PHENOTYPE

Issue 30 | Trinity Term 2018

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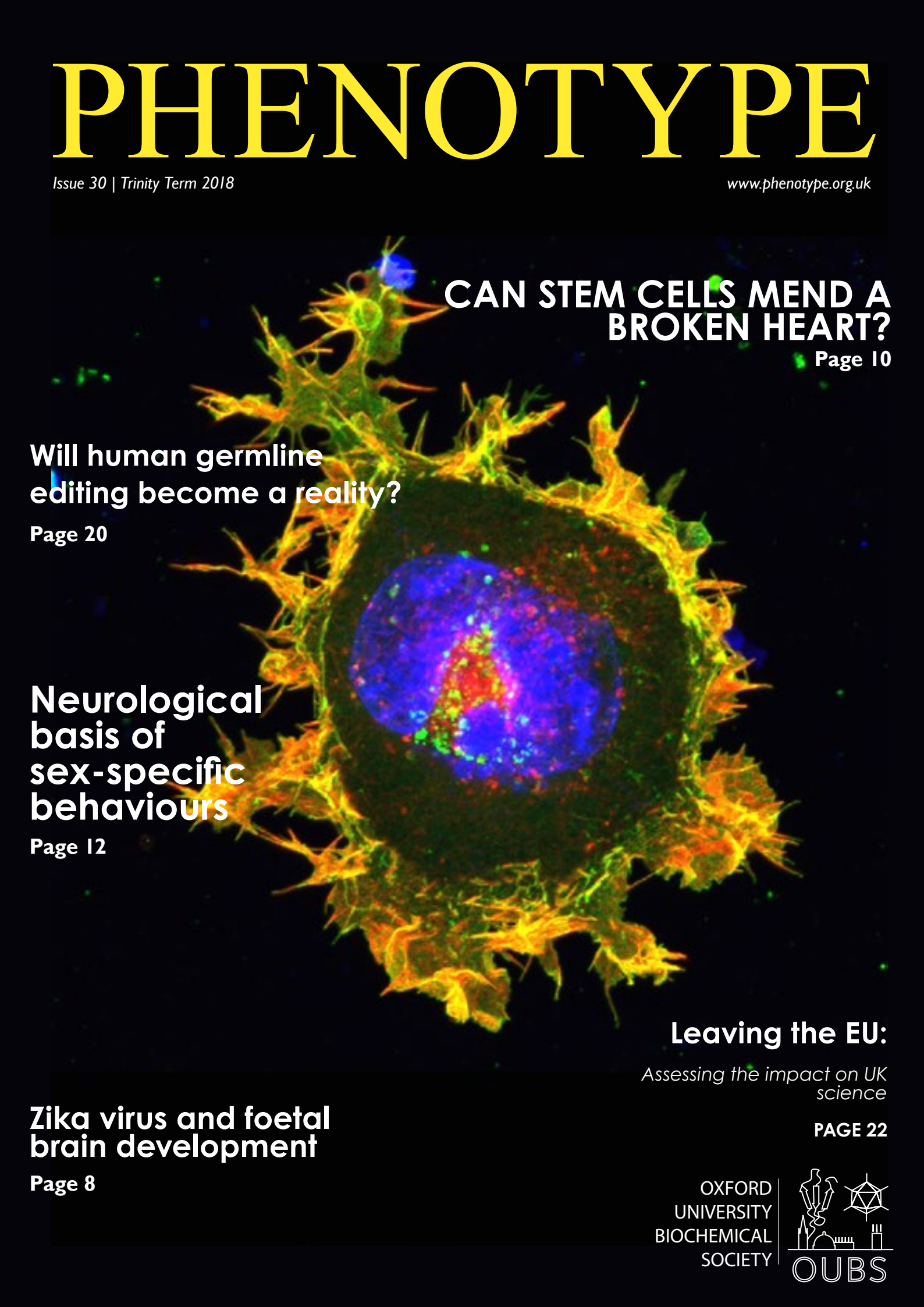


CAN STEM CELLS MEND A BROKEN HEART?

Page 10

Will human germline editing become a reality?

Page 20

Neurological basis of sex-specific behaviours

Page 12

Leaving the EU:

Assessing the impact on UK science

PAGE 22

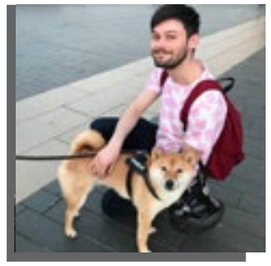
Zika virus and foetal brain development

Page 8

OXFORD UNIVERSITY BIOCHEMICAL SOCIETY

OUBS

LETTER FROM THE EDITOR



"Development has always been a science of syntheses and relationships, and these will be major themes for all sciences in the 21st century. Developmental biology will become a "biology without borders." The new developmental biology may be simultaneously molecular, ecological, evolutionary and physiological. I would be surprised if it were not." – Professor Scott Gilbert

Welcome to the 30th issue of Phenotype!

After many months growing in utero, we are excited to share the articles that our contributors and editors have so expertly developed.

As the above quote suggests, Development is relevant to every aspect of biology. You will find many examples of Developmental Biology's breadth and impact within this edition.

Turn to page 14 for an insightful article by Vincent Frontera and Emanuele Azzoni on dissecting the complex lineage map of blood formation, and read Abigail Wilson's discussion on the potential of stem cells to regenerate damaged cardiac tissue on page 10.

The brain is an enormously complex organ, and disruptions to its development have a devastating impact. Rohan Krajeski argues for the importance of establishing a reliable animal model of Primary Microcephaly on page 15, while Samuel Gerard tells us about how Zika virus affects fetal brain development on page 8. Carolina Rezaval talks about sexually dimorphic behaviours and what they can tell us about brain organisation and function on page 12.

On page 3, Professor Clive Wilson and his team investigate the role of exosomes as multifunctional intercellular signals in development, and their relevance further afield in reproduction, cancer, and neurodegeneration.

You can find our 5' interview on page 30, where Stefania Monterisi asks Professor Elena Seiradake about her cutting-edge research, career, and mentorship.

Moving on from animal life, Thomas Gate discusses on an exciting example of bacteria influencing the development of specialist tissues in plants, on page 17.

Aside from our Features articles, there is much to read in our Regulars and Science & Society sections. Patrick Inns considers the potential impacts of Brexit on UK science on page 22.

Don't forget to enter our SNAPSHOT Image competition! Read about our latest winner on page 2, and enter this term's competition for the chance to have your research image on the front cover of Phenotype, and to win a £50 voucher from Oxford University Press. On the back of this issue you will find our Developmental Biology themed crossword, for a bit of light entertainment.

I hope you enjoy this issue, and your Trinity term! If you are interested in getting involved with Phenotype, please contact us at oxphenotype@googlemail.com. We are always happy to expand our team, regardless of your experience.

Jack Cooper
Editor-in-Chief

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Issue 30 **Trinity 2018**

CONTENTS

FEATURES

SCIENCE & SOCIETY

REGULARS

ON THE COVER

"This is an image of a human embryonic kidney cell, which overexpresses epidermal growth factor (EGF) fused to a green fluorescent protein (EGFP). The cell has been stained for the EGF (green) and the actin cytoskeleton (red). The nucleus has been stained with DAPI (blue). The image has been acquired on an Olympus FV100 using a 63x objective with oil immersion.
EGF is a transmembrane protein, which is cleaved and released at the plasma membrane, generating soluble EGF. Upon binding to is receptor, EGF regulates cell proliferation, migration and differentiation."

Boris Sieber, DPhil Candidate, Sir William Dunn School of Pathology.

RESEARCH HIGHLIGHTS

Subset of early radial glial progenitors that contribute to the development of callosal neurons is absent from avian brain

by Alice Lightowlers

Fernando García-Moreno and Zoltán Molnár, PNAS, 2015

The corpus callosum, the largest white matter (nerve fibre) structure in the human brain, is the major route for neural connections between the cerebral hemispheres. This evolutionarily recent addition to the cortical structures is not present in the brains of birds and reptiles but is common to most mammals. In a recent study from the Molnár lab, they investigate a subpopulation of neurons present in both mice and chicks which they hypothesize contribute to the formation of the corpus callosum in mammals.

Radial glial cells (RGCs) are crucial progenitor cells in the developing nervous system. RGCs are multipotent and self-renew until they begin to participate in neurogenesis around embryonic day 12 (E12). Until recently, most of the evidence indicated a sequential model of RGC neurogenesis, where all RGCs contributed equally to the formation of both upper and lower layers of the cortex. However, new findings suggest that some subpopulations of RGCs in mice contribute only to the upper layers of the cortex, known as fate restriction. In this paper, the authors track a subpopulation of RGCs expressing Emx2, a key neuronal transcription factor, in the developing mouse neocortex and chick forebrain.

To achieve this, they used in utero electroporation to introduce a construct with green fluorescent protein (GFP) expressed under the Emx2 enhancers to the developing embryos. The neural development of the embryo was monitored from E12, to postnatal day 16 (P16). In mice, they found that GFP-labelled Emx2+ RGCs were delayed during the first stage of neurogenesis, as determined by their absence from the cortical plate, and later went on to produce upper layer callosal neurons. However, no such delay was found in the homologous Emx2+ subpopulation in the chick forebrain.

The group hypothesize that this delay in neurogenesis of a subpopulation of RGCs contributed to the evolution of the corpus callosum. This study provides a glimpse into the evolution of the mammalian brain and the development of

Recurrent Circuitry for Balancing Sleep Need and Sleep.

by Laura Garmendia Sanchez

Donlea *et al.* (2018) *Neuron* 97(2):378-389

Recent work in the Drosophila fruit fly has provided numerous insights into the neuronal circuitry used by the brain to generate sleep need and induce sleep. Activity in a small group of neurons projecting to the dorsal fan-shaped body (dFB) of the fly brain is known to be sufficient to induce sleep; as sleep pressure builds up during wake, these neurons switch from an electrically silent state to electrical excitability in order to promote sleep. But, what are the downstream circuit mechanisms used to regulate sleep based on the electrical excitability of the sleep-promoting dFB neurons?

To address this question, Donlea *et al.* (2018) investigated the signals released by the dFB neurons, their downstream targets, and how they modulate them to control sleep. Because expression of allatostatin-A (AstA) is found near the dFB neurons, the group hypothesised that AstA might be a signal released by the dFB neurons to induce sleep. This was confirmed by investigation of AstA mutant flies and knock-down of AstA in the dFB neurons, which decreased sleep and abolished the homeostatic response to sleep deprivation.

By studying the expression patterns of AstA receptors and the phenotype of AstA receptor mutants, a small group of large interneurons, termed 'Helicon cells', was identified as a target of the dFB sleep-promoting neurons. Specifically, activation of dFB neurons and subsequent AstA signaling was found to inhibit the Helicon cells. Helicon cells were found to play a permissive role in visually guided movement, and to promote activity when depolarized. In addition, activity of Helicon cells induced rebound sleep, and they were found to excite R2 neurons of the ellipsoid body, a principal source of sleep pressure to the dFB.

Overall, this study identifies an autoregulatory loop whereby dFB neuronal activity, induced by sleep pressure, shuts down the neurons promoting activity and buildup of sleep need. The discovery of these Helicon cells provides an explanation of how activity of only a handful of neurons suffices to induce sleep in flies, as well as increasing our understanding of the intrinsic circuit mechanisms used to set the balance between sleep and sleep need.

Exosomes in Development and Disease: From Waste Disposal to Multifunctional Signal

by Clive Wilson

Clive Wilson is Professor of Cell and Developmental Genetics at the Department of Physiology, Anatomy and Genetics.

Development of all complex organisms relies on an elaborate series of cell-cell communication events, intricately orchestrated in time, which ensure that different cells, tissues and organs grow and differentiate in their appropriate positions. Extracellular protein and peptide signals are known to be major players in these processes, activating membrane-bound receptors on target cells. But secreted nano-vesicles called exosomes (1,2) have recently started to emerge as more complex signalling mediators, whose functions extend far beyond developmental biology to immunity, reproduction, endocrinology, viral biogenesis, neurodegenerative disorders and cancer.

Signalling in development

From the early 1980s onwards, studies primarily initiated in the fruit fly, Drosophila melanogaster, started to reveal that many developmental events are controlled by a small group of highly conserved, 'diffusible' extracellular signalling molecules, like those in the Hedgehog, Wnt and Transforming Growth Factor-beta families. Further characterisation of these signals revealed that generally they are not isolated soluble molecules; indeed, Hedgehog and Wnt ligands carry lipid anchors that are stabilised in membrane bilayers. This might seem a surprising strategy for an intercellular signal, but it allows these signals to hitch a ride by binding to hydrophobic molecules and structures, like exosomes.

The emergence of exosomes as multifunctional intercellular signals

Eukaryotic cells contain a complex network of intracellular secretory and endocytic compartments surrounded by membranes (Figure 1). The secretory system, including the rough ER and Golgi, traffics signals out of the cell, simultaneously expanding the plasma membrane as each compartment fuses to the cell surface. Endocytosis redresses the balance. Endocytic trafficking bifurcates, so some molecules are recycled back to the cell surface, while others end up in the late endosomes and lysosomes, where their constituent building blocks can be extracted and exported back into the cytosol. Part of that process involves the inward budding of the late endosomal limiting membrane into intraluminal vesicles (ILVs) to form multivesicular bodies (MVBs).

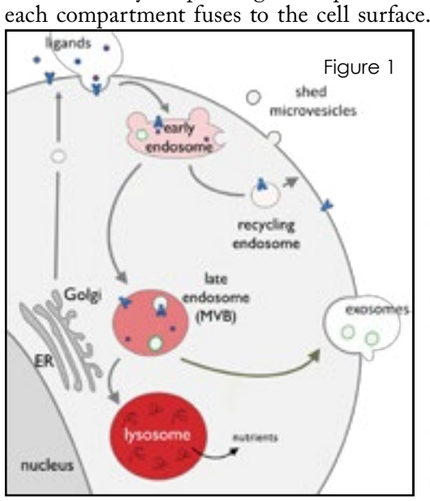

Figure 1. Secretory and endosomal trafficking. Current models suggest exosomes are secreted from late endosomal multivesicular bodies.

