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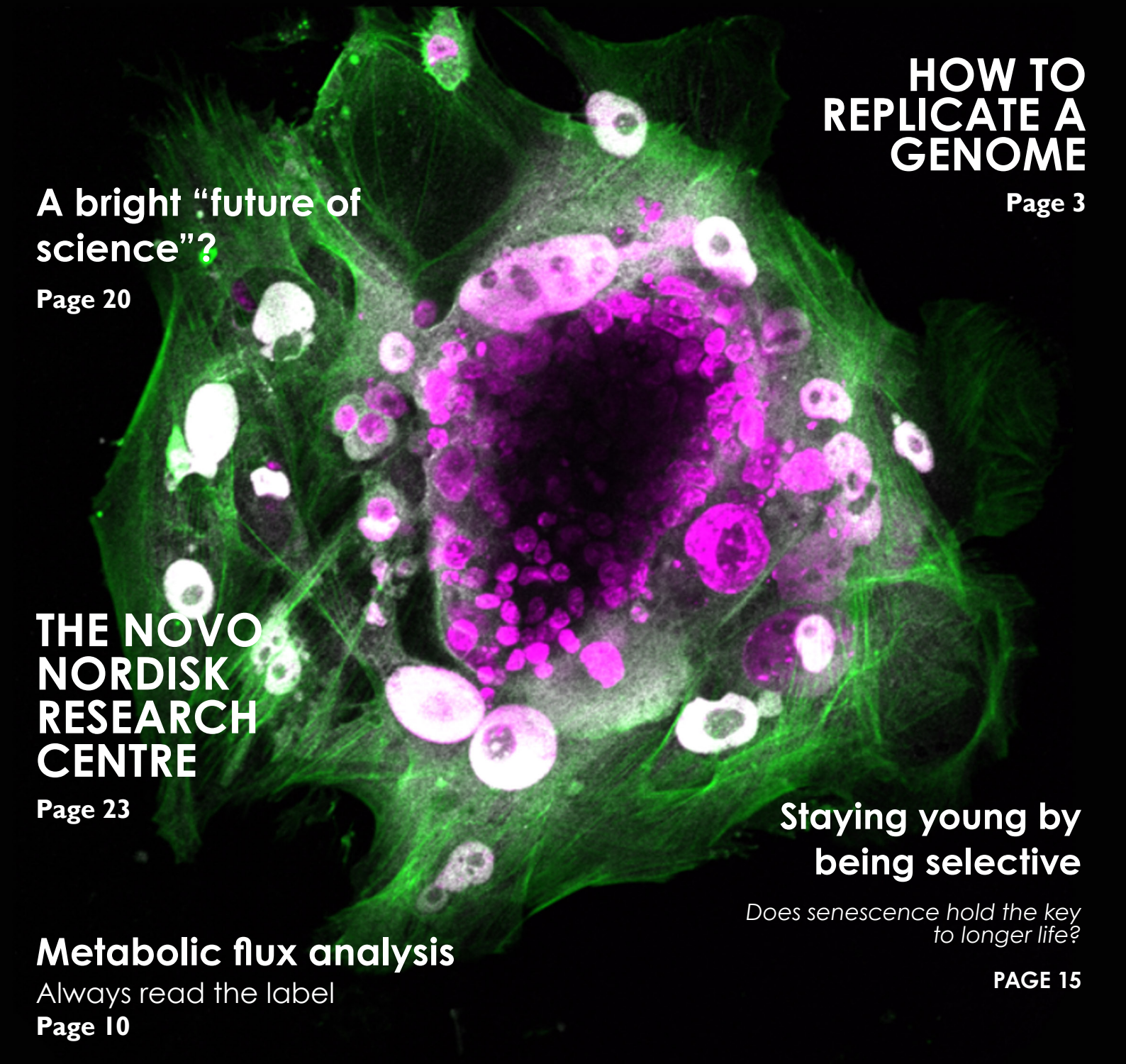

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David Acheson is an Emeritus Fellow of Jesus College, Oxford, and author of the best-selling popular maths book '1089 and All That', which has now been translated into 11 languages. His new book The Calculus Story was one of New Scientist's 'picks for Christmas'.

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Letter FROM THE EDITOR

Welcome to the 29th issue of Phenotype!

This issue is filled with exciting articles on a broad range of topics including intracellular molecular pathways, development of blood progenitor cells into mature cells, diagnostic tools to prevent heart attacks, and development of vaccines against the African Swine Fever Virus.

Professor Nieduszynski explains how cells replicate their genome on page 3, and Sheng Kai Pong describes how small non-coding RNAs respond to damaged DNA on page 5. Alice Robinson helps us understand the role of a protein complex, the retromer, in recycling transmembrane receptors from the endosomes to the Golgi network (page 8). On page 10 Thomas Gate provides an in-depth analysis of using flux maps to understand metabolic networks, and on page 14 Severin Limal provides insights into calcium imaging, a technique for monitoring neural activity in animals performing a sensory task.

You can also discover the intricacies of blood progenitor cell maturation by reading Aboukhalil *et al.*'s article on page 11.

Have you ever wondered whether senescence holds the key to longer life? Turn to page 15 to find out how to stay young by being selective. Then, turn to page 12 to read an insightful article on using cardiac troponin assays as a diagnostic tool, and page 17 for an overview of the current efforts towards developing vaccines against the African Swine Fever Virus.

Apart from our Features articles, please do check out our Regulars articles and the Science & Society section, where you can learn about the new Novo Nordisk Research Centre in Oxford (page 23), and the possible effects of Brexit on UK research (page 22).

In this issue, we celebrate the 2017 Nobel Prize in Physiology or Medicine with a circadian clock-themed infographic (page 26) and a crossword competition (on the back cover). Can you solve the crossword? The clock is ticking… send your entries as soon as possible for the crossword competition, as well as the snapshot competition (page 34), for a chance to win some amazing prizes!

My journey as Editor-in-Chief for Phenotype has come to an end. I have been very fortunate to work with an amazing team of editors and authors over the years. Their incredible work, dedication, and enthusiasm are evident in every page. Farewell, and I hope you always enjoy Phenotype as much as I have!

I now pass my duties to our new Editor-in-Chief: Jack Cooper. Please contact Jack at jack.heywood.cooper@gmail.com if you are interested in getting involved with Phenotype.

Stephanie Kapsetaki
Editor-in-Chief

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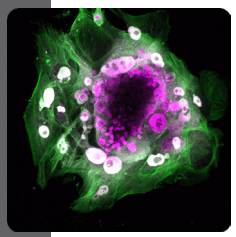
REGULARS

On the cover

The image shows blastocyst outgrowth. The outgrowth was stained for the protein YEP (grayscale) and the actin cytoskeleton (green). The nuclei (magenta) were stained with DAPI. Trophoblast giant cells, characterised by their exceptionally large nuclei, exhibit nuclear YAP localisation, whereas cells originating from the inner cell mass are devoid of nuclear YAP.



Read more on page 34 & 35!

How to replicate a genome

by Conrad Nieduszynski

Virtually every living cell contains a complete copy of its genome – the genetic blueprint for life. Maintaining genome integrity requires accurate replication of the whole genome prior to each cell division. Replication errors give rise to diversity and fuel evolution, but can also cause mutations that lead to genetic diseases, including cancer. Therefore, each time a human cell divides our ~6 billion base pair genome must be accurately copied in a process that takes just a few hours. The famous double helical structure of DNA enables a solution:

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

Watson and Crick's understated 'copying mechanism' has stood the test of time and we now have a molecular understanding of how DNA is replicated in bacterial and eukaryotic cells. However, in addition to maintaining accurate DNA replication, cells must also coordinate DNA replication with cell division. It is essential that the whole genome be replicated once and only once in each cell division cycle. Two pathological scenarios must be avoided: no region of the DNA should be replicated more than once, and no region should be left unreplicated. Our research aims to understand how cells ensure that DNA replication is complete before cell division.

The sheer size of eukaryotic genomes requires that the process of DNA replication must start at thousands of sites. These initiation sites, called DNA replication origins (Figure 1), are specialised DNA sequences that act as a scaffold for assembling the proteins required for copying the DNA. Each replication origin gives rise to two bidirectional replication forks, that proceed away from the origin to replicate the flanking DNA. Therefore, complete genome replication requires the activation of sufficient and appropriately distributed replication origins in every cell cycle. Failure to activate sufficient replication origins is associated with genome instability, including chromosome breakage and rearrangements.

My research group has identified the location and activity of replication origins in model organisms. Primarily, we work with baker's yeast (*Saccharomyces cerevisiae*), which offers powerful genetics, is one of the most established model systems, and has all the steps of genome replication conserved with that of humans. A major advantage of working with *S. cerevisiae* is that it was the first eukaryote to have its genome completely sequenced, and ever since it has been at the forefront of genomics research. Exploiting the advantages of yeast genomics allows us to make advances that accelerate experimentation in human cells, which may ultimately help treat human diseases.

When a region of the genome replicates, it changes from one to two copies per cell. Recent advances in genome sequencing essentially provide a precise counting machine, allowing us to accurately determine the time at which each region of the genome replicates (Figure 2A). In this way, we have been able to identify replication origins as the earliest DNA sequences to replicate and then track the movement of replication forks as the cells complete genome replication (2). Not all replication origins activate simultaneously; they rather activate in a characteristic order, such that different regions of the genome are replicated at different times (Figure 2B). A further complexity is that of the many origins available, only a subset is active in each cell division cycle, referred to as the Jesuit model: 'Many are called but few are chosen'. Therefore, to understand how genome replication is completed, we have to determine not only the location of replication origins but also understand what determines the differences in origin activity.

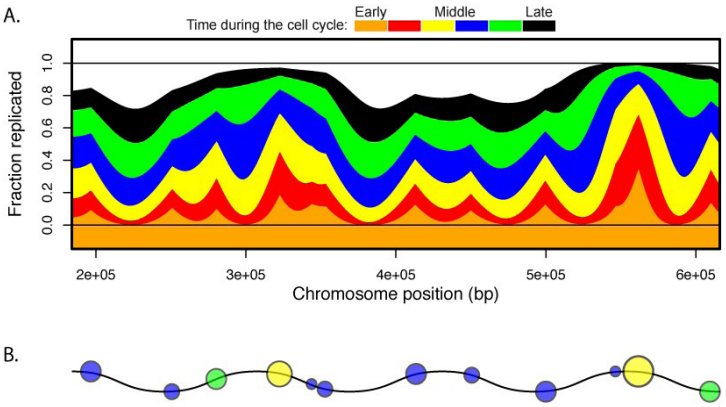


Figure 2. The dynamics of genome replication. (A) Deep sequencing measures the extent of DNA synthesis at each chromosomal location and time point through the cell cycle. Replication origins are the earliest sequences to replicate and therefore give peaks, with larger and earlier peaks corresponding to greater activity. (B) A cartoon characterising the location of replication origins (circles), when they are active (yellow is early; blue later) and how active they are (circle size).

What makes some replication origins highly active and the

Figure 1. Chromosome replication mosaic by Dunn School alumna.

others rarely active? One possible explanation could be the differences in the genome sequence of different origins. To address this, we used comparative genomics, an approach that involves comparing genome replication in a range of yeast species. Using this approach, we found that the origin sequences that recruit the replication initiator protein (Origin Recognition Protein, ORC) are evolutionarily conserved, referred to as a phylogenetic footprint (Figure 3). Scanning the genome for ORC-binding phylogenetic footprints allowed the pinpointing of each replication origin (3). We also discovered that replication origin activity is generally evolutionarily conserved: if an origin is highly active in one species the equivalent origin is similarly active in other species (4). However, just occasionally the activity of a replication origin differs between species and this offers us the opportunity to discover what causes such difference in activity. Understanding these mechanisms is important, since activation of an appropriate number of origins is critical: too many and the cell cannot supply sufficient precursors (e.g. nucleotides) and replication errors are made; too few and genome replication may not be completed on time.

Each region of a genome replicates at a characteristic time in S-phase, but is a region's replication time physiologically important? To address this question, we turned again to comparative genomics, this time assessing a much wider evolutionary range of yeast species. We discovered certain regions of the genome that replicate early in all of the species we examined – suggesting an evolutionary selective pressure for early replication (5). These regions included the centromeric region of eukaryotic chromosomes and certain individual genes, such as those encoding the histone proteins. Follow up experiments indicated that early centromere replication is important for stable chromosome inheritance (6) and early histone gene replication contributes to appropriate gene expression (5). Therefore, DNA replication time can be important and changes in replication time, for example during carcinogenesis, could potentially contribute to disease progression.

In an attempt to study the consequences of insufficient replication origin activity, we turned to an archaeon, *Haloferax volcanii*, for which our collaborator, Thorsten Allers (University of Nottingham) has developed genetic tools. The main chromosome of *H. volcanii* has just four replication origins; sufficiently few that we could try to delete most of them. Unexpectedly, we found no detriment to deleting origins and in fact the more origins we deleted the faster the cells grew. Eventually, we were able to delete all of the origins from the main chromosome with no obvious defect and accelerated growth (7). These findings challenged our assumptions about the requirement for replication origins. We now have to determine exactly how *H. volcanii* survives without replication origins and whether similar pathways exist in other organisms or in cancer cells.

There remain fundamental questions about the regulation of genome replication that we continue to study in model organisms and also cultured human cells. Our current research is focused on three areas: cellular heterogeneity, identifying the mechanisms responsible for differences in replication time, and determining how cells ensure that genome replication is completed. Insufficient replication origin activity contributes to genome instability and human diseases, including cancer. Therefore, identifying the mechanisms that act in healthy cells would be a critical

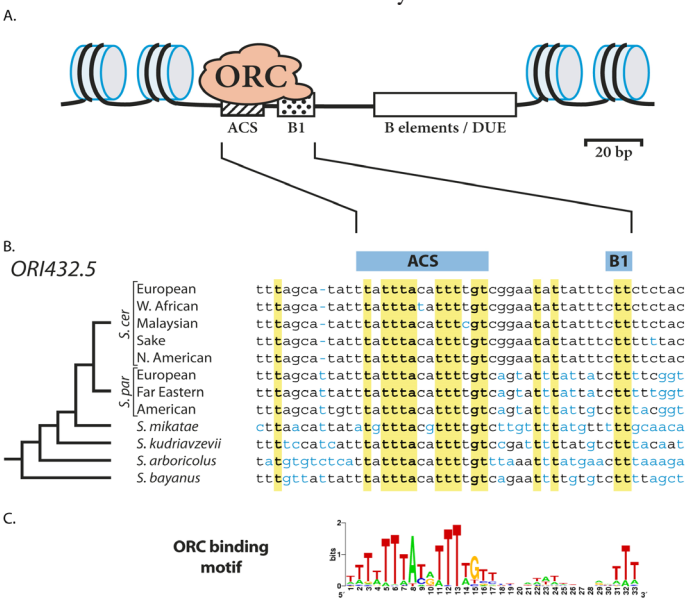


Figure 3. DNA replication origin structure. (A) Replication origins consist of a nucleosome-depleted region bound by the Origin Recognition Complex (ORC), referred to as the ACS-B1 in budding yeast. Additional elements that include a helically unstable region (the DUE or DNA Unwinding Element) promote the loading of additional replication factors. (B) An example of a phylogenetic footprint at a budding yeast replication origin with absolutely conserved bases highlighted in yellow. (C) A motif representation of the ACS-B1 element that is bound by ORC.

first step to understanding what goes wrong during disease development and how it might be treated.

Conrad Nieduszynski is an Associate Professor of Cell Biology in the Sir William Dunn School of Pathology.

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Transcription and genome instability: RNA-dependent damage response

by Sheng Kai Pong

Sheng Kai Pong is a DPhil student in Dr Monika Gullerova's research group at the Sir William Dunn School of Pathology.

In addition to being an intermediate between DNA and proteins, RNA can impose a layer of regulation on biological processes. This is commonly demonstrated by regulatory small non-protein-coding RNAs (ncRNAs). Recently, small ncRNAs have been shown to be involved in the mediation of DNA damage response (DDR) in higher eukaryotes, painting a more complex picture of the interplay between transcription and DDR. Are these processes mutually exclusive? If not, how do they interact with each other? This article discusses the crosstalk between transcription and DDR.

DNA damage response and small non-coding RNAs

The hereditary information of our cells is stored in the form of DNA. Cells are constantly subject to endogenous and exogenous stresses that may perturb the stability of their genome. These perturbations can cause informational or structural changes to the genome, leading to dire consequences such as cell death or cancer formation. Therefore, it is of paramount importance that cells protect their genome integrity. To this end, organisms have evolved DDR, a series of mechanisms that detect, signal and repair DNA damage. DDR is initiated and carried out by a complex signalling cascade involving a number of proteins, which are meticulously regulated as they need to sense and fix DNA damage as quickly and accurately as possible. The prevailing view of DDR is that the process primarily involves the action of proteins.

Only around 2% of the human genome is transcribed into messenger RNAs (mRNAs), which directly code for proteins. The rest of the genome generates a repertoire of RNAs that do not have protein-coding capacity, the ncRNAs. For example, microRNAs (miRNAs), a group of 18 to 25 nucleotide-long (small) ncRNAs, carry out post-transcriptional gene silencing, together with the RNA-induced silencing complex (RISC), by depleting levels of mRNA through complementary binding. miRNA exemplifies a means by which the non-coding component of the DNA can contribute to the regulation of gene expression, which eventually affects various biological processes (1). Recently, novel classes of small ncRNAs have been shown to be directly involved in mediating DDR.

