2016

# Babraham Institute
# Annual Research Report

Babraham Institute

# Life sciences research for lifelong health


Babraham Institute

**The Babraham Institute undertakes fundamental research to generate new knowledge of biological mechanisms underpinning ageing, development and the maintenance of health. Our research creates impact by uncovering a new understanding of biology, often gained through the development of innovative research methods, to inform future healthcare.**

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# Director's welcome

Welcome to the Babraham Institute Annual Research Report, which provides a summary of the Institute's key research foci, progress and activities in 2016. The Babraham Institute's vision is to build upon our pre-eminence as a bioscience research institute, making seminal contributions to understanding that enhance lifelong health and wellbeing whilst addressing strategic imperatives within the BBSRC Strategic Plan.

This collection of 2016 research reports demonstrates how our researchers are tackling challenging problems facing an ageing global population by developing and applying new technologies and pioneering approaches. The four research features included in this report highlight discoveries made in 2016 – all with importance for uncovering the basis of health and disease.

Research features:



Professor Michael Wakelam
Institute Director

## About the Institute

The fundamental underpinning ethos of the Institute is excellence in science, producing outstanding outcomes in both increased knowledge and application. Our strategic overhaul over the past ten years has focused the Institute's science into four interconnected and collaborative programmes (Institute Strategic Programmes; ISPs): Immunology, Signalling, Epigenetics and Nuclear Dynamics. Together these contribute to the Institute's overarching strategy of determining the mechanisms underlying developmental, immunological and ageing processes. To support this, we have invested heavily in expanding computational and systems biology approaches, including group leader recruitment to extend our expertise in this area, and today all research groups have their own, or access to, extensive bioinformatics support.

Each group's individual research programme contributes to one or more of the four ISPs. They bring their individual, but complementary expertise to bear on key research questions centred around understanding the fundamental biology of human life from development to how we age.

## Developing global and local partnerships

Collaboration and cooperation is central to Babraham's approach. This is illustrated by a glance at our interactions throughout 2016: hosting over 90 visiting researchers within the Institute's labs to share knowledge and expertise, 23 international academic collaborations with 162 organisations in 32 countries, and 115 commercial projects with 61 organisations. The range of our international impact will be further expanded through projects funded in 2016 by the Global Challenges Research Fund. Through these projects the Institute's strengths in fundamental biology and ageing research will be utilised to address the needs of developing countries and promote improved welfare and economic growth.

The co-location of the Institute with the 60 commercial life science companies that, together with us, form the Babraham Research Campus, creates a fruitful environment for mutually beneficial collaboration. We have been pleased to see the Institute's interactions with campus-based companies continue to grow during 2016, with the Institute currently participating in a total of 29 research collaborations with 16 campus-based organisations. Pages 68-69 present some of these collaborations in more detail.

**90**
VISITING RESEARCHERS

**23**
INTERNATIONAL ACADEMIC PROJECTS WITH 162 ORGANISATIONS

**115**
COMMERCIAL PROJECTS

'The fundamental underpinning ethos of the Institute is excellence in science'

## Achieving excellence

The Institute's science output remains at an excellent level. The following pages highlight some of the 93 papers published in 2016. As of 1st February 2017 our scientists have published 623 papers since 2011 with 112 in Nature, Science and Cell family journals. These papers have been cited more than 11,000 times. A Leiden Ranking* exercise, carried out on behalf of the EU-LIFE alliance of European research institutes in 2016, ranked the Institute's publication output from 2010-2013 higher than any university in the biomedical science arena, with the exception of the Massachusetts Institute of Technology.

Excellent science requires outstanding science capabilities and the Institute is fortunate in having superb core facilities. Establishment of these facilities has promoted novel scientific directions, particular examples include combining the Institute's biological chemistry and imaging expertise to understanding the dynamics of TOR-regulated autophagy (1) and the organisation of genomic and epigenomic regulation elucidated through application of next-generation sequencing and bioinformatics approaches (2). Further examples are included in the individual reports, both from the research groups and the nine core facilities. These highlight our experimental strategy whereby we develop novel methodologies to answer fundamental research questions. In addition to making these fundamental advances in knowledge we aim to exploit their potential where possible through commercialising our research.

'Collaboration and cooperation is central to Babraham's approach'

## Contributing to strategic research priorities

The Institute's ISPs form the central pillar of the Institute's alignment and input to the BBSRC's Strategic Research Priority: Bioscience for Health. The Institute's research addresses the majority of the Strategic Plan's health priorities and is clearly mapped onto the three enabling themes: Enabling Innovation, Exploiting New Ways of Working and Partnerships. In targeting research on the Global Grand Challenge of ageing, we recognise that by 2050 25% of the UK population will be more than 65 years old. However, whilst lifespan has increased, healthspan has not improved in a corresponding manner. Over the past five years we have refocused our research and recruited to target this area. Critically, being able to respond to this challenge requires an understanding of lifelong health. Thus, the Institute's mechanistic approach will significantly increase understanding of the importance of epigenetic regulation of ageing, of the mechanisms of nutrient sensing and how the regulation and function of the immune system is particularly central to an enhanced healthspan.

## Publications


1. Press release October 2016: Real-time imaging uncovers mTORC1 dynamics www.babraham.ac.uk/news/2016/10/realtime-imaging-uncovers-mtorc1-dynamics
2. Javierre, B.M. *et al*.. (2016) Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. *Cell* 167: 1369-1384


* www.leidenranking.com

## Developing scientific leaders

Our scientific aims are coupled to a clear commitment to training and knowledge exchange. We benefit from an outstanding cohort of group leaders, many of whom have developed their scientific careers at the Institute. In particular this year, Immunology programme leader Dr Martin Turner was awarded a Wellcome Trust Investigator award and Immunology group leader Dr Michelle Linterman became an EMBO Young Investigator.

