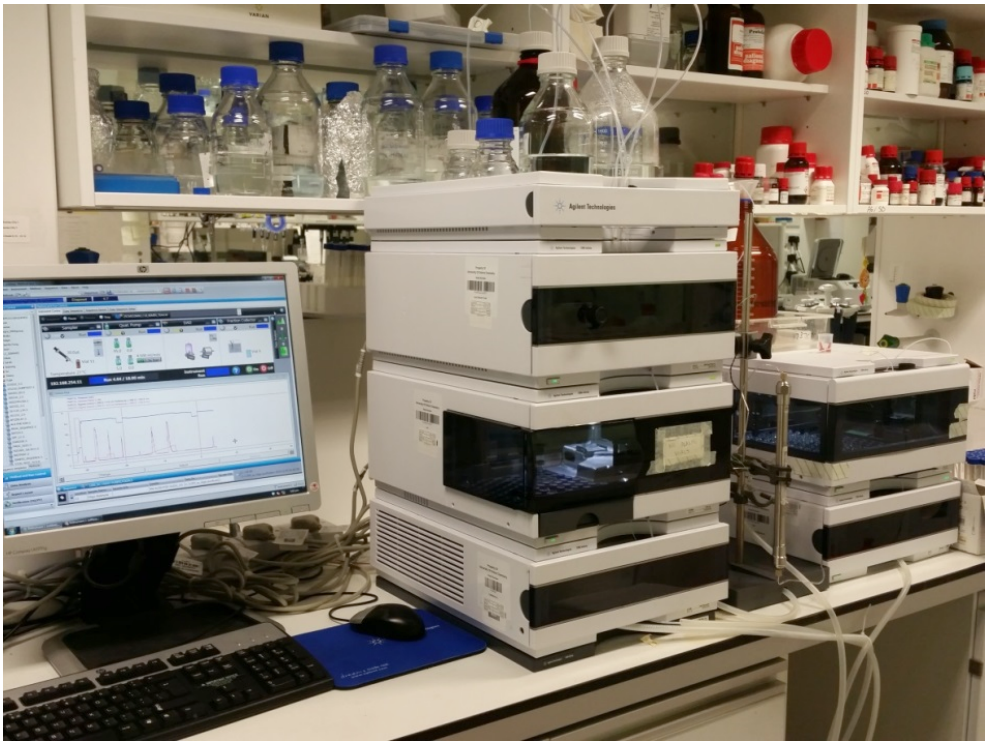

Figure 1. Picture of HPLC from Professor Hagan Bayley's group, Chemistry Research Lab, University of Oxford.

products. The need for better resolution and high-speed analyses of non-volatile samples led to the development of HPLC in the 1960s. HPLC was achieved by reducing the packing-particle diameter below 150 μm and by using pressure to increase the flow rate of the mobile phase (2).

Along with the advent of HPLC came a desire for reproducibility in the separation technique, which led to the first column-packing materials featuring standardised adsorption strengths. Particle sizes of the standardised adsorbent material were further reduced to about 10 μm and the particles were packed into pressure-stable columns operated at constant linear velocities. The first commercial HPLC was manufactured by Waters Corporation in 1969. Since then, incremental evolutions have generated an extremely powerful tool capable of high accuracy and precision. While instrumental developments such as improved pumps, gradient formers, valves, and detectors have all played a significant role in the advancement of HPLC, the evolution of particle technology and columns has been central. After the introduction of porous layer particles, there has been a steady trend toward reduced particle size to improve efficiency. However, decreasing particle size renders the preparation of uniformly-packed columns more challenging and makes it difficult to create the excessive pressure drop needed to force the mobile phase through the column. A result of this trend in reducing particle size and increasing pressure is Ultra Performance LC (UPLC), released in 2004 by Waters Corporation, which features increased resolution and run times up to ten times shorter than those of existing HPLC systems. The technique uses specially designed columns that contain particles as small as 1.7 μm in size and pressures that exceed 1000 bar. Standard HPLC typically uses column particles with sizes ranging from 3 to 5 μm and pressures of around 400 bar. The UPLC system is faster and more sensitive than traditional HPLC but suffers from quick column ageing and degradation due to the high pressure used (1, 2). In contrast, Fast Protein LC (FPLC) uses low pressures of up to 5 bar to allow the separation of sensitive proteins and other biomolecules. The most common FPLC method is ion exchange, in which proteins bind the solid phase by charge interactions in the presence of the initial solvent but can be displaced and eluted out using a second solvent (1).