A direct role for small RNAs in DNA damage response

In 2012, Francia *et al.* reported "an unanticipated direct role of a novel class of ncRNAs in the control of DDR activation at sites of DNA damage". Upon reducing the levels of Drosha and Dicer, key proteins in miRNA biogenesis, they observed impaired DDR activation in zebrafish, mice and human cells (2). The authors excluded miRNA-mediated gene silencing as a possible reason for the observation because reducing the levels of effectors that are components of the RISC, a downstream complex that carries out gene-silencing functions of miRNAs, does not affect DDR. Interestingly, small ncRNAs that are generated from the sequence of the damage sites were detected. The authors named the small ncRNAs, DNA damage response RNAs (DDRNAs). In the absence of DDRNAs, DDR was less efficient.

> *"an unanticipated direct role of a novel class of ncRNAs in the control of DDR activation at sites of DNA damage"*

In miRNA biogenesis, Drosha is responsible for processing primary transcripts generated from miRNA genes into miRNA precursors in the nucleus. Dicer cleaves the miRNA precursors into mature miRNAs in the cytoplasm. Given that both Drosha and Dicer are involved in RNA-dependent DDR, it is proposed that DDRNAs are products of these two proteins. A follow-up study by the same research group showed that Drosha, Dicer and DDRNAs altogether are essential for the recruitment of secondary DNA repair factors, which are crucial for the amplification of DDR, but not the primary factors necessary for detecting and sensing DNA damage (3).

Another independent study by Wei *et al.* showed that plants with Dicer-like protein mutants have compromised repair of double-strand breaks (DSBs), the most dangerous DNA lesion (4). The authors observed small ncRNAs, which they called DSB-induced small RNAs (diRNAs), in plants in which DSBs were induced. They found that diRNAs work alongside Argonaute 2 (Ago2), a principal component of RISC, to facilitate DSB repair. diRNAs do not affect the phosphorylation of the histone variant H2AX, an event that occurs very early in DDR, echoing the results found by other studies that small ncRNAs are not required for early DDR events. In human cells, the researchers showed that depleting the levels of Dicer and Ago2 results in impaired DSB repair, thereby linking the mechanism in plants to the mammalian system.

Recently, a paper by Burger & Gullerova demonstrated that when DSBs are induced in human cells, Dicer is phosphorylated, re-localised into the nucleus, and recruited to DSB sites (5). Mutations in the catalytic

domain of Dicer cause accumulation of double-stranded RNAs (dsRNAs) in cells with DSBs, suggesting that Dicer processes these dsRNAs. The dsRNAs detected in human cells after DSB induction may be formed by the hybridisation of complementary transcripts produced at sites flanking the DSB.

These studies suggest a direct role for small ncRNAs in the mediation of DDR. DDRNAs in zebrafish, mice and human cells, which are dependent upon Drosha and Dicer, recruit secondary repair factors to the damage site, effectively amplifying the DDR signal, while diRNAs in plants associate with Ago2 to facilitate the repair mechanism (Figure 1). However, many questions regarding the molecular mechanism of RNA-dependent DDR remain. Are DDRNAs and diRNAs products resulting from Dicer and Drosha processing of dsRNAs generated from the damage sites? How does RNA polymerase (Pol) II recognise the damaged sites and transcribe close to or from the sites? More research needs to be done to elucidate this newly discovered role of ncRNAs in DDR.

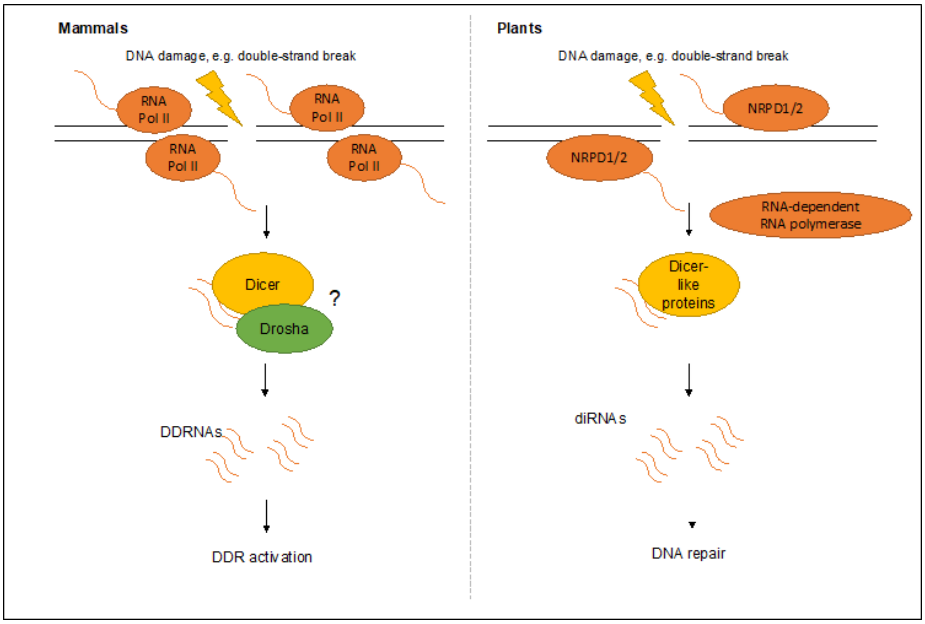

Figure 1. The biogenesis of DDRNAs in mammals and diRNAs in plants, and their involvement in DDR and DNA repair.

Interplay between transcription and DNA damage

The notion that ncRNAs are transcribed in the vicinity of DNA damage sites is contrary to the popular belief that transcription is shut down where DNA damage occurs. The rationale behind this belief is straightforward, that transcribing damaged DNA can generate aberrant RNAs or proteins that may harm the cell. This is certainly the case in Neurospora crassa where endogenous short-interfering RNAs, another class of ncRNAs, suppress transcription from damaged sequences. In mammalian systems, however, recent evidence has shown that non-canonical transcription occurs in the vicinity of DNA damage sites, even though DNA damage appears to repress transcription globally (6).

How can we reconcile these two contrasting findings, that transcription is suppressed where DNA damage occurs and that small ncRNAs are transcribed close to the damage sites? It is proposed that during DNA damage, the chromatin structure loosens up and becomes accessible to DDR and DNA repair factors. Before repressive epigenetic marks that suppress transcription are spread throughout the damaged region, transcription factors and RNA Pol II can exploit the 'open' structure to generate primary transcripts that are then processed by endonucleases Drosha and/or Dicer into small ncRNAs to ensure proper activation of DDR. This model explains how transcription can occur during DNA damage, but more research is required to support it.

Both transcription and DDR are highly robust and tightly regulated cellular activities that involve many components. Thus, precise regulation of the interaction between transcription and DDR is crucial for the cell to maintain its genome integrity. Understanding the molecular mechanism of RNA-dependent DDR can provide insights into this intricate relationship between these two fundamental cellular processes.

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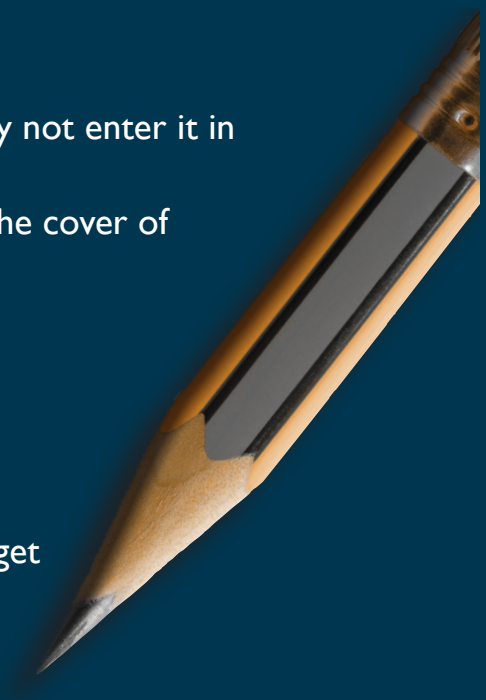

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THE RETROMER COMPLEX

understanding its coordination of active signalling by the endosome

by Alice Robinson

The retromer complex is a highly conserved, key endosomal sorting machinery that was first discovered in 1998 in *Saccharomyces cerevisiae* (baker's yeast) (1). Since its discovery, retromer has been identified as having a role in numerous mammalian physiological processes, including the active regulation of membrane composition (2). Retromer function has been divided into two roles, whereby the major and moderately well-defined function of the retromer complex is its role in the endosome-Golgi retrieval pathway. Alternatively, the minor and relatively poorly understood role details its involvement in the recycling of endosomal cargo back to the plasma membrane. In recent years, this minor retromeric function has attracted much interest, and alongside other protein machinery is challenging the long-standing view that endocytosis acts simply and passively to down-modulate the number of proteins at the plasma-membrane. Instead, an alternative paradigm is proposed, whereby the endocytic network, and more specifically the endosome, are able to actively control receptor signalling (3).

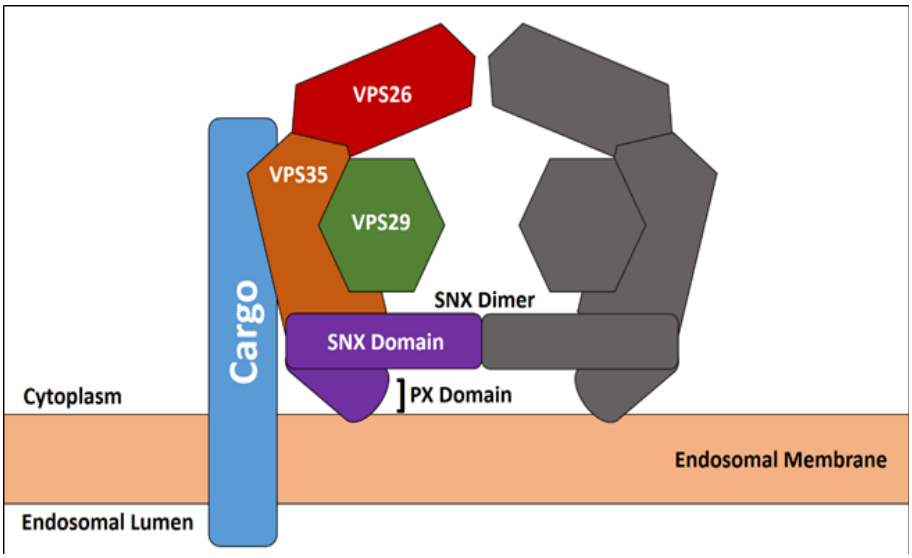

Figure 1. The structure of the retromer complex, whereby core trimeric proteins Vps26-35-29 are shown to interact with the cargo, while the SNX dimer is shown to form following dimerization of two retromeric subunits.

Structurally, retromer is a heteropentameric protein complex consisting of two distinct sub-complexes, including a stable core trimer of Vacuolar Protein-Sorting (VPS) proteins, VPS26-VPS35-VPS29, and a variable sorting-Nexin (SNX) dimer (Figure 1). However, while the retromer complex is conserved across eukaryotes, numerous differences in higher eukaryotic subunits provide variation in retromeric function, cargo recognition and binding sites (2).

Despite its structural differences, for all eukaryotes, retromer is recruited to the endosome in component form, prior to its functional assembly at the endosomal membrane. This process is largely controlled by the small guanosine triphosphatases (GTPases) Rab5 and Rab7, recruiting SNX domains and the Vps-trimer, respectively, before retromer can interact with, and determine the fate of, endosomal cargo (4). Furthermore, retromer-mediated trafficking is assisted by a broad range of accessory proteins, giving rise to recruitment of cargo, membrane scission and fusion, docking and transport along the cytoskeleton (5).

Considering that retromer function can be broadly divided into two categories, the function of a single retromer is determined by the type of SNX-dimer present. To date, three distinct forms of retromer have been identified: the SNX-BAR-retromer, which includes any SNX protein containing BAR domains, the SNX3-retromer and the SNX27-retromer. However, while the SNX-BAR and SNX-3-retromers are involved in retrograde transport, albeit by different mechanisms, the SNX27-retromer solely mediates shuttling of endosomal cargo to the plasma membrane, bypassing any involvement with the TGN (Figure 2; 2, 3).

Contradictory to the classical belief that the Vps-trimer exclusively mediates cargo selection, there is increasing evidence to suggest SNX-dimer proteins act as adaptor molecules for cargo selection. For example, the presence of two specific domains, known as FERM and PDZ, encoded for within the SNX27-retromer, correspond to molecular motifs encoded for within endosomal cargos destined for recycling back to the plasma membrane. This simple cargo recognition demonstrates how the SNX-dimers not only determine the trafficking pathway utilised, but also provide diversity in cargo recognition (2). However, while the function and our ability to assign proteins to a specific retromeric fate are well-defined, a comprehensive understating of the molecular regulation and direction of cargo back to the plasma membrane is lacking.

Therefore, while the structure and function of the retromer complex has been thoroughly investigated with success, exploration and definition of its mechanistic action, especially that of its minor role, has seen much less progress. Such a lack of molecular insight not only limits our ability to understand how membrane composition is regulated, but also hinders our ability to identify key molecular targets and develop appropriate molecular tools for the successful targeting of retromer dysfunction-related pathologies, including a range of developmental and neurodegenerative diseases.

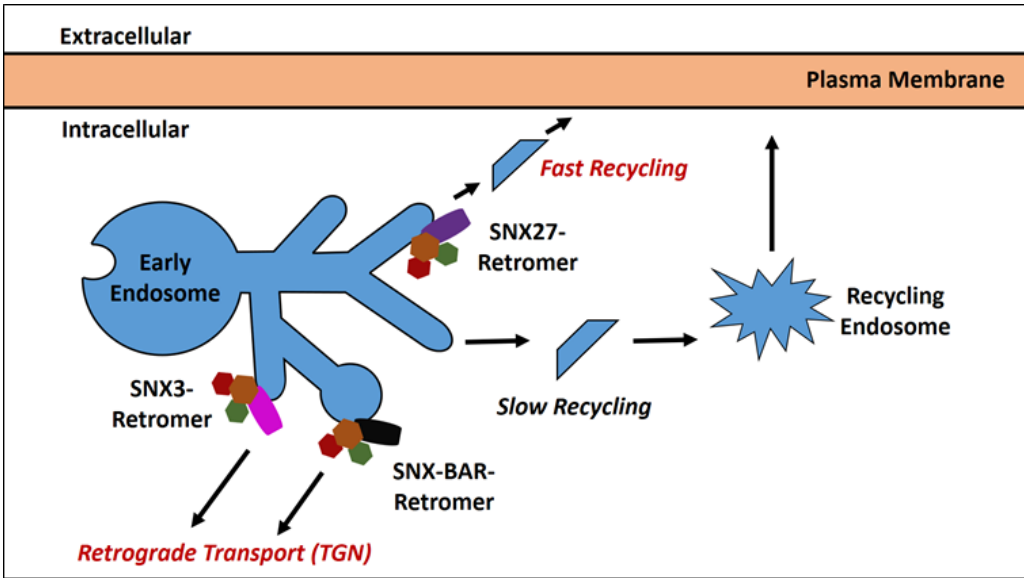

Figure 2. Schematic representation of retromeric function, highlighting the major (retrograde transport) and minor (fast recycling) roles of the retromer complex (red).

More recently, increasingly relevant studies have focused on the endosome-to-plasma membrane trafficking of endosomal cargo. These studies have not only demonstrated how specific endosomal cargo interacts with both stable and variable domains of the retromer complex, but have also provided evidence for retromer-cargo interactions that allow the endosome to function as an active signalling platform for receptor sorting and signalling.

One key example is the role of retromer in interferon (IFN)-induced JAK/STAT signalling and downstream gene transcription. While the IFN receptor complex (IFNAR) forms at the plasma membrane, downstream JAK/STAT signalling is only activated following translocation of IFNAR to the early endosome. Interaction of IFNAR2 with core trimeric protein Vps35 sees separation of IFNAR1&2 subunits, resulting in termination of JAK/STAT signalling and trafficking of IFNAR subunits to appropriate cellular locations; be that lysosomal degradation of IFNAR1, or fast recycling to the plasma membrane of IFNAR2 for further rounds of signalling. However, in the absence of retromer, IFNAR subunits do not undergo spatio-temporal separation, and as a result, abnormally prolonged JAK/STAT signalling results in altered downstream gene transcription, including upregulation of numerous inflammatory genes. Therefore, in the presence of an appropriate retromer-receptor interaction, the endosome is not only able to control receptor sorting, but is also able to actively modulate downstream signalling (3).

A second example is retromeric modulation of the parathyroid hormone receptor (PTHR), a class B G-protein coupled receptor (GPCR). Following stimulation, the PTHR is internalised to the early endosome, where agonist and receptor dissociate due to the acidic environment. However, while the PTHR contains a PDZ-binding ligand making it suitable for SNX27-retromeric recycling, later studies proved its recycling back to the plasma membrane was in fact critically dependent upon interaction with core trimeric protein Vps26 (2).