We remain committed to training the research leaders of tomorrow with 25% of the present group leaders being tenure track. We are also committed to the training of graduate students, who are registered for PhD degrees at the University of Cambridge, and the continuing professional development of all staff. We aim to be an inclusive and supportive employer as demonstrated by the award of a Silver Athena SWAN Award in 2015.

I hope you enjoy discovering more about the Institute and our research as you read this report.

Michael Wakelam
Director

**February 2017**

# Performance in 2016

OUR COLLABORATIVE ACADEMIC PROJECTS UK AND WORLDWIDE

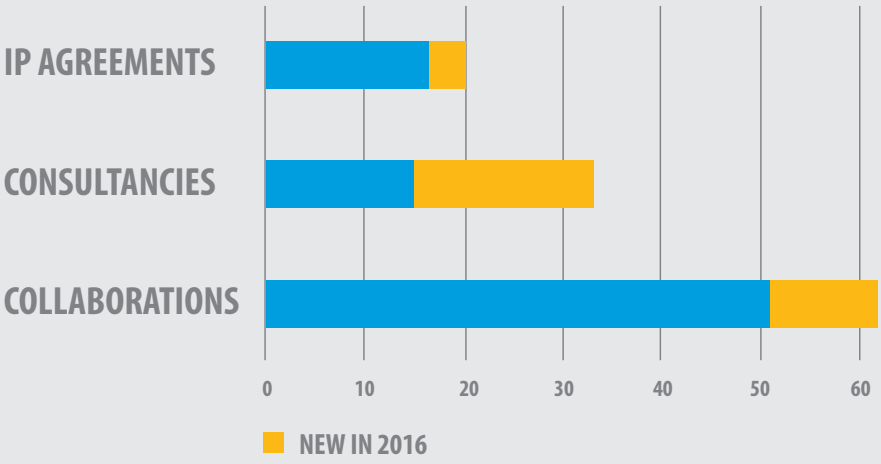
## Working with others in 2016

**33** ACTIVE PROJECTS

**33** COUNTRIES

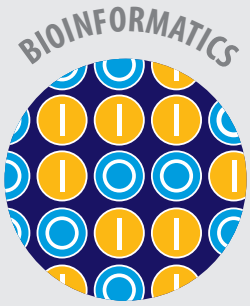
**165** ORGANISATIONS

## Working with commercial partners

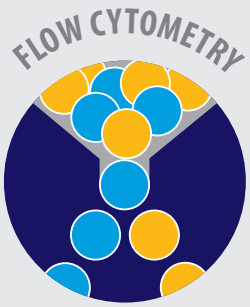



## People we've trained in our scientific facilities this year

**634**



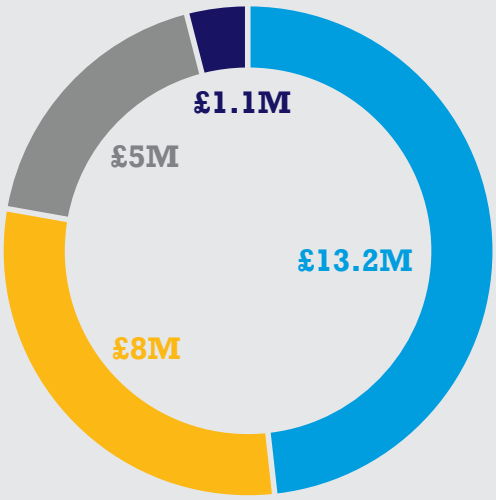

**479**




**129**


**26**

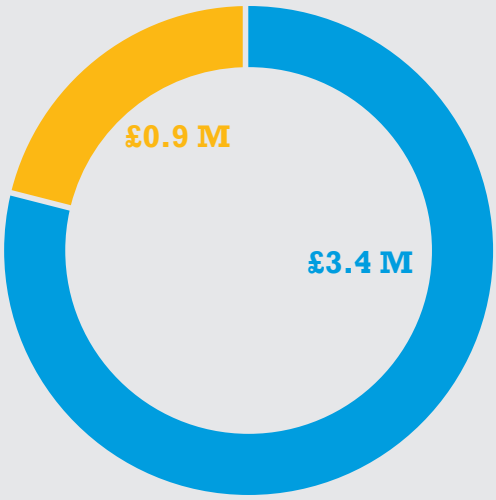
# 2016 income




**£27.3M**

- Core ISP grants & BBSRC non-grant income
- Competitively awarded grant income in 2016
- Income from services provided by the Institute
- Other

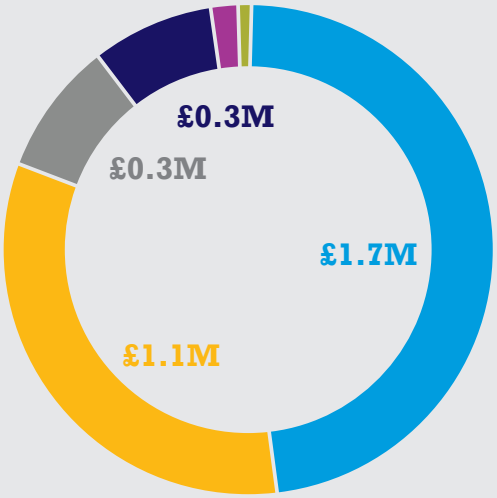
# Value of all grants awarded in 2016




**£4.3M**

- UK funders
- International grants*

# Value of UK grants awarded in 2016




**£3.4M**

- Wellcome Trust
- BBSRC
- CRUK
- Industry
- MRC
- MND Association

*International grant sources:
NIH (USA), EU (Horizon 2020), King Abdullah University of Science and Technology (Saudi Arabia)