Other advances in the history of HPLC include the development of chiral stationary phases in the 1980s that allowed for the separation of enantiomers and provided a cost-effective strategy for making enantiopure pharmaceuticals. Another significant milestone came in the 1990s when the separation method was coupled to a mass spectrometer (LC-MS), which acts as a high-sensitivity detector that provides information about the exact mass of the analyte(s). LC-MS is routinely used in pharmacokinetic studies of pharmaceuticals (3). In the last decade, miniaturised HPLC systems known as micro- and nano-LC have been produced to enhance the detection of target biomolecules in low concentrations. The systems feature narrow columns that require small amounts of precious samples, low solvent consumption (which decreases sample dilution), and provide the higher sensitivity needed to detect low-concentration analytes. The low flow rates of micro- and nano-LC systems make them ideal partners for MS (1). Nano LC coupled with tandem MS has become routine in the field of proteomics, where the analysis of complex samples with a range of component concentrations is necessary. Researchers are also exploring the clinical applications of LC. The technique can be used to selectively remove pathogenic compounds present in blood: the patient's blood or plasma is circulated extracorporeally over a packed column, which selectively binds and extracts endogenous and/or exogenous noxious compounds (1).

HPLC is an indispensable analytical tool in most labs and has been one of the defining separation techniques of the last 50 years. The technique boasts sensitivity and reproducibility, although the systems can be difficult to maintain and operation of newer HPLC versions is not always straightforward. LC methods cover the broadest range of applications imaginable today, and its range of uses will likely continue to expand. The largest area in need of further research is the field of life science chromatography, where dealing with unstable and sometimes transient target molecules present in an extremely wide range of concentrations poses a challenge to the available technology. Multidimensional LC systems that couple different separation techniques with online sample handling and detectors such as mass spectrometry are becoming increasingly common and offer the gain in sensitivity and selectivity that is fundamental for the analysis of complex samples. Multiple detectors per system may become standard, and computer-generated optimisation of HPLC conditions will advance along with computer technology. Regardless of the evolution and application of HPLC in the future, the technique will remain at the forefront of laboratory separation tools.

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FEATURED SEMINAR:

One for all and all for one - in pursuit of a broad-spectrum antiviral

by
Burcu Anil Kirmizitas

An OUBS seminar by Nicole Zitzmann

Nicole Zitzmann is the Head of the Antiviral Research Unit and Director of the Glycobiology Institute at the University of Oxford. Nicole's recent talk in the OUBS Michaelmas seminar series gave an interesting overview of her group's work, which focuses on the treatment of a variety of viral infections including dengue, Zika, Hepatitis B and HIV using broad-spectrum antivirals.

One of the most common methods of antiviral therapy is to target proteins that the virus uses to enter a host cell. A major problem with this approach is the high mutation rate of viruses, which often leads to structural changes in these proteins that can render them resistant to the drugs used. Researchers in the field believe that a way to combat this issue would be to instead focus on the host cells and the molecules within them that are necessary for the virus to survive and spread. In addition to eliminating the difficulties of working alongside viral mutations, this method could also enable a wide array of viruses to be inhibited with just one tool.

During her seminar, Nicole told us about her group's studies focusing on the endoplasmic reticulum (ER). The ER is equipped with various protein 'quality control' (QC) mechanisms that assist in the correct protein folding, placement of post-translational modifications and maturation of proteins for secretion. Enveloped viruses are highly dependent on a specific aspect of this QC machinery, protein glycosylation, for their infection and propagation. The virus surface glycoproteins rely on ER α-glucosidase II (α-GluII) as the main ER QC glycosyl hydrolase. This enzyme admits 'glycoproteins-to-be' into the ER QC and is responsible for some of the glycosylation steps and assisting in the protein folding that follows. It then releases the finished glycoproteins out of the ER when they are ready to be secreted (1). The importance of this enzyme in viral glycoprotein folding has made it an appealing therapeutic target; once inhibited, the enzyme starts to release misfolded proteins, which results in reduced virion infectivity.

Nicole's group has been working on designing derivatives of iminosugars to inhibit α-GluII. These 'glycomimetics' have proven to be effective against a range of viruses, with two of the known α-GluII inhibitors in clinical trials for dengue fever (2,3). In addition, iminosugars are already being used as inhibitors of other ER glycosylation enzymes in order to treat non-viral diseases. For instance, Miglitol and Miglustat are used in the treatment of diabetes and Gaucher's disease, respectively. However, this raises an important issue: if there are multiple enzymes in the ER that can be targeted by different iminosugars, how could Nicole's lab avoid targeting them with their iminosugar derivative intended to inhibit α-GluII? They believed a better understanding of the structure of α-GluII and its possible interactions with different iminosugars may help them design a novel iminosugar derivative with improved specificity. Although it proved difficult to crystallise, Nicole's group produced a series of α-GluII crystal structures, including the enzyme alone and in complex with various iminosugars (4). While the binding site of α-GluII did not look distinctively different than other iminosugar target enzymes, Nicole indicated that there is the possibility for designing new inhibitors using the structural information at hand.

Nicole's group is also gathering information on the other enzymes that work in the ER QC process. One of these enzymes is UDP-glucose:glycoprotein glucosyltransferase (UGGT), which is responsible for detecting, and subsequently correcting, misfolded proteins. Understanding how this protein can perform two distinct jobs will help in their efforts in targeting the ER QC machinery for the treatment of viral infections. Given that the group now have a crystal structure for this large 7-domain protein, we are eager to see their next set of results, which will no doubt be as exciting and interesting as the ones presented in this OUBS seminar.