Similarly to PTHR, another example of retromeric modulation is the recycling of the β2-adrenergic receptor (β2AR) between the plasma membrane and endosome in the presence of a β2AR agonist. A comprehensive mechanistic understanding for this control was uncovered when the SNX27-retromer was identified as an essential adapter required for the targeting of β2AR to retromeric tubules, for fast recycling back to the plasma membrane and re-sensitisation of cells. For β2AR ligands unable to interact with SNX27, a phenotype of non-recycling to the plasma membrane was observed, thus highlighting the active modulation of β2AR signalling by retromer, at the endosome (6).

Therefore, if the endosome is indeed able to actively control signalling outputs, the importance and necessity of further investigation into the relationship between endosomal associated receptors and the retromer complex is clear. However, it is important to note that the retromer complex is one of potentially multiple controls of endosomal signalling. Therefore, investigations into the retromer complex and alternative endosomal signalling controls, such as retriever, will not only provide vital molecular clues about the poorly understood signalling control, but also aid more complete characterisation of how the endosome actively modulates protein sorting and signalling.

Alice Robinson is a Research Assistant in Professor Monaco's research group at the Kennedy Institute of Rheumatology.

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By Thomas Gate

Metabolic flux analysis

always read the label

Thomas Gate is a fourth year Biochemistry undergraduate student in Nick Kruger's research group at the Department of Plant Sciences.

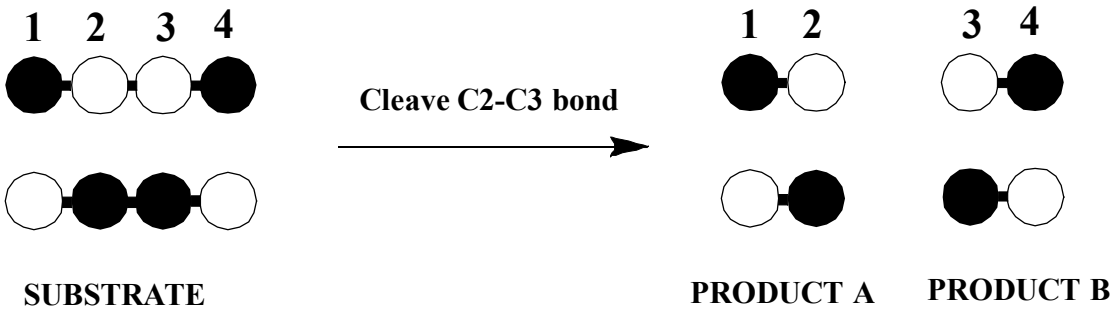

Figure 1. Hypothetical reaction with differently labelled 4-carbon molecules broken into two 2-carbon products with different labelling patterns.

Metabolism is not simply a chain of linear reactions. It is, instead, more accurately represented as a network. These networks can be investigated by a process called metabolic flux analysis (MFA). In this technique (1), an organism is fed a substrate containing both 13-carbon (C) and 12-C atoms. Then, downstream reactions in the metabolic network transfer the carbon atoms from the substrate to measurable products in the organism, like amino acids. The distribution of 12-C and 13-C in a product is characteristic of the labelling pattern of the substrate, the network structure and the fluxes of the reactions within the network. Therefore, this provides a means for the fluxes of reactions in the metabolic network to be calculated.

To carry out MFA, the metabolic network first needs to be designed in silico. Network design involves specifying only how the reactions rearrange the carbon skeleton of the substrate to form the product. Next, a substrate labelling strategy needs to be optimised. As shown in Figure 1, different substrate labelling patterns lead to different product labelling. As the fluxes for the reactions in the network rely on using the information in the labelling pattern of the products, different substrate labelling strategies will allow distinct information to be gained about the network. Simulations can be run using varied combinations and proportions of differentially labelled substrate to optimise the precision with which the flux map for the metabolic network as a whole can be determined (2). A complication of the simulations of such substrate labelling experiments arises due to natural abundance of the 13-C isotope (1.1%) and impurities in labelled substrates, as often only 99% purity is attained.

Once the optimal substrate labelling strategy has been determined, the wet work can begin where the organism is fed with the labelled substrate and left growing until a steady state is reached, with no change in substrate and product concentration of individual reactions in the network. At this point, the cells are harvested and the contents of the cell (including amino acids which are often the products to be analysed) are extracted, processed and then sent to be analysed by nuclear magnetic resonance and mass spectrometry. From these two methods, information about the incorporation of the labelled and unlabelled carbon into products can be extracted and used to estimate the flux map of the network. A software, in my case INCA, that generates random flux maps, and predicts a product labelling pattern for each map is used to find the best fitting flux map for the network model. The software then compares this product labelling pattern to that obtained experimentally, and then keeps altering its flux map until the predicted and obtained product labelling patterns match.

Flux maps can provide very useful information about both prokaryotic and eukaryotic metabolic networks. For example, flux maps were used to identify highly active futile cycles in the bacterium Corynebacterium glutamicum (3) which were wasting ATP and reducing industrial lysine production. Currently, in my project in the Kruger lab, I am using MFA to compare the flux maps of the soil bacterium Azorhizobium between aerobic non-nitrogen fixing conditions and microaerobic nitrogen fixing conditions to better understand the metabolic changes that occur during switching from one condition to the other.

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Deciphering the complexity of blood progenitor cells

By Zahra Aboukhalil, Bilyana Stoilova & Dimitris Karamitros

Zahra Aboukhalil, Bilyana Stoilova & Dimitris Karamitros are researchers at the Weatherall Institute of Molecular Medicine.

Blood production by haematopoietic stem and progenitor cells is complex, with multiple proposed models of differentiation. Using single-cell technologies we are uncovering the ways in which blood progenitors generate mature cells. We have discovered differentiation occurs primarily through single-cells that only produce one mature cell type, and rarely through progenitors that retain the ability to generate multiple cell types. Dysregulation of haematopoiesis can lead to blood cancer, and an improved understanding of these fundamental processes may provide insight into how to target the disease.

10 billion new, blood cells are produced every day. This process, known as haematopoiesis, generates a range of cell types. These include red blood cells that carry oxygen to tissues, platelets that are responsible for blood clotting, and white blood cells that fight infection. This process is tightly regulated and its dysregulation can lead to haematopoietic deficiency, immunodeficiency, and blood cancer (leukaemia).

At the top of the blood cell hierarchy are haematopoietic stem cells (HSCs) (Figure 1). HSCs have the unique ability of both producing more of themselves (self-renew) and generating the range of mature blood cells. Downstream of the HSCs are several intermediate, progenitor cells. These have a decreased ability to self-renew and are more restricted in the types of cell they can produce. These progenitors may generate the bulk of blood cells, while HSCs remain mostly inactive (1). However, the exact mechanisms of haematopoietic differentiation remain unclear.

Traditionally it was thought that haematopoietic differentiation occurred through discrete progenitor cell types that bifurcate in a tree model of differentiation. These studies were based on bulk populations of progenitors cells that have shown the potential to produce multiple blood cell types. However, recent advances in technology now allow us to study cells at the single-cells level, uncovering a previously unknown heterogeneity among the individual cells.

It has come to light that many progenitor populations are a heterogeneous mix of single-cells. Previously, multiple potentials had been seen from a bulk population of cells. It is now proposed this often arises from a collection of single-cells, each with a distinct differentiation potential. When analysing many cells together, these scenarios cannot be distinguished. But by studying single-cells, we are able to make this distinction.

In haematopoiesis, our understanding of differentiation from HSCs to mature blood cells has advanced. Many researchers have proposed that downstream of HSCs there is a pool of progenitors, each of which is only able to generate one mature cell type (2). This abolishes the idea

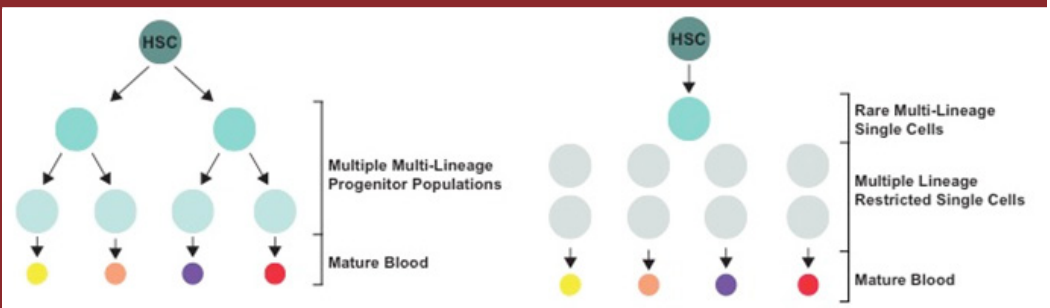

Figure 1. Mechanisms of haematopoietic differentiation: The schematic on the left depicts a classic model of differentiation with discrete progenitor cell types that bifurcate in a tree structure. The schematic on the right depicts an updated model where rare single cells have multi-lineage ability, but the majority of single cells are lineage restricted.

We have shown, using pioneering single-cells techniques, that the differentiation mechanism is complex (3). We have studied around 5,000 single haematopoietic progenitor cells both functionally and by gene expression. The vast majority of single-cells do only produce one cell type. However, we uncovered rare progenitor cells that possessed the ability to generate multiple cell types.

Our work shows that HSCs are not the only cells in haematopoiesis that generate multiple mature blood cell types. Rare progenitor cells retain this ability. In addition to these rare cells are the majority of progenitors, each of which are primed towards their goal output. These single-cells have restricted their differentiation potential and are fated towards one cell type.

There must be, tight regulatory mechanisms controlling this fine balance between lineage-restricted and multi-lineage single-cells. When this regulation is lost, cancer can cripple the system. Acute myeloid leukaemia (AML) is the most common acute adult blood cancer and in patients cancerous progenitor-like cells, instead of generating healthy, mature blood, propagate the disease. By studying how these healthy progenitors generate their vital mature output, we may provide insight into targeting their diseased counterparts in AML.

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CARDIAC TROPONIN ASSAYS:

a versatile diagnostic tool that is simple, yet not so simple

By Kyung Chan (KC) Park

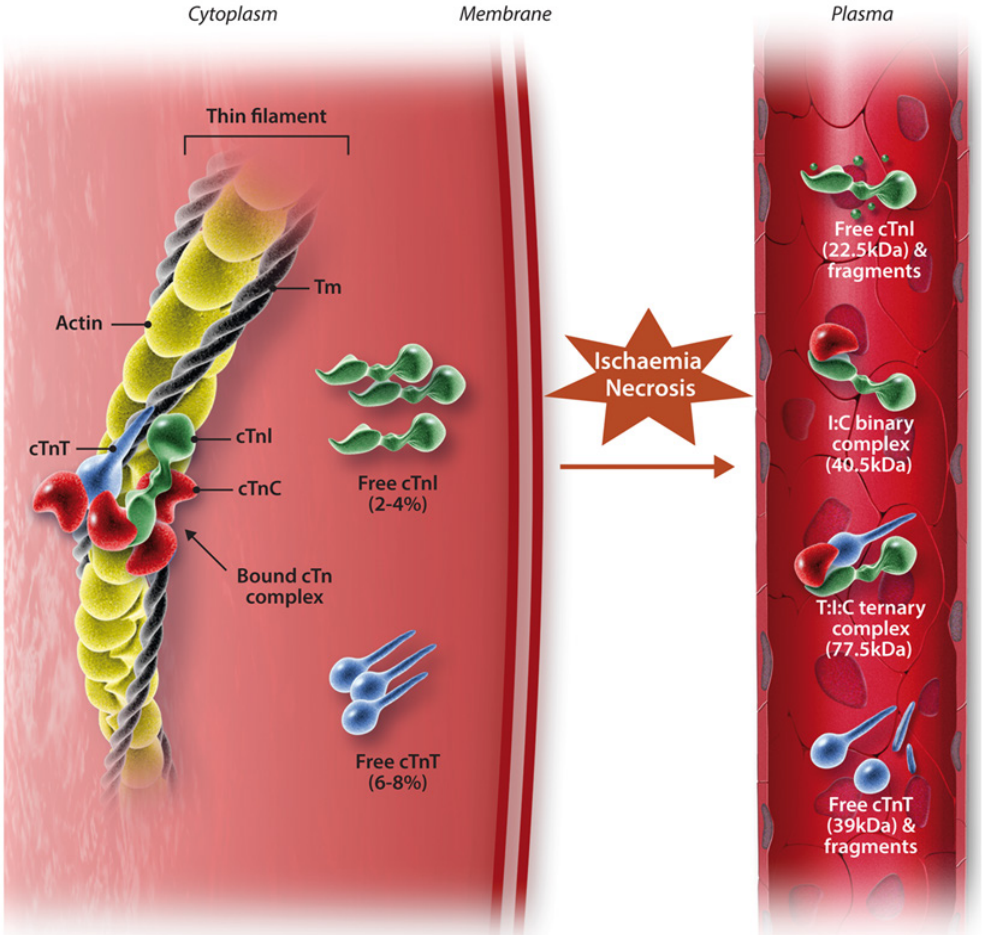

Figure 1. Structure of the cardiac troponin-tropomyosin complex and the forms of troponin released following myocardial necrosis. Not all troponin is bound to the myofibril. ~2–4% and ~6–8% of cTnI and cTnT respectively exist either unbound in the cytosol, or 'loosely bound' to the sarcomere. These forms are released first. As well as free-forms of troponin, complexed forms of troponin are detected in serum. Figure from Park *et al.* (5) under the Creative Commons CC BY licence.

Cold sweat, shortness of breath, chest pain, and an impending sense of doom. These are some of the common signs of an acute myocardial infarction (AMI), more commonly known as a heart attack. It is critical that blood supply is restored promptly to prevent further death of myocardium. As such, it is essential an AMI is confirmed (or excluded) as soon as possible to determine the appropriate treatment pathway. But how can you tell if someone is having a heart attack? The cardiac troponin (cTn) has been the gold-standard indicator of myocardial necrosis for the past decade, and is a routine test used for the diagnosis of AMI. However, cTn is frequently elevated in non AMI-related conditions, and in chronically unwell patients these levels of cTn are correlated to prognosis.

Finally, it is disconcerting that a rise in cTn can be detected following extreme exercise. Yet, available evidence reinforces the view that troponin is only released from cardiomyocytes following irreversible cell death.

The heart and skeletal muscles are striated. That is, they are marked by transverse dark and light bands featuring repeating contractile units, called sarcomeres. The sarcomere consists of thick myosin filaments and thin actin filaments. The heartbeat is initiated by depolarisation and is modulated by the interplay of Ca2+ ions with regulatory sites on the contractile apparatus. Actin filaments have a double helical structure, and these regulatory sites—the troponin complex—lie in the groove of the helix. Troponin was discovered in 1965 by Ebashi and Kodama as a single homogeneous protein (1). It was not until 1973 that Greaser and Gergely identified the three subunits of troponin: 'I' for inhibitory (TnI), 'T' for tropomyosin (TnT) and 'C' for calcium (TnC) (2).

There are isoforms of troponin that are expressed selectively in cardiac muscle (cTnI and cTnT), which led to the development of one of the most successful diagnostic tests in clinical practice–the cTn assay. The detection of cTn at a concentration above the population-based threshold (the '99th percentile') is a prerequisite in the diagnosis of acute myocardial infarction (AMI) as per the 'Universal Definition of Acute Myocardial Infarction' (3). An AMI occurs when there is a reduction of coronary flow, resulting in myocardial ischaemia and necrosis (death of cardiomyocytes). As the myocyte dies, its membrane is disintegrated, leading to leakage of cellular contents and spill-over of troponin into the circulatory system (Figure 1).

Most cTn assays are non-competitive enzyme-linked immunosorbent assays (ELISA), utilising the high specificity of antibodies. Thus, cTn is a highly selective and sensitive readout of cardiomyocyte death. However, it is important to emphasise that cTn is indeed an excellent assay for cardiomyocyte death, but herein lies a problem. An AMI is not the only source of cardiac injury. Whilst cTn is the gold-standard clinical indicator of myocardial necrosis, it is frequently elevated in acute conditions of extra-cardiac origin (e.g. sepsis, pulmonary embolism); >50% of patients presenting to the Emergency Department with chest pain have a 'positive troponin', yet show an absence of any clinical signs of acute myocardial ischaemia (4). Furthermore, cTn is elevated in a myriad

of chronic diseases (e.g. chronic renal failure). These elevations however, are important and have value, since cTn levels in chronically unwell patients are directly related to prognosis (5). For practical reasons, there have been no prospective studies altering patient's treatment to determine whether it has an effect on troponin release. As such, the mechanisms resulting in cTn release in chronic diseases are speculative and largely based on inferences from clinical trials and animal models (Figure 2).

In recent years, evolution in assay technology has ironically made the interpretation of cTn results more difficult. Whilst conventional cTn assays had an analytical sensitivity in the picomolar range, new high-sensitivity cTn assays (hs-cTn) can detect troponin in the femtomolar range. It is impressive that the biological variation of troponin can be detected in healthy individuals over a 4-hour period (6). However, it is highly disconcerting that in using these assays, a rise in troponin can be detected after extreme exercise (7). Such findings have reinforced a viewpoint that troponin is capable of being released with reversible cell injury—without necrosis, or even cell death: for example, due to an increase in membrane permeability of the release of membranous blebs (8). However, it is important to note that the majority of these studies use alternative readouts of cardiomyocyte death (e.g. lactate dehydrogenase assays, immunohistochemistry) that are significantly inferior in analytical sensitivity versus cTn (5, 7); that is, cell death occurs which is not detected using such methods. A new experimental study substantiates this idea, whereby hs-cTn assays detected troponin elevations from necrosis of a few milligrams of myocardium: an amount beyond the resolution of any imaging technique (9).