In the 1980s, it was discovered that in maturing red blood cells, these MVBs could fuse with the plasma membrane, releasing their vesicles into the extracellular fluid as exosomes of between 30 and 150 nm in diameter. At the time, it was postulated that this provided a mechanism to dispose of unnecessary membrane proteins, such as the transferrin receptor that is used during differentiation to recruit iron for haemoglobin synthesis. But over the years, it has become increasingly apparent that exosomes carry numerous bioactive receptors and ligands at their surface, and internally, they house intracellular signalling molecules and nucleic acids, particularly mRNAs and microRNAs that can modulate the expression of multiple target transcripts (Figure 2). Preparations of extracellular vesicles containing exosomes have been shown to reprogramme fundamental properties of cells to which they are added, such as growth, migration and differentiation, and to mediate signalling by most of the major developmental ligands. They can signal at the cell surface, or release their cytosolic contents into the cell by fusion with the plasma membrane or with endosomal membranes following endocytosis. So just how important are exosomes?

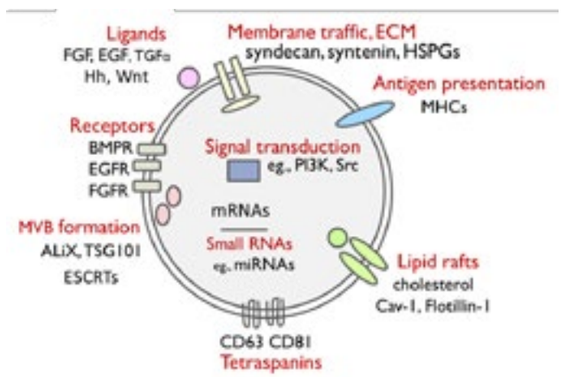

Figure 2. The complex cargos of exosomes. Ligands, receptors, intracellular signalling molecules and RNAs are all routinely found in these structures.

The problem(s) with exosomes

Exosome research is one of the minefields of modern bioscience (1). One central problem is that even the very best isolation methods enrich for all kinds of small extracellular vesicle (sEV), most notably small microvesicles that have budded off the plasma membrane directly. Exosomes appear to carry more specific cargos at their surface than these microvesicles. These cargos can be used as tags to pull down exosomes with antibodies, enriching for specific exosome subtypes, but once isolated, it is difficult to recover them in intact form after pull-down to perform assays for biological activity.

A classic approach to test the function of a biological process is to specifically inhibit it. But the genetic tools available to do this for exosomes are extremely blunt: blocking components of the Endosomal Sorting Complex Required for Transport (ESCRT) complex, which co-ordinates the formation of ILVs, also has effects on the maturation of endosomes and is involved in other cellular events, such as cytokinesis. Knocking down specific Rabs, small GTPases that mediate distinct trafficking pathways in the cell, can block exosome secretion, but also simultaneously affects other secretory processes mediated by MVBs.

Furthermore, in assessing the functional activities of exosomes, a major issue is deciding how many exosomes to add and how to apply them, whether the experiments are performed in vitro or in vivo. The best studies in the field typically report at least three key observations: (i) a function attributed to sEV preparations; (ii) an exosome protein or miRNA inside the exosome that plays a key role in this function, and (iii) the ability to block the signalling by inhibiting specific ESCRTs or Rabs. However, many reports do not employ all of these approaches and the key experiments are rarely reproduced in vivo, making it difficult to assess the full significance of these studies.

Exosomes in cancer

Problems aside, there is now a large number of publications that claim important roles for exosomes in cancer (3). For instance, circulating exosomes from the primary tumour are reported to reprogramme distant cells so that they form a pre-metastatic niche, which provides a home for migrating cancer cells in metastasis. The exchange of exosomes between cancer cells and the surrounding 'normal' stroma is thought to affect both stromal and tumour cells, promoting the adaptation of the tumour to different microenvironments. These changes are central features of tumour progression, so it is little wonder that interest in cancer exosomes, how to detect them in clinical tests, and how to inhibit them, all represent priority areas for oncological research.

The cellular targeting of exosomes is another area of active interest, particularly since it might pave the way for using exosomes in the delivery of bioactive disease-suppressing molecules or drugs. In this regard, elegant in vivo studies showed transfer of the mRNA encoding the DNA recombinase Cre in extracellular vesicles from cancer cells, providing proof-of-principle evidence that targeting can be detected with loxP reporter constructs. This approach could be combined with genetic screening methods to unravel the processes involved (4).

"in cancer, loss of cell polarity could lead to secretion of this vesicular weaponry into the bloodstream of a patient"

Exosomes in development

Studies using some of the approaches discussed previously have already indicated that the range and effects of developmental signals like Hedgehog can be dependent on exosome packaging (2). Our interest in this form of signalling emerged when Laura Corrigan, a DPhil student in our group, discovered that prostate-like cells, called secondary cells, in the accessory gland of the male fruit fly secrete exosomes into seminal fluid, which are transferred to females upon mating (5). Like human prostate exosomes, they fuse to sperm when combined after mating, and this may affect sperm function. But more spectacularly, they appear to be involved in reprogramming of the female's brain, so that she rejects other male suitors and continues to use the sperm she has stored from the first mating to generate new progeny.

The idea that exosomes might be used for inter-organism communication is indeed appealing. It may allow an animal to completely override the cellular signalling systems in another animals, promoting its own interests (as in the secondary cell example of sexual conflict) or reprogramming neonatal physiology (as in breast milk). It is intriguing that in cancer, loss of cell polarity could lead to secretion of this vesicular weaponry into the bloodstream of a patient.

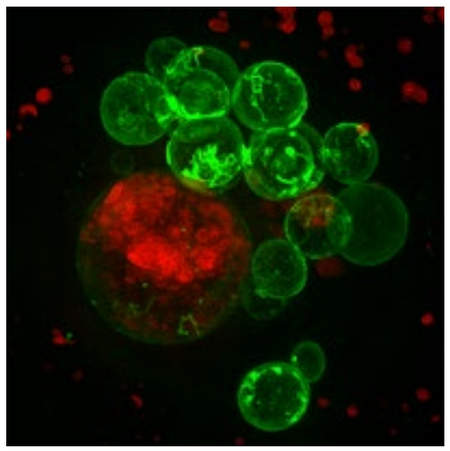

Figure 3. Super-resolution 3D-SIM reconstruction of a single Drosophila melanogaster secondary cell. The large intracellular compartments are labelled by a GFP-tagged version of the human tetraspanin and exosome marker CD63, which highlights complex membranous structures inside each compartment. One large acidic compartment is labelled by the dye LysoTracker Red. At the same scale, secretory and endosomal compartments in most other cells would appear as an unresolved dot (image from B. Kroeger).

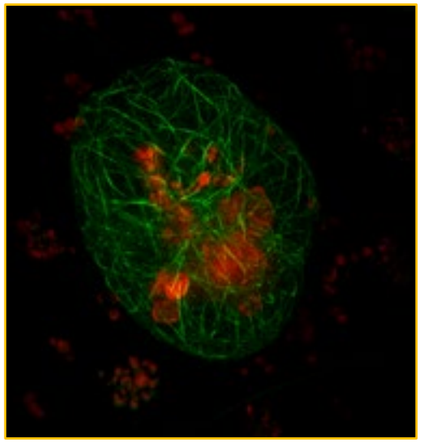

Figure 4. Super-resolution 3D-SIM reconstruction of a single Drosophila melanogaster secondary cell. The microtubule cytoskeleton is marked with a GFP-tagged binding protein and acididc compartments are labelled with LysoTracker Red. The large secretory compartments are enveloped in a microtubule cage through which they must pass during secretion (image from B. Kroeger).

Back to basics – how do you make an exosome?

Drosophila secondary cells have another remarkable property that has transformed the direction of our group's research in a profound way. Their secretory and endosomal compartments are extremely large, with a volume perhaps 10,000 times greater than other fly or human cells (5). Yet, the basic mechanisms that regulate trafficking and secretion via these compartments seem very similar to other secretory cells in higher organisms. Exosome biogenesis can normally only be visualised using electron microscopy because the size of endosomal compartments is near the limit of resolution for fluorescence microscopy. In secondary cells, imaging can be undertaken in living tissue with confocal, wide-field (6) and super-resolution (3D-SIM) microscopy, employing the diverse range of fluorescent protein markers available in flies (Figures 3 and 4). This work, developed by Ben Kroeger in my group, has been greatly facilitated by Ian Dobbie and his colleagues at the Wellcome Trust-funded MICRON facility in the Science Area.

An added bonus for our studies is that in the first few days after a male emerges from its pupal case, it goes through a form of puberty, during which it becomes increasingly fertile. Over the same time period, the first large exosome-forming compartments are set up in secondary cells, allowing us to visualise the process by which an exosome-secreting cell develops. Capitalising on these features, we have started to unravel the biology of exosome biogenesis, collaborating with others in the Medical Sciences Division, most notably the groups of Deborah Goberdhan (DPAG), Adrian Harris (WIMM) and Freddie Hamdy (NDS), searching for evolutionary conservation in the mechanisms we identify.

Our most recent unpublished work reveals that exosomes are not only made in late endosomes, but also in other compartments in the cell, and each of these compartments generates different exosome subtypes. The same mechanisms are present in human cancer cells, where these different exosome subtypes appear to be involved in adaptive responses and tumour progression. Proteomics analysis of these human exosome subtypes has already revealed putative subtype-specific regulators that we are now testing in both systems. One way in which this work might translate quite rapidly into the clinic is if subtype-specific combinations of tumour exosome markers can be identified and used to screen serum samples for responses to drugs or as a diagnostic/prognostic indicator in patients.

Intraluminal vesicles, exosomes and disease

As we zero in on the inner workings of exosome-forming compartments and genetically manipulate cells to inhibit exosome biogenesis, it is becoming increasingly clear that such manipulations can have major effects on the compartments themselves and their functions. We observe changes in secretory biology that we believe are relevant to other endocrine and exocrine cells, as well as a major disruption in the mechanisms by which late endosomes and lysosomes are acidified. This highlights the potential importance of intraluminal vesicles, even before they are secreted as exosomes, while also adding to a growing awareness that exosomes could be new players in both intracellular and extracellular signalling. Tantalisingly, ILVs have been implicated in the assembly of amyloid fibres associated with neurodegenerative disorders such as Alzheimer's disease (7), while ILV biogenesis pathways can be hijacked by viral packaging pathways or used in viral evasion from the immune system (8). Indeed there is a potential role for exosomes and EVs in almost all areas of developmental signalling and biomedicine. With a real need to combine basic and clinical science to understand and be able to exploit exosome biology, Oxford is well positioned to make contributions to this exciting and growing field, with annual Oxosome (Oxford Extracellular Vesicle Group) meetings held to discuss developments in this area.

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GENE DELIVERY SYSTEMS IN DEVELOPMENTAL BIOLOGY

by Veronika Hartleb

Veronika Hartleb is a visiting researcher in the Gene Medicine Research Group in the Radcliffe Department of Medicine.

Broadly speaking, developmental biology studies cell growth, differentiation and morphogenesis, which is the formation of organs and tissue (1). Research in developmental biology leads to advancements and improved understanding in many areas including normal development, cancer, birth defects, stem cells and their use in regenerative medicine, and the influence of the environment on development. For instance, birth defects are extremely common, affecting one in 33 newborns in the US (2). However, since human foetuses cannot be experimented on for ethical reasons, the advancement of research lies in non-human model organisms.

Developmental biology is frequently applied to cancer, as it can be regarded as a disease of altered development. Often the genes that are responsible for normal development such as those involved in cell signalling, cell cycle and cellular differentiation also promote cancer growth. For example, the Wnt and hedgehog genes, important in cancer, were first identified as being crucial for development in Drosophila melanogaster (3).
Findings from developmental biology can also be exploited to advance regenerative medicine. An interesting example of this is the salamander, which can regrow whole limbs; scientists are trying to understand the mechanisms involved in this process, in the hope of exploiting them for clinical purposes.

For all these applications, the delivery of exogenous genes to cells and embryonic model systems is crucial. This is often achieved by introducing a coding sequence of DNA under the control of either a ubiquitous promoter, such as cytomegalovirus (CMV) or human ubiquitin C (hUbiC), or a cell-specific promoter, such as human cytomegalovirus enhancer/elongation factor 1alpha (hCEFI). These promoters can be linked to fluorescent reporter constructs, such as eGFP, in order to study gene expression at the transcriptional level. This method is especially useful for studying the embryonic development and to unravel the molecular mechanisms that lead to disease even later in life. It can be carried out in vivo by targeting either germ or somatic cells.

There are two methods commonly used to deliver genes to cells and animal models: viral and non-viral. Viral methods e.g. lentiviruses, gamma-retroviruses or adeno-associated viruses, deliver specific genes to cells of interest under a viral promoter. They have the advantage of often leading to strong, long-term expression of genes, which is necessary to study a subject's development or tissue regeneration.
In salamanders, a viral gene delivery system based on foamy viruses shows long-term stable expression of genes in groups of cells capable of regeneration called blastemas, originally thought to be composed of stem cells but recently identified to include memory cells (4).

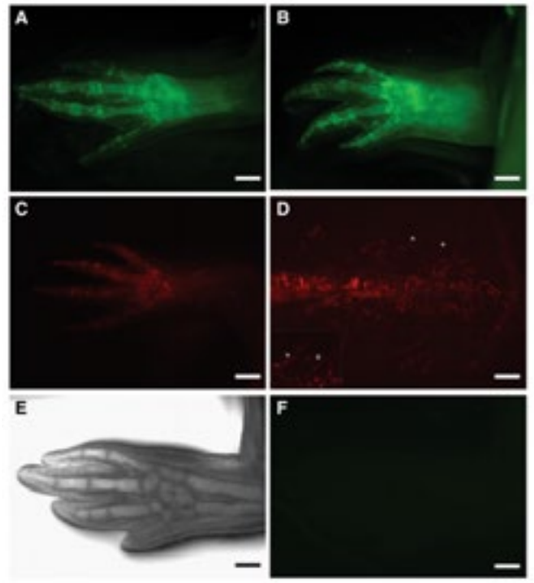

Figure 1. Salamander limbs transduced with foamy virus (FV) to demonstrate the difference between original limbs and regenerated limbs and the increased efficiency of FV over lentivirus (LV) for gene delivery. (A) Original limb injected with FV-eGFP under human ubiquitin C promoter (hUbiC). (B) Regenerated limb post injury, visualised using FV_hUbiC_eGFP. (C) and (D) Limb and tail injected with FV_hUbiC_DsRed. (E) Bright-field image of limb injected

Alternatively, microinjection is commonly used to study embryonic and neonatal development, such as in chick embryos. This simple but very effective method has been exploited to study many developmental defects, such as Hirschsprung's disease, cleft lip and/or palate and primary ciliary dyskinesia (5).

