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LETTER FROM THE EDITOR

Welcome to the HT19 issue.

This issue is special on several counts. Firstly, in addition to our regular sections, we have a new section, 'Aspire to Inspire' which focuses on Women in Science, with a special emphasis on the personal struggles that have powered their science and lives. Secondly, through this issue we also celebrate the spirit of collaboration and teamwork. Researchers from across the world-in particular our close neighbours from Cambridge, have contributed articles, thus widening the spectrum of the Phenotype family. I hope our ties within the scientific community continue to get stronger with the passage of time.

On page 4, Dr. Alberto Lopez Baena and Claire Hill describe novel roles for death-associated caspases. On page 16, join Dr. Maria Blanca Torroba on an exciting journey involving neural crest cells. Turn to page 13, where Iqbal Dulloo touches upon the emerging roles of inactive proteases.

On page 9, Errin Johnson describes a new technique in Electron microscopy for high resolution characterisation of biological samples, and on page 14, Anna Caballe discusses the past, present and future of various super-resolution light microscopy techniques.

In conjuction with our spirit to promote collegiality, we have Indu Santhanagopalan and Katharina Kessler, our colleagues from Cambridge, discussing diverse facets of their research. On page 11, Indu writes about alternative lifestyles of photosynthetic microbes, while on page 18, Katharina shares the story of IHAT, a novel iron supplement and it's journey through various phases of a clinical trial. Turn to page 21, where I speak about my experience at the RisingWISE workshop, an Oxbridge initiative to promote entrepreneurial spirit amongst women scientists.

On page 26, Samantha Moore has crafted a beautiful Research Infographic, vividly illustrating the events leading upto the discovery behind the 2018 Nobel Prize for Physiology and Medicine.

On page 25, Swathi Lingam writes about an efficient way to degrade plastic, and on page 22, Komal Yasmin interviews Prof. Garmen about scientific outreach.

As part of the Aspire to Inspire initiative, we celebrate the personal and professional achievements of various women scientists from academia and industry. We have Laura Hankins on page 34, describing Oxford's celebration of 100 years of the Suffragette movement. On page 35, Fiona Neelson shares her story behind Repositive and on page 36, Hannah Nazri writes about what helped her spearhead the Kalsom movement. On page 32, read a brave account of Priti Gupta's travels with her tumor. We hope these stories will inspire you to strive for your goals with renewed motivation. For our next issue, we invite entries and nominations for this section, from both men and women.

Most successful initiatives have always been driven by teamwork. This holds true for *Phenotype* as well. This magazine owes its existence and popularity to all the authors and editors, who quite painstakingly and voluntarily contribute their time and effort. The idea behind *Phenotype* was conceived and executed by a dedicated group of PhDs and Post-Docs. Watch out for our next issue that will explore the history of this magazine in detail.

Sonia Muliyil

Editor-in-Chief

Phenotype is also available to read online via our website: **www.phenotype.org.uk**

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EDITORIAL TEAM

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ASPIRE TO INSPIRE

CASPASES AND THEIR ROLES BEYOND DEATH

By Alberto Baena-Lopez and Claire Hill

Alberto Baena-Lopez is a Principal Investigator at the Sir William Dunn School of Pathology and a Career Development Fellow of CRUK and Oriel College.

Claire Hill is a third year Interdisciplinary Bioscience PhD student (BBSRC Doctoral Training Partnership) in Luis Alberto Baena-Lopez's lab, in collaboration with Prof. Dave Carter (Oxford Brookes).

The term apoptosis, coined in 1972 (1), refers to one of the major genetically programmed forms of cell death. Apoptotic cells have characteristic features such as a progressive stop of all essential cellular functions, nuclear condensation, DNA degradation, and cell fragmentation into small membrane-bound vesicles that are shed as apoptotic bodies (1). Apoptosis is a vital cellular process that allows defective or unnecessary cells to be eliminated, thus ensuring cell replacement, proper development and tissue homeostasis. Central to the process of apoptosis are an evolutionarily conserved family of cysteine-aspartic proteases, called caspases. The genetic control of caspase-mediated apoptosis was delineated by Ellis and Horvitz in 1986 (2). Evolutionary conservation of caspase enzymatic activity was later shown in 1993, when the structural and functional similarities between mammalian interleukin-1β-converting enzyme (ICE or caspase-1) and the C. elegans gene ced-3 were discovered (3). Since then, many proteins with analogous properties have been identified across many species, and research efforts have focused on understanding their regulation and activity during apoptosis. Paradoxically, recent research has now uncovered the involvement of caspases in a large repertoire of cellular activities, beyond their role in apoptosis (Figure 1). These new, non-apoptotic roles appear to support core cellular functions such as cell proliferation, metabolism, cell differentiation, secretion and cell migration, via mechanisms that are not yet fully understood.

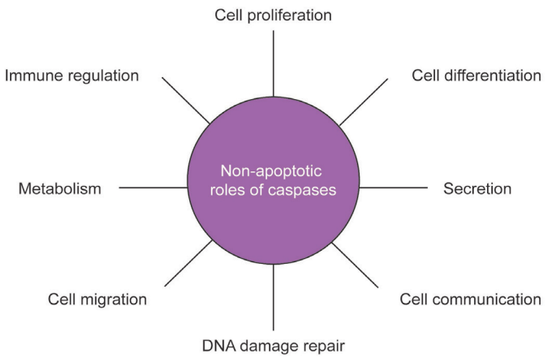

Figure 1. Non-apoptotic roles for caspases in normal cell function, which when deregulated, can lead to diseases such as autoimmunity, neurodegeneration and cancer.

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Furthermore, if deregulated, these novel caspase functions can instigate and facilitate the progression of multiple diseases, such as autoimmunity, neurodegeneration and cancers. These findings are opening up new and exciting biological questions, encouraging scientists to look beyond the original role of these enzymes.

Due to the easy recognition of apoptotic cells, and readily available cellular manipulations to trigger apoptosis, research into non-apoptotic caspase roles has been overshadowed for many years. However, recent technical advances in the detection of caspase activation in vivo, using simple model organisms such as Drosophila melanogaster (4) (Figure 2), along with the functional conservation of caspases across species, are facilitating the analysis of non-death related caspase functions. In the laboratory (www.caspaselab.com), we utilise Drosophila melanogaster

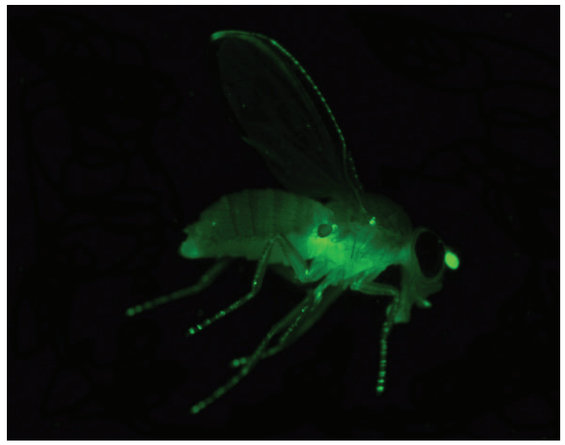

Figure 2. Non-apoptotic caspase expression (green) in an adult Drosophila melanogaster (fruit fly).

and mammalian cells as models to investigate caspase biology, in both apoptotic and non-apoptotic situations. We are particularly interested in uncovering the molecular networks connecting caspases with the regulation of stem cell properties, as well as the potential association of these functions with the origin and/or progression of diseases such as cancer.

One hallmark of cancer cells is their ability to evade cell death, and therefore strong connections have been made between caspases and tumorigenesis. The ability of cancer cells to circumvent death often relies on their high level of anti-apoptotic factors, such as BCL-2, and/or their deficiency in pro-apoptotic proteins, including Bcl-2 associated X protein, BAX (5). Nevertheless, this is not the only biological process regulated by caspases that can incentivise the appearance and expansion of malignant cells. As previously mentioned, caspases can decisively influence the dynamics of self-renewal and differentiation of stem cells (6). Fujita and collaborators vividly illustrated the implication of caspases in the regulation of stem cell properties. They elegantly demonstrated that caspase-mediated degradation of the transcription factor Nanog in mouse embryonic stem cells, precedes their exit into the differentiation pathway (7). Caspase cleavage of Nanog is also essential for human stem cell differentiation, further highlighting the importance of caspases in this biological process. It is therefore not surprising, that deregulated caspase activity, and ultimately stem cell function, often sits at the heart of tumour development. Several projects in the laboratory are intensively studying the crosstalk between caspases and specific signalling pathways implicated in the regulation of stem cell properties. Our experiments indicate that the regulation of caspase-mediated stem cell properties is highly tissue-specific; requiring the intersection of caspases with specific signalling pathways within each cell type. Our findings prove the versatility of these enzymes to interfere with a large repertoire of cellular events, whilst also exemplifying the complexities involved in gaining a full understanding of caspase biology.

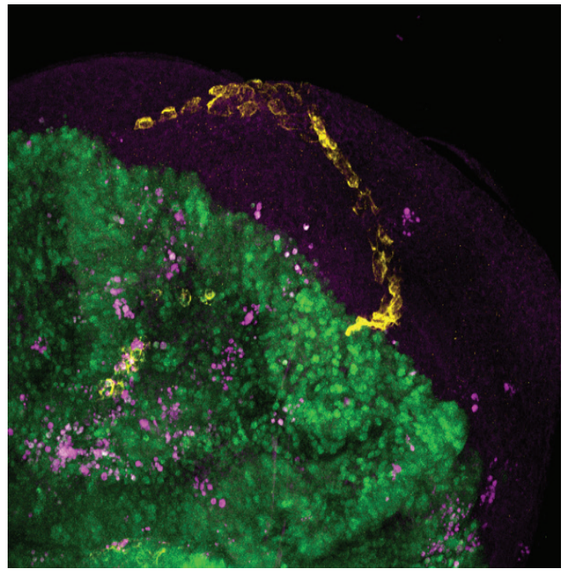

Figure 3. Imaginal wing disc from Drosophila melanogaster with tumoural cells (green) and DCP-1 (Death Caspase-1) activity (magenta) highlighted. Immune surveillance cells in Drosophila, called haemocytes (yellow), are present at this area of cell competition to clear away dead cell debris. We are currently studying the role of caspases in haemocyte recruitment in the tumoural setting. Image courtesy of Derek Xu.

Another hallmark of cancer cells is their ability to manipulate signalling pathways and influence the tumour microenvironment, allowing them to proliferate and evade immune system detection. Again, caspases have been shown to contribute to these cellular processes in tumour cells. For example, caspases appear to modulate the recruitment of immune cells to tumours by finely adjusting the extracellular levels of reactive oxygen species, which leads to apoptosis-induced proliferation in neighbouring healthy cells (8). Although our results, to a large extent, confirm previous findings, they also suggest that this function of caspases is not universal, but highly dependent on the tumour origin. Current projects in the laboratory are trying to elucidate the basis of this functional diversity and the underlying molecular mechanisms involved.

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Many of the cellular functions mediated by caspases depend upon cell communication mechanisms. One method used by cells to communicate between neighbouring and even distant cells are extracellular vesicles (EVs). EVs are a heterogeneous group of membrane bound carriers, categorised depending on their size and origin. EVs are present in many biological fluids and carry RNAs, proteins and lipids, to be taken up by recipient cells (9). EVs play important roles in normal cellular processes, such as development, immunity, and neuronal communication (10). Research indicates the association of caspases with the loading, release and uptake of EVs. In particular, it has been shown that caspase-8 controls the secretion of lysyl-tRNA synthetase (KRS) from cancer cells. This alters the tumour microenvironment and inflammatory responses, creating a favourable niche for cancer cell survival and proliferation (11, 12). Research has also shown that the anti-apoptotic protein Bcl-xl is cleaved by caspase-3 within exosomes (a subgroup of EVs). Bcl-xl cleavage is required for these exosomes to be taken-up by myeloma and lymphoma cells, thus contributing to their uncontrolled proliferation (13). The fundamental role of EVs during inter-cellular communication, particularly in the development and progression of pathologies such as cancer, is beginning to be more widely explored; however, the molecular mechanisms regulating EV loading and release remain largely unknown, owing to technical limitations. Some progress has recently been made to study EV-mediated communication in complex biological systems, with the development of a Cre-LoxP model in mice (14-17). We are adapting this model for Drosophila melanogaster to gain an evolutionary perspective regarding the molecular mechanisms involved in EV-mediated communication and the potential crosslink with caspase activity.

From a therapeutic perspective, caspase activation has been one of the main strategies used to kill cancer cells. However, it is likely that some of these treatments will need to be revised in the near future, as these novel non-apoptotic functions may be having unexpected impacts on patient recovery and/or relapse. This does not reduce the potential for caspases to be used as therapeutic targets, but highlights the need for a more refined approach, taking into consideration both apoptotic and non-apoptotic caspase functions. Along these lines, one specific project within our group is currently focused on investigating the implication of caspases in the DNA repair process and the molecular mechanisms that may aid tumour suppression. Thor-

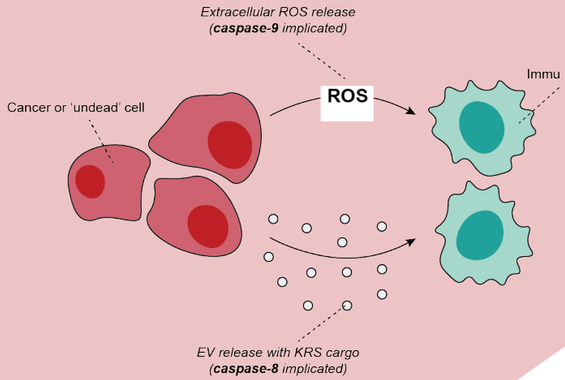

Figure 4. Schematic of how caspase-9 has been implicated in the release of ROS (reactive oxygen species) from 'undead' cells to recruit immune cells, which ultimately leads to apoptosis-induced proliferation in neighbouring healthy cells. Cancer cells have been shown to modulate the activity of neighbouring cells through the release of specific cargos via extracellular vesicles (EVs). Caspase-8 controls the secretion of lysyl-tRNA synthetase (KRS) from cancer cells to alter inflammatory responses, creating a niche for

ough this research, we hope to identify molecular factors that associate with caspases to play vital roles in the decision between cell-death or DNA repair routes.

In summary, we are investing a substantial amount of effort into developing tools that will enable us to manipulate and visualize caspases in vivo, with the ultimate aim of uncovering caspase involvement in a wide range of biological processes, which are not related to cell death. We hope our research will begin to provide answers to some of the key questions in the field such as, what molecular mechanisms are involved in the regulation of these newly discovered roles for caspases? What is the subcellular localisation of caspases during their non-apoptotic activities? What signalling pathways do caspases interact with to regulate specific cellular functions? We hope that by providing answers to these questions, we will contribute to building a comprehensive understanding of caspase biology, highlighting their primary roles and ultimately, improving our ability to harness their therapeutic potential.