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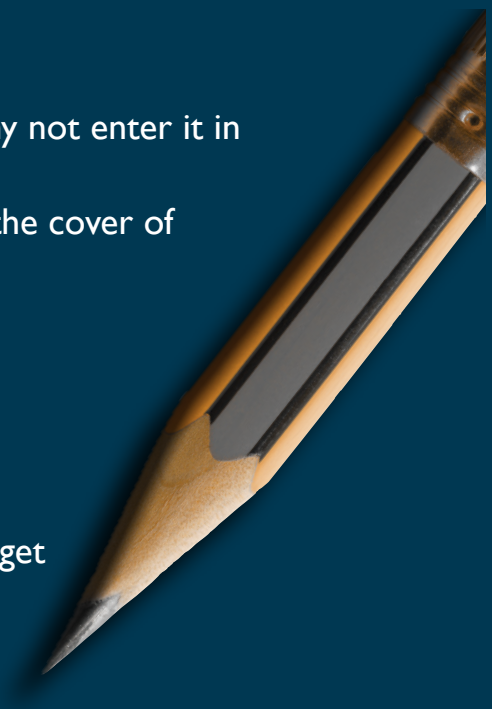

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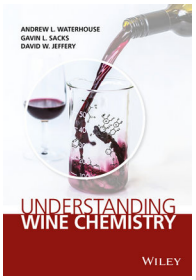
BOOK REVIEWS

Understanding Wine Chemistry

Andrew L. Waterhouse, Gavin L. Sacks, David W. Jeffery
ISBN: 978-1-118-62780-8 Wiley Blackwell (2016)
470 pages: Hardback £85/ eBook £76.99
Reviewed by Cristina Marculescu



Wine has been part of our civilization for over 6 millennia, as suggested by archaeological evidence of wine production found in Iran (c.5000 BC), Greece (c.4500 BC) and Armenia (c.4100 BC). But how much do we know about the true complexity of this drink? And where does this complexity come from? Can we control it or is it all magic? These are just some of the questions that the book, 'Understanding Wine Chemistry', is trying to answer.

Wine is a mixture of hundreds of different molecules in a constant state of flux, which means that it is a living, breathing thing. The composition of wine, both in term of compounds but also their concentration, at any given moment is reliant on endless factors such as the climate/soil where the grapes were grown, how production was carried out, storage conditions and last but not least, how long the wine was exposed to air before being savoured.

The three authors of the book, all experts in wine science, firstly take the reader step by step through the wine components and their reactions, followed by the chemistry of wine production. They then finish with a review on how modern analytical techniques have allowed recent advances in wine chemistry.

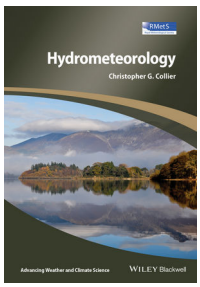
The best way to describe this book is as the most up-to-date encyclopaedia of wine. It contains a great deal of information but a large portion of it is presented in graphical form, allowing the reader to go through it easily and without prior knowledge.

The final section of the book contains information about current and emerging techniques for the detection of fraudulent wines. Additionally, it gives a review on the current understanding of the sources of reduced aromas that occur at times during wine storage, as well as optimising white wine aromas.

So, whether you are a wine producer, scientist or just wine enthusiast, sit back, relax and enjoy the read - maybe even whilst drinking some of your favourite wine.

Hydrometeorology

Christopher G. Collier
ISBN: 978-1-118-41498-9 Wiley Blackwell (2016)
376 pages: Hardback, £100 / Paperback, £47.50
Reviewed by Vasiliki Economopoulos



In today's world, with more frequent and extreme weather events such as flooding and severe storms that affect many people, climate change and weather forecasting has come to the forefront of many people's lives. Being able to provide accurate forecasts on significant weather events is essential to protect both people and property.

"Hydrometeorology", Collier's contribution to the series "Advancing Weather and Climate Science", provides the reader with an excellent reference on the subject. It includes mathematical details for many of the processes discussed, as well as problems for the reader to work through to check understanding.

Historically, hydrological and atmospheric sciences have remained fairly separate areas of study even though both sciences play heavily into meteorological modelling and now overlap greatly in practice. The separation of these fields leads to limitations in weather modelling, resulting in forecasts that are accurate for only a few days. In "Hydrometeorology", Collier brings these two areas of research together, allowing the reader to begin to understand these two areas as a single science.

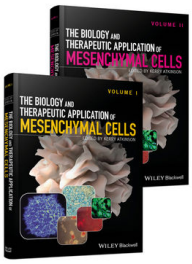
In this book, Collier provides in depth overview of the hydrological cycle and its implications in normal weather patterns as well as extreme weather events, such as thunderstorms, hurricanes, flooding, and drought. The author also gives detailed explanations of the physics involved in precipitation, evaporation and transpiration, snow and ice formation, and their specific meteorological impacts. He goes on to discuss current measurement techniques: from rain gauges and radar systems, to satellite based sensing systems.