In summary, developmental biology is a broad field covering cell growth and differentiation and development of tissues and organs. It therefore also plays a role in cancer research, birth defects and regenerative medicine, often using viral vectors to deliver genes containing a fluorescent reporter. Alternatively, naked DNA can be injected into embryos of animal models to mirror these diseases.

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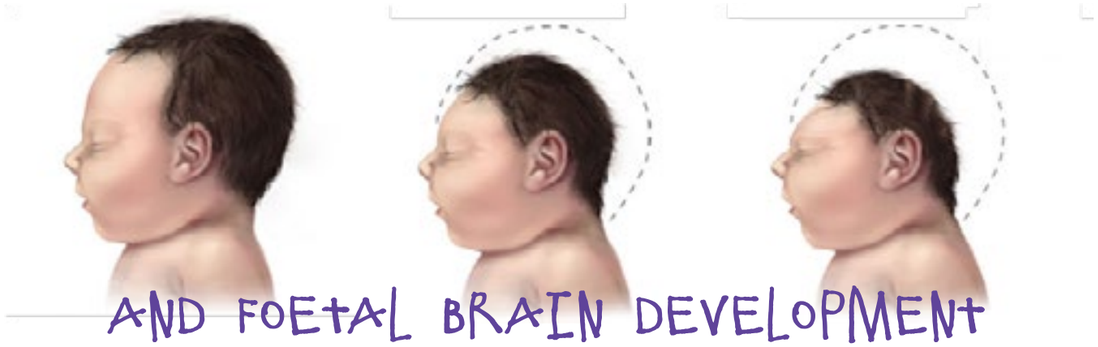
ZIKA VIRUS



AND FOETAL BRAIN DEVELOPMENT

by Samuel F. Gerard

Figure 1. Typical head size (left) vs microcephaly (middle) and severe microcephaly (right). The malformation of cortical development is characterized by a reduction in head circumference and brain size due to a reduced number of neurons or neural progenitors. Adapted from CDC website (1).

The recent outbreak of Zika virus (ZIKV) has been associated with a rising number of microcephaly cases in endemic countries, primarily in Brazil. Since the beginning of the crisis, researchers have been trying to determine whether the viral infection of pregnant women was in fact the causative agent of congenital brain disorders in newborns. In this short article, the history of ZIKV and recent significant advances that shed light on direct effects of the virus on foetal brain development are described.

ZIKV is a flavivirus related to the dengue and yellow fever viruses. It was first isolated in 1947 from an infected Rhesus monkey in the Zika forest of Uganda. In 1952, the virus was recovered from humans and found to be mainly transmitted by Aedes africanus mosquitoes. Over the next decades, ZIKV spread across Sub-Saharan Africa and South-East Asia, causing benign illnesses in humans, often characterised by mild fever. Until 2007, only 14 cases of ZIKV disease in humans had been documented globally. Yet, 2007 marked the first ZIKV outbreak in the Pacific Island of Yap, with more than 70% of residents infected by the virus. Likewise, from 2013 to 2014, ZIKV reappeared in the Pacific Islands and triggered epidemics with associated cases of Guillain-Barré syndrome, a rare neurological disorder. Furthermore, ZIKVs were detected in two mothers as well as in their newborns, highlighting possible transplacental transmission of the virus. In early 2015, as ZIKV spread more globally through Aedes mosquito vectors, a high number of infections were reported in Latin America with north-eastern states of Brazil reporting around 7000 cases of mild ZIKV-related disease. The same year, Brazil experienced a significant rise in birth defects, most notably microcephaly cases among newborns, characterised by anomalously small heads (Figure 1). By the end of 2015, Brazil estimated up to 1.3 million cases of ZIKV infection. Following its first emergence in the continent, ZIKV continued to disseminate throughout South and Central America, together with the Caribbean Islands. In the beginning of 2016, ZIKV was detected in amniotic fluid of foetuses diagnosed with microcephaly and impaired brain development. Subsequently, ZIKV RNA was found in the brain tissue of newborn babies and in miscarried foetuses from pregnant mothers suffering from mild ZIKV-related symptoms. Although over 80% of ZIKV infections in humans are asymptomatic, the ever-increasing number of ZIKV-related cases of microcephaly and neurological abnormalities prompted the World Health Organization to declare a Public Health Emergency of International Concern on 1st February 2016 (2). Since then, additional ZIKV-linked congenital disorders have been reported in dozens of countries and the virus has spread in over 70 countries and territories globally, including the United States.

Evidence of the association between ZIKV infection and congenital disorders emerged from case reports and epidemiological studies. Yet, these observations alone do not prove causality, as the viral effect on brain development might be indirect. In the past three years, several major biological studies have made significant headway in explaining how the virus directly affects a baby during its development in the womb.

To investigate this, the first major challenge was to model ZIKV pathogenesis to identify viral tropism (Figure 2). As live infected human foetal tissues are not accessible and because of the variability of post-mortem tissues, one way to address these questions was through the rapidly-advancing stem cell field.

To mimic the building blocks of the human cortex, cortical neural progenitor cells (NPCs) were derived from human induced pluripotent stem cells (iPSCs) in monolayer cultures. These NPCs are precursors for neurons and astrocytes, which are required for cortical development. Upon exposure to ZIKV, 90% of the cells were infected, many of which died, while others exhibited downregulation of genes controlling cell division and an increase in apoptosis-related gene expression. This first report of a human iPSC-based model of ZIKV infection constituted evidence of a plausible direct link between ZIKV and impaired brain development and, together with clinical studies, led the United States Centers for Disease Control (CDC) to declare that ZIKV causes microcephaly and other severe foetal brain disorders.

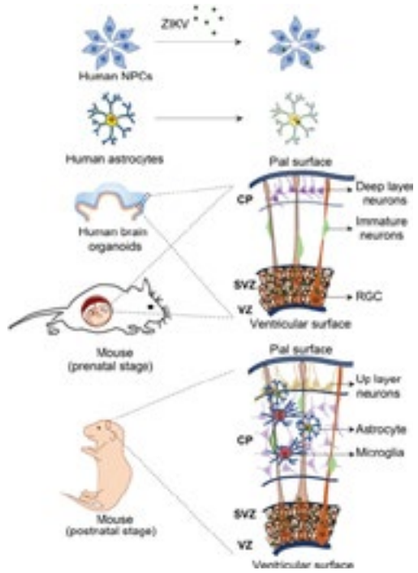


Figure 2. In vitro and in vivo ZIKV infection models. NPC and astrocyte monolayers, 3D brain organoids, and animal models, such as mouse models at multiple developmental stages, have led to a better understanding of the virus' impact on the developing brain (4).

To better study ZIKV infection during brain development, another system was required to model the various progenitor and neuronal layers of the developing cortex. This was achieved through the development of iPSC-derived tissues that self-assemble into an ordered structure with diverse cell types, similar to that of the foetal brain. This 3D mimic, or brain organoid, allowed the study of the relationship between ZIKV exposure and cortical layer thickness. In particular, this model was used to compare the infection efficiency of ZIKV for different cell types, layers and areas inside the complex organoid. In addition, brain organoids of different stages could mimic the development of the foetal brain during the first two trimesters of pregnancy and allow the determination of the outcome of short and long-term infection. Using this model, the pathological effects of ZIKV infection were described. Several groups showed that ZIKV infection reduced the growth of brain organoids. Most importantly, a study of ZIKV-exposed forebrain organoids at different stages of their development highlighted the preferential viral infection of NPCs, including radial glial cells (RGCs), compared to intermediate progenitors and immature neurons (3). Interestingly, outer RGCs are thought to direct primate and human cortical expansion. Upon infection, NPCs were shown to turn into virus factories, enhancing infection. NPC proliferation was abrogated and cell deaths of infected NPCs and non-infected neurons were enhanced. The resulting reduced thickness of the NPC and neuronal layers was similar to the microcephaly phenotype. These organoid studies were further supported by mouse and non-human primate animal models and the use of human foetal brain tissues.

Stem cell platforms, complemented with biochemical studies, have also contributed to uncovering the mechanisms of ZIKV pathogenesis at the protein level. For instance, human foetal brain tissue and single-cell RNA sequencing showed that a receptor protein called AXL was highly expressed in the developing foetal cortex, particularly in RGCs and brain capillaries. As ZIKV was previously shown to use the AXL receptor to infect skin cells, it was suggested that ZIKV used AXL to enter NPCs in the foetal brain too. However, ZIKV was subsequently shown to infect and kill AXL-knockout human NPCs in iPSC-derived and cerebral organoid models, suggesting that other molecules must be involved in the viral entry process. More recently, AXL was found to be expressed by microglial cells and astrocytes in the human developing brain, to bind ZIKV through Gas6-bridging, and to mediate viral entry into glial cells but not NPCs (5). Finally, researchers recently showed that a region of ZIKV RNA binds to the RNA binding protein Musashi-1 (MSI1), disrupting MSI1 binding to its endogenous targets, therefore leading to a deregulation of factors involved in stem cell function. In particular, MSI1 was found to be highly expressed in NPCs and its binding to the viral genome enabled effective ZIKV replication (6).

Conclusion

Thanks to the advances in stem cell technology, the causal relationship between ZIKV infection and impaired brain development is now well-established. ZIKV exhibits tropism for NPCs and foetal ZIKV infection triggers disruptions to the cell cycle, apoptosis and differentiation in the developing brain. Nonetheless, the factors that make the immature brain vulnerable to ZIKV are still poorly understood and further research efforts are required to uncover the complete mechanisms of ZIKV pathogenesis.

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Can stem cells mend a broken heart?

by Abigail Wilson

Abigail Wilson is a British Heart Foundation funded DPhil student in Professor Sitsapesan's group in the Department of Pharmacology.

Following a heart attack, the human heart is devastated by the death of cardiomyocytes and their replacement by fibrotic scarring which has no ability to contract like normal cardiac muscle. This damage is currently irreversible and incurable. Although some animals can regenerate their hearts, this capacity has been lost in mammals. It is a tantalising prospect that we could regenerate damaged tissue - could stem cells be the future to fixing a broken heart?

Humans can be heartbroken - are fish the lucky ones?

Why do we see a disparity between fish and mammalian cardiac regeneration? Two similar and elegant studies in 2010 gave evidence of why fish have a superior heart regeneration ability over mammals (1,2).

A zebrafish strain was generated with two transgenes in an inducible Cre-loxP system, allowing researchers to follow the regeneration of the heart with a simple red or green colour comparison. These zebrafish contain both the DsRed gene sequence, flanked by two loxP sites, and the GFP gene, which is initially inactive. These fish also have a sequence that encodes for a Cre recombinase, but this is only activated by tamoxifen treatment, and specifically within mature cardiomyocytes.

The first gene caused all cardiac progenitors and cardiomyoctyes to produce a red fluorescent DsRed protein. Firstly, the zebrafish were temporarily treated with tamoxifen, causing the inducible Cre-loxP system to replace the DsRed gene with GFP in cardiomyocytes, resulting in differentiated cardiomyocytes fluorescing green. Next, the apex of the zebrafish heart was then amputated. Following this injury, the zebrafish heart acts to repair the damage. Interestingly, it was found that newly regenerated heart tissue was populated solely with green cardiomyocytes, indicating that the regenerated cells came only from pre-existing green cardiomyocytes dividing, and not from the red stem cell progenitor cells differentiating (Figure 1).

An analogous experiment in mice was used to compare the mammalian mechanisms of cardiac regeneration. Experimenters used a progenitor cell that produces an intense blue colour (due to the activity of a β-galactosidase), and an inducible GFP in cardiomyocytes (3). Upon tamoxifen induction, the green:blue ratio was 80:20. Over normal ageing, there was no change in these values, indicating no renewal from progenitor cells. Cardiac injury in the mouse caused the ratio to shift to 65:35 in the damaged zone of the heart. In contrast to the zebrafish model, this suggests that the newly generated cells were in part recruited from the blue progenitor cells, and not completely regenerated by neighbouring cardiomyocytes.

This interesting experimental design highlighted that the limited ability for cardiac regeneration in mammals involves induction of cardiac progenitor cells, whereas in zebrafish cardiac regeneration occurs from pre-existing cardiomyocytes. This explains the evolutionary difference

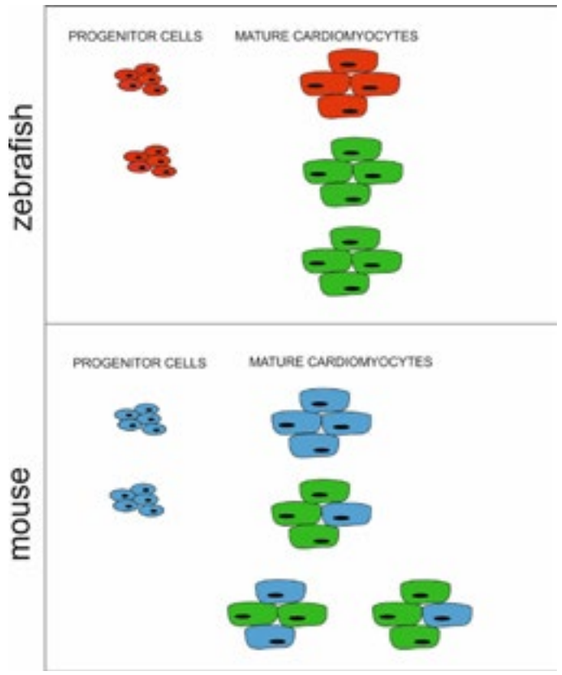

Figure 1: Genetic fate mapping in cardiac regeneration (4) *Image adapted from Laflamme M & Murry C (2011) Heart Regeneration. Nature 473:326-335*

in regenerative capacity between mammals and fish. However, does the evidence of some regenerative capacity from progenitor cardiac cells give us hope for human heart regeneration as a potential therapy.