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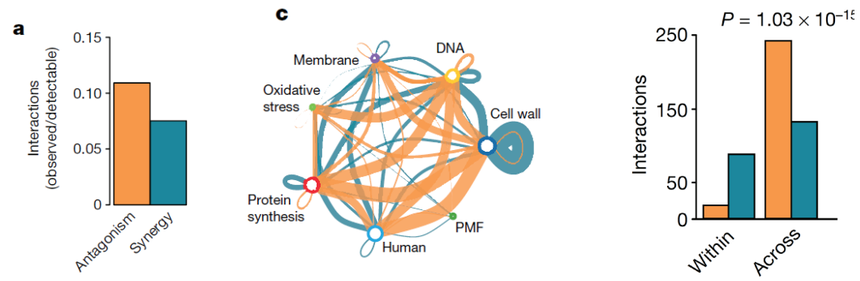
COMBINATIONS TO COMBAT BACTERIA

Can combination therapy buy us time in the fight against resistant bacteria?



Left: Brochado et al. observed more antagonism than synergy. Middle: The network shows antagonisms and synergies between drugs grouped by the cellular process they target. The strong synergistic effect observed between drugs targeting the cell wall is predominantly due to β-lactam antibiotics. "Human" describes other compounds like food-additives. Right: The graph quantifies the interactions of the network: antagonism almost exclusively occurred between drugs targeting different processes. (From Brochado et al., 2018)

By Hannah Behrens

Hannah Behrens is a PhD student in the Kleanthous lab in the Department of Biochemistry.

The interplay between different antibiotics, and between antibiotics and other drugs, is much more complex than previously assumed. New studies found that while some combinations improve efficacy, the majority decrease efficacy, and some even speed up the development of resistance. The situation is further complicated by these effects being species specific. Can a better understanding of drug combinations help us to slow the development of resistance and buy us time in the fight against super bugs?

The whole is more – or less – than the sum of its parts

Traditionally, bacterial infections were treated with a single antibiotic. However, some pathogens, like Mycobacterium tuberculosis, , routinely require combination therapy of at least two antibiotics. The question of whether combinations of antibiotics are better or worse than just the sum of the individual effects had not been addressed until earlier this year.

Elif Tekin et al. (1) selected eight antibiotics, all acting by different mechanisms, and combined two, three, four or five of them. A total of 20 000 combinations was tested against E. coli; surprisingly, some of them showed net synergy, which is when the combination acts better than the sum of the two individual antibiotics, some instead showed antagonism, which is when the combination is less effective than the expected sum of the individual effects. Much more research is needed on the topic, but understanding combinations of antibiotics and choosing them wisely might buy us some time in the fight against super bugs.

"There is a tradition of using just one drug, maybe two," explains Pamela Yeh, one of the authors of the study and Assistant Professor at the University of California. "We're offering an alternative that looks very promising. We shouldn't limit ourselves to just single drugs or two-drug combinations in our medical toolbox. We expect several of these combinations, or more, will work much better than existing antibiotics." Apart from exploiting synergistic combinations, avoiding antagonistic ones will also be essential.

Can other drugs and food influence efficacy?

Ana Rita Brochado and her colleagues took these thoughts one step further. If combining antibiotics with each other influences their efficacy, what happens when antibiotics are combined with other drugs and even food additives? After all, a patient taking antibiotics, might also take other medication and consume food additives. Looking at the effects on E. coli, Salmonella enterica and Pseudomonas aeruginosa, the international group of researchers tested 3000 pair-wise combinations (2). Synergy occurred most often between antibiotics of the same classes, while antagonism occurred mostly between different types of drugs. The researchers observed more antagonism than synergy and found that 70% of drug–drug interactions were species specific. This means that if we truly want to understand antibiotic combination therapy, a lot more research is needed.

A third effect besides synergy and antagonism was observed by Min Jin and his colleagues: the antidepressant fluoxetine accelerates the development of resistance when combined with antibiotics (3). Compared with a control of chloramphenicol, amoxicillin or tetracyclin alone, the addition of fluoxetine to each of

these antibiotics, resulted in up to 50-million-fold increased resistance. Genomic, transcriptional and proteomic analysis suggest that fluoxetine increases the mutation frequency, by creating reactive oxygen species which mediate mutagenesis, including deletions, insertions and substitutions. Mutants with up-regulated efflux pumps, especially the AcrAB-TolC pump, the YadG/YadH transporter, a Tsx channel and the MdtEF-TolC pump, displayed increased resistance to the tested antibiotics.

The resistance-accelerating effect of fluoxetine is particularly concerning, considering that it is one of the most widely used antidepressants.

The resistance-accelerating effect of fluoxetine is particularly concerning, considering that it is one of the most widely used antidepressants. "Fluoxetine is a very persistent and well-documented drug in the wider environment, where strong environmental levels can induce multi-drug resistance," notes Jianhua Guo, one of the researchers involved in the study. "[It] has previously been an invisible factor in the spread of antibiotic resistance," adds his co-author Min Jin. "We should consider this a warning".

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Triclosan, friend or foe?

The same researcher found that triclosan, an antimicrobial compound used in soaps and toothpaste, lead to increased resistance in E. coli (4). However, this resistance-increasing effect was only observed at one concentration of triclosan; higher and lower concentrations had no effect. Moreover, the increase in resistance was only 6.7-fold – significantly lower than the 50-million-fold change observed with fluoxetine.

The effects of triclosan are controversial because a different research group, led by Christopher Waters from Michigan State University in the USA, found that triclosan acts as an adjuvant for the elimination of P. aeruginosa, Burkholderia cenocepacia and Staphylococcus aureus (5). Triclosan or the antibiotic tobramycin alone were not effective, but when combined, they resulted in a 100-fold reduction in viable cells within P. aeruginosa biofilms. Pseudomonas are a common complication in hospital acquired infections, and natural and acquired resistance put them at the top of the WHO's list of pathogens against which we urgently need new antibiotics (6).

Synergetic effects were also observed between triclosan and gentamicin, and triclosan and streptomycin.

A more general approach to how combining antibiotics affects the development of resistance was taken by Suzuki et al. in 2017 (7). As with the other large scale studies, some combinations had positive or negative effects on the development of resistance and the fitness of the resistant strains. The authors hope to be able to choose antibiotic combinations such that strains resistant to both will have low fitness.

Moving towards personalised medicine

Now that we realise that species-specific effects occur even for broad spectrum antibiotics, we need species-specific research, diagnostics and therapy decisions. Personalised medicine has arrived in the field of antibiotics! Now it is time not only to personalise treatments for each patient but also for each bacterium. Eventually, all these combinatorial datasets and their different aspects need to be integrated with one another. Perhaps then, we can design antibiotic combination therapy specific to each bacterial species, while taking into account efficacy and development of resistance.

A race against time

All six studies evaluated anti-microbial effects in vitro. While all substances tested are already known to be safe for use in humans, the effects of combinations have yet to be tested in vivo. In the presence of hundreds of other components in our bodies, synergistic, antagonistic and resistance-enhancing effects may or may not occur.

Because effects differ between species, thousands of combinations of drugs need to be tested for hundreds of clinically relevant species – first in vitro, then in vivo. Nonetheless, this process would probably be faster and cheaper than developing new antibiotics, especially if automated large-scale screens are employed. By avoiding antagonistic and resistance-enhancing combinations and promoting synergistic ones and those slowing down resistance, humanity could gain some time in the race against antimicrobial resistance. Eventually we will need both, a good understanding of antibiotic combination therapy and new antibiotics.

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ELEMENTAL BIOLOGY

Using energy dispersive x-ray spectroscopy with scanning electron microscopy for high resolution characterisation of biological samples

By Errin Johnson

Errin is head of the Dunn School Electron Microscopy Facility.

Recent years have seen a surge of progress in biological electron microscopy (EM). This has been spearheaded by the cryo-EM juggernaut (reviewed by Bai et al 2015 (1)) and also assisted by developments in EM-specific genetic tags, correlative microscopy (see de Boer et al 2015 (2); Hauser et al 2017 (3)) and volume microscopy (reviewed by Kremer et al 2015 (4)). The latter technique uses a scanning electron microscope (SEM) with either an in situ microtome (Gatan 3View system) or a focussed ion beam (FIB) to serially slice a sample in between image acquisition with the electron beam, to produce a high resolution volume that can then be used to reconstruct cellular ultrastructure in 3D.

In fact, FIB-SEM was routinely used in material science and engineering applications for many years before it entered the mainstream of biological EM, where it is now employed not only in volume EM studies but also in cryo-lamellae preparation for cryo-EM (5). Now another SEM-based technique known as energy dispersive x-ray spectroscopy (EDS, also referred to as EDX) is poised to follow the same pattern. This is made possible by a new generation of sensitive and fast x-ray detectors which enable the chemical composition of cells and tissue in the SEM to be mapped beyond 10 nm resolution.

Principles of EDS microanalysis in the SEM

In an SEM, a focussed beam of high-energy electrons is scanned across a sample. At each point along the scan there are multiple interactions between the electron beam and the atoms at, and just below, the sample surface. As a result, several different signals are generated from the sample which, if you have the appropriate detectors available, can give you information regarding topography (secondary electrons, producing the 'traditional' SEM image), variations in atomic number (back scattered electrons), cathodoluminescence properties (photons), internal ultrastructure (transmitted electrons, for thin samples) and chemical composition (characteristic x-rays).

Characteristic x-rays are emitted from an atom when one or more of its electrons transition from a higher to a lower energy state. In SEM, when an electron from the beam hits an atom, part of its energy can be transferred to an inner shell electron, which then has enough energy to exit the atom as a secondary electron. The resulting vacancy is filled by an electron transitioning down from a higher energy, outer shell. When this occurs, a characteristic x-ray with an energy equivalent to the difference in energies between the inner and outer shells (or sub-shells) involved in the transition is emitted. These energy differences are specific to each element, such that measuring the energy of characteristic x-rays generated by the sample enables you to identify the chemicals present within the sample.

This x-ray measurement is performed using an EDS detector, which is inserted as close as is safely possible to the sample surface. This will usually have a take-off angle of between 30 to 40 degrees in order to maximise the number of x-rays the detector collects. The energy of a characteristic x-ray is measured by the amount of ionisation produced when it strikes the silicon drift detector, which outputs the signal as a spectrum of x-ray counts versus energy. This enables the distribution of elements across the sample to be mapped and quantified. The higher the number of x-rays you detect, the better the ratio between your characteristic x-ray peaks and the background level of continuous x-rays generated by other beam-sample interactions and the more robust your chemical identification and quantification are.

Specific requirements for biological SEM-based EDS

Microanalysis of biological samples using SEM-based EDS can be more problematic than for materials samples for several reasons. Firstly, soft samples, such as cells and tissue, require extensive processing to ensure that they are stable when exposed to high vacuum conditions in the SEM and to the electron beam. This processing, particularly the ethanol dehydration steps, can affect the distribution and concentration of elements in the sample. Heavy metal stains (e.g. uranyl acetate and osmium tetroxide) are used to contrast cellular membranes and constituents in EM. While these stains may mask weak characteristic x-ray signals from low abundance elements, omitting them altogether can make it challenging to correlate the x-ray signal with poorly contrasted ultrastructure. Weak x-ray signals may also be drowned out by the signal from conductive coatings; thin layers of gold, platinum or carbon are typically applied to SEM samples to reduce their reactivity to the electron beam. However, even with a conductive coating, beam sensitivity can be a significant limitation to producing high quality elemental maps. This is because the beam energies required to generate characteristic x-rays from many elements are often an order of magnitude higher than that normally used for routine SEM imaging of biological samples without charging artefacts. This, combined with long acquisition times to collect sufficient x-rays for meaningful quantification, often results in sample drift, reducing the spatial resolution of the elemental maps.

Long acquisition times and high beam energies and currents were also, until recently, a requirement of EDS detectors themselves. Traditionally, the detector crystal is maintained under vacuum and is isolated from the SEM chamber by a window. How-

ever, this window can attenuate low-energy x-rays emitted from lighter elements commonly found in biological samples, such as nitrogen, oxygen, sulphur, and sodium. There is now a new generation of windowless detectors available specifically designed to enable detection of low-energy x-rays using much lower electron beam energies and currents. This design, coupled with new detector geometry as the detector can be placed closer to the sample with a larger collection angle, increases sensitivity to low energy x-rays by 10 to 30 times. With improved electronics and computing power, faster collection and real-time data processing are also possible. Now x-ray maps with a better signal-to-noise ratio in the lower end of the x-ray spectrum, and with a spatial resolution close to that of the SEM, can be obtained from biological samples in shorter times and with less sample damage. This enables even low abundance elements to be quantified and their ultrastructural location(s) within the cell to be accurately pinpointed by overlaying the elemental x-ray map with the corresponding SEM image (which can be simultaneously acquired).

By coupling improved detector capabilities with new optimised sample processing protocols and raising general awareness of the technique as a viable tool for microanalysis of soft samples, the stage is set for SEM-based EDS to become much more routinely used in bio-relevant research.

Applications of biological EDS

Indeed, there are diverse applications for SEM-based EDS, as well as for other microanalysis techniques (reviewed by Pirozzi et al, 2018 (6)), across many research areas, including cell biology, biomedicine, bioengineering, microbiology, environmental research and plant sciences. For instance, EDS can be used to identify and quantify particulates from environmental pollutants both on the surface of samples (e.g. leaves from plants in heavy traffic areas) and within tissues (e.g. investigating the role of environmental nanoparticles in neurodegenerative diseases). Metal accumulation and depletion can be quantified by EDS in bacteria, mammalian culture cells and in healthy and diseased tissue (e.g., in cells of the gut). Furthermore, subcellular compartments can be characterised using EDS microanalysis for their accumulating metals, such as iron and caesium, as can degradation products from biomedical implants (e.g., patient-derived nanoparticles after hip replacement). This technique also facilitates identification of specific cell types and organelles through the use of exogenous tracers (e.g. gold- and cadmium-labelled antibodies) or, in some cases, by using their elemental 'fingerprint' for label-free identification. For example, Scotuzzi and co-workers use SEM-based EDS (7), which they refer to as 'ColorEM', to identify cells in rat pancreatic tissue by the elemental content of their granules, as the glucagon granules in alpha cells are rich in phosphorous and the insulin granules in beta cells are rich in silicon.

In the Dunn School EM Facility, we are applying EDS to the study of Type 1 congenital dyserythropoietic anemia (CDA-1) in collaboration with Veronica Buckle's group at the WIMM. This disease is characterised by erythroblasts exhibiting a distinctive 'spongey' nuclear phenotype by TEM and we are in the process of using EDS to determine whether the 'holes' in the heterochromatin are improperly packed chromatin or protein by quantifying their relative nitrogen, phosphorous and sulphur levels (Figure 1).