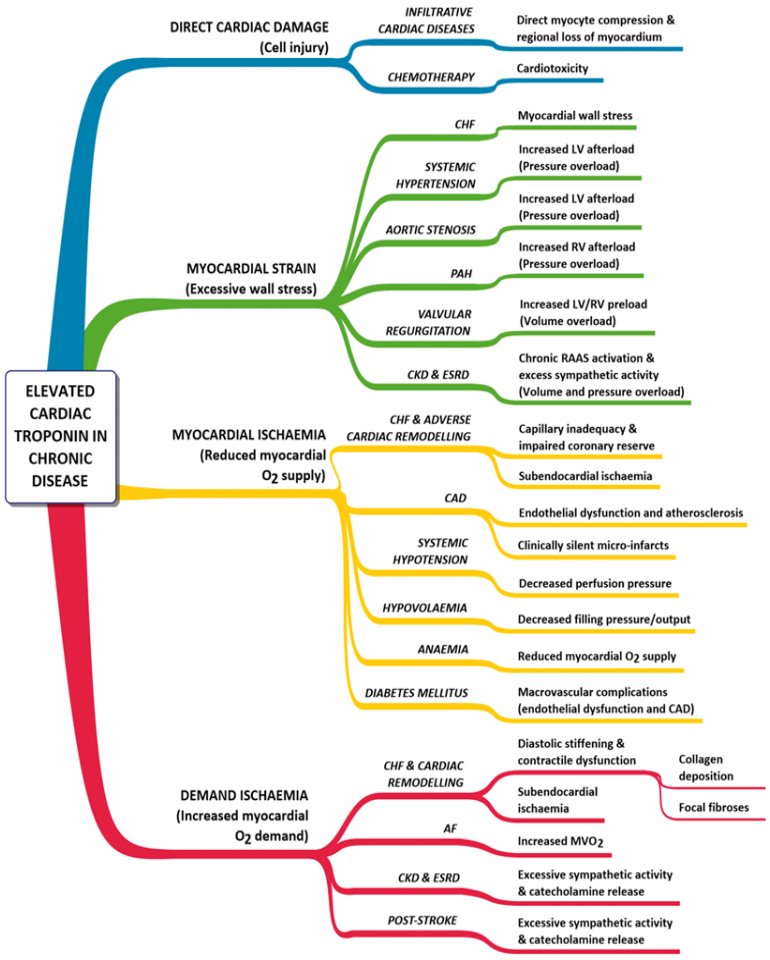

Figure 2. Presumed mechanisms for elevated cardiac troponin (cTn) in chronic diseases. Elevated cTn has been documented in the conditions listed. AF, atrial fibrillation; CAD, coronary artery disease; CHF, chronic heart failure; CKD, chronic kidney disease; ESRD, end-stage renal disease; LV, left ventricle; MVO2, myocardial oxygen consumption; PAH, pulmonary arterial hypertension; RAAS, renin-angiotensin-aldosterone system; RV, right ventricle. Figure from Park *et al.* (5) under the Creative Commons CC BY licence.

When the cTn assay was first developed, the rationale behind it was relatively simple: myocardial necrosis and cell death during an AMI leads to membrane disruption and troponin release which is detected in serum. Measuring cTn is a fundamental criterion in diagnosing an AMI. Despite interesting theories, available evidence reinforces the view that troponin is only released from cardiomyocytes following irreversible cell death. Any detectable cTn is reflective of myocardial injury, although in a majority of cases (such as in chronic diseases), the mechanisms of release are unclear. However, such releases should not be ignored as cTn levels are correlated to overall prognosis, irrespective of the underlying disease.

Kc Park is a British Heart Foundation DPhil Student led by Associate Professor Pawel Swietach and co-supervised by Dr Nicola Smart in the Department of Physiology, Anatomy & Genetics.

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CALCIUM IMAGING IN AWAKE ANIMALS

A new window into the mind?

By Séverin Limal

Since its early days of development, multiphoton calcium imaging has allowed us to simultaneously image neural activity in tens, if not hundreds, of neurons in a single field of view in an animal's brain. By expressing calcium-binding fluorescent molecules, we are able to track changes in calcium concentration in neurons, used as a proxy for neural activity. When a neuron fires an action potential, cytoplasmic calcium ion concentration increases, increasing the calcium indicator's fluorescence. Therefore, changes in a neuron's fluorescence can be used to monitor its activity. While the technique was initially implemented using in vitro preparations or anaesthetised animals, more recent developments have allowed for imaging of awake animals over time (2; Figure 1), enabling us to monitor patterns of neural activity in animals performing a sensory task.

Thanks to long-lasting imaging, it is now possible to image the same brain area over the course of several days or weeks. This can shed light on essential processes such as habituation (a decrease in typical response to a repeating stimulus, like learning to ignore the repetitive 'tick-tock' of a clock). However, it can also be applied to more complex paradigms, like comparing the same subject's neural responses to a specific stimulus while it learns to associate the stimulus with a reward. By targeting brain areas known to be involved in learning and memory or reward prediction, we have the ability to measure how a stimulus' representation changes with its associated value, or at least the learning process underlying the change.

One of the main limitations of the technique resides in the calcium indicator kinetics. There is both a delay in calcium ion build-up as well as conformational changes in the indicator. This means that temporal resolution equivalent to that of electrophysiological recordings cannot be achieved, reducing insight into the timing and patterns of a specific neural response. However, very few techniques allow for the simultaneous imaging of hundreds of neurons and the subsequent insight into their spatial layout. Furthermore, while temporal resolution may not be optimal, it remains sufficient to estimate spike train patterns (how neurons encode information) (3).

How does this relate to an animal's perception or train of thought? The short answer is we do not know. We cannot ask it what it is thinking, but we can infer this from its behaviour. We can train a subject to recognise a particular stimulus and associate it with a reward. More importantly, we can image the same awake animal and apply machine-learning algorithms to the extracted data to decode its behaviour or perception (4). In short, computational tools are being developed in order to 'read a subject's mind', or to decode neural codes and provide a correct estimate of an animal's behaviour or response to stimuli to which it is exposed.

At present, this means we can decode simple stimulus representation, but as the technique develops, we can hope to decode more complex representations or motivational states. Eventually, through measuring changes in fluorescence in the brain, we may be able to assemble a picture of a subject's environment, or even its motivational state – a literal window into the mind.

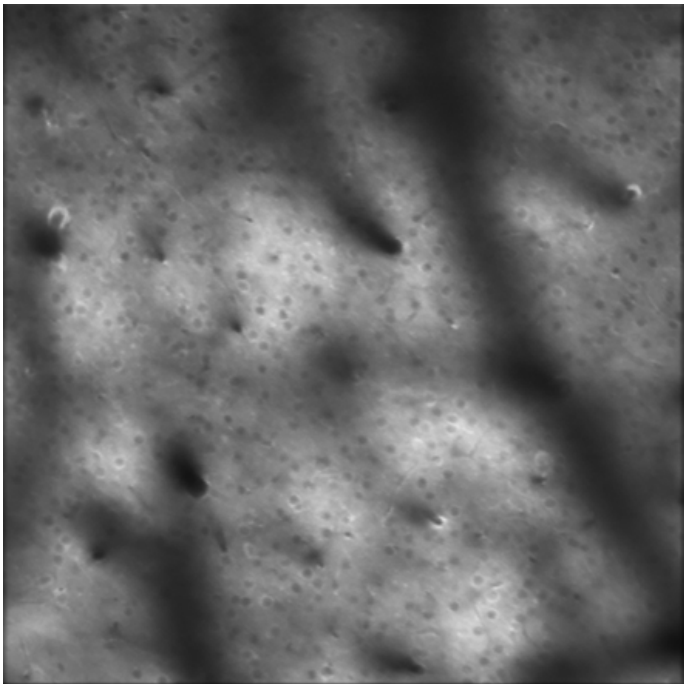

Figure 1. Average image in a 2-photon field of view. Every 'doughnut' is a neuron. Activity patterns can be extracted from changes in fluorescence in every neuron across an imaging session.

Séverin Limal is a DPhil student with Dr Michael Kohl, Dr Kerry Walker and Prof Andrew King's research groups at the Department of Physiology, Anatomy and Genetics.

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Staying YOUNG by being selective: does senescence hold the key to longer life?

By Paul J. Rutten

Cellular senescence, long understood as an anti-tumorigenesis mechanism, has recently been linked to the process of ageing with startling implications for therapeutics. Mouse studies have not only shown that senescence is partly responsible for many of the effects of ageing, but also that removing senescent cells can retard these effects and even extend lifespan. Several companies are now working to replicate these results in humans, but many challenges remain to be overcome.

Like many ground-breaking discoveries, it started with an unexpected but serendipitous result. In 2000, Jan van Deursen and his group at the Mayo Clinic in Minnesota were studying the pathways that lead to cancer (1). They had created transgenic mice expressing low levels of the BubR1 mitotic checkpoint protein. Insufficient levels of BubR1 causes premature separation of sister chromatids during replication, resulting in aneuploidy in the daughter cells: one will get too much DNA, and the other too little. The imbalance is slowly exacerbated with each new division, and Van Deursen and his group expected mice with this progressive genetic aberration to be very prone to cancer. To their surprise, the mice instead exhibited a very severe form of premature ageing, called progeria.

When Van Deursen and his group investigated this, they found that knocking out BubR1 significantly changed the expression level of two genes, called p16Ink4a and p19Arf, in many tissues (1). Both genes had previously been linked to cell senescence, and the BubR1 knockdown mice were accumulating an abnormally large number of senescent cells.

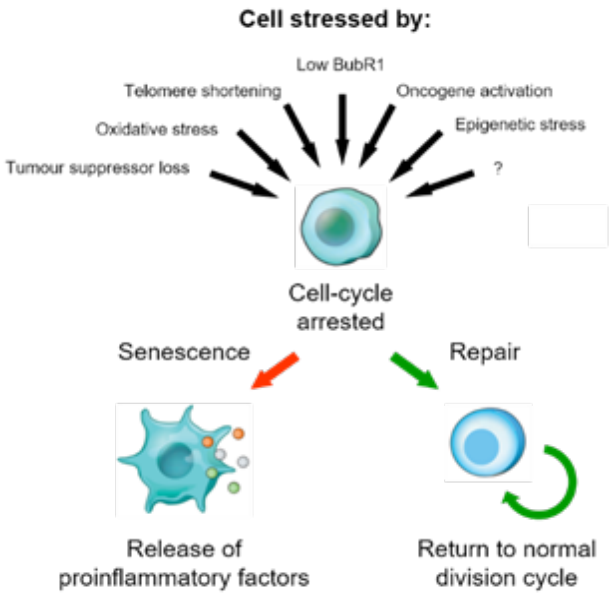

Figure 1. Cell cycle arrest leading to senescence can be caused by a large variety of signals. Once division stops, cells may repair themselves or irreversibly senesce and begin releasing pro-inflammatory factors promoting their clearance by the immune system. Figure adapted from Van Deursen (3).

First described by Hayflick and Moorhead in 1961, senescence is a process by which cells irreversibly stop dividing and undergo widespread changes in both their metabolism and chromatin structure (2). It is believed to be primarily an anti-cancer mechanism; cells which are not dividing are at much lower risk of becoming cancerous (3). This is supported by many of the signals and stresses that appear to induce senescence in a cell (Figure 1). The most well-known trigger is insufficient telomere length (DNA repeats at the ends of chromosomes which shorten with each cell division). Many cells stop dividing once their telomeres become too short. Several senescence triggers appear to be associated with the DNA damage response and are frequently mediated by the p53 tumour suppressor protein. Other triggers are the result of much more specific tumorigenic events, like the loss of the PTEN tumour suppressor gene (4). A common theme amongst many of these stresses is that senescence is a 'failsafe' process that cells activate to prevent themselves from becoming tumorigenic. However, senescence has also been associated with other important processes including wound healing and development. Critically, many different cell types secrete pro-inflammatory factors upon becoming senescent to trigger the immune system to attack them. Clearance of senescent cells is therefore a naturally occurring process, but may become compromised with age as immune function diminishes.

Investigating their mice, Van Deursen and his group showed that the build-up of senescent cells was causing many of the observed ageing phenotypes. They also demonstrated that knocking out p16Ink4a, thus decreasing the production of senescent cells, boosted the healthy lifespan of their transgenic mice and delayed many of their ageing symptoms. This was a major breakthrough, suggesting a connection between senescence and the process of biological ageing at the organismal level. Hayflick and Moorhead had originally postulated such a link, but without any evidence their theory had been largely ignored for decades. Van Deursen's results suggested that many of the effects and degenerations we associate with ageing might be due to the accumulation of senescent cells in our tissues. Suddenly, a phenomenon which had received relatively little attention for decades was in the spotlight. The next question was clear: could senescent cells be removed to slow down or even reverse ageing?

In 2011, Van Deursen's group published a paper showing exactly that (5). They generated transgenic mice in which they could selectively clear senescent cells (Figure 2). The clearance of senescent cells as the mice aged, significantly

improved muscle function, reduced loss of adipose tissue, and substantially delayed the formation of cataracts. They had invented a completely new kind of therapy: senolytic drugs which specifically cleared senescent cells.

Within a month of publication, Van Deursen and seasoned biotechnology entrepreneur Nathaniel David founded Unity Biotechnology, a start-up dedicated to creating senolytic therapies for humans. As of October 2017, 14 compounds with senolytic activity had been discovered. Interestingly, some chemotherapy drugs currently in use have been found to have senolytic activity, possibly revealing a new mechanism of action. Several senolytics have shown considerable promise in treating mice (6). Zhu *et al.* showed in 2015 that a single dose of a senolytic drug was able to improve cardiac function in mice within days (7). Even more promisingly, Van Deursen's group was able to extend the lifespan of healthy wild-type mice by up to 25% with regular treatment (8).

Although many of these results are encouraging, considerable scientific challenges remain. Senescent cells are as varied as cancer cells, so it is very unlikely that a single 'magic-bullet' drug will be found to clear them all. Nor is such a widespread attack likely to be a good idea; far from being harmful, many may be performing a crucial function. Instead, current efforts are focusing on developing therapies that target specific ailments by attacking senescent cells in a particular organ or tissue. The Mayo clinic is currently recruiting participants for a clinical trial to treat chronic kidney disease using a combination of senolytic drugs. Another challenge with these therapies is frequency of dosing and whether senescent cells could develop resistance. The plausibility of senolytic therapies therefore remains hotly debated. Despite apparent successes in mice, many researchers remain sceptical that any therapy, no matter how innovative, can truly make a dent in the ageing process.

At present it seems unlikely that senescence will revolutionise human lifespans anytime soon. Researchers in the field are quick to point out that nobody should be taking existing drugs with senolytic activity in the hope of slowing their ageing. By their very nature, trials to test for an increase in human lifespan are challenging and are hampered by the fact that ageing is not considered a disease by many drug regulatory bodies, including the Food and Drug Administration (FDA) in the US. Nevertheless, even if senolytics may not hold the key to the fountain of youth, improving our understanding of senescence could pave the way for novel treatments and give researchers a completely new perspective on ageing.

"At present it seems unlikely that senescence will revolutionise human lifespans anytime soon"

Paul Rutten is an Oxford Interdisciplinary Bioscience DTP DPhil student in Professor Philip Poole's research group at the Department of Plant Sciences.

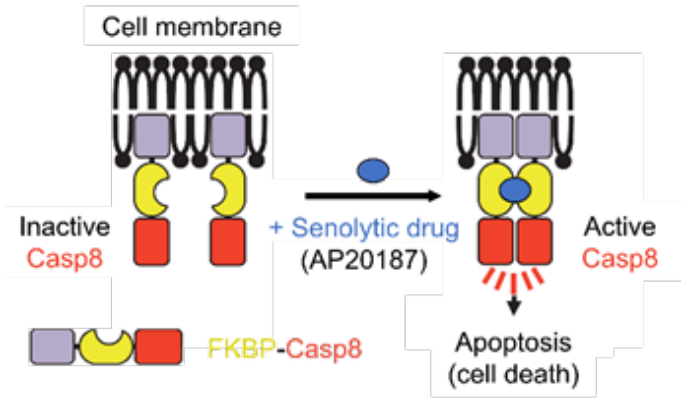

Figure 2. The first senolytic 'therapy' to be designed in mice. Researchers created an artificial membrane protein and placed it under the control of the p16Ink4a promoter in transgenic mice. Many cells activate this promoter upon senescing, causing the production of myristoylated (grey) FKBP-Casp8. Exposure to AP20187 causes the membrane protein to dimerise, activating Casp8 and inducing cell apoptosis. This system allowed specific killing of senescent cells in mice on treatment with AP20187. Adapted from Baker *et al.* (5).