# 2016 successes

**35**
PUBLIC ENGAGEMENT EVENTS

INVOLVING

**91**
RESEARCHERS

**12,000**
PEOPLE ENGAGED

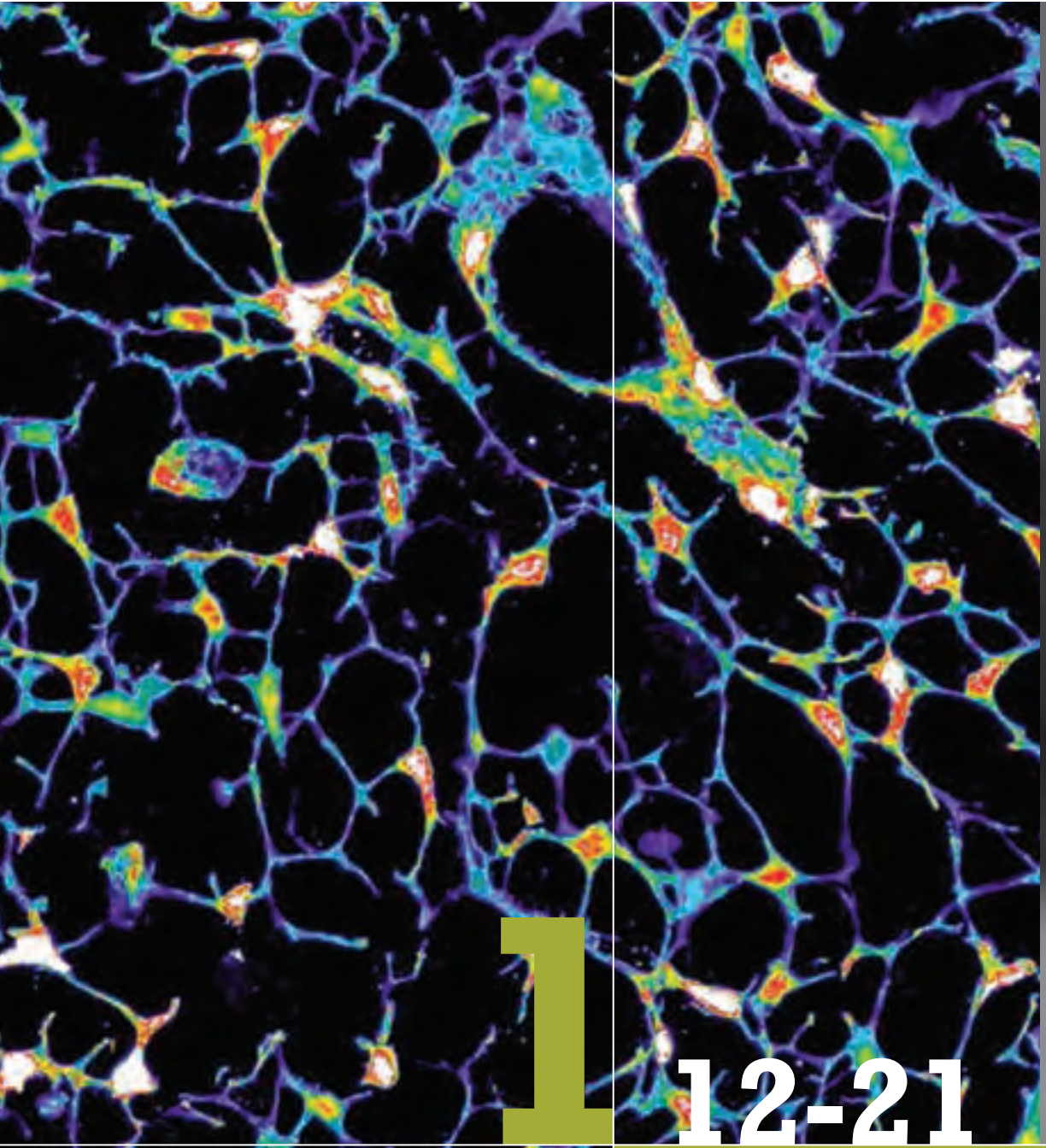
**93**
PUBLICATIONS

**73**
RESEARCH PUBLICATIONS

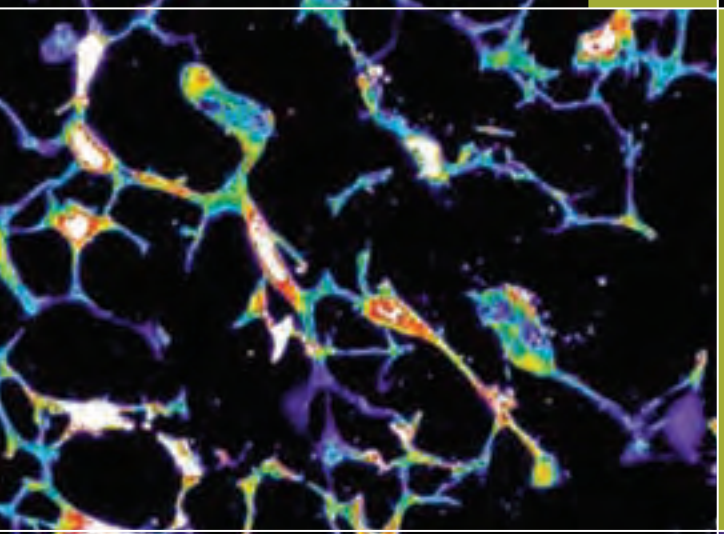
**20**
REVIEWS

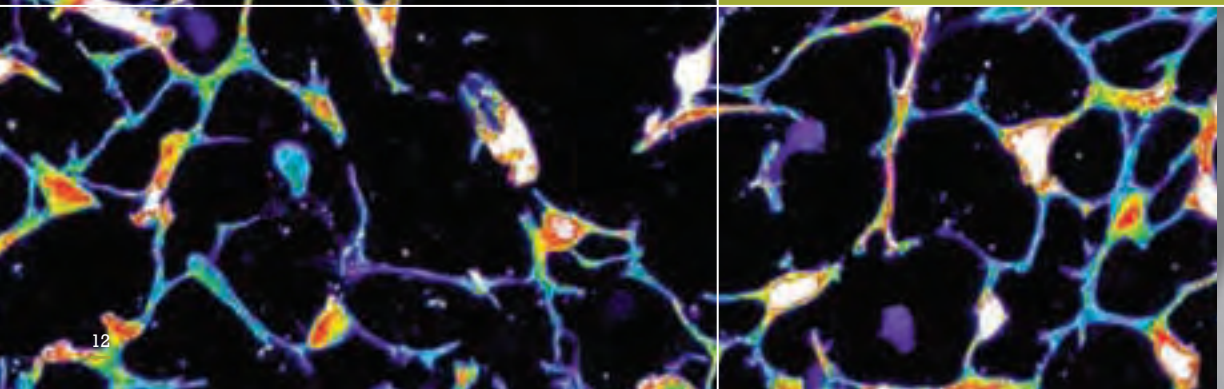
BBSRC EXCELLENCE WITH IMPACT COMMENDATION WINNER