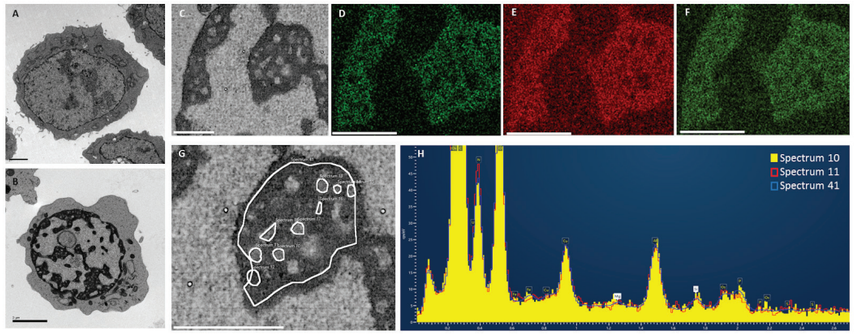

Figure 1. Using EDS microanalysis to study Type-I congenital dysmorphic anaemia (CDA-I). TEM of healthy (A) and CDA-I (B) erythroblasts. Both euchromatin and heterochromatin appear abnormal in CDA-1 nuclei, with the heterochromatin exhibiting distinct holes/inclusions. To determine whether these holes contain protein or DNA, SEM-based EDS microanalysis was employed (C-H). An example of a CDA-I nucleus imaged using the secondary electron detector (C) and then with the Ultim Extreme EDS detector, which produced maps of the phosphorous (D), nitrogen (E) and uranium (F) distribution. Because X-ray spectra are collected at every pixel, post-analysis extraction and quantification of spectra from regions of interest (G) is possible, such that the spectrum from a specific area of the heterochromatin can be compared to that from the holes within it (H). Preliminary results indicate that there are differences in nitrogen and phosphorous levels between holes and relative to the entire heterochromatin area. Scale bar represents 1 um.

All of the techniques mentioned here, including EDS with our new Ultim Extreme detector from Oxford Instruments, are available through the Dunn School EM Facility and the Central Oxford Structural Molecular Imaging Centre (COSMIC). Please contact me (errin.johnson@path.ox.ac.uk) for further information or to setup a meeting to discuss how SEM-based EDS or, indeed, EM in general, could benefit your research. Other microanalysis techniques, such as nanoscale secondary ion mass spectrometry (NanoSIMs) and electron energy loss spectroscopy (EELS), which can be applied to biological samples (see Pirozzi et al, 2018 (6)) are available through the David Cockayne Centre for Electron Microscopy in the Department of Materials.

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ALTERNATIVE LIFESTYLES OF PHOTOSYNTHETIC MICROBES

By Indu Santhanagopalan

Indu Santhanagopalan is a postdoctoral researcher in Howard Griffiths' lab working on the TIGR2ESS (Transforming India's Green Revolution by Research and Empowerment for Sustainable food Supplies) project at the Department of Plant Sciences, Cambridge.

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the world's most abundant protein, forming the basis of almost all life on Earth. It catalyses the major step of the C3 photosynthesis (Calvin-Benson-Bassham cycle), which helps convert carbon dioxide (CO2) to energy-rich molecules such as glucose in photosynthetic organisms. Despite its pivotal role in the biosphere, Rubisco is an inefficient and promiscuous enzyme. In addition to the carboxylase activity which forms the basis of photosynthesis, oxygen (O2) can also act as a substrate for Rubisco, leading to the formation of phosphoglycolate (Fig. 1A). Phosphoglycolate is recycled through metabolic repair steps of photorespiration, leading to loss of fixed carbon as CO2. The inability of Rubisco to prevent its own reaction with O2 and low affinity for CO2, greatly reduces the photosynthetic capacity of organisms.

This inefficiency of Rubisco can be traced back to its origins in a CO2-rich, O2-poor atmosphere over 2.4 billion years ago. The levels of O2 and CO2 are estimated to have been around .01-0.1 and 100 times the current atmospheric levels respectively, during this period. Further, the extent of dissolution in water for CO2 and O2 are different, with CO2 being approximately 30 times more soluble than O2 in sea water. This environment had selected forms of Rubisco that could operate quite effectively with low affinities for CO2 and high affinities for O2, and lead to declining CO2 and rising O2 levels in the atmosphere over geological time scales. Around 450 million years ago, O2 and CO2 levels were equimolar in marine water. It is suggested that this threshold for atmospheric O2 levels, where it overtakes the level of CO2 in water, could be one of the drivers for land invasion by plants. It is also worth noting that affinities for O2 and CO2 for modern Rubisco molecules in plants and algae appear to be tuned, such that they experience a competition of 1:1 between O2 and CO2 molecules. The evolution of Rubisco in changing atmospheres over billions of years described above has been reviewed in detail by Griffiths et al. (1). Rubisco molecules in different organisms having undergone modifications to reflect changes in the O2:CO2 ratios, but they still remain largely inefficient owing to their origins in an atmosphere of different composition.

Despite all its caveats, Rubisco is responsible for the production of >99 % of all organic carbon, which supports life on Earth. Nearly 50 % of the productivity of oxygenic photosynthesis is attributed to the hydrosphere. An incredible feat, considering that 3 PgC(1015 grams of carbon) of aquatic biomass (primarily consisting of microbes) delivers an annual net primary productivity of 47.5 PgC, relative to the 56.4 PgC produced by 610 PgC of terrestrial plant biomass

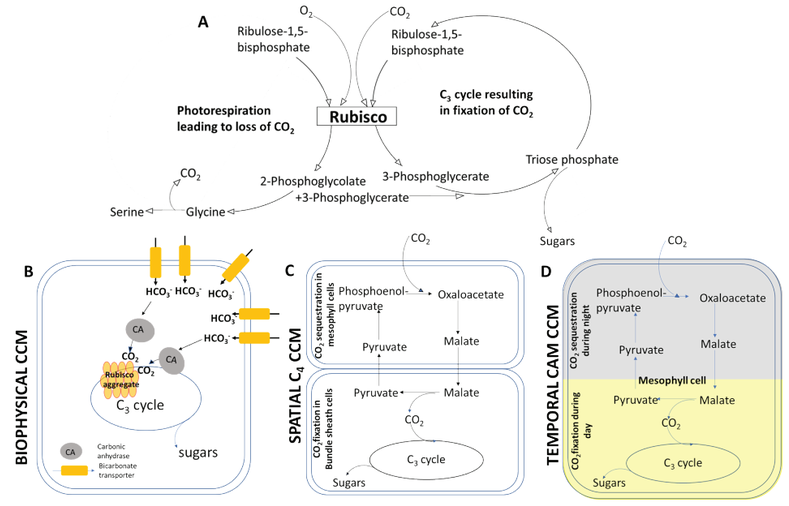

Figure 1. Schematics representing (A) photorespiration and C3 cycle in C3 plants; biophysical, spatial and temporal CCM in photosynthetic microbes (B), C4 plants (C), and CAM plants (D) respectively.

(1). The lower access to dissolved CO2 in bodies of water, the result of low solubility and diffusivity and the vast extent of surface boundary layers, only serves to make this a more amazing achievement for the aquatic microbes, primarily cyanobacteria and eukaryotic algae. This raises the question: how do these microbes manage to punch above their weight? The answer lies in the emergence of carbon concentration mechanisms (CCM) around 410 Ma coinciding with an O2:CO2 ratio turning >1 in marine water that helped increase the concentration of CO2 surrounding Rubisco.

Although these CCM strategies employed by algae and cyanobacteria evolved independently and they employ distinct components, both invoke the following three key pillars (Fig. 1B)(2): (i) biophysical inorganic transporters operating in parallel across membranes to increase the inorganic carbon pool by 40-fold (chlorophytic algae) to 400-fold (Cyanobacteria); (ii) carbonic anhydrases placed strategically to assist in bicarbonate interconversion or regeneration of CO2 close to Rubisco; and (iii) a microcompartment for Rubisco aggregation, like the capsular cyanobacterial carboxysome or algal pyrenoid in the chloroplast (Fig. 2), from which leakage of CO2 is minimized. It must be noted that while cyanobacterial CCMs are constitutive, algal CCMs are inducible by environmental cues such as light, pH and CO2 availability. The orchestration of CCM in diverse systems requires the interplay of several proteins. The increasing availability of genome sequences for several microbes and structural information for various CCM components will shed more light on the intricacies of the processes that establish this phenomenon. In this regard, the extensive mutant library for the model green alga Chlamydomonas reinhardtii (3) provides tools to investigate the various factors that come into play in establishing CCM. The recently identified role for the linker protein EPYC1 in bringing Rubisco molecules together within pyrenoid compartments has provided more cues to researchers trying to engineer CCM in commercial crop plants (4).

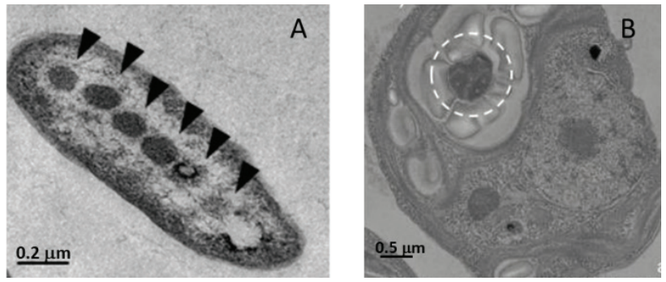

Figure 3. Electron micrograph of (A) carboxysomes (shown by arrows) in Halothiobacillus neapolitanus (By Gonzalez R and Kerfeld C, https://www.kerfeldlab.org/images.html) and (B) pyrenoid (encircled) in chloroplast of Chlamydomonas reinhardtii (6)

Most land plants including several commercial crops are dependent on C3 photosynthesis, with considerable losses owing to photorespiration. The separation of CO2 sequestration from photosynthetic machinery either spatially (in C4 plants such as maize) or temporally (in CAM(Crassulacean acid metabolism) plants such as cacti) minimizes photorespiration in some land plants (Figs 1C and 1D). Engineering of CCM in C3 plants that are limited in their crop yield by inefficacies of photosynthetic pathways would act as a means of increasing their productivity. With the world headed towards a human population of 9 billion by 2050, there is a dire need to increase crop yields. Bruinsma estimates a requirement of at least 70 % increase in current productivity to meet our needs in 2050 (5). The genetic modification of C3 plants to introduce a mode of carbon concentration offers a possible means to enhance agricultural production. Engineering of the C4 or CAM pathways in C3 plants would require regulated expression of several genes in a cell-specific manner, whereas introduction of cyanobacterial or eukaryotic CCM might be simpler routes to enhance photosynthesis. While cyanobacterial CCM has the advantage of being both more efficient and better understood, the algal CCM, owing to its eukaryotic nature, is evolutionarily closer to higher plants. The establishment of either of the microbial CCMs in higher plants would require the engineering of the three key pillars (mentioned above), in addition to ensuring the chaperone-aided folding and assembly of engineered Rubisco, and deactivation of native carbonic anhydrases that might lead to CO2 leakage. The identification of the minimal number of components for CCM establishment, regulation of expression of the identified components to ensure maintenance and propagation of sufficient numbers of pyrenoids or carboxysomes in the chloroplasts of the engineered plants are challenges faced by the researchers working in this field. With rapid strides being made to unravel the various phenomena underlying CCM, the possibility of engineering higher plants which are photosynthetically efficient might be a reality very soon.

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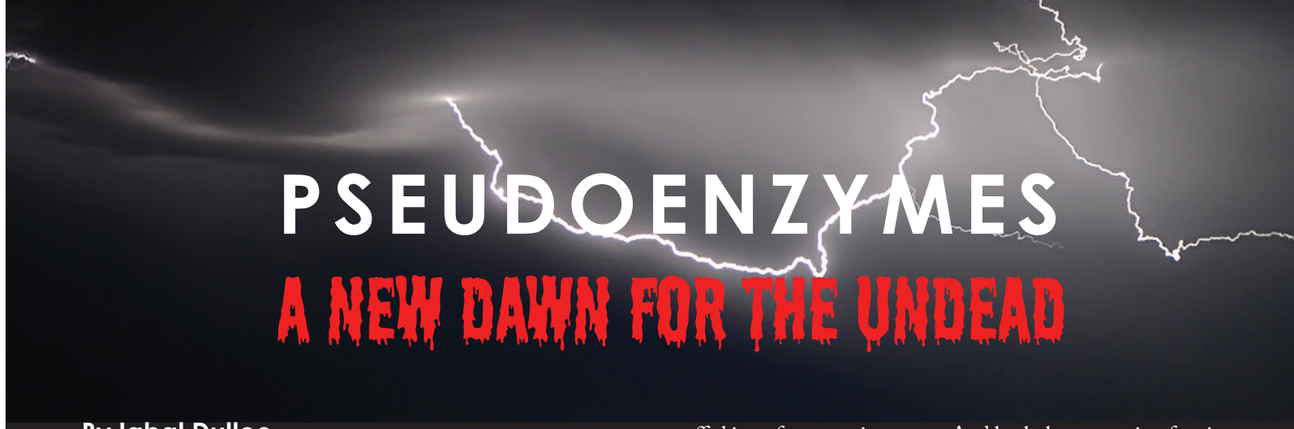

PSEUDOENZYMES

A NEW DAWN FOR THE UNDEAD

By Iqbal Dulloo

Iqbal Dulloo is a postdoctoral research assistant in Matthew Freeman lab, Sir William Dunn School of Pathology.

One of the defining turns in biology over the past couple of decades has been our ability to sequence and annotate genomes of multiple organisms in a precise and detailed manner. An unexpected discovery has been the prevalence of genes coding for pseudoenzymes or 'zombie' or 'undead' proteins. These proteins are structurally quite similar to their active cognates but lack essential catalytic residues to be active. The existence of pseudoenzymes was first inferred through a direct comparison of the sequences of lysozyme and β-lactalbumin around 50 years ago (1). Since then, many pseudoenzymes have been identified throughout all kingdoms of life and comprise of around 10% of proteomes, illustrating their likely vital importance in biological processes (2).

Unlike their active equivalents, barely any attention has been paid to pseudoenzymes as they were considered to be evolutionary vestiges in cellular signalling. Detailed analysis has now shown that almost every family of enzymes in the human genomes includes potential catalytically-dead members (3). Examples of some of the most well-studied are pseudokinases, pseudophosphatases and pseudoproteases. Several bioinformatic analyses supports the hypothesis that most pseudoenzymes have evolved from their ancestral active counterparts, likely via gene duplication, evidenced by the mutations of critical catalytic residues. Remarkably, this evolution has led to many instances where the enzyme and the inactive cognate function in the same signalling pathways, pointing to a likely regulatory role of the pseudoenzyme on its active counterpart. Conversely, in some cases, gene duplication has led to the formation of a protein that contains both active and inactive pair, an example being the Janus kinases, where the inactive module has an important regulatory role for the enzyme (4). It is noteworthy that pseudoenzymes cannot be converted back into an active form through simple mutagenesis, indicating that these proteins have acquired additional functional variations to their sequence and/or structure.