Collier details forecasting models for precipitation, inland flooding, and coastal flooding. The reader has the opportunity to learn about the specific model requirements and the advantages and limitations of each different model type. Finally, the author discusses drought, global circulation, and climate change in the context of hydrometeorology.

Overall, this book is excellent for those studying meteorology or those with a strong physics background who are interested in learning more about different weather processes and how these are modelled.

The Biology and Therapeutic Application of Mesenchymal Cells - Set

Kerry Atkinson (Editor)
ISBN: 978-1-118-90751-1 Wiley Blackwell (2017)
1048 pages: Hardback, £400
Reviewed by James R. O. Eaton



The seminal studies of Friedenstein and coworkers in the 1960s and 1970s laid the foundations of the mesenchymal cell field. Since then this area of biology and medicine has grown exponentially and many studies have been conducted on these cells *in vitro*, *in vivo* and even in the clinic. Kerry Atkinson's book "The Biology and Therapeutic Application of Mesenchymal Cells, volumes I and II" compiles several decades of work into a clear and concise text that focuses extensively on our present understanding of mesenchymal cells and where the field may go.

The book is divided into eight sections, which are each split into several chapters, and begins by covering the in vitro research of mesenchymal stem cells before detailing more clinical applications, ensuring that upon completion the reader will be well versed in all aspects of mesenchymal cell research.

The author introduces the subject with clear definitions of mesenchymal stem and stromal cells, as well as their nomenclature, followed by a detailed explanation of the cells' isolation, cultivation and characterisation. The molecular and cellular biology of mesenchymal stem cells are discussed extensively, with several topics covered, ranging from the epigenetic regulation of these cells to their use as treatments for lung disease, asthma and allergic rhinitis.

The book then seamlessly shifts to a more clinical stance and covers the bioengineering and GMP production of mesenchymal stem cells before explaining their therapeutic applications, making this book suitable for those who wish to learn more about using mesenchymal stem cells in an industrial setting. The book ends with a chapter looking to the future, covering the most recent developments and challenges that will be faced in the mesenchymal stem cell field.

Each chapter is fairly self-contained, so this book can also be used as a quick reference tool. It would suit someone who has a good basic understanding of stem cell biology and wants to learn more about the recent developments in our understanding of mesenchymal stem cell biology and therapy.

Insight into founding a start-up company

EduChroma:
Pioneering personalized career mentoring

by
Sundeep Teki

EduChroma is a career mentoring start-up for students, early-career researchers and working professionals, launched in 2016. It empowers clients to realise their career aspirations through strategic advice and personalised mentoring. In order to fulfil this goal, EduChroma is building an online community of expert mentors who have significant experience in various disciplines and can provide personalised guidance and career advice to mentees in the same field as them.

Motivation

The idea behind EduChroma was conceived in 2008 just after I completed my Master's in Neuroscience at Oxford University. My year at Oxford confirmed my interest in the subject, and I decided to pursue a research career in neuroscience. However, with a background in Electronics engineering, I was uncertain about pursuing a PhD, whether I was ready for it, and whether I needed more research experience before applying. I therefore applied everywhere funded PhD positions were advertised, which in retrospect is not an advisable strategy. Not surprisingly, all (twenty) applications were unanimously rejected! Although I was surrounded by top neuroscientists at Oxford, I did not know who I could turn to for advice on simple matters like how to make a decent PhD application. I realised that whilst there was no dearth of research experts at a global learning centre like Oxford, there was a lack of guided career mentoring for candidates like me. Furthermore, this problem is endemic in academia and centrally administered career services only provide generic advice without considering the highly personalised background of each candidate, and their skills and experience.

Fast forward to 2015, now as a Wellcome Trust Postdoctoral Fellow at Oxford University, I became acutely aware of the lack of appropriate mentoring opportunities for students and early career researchers, leading to immense uncertainty and anxiety about future career prospects. After a lot of thought on how to effectively address this problem, and developing our business strategy, EduChroma was launched in January 2016.

Services

Through EduChroma, students receive targeted advice on university and scholarship applications at both undergraduate and postgraduate levels; mentoring and research support during PhD and postdoctoral stints; advice for competitive grant applications; and most importantly, personalised advice on how to strategically plan and grow in one's career.

EduChroma has already helped several students and researchers from leading institutes like Oxford, Cambridge, UCL, Imperial College in the UK; UPMC and CNRS in France; Universities of Hamburg and Cologne in Germany; as well as premier institutes from Asia including the Indian Institute of Science and Indian Institute of Technology. EduChroma has also advised clients on highly competitive grant applications to global funding bodies like the Wellcome Trust and the European Research Council.

Vision

A start-up journey is full of challenges: it was difficult to pitch EduChroma as an entity distinct from ubiquitous, run-of-the-mill career 'counselling' companies that have no qualifications from, nor experience and knowledge of specific industries, such as academia, and employ outdated methodologies providing a nebulous and static view of one's career interests. A continuing challenge is to find highly motivated mentors with stellar credentials who possess essential personal traits like patience, perseverance and empathy, and have a genuine interest in helping the client succeed. EduChroma's long-term vision is to build a dedicated team of in-house mentors who share its values; scale up our services using appropriate technologies; secure external funding and sponsorship to achieve our mission; and most importantly, raise awareness that it is absolutely imperative to support the next generations in their professional and personal lives.