Hope for us humans

Despite the experimental evidence that mammals have little capacity to regenerate damaged cardiac tissue, is there still hope for us humans? An open phase IIa trial conducted in 2012 showed promising results for the use of stem cells for cardiac regenerative potential. In this trial, patients were injected with a suspension of a mesenchymal precursor cell type into areas of damaged myocardium that could not otherwise be rescued by surgical revascularisation during coronary artery bypass grafting (5). Mesenchymal stem cells are precursors to many cell types including cardiomyocytes. These cells were isolated from healthy donor bone marrow by a UK biotech called Cell Therapy and marketed as immunomodulatory progenitor (iMP) cells, or "HeartCel". The intervention was deemed safe and well tolerated, and results showed a remarkable improvement in myocardial contractility and a significant amelioration in the left ventricular scar area 12 months after treatment (5). Although this study was limited to 11 patients, it holds a lot of hope for utilising stem cells in regenerative therapy. Cell Therapy has since rebranded itself as Celixir, and in January 2018, the company announced approval of a clinical trial application for the larger, global phase IIb trial with HeartCel. This study aims to be completed in 2020 with market entry in 2021. It will be exciting to follow this pioneering study with the hope to use stem cell technology to improve the quality of life for many patients suffering from heart failure.

"patients were injected with a suspension of a mesenchymal precursor cell type into areas of damaged myocardium that could not otherwise be rescued"

"It will be exciting to follow this pioneering study with the hope to use stem cell technology to improve the quality of life for many patients suffering from heart failure."

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NEUROBIOLOGICAL BASIS of sex-specific behaviours

by Carolina Rezaval

Drosophila melanogaster female. Artwork by Joane C. Carvalho, Evolutionary and Developmental Biologist, Portugal

Sex-specific behaviours are prevalent across the animal kingdom, particularly in activities related to reproduction, such as mate selection and offspring care. There are a number of beautiful examples of gender-specific mating rituals in nature, including sophisticated dances and ornamental displays. In some species of frogs, songbirds and crocodiles, males produce a courtship song that is thought to attract females by advertising desirable attributes. Whereas in some species of spiders, males use gifts of food to entice females. It is thought that females use these male courtship displays to evaluate the fitness of potential mates and decide whether to copulate or not. This variation in behaviour between sexes is essential for reproductive success, and the intriguing question here is to understand how these differences in behaviour arise.

Sex-specific behaviours are usually innate and are believed to reflect gender differences in the nervous system. It is possible that, in the most extreme case, an entire neural circuit unique to one sex might generate a sex-specific behaviour. Alternatively, sex differences may reside in sensory or motor neurons that are connected to shared neural circuitry. However, given that many non-reproductive behaviours are common to both sexes, these pathways are likely to be similar between males and females, and sex differences may instead arise from intermediate neurons in the brain. Knowledge of this underlying neural circuitry will help further our understanding of the cellular basis of sex-specific behaviours.

The fruit fly Drosophila melanogaster has proved an ideal model for studying the neuronal bases of sexually dimorphic innate behaviours, as their sexual behaviours are robust and highly stereotypical. During courtship, the male chases the female while vibrating one wing in order to perform a species-specific song, whereas females do not court.

"As a result of available sophisticated genetic tools, we have gained insight into the genes and neurons that control sex-specific behaviours in fruit flies."

As a result of available sophisticated genetic tools, we have gained insight into the genes and neurons that control sex-specific behaviours in fruit flies. Previous work has indicated that the neuronal circuitry expressing the sex determination genes doublesex (dsx) and fruitless (fru) controls all aspects of male courtship (1). Notably, activation of male-specific dsx/fru+ neurons has been shown to initiate courtship behaviour in males. Therefore, it has been proposed that the presence of these male-specific neurons could explain why males court and females do not. However, other studies have challenged this notion. For example, direct stimulation of fru+ neurons in the thorax has been shown to elicit singing in female flies (2), demonstrating that neurons capable of male-like song generation are indeed present in the female. These observations led to the hypothesis that females do not normally sing because 'command' neurons in the brain, normally required to activate the song pattern generator, are either missing or inactive. Additionally, it has recently been shown that activation of dsx+ neurons in the brain induces female flies to exhibit male-like behaviour, such as courtship song.

This finding shows that key components of the neuronal circuitry for 'male' behaviours exist in the female fly brain but remain dormant (3). Thus, sex differences in behaviour may not stem from the presence or absence of key neural circuits, but rather from how their activity is modulated. Interestingly, the female mouse brain has also been shown to contain latent circuits driving male-specific behaviours (4). Therefore, the presence of male and female-specific circuits in the brain might be a conserved feature of the nervous system.

These findings are not only fascinating, but they also remind us of the importance of taking a comparative approach to further understand brain organisation and function. Future work is likely to focus on understanding how behaviours of the opposite sex are repressed in functionally bisexual brains. Needless to say, exciting times lie ahead in this interesting field of research!

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Dr Carolina Rezaval, School of Biosciences, University of Birmingham

PRE-EXISTING IMMUNITY TO CAS9

HAS THE GENOME EDITING REVOLUTION BEEN THWARTED BEFORE IT BEGINS?

by Joost Vanhaasteren

Genome editing has the potential to effectively and permanently cure genetic diseases by correcting the underlying genetic mutation. This promise has had researchers scrambling to perfect genome editing machinery, something greatly aided by the introduction of a new type of endonuclease, Cas9. However, recent work has cast doubt on whether genome editing using Cas9 will ever be feasible in humans.

The CRISPR/Cas9 system, heralded as Science's Breakthrough of the Year in 2015, has vastly simplified the generation of research models that address the effects of specific genetic mutations. Moreover, it has amplified ambitions to get genome editing into the clinic. The first patients treated with CRISPR/Cas9-modified cells were in China, and are soon to be followed by cohorts in the United States. The company Sangamo Biosciences recently attempted the first ever in-vivo¬ genome editing study, whereby the genome editing machinery, zinc-finger nucleases in this case, were injected directly into the bloodstream. However, a recent pre-print publication led by Matthew Porteus at Stanford University has caused concern in the genome editing field (1).

What is the issue?

In its simplest form, correcting a genetic mutation often involves the use of two components, an endonuclease (a protein that can cut DNA), such as Cas9 or zinc-finger nuclease, and a DNA repair template. It is the Cas9 endonuclease that is at the heart of the present issue. The two most prominent and promising Cas9 orthologues, Staphylococcus aureus (saCas9) and Streptococcus pyogenes (spCas9) stem from two commensal bacteria that are part of the normal human microbiota. As such, it is highly likely that the immune systems of most individuals have come across proteins from both bacteria, including Cas9. Indeed, researchers found that 79% and 46% of tested individuals had antibodies against saCas9 and spCas9 respectively. Moreover, they found that some tested positive for saCas9-specific cytotoxic (killer) T-cells. This implies that cells that are targeted for genome editing, and thus express Cas9, could be targeted for destruction by the patient's own immune system. With edited cells killed off there will be no therapeutic effect, and further the immune reaction could potentially pose a safety hazard. As a result, patients testing seropositive for Cas9 proteins might be excluded from future clinical trials.

How do we proceed?

It is unlikely that ex vivo applications (genome editing cells outside of the patient's body and injecting them afterwards) will be impacted since one could simply wait for Cas9 protein expression to fade before infusing the patient with the edited cells. Such an evident solution is lacking for in vivo genome editing. Regardless, a few resolutions might indicate that all hope is not lost. While saCas9 and spCas9 are currently the most widely used Cas9 orthologues, fishing for Cas9 proteins in bacteria that humans have never been exposed to, such as geoCas9 from bacteria in thermal vents (2), will likely yield Cas9 proteins that humans have no pre-existing immunity to, allowing for at least one shot at genome editing in vivo. Another approach is to humanise the Cas9 proteins such that the body's immune system does not react as thoroughly as it would against a protein that looks more 'bacterial'. This method is in line with the observation that the expression of non-self-proteins in the liver can give rise to immune tolerisation against that protein, preventing an immune response (3). Applying this approach to Cas9 could abolish any antibody or cells that have been primed to respond to it.

Genome editing with Cas9 is not yet dead in the water, but these findings warrant a trip back to the drawing board.

Joost van Haasteren is a DPhil student in the Gene Medicine Research Group at the Radcliffe Department of Medicine.

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HOW STUDYING BLOOD FORMATION IN THE MOUSE EMBRYO CAN HELP US TO DEVELOP BETTER THERAPIES.

Vincent Frontera and Emanuele Azzoni are Post-Doctoral researchers in the de Bruijn group, MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine.

by Vincent Frontera and Emanuele

For patients with blood conditions, bone marrow transplants are one of the few therapeutic options, but there are limitations. In the de Bruijn lab, we are dissecting the complex lineage map of blood formation in the embryo. We hope that this will inform the therapeutic production of personalized haematopoietic stem cells in the future.

Haematopoietic stem cells (HSCs) are multipotent cells characterised by their ability to self-renew, and to produce all types of blood cells. HSCs reside in the bone marrow and maintain blood production (haematopoiesis) throughout the lifespan of an organism. The haematopoietic process is remarkably well-balanced and its dysregulation can lead to blood disorders, such as haemophilia, or blood cancers, such as leukaemia.

Stem cell-based treatments have been used for several decades, and require the injection of stem cells into patients who have undergone chemotherapy or total body irradiation. Depending on the origin of the stem cells used, these transplants can fall into one of two categories. In autologous transplantation patients receive their own HSCs, collected before bone marrow ablation. However, this method can be inefficient on its own if the stem cells carry the detrimental mutation(s) in question, or if the autograft is contaminated by tumour cells. In these situations, allogenic transplants are often preferred (1). These involve bone marrow transplantation from healthy donors and can, therefore, be curative. However, there is an increased risk of graft rejection (graft-versus-host diseases). Being able to generate HSCs at the bench would bypass these limitations and would constitute a "giant (therapeutic) leap for mankind". However, this approach remains technically challenging and is currently too inefficient to be employed clinically. Understanding the cellular and molecular mechanisms that underlie the generation of the first HSCs, which occurs during embryonic development, is essential to generate new insights.

In the de Bruijn lab, we are interested in understanding the complex process by which blood is formed and how the very first HSCs appear. Our model of choice is the mouse embryo, where this process has been relatively well characterised. Progenitors with limited potential are the first to emerge, and they do so independently of HSCs, which appear only later in gestation. The first lineage-restricted progenitors produce only primitive red blood cells expressing an embryonic haemoglobin. Subsequently, two different types of progenitors, with broader potential but limited lifespan, appear and generate multiple types of blood cells. Finally, during mid-gestation and in contrast to other progenitors, the first HSCs develop intra-embryonically in

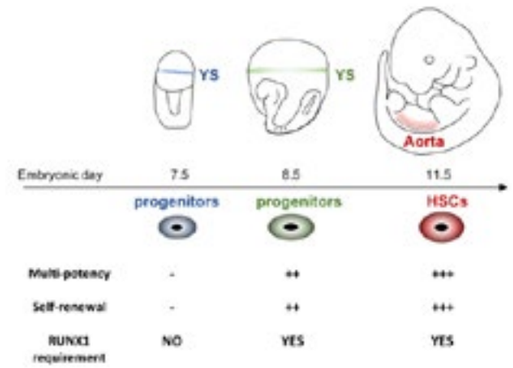

Figure 1. Emergence of haematopoietic progenitor and stem cells during mouse development. Each cell type is defined by their multipotency, self-renewal potential and RUNX1 requirement (YS: yolk sac).

the dorsal aorta and in the other major arteries (Figure 1). Embryonic HSCs are generated through an intriguing and unique process, known as the endothelial-to-haematopoietic transition. In this process, a specialised subset of the inner lining of the major blood vessels, the haemogenic endothelium, loses endothelial characteristics in order to become hematopoietic cells (2).

In our laboratory, we are interested in a critical player in the endothelial-to-haematopoietic transition: the transcription factor RUNX1. Indeed, homozygous deletion of the Runx1 gene in mice is embryonic lethal due to the complete failure of production of HSCs and other blood progenitors. The expression pattern of RUNX1 suggests a critical role for this transcription factor in the specification of endothelial cells towards the haematopoietic lineage, which has been confirmed experimentally (3). However, its regulation remains unclear. We are trying to identify the regulatory factors controlling the expression of RUNX1, as we believe that these factors could be the key signals triggering haematopoietic specification and therefore, HSC generation. Our ultimate goal is to build a complete roadmap of HSC development in the embryo as well as identify the signals and transcription factors required for HSC specification and maintenance. This work will provide the knowledge base necessary to advance HSC production in vitro for therapeutic purposes.

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PRiMARY MiCRoCEPHALY

the Importance of Establishing a Reliable Animal Model

by Rohan N. Krajeski, Research Assistant in Neuroscience & Dr Tommas Ellender Group, Department of Pharmacology

Normal Brain Development:

In humans, the embryonic period lasts from conception to gestational week (GW) 8. By the end of this phase, fundamental structures of the brain and central nervous system have already been developed. Gastrulation at GW2 forms a multi-layered gastrula, whose primary germ layers later give rise to specific tissues and organs. The neural plate, derived from the third germ layer, forms the neural tube at GW3, in a process called neurulation (2). At GW3 onwards, in a process known as regionalisation, cells acquire distinct identities based upon their spatial position. During this time, the shape and structure of the neural tube changes markedly; the anterior end expands and divides

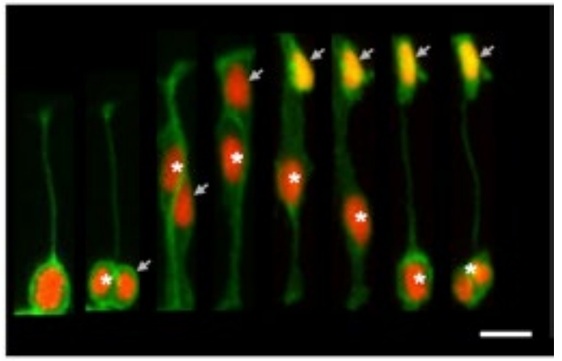

Figure 1: Asymmetric daughter fates. 9-hour time-lapse sequence, in-vivo in a zebrafish brain. A single neural progenitor expressing membrane-GFP and nuclear-RFP along the ventricular surface undergoes asymmetric division producing one neural progenitor (star) and one neuron (arrow). Neuronal fate was determined by the expression of HuC:GFP in both the cytoplasm and nucleus (the nucleus became yellow). Progenitor fate was confirmed by a subsequent division. Scale bar = 12μm (adapted, 3).

into five segments, which later corresponds to adult brain structures (2).