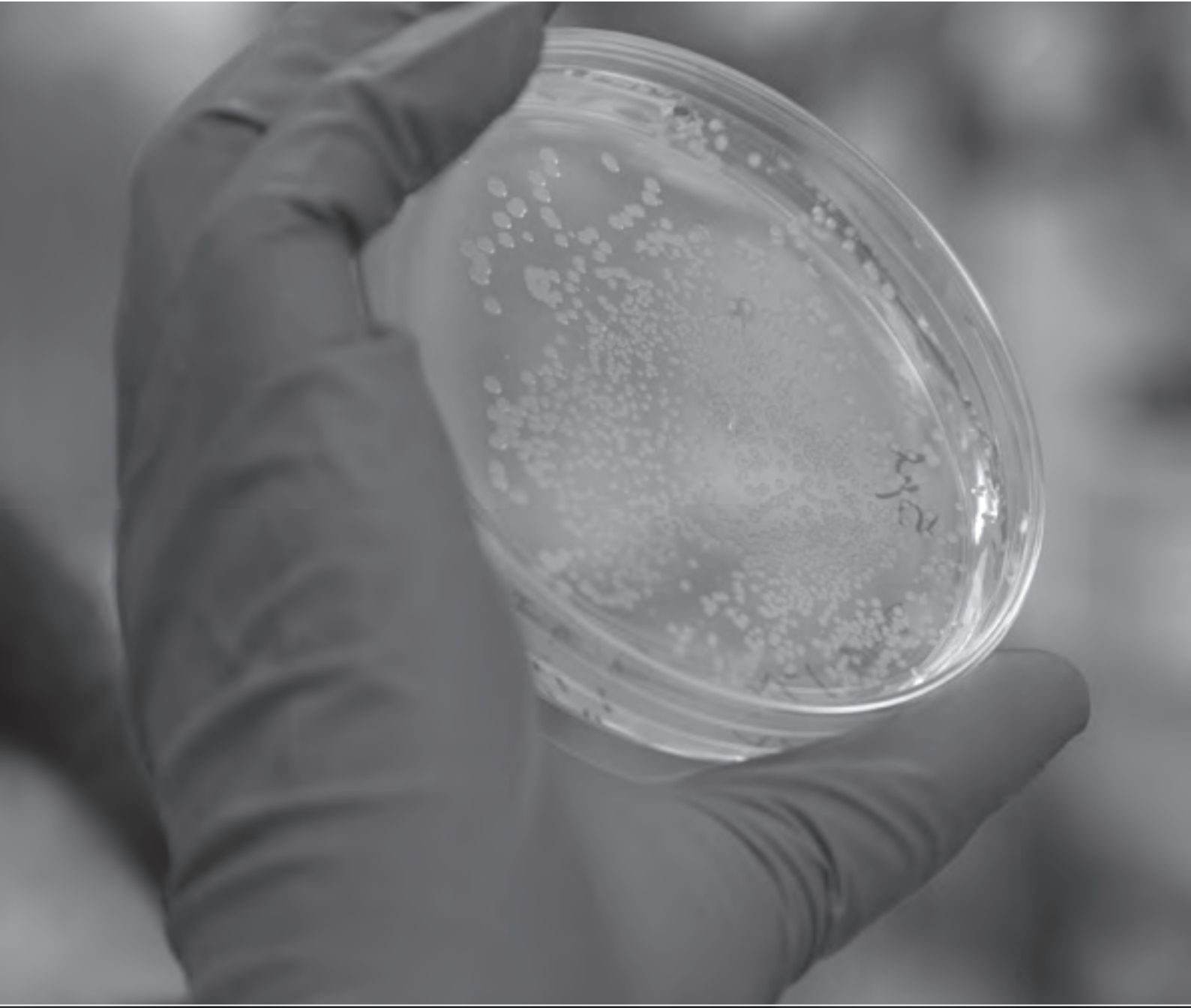
**9**
PhDs COMPLETED



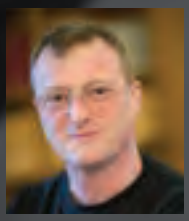
# 1 12-21 Immunology





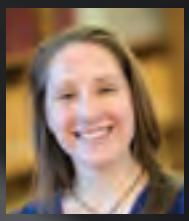


## Group leaders



Martin Turner

Geoff Butcher



Michelle Linterman

Klaus Okkenhaug

Rahul Roychoudhuri

Martin Turner
Programme leader

**Group members**

**PhD students:**

Fengyuan Hu

David Turner

Rebecca Newman
(left in 2016)

**Research assistant:**

Dr Kirsty Bates

**Postdoctoral researchers:**

Dr Elisa Monzon-Casanova
Dr Cesare Sala
(joined in 2016)

Dr Alexander Saveliev

Dr Michael Screen

Dr Ram Venigalla

Dr Alison Galloway
(left in 2016)

Dr Sarah Bell

Dr Manuel Diaz-Muñoz

Dr Helena Ahlfors
(left in 2016)

**Visiting researchers in 2016:**

Vanessa D'Angeli

Lorenzo Caracci

Fiamma Salerno

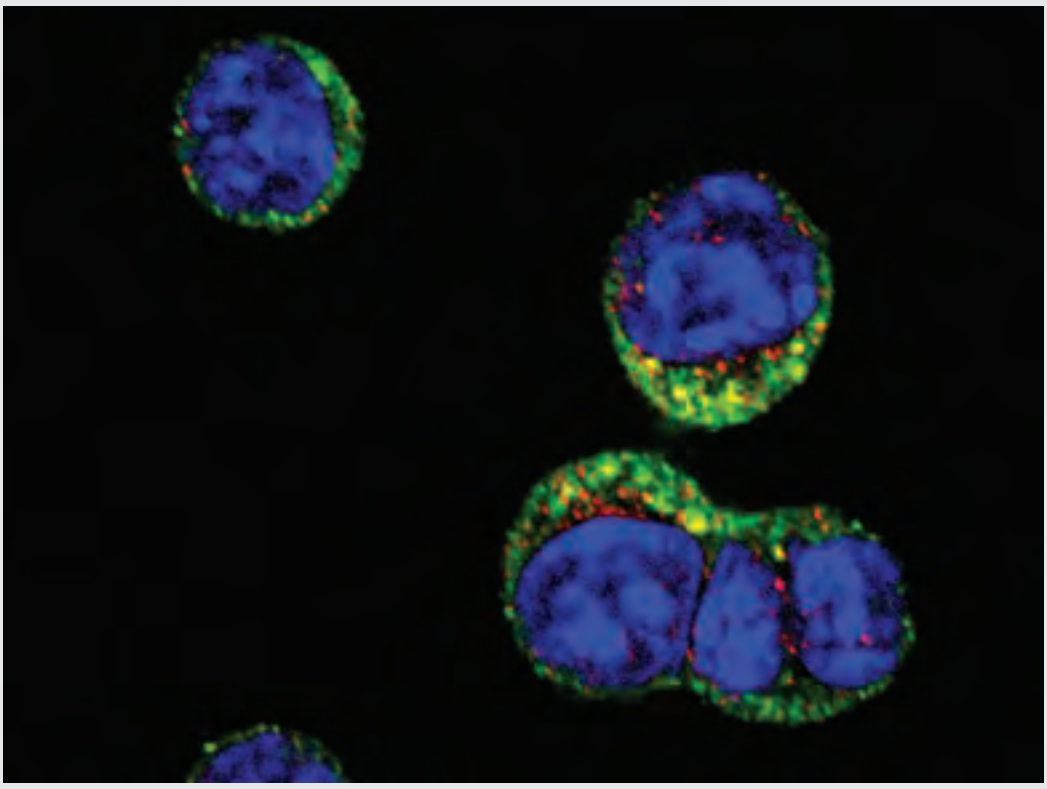
# New mechanisms of lymphocyte development

**We are working to understand the fundamental mechanisms that regulate dynamic changes in gene expression during lymphocyte development and activation. Our recent work has focused on RNA binding proteins that control the stability of messenger RNA.**

RNA binding proteins are often found within cells as part of large RNA-protein complexes that can be seen with a light microscope (see image). We have identified functions for RNA binding proteins in lymphocyte development and activation and we are now addressing the challenge of how they work mechanistically. To do this we have applied innovative technologies that measure RNA-protein interactions on a transcriptome scale and have integrated these data with studies of RNA abundance. The application of these approaches has uncovered novel mechanisms that regulate cell function.

As lymphocytes develop there are dynamic changes in gene expression that facilitate periods of quiescence to enable the assembly of antigen receptor genes by VDJ recombination and proliferation that serves to expand the repertoire of antigen receptor bearing lymphocytes. Failure to regulate this properly can lead to chromosomal translocations. We found that during B and T lymphocyte development members of the ZFP36 family of RNA binding proteins inhibit progression of the cell cycle (1). These regulators work by specifically binding messenger RNA transcripts that encode cell cycle progression factors and inhibiting their expression.

This mechanism may enforce quiescence in other cell types and has the potential to allow signalling pathways to rapidly promote cell cycle entry in quiescent cell populations.