As these evolutionary conserved pseudoenzymes lack any detectable enzymatic activity, therefore, how do these proteins exert their cellular functions? Based on what on the data so far, four main mechanisms of action have been proposed. Firstly, by acting as allosteric regulators of signalling output of their catalytically active counterparts. Secondly, by operating as molecular switches to sense signals such as post-translational modifications or ligands binding to trigger enzymatic activity on their active counterparts. Thirdly, by working as protein scaffolds for functional enzyme complexes or regulate the localisation and cellular trafficking of enzymatic partner. And lastly, by competing for either substrate binding or enzyme complex assembly due to their close structural similarity to their active counterparts. As further progress is made in understanding the molecular functions of these pseudoenzymes, it is plausible that new mechanisms of how these proteins exert their functions will be uncovered.

As our understanding of these pseudoenzymes gradually expands, more evidence is emerging in support for a pathophysiological function of several of these zombie proteins in human diseases. For example, some pseudokinases (the most-studied pseudoenzyme family) have been implicated in several disease conditions (5). Janus kinase (JAK) family of pseudokinases, which are important players in blood cell function, carry several clinical mutations in the pseudokinase domain which are directly associated with myeloproliferative disorders namely leukemias (4). Pseudokinase STRADβ binds and regulates tumor-suppressor kinase LKB1 and mutation in the latter, associated with cancers and Peurtz-Jeghers syndrome, perturb its regulation of STRADβ (6). Both pseudokinases are clear examples how pseudoenzymes are important focal points for potential therapeutic intervention.

In spite of being initially considered as uninteresting remnants in the genome, pseudoenzymes are emerging as key proteins that affect important biological processes. It seems that we are all made up of zombies running around throughout our living cells. And unlike in the movies, these proteins are not carnivorous man-eaters but important players in maintaining a healthy cellular ecosystem. Currently, a major limitation to a better understanding of these under-appreciated proteins is assaying for their functions (unlike the well-defined assays for their active counterparts) and therefore the development of new methodologies/assays are urgently needed. And as these proteins often operate in similar pathways as their active cognates, mechanistic understanding into their regulation will certainly provide new insights in the fundamental regulation of various medically-relevant pathways, likely providing unexplored interfaces for therapeutic intervention.

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SEEING IS BELIEVING

THE POWER OF MICROSCOPY IN CELL BIOLOGY

By Dr Anna Caballe

Anna Caballe is a postdoctoral researcher in the Raff lab at the Sir William Dunn School of Pathology

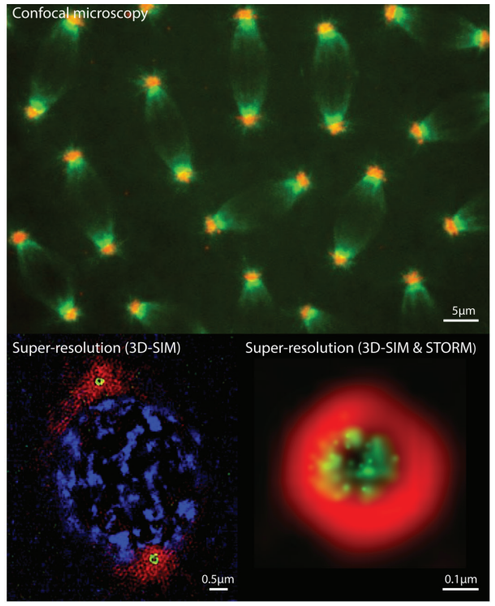
We sometimes underestimate the power that cutting-edge microscopy has in pushing the boundaries of key biological questions, and may think that the use of microscopy in research is limited to "getting a pretty picture" or seeing where our favourite gene localises.

Over the recent decades, the world of bioimaging has experienced major developments that have allowed microscopes to capture informative and beautiful images, and impactful data, making the fundamental understanding of biology intimately linked to microscopy. Some systems have the potential to visualise single molecules or image whole organisms, while others can measure force, interactions between proteins or cellular environment changes. Most of these improvements have emerged by taking discoveries from physics and chemistry into biology, surpassing long-standing limitations in bioimaging.

The ability of a microscope to separate two nearby points as distinct structures is known as its resolving power. Optical (or light) microscopes, in particular, have a restricted spatial resolution due to the diffraction limit of light. Light moves as a wave rather than a straight line; therefore, it can only be focused onto a small area and not an infinitely small point. This means that objects smaller than this area cannot be visualised. In 1873, Ernst Abbe formulated a mathematical theory that identified the physical constraining factors limiting resolution: the wavelength of light and the numerical aperture of the lens (how much light a lens can collect) (1). For many years, Abbe's rule was considered unbreakable, restricting optical lateral resolution to ~200nm. In the last decade, however, several techniques have emerged for sub-diffraction limited imaging, known as super-resolution microscopy (2). Generally-speaking, there are two different approaches: those relying on modulating the shape of excitation light, and those based on single-molecule imaging, which require the control of emitting molecules by photoactivation or photo-switching. These techniques have allowed biomedical researchers to see inside living cells in non-invasive ways, with a level of resolution similar to classical electron microscopy.

SMLM can also provide detailed information on the number of proteins within cellular compartments, diffusion of molecules and complex/dynamic molecular interactions.

For instance, Structured-Illumination Microscopy (SIM), which achieves super-resolution by generating an interference pattern, has been widely used in the last decade, reaching a lateral resolution of ~100nm. It is considered of low photo-toxicity and, as such, is useful for live-cell imaging and high throughput applications. STED (STimulated Emission Depletion) microscopy is useful for high-resolution 2D studies of high-contrast targets, like filaments, organelles or vesicles, and for deeper imaging in tissues. Single-molecule localisation (or SMLM) approaches can separate fluorophores with ~5-20nm lateral resolution. Examples of SMLM include PALM (with PhotoActivated Localisation) and STORM (STochastic blinking of dyes with Optical Reconstruction), which have helped resolve the 3D organisation of complex cellular structures like nuclear pores, chromatin, centrioles (3) or mitochondria at nanometre resolution. SMLM can also provide detailed information on the number of proteins within cellular compartments, diffusion of molecules and complex/dynamic molecular interactions. A further level of detail into protein systems can be achieved by combining single-molecule imaging with optical tweezers (highly-focused laser beams to generate small forces), for instance, for the study of processive cytoskeletal motors.



An image is worth a thousand words. Comparative images of different early *Drosophila* embryos. Note the centrosomes at the mitotic spindle poles (top, in red, spinning disc confocal microscope). Super-resolution is able to reveal the centriolar cylindrical shape within the centrosome (bottom left, green rings, 3D-SIM image) and SMLM reveals single-molecule localisation of individual centriole proteins (bottom right, green dots within the red ring marking the centriole wall, correlative 3D-SIM and STORM image from (3)).

In order to preserve native environments or large specimens while imaging, lattice-light sheet microscopy can be used, which works by repeatedly passing a thin sheet of light up and down living tissue at high speed and perpendicular to the imaging lens axis. It can image a whole zebrafish embryo over a long-period of time and also allows the visualisation of individual cells, as recently demonstrated in a zebrafish embryo (4). There are several other techniques currently emerging, including 4π microscopy with a much-improved axial resolution (using two opposing objective lenses), ultraviolet microscopy which 'sees' beyond what standard optical microscopes can image, or atomic force microscopy, a type of scanning probe microscopy that allows force measurements and extremely high-resolution imaging.

For researchers, it can be challenging to select the best-suited approach to address key biological questions. A thorough experimental design is necessary to generate the best quality samples, use the right imaging conditions and available equipment, minimise artefacts and photobleaching issues, and maximise the use of good image processing tools (2). It is key for researchers to identify how they can push the boundaries of cell biology research with new imaging techniques. Microscope manufacturers are developing systems that are increasingly more powerful, user-friendly, and able to facilitate automated image acquisition and analysis (often linked to machine learning, see (5)), thus making sophisticated microscopy more widely accessible.

It is key for researchers to identify how they can push the boundaries of cell biology research with new imaging techniques.

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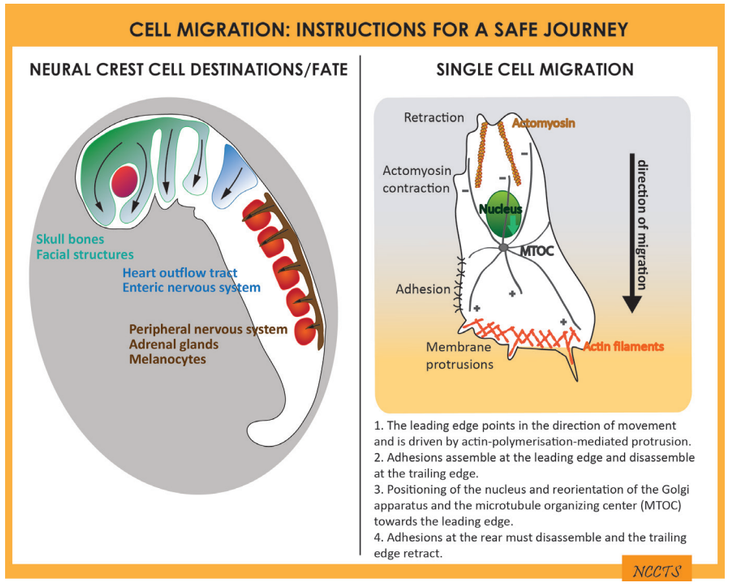
Migration of the neural crest cells: A 'bodywide' journey

By Maria Blanca Torroba

Maria Blanca Torroba is a postdoctoral researcher in Prof. Francis Szele's research group at the Department of Physiology, Anatomy and Genetics.

Welcome to the Neural Crest Cell Transport Society



Thank you for joining the NCCTS!

We run one of the largest collective migratory networks in the developing embryo. Our transport services are fully coordinated, allowing access of the registered neural crest cells (NCCs) to a myriad of tissues and organs. This only happens for a short period of time, starting around week four of human gestation, so get yourself ready as quickly as possible (1).

In this document, you can find information about the next steps to complete your membership within this selected group of highly-migratory, ectoderm-derived cells. First of all, there will be an induction session, called delamination to start defining the morphological and molecular features of an NCC, so that you can differentiate from neighbouring tissues. This will take place in the main departure station, the dorsal edges of the closing neural tube, which is the precursor of the central nervous system. It is here where you will also be informed about the possible destinations of a NCC.

Where to go

There are two main migratory routes available, depending on which part of the neural tube you begin your journey.

By departing from the most anterior part of the station, you can reach structures in the head region. This is the so-called 'cephalic neural crest cells route'. These paths will lead you to become part of the bones and cartilages of the face and neck. Additionally, you could integrate into tendons, muscles and connective tissues of the ear, eye, teeth and blood vessels.

If you are planning to visit more posterior areas of the developing embryo, then you should take the 'vagal-trunk neural crest cells route'. Some paths will lead you to the heart and to the enteric nervous system; others can guide you to become part of the peripheral nervous system, the adrenal glands on top of the kidneys or even pigment cells of the skin known as melanocytes (2). Make sure you depart from the right place!

Top travel tips

1. Once registered, the first step to become a NCC is called 'delamination', to physically separate from the adjacent ectoderm and neuroectoderm. One of the main drivers of this event is an epithelial-to-mesenchymal transition (EMT). EMT is a biological process that allows a polarised, immobile cell to assume a mesenchymal phenotype, which includes enhanced migratory capacity, invasiveness and elevated resistance to apoptosis. It is like having a special travel card.

2. After delamination, you will initially have to travel as a continuous sheet of NCCs which, shortly after, splits into streams targeting the different destinations described above. As you will be part of one of those streams, never left alone, there is no need for maps. Instead, you will wear an outer layer overlaid with membrane receptors, such as Eph, plexin/neuropilin and robo receptors, which will respond to the traffic signs along the highway (ephrins, semaphorins and slits, respectively). This is to prevent you from entering into forbidden zones while directing you to your specific final location.

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3. As previously stated, you will maintain contact with other NCCs throughout the migration process, but they will be far enough away that you are still able to move freely. You can achieve that by following two principles. The first one is called 'contact inhibition of locomotion' (CIL), whereby you will stop momentarily upon physical contact with one another, repolarise in the opposite direction and then move away. When this happens, however, you will not be able to go too far as you will be quickly attracted back to your travel companions via chemical signals in a process called 'chemotaxis'. This phenomenon of inhibition-vs.-co-attraction allows collective migration without dispersion (2).

4. Once you reach your destination, you will become an integral part of the tissue and live there for the rest of your life cycle/happily ever after.

You can plan your journey by downloading our 'Cell Migration' leaflet – an essential brochure with key information about how cell migration is regulated (3). r, repolarise in the opposite direction and then move away. When this happens, however, you will not be able to go too far as you will be quickly attracted back to your travel companions via chemical signals in a process called 'chemotaxis'. This phenomenon of inhibition-vs.-co-attraction allows collective migration without dispersion (2).

THE JOURNEY OF IHAT

A Novel Oral Iron Supplement from Concept to Phase II

By Katharina Kessler, Dora Pereira, Sylvaine Bruggraber, Andrew Prentice, Nuno Faria, and Jonathan Powell.

Katharina Kessler is a postdoctoral researcher in Jonathan Powell's lab at the Department of Veterinary Medicine, University of Cambridge

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Need for a novel iron supplement

Iron deficiency, with its associated anaemia, is the largest nutritional deficiency disorder in the world and affects more than 1 billion people – the majority of whom are children and women from resource-poor countries. Iron deficiency anaemia is responsible for an estimated loss of 35 million disability-adjusted life years (DALYs) and is linked with limited cognitive development in children as well as poor outcomes in pregnancy (1).

Iron formulations have been used medically for at least two centuries since the French physician Blaud of Beaucair introduced the first iron pills in 1832. And yet, taking iron supplements is often associated with deleterious side effects. Many of the currently used oral iron supplements lead to significant gastrointestinal side effects, such as nausea, constipation, and abdominal discomfort. Perhaps more importantly, they also negatively affect the gut microbiome, promoting the presence of potentially pathogenic bacteria at the expense of beneficial bacteria. Additionally, concerns have been raised that they increase the risk of developing colorectal cancer (2).

In resource-poor countries where parasitic and bacterial infections are often widespread, these side effects are a significant public health concern. Iron deficiency is frequently exacerbated by infections and, in turn, iron supplementation further increases the risk of infection in the intestine (3). Despite considerable effort and investment over the past 20 years to develop nutritional intervention programmes with iron supplementation and fortification, we have been unable to decrease the global burden of iron deficiency and iron deficiency anaemia.

2005 – 2006: Development in the laboratory

IHAT – a patent-protected, novel oral iron supplement

Nearly 14 years ago, whilst at the Medical Research Council (MRC) Human Nutrition Research Unit in Cambridge, Dr Jonathan Powell began to question whether the right type of iron was being used, and if in fact supplemental iron would be better off looking like food-derived iron. Most supplements provide large amounts of iron in a form that humans have not evolved with. Only a small fraction of this iron is absorbed, thereby reaching the blood circulation and organs of the body, while the majority stays in the gut, where it can be chemically active and induces side effects. Powell and his colleague Dr Sylvaine Bruggraber understood that if they wanted to find a form of iron that does not induce the same side effects, they needed to copy nature. Naturally, iron from our diet comes mainly from pulses, whole grains, vegetables, and meat. The iron in plant-based foods is generally in less accessible forms than the soluble iron that is currently used in supplements. Consequently, the iron from plants is less chemically reactive and not available to the bacteria in the gut and hence does not induce side effects.