To consult EduChroma, visit http://educhroma.com/

Sundeep Teki is a Sir Henry Wellcome Postdoctoral Fellow in the Auditory Neuroscience Group, DPAG and is the Founder of EduChroma

Science communication – to be embraced or ignored?

by Rosemary Wilson

What are your thoughts about science communication? As someone studying or working in science, do you see it as something to be done as little as possible, and then only as a CV box ticking exercise, or instead as something interesting and valuable, both to you and your audience? I confess I used to be in the former camp, but now, after actually putting science communication into practice, I am definitely in the latter.

Let me backtrack… What is science communication? It has been defined as "the use of appropriate skills, media, activities, and dialogue to produce one or more of the following personal responses to science (the AEIOU analogy): Awareness, Enjoyment, Interest, Opinion-forming, and Understanding" (1). However, definitions of what comprises science communication vary somewhat. For example, the BBSRC chooses to distinguish between science communication and public engagement, viewing science communication as "primarily a one-way communication process… it aims to inform, enthuse or inspire" compared to the more two-way discussion of public engagement (2). In fact, in recent years, science communication has developed into a profession, and a number of science graduates go on to find jobs in this sector. However, what does it really mean for someone who currently works in science research?

Science communication can take the form of a press release, a science blog, tweets, a public engagement event, a public lecture, or making something science related from cardboard and glitter with under-5s. But a key feature is making it audience appropriate. Incorrectly assessing my audience is something that has tripped me up several times!

During my PhD at the University of York and later as a postdoc at the University of Oxford I have ended up doing quite a variety of things that could be considered science communication. Mostly they have been opportunities that arose that I thought would be interesting, rather than activities undertaken with a specific plan in mind. Nevertheless, whether you chose to actively seek out opportunities or not, I guarantee that you are involved in some way, even if it's only talking to your friends or family when they ask about your course or job.

What not to do in science communication!

To highlight some things not to do, I want to pick out two events that I got involved with whilst at York, both communicating science to adults. One was a six-week evening course that I designed and ran, covering some of the biology underlying diseases. The other was an evening visit for a local society, which I helped organise and run as part of a group.

The evening course was the first time I had actively gotten involved in science communication. An email went around asking for people interested in running courses and I thought it sounded interesting. (As an aside, my supervisor was not particularly pro-public engagement, but didn't mind me doing extra things as long as it didn't affect my lab work.) Naively, I approached the course like undergraduate lectures and prepared PowerPoint slides accordingly. Thank goodness I included some videos and time for discussion. Looking back now, being further removed from lectures and at least aware that different teaching strategies exist, I would make the classes much more interactive. Nevertheless, my attendees kept coming back and even said nice things in the review at the end of the course. However, my biggest lesson was when one person pointed out that "protein is jargon". Unfortunately, it is all too easy to assume knowledge that to you may seem basic. I'm very thankful that he spoke up as it had not occurred to me that anyone would consider something like the word 'protein' to be jargon. This has stuck with me, and I think about it every time I talk about science with non-specialists. I have also often found the following quote, of unknown origin, to be useful: "assume maximum intelligence and minimal knowledge". Interestingly, these two ways in which my first foray into science communication did not go amazingly well both relate to leaning too heavily on my own prior experiences rather than trying to put myself in the audience's shoes.

> *"My biggest lesson was when one person pointed out that 'protein is jargon'."*

In contrast, an approach that I found worked really well was dedicating one week to diseases that were suggested by attendees. One man was interested in learning more about biology in general, as he had two children affected by Fanconi anaemia. This is a serious genetic disorder, causing bone marrow failure and an increased risk of leukaemia. Before starting this course I was terrified that someone was going to tell me that they had a sick relative and expect medical advice. In hindsight, no one was likely to ask advice from a PhD student who knew nothing about treating a patient. However, this instance of a family affected by a terrible disease actually turned into the most successful part of the course, as we were discussing something that the man had a genuine prior interest in, and it highlighted to the others the direct relevance of biology.

At the second event I mention - the society visit - there were some talks followed by lab tours, with these being the most successful part. People were interested in seeing the labs but were also really keen to chat. Understandably, they asked questions about things they were interested in, rather than the things I thought I should be telling them. Helpfully, their

prior level of knowledge usually became apparent from their questions.

Likewise, when our lab group went to the Natural History Museum for their recent Science Uncovered evening, conversations went best when, after my little intro spiel, the person asked a question relating to the area but also about something they were particularly interested in. For example, a man with a genetic disorder asked about how mutations occurred and how they were inherited. Another, an artist influenced by science, asked about epigenetics and epigenetic inheritance. One boy of about nine sang a song he had learnt about DNA base pairing, after which we talked about what would happen if a base was damaged. It was much more successful to be led by the things people were already interested in, and either focussing on those or using those questions to draw in other aspects of our research. Perhaps my 'revelations' sound obvious but they have helped my greatly.