*Tis11b (red) has been shown to be a component of processing bodies, RNA protein complexes, which mediate RNA decay. This image shows localisation of TIS11b with the decapping enzyme Dcp-1 (green) in activated lymphocytes. The function of processing bodies in lymphocytes is unknown and is under investigation in the laboratory.*

## Publications

www.babraham.ac.uk/our-research/lymphocyte/martin-turner

1 Galloway, A. *et al*. (2016) RNA-binding proteins ZFP36L1 and ZFP36L2 promote cell quiescence. *Science* 352(6284): 453-459

2 Vogel, K.U. *et al*. (2016) The RNA-binding proteins Zfp36l1 and Zfp36l2 enforce the thymic β-selection checkpoint by limiting DNA damage response signaling and cell cycle progression. *J Immunol* 197(7): 2673-2685

3 Newman, R., McHugh, J. & Turner, M. (2016) RNA binding proteins as regulators of immune cell biology. *Clin Exp Immunol* 183(1): 37-49

Geoff Butcher

**Group members**

**Research assistant:**
Dr Silvia Innocentin

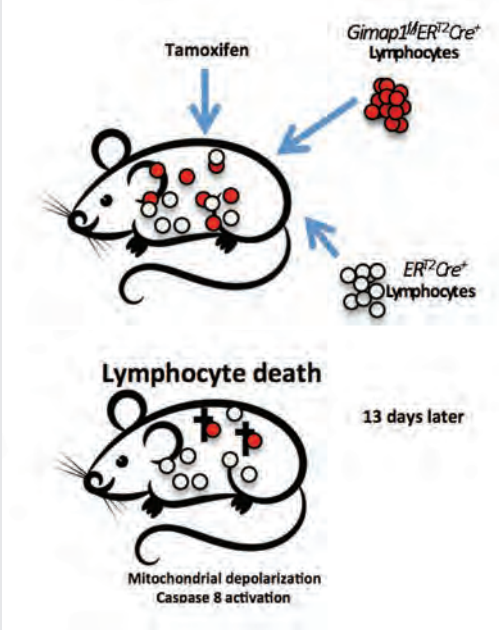
**Postdoctoral researcher:**
Dr John Pascall

# Immune GTPases in lymphocyte homeostasis

**A fully effective immune system requires stable populations of mature T and B lymphocytes (also called T and B cells). A family of GTPase enzymes, the GIMAPs, play an important but ill-understood role in the homeostasis of these lymphocytes. Uncovering the mechanisms by which the GIMAPs exert their functions would enrich our view of lymphocyte survival and offer new opportunities for pharmacological intervention in some immune-mediated diseases.**

Our current research involves studies of two members of the *GIMAP* gene family, namely *GIMAP1* and *GIMAP6*, both of which are expressed in both humans and our model species, mice. We have created lines of mice in which these genes can be selectively deleted, either under the control of tissue-specific promoters or in response to chemical inducers. Deletion of *GIMAP1* has proven to have the most profound effect of any *GIMAP* gene manipulation attempted so far: under the influence of lymphocyte-specific promoters, ablation of *GIMAP1* produces near-complete loss not only of mature T lymphocytes but also, remarkably, of B lymphocytes. Further investigations of the B cell effects both *in vivo* and *in vitro* revealed that both resting and activated B cells, as well as the establishment of B cell memory, require GIMAP1 (1). We have also investigated the nature of the survival defect in GIMAP1-deficient CD4+ T cells and shown that the death occurring after *GIMAP1* ablation is accompanied by mitochondrial depolarization and activation of the extrinsic apoptotic pathway (2).

Turning to GIMAP6, we previously reported that this GIMAP family member is recruited to autophagosomes on cell starvation, suggesting a potential role for the protein in autophagy (3). However, we were unable to demonstrate any effect of over-expression or knock-down of GIMAP6 on autophagy in our cell culture systems. We have now extended these studies by creating a mouse model in which *GIMAP6* is conditionally ablated in lymphocytes. These mice show reduced numbers of T but not B cells indicating an important role for GIMAP6 in the establishment and/or the maintenance of the mature T cell population. Moreover, analysis of proteins in these lymphocytes show changes diagnostic of disruption of normal autophagy, suggesting strongly that GIMAP6 is indeed involved in autophagic regulation.




*Ablation of GIMAP1 in mature lymphocytes results in their death in the periphery. Transfer of purified mature lymphocytes into recipient mice, followed by deletion of their GIMAP1 gene, results in cell death. Cell death is preceded by mitochondrial depolarization and activation of caspase 8.*

*The 'red' lymphocytes are from a genetically modified mouse strain and delete the GIMAP1 gene in response to the drug tamoxifen; the 'white' lymphocytes are controls in which this does not take place.*

## Publications

www.babraham.ac.uk/our-research/lymphocyte/geoffrey-butcher

1 Webb, L.M.C. *et al*. (2016) GIMAP1 is essential for the survival of naïve and activated B cells in vivo *J Immunol* 196: 207-216

2 Datta, P., Webb, L.M.C. *et al*. (2017) Survival of mature T cells in the periphery is intrinsically dependent on GIMAP1 in mice *Eur J Immunol* (2017) 47: 84-93

3 Pascall, J.C. *et al*. (2013) The immune system GTPase GIMAP6 interacts with the Atg8 homologue GABARAPL2 and is recruited to autophagosomes. *PLoS ONE* 8(10): e77782