Dr Nuno Faria and Dr Dora Pereira joined the Powell team, and developed different formulations that resemble the iron in plant-based foods. Iron Hydroxide Adipate Tartrate (IHAT) was their lead formulation, which not only mimics dietary iron derived from plant-based foods, but also consists entirely of natural food constituents thereby further enhancing its safety.

2006 – 2015: Pre-clinical and Phase 0 Studies

IHAT has the potential to improve iron deficiency without inducing side effects

In 2008 (4) and then 2015 (5), patents were filed for IHAT materials and their production. These have now been granted in multiple territories. IHAT is distinct from all currently used oral iron supplements for two reasons. Firstly, it is a powder that consists of many thousands of nanoparticles, each particle being ~5 nm in size: as such it behaves like the iron forms found in the intestine following ingestion of plants. In the gut environment, IHAT is taken up whole as nanoparticles by cells in the intestinal lining and is broken down inside these cells, mimicking what happens with iron from a natural diet. The breakdown of IHAT within the cells and subsequently the release of iron into the blood stream occurs slowly. This slow release is one of the advantages of IHAT as it limits the availability of free iron in the body (which is undesirable versus properly chaperoned iron) and, importantly, it limits free iron delivery to pathogens (6, 7).

Secondly, IHAT is less reactive than the iron in currently used iron supplements, the latter often being redox reactive. As such supplemental iron can react with oxygen-providing molecules to generate free radicals that damage the gut lining and contribute to side effects such as diarrhoea, constipation, abdominal pain, cramps and heartburn (6, 7).

Importantly, absorption studies and early nutritional trials in humans have shown that IHAT is absorbed efficiently and corrects markers of iron deficiency. This means that supplementation with IHAT should correct iron deficiency without increasing the burden from infectious diarrhoea and should result in improved overall response to iron supplementation compared to conventional soluble iron. In 2014, Powell and Pereira, on behalf of the group, received the prize for the Top Emerging Life Science Technology from the Royal Society of Chemistry.

2016 – 2019: Phase II Clinical Trial

Large trial in iron deficient anaemic young children in The Gambia

Since 2016, Pereira and Prof Andrew Prentice (MRC Unit The Gambia at LSHTM) have been testing the efficacy and safety of IHAT in The Gambia, West Africa, in a large paediatric trial (registered at clinicaltrials.gov as NCT02941081). IHAT-GUT, as the trial is officially referred to, is a double-blind, randomised, placebo-controlled trial. The trial, which was only completed in December 2018, was conducted in the Upper River Region of The Gambia. According to the most recent Gambia Demographic and Health Survey (9), the Upper River Region has the highest under-5 mortality rate in the country, the highest percentage of severely malnourished children, and the highest prevalence of malaria and anaemia in children under 5 years. Severe anaemia in children is highly prevalent and diarrhoeal diseases common. Therefore, there is a clear clinical need for safe and effective iron supplementation strategies in this region (8).

705 apparently healthy, malaria-negative children, aged 6–35 months, were included into the trial. They were randomised to one of three intervention groups: IHAT, ferrous sulphate (the current gold standard of iron supplementation) and placebo. In each group, children were treated for 12 weeks. Children in the ferrous sulphate group were given 12.5 mg elemental iron equivalent daily, in line with WHO recommendations. Children in the IHAT group received 20 mg elemental iron equivalent daily, which is the bioequivalent dose to ferrous sulphate. Children in the placebo group received ca. 30 mg pharmaceutical grade sucrose daily. The primary efficacy outcome of the trial is the proportion of children in each group who resolve iron deficiency after 12 weeks. The primary safety outcome is the burden of moderate-to-severe diarrhoea (8).

Samples and data of the trial are currently being analysed and first results are expected to be published in spring 2019.

Since 2017

Exclusive licence deal and market authorisation application

In 2017, LifeArc, formerly known as MRC Technology and working on behalf of the MRC, negotiated an exclusive license deal for IHAT with Nemysis Limited, a Dublin-based company, helping to provide strategies to manage iron deficiency/anaemia and gluten intolerance. Supported by the inventors of IHAT, Nemysis is seeking market authorisation for IHAT. Currently, regulatory toxicology studies are being undertaken and an application to the European Food Safety Authority is being prepared. Faria and Dr Katharina Kessler, working with Powell, lead on experiments to support the market authorisation.

IHAT seeks to be a safe and efficacious supplement for iron deficiency in both the paediatric and adult population and, perhaps at last, we can safely and efficiently decrease the global burden of iron deficiency and iron deficiency anaemia as a result.

Acknowledgement

The authors wish to acknowledge the long-term support of the Medical Research Council (MRC) enabling the development of IHAT as well as human absorption and repletion studies (Grant Number MC_U105960399). The paediatric nutritional trial is supported by a Bill & Melinda Gates Foundation Grand Challenges New Interventions in Global Health award [OPP1140952] and MCA760-5QX00 from the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement.

TECHNOLOGY TRANSFER

HOW DOES IT WORK IN RESEARCH UNIVERSITIES?

By Sheng Kai Pong

Sheng Kai Pong is a DPhil student in Monika Gullerova's lat at the Sir Willian Dunn School of Pathology. He was an intern at Oxford University Innovation in 2017.

During your research career, imagine that you discover or invent a technology that has the potential for wider applications beyond academic research. You would then like to take this idea forward, by either selling it or by setting up a new company to further develop the technology. The process of moving a technology from one place or purpose to another is called technology transfer, or simply 'tech transfer'.

The idea of tech transfer has become increasingly popular within research universities, as it not only generates income, but can also expand the impact of a university's academic research (1). Inventions conceived at universities are often at an 'embryonic' stage in terms of product development, but with the help of industrial and market resources, they can grow and eventually be commercialised. Technologies that are discovered or invented at universities are regarded as intellectual property (IP) that is owned by that institution. This practice was introduced in the UK in 1987, with a policy that handed over the ownership of IPs derived from government-funded research to the associated university (2). Each university has their own agreements with their researchers as to how they share the revenue gained from exploiting any newly-generated IP; for instance, depending on the total net revenue generated from licensing, the inventors at the University of Oxford can share up to 60% of the revenue among themselves, while the rest is retained by the tech transfer office and the academic department wherein the research was conducted (3).

How is the process of tech transfer carried out? Many research universities have now established their own in-house tech transfer offices, with dedicated project managers who guide researchers through the complex process of tech transfer. They are the first point of contact when a researcher approaches the tech transfer office with an idea or invention generated from their work. The project managers will have an initial discussion with the researcher(s) and request for an invention disclosure form to be completed; this form details what the IP is about, where, when and by whom it was invented, and finally the potential applications it is expected to have. The project managers will subsequently assess whether the IP has the potential to be commercialised and whether it fulfils the requirements for legal protection, either in the form of copyright or patent (4). In general, copyright protection can be applied to artistic work and software, while patents are applied to inventions and industrial processes.

Once the IP is legally protected, the next steps can be taken to exploit it. Commercialisation of IPs derived from academic research normally falls into two categories: licensing the technology to existing external companies, or establishing a 'spin-out' (or 'spin-off') company that makes use of the IP. In the case of selling the IP, existing companies may want to utilise the technology to complete or enhance their own products. In the case of setting up a spin-out company, the IP often has the opportunity to be developed into a full product with the help of further research and investments. Many university tech transfer offices have close partnerships with investment funds or 'angel investors' – wealthy individuals who provide capital for new businesses in exchange for ownership equity.

One successful example of an Oxford University spin-out company is Oxford Nanopore Technologies. It was established in 2015 after raising £0.5M in seed funding from the investment company, IP Group. The ground-breaking electronics-based DNA/RNA sequencing technologies offered by Oxford Nanopore Technologies are based on research initially conducted by Professor Hagan Bayley at the Department of Chemistry. Recently, the company has been valued at £1.5Bn after completing a funding round in Asia Pacific during March 2018.

Oxford University Innovation, the tech transfer office of the University, routinely organises seminars and talks to inform researchers about IP protection, licensing and spin-out companies. They also hold drop-in sessions in departments across the University to speak with researchers who have questions regarding their services.

With these internal tech transfer services in place, it will be easier for scientists and engineers at research universities to directly translate their scientific ideas from the lab benches to industry.

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RisingWISE

An Oxbridge initiative to promote entrepreneurial spirit amongst women

By Sonia Muliyil

Sonia Muliyil is a Post-Doctoral research fellow in Matthew Freeman's lab at the Sir William Dunn School of Pathology. She was also an attendee of the first-ever RisingWISE workshop.

Many initiatives have been undertaken to promote entrepreneurial spirit amongst women scientists, but very few can boast of doing so in a concerted manner between Oxford and Cambridge. The RisingWISE programme is a new Oxbridge network fostering long-term relationships between enterprising early-career researchers and women working in industry, emboldening these women to move beyond gender stereotypes and confidently build careers across the science and technology sector.

The RisingWISE programme was spread across three weekends between November 2018 and January 2019, in Oxford and Cambridge. More than 60 women, actively engaged in academic and industrial research, came together with a will to network and grow their personal and business ideas into a solid plan of action. The workshops were designed to inspire and strengthen the Oxbridge Women in Science and Engineering (WISE) network, to offer mentoring and leadership skills, and to help all participants to enhance their confidence and learn techniques that could be employed in their own working environments. In addition, it provided a platform for women in academia to interact with their peers in industry, in order to build future collaborations.

Despite having just finished a long working week, the participants came fully prepared to engage in in-depth discussions and hands-on workshops that across the whole weekend. Additionally, the participants had the unique opportunity to visit start-ups and entrepreneurial spaces spread across Oxford and Cambridge, with a detailed account of the resources and support available in these local hubs. All participants were divided into small groups with facilitators and mentors, who worked alongside them during the entire course of the workshop. The beautiful settings of Madingley Hall in Cambridge and Egrove Park in Oxford lent an extra stroke of magic to the event, while the bus journeys between Oxford and Cambridge served as added time to interact and exchange ideas.

Key highlights of the event included a plethora of engaging talks on confidence boosting, networking and negotiating skills, combined with workshops on writing impact statements, defending proposals and pitching ideas to a larger audience. In addition, there were a slew of talks by successful women entrepreneurs sharing their personal narratives of triumphs and tribulations.

The first day at Cambridge focused mainly on exploring and comparing the mindsets of a researcher versus an entrepreneur, and encouraged one and all to focus on a long-term mission statement. This was followed by a networking session for women in academia and industry, over drinks and dinner. The second day aimed to encourage active listening and putting one's best foot forward, using very creative group exercises (including dancing!). Post-lunch, all women worked together in small cohorts to discuss their vision and paths to impact. The day concluded with a session on collaboration –how to achieve and maintain good collaborations and be an effective collaborator.

Highlights of the second weekend in Oxford involved promoting authentic leadership and negotiating skills. Group exercises served as a good vantage point to discuss personal achievements and reflect upon difficult situations faced by individuals in their professional life. A considerable amount of time was spent on contemplation and deliberation of personal goals set by each participant.

The final weekend in Oxford commenced with a number of inspiring talks, followed by networking drinks and a formal dinner for all participants at Egrove Park. On the last day, there were group sessions centred on celebrating success stories and sharing experiences as well as talks on how to manage the media and use it effectively to promote one's endeavors. Post-lunch, group strategies and team building were put to the test, when teams were asked to pitch a start-up idea in the form of an elevator pitch. The workshop ended with a promise to meet again in six months, to discuss each individual's plan of action and to exchange notes on the impact of the work done as a collective.

Overall, the workshop helped foster new collaborations between women in academia and industry, and reconfirmed the special relationship shared between Cambridge and Oxford. One last parting thought that dominated the participants' minds was that the workshop should continue to empower more women in the years to come.

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This workshop was sponsored by EPSRC, and designed and developed collaboratively between MPLS Enterprise,Oxford and the Office of Post Doctoral Affairs, Cambridge.

For more information about the RisingWISE program, visit https://www.mpls.ox.ac.uk/enterprise/mpls-enterprise-programme-courses/risingwise-a-new-enterprise-course-by-and-for-women

INTERVIEW

PROFESSOR GARMAN

on scientific outreach

> *Uptatum, quibus consedi site es volupture nis aut quaeperio dolest, oditate ssimus.*

Professor Garman was interviewed by Komal Yasmin, a DPhil student in the Brockdorff lab.

When investigating inclusivity in research, Phenotype did not have to look further than Professor Elspeth Garman, whose career is nothing less than inspirational. She began in the male-dominated field of nuclear physics before making the leap to structural biology and has been celebrated for both her research and teaching alike at all stages of her challenging career. Indeed, Professor Garman is well recognized for her passion for both teaching and mentoring, having been presented with the 'Most Acclaimed Lecturer in Medical Sciences' award by the Oxford University Student Union in 2014, and offers mentoring to colleagues at all stages of their career, from students to post-doctoral research fellows. This extensive experience of mentoring scientists from a variety of cultural backgrounds and career stages has given her a wealth of knowledge on issues faced by scientists today. Here is a glimpse of what she had to say about inclusivity in research.

What does the idea of inclusivity in research entail for you?

To me it means always being open and including people from all over the world within my research group. In 2009, my group was particularly diverse with both male and female members originating from Mexico, Russia, India and Jordan. For me, inclusivity means that when you receive an email in the most terrible English saying `I would like to work for you', entitled 'pot-doc' instead of 'post-doc' and with no CV or funding details attached, not just deleting it and instead thinking: 'Where is this person coming from? What facilities have they got there? What can I offer them? What difference would it make to their lives if I have them with me for a year?' This has been my guiding principle; not just dismissing people on the basis of things like these is I think important.

How important is it to consider the diversity that a candidate will bring to a lab as well as their qualifications?

That's a good question, because you have to be driven by merit. But on the other hand, you also have to look at CVs while keeping in mind the background of the person. What I am looking for is potential. The most important traits for me are enthusiasm and curiosity. I believe that having a diverse staff gives the research group more breadth and is a wonderful experience for the local students as they are able to learn about other cultures and see how other people do things. I was never conscious that I was looking for a diverse research group until you asked me and then I realized that, when I get these emails, I do make a point of looking at them carefully rather than just deleting them.

Do you agree with the perception of this university as being reserved for people from elite backgrounds?