The neural tube contains neuroepithelial progenitor (NP) cells, which can generate both neural progenitors and post-mitotic neurons. NPs initially undergo symmetric division between GW3 and GW6, then switch to an asymmetric mode of division (Figure 1), producing one NP and one neuron. While the NP remains within the proliferative ventricular zone (VZ) of the neural tube, the neuron migrates away towards the pial surface of the brain. This shift to asymmetric division occurs slowly, but later a new population of NPs that divide away from the ventricle forms, further expanding the neuronal population (2).

During the early stages of development, neurons migrate via somal translocation by extending a long basal process from the cell body (within the VZ) to the pial surface. The nucleus then migrates through the cytoplasm of this process until it reaches the embryonic cortex, forming the primitive brain structure. As the brain continues to develop and becomes larger, the mode of migration changes and neurons rely on radial glial cells, a type of NP, to act as a scaffold. This forms the deepest layers of the mature brain. Neuronal differentiation occurs once neurons reach their target region and develop neuronal processes, facilitating the transmission of electrochemical signals required for neural network formation (2).

Primary microcephaly, a developmental brain disorder:

Human microcephaly is defined as a significant reduction in head circumference and is associated with significantly decreased brain volume, particularly in the cerebral cortex (4). Primary microcephaly can arise from non-genetic causes, such as alcohol consumption during pregnancy, poor prenatal care or congenital Zika virus or cytomegalovirus infections. Genetic primary microcephaly, known as autosomal recessive primary microcephaly (MCPH), occurs when the brain fails to grow to the correct size in-utero due to mutations in genes associated with centrosomal function (4). Centrosomes are microtubule-organising organelles, capable of regulating many aspects of cell division, including mitotic spindle formation and orientation, neuronal migration and cilia function. MCPH-associated centrosomal proteins regulate these cell functions (4), but it remains unknown whet her these are common causes of primary microcephaly.

Understanding abnormal spindle-like, microcephaly associated (ASPM) protein:

In humans, MCPH is mostly caused by homozygous mutations in Aspm. ASPM is particularly important during spindle organisation and orientation, mitotic progression, and cytokinesis (4). Nonetheless, only few publications have focused on Aspm expression, its orthologs and its association with primary microcephaly.

Drosophila Asp, an ortholog of Aspm, is vital for the central spindle assembly during mitosis. ASP initially localises to spindle poles, before migrating to the microtubule minus ends as the spindle dismantles in late anaphase and telophase. Interestingly, Drosophila Asp mutations cause metaphase cell cycle arrest in larval neuroblasts (5).

Fish and colleagues analysed Aspm expression during brain development in mice (6). They found that Aspm was highly expressed during symmetric proliferation, whereas the expression decreased during asymmetric division, suggesting a role in NP fate decisions. ASPM was localised around the mitotic spindle poles of centrosomes throughout mitosis, with a decreased protein intensity observed by immunostaining during telophase. Interestingly, RNAi

knockdown of Aspm resulted in abnormal centrosomal function; although centrosomes successfully localised to opposite ends of the NP during interphase, many were detached from their sister chromatids during telophase. However, unlike in Drosophila, this did not inhibit mitosis, suggesting that mitotic spindles can still function when Aspm is absent. Finally, Aspm knockdown was found to significantly alter the cleavage plane orientation of neuroepithelial cells. NPs usually divide with a vertical cleavage orientation, which is believed to enable symmetric division. Any deviation in this orientation (eg. from perpendicular vertical to parallel), is predicted to cause a premature transition to asymmetric division. In line with this, Fish and colleagues observed an early termination of symmetric division after Aspm knockdown (6), potentially leading to a reduction in the NP pool, thereby producing fewer neurons and giving rise to a 'small brain' microcephaly phenotype.

Further research from this group generated an Aspm-/- mouse model, for which newborn mice exhibited reduced brain size (7). In contrast to previous findings, truncated ASPM was found to be localised at spindle poles during metaphase, but not at the mid-body during telophase. Furthermore, Aspm knockouts did not exhibit a significant change in cleavage plane orientation or a premature transition to asymmetric division. Unlike Drosophila mutants, Aspm mouse mutants did not exhibit cell cycle arrest during metaphase or increased apoptosis. However, a reduced NP pool was still observed, the cause of which remains unknown (7).

These diverse findings raise doubts as to whether ASPM-related microcephalies result from variations in mitotic spindle orientation and therefore an impairment of neurogenesis. How ASPM function regulates NP populations remains elusive and the establishment of a reliable, reproducible animal model is needed to address this question.

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NODULE DEVELOPMENT IN *legumes*

by Thomas Gate

Plants require nitrogen for synthesis of amino acids as well as DNA and RNA bases. This nitrogen comes mainly in the form of nitrate or ammonium ions. If these are unavailable, plants can rely on forming a symbiotic relationship with bacteria which can 'fix' nitrogen, whereby N2 in the atmosphere is reduced to ammonia that is given to the plant in exchange for sugars. This symbiosis often requires the development of specialist nodules, commonly found on plant roots, which contain these nitrogen-fixing bacteria (1). The process of nodule development follows a series of steps including plant-bacteria communication and coordination of the uptake of bacteria with nodule development using plant hormones .

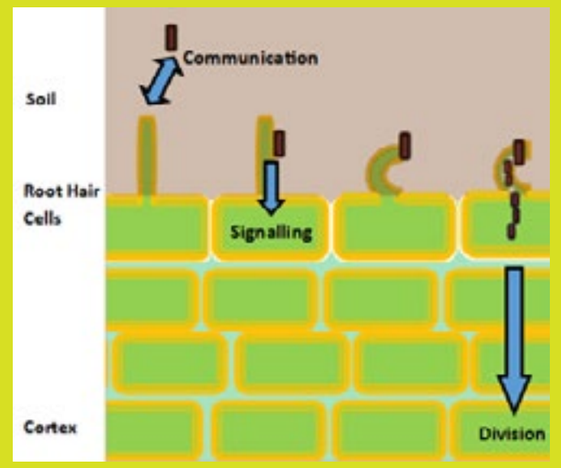

Figure 1. Initiation of nodule development. The steps of initiation are shown from left to right with i) communication, ii) signalling, iii) root hair cell curling and iv) induction of cell division.

Plant-bacteria communication starts with plant roots secreting secondary metabolites which then induce synthesis of nod factors in specific bacteria. Nod factors are complex conjugated sugars that are released by bacteria that diffuse and bind to receptors on plant root cells. Upon binding, they elicit several responses in plants including calcium spiking and root hair cell deformation. In addition to nod factors, exopolysaccharides (large extracellular sugar polymers) on bacterial surfaces are also used in signalling to initiate nodule development. Together these allow root hair cell curling and then internalisation of the bacteria into the plant root cells as infection threads (Figure 1). The specificity of these signals is important to promote uptake of beneficial symbiotic bacteria into the root whilst selecting against pathogenic microorganisms.

These signals cause transcriptional changes in the plant such as inducing enzymes which weaken the cell wall, making formation of an infection thread possible. In addition, these signals perturb auxin and cytokinin signalling, two key plant hormones, for example by increasing the expression of a cytokinin receptor in root cortex cells (2). Together, these two hormones induce cell division in the root cortex to start nodule development.
Once the infection thread reaches the cortex, the bacteria can be released into the plant cytosol of cortex cells. The plant then produces cysteine-rich peptides which cause the bacteria to swell, have leaky membranes and replicate their DNA without division to become bacteroids with 24 chromosome copies (compared to 1-2 seen in free-living bacteria). This polyploidy causes the bacteria to increase transcriptional and metabolic rates and so increase nitrogen fixation and ammonia export. The plant must then induce genes to support the metabolic needs of the intracellular bacteria, such as the gene for leghaemoglobin which maintains a low oxygen concentration to preserve the integrity of the nitrogenase enzymes.

"Different types of nodule show different developmental pathways"

Different types of nodule show different developmental pathways and the choice between them can be controlled by micro RNAs (3). For determinate legumes, the nodules have no permanent meristem (a zone of undifferentiated and growing cells) and so the cells in the nodule proliferate, differentiate and senesce in a synchronised manner. However, in indeterminate nodules, there is a gradient of differentiation with a permanent meristem at one end and a zone of senescence at the other, with a zone of nitrogen fixation in between. Further elucidation of the developmental pathways used by model organisms to form nodules will provide clues to induce nodulation in crop plants and so reduce fertiliser use.

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VACCINE DEVELOPMENT FOR ENTERIC FEVER

by Soumya Perinparajah

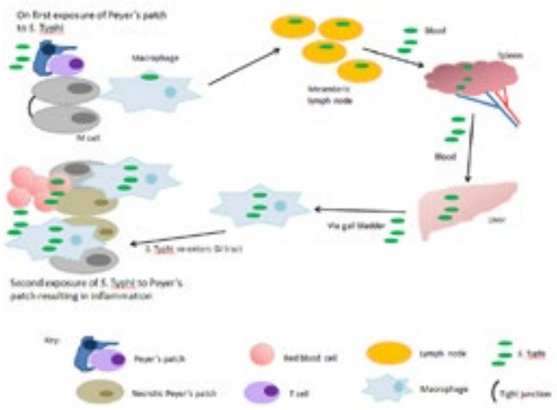

Figure 1. Schematic showing the pathogenesis of S. Typhi infection, reproduced from Everest et al. (2).

Typhoid and paratyphoid fever, known collectively as enteric fever, are both systemic infections caused by two human-restricted bacteria, Salmonella enterica subspecies enterica serovar Typhi (S. Typhi) and Paratyphi (S. Paratyphi). Both types of fever present with clinically identical symptoms and cannot be distinguished from other illnesses causing fever. Symptoms include prolonged fever, headache, nausea, loss of appetite, constipation, and sometimes diarrhoea.

Endemic to South Asia and sub-Saharan Africa, typhoid fever accounts for 75-80% of enteric fever cases, with infection occurring by the faeco-oral route via ingestion of food or water contaminated with human faeces containing S. Typhi (1). According to the World Health Organisation (WHO), there are 11-20 million cases of typhoid worldwide, associated with 128,000-161,000 deaths every year. Once ingested, S. Typhi crosses the mucosal layer of the intestine via microfold cells (M cells) and are then presented to immune cells in gut-associated lymphoid tissue, specifically Peyer's patches. S. Typhi multiply intracellularly within immune cells, eventually leading to inflammation of the mesenteric lymph nodes resulting in bacteraemia and the dissemination of S. Typhi to other organs such as the liver, gall bladder and spleen (Figure 1).

Human challenge studies, in which participants are deliberately infected with a microbe, have been invaluable in helping to uncover the mechanisms of various diseases caused by human-restricted pathogens, as well as for testing the safety, tolerability and efficacy of vaccines. The Oxford Vaccine Group conducts studies of new and improved vaccines for children and adults, with a large body of research on enteric fever based on an established adult typhoid challenge model. Preliminary studies have explored the establishment of a human paratyphoid challenge model which, in addition to addressing this gap in knowledge, highlight the need for its development to test new vaccines, especially in light of the increase in S. Paratyphi A cases seen in recent years (3, 4).

There are currently two licenced vaccines available against typhoid: a subunit Vi capsular polysaccharide vaccine (ViCPS vaccine), administered intramuscularly, and an oral live attenuated mutant vaccine made from the Ty21a mutant strain of S. Typhi (Ty21a vaccine). However, the ViCPS and Ty21a vaccines have not been approved for the immunisation of children under 2 and 6 years of age respectively.

In a recent study conducted in Oxford, we assessed the safety and tolerability of a leading typhoid vaccine candidate, the Vi-tetanus toxoid conjugate vaccine (Vi-TT), for the first time (5). The efficacy data from this study which demonstrated a reduction of up to 54-87% of typhoid infections in the human typhoid challenge model, was used to support the pre-qualification of the Vi-TT vaccine by the WHO in January 2018, and the recommendation by the WHO SAGE committee in October 2017 for use in children aged 6 months or older. It is hoped that this new conjugate vaccine will aid in the slowing down of emerging multi-drug resistant typhoid strains in endemic regions, such as the highly resistant strain responsible for the current outbreak in Pakistan, found to be resistant to cephalosporins (6).

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TACKLING REPRODUCIBILITY
Antibody Review Databases

by Ines Barreiros

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In the midst of the 'reproducibility crisis', scientists waste precious samples, time, and funds on unreliable antibodies that fail to replicate crucial results. Collaborative antibody review databases can help tackle this problem. So, if you use antibodies in your research, you need to read this article! And, if you don't, you should read it anyway and tell your antibody-using friends about it.

Antibodies are Y-shaped proteins naturally produced by the immune system to recognise pathogens. The tips of an antibody's light chains (the small arms of the Y) are highly variable and bind only to specific antigens – the pathogen molecules recognised by antibodies as foreign or toxic – in a lock-and-key mechanism. In this way, each antibody matches and recognises only one type of pathogens.

Scientists made use of this mechanism to develop a wide range of research methods now commonly used in biological sciences. These include, amongst others, Western-Blots, immunohistochemistry, ELISAs, and flow cytometry. By tagging antibodies with easily detectable compounds, such as fluorophores, their location and intensity in a sample flag the presence and quantity of biological targets of interest (Fig. 1) (1).