Michelle Linterman

**Group members**

**Masters student:**
Michael Shannack (left in 2016)

**PhD students:**
Marisa Stebegg (joined in 2016)
Alyssa Silva-Cayetano
Ine Vanderleyden

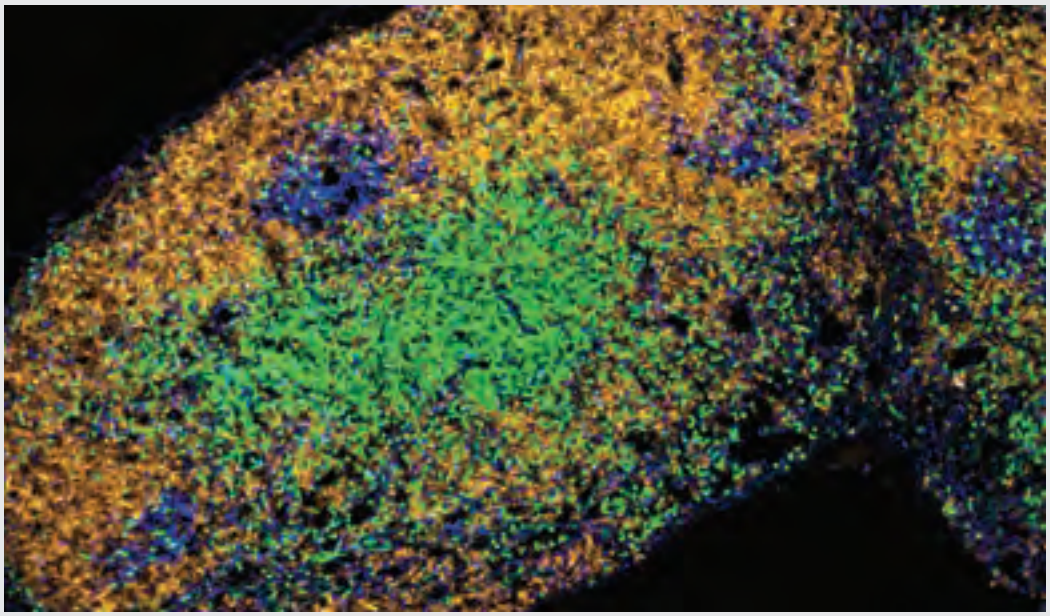
**Research assistant:**
Dr Silvia Innocentin (joined in 2016)

**Postdoctoral researchers:**
Dr Alexandre Bignon
Dr Edward Carr
Dr Danika Hill
Dr Wim Pierson
Dr Louise Webb

**Fellow:**
Dr Alice Denton

**Visiting researchers in 2016:**
Patricia Ame-Thomas
Noudjoud Attaf
Elizabeth Wallin

# The ageing immune response to vaccination



*This image shows germinal centres (blue staining, surrounded by yellow) in a lymph node 14 days after immunisation. Staining: Blue=Ki67, Yellow=IgD, Green=CD3.*

**Our ageing population creates a new challenge for medical science; to facilitate healthy ageing. With age, the function of the immune system declines, rendering older people more susceptible to infections and less able to benefit from vaccination. Our research aims to understand how the immune system changes with age, to determine if we can improve immune function in older persons.**

Ageing dramatically affects the function of the immune system, resulting in increased susceptibility to infections and increased infection-related morbidity and mortality in older members of our communities. Notably, there is already an established intervention that can prevent a range of potentially life-threatening infections – vaccination. It has been observed, however, that older individuals often do not generate protective immunity after vaccination.

At the heart of the immune response to vaccination is the germinal centre (GC) – a dynamic structure that forms in secondary lymphoid tissues after immunisation and produces long-lived plasma cells, which secrete antibodies that block pathogens from establishing an infection, and memory B cells. A defining property of the GC is the collaboration of multiple cell types: proliferating B cells, T follicular helper cells (Tfh), T follicular regulatory cells (Tfr) and follicular dendritic cells to produce effector B cells of higher quality.

With age, the magnitude of the GC response decreases resulting in impaired production of plasma cells, lower serum antibody levels and consequently, decreased protection against subsequent infection. The aim of our research is to understand the age-dependent cellular and molecular changes in the GC T cells, Tfh and Tfr cells, and the alterations in the microenvironment that are responsible for the impaired GC response following vaccination of older individuals. Our research uses both mice and human studies to combine the tractability advantages of mice with the physiological relevance of studying humans. In the past year we have characterised the age-dependent changes in the human immune system in 670 healthy individuals (2). We found that only some immune cells are affected by ageing, and, of those that are, the changes occur gradually over the course of the lifespan rather than in the later years of life. We are in the process extending these studies to human influenza vaccination studies, to determine which changes are associated with poor vaccine responses in older persons.

## Publications

www.babraham.ac.uk/our-research/lymphocyte/michelle-linterman


1 Vinuesa, C.G. *et al*. (2016) Follicular Helper T cells. *Annu Rev Immunol* 20;34: 335-68. doi: 10.1146/annurev-immunol-041015-055605

2 Carr, E.J. *et al*. (2016) The cellular composition of the human immune system is shaped by age and cohabitation. *Nat Immunol* 17(4): 461-8. doi: 10.1038/ni.3371

3 Aloulou, M. *et al*. (2016) Follicular regulatory T cells can be specific for the immunizing antigen and derive from naive T cells. *Nat Commun* 28;7:10579. doi: 10.1038/ncomms10579

Klaus Okkenhaug

**Group members**

**PhD students:**

Rafeah Alam

Christina Courreges (joined in 2016)

Fiorella Cugliandolo (joined in 2016)