This, to me, is a myth. I went to Durham University and when I decided to apply to Oxford to do a DPhil in Nuclear Physics, I was told by everybody that there was no use in applying to the University of Oxford since it only takes its own former undergraduate students on as research students. However, when I came here, none of the 12 other new research students in nuclear physics that year were Oxford graduates. I am well aware that such a reputation exists and when you talk to undergraduates they also admit that, prior to visiting on open days, they also thought that Oxford was a university for rich people. There are however a variety of scholarships such as the Moritz-Heyman scholarship aimed at aiding around 100 students a year from under-privileged backgrounds applying to Oxford. Nevertheless, it is true that universities like Harvard have huge endowments to support students, which we do not have.

What advice do you have for students, especially those from developing countries, who may feel that their prior education is inadequate but are too shy to ask for help?

This so called "imposter syndrome" is very common. It's not just a problem faced by international students but by everyone. I faced it when I arrived here. I did not know about the college system and the culture of formal dressing; it was all alien to me. My parents never went to a university, I came from the north of England and to me it was another land here. Being in nuclear physics, a male-dominated field, I thought I would never make it. Later, I switched from nuclear physics to structural biology. I had no experience in biology, so when I walked into the lab on the first morning I made the decision that if I didn't understand anything I would ask about it. I did not care if I looked stupid, as they had taken me on for this job and if I didn't ask I would never be able to learn. What I discovered was something magical. Whenever I asked a question, several people would admit that they didn't know the answer either. The result is a discussion in which everyone comes together to understand the question, which I find really exciting. I have the same culture in the journal clubs at my lab. It takes a person at the top to be prepared to make themselves vulnerable and then everyone can learn.

International students can feel discouraged from applying to Oxford due to the apparent lack of funding, which in turn reduces diversity. What advice do you have?

I was involved in the EPSRC-funded Doctoral Training Program for 5 years (2009-2014). About 80 graduates were interviewed annually for the studentship places, many of them international students, and the question of funding arose very often. The international students who were among the top interviewees and whom we could not fund from the EPSRC grant would be nominated for the Clarendon fund, and each of the DTC programs would always be awarded one scholarship. In addition, there are a number of students funded by their governments. I have worked with students funded by scholarships such as those offered by the governments of Brazil, Australia and Mexico. The University of Oxford graduate application form also contains a webpage advertising a range of funding opportunities. I was the Tutor for Graduates at Brasenose for 4.5 years (2013-2018), where we have over 200 graduates from around the globe and the college dedicates an enormous amount of funding for graduate students. We had special scholarships for some students from Singapore and Cyprus amongst others, so it requires some research. However, some grants come in exchange with a condition to work substantial teaching loads back in the home country afterwards in return for every year spent abroad, which can be quite restricting. It is difficult to find funding, but it is not impossible.

New graduate students are not adept at networking, which can not only make them feel excluded but also prove detrimental for their career progression. How can you help students in this regard?

At the beginning, very few graduate students in science know how the scientific community works. For the post-doctoral researchers and DPhil students working in my group, I like to ask them what conferences they are would like to attend and I advise them to go to at least one in a year, but not just to talk to people from Oxford they already know while they are at these conferences and to put themselves out there. The only way to network well is to risk being rebuffed. You have got to go to a conference, talk to people, stand beside your poster and be enthusiastic. Such activities are a very important part of a researcher's training and career.

How do you think the university is dealing with the issue of encouraging women in science?

There is a huge attrition rate as you progress up the hierarchy. There is an excellent gender balance at the undergraduate student level, at the graduate level it is good, post-doc is not too bad but then there is a huge fall-off. What is interesting is that for the Athena SWAN application for this department, there was a very careful analysis conducted of the applications received for posts over the last ten years and proportionately if they apply the women are as successful as the men,. However, many fewer women apply. What I have observed generally is that men look at a job description and think, I fulfil 60% of the criteria, I will apply, whereas when women look at a job description, they think they fulfil (only) 85% of the criteria and hence do not apply. Thus, women need to be encouraged to make applications. I think fear of rejection and self-doubt are often more prevalent in women. The university and colleges are making huge efforts in this regard, but people are in lectureship posts for 40 years and so it not easy to quickly shift this trend.

How important is public engagement and how does it contribute to improving the diversity in science?

Public engagement is very important. I have been involved in organizing the Biochemistry Department's contribution to the Oxford Science Festival in the Town Hall for the past 4 years. We also had a stall in Broad Street in October to demonstrate some simple experiments with microscopes. You have to believe that you are sparking interest in some child, somewhere. On the radio show 'The Life Scientific', scientists are asked what inspired them to study science and there are some amazing responses, such as 'I was four and I was passing this person with a telescope looking at the moon' or 'when I lost my helium balloon and it went up, my uncle told me that helium was light, and that sparked my interest in science'. So, you never know when you are making a memory. Public outreach is important for diversity as well. Last year we held a European Researchers' night at the History of Science Museum, including a quiz for members of the public. Each table had a researcher to help get the discussion going, and people took great interest in having a one-to-one chat with researchers and learning about their research. These outreach activities take time and effort, but I try to ensure that they are encouraged in the Department.

Visit http://www.bioch.ox.ac.uk/about/outreach to find out more about outreach events led by the Department of Biochemistry, and http://www.ox.ac.uk/admissions/graduate/fees-and-funding/graduate-scholarships for more information about graduate scholarships.

We've all heard about

veganuary

...but what about lab animals?

By Paige Chandler

Paige is a DPhil student in the Neurobehavioural Genetics group at the Mammalian Genetics Unit, MRC Harwell.

In October, a paper published in Nature outlined in no uncertain terms that meat consumption must fall in order to mitigate disastrous climate change (1). It is predicted that, if demand for animal products remains the same, by the end of the century the global temperature will increase by 3^0C, putting the continuing survival of humanity at risk (2).

Always a cynic, I scoffed at the reports. "What about the big corporations?" I complained to my friend, "As if you or I becoming vegan would save the world!" My friend, forever a wise voice in my life, commented "Sure, and that's what everyone is telling themselves, which is why nothing changes." The next week, I went vegan.

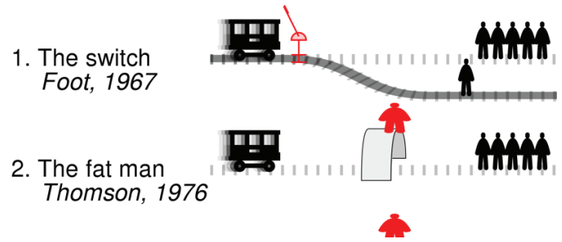
I began to engage with 'vegan media' - places like the vegan forum on the website Reddit and documentaries on Netflix and YouTube - to educate myself about animal agriculture (and what the hell you're supposed to do with tofu). The more I read and watched the more uneasy I began to feel, as I am an animal researcher who conducts behavioural work with mice on a daily basis.

The controversial and thought-provoking documentary Dominion (available on YouTube) quotes a 2015 study by the National Institutes of Health in the USA which found that 95% of drugs that showed promise in animal models of disease went on to fail in human clinical trials (3). Within the context of the documentary, which showcases horrifying cruelty towards 'food', 'fur' and 'entertainment' animals by humans, it is clear that the makers of Dominion mean to show 'laboratory' animals as being equally exploited.

...95% of drugs that showed promise in animal models of disease went on to fail in human clinical trials.

They have a point. The basis of scientific animal research conducted by humans is 'speciesism', the idea that we as humans give ourselves greater rights than those of other species. As an animal researcher, it is a fact that, in order for my research to potentially save human lives in the future, I personally sacrifice the lives of mice.

Consider the famous thought experiment 'The Trolley Problem'. A runaway trolley is heading towards five incapacitated people on a track. You have the ability to throw a switch which changes the course of the trolley to a fork in the track, with only one incapacitated person on this alternative track. Would you throw the switch? Most people would, to save the majority with the (potential) sacrifice of a few. An adaptation of this problem is 'The Fat Man' – instead of a switch, you and a very large man are on a bridge over the track. You know that the man is large enough to stop the trolley in its tracks. Would you push the large man off the bridge to his certain death to save the five on the track? Most people would balk at the idea, however this is the fundamental idea underpinning animal research. In 'the switch', the death of the single person on the other track is an unfortunate side effect. However for the single large man to stop the trolley, he must die – and for biomedical science to progress, so must the mouse. As an animal researcher, I must face my role as a 'pusher', everything a vegan should surely stand against.



The key question presented in documentaries like Dominion is: what exactly does give us the right? The truth is that the vast majority of us do not need to consume animals in order to live. A healthy lifestyle can be maintained on a plant-based diet. However, if all animal-based research was banned tomorrow, could we ensure the survival of humanity? While the probability of successfully developing a promising drug from animal studies through human clinical trials is low, a small proportion of these programmes do lead to the development of important new medicines. Furthermore, we gain important biological insight from such studies regardless of their outcome. I am not going to eat meat, or quit my research, any day soon.

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THE PLASTIC CRISIS

BACTERIA to THE RESCUE?

By Swathi Lingam

Swathi Lingam is a postdoctoral researcher in Heidi de Wet's lab at the Department of Physiology, Anatomy and Genetics

Plastic: a ground-breaking invention at its advent and currently choking the planet due to unmonitored accumulation. As of June 2018, the statistics show that roughly 8.3 billion tons of plastic have been generated in the last 70 years, of which only 9% have been recycled, 12% have been incinerated and a whopping 79% have ended up in landfills or oceans (1). It is estimated that it takes up to 500 years for some types of plastic to be naturally degraded and current methods of disposal fall short of the urgent requirement to clear plastic from our environment.

Polyethylene terephthalate (PET) is the most ubiquitous of the polymeric plastics manufactured. It is an inert aromatic polymer that is resistant to biodegradation, which leads to its accumulation worldwide. There could, however, be good news on the horizon. In 2016, a group of scientists in Japan identified a bacterium known as Ideonella sakaiensis 201-F6, which can use PET as a primary carbon source for its growth (2). By sequencing the genome of I. sakaiensis, this group of scientists was able to identify a putative enzyme able to degrade PET films in the laboratory. Further investigation revealed that this enzyme functioned as a PET hydrolase, leading it to be named 'PETase'. The PETase degrades PET into the harmless monomers terephthalic acid (TPA) and ethylene glycol (EG) (2).

Recently, UK-based scientists in collaboration with several international research groups, achieved the crystallisation of recombinantly expressed wild-type PETase, allowing its characterisation as a canonical β/β hydrolase (3). Interestingly, introduction of key mutations in the active site improved PET degradation significantly. Additionally, this study demonstrated that PETase can degrade another aromatic polymer known as polyethylene 2,5-furandicarboxylate (PEF), which is emerging as a promising sustainable replacement for PET.

While this research is promising, further work needs to be done to elucidate the binding mechanism of the enzyme to PET and to optimise its activity. Furthermore, a scale-up of its production would be required to prove its potential for the degradation of plastic waste on an industrial scale. The use of bacteria to facilitate the degradation of certain plastics is a burgeoning and exciting new field that may be the answer to the current plastic pollution crisis. These recent discoveries from the lab could, therefore, have significant positive repercussions for the environment worldwide.

1. Trowsdale R, et al. (2017) Seven charts that explain the plastic pollution problem. Available at https://www.bbc.co.uk/news/science-environment-42264788 [Accessed 21st January 2019]
2. Yoshida S, et al. (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351 (6278): 1196–1199.
3. Austin H, et al. (2018) Characterization and engineering of a plastic-degrading aromatic polyesterase. Proc Natl Acad Sci U S A 115 (19): E4350–E4357.

IMMUNE CHECKPOINT THERAPY

RELEASING THE IMMUNE SYSTEM'S BRAKES

By Samantha Moore

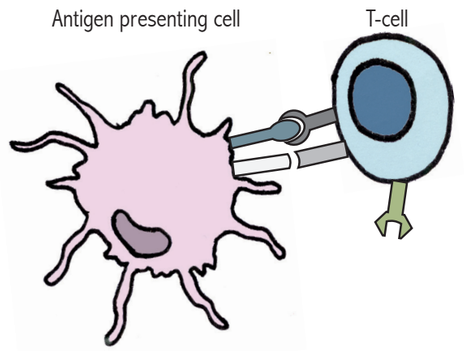
The 2018 Nobel Prize for Physiology or Medicine was awarded to James P. Allison & Tasuku Honjo. In parallel, the pair revealed the utility of checkpoint inhibitors in releasing the immune system's brakes, thus harnessing immune responses to treat a range of cancers. These discoveries mark an entirely new approach to cancer treatment.

CTLA-4

James P. Allison
University of Texas,
MD Anderson Cancer Center

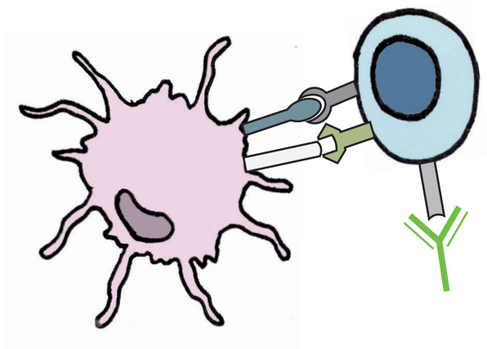
1987 -
CTLA-4 first identfied from cDNA cloned from CD8+ T cells.

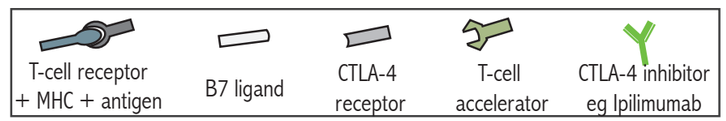
1991 -
CTLA-4 identified as part of T- cell stimulatory pathway through binding to B7 ligands.



1995 -
CTLA-4 shown to have opposing effects to CD28, acting as brakes on T-cell activation.

1996/1997 -
Anti-CTLA-4 antibodies developed. Results in rejection of tumours in mice.





2010 -
Clinical trial in metastatic melanoma yielded increased survival time by 10 months.

2011 -
FDA approved Ipilimumab for metastatic melanoma. First checkpoint inhibitor to reach patients.

2015 -
Combined PD-1 and CTLA-4 blockade shown to increase progression-free survival in metastatic melanoma.

PD-1

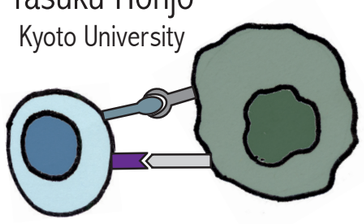
Tasuku Honjo
Kyoto University

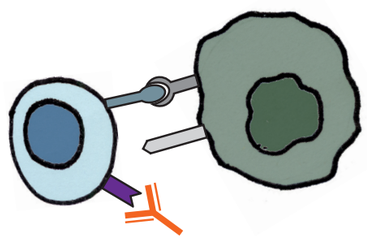
1992 -
PD-1 discovered due to its role in programmed cell death.

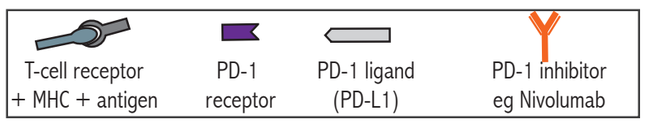
1999 -
Identification of PD-1 as an immunoinhibitory factor. PD-1 ligand (PD-L1) also identified.