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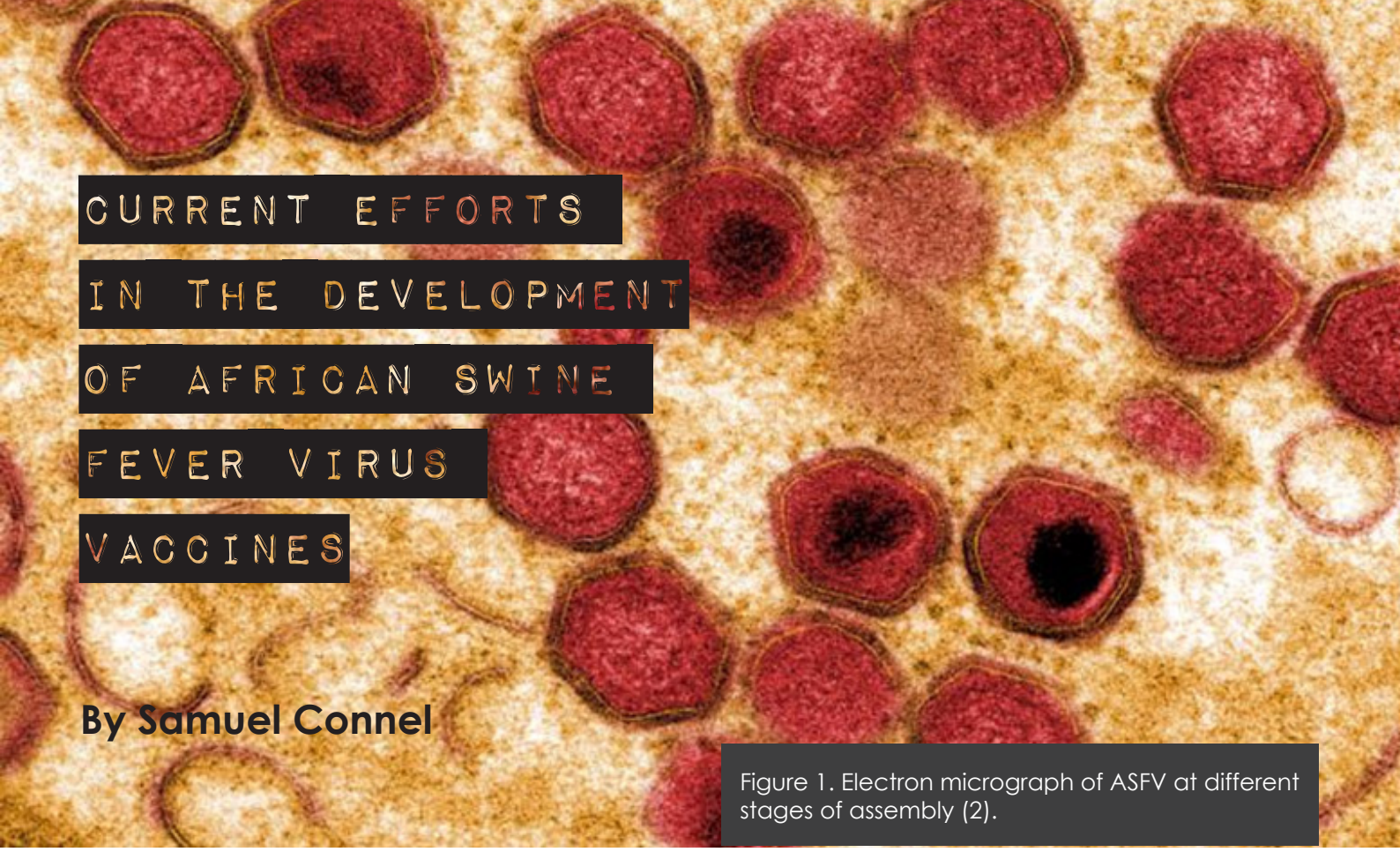

CURRENT EFFORTS IN THE DEVELOPMENT OF AFRICAN SWINE FEVER VIRUS VACCINES

By Samuel Connel

Figure 1. Electron micrograph of ASFV at different stages of assembly (2).

The potential impact of animal infectious disease on humans is often overlooked. Not only do livestock outbreaks impact animals, but they can have economic and food security consequences. These effects were clearly visible in 2001 during the Foot and Mouth Disease virus epidemic, which resulted in the culling of 10 million animals and a loss of £8bn to the UK economy. There is increasing concern that emerging viral infections, including African Swine Fever Virus (ASFV), pose a stark threat to agriculture.

Infection with ASFV results in an exceptionally severe viral haemorrhagic disease of domestic pigs, with virulent strains showing an unprecedented lethality of up to 100%. The virus has long been established in sub-Saharan Africa where infection is seen in pigs and asymptomatically in its natural host, the warthog. However in 2007, ASFV gained a foothold in the Caucus regions, and has since disseminated through wild boar populations into Eastern Europe, which poses a threat to domestic swine as a viral reservoir. Cases have been observed in the Baltics, Poland, and recently the Czech Republic. Due to ASFV's contagiousness and its extreme lethality, there is growing concern that ASFV poses risk of a lasting and devastating effect on European pork industries (1).

ASFV is an enveloped double-stranded DNA virus, with an icosahedral structure, and is the sole member of Asfarviridae family (Figure 1). The virus possesses a covalently closed linear genome which is uncharacteristically large for a virus at 170-193 kb, encoding approximately 160 genes. Replication occurs in the cytoplasm of macrophages (2). Transmission in warthogs occurs via an ancient sylvatic (wild) cycle involving the soft-bodied tick vector Ornithodoros, making ASFV the only known DNA arbovirus. In populations of domestic swine, however, transmission occurs via direct contact with infected animals or meat.

No vaccine is available and current control measures are limited to culling and restriction of livestock movement. However, this relies on meticulous disease reporting and laboratory testing, methods which are unsuitable for sub-Saharan Africa, and unsatisfactory for the EU. Control of viral disease using vaccination is widely regarded as the most efficient and cost effective method. Thus continued efforts to develop an efficacious ASFV vaccine are of paramount importance in preventing an epidemic.

Numerous approaches to vaccine development have been trialled since the 1960's, however despite extensive research efforts, none have proved successful enough for commercial production. Although there is evidence for both cellular and humoral responses being required for protection, little is understood about mechanisms of natural protection, complicating vaccine development. Several approaches are being used to produce candidate vaccines.

"Numerous approaches to vaccine development have been trialled since the 1960's, however despite extensive research efforts, none have proved successful enough for commecial production."

Inactivated viral vaccines

Candidate vaccines have not conferred significant protection, even in the presence of adjuvants. This may be due to the requirement for cellular immunity for protection, whereas inactivated vaccines typically induce a humoral response.

Subunit vaccines:

Vaccination with viral vectors expressing viral proteins in order to induce immune response has been proposed. Numerous ASFV proteins have been identified as targets for antibody neutralisation, such as essential structural proteins and immunogenic antigens p30 and p54. Trials with baculovirus expression vectors have shown limited promise, and generated conflicting results with heterologous ASFV strains. The viral transmembrane CD2 homologue (CD2v) binds to an unknown cellular surface molecule, inducing erythrocyte aggregation around infected macrophages, thought to enhance viral dissemination via the circulatory system, or 'hide' infected cells from destruction. DNA vaccines of plasmids expressing fusions of p30, p54 and CD2v induced an ASFV specific CD8+ T-cell response, but not neutralising antibodies, and only conferred partial protection (3).

Live attenuated vaccines

Live attenuated vaccines (LAVs) have so far been the most promising. Despite the lethality of virulent ASFV strains, the naturally-occurring attenuated isolate OURT88/3 was identified in 1988. Immunisation with OURT88/3 confers CD8+ T-cell mediated protection against lethal challenge with homologous and heterologous ASFV.

Despite protection of 60-100%, depending on immunisation route and dose, the risk of side effects such as transient fever, low viraemia and arthritis with LAVs has prevented their commercialisation.

Approximately 30% of the ASFV genome is formed of multi-gene family genes (MGF). These are 5 clusters of paralogous genes, believed to have arisen via gene duplication, a method of rapidly adapting to selective pressure observed in DNA viruses. Although MGF genes are highly conserved, there is significant variation between the number of genes within each cluster in different viral strains (2).

Despite the function of proteins encoded by MGF genes currently being unknown, there is increasing evidence that they may play a role in virulence by subverting the interferon (IFN) response, enhancing survival (4). Attenuation of OURT88/3 is due to a large contiguous deletion of 8 MGF genes (MGF360-10L, 11L, 12L, 13L, 14L, MGF505-1R, 2R, 3R). Both OURT88/3 and Pr4 (another isolate lacking a number of MGF genes) showed increased IFN-β and IFN-stimulated gene expression in vitro compared to virulent counterparts (5). Furthermore, virulent viruses possessing these genes show increased replicative efficiency in the presence of IFN-α. This knowledge provided the rationale for the development of a recombinant virus vaccine, deleting the 8 MGF genes absent in OURT88/3 from virulent strain Benin97/1, termed BeninΔMGF (Figure 2). Inoculation with BeninΔMGF conferred significant protection against lethal challenge (6). Deletion of other genes involved in immunomodulation has also shown promise. Immunisation with a deletion mutant lacking early gene DP148R conferred protection, and showed replicative capability yet significantly reduced pathogenesis (7). Furthermore, deletion of a combination of virulence factors reduced virulence and improved safety relative to single gene deletion, as demonstrated with DP96R and 9GL deletion from the virulent Georgia07 strain.

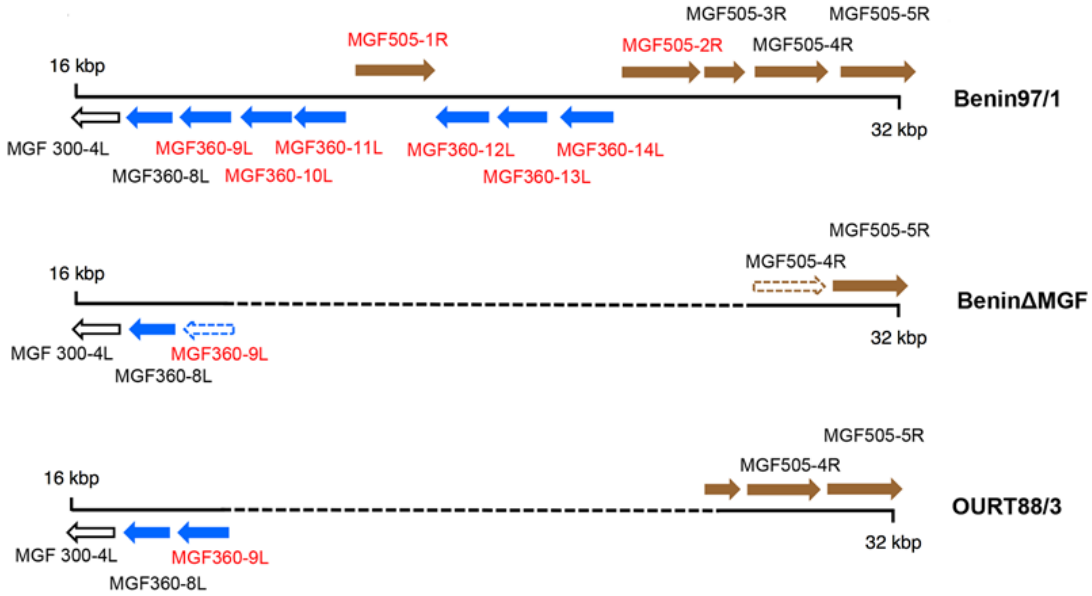

Figure 2. Deletions of MGF360 and MGF505 genes in attenuated isolates. Adapted from (6).

These studies indicate that deleting virulence genes and IFN interactors shows potential as an effective ASFV vaccine strategy. Currently extensive work is being performed to identify and elucidate ASFV modulators of the IFN system with the aim of rationally developing a recombinant LAV that finely balances efficient viral replication and immunogenicity, but avoids clinical presentation. Further testing of recombinant LAV is required to assess cross-protection against varying genotypes.

Additional issues remain to be addressed. The dissemina-

tion of the vaccine virus through wild boar populations in inoculation programmes may be challenging. Bait laden with vaccine could provide a solution, a method proven successful in the vaccination of foxes against rabies virus. The inability to distinguish between inoculated animals and those naturally infected is also a concern, as it would hinder disease monitoring and prevent movement of meat products due to perceived risk of further spread.

Despite the efforts of the past 60 years, an ASFV vaccine remains elusive. Development of LAV currently provides the greatest potential, but deeper knowledge of roles of viral genes and immunogenicity over varying doses is essential if a vaccine is to be deemed safe and sufficiently efficacious for commercialisation.

"Despite the efforts of the past 60 years, an ASFV vaccine remains elusive. Development of LAV currently provides the greatest potential..."

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A bright 'FUTURE OF SCIENCE'?

By Sonia Muliyil

The Future of Science Symposium, held on 28th September 2017 by the Dunn School Graduate Student Association, was truly a grand event. The day kicked off with a series of talks from stalwarts in academia, industry and government policy-making, who shared their thoughts on what could define the future of science. Sir John Walker, Nobel Laureate and Emeritus Director of the MRC Mitochondrial Biology Unit Cambridge, stressed the need for fundamental discoveries that can be utilised for the benefit of mankind. He discussed the biggest challenges currently facing mankind: the energy crisis, ageing-associated neurodegeneration, and the resistance of pathogens to drugs. He pointed out the requirement for stable and long-term research funding, coupled with freedom from the influence of scientific policy makers. Changing gears, Sir Walker moved on to speak about scientific giants who inspired him at different stages of his career, naming Edward Abraham, Frederick Sanger and Max Perutz as his three main heroes. Finally, touching upon the subject of his own Nobel Prize, Sir Walker acknowledged his younger collaborators for all the fame and glory that he has received during his career. His parting words to the audience were "You are the future of science".

"You are the future of science".

Immunocore CEO Dr Elliot Forster began by discussing the exciting scientific milestones being achieved across the world. These included the gene editing of human embryos in China, the development of a fully functional kidney from nephron-like cells in Australia, and, closer to home, the UK's 100,000 Genomes Project. Dr Forster believes that the life sciences can deeply benefit from technological advancement. To exemplify this statement, he shared the newest technologies being developed by Immunocore to engineer T cell receptors in order to kill cancer cells. When asked about how to find your own niche within the biotechnology sector, Dr Forster emphasised the importance of focusing on challenging areas and using state of the art tools, and of protecting new technological breakthroughs with the aid of patents.

Dr Ruth McKernan, Chief Executive of Innovate UK, started her talk by stating that the best way to predict the future of science is to make it yourself. She spoke of her personal journey in science, which involved working for a number of companies including Merck and Pfizer, as well as a short sabbatical with the newspaper The Independent. She discussed the different perspectives and intellectual insights that she gained as part of these ventures, which helped her pen down her thoughts and combine her personal and scientific experiences in the form of a book titled Billy's Halo. Dr McKernan then went on to describe the aims and scope of Innovate UK, which drives productivity and growth by supporting businesses. She enlightened the audience about the various funds and grants available to support researchers to start their own small enterprises, and highlighted that the themes gaining maximal funding are precision and discovery medicine, improvement of agricultural productivity, and advancements in therapeutics. When asked about the relative responsibility of research versus industry to promote basic sciences, Dr McKernan pointed out that the motivation behind these two professions are distinct. The driving force in research is curiosity coupled with monitoring of the rigour and impact of the work being performed. In contrast, industry jobs are focused on new ways to deliver business and create a profit for shareholders. She ended with a statement that there is a renaissance in the life sciences which needs to be capitalised on, a sentiment echoed by two speakers of the morning session.

Image depicting the group discussion led by Dr
The image has been provided by Mariya Lobar

After a coffee break, the sessions reconvened to discuss policy making and the relevance of outreach events in garnering funding for research, as well as the importance

r Ben Goldacre, Dr John Tregoning, Dr Jo Dally and Story Sylwester.
novska from the Dunn School Graduate Student Association.

of raising one's voice for the betterment of science (Fig. 1). This discussion was led by a diverse group of speakers from different walks of life, including Dr John Tregoning (an academic and blogger), Dr Ben Goldacre (an academic and bestselling author), Dr Jo Dally (Head of Policy, Research at the Royal Society), and Story Sylwester (March for Science coordinator).

The afternoon session began with Dr Ruxandra Draghia-Akli, Vice-President for Scientific Affairs at Merck Co. Global Vaccines Group, describing the value and impact of vaccines. She commented on the fact that vaccine development can be a long and drawn-out process, owing to the influence of various social and economic issues. She also brought to light the possibility that rare events may escape detection during vaccine clinical trials, thus stressing the need for post-licensure monitoring. She then infused a ray of hope by explaining how agencies like the International Rare Diseases Research Consortium are actively working towards reducing the average time to diagnose a rare disease from ten years to a year. Dr Draghia-Akli also spoke of the European Lead Factory and its efforts to build the first compound library and screening centre, which aims to resolve a number of common issues in drug discovery that exist within the European Union. She ended her talk by reiterating the need for new drugs to tackle 'bad bugs'.