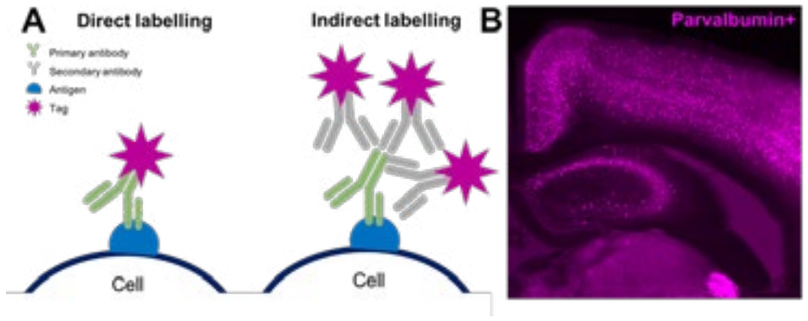

Figure 1. Immunolabeling. Biological structures can be immunolabeled directly, (A – left) with tagged antibodies or indirectly (A – right) using tagged secondary antibodies, which bind to the primary ones. (B) Immunolabeling example in which neurons

Despite their unquestionable value in biological research, antibodies can be exasperating. If you have had to use them in your experiments, then you are probably familiar with how frustrating it can be finding the appropriate antibodies and testing them. Even when you are lucky to find in the literature, an article that has successfully used exactly the antibody you need and on the same application, often the methodology is not detailed enough, or you are simply unable to reproduce the reported results.

In addition to insufficient protocol details, there are other common problems weakening the trust in currently available antibodies: cross-reactivity, when antibodies recognise proteins other than the ones they were supposed to; performance variability between batches, even from the same supplier; and wrong application of an antibody to an experiment in which the experimental conditions will change its binding ability (2). This significantly slows down scientific progress by making researchers waste samples, time, and funds.

To help tackle this problem, various projects developed collaborative online-tools and platforms. The antibody review databases pAbmAbs (www.pAbmAbs.com) and Antybuddy (www.Antybuddy.com), are the result of such initiatives, led by Professor Glerup (Aarhus University, Denmark) and Dr Barone (University of Sheffield, UK), respectively. On these platforms, scientists can search for antibodies they are considering for their experiments and rate the ones they have previously used. A quick antibody search will return brief reviews, example results, short protocols and dilutions used in various applications, previously tested by independent researchers.

As a bonus, when you submit an antibody review, you are not only helping research evolve better, faster, and cheaper; you also enter a cash prize draw or, if your antibody is from certain suppliers, you are rewarded with Amazon vouchers or antibody discounts.

In addition to being directly useful for researchers, such platforms pressure manufacturers to have higher standards for antibodies production and provide more details on the applications they have been tested for along with how they should be used for reliable results.

Reproducibility is critical for science progression and it is one of the biggest challenges researchers face nowadays. Collaborative initiatives that advocate for further transparency such as antibody review databases can help us tackle this problem, but they need the cooperation of the scientific community. So, if you use antibodies in your research, please don't forget to submit your reviews!

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WILL HUMAN GERMLINE EDITING BECOME A REALITY?

by Anne Turberfield

Genome editing, the rewriting, insertion or deletion of a specific DNA sequence, is a powerful technique with the potential to improve human health. Recently, a new genome editing tool, the CRISPR-Cas9 system, has emerged. This is particularly efficient and easy to use, and therefore is revolutionising research into the roles that individual genes play in normal biology and disease. Rapid advances in CRISPR-mediated genome editing have also led to the first clinical trials using this technique, to either correct a disease-causing gene or to increase the body's ability to fight a disease, with more trials planned for this year. While these current gene therapy applications involve editing the patient's somatic (non-reproductive) cells, recently there has been intense international interest in the potential use of CRISPR-mediated genome editing to prevent the inheritance of debilitating diseases—for example, in rare cases where a genetically defined disease would otherwise be passed on to all offspring.

This concept stems from groundbreaking new research in which the CRISPR-Cas9 system is used to edit the genome of cells of the early human embryo, which carry the inherited germline DNA. For example, work from Kathy Niakan's lab at the Crick Institute targeting the Oct4 gene in human preimplantation embryos has revealed a very early role for Oct4 in human development (1). Such research involving human germline editing has sparked major controversy. On one hand, it can provide unique insight into early human development that cannot be fully modelled using other systems, which could ultimately lead to advances in fertility treatment. On the other hand, any genome edits made in eggs, sperm, or the early embryo would be passed on to future generations if made in a clinical context, raising a number of important ethical, safety, and regulatory concerns.

One concern for the clinical application of germline editing is the possibility that unwanted DNA editing may occur in addition to the desired edit, which would also be inherited. The technology may also have other unanticipated and heritable effects on the cell. In addition to these safety considerations, some argue that the generation of heritable genetic changes crosses an ethically inviolable line, while others raise social and religious concerns. Genome editing of human embryos is still in its infancy and faces major technical barriers, so potential therapeutic applications are not yet feasible. It remains illegal to edit the genomes of embryos used in fertility treatment in the United Kingdom. Nevertheless, further experience with genome editing of human embryos in a research context will enable the refinement of this technology to make it more efficient and accurate, providing the opportunity to understand its potential future clinical harms and benefits.

Criteria for clinical trials using heritable genome editing

- Absence of reasonable alternatives
- Restriction to preventing a serious disease or condition
- Restriction to editing genes that have been convincingly demonstrated to cause or strongly predispose to that disease or condition
- Restriction to converting such genes to versions that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects
- Availability of credible pre-clinical and/or clinical data on risks and potential health benefits of the procedures
- During the trial, ongoing, rigorous oversight of the effects of the procedure on the health and safety of the research participants
- Comprehensive plans for long-term multigenerational follow-up that still

Figure 1. Criteria for clinical trials using heritable genome editing, set out in the 2017 report by the National Academy of Sciences and National Academy of Medicine on human genome editing.

"One concern for the clinical application of germline editing is the possibility that unwanted DNA editing may occur"

The rapid pace of developments in germline editing means that its use during fertility treatment to prevent debilitating diseases could become a reality, particularly as thousands of inherited diseases are caused by single gene mutations and thus represent relatively simple targets (2). Therefore, it is important that we begin to consider the conditions in which clinical germline editing might be permissible in the future. In February 2017, the US National Academy of Sciences and the National Academy of Medicine released a report which proposed a list of stringent criteria to govern clinical trials using heritable genome editing, shown in Figure 1 (3). These include the restrictions to preventing a serious disease or condition, and to converting genes to versions that are prevalent in the population and associated with normal health. These criteria would prevent the use of genome editing to generate 'designer babies' engineered to confer traits that are not related to disease prevention. While it is not possible to enhance traits such as intelligence for which we do not fully understand the genetic contributions, George Church has listed 'rare protective gene variants of large impact', such as an LRP5 variant that confers high bone mass, which might pave the way to human enhancement (4). Therefore, regulators must also consider these highly controversial applications.

We need a broad reaching and inclusive discussion of the ethical, social, regulatory and safety implications of human germline editing. In the UK, we can draw on the example of the extensive public consultation that preceded the recent legalisation of mitochondrial replacement therapies. This will enable the evaluation of whether, in the future, these issues might be addressed sufficiently to justify the legalisation of genome-editing of the human germline for specific clinical applications.

"We need a broad reaching and inclusive discussion"

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LEAVING THE EU

Assessing the impact on UK science

by Patrick Inns

The UK science industry is incredibly valuable. A report by Oxford Economics estimated that UK research, development and innovation contributed 2.3 – 2.6% of total GDP to the economy in 2013 (1). In addition to being economically valuable, UK research is of incredibly high quality: with only 0.9% of the population of the world, the UK generated 15.2% of the most highly-cited articles in 2014 (2).

How will leaving the EU affect this valuable, high quality industry? To answer this question, we must first understand the role the EU currently plays in UK research.

How does the EU currently support UK science?

Monetary funding is the most obvious example of direct support for UK research. The European Commission distributes funds for research through a variety of programmes. Most of this funding is awarded through a framework programme (FP) known as Horizon 2020, with a total budget of €74.8 billion from 2014 to 2020 (3). The aim of Horizon 2020 is to promote international collaboration and excellence in research. This aim is achieved through two funding bodies that distribute a considerable portion of Horizon 2020 funds: Marie Skłodowska-Curie Actions (MSCAs) and the European Research Council (3). MSCAs give individual researchers international experience, by providing funding for researchers who move country to gain experience in a host research organisation in the EU (4). The European Research Council promotes high quality research by providing grants based solely on academic excellence (5).

Although the UK is a net contributor to the EU budget, during 2007 – 2013 the UK contributed €5.4 billion specifically for research, development and innovation to the EU but received €8.8 billion in funds for this same period, a net gain of €3.4 billion (3).

Research conducted in the UK is highly collaborative, with 48% of all articles from the UK being the result of international collaboration in 2012 (6). The EU has multiple projects that promote this international collaboration, from building a European Research Area (ERA), which will act as a free market for researchers, to current collaborative projects, such as the European Atomic Energy Community (EURATOM) and research partnerships that span the public and private sector (3). This international collaboration is further aided through policies such as the free movement of people, which allows EU citizens to settle in the UK with relative ease and contribute to the scientific industry of the UK. This free movement is also hugely beneficial to active UK researchers, 72% of whom published articles working at institutions outside the UK between 1996 and 2012 (6).

UK science outside the EU

In a 2014 report detailing the UK government's plan for science, the EU played a key role. The report stated that the UK was "the top beneficiary from the EU Framework Programme" and would "work closely with the European Commission to establish frameworks for innovation in the EU" (6). It was claimed that the UK was heavily involved in "the development of the ERA roadmap" and "will seek out the priorities for deepening the ERA as a single market for research and knowledge" (6).

"[a 2014 report] stated that the UK was "the top beneficiary from the EU Framework Programme"

In September last year, however, the government published a 'future partnership paper' on its plan for science in the UK after leaving the EU. This paper abandons many of the policies outlined in the government's 2014 report and fails to address the most pressing issues raised by the withdrawal of the UK from the EU. On membership of future framework programmes, the new paper stated that "future association arrangements will be discussed as part of the EU's negotiations on the next Framework Programme" (7), making no commitment on the funding body that distributes the vast majority of EU science funds (3). On the ERA, which was part of the government's science policy in 2014, it is now stated that "The UK would welcome discussion as to how this [the ERA] might apply in future" (6,7). The government's position on MSCAs, which distribute funds in a manner that promotes international collaboration, is similarly non-committal (7). It is worth noting that in the period of 2007 – 2013, 78% of EU science funding for the UK was distributed by FP7, the predecessor to Horizon 2020 (3). It is hence worrying that the UK has not committed to any scenario that would allow us to retain access to those funds that support so many UK researchers and institutions. The current lack of clarity also makes it difficult to fully assess the impact leaving the EU will have on the science industry. In the best-case scenario, the UK would retain access to FP funding, through gaining 'associated country' status. However, associated countries do not have any negotiating position with regards to EU science funding (3), so the UK would therefore not be able to effectively influence how EU funds are distributed, inevitably harming the national science industry. The alternative, of not seeking associated country status, would be significantly more damaging to the science industry, as this could cause the UK to lose access to EU collaborative communities such as the ERA and billions of Euros in FP funding (3).

Although the UK government has no plans on future FPs and the ERA, the government has stated its plan to end the free movement of people (7). This decision will place a barrier to international collaboration that the EU facilitates, as for non-EU citizens current visa applications for settlement can, in some cases, take up to six months to process (8). If such a system was introduced for EU citizens, UK institutions would be less effective at hiring skilled EU researchers. It would also be likely that UK researchers, who we have seen benefit from international mobility, would also face similar issues when settling in the EU. Despite the UK government's plans to end free movement, the government has failed to publish their policy on EU migration and specifically what system will replace free movement (9). This position on free movement is increasingly worrying when it is considered that Switzerland, an associated country, was only able to participate fully in Horizon 2020 upon acceptance of the free movement of people from Croatia (10). This indicates that the current rigid position the government is taking to free movement could risk the full involvement of the UK in future FPs that, like Horizon 2020, will be responsible for distributing billions of Euros in research funding (11).

Concluding remarks

Science in the UK will survive leaving the European Union, however there is no question that it will be weakened. The EU provides generous funding and access to a world class collaborative community, and the UK risks access to this by leaving the EU. Despite the huge impact that this could have on UK science, the government's plans are woefully lacking. No commitments have been made on future framework programmes, specific European funding bodies, or the European Research Area, which previously played a key role in the science policy of the UK government. The plans that the UK government has briefly outlined will likely harm the science industry: leaving collaborative projects such as EURATOM and ending the free movement of people will damage the international collaboration that science thrives on, and calls into question the access of UK researchers to future framework programme funding.

"Despite the UK government's plans to end free movement, the government has failed to publish their policy on EU migration and specifically what system will replace free movement"

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Nine losses, one win: putting science into perspective

by Evangelia Myttaraki

Many have tried to address the question of what it takes to be a 'good scientist'. Fortunately, many others have attempted to provide an answer. Yet why isn't the everyday life of a scientist much easier?

During his career, mathematician Richard Hamming proposed many factors that distinguish a good scientist, exploring the concept of 'the difference between those who do and those who might have done'. His ideas were later summarised as 'ten simple rules for doing your best research' by Prof Erren and colleagues (1; Figure 1). According to these rules, good preparation, dedication, courage and hard work are some key factors that make a scientist great. Dr Giddings, from the University of North Carolina, extends these notions further (2). Interestingly, luck is addressed by Giddings and many other scientists, as an inseparable part of scientific work.

I find that junior scientists struggle to define what makes a 'good scientist'. In addition, I find it unbelievable how many young scientists, medical students and junior doctors face the symptoms of impostor syndrome: chronic self-doubt and fear of being discovered as an intellectual fraud (3). Discouragement is inevitable in a competitive system, but are these fears self-inflicted, or is the system emphasising failures to the detriment of young scientists' self-esteem?

From the moment you enter the scientific world, it can feel as if your life has been turned into an arena, full of constant psychological distress over research projects, grant applications, conference presentations, and peer-reviewed publications. Some insist that intensity and vigorous planning are drivers of success, including motivational author Brian Tracy (4). However, psychologists instead claim that pushing young adults to their limits does not help express their potential, but is instead detrimental to their personal and professional future, exacerbating psychological distress (5). Maybe this is an indicator that the educational system needs, somehow, to step down.

"pushing young adults to their limits does not help express their potential, but is instead detrimental to their personal and professional future"

I believe that the road to success depends on three key factors: information, skills and practical support. The first should be available, the second should be taught, and the third should be requested. Young scientists need to focus on areas within their fields which offer the most opportunities and promise for the future. They need to continually develop their scientific skills, and they need to request practical support from supervisors and advisors in order to make the most of their experiences.