Whilst I hope these events have benefitted those who attended, they have also benefitted me. I haven't had brilliant ideas about my work from these events but I have often seen a new perspective on science and how it sits within our society. I think having one toe outside the 'ivory tower' is incredibly important for appreciating how people see and relate to science. In addition, science in general is currently facing many challenges and it is important for the future that we justify our work (and ultimately jobs) to show its importance and relevance to those who, even indirectly, provide funding and support. But more than that, I believe the study of science is a valuable thing, not just for the often unexpected advances this can lead to, but also for simply learning more about the beautiful complexities of our world. Engaging with people, being there to answer questions, and talking a little bit about what we do is part of our responsibility and also allows people to see the joys of science. Why would we want to keep them a secret?

"They asked questions about things they were interested in, rather than the things I thought I should be telling them."

More personally, I also find that talking to people about science in these kinds of situations is fun, makes me forget that assay I have spent weeks troubleshooting, and instead helps me take a step back and remember that science is fascinating and that I am very lucky to be able to do this as a job.

Rosemary Wilson is a postdoctoral researcher at the Wellcome Trust Centre of Human Genetics

References

1. Burns T W, et al. (2003) Science communication: a contemporary definition. Public Understanding of Science 12(2):183¬–202.
2. BBSRC Public Engagement Training The Handbook. Available at http://www.bbsrc.ac.uk/documents/pe-training-handbook-pdf/ [Accessed 28th November 2016].

5' with... Dr Sebastian Nijman

by Chandan Seth

Dr. Nijman is a Principal investigator at Ludwig Institute for Cancer Research in Oxford, Director of Functional Genomics at the Target Discovery Institute and adjunct Principal Investigator at the Research Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences. He studied for his MSc and PhD in Medical Biology at the University of Utrecht and carried out post-doctoral research at the Broad Institute of MIT and Harvard.

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

I am not sure that I 'decided' to become a scientist. Ever since my childhood I have had an interest in knowing how things work. Nature and Biology have had a particular attraction. I was fascinated by the cartoon series "Il etait une fois…" (Once upon a time…) explaining in a graphical, mechanistic manner how human bodies worked. Later, I watched literally all nature documentaries on TV. Jacques Cousteau and David Attenborough are among my childhood heroes.

How did you get interested in working on genetic interactions and synthetic lethality (SL)?

During my PhD I read many papers about high throughput yeast genetics, including SL screens. I was enthralled by the systematic approach and that one could infer gene function by network analysis. Amazing stuff. The notion that SL could also be exploited for cancer therapy, as proposed by Stephen Friend and Lee Hartwell put two and two together.

What do think is the big challenge the scientific community is facing?

Perhaps the biggest threat to science is the constant push from politics and funders to work on projects that deliver 'value'. These days, basic biology in model organisms has become almost unfundable. This is a real problem in the long run as nearly all fundamental discoveries on which translational research is built stems from this type of curiosity driven, basic research. We should be more like Jacques Cousteau: just dive into the unknown and see what we find…

What do you like most about pharmacogenomics?

I like pharmacogenomics because it is rooted in fundamental science and systems biology, but at the same time there is a potential to impact human lives.

Where do you see the field of pharmacogenomics going in next five years?

"It is difficult to make predictions – especially about the future." (Niels Bohr)

What are you most proud of in your career so far?

I am proud that I have been able to inspire (at least some of) the people that have passed through my lab to continue a career in science.

What has been the biggest challenge you have faced during your career?

Dealing with disappointment is a constant challenge. Even when things are going well, most things you try and work very hard for fail. Experiments more often fail than yield insightful results, most of the grants that I have written were not funded; rejection letters outnumber publications. You need to be a bit of a masochist to do this job and cherish the moments when you do succeed.

What advice would you give to your junior researchers in your field?

Associate yourself with the best people you can find. Don't get comfortable. Don't accept mediocrity.

Who has inspired you the most during your career?

I have (had) many inspiring mentors. In fact, one should strive towards only working with people that inspire you

What scientific discovery in the last five years has impressed you the most?