One of the most awaited talks of this session was given by the Editorial Director of Nature, Dr Ritu Dhand. She spoke of how Nature has been experimenting with various peer review and open access options to make scientific publishing more transparent and accessible to the scientific audience at large. Dr Dhand spoke in detail about new strategies that have been tested across various journals belonging to the Nature publishing house. These strategies include publishing anonymised referee reports online, as well as opening up the peer review procedures by introducing double-blind peer review in Nature Geoscience. She also spoke of how the publishing house is actively working towards improving data accessibility through a combinatorial approach. This involves the introduction of concepts such as SourceData, a platform that allows researchers and publishers to share source data underlying scientific figures, which is now mandatory for all submissions to Nature, and Protocol Exchange, which will allow researchers to share an in-depth description of their methods either publically or privately. To combat problems associated with impact factor ratings, all Nature journals have also started to assign an Altmetric score to each paper. The score is calculated using the paper's ratings on social media, the general public interest generated and the feedback received, and does not take into account the institution where the research was conducted. Additionally, some Nature journals such as Nature Communications are 100% open access, allowing free access to all published articles. Dr Dhand also spoke of other recent additions, which include adding a research summary for each paper to render articles accessible to a broader audience. Dr Dhand ended her talk by stating that scientific publishing has become highly dynamic and creative in its approach, such that it is almost impossible to predict the direction in which it will evolve.

Overall the symposium was a great success, bringing together people from different avenues of science to share their thoughts, criticisms, and ideas about the future of science. It was inspiring to note that almost every speaker felt that science is currently undergoing a phenomenal change, with young minds rising to the challenge of driving science forward.

"...science is currently undergoing a phenomenal change, with young minds rising to the challenge of driving science forward."

A recording of The Future of Science Symposium is available online via Oxford Podcasts
http://podcasts.ox.ac.uk/series/future-science-symposium

Sonia Muliyil is a HFSP Postdoctoral Researcher in Matthew Freeman's research group at the Sir William Dunn School of Pathology.

WHAT DOES BREXIT MEAN FOR UK SCIENCE

By Elizabeth Dellar

As a research nation the UK has a big global impact, accounting for 15.2% of the top 1% of most highly cited papers despite only 2.7% of global R&D expenditure (1). A key part of this prominence is its central position in the global network of collaboration, to which many see Brexit as a threat.

Science is reliant on a diverse workforce

A primary concern is maintaining access to the best global talent. In 2015/16, 32% of academics in scientific disciplines were from outside the UK, a percentage which rises to 56% in chemical engineering (2). Large proportions are from the EU and are thus faced with uncertainty around their and their families' future rights to live and work in this country. Whilst the UK is, and will surely remain, a great place to do science, excellent science alone is not enough if it does not come with some security.

This insecurity feeds forward. Is the UK presenting itself as a good place to build a career in the long-term? It seems not; the Wellcome Trust Sanger Institute has already seen a near 50% drop in PhD applications from non-British EU nationals (3). This starts to undermine the key strength of UK science, the fact that it is so international. Not only are diverse teams linked to increased performance, impact and innovation, the continued international flow of students and scientists helps the UK retain a central place in the global collaborative network.

"Not only are diverse teams linked to increased performance, impact and innovation, the continued international flow of students and scientists helps the UK retain a central place in the global collaborative network"

EU funding is about more than just the numbers

Taking a look at funding, in purely financial terms the UK is net receiver of R&D investment, although is a net contributor to the EU overall. This EU investment accounted for 17% of funding for scientific research in higher education institutions in 2013/14, an increase from 8% in 2007/2008 (4). In that period UK government funding decreased slightly, so it is EU funding that has allowed the total to rise, funding to which our future access is uncertain. Whilst there is support across political parties for increasing long-term investment in science, there is some concern that, amongst many voices, science might lose out.

However, the value of being a part of EU Framework Programmes is more than just a net quantitative balance. The less tangible benefits; the influence in shaping scientific priorities across the EU, the prestige and the talent that these grants attract, and the enhanced ability to be involved in big collaborative science, are all part of what maintains UK's scientific reputation abroad.

Continuity of regulation is key for industry

A final important feature of EU membership is the continuity of regulations in a wide range of areas such as data protection, product safety and clinical trials, to name just a few. Whatever the final Brexit deal, the UK will have to follow EU regulations to continue to trade and collaborate with its members, but will no longer be contributing to determining what those regulations are. Regulation is particularly important to businesses, which make up 66% of total R&D investment in the UK (5) but are increasingly seemingly looking elsewhere. The UK needs to do more to mitigate this risk, to actively incentivise existing companies to stay, and to showcase that it is, and can remain, a great place to do business.

"the value of being a part of EU Framework Programmes is more than just a net quantitative balance"

Elizabeth Dellar is a DPhil student in Dr Alberto Baena Lopez's research group at the Dunn School of Pathology, working with Dr David Carter in the Department of Biological and Medical Sciences at Oxford Brookes University.

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THE NOVO NORDISK RESEARCH CENTRE OXFORD

Rendered image of the new BioEscalator building, which is currently under construction. Source: BioEscalator Oxford

Earlier this year, Oxford University announced the launch of a landmark collaboration with Novo Nordisk, building on its longstanding relationship with this leading diabetes therapeutics company. The alliance will take the form of an academic-industry hybrid research institute, the Novo Nordisk Research Centre Oxford (NNRCO), which will receive an estimated £115 million investment from Novo Nordisk over a period of ten years. The centre will focus on innovation in early-stage diabetes research, and will employ up to 100 researchers [1]. To find out more, we got in touch with the head of NNRCO Professor James Johnson, who is renowned for his research into the fundamental biology of pancreatic islets, insulin action, and diabetes.

What is the scientific mission of the Novo Nordisk Research Centre Oxford?

The NNRCO has multiple missions. Scientifically, our mission is to make fundamental discoveries in the field of type 2 diabetes that will result in the identification of new therapeutic targets that will eventually move to the clinic and benefit patients. We also have the mission to participate in the academic space, helping to train the next generation of scientists in our field. More broadly, we seek to reduce the barriers to true collaboration between academia and industry in discovery research.

How was the idea of creating a collaborative research centre born?

I think it's fair to say that there are a few examples around the globe where pharmaceutical companies have sought to increase their proximity and connections to universities. However, I think the vision of a highly collaborative strategic alliance between the University of Oxford and Novo Nordisk is unique. Novo Nordisk has a long relationship with the University, dating back around 30 years, which has included supporting the Oxford Centre for Diabetes Endocrinology and Metabolism (OCDEM), the Novo Nordisk Fellowship program and now NNRCO. NNRCO was the brainchild of Sir John Bell at the University of Oxford and Mads Krogsgaard Thompsen, the CSO of Novo Nordisk. They developed a relationship outline that stresses collaboration and co-creation with the goal of leveraging the complementary strengths of both organisations. When I was approached about the possibility of leading this initiative I was unaware of these details, but proposed these same core principles as my own vision for the institute.

What is innovative about the NNRCO research concept?

The innovation in the NNRCO concept comes from our efforts to build a new type of hybrid institute. This means that our scientists will join the University as guests in its mission of basic pre-competitive research when we are collaborating, including as Visiting Professors and Industrial Visiting Fellows. While the alliance does not compel University laboratories to collaborate with NNRCO, we expect that that the opportunity to work with the outstanding team of scientists we are recruiting will make collaborations attractive win-win situations.

How will the NNRCO encourage cross-fertilisation between academics and industry experts?

Cross-fertilisation will come from increased interactions

(seminars, workshops, informal chats) around the University, as well as from true scientific collaborations. When I say true collaboration, I mean scientists working shoulder to shoulder on the same problems. In the past, pharma companies and academics have worked largely separately, although sometimes on identical questions. In the basic science and discovery science spheres of research, I don't believe this secrecy has any benefit. I will encourage a culture of openness and sharing at NNRCO and hopefully this will become the norm.

Where will the centre be based, and what will the new facilities be like?

Currently we are based in temporary offices and labs in the Centre for Cellular and Molecular Physiology building adjacent to the Wellcome Trust Centre for Human Genetics on the Old Road Campus. Next summer we will move into a brand new space in the nearby BioEscalator Building.

Why was type 2 diabetes chosen as a focus for NNRCO, and what are the research themes?

Type 2 diabetes was chosen as a focus because it is the major research priority for Novo Nordisk, and because the University of Oxford has a very strong reputation in diabetes research. Specifically, we aim to discover new protein targets that can be developed into more effective drugs. This means we have to understand the fundamental biology of type 2 diabetes better, with an emphasis on the secreted proteome (ligands like hormones, growth factors and cytokines) and all of their plasma membrane receptors (the receptorome). We will focus on key tissues that are important for glucose homeostasis, that exhibit defects in type 2 diabetes, including genetic defects, and that are druggable. Initially we will focus on pancreatic islets, liver, adipose tissue and muscle, in that order of importance.

Will there be the opportunity for start-up ventures to become part of the NNRCO ecosystem?

Generally, we hope that NNRCO enriches the life sciences research ecosystem in Oxford. This should tend to increase commercial activity at all levels, and Novo Nordisk's experience in commercialization may provide additional value.

What will the balance between clinical and non-clinical researchers be?

Almost all research will be basic, non-clinical research aimed at target identification and understanding the fundamental biology of type 2 diabetes. However, we will draw information from human genetics and clinical trials, as well as focus on human cell models when possible.

Will the NNRCO also be recruiting DPhil students?

We will participate in the training of DPhil students indirectly, in co-supervision arrangements with academics in Oxford and elsewhere.

What is your vision for the NNRCO in five years' time, and further into the future?

In five years, I want NNRCO to be known as a world-class hub of innovation, including both its collaborative relationship to Universities and in the scientific research being done. I want the institute to be diverse, open, collaborative and fun, and to be known as a great environment to do science in. Further into the future, I would like to know that we made key discoveries and paved the way for new drugs that improve the lives of patients with type 2 diabetes.

Professor Johnson was interviewed by Anne Turberfield.

Novo Nordisk employees on a visit to the BioEscalator construction site, future home of the Novo Nordisk Research Centre Oxford.

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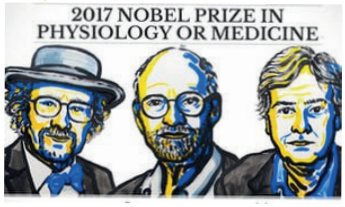
CIRCADIAN CLOCK

By Laura Garmendia Sanchez



2017: The 2017 Nobel Prize in Physiology and Medicine was awarded to Jeffrey C Hall, Michael Rosbash and Michael W Young for their work on the molecular mechanisms controlling circadian rythms.

NON-TRANSCRIPTIONAL OSCILLATIONS

2011: Akhilesh Reddy and John O'Neill showed that non-transcriptional oscillations occurred in human red blood cells. In these cells, circadian rhythms of antioxidant proteins were sustained by redox reactions.

2005: Iwasaki's group showed that, in cyanobacteria, the biochemical oscillations catalysed by several clock proteins can occur even when the transcription-translation feedback control is absent.

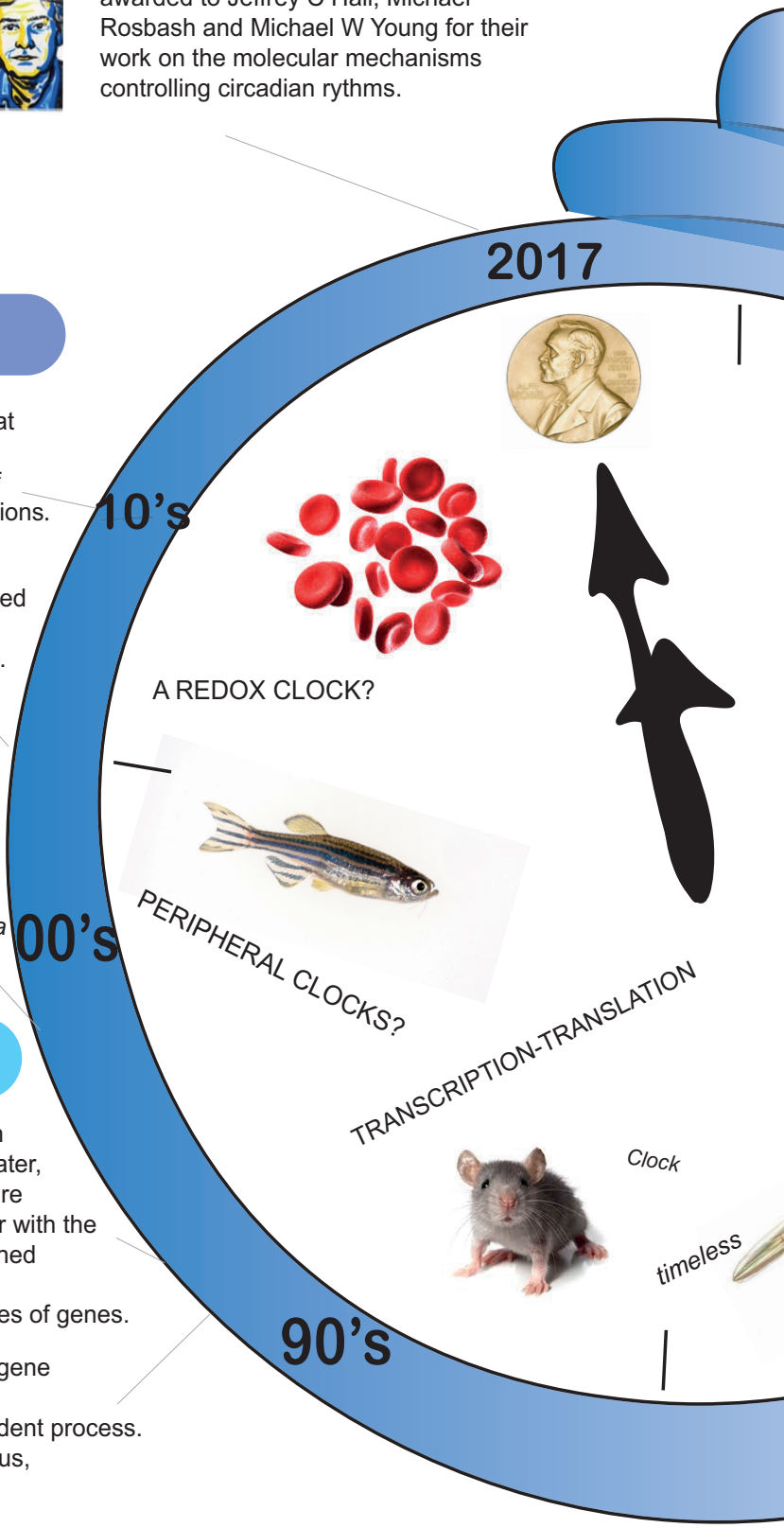
DECENTRALISED CLOCKS: ZEBRAFISH

1998: Research in zebrafish showed that almost all cells in this organism contain a functional circadian clock. This, together with findings about peripheral oscillations in *Drosophila* and in some mammalian organs challenges the view of the master oscillator.

A TRANSCRIPTION-TRANSLATION NEGATIVE FEEDBACK LOOP

1997: the *Clock* gene was identified in mice. Soon after, its homologue was isolated in *Drosophila*. Later, other clock components and their homologues were identified in both species. These findings, together with the discoveries by Young, Rosbash and Hall, established a model of the molecular clock based on the transcription-translation feedback control of a series of genes.

1994: Michael Young discovered a second clock gene called *timeless*. The protein product of timeless (TIM) is degraded through a light-dependent process. Young showed that TIM and PER enter the nucleus, blocking their own transcription.



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What are circadian rhythms? Key concepts:

Circadian rhythms are biological processes that display endogenous oscillations of about 24 hours and are entrained to the local day-night cycle by environmental cues. Biological clocks regulate key aspects of our physiology and behaviour, such as metabolism and sleep, adapting them to the local light-dark cycle. When our internal clock does not align with the local timing, this can affect our physiology, as it occurs during 'jet lag' when travelling. Several studies indicate that this mismatch between our clock and our environment can have serious detrimental effects in health.To understand how circadian clocks work, numerous models have been used, including plants, neurospora, cyanobacteria, rodents, flies and fish.