2005 -
PD-1 blockade shown to prevent spread of poorly immunogenic cancer cells through the bloodstream in mice.

2012 -
PD-1 antibody clinical trial shows decreased tumour sizes in range of cancer types, including lung and renal cancers, and melanoma.



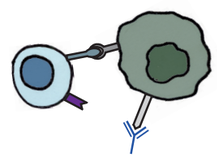




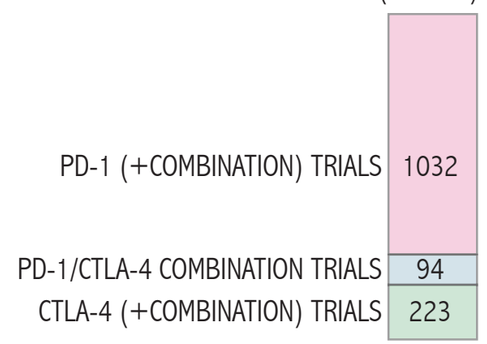
FUTURE DIRECTIONS

-COMBINATION THERAPIES

- OTHER CHECKPOINT INHIBITORS



EG. PD-L1 INHIBITORS



5' with... Prof Alison H. Banham

Interviewed by Vasiliki Economopoulos

Prof Alison H. Banham is currently Professor of Haemato-oncology at the University of Oxford and Head of the Nuffield Division of Clinical Laboratory Sciences. She is also the Vice President of the European Network of Monoclonal Antibody Producing Laboratories. She is based at the John Radcliffe Hospital where her laboratory focuses on developing anti-cancer diagnostic and therapeutic monoclonal antibodies, and studies the role of the family of forkhead transcription factors in haematological malignancies.

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

My father was a biochemist and so I was exposed to his laboratory and colleagues from an early age. My parents were passionate about natural history photography and I shared their enthusiasm for biology, which probably accounts for it being my 'best' subject at school. Therefore, my decision to become a scientist was a gradual evolution that was influenced by a curiosity about the natural world, a familiarity with and an aptitude for science.

How did you decide that you wanted to work in haemato-oncology?

I got married during my DPhil and, as my husband had a permanent post, I needed to find a postdoctoral position locally (in Oxford). I initially struggled to get a job, because I could not convince employers that I would finish within 3 years and my DPhil was very specialised. Dr Molly Dewey in Plant Sciences kindly taught me to make monoclonal antibodies during a 6-month internship and it was the skills I developed with her that enabled me to get my first postdoc position in virology at the Sir William Dunn School of Pathology. I later joined Prof David Mason's lab, at the John Radcliffe hospital, where the team made monoclonal antibodies to help diagnose blood cancers. It was an exciting time in the field and being funded by a rolling programme grant gave me the continuity needed to establish my research track record and international reputation, while also having two children.

What is the best career advice you have ever received?

My advice is that when you want to join a new laboratory, insist on being able to look round and talk to other people in the group to get a feel for what the work environment is really like. Don't just visit with the group leader.

Also, when competing for a tenured post, my father advised me not to focus too much on justifying what I had done to deserve the position, but instead to highlight what more having the position would enable me to achieve in the future.

What has been the biggest challenge in your career?

My biggest early challenge was developing my research identity and independence, while still working in someone else's laboratory. The project that enabled me to do this was partly a lucky find that matched my previous expertise. However, I had to take considerable risks to pursue and hold onto it.

While children are seen as a barrier to women achieving senior positions, this is not the only barrier that women in research face. I have found coping with menopause symptoms to be equally challenging. Not just the expected hot flushes, but the surprisingly common symptoms of fatigue, anxiety and difficulty concentrating, which have made a visible senior leadership role a bigger challenge than I initially expected. Caring roles in later life, such as looking after elderly parents, can also impact on career progression. I feel that research is like distance running, you need to pace yourself to stay strong and succeed, especially to secure a senior position.

What have been the most important moments in your career?

Getting into Oxford for my undergraduate degree was a huge moment for me. My school was not supportive of my application, as I was not one of their stellar academic achievers and I only applied because my parents encouraged me to give it a go. I was one of only two girls from my school to be admitted to Oxford that year. Being willing to risk failure and try was a key early lesson.

I was a senior postdoc, when our funding body indicated their intention for the current award to be Prof Mason's last Programme grant before his retirement. The team were keen to continue our research and so myself and another postdoc spoke to the head of our funding body. He reassured us that they would find a bright young chap to look after us. Being willing to say that this was not what we were looking for and that we wanted the chance to run the group ourselves led to us being given the opportunity to apply for independent funding, our posts were underwritten by the charity and we received invitations to apply for independent Programme grant funding in our own right. Again, being prepared to take risks as well as asking for what you want are important for success.

How do you think the treatment of blood cancers will change in 20 years' time?

At the moment we have a huge array of new treatments being developed. Understanding how these can be combined and the contribution of specific driver mutations in individual patients to treatment response and relapse will transform the use of targeted therapies. I think that reactivating patients' immune systems to facilitate immunotherapy will also be important.

In your opinion, what makes a good scientist?

You have to love and be passionate about research, curious, self-motivated and persistent. Having high standards and remaining critical is essential, even if this costs you the occasional paper along the way. The quality of your work is much more important.

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

I enjoy working with other people and helping them to solve their own problems. I have a foster daughter and could easily see myself as a Social Worker or Counsellor.

If you were starting your career again, what you would do differently?

I would try to be braver about taking opportunities that push me outside of my comfort zone and push boundaries more.

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SNAPSHOT

Congrats to Rita for winning our Michaelmas 2018 Snapshot cover contest!

Rita Alonaizan received her Bachelor of Science degree in Biomedical Sciences – Developmental Biology from University College London in 2013 and was awarded a Master of Science degree in Clinical Embryology from the University of Oxford in 2015. Rita is currently a final year DPhil candidate supervised by Professors Carolyn Carr and Nicola Smart at the Department of Physiology, Anatomy, & Genetics, University of Oxford. Her research aims to better define cardiac nonmyocyte populations found in the adult mouse heart by detailed examination of their expression profile at the single cell level, and to optimise cell therapy for cardiac repair following myocardial infarction by harnessing advances in the field of microRNA therapeutics.

The image shows an adult mouse heart section positive for the fluorescent protein tdtomato (red). The nuclei were stained with DAPI (blue).

You could be our next Snapshot winner!

The next theme is Neuroscience

Send us your high quality scientific photos for a chance to be featured on the next cover of Phenotype

Pitch us your illustrations

We are looking for original artwork to accompany our articles

Entries should go to oxphenotype@googlemail.com

TRANSCRIPTIONAL REGULATION

A rainbow poem by
Dr Jayanthiny Kangatharan

Multiple
Transcription factors can,
Do and will modulate
The transcriptional
Signatures, so
Improving somatic
Care and longevity

Dr Jayanthiny Kangatharan is a postdoctoral research assistant at Harvard University, and the author of *Multilingual Neuropoetry*.

"I always have been interested in bringing the arts and the sciences together and it is in that context that I reintroduced the idea of Neuropoetry and revamped it a little to make it fun and interactive.

Usually I create poems in form of riddles, where one has to guess what the poem is all about but in this case, I formed a regular poem for the 'Rainbow poem' type that I invented.

This poem type is like any other short poem type such as a Haiku, and therefore it is much simpler than, for example, a Pantoum or Sonnet.

Here is the rationale behind the Rainbow poem: Just like the rainbow has seven colours, the Rainbow poem form is seven lines long. Usually, each line starts with the first letter of each of the seven rainbow colours that are red, orange, yellow, green, blue, indigo, and violet. The number of letters in each of these colours corresponds to the number of syllables in each line."

Parmanu MY UNWANTED COMPANION

परमाणु,

"She is rare, huge, tenacious and fierce. Her origin is unknown and her future is unpredictable. She believes in reincarnation. Her discovery was unbelievable. In a million, she chose me and transformed my life. She thinks I am special!"

By Priti Gupta

Back in 2014 while playing ultimate frisbee, Naveen told me that the left side of my back below the scapula seems a bit swollen. That was the first time Parmanu's presence was noticed. As she was not hurting at that time, I ignored her assuming that perhaps my back was always asymmetrical like that. She remained undiscovered and continued building a cozy home for herself in my body.

While thriving inside me, she was working hard and fast to change my internal structure and at the same time, she wanted to remain uncovered until she was well settled. She was silently constructing a home for herself, but could not resist straining her surroundings. At the beginning of 2015, Parmanu's diligence started showing up as my pain. Parmanu was cautious but could not avoid pulling my intercostal nerves which resulted in incidences of sharp stabbing pain below my front ribs. The doctor in Mumbai did not doubt her as the origin for this pain. He attributed it to my cycling posture as I was cycling long distances often in those days. Thus, Parmanu went undetected almost like an undercover agent.

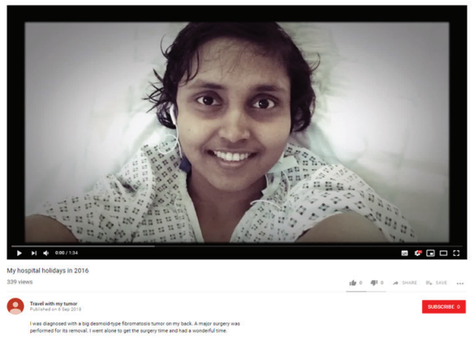
Unaware of my silent associate, I moved from Mumbai to Cambridge in mid-2015. While I was hustling to adjust in a new country, Parmanu was hustling harder to adjust in me. She soon became big and malicious with an unsatisfiable desire to conquer more. She knew it wais about time to build the hype for her grand announcement. She started hurting me and I could not even sleep on my left side. In the beginning of 2016, I decided to consult a general practitioner nearby, but not with any strong suspicion. The doctor examined me, prescribed a few scans and, surprisingly, did not clearly say anything to me. A few days later, my pigeon-hole saw the first of many future letters letter of the many in future from the oncology department of Addenbrooke's hospital. The letter informed me about the schedule of four scans (MRI, CT scan, X-ray, and ultrasound) and a blood test. Like a grand promotion of an event, Parmanu slowly started dropping hints with each scan result and the oncology team started speculating as to her identity. Is she just a lipoma or is she a soft tissue sarcoma? Is she malignant or is she benign? I was curious and puzzled as a clear conclusion about Parmanu's identity was not made yet. The oncology detectives referred my case to their colleagues at the Royal Orthopaedic Center in Birmingham. It was time for Parmanu to get some royal treatment. Although I was confused at seeing this rapid referral, I was mentally prepared to deal with whatever Parmanu had to offer me. The doctors at the Royal Orthopaedic Center did a biopsy to get a sample of Parmanu to establish her true identity. Two weeks later, I got a call from Birmingham while I was working in my research lab. The not-so-unexpected (by now) colossal announcement, which Parmanu planned, was finally made.

Her identity card read like this:

With this revelation, just when Parmanu was feeling a sense of accomplishment and I was feeling a sense of uniqueness, another announcement was made. As Parmanu was huge by now, the best-suggested treatment was to uproot her, with a margin even though that will would be immensely painful for me. A major surgery was planned to get rid of her. Parmanu started throwing tantrums against this decision leading to more radiative pain around her. Her idiocy left no choice than to eliminate her. The pain she was giving was unbearable and unforgivable! Parmanu and I finally parted in April 2016.

Name: Parmanu (परमाणु, a Hindi word which means atom)
Family: Name Desmoid-type fibromatosis tumor
Address: Left posterior chest wall, Body of Priti Gupta
Size: Big
Ethnicity: Rare kind of aggressive tumor (2-4 in a million)
Birthcause: Unknown
Skills: High recurrence rate (~ 30-50%)
Allergic to: Not precisely established--therapy varies from host to host (low-medium success rate)

Parmanu's departure scarred me for life. She left, but created a big void in her place. Her removal resulted in a permanent loss of strength. My left shoulder and arm could not function properly for almost three months. But I was cheerfully bearing all these discomforts as she was gone for good. Her mysterious origin, hostile behavior, and successful treatment are yet to be understood fully. I donated Parmanu as my contribution to medical the research studies.



Here is a glimpse of the amazing fun I had during the surgery period at the Royal Orthopaedic Center in Birmingham. I went for my surgery alone, hence spent a lot of time learning origami. I made a paper bouquet for the hospital staff as a thank you token and also created one flower each for all the fellow-fighters in the ward.

So, was it the end of Parmanu's story in my life? No!

Like thea story of any haunted house, in September 2016, my MRI scan indicated the void Parmanu created wais not empty anymore. Someone unusual started living in that space. Was she back? Doctors did not confirm her return. Entities like Parmanu mimic scar tissue and I was still recovering from my surgery with a big scar on my back. With mild suspicion but strong optimism, I went to India for some time. During a month-long mountaineering course in the Himalayas, both Parmanu and I were on an arduous journey to reclaim what we lost. Oblivious to her recent expedition, sometime later in Bangalore, a familiar pain started emerging from inside. She was silently reaching out to me once again. She had infiltrated deeper than evaluated by the medical team. She regenerated from her remnants. A new year - a new launch for the tenacious Parmanu. The oncology team confirmed that she wais bigger and more aggressive than the last time.

Parmanu, by nature, has a high recurrence probability. So, going under the knife once again was not advisable. I was referred back from Birmingham to Cambridge. The second plan was to weaken Parmanu so that she eventually shrinks and hopefully disappears. But there wais a catch in it. There are no sure and secure medical protocols to achieve this goal. For my Parmanu, I started a hormonal and anti-inflammatory medical treatment. Another MRI was done after three months and Parmanu was still unaffected. My medicine doses were doubled and so were the side-effects.

Setting aside this mess which Parmanu created, I moved to Berlin in May 2017 for my career development. The oncologist in Berlin decided to continue the treatment and I decided to keep Parmanu secondary in my life. It was tough! While I was finding my feet in a new place, I went through many painful days and sleepless nights due to the strong side effects of the therapy in addition to Parmanu's radiating pain. Mentally, I was a strong fighter but physically not. Parmanu was winning this battle against the therapy. She was progressively expanding her kingdom and my overall body health was deteriorating. After sSix more months and, another MRI and it was obvious that the medication was not working. Finally, this battle was called off. With my oncologist's consent, I decided to see how I am was doing without any medication for a while. To my surprise, I did fairly well and could tolerate the occasional pain due to Parmanu and the fading symptoms of the previous therapy.

Another MRI after four months, and Parmanu was still growing. She was huge by tumor standards, so another major surgery was proposed. It was difficult to surgically remove her successfully. A clear margin was an absolute necessity. To achieve this margin, the cancer surgeon suggested to remove 5-6 rib bones, a part of ny lungs and definitely a big chunk of my left shoulder muscles permanently. This will would certainly reduce the probability of relapse from high to not-so-low, but a real assurance was not given. Visualizing the quality of my life after this surgery, Parmanu threw me off-balance for the very first time. In addition to this conundrum, the previous treatment led to the development of a big cyst in my ovary which my gynecologist suspected was another tumor. My evergreen optimism was came crashing down and I was finding it difficult to pull it together. Thankfully, the tumor marker test was negative in my ovary, but it did result in something I have to annually keep an eye on.