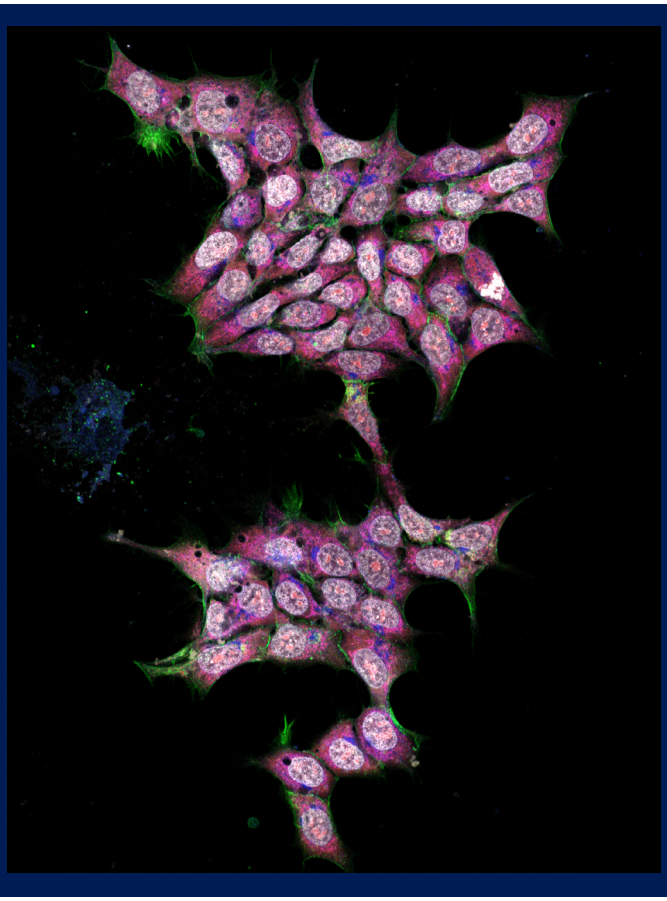
I am intrigued by the notion that the microorganisms we carry with us have a much more profound influence on our biology than we ever thought possible. I don't think anybody saw that coming.

If not science, which field would you have been in?

A carpenter perhaps – just like my grandfather.

SNAPSHOT Research Image Competition

This issue's winner is...



Dr. Chandan Seth,
Ludwig Institute for Cancer Research

This issue's winner of our SNAPSHOT competition is Chandan Seth with the image titled *Dancing Bats*.

The image is of mammalian haploid cancer cells (Hap1), which have been stained for mitochondria using MitoHealth (red), actin cytoskeleton using Phalloidin Alexafluor 488 (green) and CellMask used to stain the cytoplasm (blue). The cell nuclei are depicted in white and were stained using DAPI. The image was acquired on a Zeiss 710 confocal microscope using a 63x objective with oil immersion and 0.6x zoom.

Dr. Chandan Seth is a postdoctoral fellow within Prof. Sebastian Nijman's group at the Target Discovery Institute & is a part of Ludwig Cancer Research. Dr. Seth's research is focused on using a multi-omics approach to learn more about tumour cell plasticity and heterogeneity at the single cell level.

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SNAPSHOT
Research Image Competition

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

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT**?
We are now accepting entries for pictures to be featured on the cover of the Trinity 2017 issue of *Phenotype*.

To enter, send images to heather.booth@st-annes.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

The deadline for the competition is Friday 10th March 2017.

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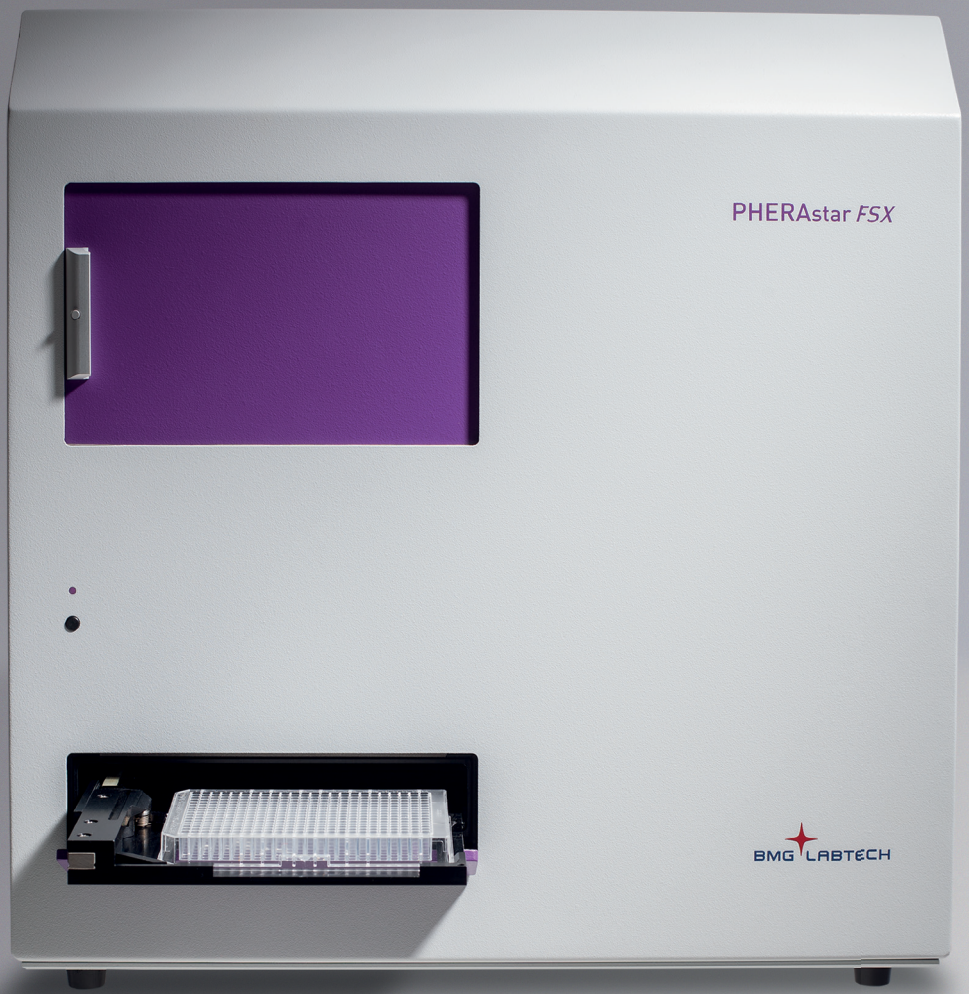