EARLY WORK ON CIRCADIAN RHYTHMS: FROM PLANTS TO MOSQUITOES

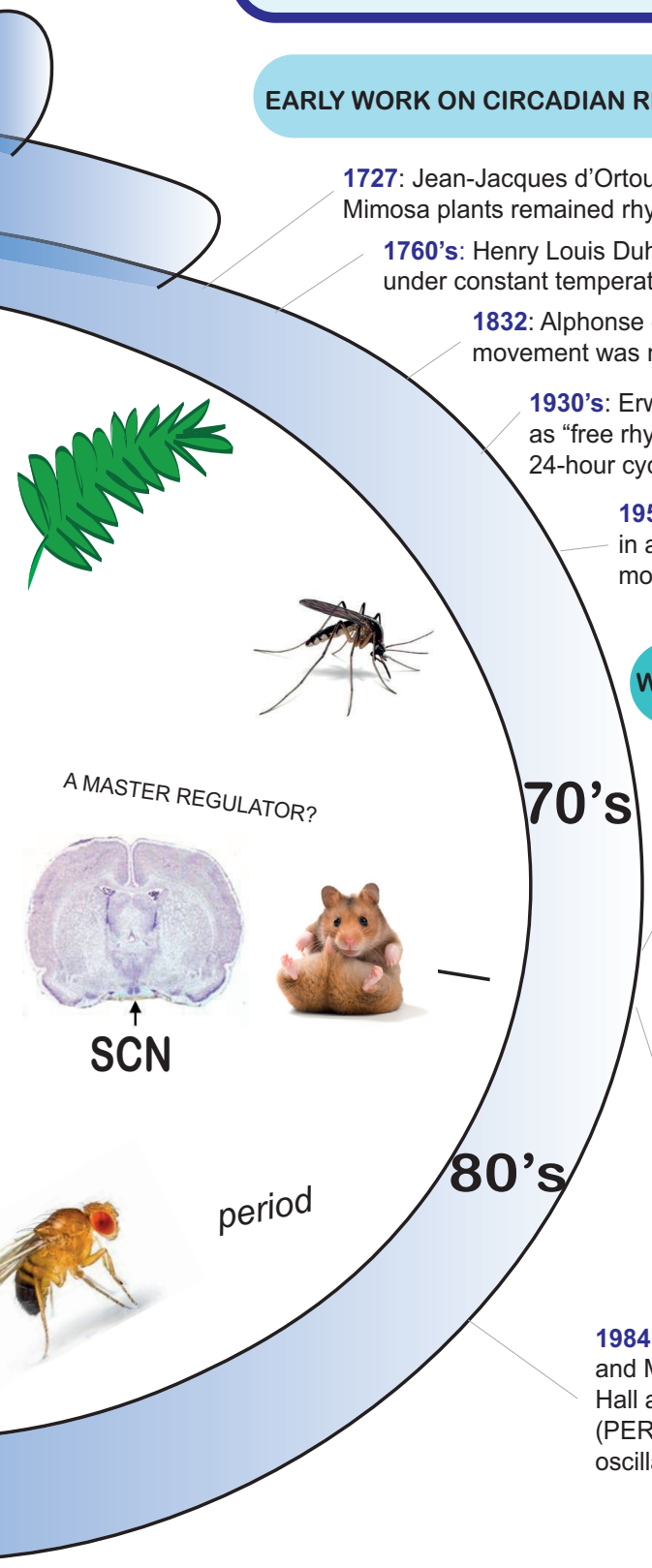
1727: Jean-Jacques d'Ortous de Mairan observed that the leaf opening and closing processes of Mimosa plants remained rhythmic even in constant darkness.

1760's: Henry Louis Duhamel found that this rhythmicity also persisted under constant temperature conditions (temperature compensation of circadian rhythms).

1832: Alphonse de Candolle observed that under constant darkness the period of leaf movement was not 24 hours exactly, but had slight variations.

1930's: Erwin Bünning's plant studies established the concept of circadian rhythms as "free rhythms". Environmental cues are necessary to "entrain" them to the local 24-hour cycle.

1950's: Colin S. Pittendrigh studied light entrainment of circadian rhythms in a range of species, including mosquitoes and flies. This established the model of the "phase-response curve" to light.



WHERE IS THE CLOCK? THE MAMMALIAN MASTER REGULATOR

1970's and **1980's**: lesion studies in rodents indicated that a small cluster of cells in the mammalian hypothalamus, known as the suprachiasmatic nucleus (SCN), had a critical function in the control of circadian rhythms. Michael Menaker's laboratory discovery that transplantations of SCN between wild type and short period *tau* golden hamster mutants always restored the period of the donor, establishing the SCN as the 'master clock'.

EARLY FLY STUDIES ON THE MOLECULAR CLOCK

1970's: Seymour Benzer's group developed the first *Drosophila* screens for clock mutants. They identified a gene, *period*, which controlled circadian rhythms.

1984: Jeffrey Hall, Michael Rosbach and Michael Young isolated the gene *period*. Later, Hall and Rosbach discovered that the protein encoded by *period* (PER) accumulates during the night and is degraded during the day, oscillating over a 24-hour cycle.

BioID and BRET

A comparison of two proximity-based assays for the detection of protein-protein interactions and their advantages over conventional methodologies

By Alice Robinson

Protein-protein interactions (PPIs) play an essential role in sustaining cellular function. Identification and characterisation of PPIs and the environment within which they interact not only helps us to define the importance of specific molecular pathways, but also allows for the appropriate development of drugs against specific molecular targets. However, while conventional assays for the identification and characterisation of novel and candidate PPIs are rather limited, a new class of techniques, known as proximity-based assays, are showing exciting potential to revolutionise our ability to identify and characterise physiologically relevant PPIs.

Over the years, yeast two-hybrid (Y2H) and co-immunoprecipitation (Co-IP) have been accepted as gold-standard techniques for the identification of PPIs, however, both suffer from significant limitations (1). For Y2H, the greatest limitation is the necessity to perform the assay within a yeast cell nucleus, which proves highly problematic for proteins requiring cell-specific processing or compartmentalisation such as membrane proteins (2). On the other hand, the greatest limitation of Co-IP arises as a result of protein-complexes being detected only once a cell has been lysed, which not only limits how useful Co-IP may be for looking at transient interactions within a dynamic cellular environment, but also raises questions about their accuracy and relatability to in situ interactions.

In an attempt to overcome the limitations of conventional methods, the past two decades has seen interest focus upon the detection of interactions in real-time and in situ, often using proximity-based assays to accurately identify biologically-relevant PPIs (1,2).

One such example is BioID, an in situ proximity-dependent biotinylation assay, developed by Roux and colleagues in 2012 (1). A mutated *Escherichia coli* biotin ligase (BirA*) is first fused to the protein of interest (POI). BirA* then labels proteins which associate with the POI with biotin. BioID, therefore, allows for the detection of transient interacting partners through proximity-dependent biotinylation of proteins found within 10 nm of BirA*, a distance that suggests interaction with the POI (Figure 1) (1). Biotinylated proteins, namely the POI and its interacting partner(s), can then be isolated by biotin-affinity capture using streptavidin, a high affinity binding partner of biotin, before identifying interacting partners of the POI using western blot and mass spectrometry (1). Since the initial development of BioID for the Human Lamin-A protein in 2012, BioID has been successfully optimised to investigate the interactome of over 140 proteins (4), a success largely due to its superiority over more conventional techniques used to identify PPIs.

A second example of a real-time, proximity-dependent assay for the identification of PPIs is Bioluminescence Resonance Energy Transfer (BRET), which was developed by Xu Yao and colleagues in 1999 (2). BRET operates through a proximity-dependent energy transfer between a primed bioluminescent-tagged donor protein (e.g. Luciferase) and a fluorophore-tagged acceptor protein (e.g. GFP), to capture real-time interactions between two POIs (Figure 2) (2). Similar to BioID, the change in fluorescence observed for BRET occurs when the bioluminescent donor is within 100 Å of the fluorophore acceptor, a distance close enough to suggest interaction between two POIs (2,3).

However, while BRET has also proven useful, the requirement to use a sensitive plate reader with correct filters for visualisation of relevant emission spectra (2) renders BRET less accessible due to the expense and limited ap-

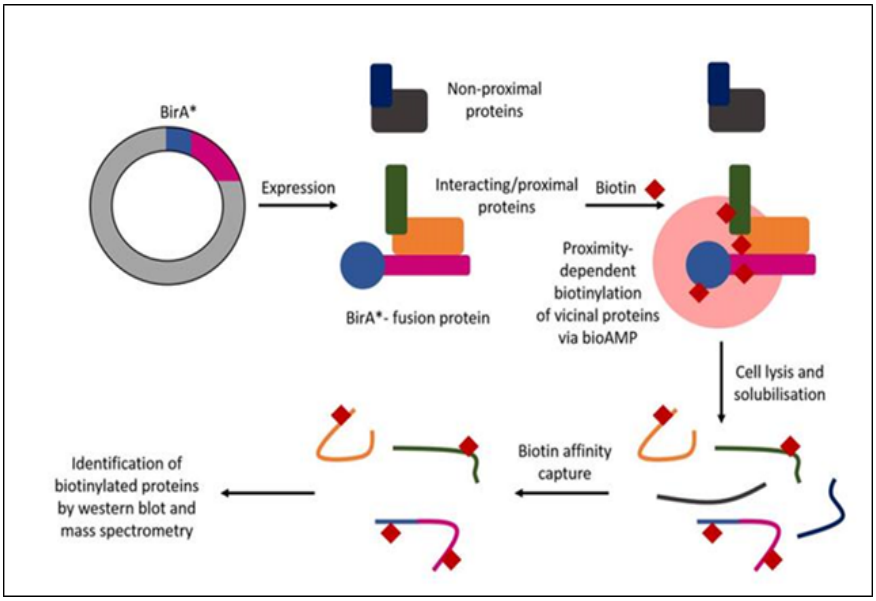


Figure 1. Cartoon representation of BioID. Expression of the BirA*-recombinant vector in a stable cell line results in biotinylation of neighbouring proteins to the POI, in a proximity-dependent fashion, following treatment of transfected cells with biotin, leading to the formation of biotinoyl-5'-AMP. Unlike the native biotin ligase BirA, biotinoyl-5'-AMP is only loosely bound to the active site of BirA*, and it therefore diffuses away, indiscriminately biotinylating lysine residues of proteins within 10nm of BirA*. Biotinylated proteins may then be isolated and identified by western blot and mass spectrometry.

plication associated with such machinery. Alternatively, BioID requires materials commonly found or relatively cheaply purchased and re-purposed within standard molecular laboratories, and is therefore much more accessible.

Furthermore, BioID is able to identify multiple interacting partners in one assay, with the potential to identify known, candidate and novel interacting partners, due to the promiscuity of biotinoyl-5'-AMP biotinylating lysine residues of any protein within 10nm of BirA* (1). BRET, however, requires in-depth knowledge of a specific interaction in order to place bioluminescent and fluorescent tags appropriately, to reside within a distance of < 100 Å, when the POIs interact (2). Therefore, BioID holds much greater potential to detect large numbers of interactions, while BRET is limited to investigating a single PPI per assay.

Yet, while BRET holds much narrower scope for the number and type of PPIs identified, the greatest advantage of BRET over BioID is its ability to study a PPI in real-time. This not only permits investigation of interaction dynamics, including confirmation of a transient or stable PPI, but also allows for real-time observations, following manipulation of the environment within which POIs reside (2,3). BioID, on the other hand, would require multiple assays and data extrapolation to attain anywhere near as much information for characterising a PPI as BRET. Furthermore, as BRET is performed in real time and in living cells, this avoids potential signal attenuations that occur as a result of cell lysis, protein solubilisation or during purification (5). Therefore, while BRET requires in-depth spatial knowledge of an interaction, it holds much greater potential for confirming and characterising a single interaction than BioID could ever achieve.

While BioID and BRET are advantageous over conventional assays, one limitation they share is the potential blindness for interacting partners found outside their labelling range. As BirA* and the bioluminescent/fluorescent tags may only be fused to the N or C terminus of the POIs, their labelling range may not detect interactions at alternate ends of the protein, especially those which are particularly large or with unique conformations, meaning a negative result does not prove non-interaction (2).

Overall, proximity-based assays for the detection of PPIs hold numerous strengths over conventional methodologies. Two examples of proximity-based assays, BioID and BRET, are differentially suitable for the type of PPI investigated, with BioID most advantageous for screening of novel PPIs, while BRET is better suited for in-depth characterisation of a specific PPI. Owing to their potential, more recently BioID and BRET have been optimised to investigate functional interactions (6) and the regulation of both cellular and receptor dynamics, for example, ubiquitination (5). BioID and BRET provide powerful examples of proximity-based assays that are revolutionising our ability to accurately characterise biochemically relevant PPIs, aiding both our overall knowledge of cellular pathways and the process of drug development against specific molecular targets.

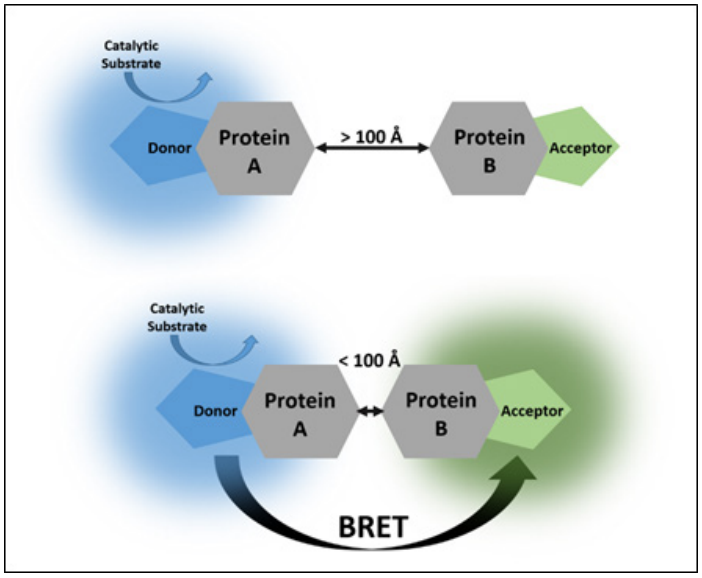


Figure 2. Schematic representation of BRET. Addition of a catalytic substrate causes the bioluminescent donor, tagged to the POI (Protein A), to emit a wavelength of light capable of exciting the fluorophore acceptor, tagged to the candidate interacting partner (Protein B), when the donor is positioned within < 100 Å of the acceptor. At this distance bioluminescence is lost, being replaced by fluorescence, indicative of the PPI occurring. Such changes in emission wavelength are detected by a sensitive plate reader.

Alice Robinson is a Research Assistant in Professor Monaco's research group at the Kennedy Institute of Rheumatology.

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The Oxford BIOMOD team. From left to right: Hannah Cornwall, Sam Garforth, Martin Veselý and Jordan Juritz (Physics).

UK's first BIOMOD team gets Gold

Oxford University's team has won Gold status at the annual Jamboree finale of the international Biomolecular Design (BIOMOD) competition (http://biomod.net/).

BIOMOD is an annual international competition for student research in biological nanotechnology. The student-driven project offers undergraduates a unique scientific experience spanning experimental design and benchwork to academic presentations and outreach. From July to September each year, small teams from around the world compete by designing and doing their own research projects, before presenting results to their peers at the 'Jamboree' conference at the University of California in San Francisco (UCSF). BIOMOD teams design and build structures and machines out of the basic molecules of life: DNA, RNA and proteins.

"It was fantastic working on a team project, not to mention presenting our work in San Fran. It's a once in a lifetime student opportunity!"
- Hannah Cornwall

This year, Oxford fielded the first UK team to participate in BIOMOD. This interdisciplinary team comprised of Hannah Cornwall (Medicine), Sam Garforth (Biochemistry), Martin Veselý (Biochemistry) and Jordan Juritz (Physics), with mentoring from DPhil students Robert Oppenheimer (SynBioCDT) and Paul Rutten (BioDTP). In the past, BIOMOD teams have worked on projects as diverse as building biosensors out of interacting RNA strands, making molecular motors from kinesin proteins walking upon microtubules, constructing enzyme cascades protected within artificial membrane compartments, and creating nanoscale circuits by positioning gold-nanoparticles on DNA nanostructures.

The Oxford BIOMOD team wanted to work on a project with in vivo applications, so they designed genetic switches that regulate protein translation through conformational switching of mRNA secondary structure (called ribo-

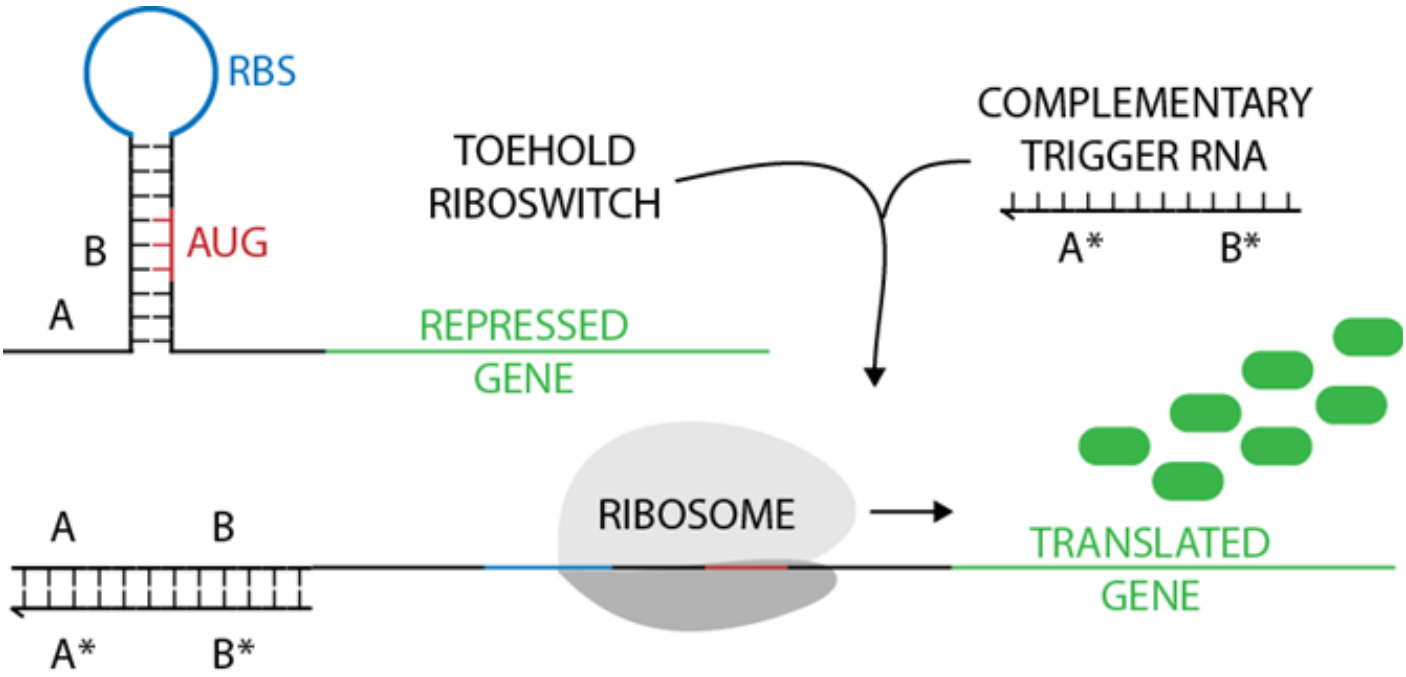

Figure 2. Riboswitches regulate gene expression. A riboswitch makes translation initiation conditional upon external factors. Without the 'trigger' the mRNA contains secondary structure preventing translation, but with the 'trigger' ribosomes bind and begin translation.

switches). For example, the translation of a protein from mRNA may be repressed by secondary structure around the ribosome-binding site (RBS) and start codon. However, when a complementary strand hybridises around the RBS, the secondary structure may be removed, permitting translation (Figure 2). Many variations on this design have been explored, including aptamers and protein-binding motifs.