Thomas Edison once said "I have not failed. I've just found 10,000 ways that won't work". Or in other words, 'nine losses, one win'. This is usually the norm in the scientific world, where patience and perseverance are key. It is patience that is needed to become confident, to not become disillusioned by difficulties, to persist, to enjoy the process, to be inspired and to be committed. Above all, patience is needed to become truly patient!

To accept failure, and rise above it, is a virtue. However, an inspirational talk or a pat on the shoulder is hardly what anybody needs. Of course, there is no magic recipe, but what young scientists might really need is to be distracted from their work. After all, integrating science into a well-balanced life and putting matters into perspective is the best thing a 'good' scientist can do.

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CLASSIC KIT

The Western Blot

by Lucy McDermott

If you ask a researcher to name their favourite lab technique, the Western blot would, perhaps, not be their first choice. The lengthy protocol and capricious reliability of this analytical protein method do little to endear. Nevertheless, the Western blot is a laboratory staple and fundamental to modern protein research.

Development

The Western blot was first described in 1979 by two competing laboratories, although the name itself was not coined until 1981 (1). The drive behind its development was the need for a sensitive, visual assay to characterise the antigen specificity of monoclonal antibodies. The methodological theory is simple and a natural sequitur from the DNA-based Southern blot, developed 4 years earlier (2).
Protein lysates from whole tissue or tissue culture extracts are resolved on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred to an adsorbent membrane under the influence of an electric current. The transferred proteins are blocked to prevent non-specific binding, after which they are incubated with the primary antibody of interest. Detection of probes can be performed using fluorescence- or radioactivity-based methods, however, the canonical and most common means of detection is via the use of chemiluminescence.

Using the chemiluminescence method, the blot is incubated in species-matched secondary antibodies conjugated to horseradish peroxidase (HRP). The HRP is used to cleave a chemiluminescent agent and the reaction product, luminescence, is related to the amount of protein present. The reaction is captured either with autoradiographic film or a CCD camera. The latter is a more sensitive and accurate means of detection. Aside from a binary protein readout, blots can be used as a semi-quantitative measure of protein abundance via the quantification and comparison of band densitometry.

Advances in Methodology

The pervasiveness of the Western blot is a testament to its utility, and in many labs the technique has not deviated far from the originally described method. However, concerns about reproducibility and the general drive to increase throughput are prompting changes to the classic technique.

One of the main frustrations of the Western blot is the inability to multiplex, that is, to probe for multiple antigens simultaneously. This problem has been addressed using fluorescently labelled secondary antibodies, permitting the simultaneous detection of multiple targets via the use of fluorophores with non-overlapping excitation-emission spectrums. This approach is particularly useful for visualising loading controls, or, for instance, characterising the phosphorylation status of the target antigen. It removes the need to strip the membrane, a practice that can adversely affect accurate quantification.

Schematic of Western blot methodology.

A more recent advance in the field is the development of single-cell Western blots; single-cell technologies are the product of increasing appreciation of cellular heterogeneity. In 2014, Hughes *et al* described a method enabling simultaneous analysis of 2000 single cells (3). The technique uses a polyacrylamide-coated slide with patterned microwells suitable for isolating single cells. The individual cells are lysed in situ and an electric field is applied across the submerged slide, electrophoresing proteins through the microwell walls and into the polyacrylamide gel sheet. In lieu of membrane transfer, the proteins are instead immobilised in the gel by UV covalent crosslinking and antigens are probed in the gel (in-gel immunolabeling). The original method suffered from several limitations, such as a diffusive loss of protein from the wells and cell handling losses in sparse samples, however, later work from the same group has attempted to address both with some success (4). Perhaps the main advantage of the system is the miniaturization and potential for automation since both qualities lend themselves for use in clinical diagnostics. Indeed, early translational uses of the technology have been in characterisation of circulating tumour cells from liquid biopsies (5).

The improvements in the workflow and sensitivity welcome revisions. After nearly 4 decades of use, few challengers have emerged. It seems likely that the Western blot will remain the workhorse of protein biology for years to come, thus securing its status as a truly classic bit of kit.

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BIOART

by Gurmeher Chadha

BioArt is a way for the thinkers of our time to artistically express the political, social and cultural perspective of the fast-moving biotechnology to raise consciousness and start a conversation to bridge a gap between science and the world.

Life is being understood at molecular levels, now more than ever, emphasizing on genetic engineering and nanotechnology. Here are some other examples of what bioart is these days. Reading about these 'new' creations using biolotechnology and art sparks some ethical dilemmas, but that is what bioart aims to do, to start a conversation about where we are heading as a species.

Alba the GFP bunny

by Eduardo Kac

Image: Eduardo Kac, GFP Bunny called Alba, 2000

His most well-known work that sparked a debate over bioart was the GFP bunny named Alba. Kac had allegedly commissioned a French laboratory to create a green-fluorescent rabbit; a rabbit implanted with a Green Fluorescent Protein (GFP) gene from a type of jellyfish, which is a commonly used marker in labs.
Under a specific blue light, the rabbit fluoresces green. GFP Bunny is an example of Kac's continuously evolving reflection on the relationship between humans and animals. In his own words 'The "GFP Bunny" project is a complex social event that starts with the creation of a chimerical animal that does not exist in nature (i.e., "chimerical" in the sense of a cultural tradition of imaginary animals, not in the scientific connotation of an organism in which there is a mixture of cells in the body).

Image: Eduardo Kac and the GFP bunny
Photo: Chrystelle Fontaine

Inigo Manglano-Ovalle uses DNA extracted from clients' hair to produce portraits. His DNA portraits use genes to show social connections - friendship instead of blood. Here the DNA becomes the real repository of one's own identity, making other indexes such as blood and skin colour totally meaningless. Manglano-Ovalle's technologically sophisticated sculptures and video installations use natural forms such as clouds, icebergs, and DNA as metaphors for understanding social issues such as immigration, gun violence, and human cloning

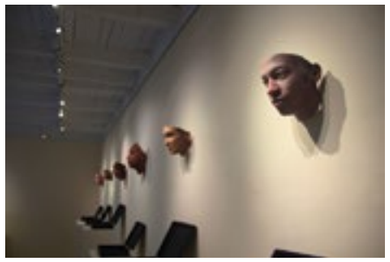

Image: http://deweyhagborg.com/projects/stranger-visions

Regenerative Reliquary 2016

by Amy Karle

Image: https://www.amykarle.com/project/regenerative-reliquary/

Amy Karle's interests were driven by people with limb differences and her friend who is need of a double lung transplant and bone marrow therapy. This drove her to find a way to generate bones and body part's using an individual's own cells.

Karle uses stem cells since it reduces the chance of transplant rejection. She uses an Ember 3D printer and designs a hand skeleton in PEGDA hydrogel, which serves as a template for cell growth. Human stem cells are embedded into the hydrogel, which grows into a tissue, and mineralizes into bone. The hydrogel disintegrates over time. In her own words, she said "the benefit of making this as art is that I can test some of these theories and technologies, develop materials and processes, and experiment outside of the scope of protocols that would have to be followed if this was to be developed as an implant".

Image: Eduardo Kac's gene is only expressed in the red veins of this genetically modified petunia.
http://www.ekac.org/nat.hist.enig.html

Edunia

by Eduardo Kac

Kac took a gene that identifies foreign bodies from his own blood and inserted it into a petunia. Kac's gene produces a protein only in the red veins on the petunia's pink petals, creating the image of human blood flowing through the veins of Edunia. His thoughts were, that the redness of blood and the redness of the plant's veins is a marker of our shared heritage in the wider spectrum of life.

Stranger Visions

by Heather Dewey-Hagborg

Heather Dewey-Hagborg collected hairs, chewed up gum, and cigarette butts of strangers from the streets, public bathrooms and waiting rooms of New York City and extracted DNA from them and analyzed it to computationally generate 3D-printed life size coloured models, which represented what those individuals might look like. The project was meant to call attention to the developing technology of forensic DNA phenotyping, the potential for a culture of biological surveillance, and the impulse towards genetic determinism. This prediction of biological surveillance came true when 2 years later, Parabon Nano Labs launched a service they called DNA "snapshot" to police around the US.

Artists have also used blood to make a statement about the AIDS epidemic. Canadian Artist Jana Sterbak drew with a pen she filled with HIV-seropositive blood. New York artist Jordan Eagles' Blood Mirror is a reaction to the ban on gay and bisexual men donating blood, regardless of whether they are HIV positive. Eagles received 59 blood samples from gay, pansexual, and transgender men. Their blood was used by Eagles to envelop a mirror, where guests stare into the blood and see their own reflection staring back at them.

(http://annex.u ma.umich.edu/post/166258941922/bioart-making-a-statement-with-genes-and-blood)

Vanitas: Flesh Dress for an Albino Anorectic (1987)

by Jana Sterbak

This dress was constructed with 50 pounds of flank steaks, sewn together and hung on a hanger. The artist was trying to express the contrast between vanity and decomposition, relating it to human vulnerability. It was more commonly referred to as a 'meat dress' and sparked major controversy and was deemed as an insult, given the early 1980's recession. Lady gaga wore a similar dress.

Dorothy Nelkin, an American sociologist of science, has talked about the symbolic associations of blood, one of the most valuable commodities in the world (when petroleum is 40 dollars/barrel, an equivalent quantity of blood is worth $67,000). She mentions a handful of blood-based artworks, such as Mark Quinn's head, Jana Sterbak's pen, and Eduardo Kac's biobot.

Self

by Marc Quinn

He used ten pints of his own blood to create a cast that is immersed in frozen silicone. He made this when he was an alcoholic and he wanted to express the notion of dependency to survive. The head needs to be connected to electricity to maintain its appearance, which is why this self-portrait has a personal but social agenda.

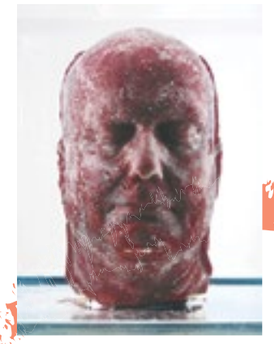

Image: Self 2001 http://marcquinn.com/artworks/self

Artwork by elle Styler

Figure 1: Ten Simple Rules for Doing You
according to Hamming

Voices From Oxford conduct a Science Communication Masterclass at the Dunn School

Written by Sonia Muliyil, HFSP Post Doctoral Researcher in Matthew Freeman's group and head of the News desk, Sir William Dunn school of Pathology

Photos by: David Edwardson, Voices from Oxford

Voices from Oxford (VOX) in collaboration with the newly formed Pathology News desk team organised a Masterclass on Science communication at the Dunn school of Pathology on the 15th of February. This masterclass was aimed at giving interested PhDs and Post Docs a sneak peak into the world of media and communication with a special emphasis on how to communicate their science to the general public.

The main speakers of this masterclass were Professor Denis Noble (Co-Founder of VOX and author of "Music of Life"), Dr. Sung Hee Kim (co-Founder and Director of VOX) and Professor William James (Editor-at-large of VOX and a Dunn school group leader). The talks began with Prof. Denis Noble stressing on the need for all scientists to develop good vocational skills to communicate their science. He voiced his beliefs that all grant applications and science funding depend on the ability of the general public to grasp the fundamentals of what we do. He also expressed the need for using analogies and metaphors to communicate complex problems or ideas. He gave a first hand demonstration of the above by helping members of the audience convert their scientific project ideas into daily anecdotes and metaphors, which could be easily imbibed by the general public.

Dr. Sung Hee Kim shared a very different perspective on the subject at hand. Her experience with the print and visual media, especially interviewing scientists and public figures of interest, helped her gauge at science communication from a very different angle. She shared a poem by Rudyard Kipling, which spoke of the 6 W's namely: Who, What, Where, When, Why and How. She stressed on how the use of these 6 W's can help communicate the essence of a story quite effectively. She also emphasised the importance of compiling the gist of a story in the headline and the opening lines of a story. An additional input was to avoid scientific jargon and instead, use a more conversational style for communicating stories in print. However, at the same time , she cautioned the audience of avoiding a patronising tone. She also suggested using a friend or a partner as a medium to practice one's speech. Finally, she also touched upon how to prepare Press releases and the importance of communicating one's science through local newspapers which could be picked up by the National dailies. Her parting words to the audience, keen on honing their skills in science communication, were: Practice, practice and practice.

The final session of the day was chaired by Prof. William James , who conducted an open circle discussion with the audience. Most of the discussion was centred around how important it is to decide the target audience as well as to pitch it in the right manner. He also emphasised the need for cultivating networking skills amongst members of the scientific community. He also spoke of the importance of converting one's research stories into publications and using it as a base to build on scientific outreach. When asked by an audience member how to communicate fundamental research to laymen, his suggestion was to find a facet of one's research which could be of potential interest to a larger group.

The masterclass ended with the potential of having similar training sessions and workshops at the Dunn school to enable scientists better communicate their science. It also ended with the possibility of forging a liaison between VOX and the Pathology newsdesk.

5' WITH...

Elena Seiradake

by Stefania Monterisi

When did you first decide that you wanted to be a scientist? And how did you decide that you wanted to work on the particular topic you are currently doing your research?

I think I have been always a scientist, in the sense that I have always been curious and interested in understanding the things around me. I like to make sense of complex phenomena by finding out patterns. Of course, by this definition, there is probably a little scientist hidden in most people! The moment I really got hooked was at the end of my PhD, when I started working on brain receptors. I find it amazing that these proteins are able to wire up a brain that can think and feel and make decisions.

What has been the most important moment of your career so far and have you had any particularly memorable

The key moment in my career was when I realised that I could do my research not just in my immediate environment, and that one can achieve so much more by collaborating with others world-wide. I love looking at a complex question from many angles, and combining many cutting-edge techniques to do research. Discussing and working with other people to do this can be so much fun and lead to exciting results.

What is the best advice you have ever received?

I have received a lot of good advice from many people. I think the advice to apply for a post at the Department of Biochemistry was amongst the most important. Some of my most wonderful professional mentors have been Stephen Cusack (PhD mentor), Yvonne Jones (postdoc mentor), Mark Sansom (PI mentor), Rüdiger Klein (wonderful collaborator). Also, Cathy Pears and Elspeth Garman are superb mentors to women at my department. Many other people have provided me with excellent advice throughout my career, and I am immensely grateful to them.