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PHENOTYPE crossword

Fish challenges you to this latest cryptic crossword! Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to heather.booth@st-annes.ox.ac.uk. Entries received before the 10th March 2017 will be entered into a prize draw to win one of the books reviewed in this issue.

The winner of the crossword competition will receive their choice of one of the books reviewed in this issue, kindly provided by

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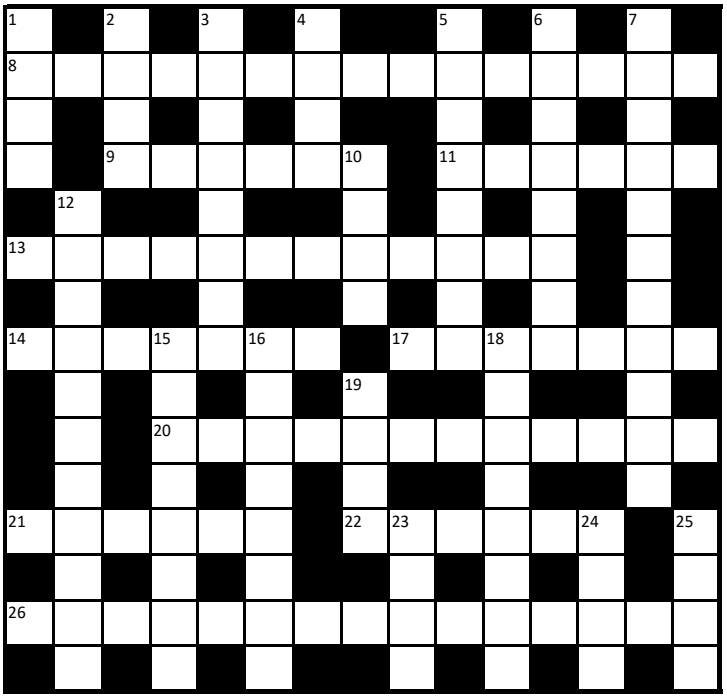

ACROSS

8. Distinguishing one thing from another is how **10 19**s develop (15)

9. These Arab citizens are, at heart, from an isle . . . (6)

11. . . . that is small, Anglican, endlessly nice and picturesque (6)

13. Exam on incorporating - without identification - steroid, drug and hormone (12)

14. Get bad round of applause when casual (7)

17. Collection of information is encoded in grid at a set frequency (7)

20. Compute inverse cubes while in servitude (12)

21. The Left supports news outlets who are towards the middle (6)

22. Hide under air mattress, say? (3,3)

26. Handling bedtime with difficulty in the Centre (4,2,3,6)

DOWN

1. Trim top of shrubbery along boundary (4)

2. Assuming that's the case, inhale mixture of fluorine, iodine, oxygen and sulfur (2,2)

3. Drunk Amaretto, causing a cancer derived from **10 19**s (8)

4. Joe is disembowelled, Jodi cut in half by Space Knight (4)

5. Exodus of people who spoke back to Parole Officer at the Royal Academy (8)

6. Alien will create, recreate, and so on (2,6)

7. To power, add titanium; it's a property of **10 19**s (11)

10,19. Science, technology, engineering and maths taught in small room - from which everything can grow? (4,4)

12. Confusingly, (real) news about elf is characteristic of **10 19**s

15. Meeting of political candidates with heads of Harvard Uni becomes very hurtful (8)

16. Daintily eating while using nickel, boron to make gaudy jewellery (8)

18. Stumble onto 50 + 0 + 1 + 500 = **19** with 3n, perhaps?

19. See **10**

23. Serve chilled, with frosting? (4)

24. Broaden work on drug swallowed while yawning (4,4)

25. See **24**

Answers to the crossword from Issue 25, Michaelmas 2016:

Across: **1**.Cuff **3**.Beta lactam **9**.An element of RISK **11**.Unison **12**.Abscess **15**.ATG **16**.Oregon **17**.Mere **19**.Inch **21**.Aikido **22**.Ski **24**.Tablets **25**.Agouti **28**.Confrontational **29**.Dummy texts **30**.Akin

Down: **1,26**.Clavulanic acid **2**.Fleming **4**.Even **5,13**.Antibiotic resistance **6**.Affect **7,27**.Make fast **8**.Aerosol **10**.Issue **13**.Resistance **14**.Penicillin **18**.Hodgkin **20**.Chain **22**.Sputnik **23**.Florey **26**.Acid **27**.Fast