Riboswitches have great potential for controlling gene expression because RNA-RNA interactions are easier to manipulate than the protein-DNA interactions responsible for gene transcription. As a result, riboswitches have been used to create highly complex networks of gene expression (1). Such fine control over biological processes could have important applications in synthetic biology, medicine and industry. However, designable riboswitches currently lack the cooperativity of some natural mechanisms for regulation of transcription. They do not have sharp 'on-off' switching behaviour around the threshold concentration of an input molecule, which limits the potential for layering multiple switches with sensitive responses.

"BIOMOD Oxford was a great opportunity to develop novel ideas into projects and push the boundaries of biotechnology." – Martin Vesely

The Oxford BIOMOD team rationally designed a cooperative riboswitch for a sharper 'on-off' response to input signals. Their system has two innovations: 1) more than one 'trigger' strand binds to open the riboswitch; and 2) it is difficult for the first 'trigger' to bind, but easy for subsequent 'triggers'. This results in an 'all-or-nothing' behaviour, which is characteristic of cooperativity in haemoglobin, ion channels and many other natural systems. In the summer of 2017, the team designed new riboswitches, confirmed their cooperativity in vitro, and began in vivo characterisation of gene expression. Full details of the project can be found on the team's website (https://oxfordbiomodteam.wixsite.com/riboxswitch).

At the competition, the team achieved Gold status. This means that the judges agreed their project was scientifically interesting and feasible, their work was clearly communicated and executed, and that in their first year they performed better than 50% of other teams worldwide.

The team received extensive support from industry sponsors, SynBio.Oxford (the Oxford University Society of Synthetic Biology), and the University of Oxford. The full list of their sponsors can also be found online (https://oxfordbiomodteam.wixsite.com/riboxswitch/sponsors).

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Robert Oppenheimer is a DPhil student in Professor Turberfield's research group at the Department of Physics.

5' WITH... Dr Kerry Walker

by Mariangela Panniello

What made you become a scientist?

I started university with the plan to become a medical doctor, but after the first couple years of my undergraduate program in Behavioural Neuroscience, I was hooked on a career in scientific research. I was excited by the idea that our own experiments could expand upon everyone's knowledge of the brain, and this still motivates me today. By understanding the brain, we can better understand ourselves and one another.

Why do you think audition is so fascinating?

Audition forms the primary gateway for communication between individuals, be it through animal calls, speech, or music. We can recognise the same melody played on different instruments, and the same word spoken by people with different accents and voices. This is a difficult problem for computers to solve, as you will notice if you watch closed captioning on live television. However, our brain accomplishes this task effortlessly, and we still do not fully know how.

"We can recognise the same melody played on different instruments, and the same word spoken by people with different accents and voices. "

What is your research group working on at the moment?

My group investigates how nerve cells in the brain, mostly in the auditory cortex, represent important features of sounds, such as their pitch, spatial location, or association with non-auditory (e.g. tactile) sensations. We ultimately want to understand how each of these cues are represented in the brain and how this information is combined to form one auditory object, like your mother's voice. To answer these questions, we record the responses of large numbers of nerve cells in the brain, using 2-photon imaging and microelectrodes. We also train animals on sound identification tasks, to better understand how their hearing compares to our own. Finally, we use computational models to make new hypotheses about how the brain can extract useful information from sounds.

What do you think was the most relevant neuroscience discovery in the past 10 years?

Our understanding of memory formation has benefited from several remarkable breakthroughs in the past decade. During this time, it has been demonstrated that we can selectively erase or implant a given memory within an animal's brain, and even transfer a memory from one animal's brain to another. For example, Ramirez *et al.* (2013) created a false fear memory in rats by activating specific patterns of brain cells while rats explored a maze; this used to be the stuff of science fiction! It is opening up potential new avenues for treating disorders, such as drug addiction and PTSD (Post-Traumatic Stress Disorder), however also presents difficult ethical questions about mind control.

"By understanding the brain, we can better understand ourselves and one another."

"The best way to protect women and minorities against unconscious bias is to make ourselves aware of it."

What do you think universities should do to promote and protect the careers of women in STEM (Science, Technology, Engineering, and Mathematics)?

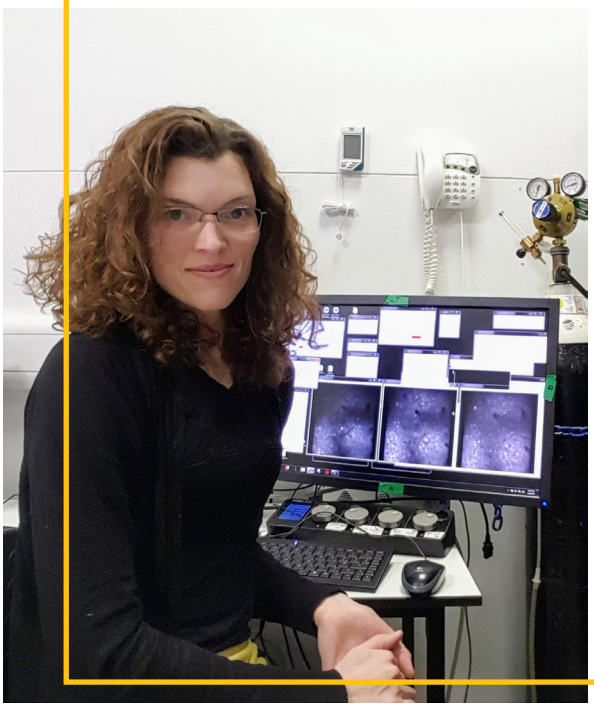
Most people working in universities agree that women and men are equally capable as scientists and engineers. Nevertheless, unconscious bias is real, as many scientific studies have demonstrated. The best way to protect women and minorities against unconscious bias is to make ourselves aware of it. In my personal experience, and in that of many of my colleagues, the single biggest issue for women in STEM is childcare. Therefore, universities need to provide the best maternity cover possible, offer on-site daycare, and be respectful of the hours that parents can and cannot be at work. Of course, this issue applies to mothers and fathers alike, but statistics show that they disproportionately affect women.

What do you think are the big challenges the scientific community is facing at the moment?

We are fortunate to be living in a prolific scientific era. Hundreds of scientific articles are published in peer-reviewed journals every day from labs all over the world. The problem is that it is impossible for any one person to keep up with reading this expanding literature. We need a reliable way of summarising this information overload.

What do you think universities should do to promote and protect the careers of women in STEM (Science, Technology,

Most people working in universities agree that women and men are equally capable as scientists and engineers. Nevertheless, unconscious bias is real, as many scientific studies have demonstrated. The best way to protect women and minorities against unconscious bias is to make ourselves aware of it. In my personal experience, and in that of many of my colleagues, the single biggest issue for women in STEM is childcare. Therefore, universities need to provide the best maternity cover possible, offer on-site daycare, and be respectful of the hours that parents can and cannot be at work. Of course, this issue applies to mothers and fathers alike, but statistics show that they disproportionately affect women.



Dr Kerry Walker is an early Career Research Fellow based in the Department of Physiology, Anatomy and Genetics. Her research group uses a range of techniques, from electrophysiology to computational methods, to understand how the brain processes sounds that humans and other animals use to communicate.

SNAPSHOT
Research Image Competition

This issue's winner is... Christophe Royer

Christophe received his Bachelor of Science degree from the University of Strasbourg, France in 2002 and was awarded a Masters Degree in Molecular Pharmacology the following year. He earned a PhD from University College London in 2008 after investigating the cross-talks between Wnt signalling and the tumour suppressor ASPP2. He moved to the University of Oxford with the Ludwig Institute for Cancer Research to pursue postdoctoral studies, investigating the role of apicobasal polarity during central nervous system development. In 2013, he joined the laboratory of Shankar Srinivas as a James Martin Stem Cell Research Fellow. His research interests now lie in understanding the mechanistic role of cellular architecture and polarity proteins in directing processes such as cell fate decision or cell movement during early embryonic development. Imaging techniques and the use of genetic tools such as mutant mouse lines in combination with fluorescent-labelled transgenic lines are at the heart of his research and the Srinivas group.

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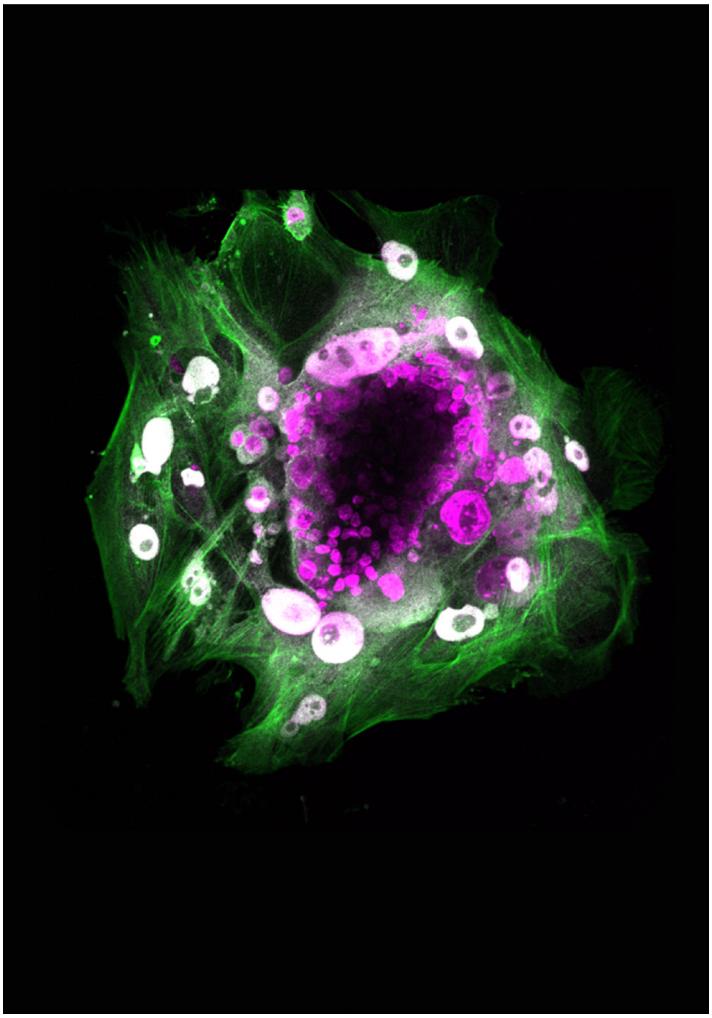
SNAPSHOT
Research Image Competition

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

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT?**
We are now accepting entries for pictures to be featured on the cover of the Trinity 2018 issue of *Phenotype*.

To enter, send images to jack.heywood.cooper@gmail.com with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

The deadline for the competition is Friday 10th March 2018.



The image is of a blastocyst outgrowth. A three-day-old mouse embryo at the blastocyst stage containing a knockin point mutation in the protein phosphatase binding site of ASPP2 was plated onto a gelatine-coated glass bottom dish and left to grow for several days, allowing the outside layer of the blastocyst, the trophectoderm, to expand and form trophoblast giant cells. The outgrowth was stained for the protein YAP (grayscale), the actin cytoskeleton (green). The nuclei (magenta) were stained with DAPI. Trophoblast giant cells, characterised by their exceptionally large nuclei, exhibit nuclear YAP localisation, whereas cells originating from the inner cell mass are devoid of nuclear YAP.

PHENOTYPE crossword

Our latest crossword is a tricky one. Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to jack.heywood.cooper@gmail.com. Entries received before the 10th March 2018 will be entered into a prize draw to win one of the books reviewed in this issue.

ACROSS

1.With caloric intake, Dick can adjust his body's natural rhythm? (9,5)

5. See 1

9. Go twice as fast for twice as long? (6-4)

10. Cereal described by **27's** musical partner (4)

11. Desmond in a dress? (4)

12. Central Asian turns up in Côte d'Ivoire, Zambia (5)

13. Greek character is raised up, decapitated in old Zimbabwe (8)

14. Agree to untie final knot (4)

15. A French participant in yoga is endlessly childish? (5)

18. Bette goes topless for time-waster (5)

22. Plant absorbing iron and radon (4)

23. When Timothy briefly touches Helen's heart, two seconds can last forever (8)

24. A star is rising in space (5)

25. Solid verbal agreement . . . (4)

27. . . . contains passage describing yield (4)

29. Egyptian city once annexed by a country to raise its atmosphere (10)

29. Getting cosy with a Dane is hot but upsettingly sulphurous? (5)

30. Unexpectedly, it hit the Right which clue this clue is? (9)

DOWN

1. Company reaches agreement with Russian on ending passage (4)

2. Philosopher removes the top of her wedding dress (8)

3. Tabling an amendment (not new) is wearing (8)

4. Description of someone's body using a euphemism? (1,6,2,6)

6. This part is unfinished - has to be unscrewed, have bearings reversed (5,3)

7. Nice and tidy model of nucleoside (8)

8. Giving some time in case anger is rising (6)

12. A city for everyone (3)

16. Risk analyst skips performance, having no part; instead, writes eulogy (8)

17 It's annoying to require fish (8)

18. During the **8** of carbon intake, an automatic reaction (8)

19. Hang around with friend who's stripped down to their underwear (8)

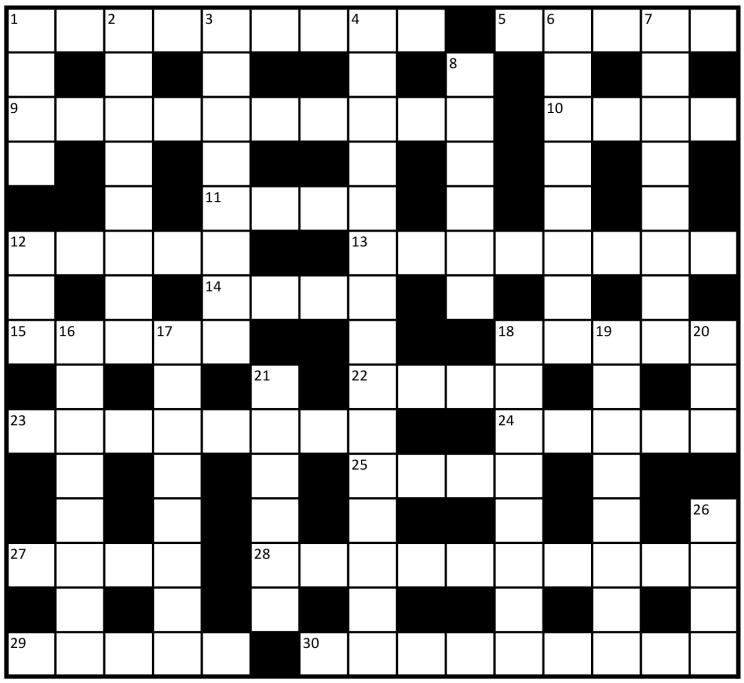
20. Short lady has initially taken superoxide or hydroxyl radical, perhaps? (3)

21. Spooner will allow binge to cause **1,5** disturbance? (3,3)

26. Try to hit the party (4)

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

WILEY-BLACKWELL



Answers to the crossword from Issue 28, Michaelmas 2017:

Across: **1,14.** cap **3.** astray **8.** clean **9.** agarose **10.** greed **11.** enough **12.** gab **14.** off **1 16.** ace **17.** fettucine **18.** EBB **20.** bop **21.** rio **22.** corona **23.** grain **25.** opens up **26.** vines **27.** a first **28.** nan

Down: **1.** changes **2.** peace of **3,7.** a load **4.** the right frame of **5.** at the back of your **6,7.** georgia **7.** See 3,6,8 **8,7.** carolina **13.** keep an open **15.** read ones **19.** be of one **20.** bears in **23.** great **24.** hive