What is the biggest challenge in your career and are there things overall that you would do differently?

I think the biggest challenge was applying for a PI post. I procrastinated as long as I could, as I was happy as a postdoc and I didn't think I was competitive. In total my postdoc adds up to over seven years. Waiting too long is dangerous because there are

In your opinion, what makes a good scientist?

Good scientists come in many shapes and types and styles, I guess it is one of the most inclusive professions. I think some of the characteristics that tend to be overrepresented amongst scientists include being curious, adventurous, imaginative, hard-working, focused and being good at abstract thinking, collecting good data and being able to draw conclusions from them. Being honest and fundamentally humble (i.e. critical of one's own ideas) are also really important, I think. Some social skills are required too, especially at later stages of one's career, as it helps to be a good manager, supervisor and collaborator.

How do you imagine biological research will change over the next twenty years, and anything in particular in your field?

The technological advances (new reagents, robotics, and developments in IT) are speeding up research at a mind-boggling rate, and as a result the projects we are able to tackle become ever more exciting and ambitious. It is a very exciting time to be a researcher! It will be important that society keeps up with this immense influx of new information, so public outreach is incredibly important, as are discussions about research policies and ethics.

In my field, I hope we will start grasping some of the important principles of how the brain works. A lot of progress has been made but we need to understand more fully how the brain works at the molecular and cellular levels. This will help treat the many neurodevelopmental diseases there are. Also, about half of all people develop mental health issues at some point in their life, and it is very hard to help them at the moment. It would be amazing to understand more of what happens when things go wrong in the brain, and be able to help those people.

Which kind of advice you would give to somebody who would like to pursue an academic career – anything specific for women?

Don't be scared of it! I think that it has never been as good for women as it is now. There are currently many more women in academia when compared to the past. Therefore, great female mentors and examples are available. I think seeking (and later giving!) help generously is important. It is also a relatively flexible profession in which one can manage one's own time to a large extent. The flexibility helps combining personal life with work.

Elena Seiradake is an Associate Professor, Fellow, and Tutor in Biochemistry at Somerville College, Oxford.

Having grown up in Greece, Germany and the US, she graduated from the University of Konstanz in Germany, got a PhD for work in the Cusack lab at the European Molecular Biology Laboratory (EMBL) in Grenoble, and worked as a postdoc in the lab of Yvonne Jones at Oxford. She joined the Biochemistry Department in 2014 as an MRC-funded independent group leader to study the structure and function of cell surface receptors in neural and vascular development.

In 2016 she was awarded the prestigious Wellcome Trust Senior Research Fellowship to investigate the role of adhesion G-protein coupled receptors (GPCRs) in brain development.

She was elected into the 2018 EMBO Young Investigator Programme.
Her long-term aim is to understand the functions of distinct receptor-ligand complexes through a detailed knowledge of their structures and signalling properties during neural and vascular development of the brain.

Confessions of a human lab rat

by anonymous

This past summer I began giving up my body to the scientific community for a fiver at a time. As a right-handed, English speaker with 20/20 vision and no mental health struggles I came prepared with the skills I needed to be involved in this kind of research—none.

The first time I walked out of an experiment with a crisp note and the feeling of a job well done, I treated myself to a nice lunch. Not a high tea at the Randolph but something beyond my usual £3 meal deal. I was pleased with myself for a moment, selfishly satisfied by my good deed. "This is just a one-time thing," I told myself.

I don't remember how I came across the second study, one that spanned multiple days and involved an fMRI, memory training, and virtual reality game meant to test my recall. It was a step up from the incredibly simple video experiment prior. On the last day, I arrived at the John Radcliffe hospital and changed into a pair of scrubs. I was asked an array of questions: are there any pacemakers, metal, wires, or leftover bullets anywhere in my body? Have I survived any explosions? Naturally the answer was no. Up next was what I had only seen in medical TV dramas, the giant tube that usually meant the patient was in trouble. I was excited to try the scanner without suspicion of a brain tumor. The disapointing part was not being allowed to see my brain, even for fun.
For three hours I wasn't allowed to move more than my fingers to play the game. The task itself was simple but by the end I felt drained. Being motionless was hard work, but boy did it pay off in the end.

From there I found more studies; adverts pinned to bulletin boards, ones posted on departmental websites. They always say "volunteer", but I like to think of it as micro-contract work these days.

I stayed in Oxford for the summer instead of going home, so I found myself with more free time than I needed. Soon the experiments were routine. One MRI became four, and the radiologists started to remember me. By August I had a study booked nearly everyday, if not multiple. Most of the time I was assigned to doing simple computer tasks reminiscent of 90s arcade games followed by survey questions. It was easy money. My student debt diminished with every experiment, along with my stress. I was surprised by my new sense of purpose—contributing to the greater good in a strange and interesting way.

Despite the extra money I still wouldn't splash out on a much needed spa day or vacation. As a high achiever, sitting still and relaxing is a concept I struggle with. MRIs became a form of meditation, a blissfully productive hour or two to just lie still. It wasn't permissible to wriggle, squirm, or scratch. The radiologist would give me pillows, arm rests, and then tuck me in with a blanket before the three second joy ride into the tube. In the higher powered 7 Tesla scanner it's normal to experience vertigo for the first minute. If I tried hard enough I could imagine I was on a rollercoaster.

Then I discovered EEGs and the joy that is having someone massage your head with a stick while they apply electrodes and connective gel. Another task I found oddly relaxing.

I made my rounds through experimental psychology and experimental psychiatry until I finished all the studies I was eligible for. I wanted and needed more. My financial situation took a turn for the worst as my savings dwindled to summer housing costs. Clinical trials paid triple the amount of any normal study. The catch was that nearly all of them involved vaccines. My lifelong phobia of needles was practically screaming no—I had even skipped getting the tetanus jab because of it. Still, I signed up.

"Clinical trials paid triple the amount of any normal study."

The problem was not with blood. My many years with a uterus has alleviated any unease in that area. Skin puncturing was what got me. Tattoos, injectable vices, or anything more than the two holes in my earlobes were out of the question. This made me an asset in the medical testing world. Aside from a mean Twirl Bar habit, I was of relative health and purity. On the day of the first trial session, I peeled off the lidocaine gel patch I had applied myself that morning, tapping my arm to make sure I couldn't feel a thing, and squeezed my eyes shut as the needle slid into the crease of my elbow. Two, three, and then four tubes were taken. The nurse tried to distract me with a conversation about the weather. I agreed that it had been rather cloudy lately as she finally pulled the stick out of my vein. No tears, no drama. I felt victorious, sneaking a glance at the viles of my blood sitting on the cart.

The world began to soften as my hearing faded along with my vision. "This is how it ends," I thought as I melted into the chair. The nurse pushed me onto the hospital bed before I could fall completely. Fainting was new feature I hadn't yet discovered in myself. A later experiment prompted a blood glucose reading by a tiny lancet, which felt like a dream compared to funneling blood from my arm. With a snap the device pinched, and she began milking the blood from my finger to get it on to the test strip. What I've discovered through these tests is that my pain threshold is very low. As the researcher left the room to get me a plaster, I once again felt the transition from person to limp noodle begin. I was prompted to eat a chocolate muffin, which to my luck was an actual task in the study, and within a few bites it was business as usual.

Early in the Michaelmas term I started seeing sleep studies posted on the bulletins. Back home I had heard you could have a sleepover in a zoo or a museum, but a lab? Now that was something special. I took the eligibility surveys for all, getting rejected each time. I was disappointed, wondering where I went wrong. Was I no longer the perfect test subject? Nine MRIs and seven EEGs later I was a defective guinea pig.

"Nine MRIs and seven EEGs later I was a defective guinea pig. "

When an email came through about a long-term study using a FitBit I eagerly signed up and was given a place. Like any health minded adult, I've lusted after one all while being put off by the price—I couldn't spend that kind of money to monitor how inactive I was. They would pay me to wear a FitBit for eight weeks. Easy! Each step I took felt more productive; I walked more, I stopped resenting the stairs in the Radcliffe Camera, and I started getting more sleep. We would all be monitored by the researchers with some prompts and incentives thrown in. Rule number one: don't take it off.

By the end of week one it felt like a parole bracelet.

During project FitBit another opportunity came up. Once again, I made the trip to the JR for an MRI. The pre-scan activity took place in an interrogation style room. A single wire was attached to the inside of my wrist as I fixated on a pain scale. Then I realized, the FitBit would have to come off. I cheerful shoved it in my pocket and delighted in my shackle free arm. Thirty-plus electric shocks later, the definitive conclusion was still that I lacked fortitude.

Eventually I lost count. "How many have studies have you done now?" asked the researchers who I'd see regularly. As I write this my guess is thirty-five. Some treat it as though I'm partaking in something sleazy or unethical. I believe the research that Oxford conducts is for the better, whether it's how to better understand social bias, or eating disorders, or cancers. Even though I let go of my earlier life dream of being a doctor in favour of my greater passion, these experiments have filled the scientific gap in my heart that I don't get through my course. I no longer have to rely on WebMD or Grey's Anatomy for casual entertainment, instead I get to be a human lab rat. It's a job someone has to do, so why not me?

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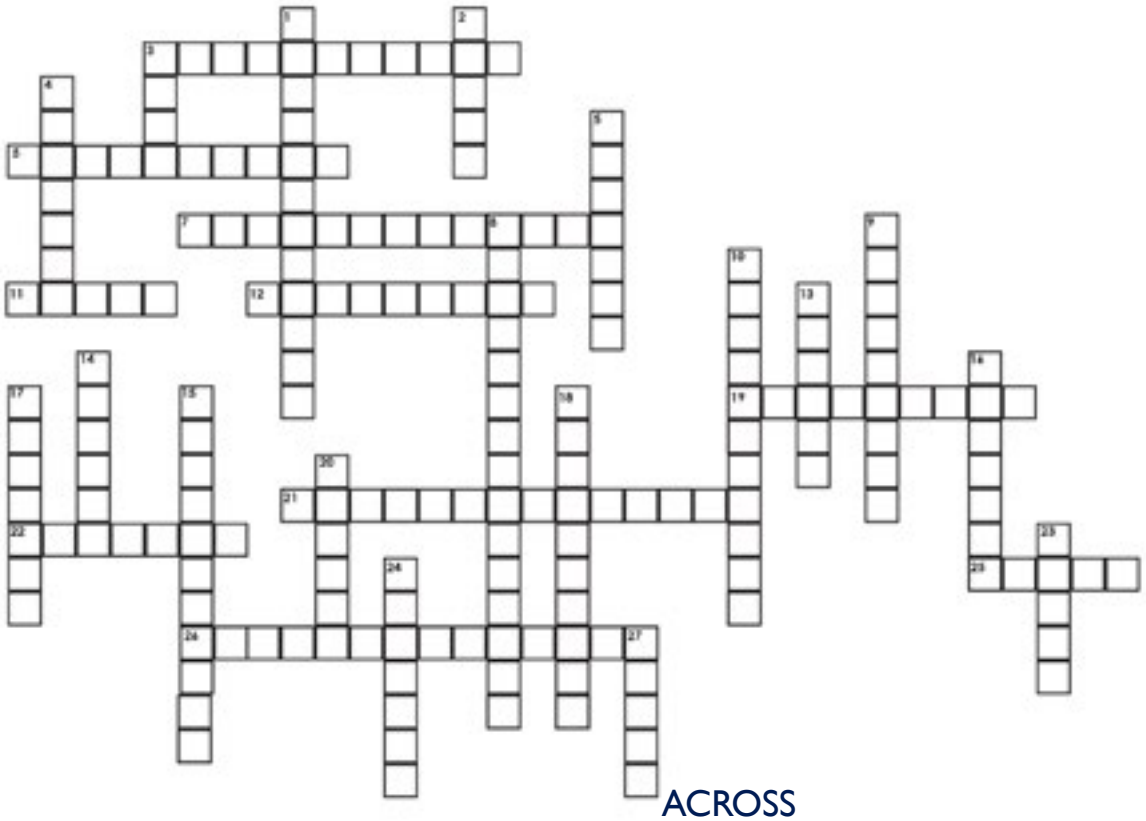

DOWN

1. The generation of neurons from neural stem cells
2. Mus musculus
3. The smallest structural and functional unit of an organism
5. The field of biology that studies the processes by which multicellular organisms grow and develop, controlled by their genes
8. Developmental Biologist who received her Nobel Prize for Physiology or Medicine in 1995 for work concerning the genetic control of embryonic development
9. The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment
10. Highly migratory cell population found in vertebrates
13. A diploid cell resulting from the fusion of two haploid gametes
14. An immature egg cell of the animal ovary
15. The study of heritable changes in gene expression that do not involve changes to the underlying DNA sequence
16. Cell division that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus
17. Cell division that results in four daughter cells each with half the number of chromosomes of the parent cell
18. The animal model in which the GAL4/UAS system is primarily used
20. this can be regarded as a disease of altered development
23. Animals renowned for their ability to regenerate limbs
24. An animal model which was particularly important in early tissue transplantation experiments. Originates from South Africa.
27. A highly conserved juxtacrine signalling pathway. Used to regulate an array of cell fate decisions in development

ACROSS

3. The author of this issue's PI article
6. The process of forming nodules and especially root nodules containing symbiotic bacteria
7. The field of biology that studies the processes by which multicellular organisms grow and develop, controlled by their genes
11. The first organ to function in the embryo
12. A model very important to regenerative research
19. Programmed cell death
21. The organ system most commonly affected by congenital defects
22. Embryologist who was awarded the Nobel Prize for Physiology or Medicine in 1935 for his discovery of embryonic induction by organisers
25. Male gametes
26. The process by which cells, tissue, and organs acquire specialized features, especially during embryonic development

SOLUTIONS

DOWN

1. Neurogenesis
2. Mouse
3. Exosome
5. Biogology
8. Nusslеinvolhard
9. Phenotype
10. NeuralCrest
13. Zygote
14. Oocyte
15. Epigenetics
16. Mitosis
17. Meiosis
18. Drosophilia
20. Cancer
23. Newt
24. Xenopus
27. Notch

ACROSS

3. Clive Wilson
6. Nodulation
7. Developemental
11. Heart
12. Zebrafish
19. Apoptosis
21. Cardiovascular
22. Spemann
25. Sperm
26. Differentiation