On realizing that I was quite hesitant to go for surgery, my oncologist arranged an appointment with a colleague of hism who is a desmoid surgery specialist at the famous "Institut Curie Hospital" in Paris. Parmanu, Naveen and I went to Paris in March 2018 for to seeking the opinion of Dr. Sylvie Bonvalot who heads the Sarcoma and Complex Tumor Surgery Unit at Institut Curie. She told us that statistically speaking surgical treatment does not work and desmoid tumors usually relapse and that too aggressively. Hence, she recommended (that too strongly) that Parmanu should not be taken out surgically. She also told us that the studies have shown that these tumors first grow in size, stabilize at some point and may start shrinking thereafter, but the duration of this process is unknown and varies a lot from one case to another. She hads the opinion that if the tumor wais stable (not growing), the best treatment is to leave it untouched and follow a 'wait and watch' policy. Unfortunately, my Parmanu was growing fast and causing a lot of pain. According to her, my Parmanu had grown by 30 percent over the past one year. Therefore, "'wait and watch" was not among the safest options for me. She suggested that the best way to take care of Parmanu was to use a combination of tyrosine kinase inhibitor targeted therapy and chemotherapy. By the way, thanks to Parmanu, I also met the French President, Mr. Emmanuel Macron, at the hospital. He visited the institute the same day to discuss artificial intelligence research. I was overjoyed to talk to him and my perennial optimism was restored.

After reading a lot and discussing with my doctor about viable medical therapies, I have learned one thing: that all the therapies are quite aggressive and have a lot of unwanted short-term as well as long-term unwanted side effects. Opting for the stronger chemicals definitely brings horrible side effects and a terrible quality of life. On top of that, the outcome is unpredictable. , it may or may not work? It made me think that the pain and subsequent problems due to therapy may not be any less than what Parmanu had already delivereds!! I felt that living with Parmanu may might be better than living with both Parmanu and the side effects of the medicine. And I chose to live a slightly better life as long as I can. My doctor in Berlin agreed to continue the "'wait and watch"' policy for some more time. Unfortunately, my recent MRI results show that Parmanu has not settled so far and is acting in a greedy manner, wanting more space. In the process of occupying more space knowingly or unknowingly she is causing more pain. Hence, so my doctor has decided to try targeted therapy on Parmanu. Soon, both Parmanu and I will be under attack once more. It was tough and it is going to be tougher. But while I can, I am trying to check as many boxes in my wish list before it is too late. This blog is going to be a chronicle of all the little adventures which I have done been on over time with Parmanu and the plans we have for the future.

"Parmanu lives close to my heart and hence I share a very personal bond with her. She definitely started as a stubborn and undesirable companion, a constraint in my life, but finally, we have started going well together as we both don't have a choice. And I must say that she has brought me new experiences. We both growo stronger with each passing day: she in my body and me in my spirit. She has taught me to live life as if it is the last moment, sharing love, having compassion, and spreading happiness. I am among those few in a million people who host friends like Parmanu. Maybe I am special!"

Why the name 'Parmanu'?

'Parmanu' is a Hindi word परमाणु which means atom. Naveen, my best friend and an immense source of motivation, gave my tumor this name to make her sound less scary and more friendly. He says it's just a tiny tumor in this universe and not strong enough to shatter one's willpower to live!

Oxford Remembers the Women Who Dared

By Laura Hankins

DPhil student in the Raff lab at the Sir William Dunn school of Pathology.

Michaelmas Term 2018 saw the University marking a significant anniversary in the history of women's voting rights. On 14th December 1918, British and Irish women over the age of thirty were able to cast a vote in a general election for the first time. To celebrate the centenary of this event, the University of Oxford Women in the Humanities forum organised the flying of a reproduction of the Oxford Women's Suffrage Society (OWSS) banner above departments and colleges across the city. This occasion was complemented by lectures and by an exhibition curated by the Bodleian Libraries, celebrating the achievements of women across history leading up to the winning of the vote. One case of particular relevance to today's scientists is the women who, during the First World War, took up roles as researchers and medics. These were some of the 'women who dared'.

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The OWSS was founded in 1904 to champion the cause of women's suffrage, with its first meeting being held in Somerville College. Subsequent meetings were held in other colleges, the town hall, and even the homes of members of the movement who were active in the city. In 1911, a society for students was also formed. Their beautiful banner, depicting the spires of Oxford's iconic architecture, has also been reproduced to celebrate the events of 1918. The establishment of these groups in the early 20th century strengthened the movement in the city, but Oxford was already entwined in the struggle for representation. Organisations including the Oxford Women's Liberal Association were early advocates of women's suffrage, while The Association for Promoting the Higher Education of Women in Oxford focused on improving women's access to tuition, an issue that was especially pertinent in a city that was home to an ancient university. Indeed, Emily Wilding Davison, who went on to become a prominent suffragette, studied English at the University in the 1890s but was barred from graduating after the completion of her exams since Oxford degrees were closed to women.

Despite these challenges, women pushed to continue their studies, with many becoming accomplished in the Sciences. However, employment in this sector was often solely available in female-only institutes. This changed when women began to take up the jobs of men who had gone to fight in the First World War. The example that springs to mind is that of women working in munitions factories, but a largely overlooked group were also taking up roles as medics, scientists and engineers. Last year, a lecture was held at the Weston Library to explore the stories of these forgotten women. It was titled A lab of one's own: science and suffrage in the First World War and was presented by Prof. Patricia Fara. Women certainly made a huge contribution to the war effort, with some historians considering the societal effects of the war to have been a key factor in women winning the vote on 6th February 1918. However, many who had worked in the scientific sector were forced out of their new roles after the war concluded.

The regressive removal of women from the workplace, alongside the fact that universal suffrage was still a long way off, demonstrates that there was progress yet to be made. This remains true today. Sadly, no original OWSS banners have survived; the flags seen flying across Oxford on 14th December were reproductions. However, an exhibition at the Weston Library entitled Sappho to Suffrage: women who dared features original objects that flesh out the achievements of women across history, including suffrage campaigners. The display also features a larger replica of the OWSS banner, for viewing up close, as well as other exhibits pertaining to local suffrage history. It is hoped that these reproduction banners will be unfurled again each year in March. Perhaps they, like their original predecessors, will watch over more advancements for women in science.

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In conversation with the CEO of Repositive

Fiona Neelson

"Keep your eye on your long-term vision and ask for feedback and inspiration all the way."

What is the story behind Repositive?

Repositive is a company on a mission: We connect researchers in genetics with the data they need to speed up their biomedical research - resulting in better treatments for patients with cancer and genetic diseases.

My personal commitment to this mission stems from my experience of witnessing close family relatives, including my mother, go through the terrifying ordeal of standard of care cancer treatment of operation, radiation and chemotherapy - well knowing that none of these treatments were precise in the sense of Precision Medicine, and that we can do better. By understanding the molecular mechanisms of disease, including genetics, we can choose therapies that have a higher chance of working and effectively treating and potentially curing the patient. My mother was one of the lucky ones - her treatment worked and she recovered. I would like to see Precision Medicine become a reality, a future where medicines are prescribed because we know they are the right choice for that specific patient - no longer a game of chance - precision medicine for their specific cancer.

What kept you going through difficult times?

When I time and time again rediscover the day-to-day work of building a business is a rollercoaster of successes and failures, I bring myself back to balance and calm by taking a bigger perspective. This may be difficult and feel like a tough job, but I know that by putting in the work, I am contributing to achieving our mission and make our vision a reality. There is nowhere else I would rather be than working to achieve this. By the hard work our team puts in today, I know that we are helping the researchers and the patients of tomorrow.

How do you define success?

Our company, our product and our business model has been through a series of iterations. Every iteration has been a series of achievements, making it over a hill, only to see the bigger challenge, the mountain looming on the other side. As a hiring manager I am only successful if I manage to make the hire, as a CEO of a start-up with no cash, I am only successful if I convince investors to invest in our plan, as a commercial company, we are only successful if we sell our products and services and make a profitable business. However, the real success counter for me is every time I see that the work that we do at Repositive has a positive impact on the quality of research and improving the prospective outcomes for patients.

What future do you envision for Repositive?

In the big scale of things, Repositive is - although already 4 years old with lots of achievements to date - still a long way from achieving the fullness of our business and product vision. We are already international, we have customers in biopharma and contract research organisations all over the world, and we have enabled a large and growing community around genomic data access. In another three to five years, we will have enabled new drug discoveries not only in cancer (by our platform marketplace for translational cancer models) but also in other disease areas where we will have successfully connected thousands of researchers to the right genomic data assets to power their research.

What would be your message to all budding women entrepreneurs?

Take the long-term view. Know why you want to be an entrepreneur. I do not recommend becoming an entrepreneur without a strong reason for what you want to achieve. Know that it will take time and effort and not every part of the way will be enjoyable. Keep your eye on your long-term vision and ask for feedback and inspiration all the way. The sooner you realize your own strengths and weaknesses, the sooner you can build yourself a support network and advisers and a team that complements your skills and strengths. Asking for help when you need it is a strength in itself. Do not beat yourself up if your first attempt does not work, just zoom out again to your long-term vision and try again.

IN THE PURSUIT OF A GREAT EDUCATION AND CAREER

By Hannah Nazri

I did not always know that I wanted to study medicine. Admittedly, at some point growing up, I thought of becoming a doctor, but I also considered becoming an accountant, a lecturer and a slew of other things. The most defining moment in my life was when I was 14 and diagnosed with Rocky Mountain Spotted Fever of all things, and a prescribed tetracycline saved me. At that moment, I knew that I could potentially study medicine. But I did not start medical school thinking that I wanted to pursue a particular specialty. While some people knew that they wanted to do neurosurgery, I just did not know what I wanted; and even if I did know now, I still wanted to do a multitude of other things. Most people told me that I was wasting my time. In medical school, I took a year off between second and third years to study a medical physics degree and, as this is extremely uncommon in my native Malaysia, there were extensive rumours of me changing my degree and quitting medicine. The reason why I chose to study this degree was because I had briefly considered radiology as a career option and obtaining this BSc would be useful for the Royal College exams. I also wanted a way out in case I lost interest in medicine and would like a change of careers. After all, I would still have spent three years to get a degree and, even if I had quit, why is it such a taboo to change your mind instead of doing something that (people might think) could suit you better?

Fast-forward to the present, I am now a qualified doctor with a few years of practice under my belt and doing a DPhil in Obstetrics & Gynaecology at the University of Oxford. Prior to starting my DPhil, I spent a year in core medical training and realised that it was not my cup of tea. Importantly, the year before I became a core medical trainee, I was studying for my MSc in Clinical Embryology and became interested in research. 2017 was a stressful year for me, I contemplated leaving core medical training, which meant leaving my source of income, to pursue a DPhil. I received many angry emails dissuading me from leaving the training programme and, even not too long ago, I was told to quit my DPhil and return to Malaysia, where a job would be quickly available for me. I found that notion incredulous – if it is so easy, why am I working so hard for it? I had a partner who once told me that I changed; who would not change under the insurmountable stress of finding a scholarship, a locum doctor job and excelling in their DPhil?

Getting degree after degree can be a waste of time, if one uses those degrees as badges of honour. The same if someone travels to over 50 countries, taking countless photos but have no recollection of their favourite places or things they did, but have favourite photos (where they think they look nice) against a tropical landscape or historical monument that they may not remember. It can be pointless. As pointless as it may be asking me to go on this 'doctor-conveyor belt' without thinking whether it resonates with my interests and priorities. I would like to live a meaningful life and, in order to do so, I need time to decide. I love soaking into the moment and trying to learn as much as I can about things around me, even if sometimes I like lying down and not think about anything. To me, there is not much point in running from one goalpost to another for no other reason than being told to do so.

Since I was young, I was taught not to waste. With the education and knowledge that I have, I treat patients, encourage others to achieve their ambitions through The Kalsom Movement: a Malaysian-registered charity in which I am the Chair of Board of Trustees; and pursue research to find answers to pressing questions about endometriosis. I am passionate about being a useful, contributing member of the society. However, is my education a waste when my contributions are not just limited to certain geographical borders?

Pursuing my DPhil posed other unique challenges as I grew older. As a woman in her early thirties, my social media feed is full of weddings and births. "Your biological clock is ticking," as some people would cheerily say to me, as if my life was incomplete without a husband and no children. As a woman, we tend to overthink, and it is not too far-fetched to assume that most of us will think about our future family when considering taking up a promotion or a new job1. We overplan years in advance and can potentially stop ourselves from "taking up too much" because it will impact our future, yet-to-come-into-existence family units, as it happened to many of my friends who did not even consider medicine as a degree. An acquaintance made a point to tell me that he would expect his wife to quit her job in order to bring up the children, because he did not trust the day-care system. (How about him quitting his job if he did not trust the day-care system?). Men do not tend to think about future families when considering a promotion or taking up more responsibilities in the workplace, as traditionally the wife would make up for any shortfalls in the family. In fact, 46% of men of Princeton's Class of 2006 expected their wives to interrupt her careers2. Women in medicine are still being told to carefully choose their specialities to avoid the disappointment of a career away from their children, in which they should be the main caregivers. In fact, I was struck to learn that half of the men stated "their children" as hobbies in a survey carried out during a company retreat1b.

What is also quite surprising is that some men in traditional societies prefer their children to be delivered by female obstetricians3,4. Female obstetricians are not born or plucked out of a tree like a fruit, they are made through years of hard work and time away from their families too. Some of these women doctors wake up as early as 5am to prepare breakfast, wake up their children and get them ready for school, and to make sure that their husband's shirts are ironed before heading off to work at 8am for a gruelling 12-hour shift. If the female workforce has stepped up and contributed to the economy, why can't men also step up and help their wives? The idea of men as main providers and women as main caregivers is dated and sexist – what if the man has a chronic condition and is unable to work full-time? Does that make him less of a man? Of course not. Times are changing and both men and women need to play their part in this.

Finally, there is no such thing as perfect timing. There are no specific milestones that one must adhere to. While there is value in planning, one needs to remember that plans depend on many factors often beyond our control. The ability to ride the waves of life is a more valuable skill then having many university degrees could have. I took many risks to be where I am today. I was once told that medicine was beyond me because my father could not afford it. If we were to tell every child from a disadvantaged background that many things are off-limits to them, most of us would not have gone to become doctors or gone to Oxbridge for a DPhil. If you want to be exceptional, expect to face difficulties because nothing great comes easy. Be ready to challenge the status quo. In the words of Jim Rohn: "If you are not willing to risk the unusual, you will have to settle for the ordinary."

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1b. Sandberg, S. and Scovell, N. (2013). Lean In: Women, Work, and the Will to Lead. New York: Alfred A. Knopf, Chapter 8: Make Your Partner a Real Partner.

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