Saad Idris

Ai Hui Doreen Lau (joined in 2016)

Lyn Lim (left in 2016)

Daisy Luff

**Research assistants:**

Valentina Carbonaro (left in 2016)

Bahram Firouzi (joined in 2016)

**Postdoctoral researchers:**

Dr Hicham Bouabe (left in 2016)

Dr Krishnendu Chakraborty (joined in 2016)

Dr Fabien Garcon

Dr Birthe Jessen (joined in 2016)

Dr Amy Macqueen

Dr Priya Schoenfelder

Dr Anne-Katrien Stark

**Fellow:**

Dr Anita Chandra

# PI3K in immunity, infection and cancer

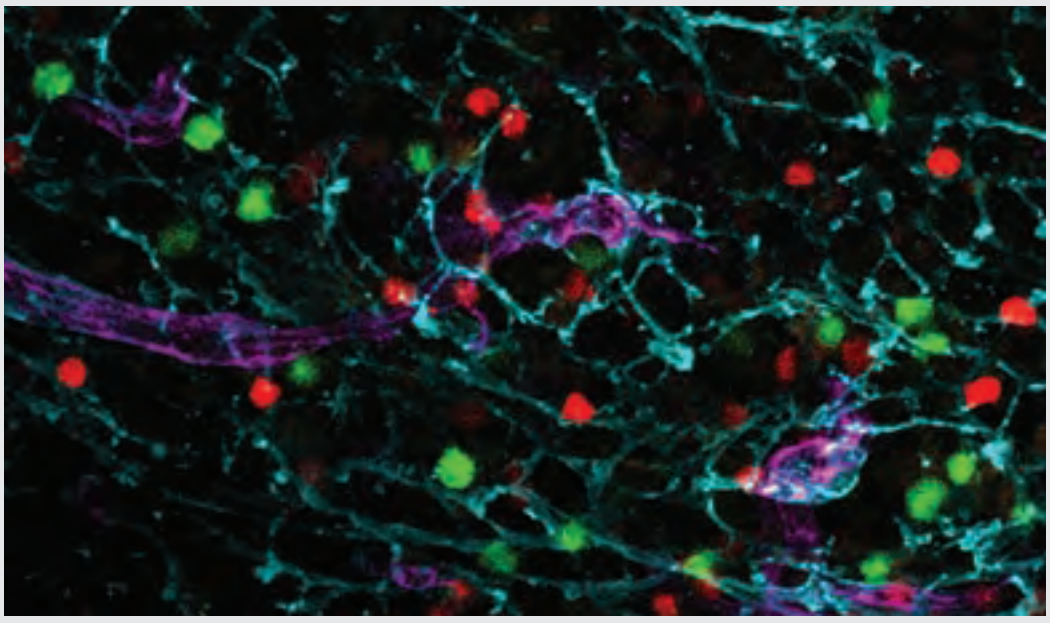
**The PI3K family of enzymes control many aspects of immune function. PI3Ks are required for the normal development and homeostasis of lymphocytes, as well as for the immune system to respond to and protect against infections and cancer. We aim to better understand how drugs that target specific PI3Ks can best be exploited for clinical benefit.**

We aim to understand how a family of enzymes called the PI3Ks control the development and function of the immune system. Much of our work has focused on the role of the PI3Kδ isoform which is highly expressed in cells of the immune system. PI3Kδ is the target for the drug Idelalisib which is now approved for the treatment of certain B cell malignancies. However, many questions remain about the impact of PI3Kδ inhibition on different immune cell types in health and diseases. Moreover, the recent discovery of patients with a primary immunodeficiency caused by activating mutations in PI3Kδ (called APDS) raises further questions about the physiological role of this enzyme.

Typically, PI3Ks are considered to signal via kinases such as AKT and mTOR to regulate gene expression. However, PI3Ks also control a number of proteins involved in cell biological processes such migration and homing to different tissues. In the lymph nodes, T cells interact with antigen presenting cells (APCs) which present peptide antigens in the context of major histocompatibility molecules. Our published research this year showed that PI3Kδ signalling in the T cells has profound impacts on the cell shape changes that occur when a T cell is activated. Moreover, we found a specific requirement for PI3Kδ to regulate the integrin LFA1 that facilitates the interaction between T cells and APCs (1). These findings have implications for how PI3Kδ inhibitors affect T cell activation.

Following on from the discovery of APDS, we continue to investigate why hyperactivation of PI3Kδ leads to immunodeficiency. We recently reviewed the progress in understanding this complex disease (2). Ongoing work considers the effect of hyperactive PI3Kδ in different immune cell subsets and in the context of bacterial and viral infections. We hope this work will help guide the treatment of this syndrome and also uncover fundamental new insights into how PI3Kδ regulates susceptibility to infections.

Although PI3Kδ inhibition was initially thought to lead to immune suppression, we have found that PI3Kδ-deficient mice are resistant to several types of infections and can mount strong anti-tumour responses. We have shown that PI3Kδ plays an important role in regulatory T cells that suppress anti-tumour immune responses. We are currently evaluating the role of PI3Ks in different components of the tumour stroma and have recently reviewed progress in this area (3).



*T cells (red and green) migrating through a tumour infiltrated with blood vessels (purple)*

## Publications

www.babraham.ac.uk/our-research/lymphocyte/klaus-okkenhaug


1 Garcon, F. & Okkenhaug, K. (2016) PI3Kdelta promotes CD4(+) T-cell interactions with antigen-presenting cells by increasing LFA-1 binding to ICAM-1 *Immunol Cell Biol* 94(5): 486-95

2 Lucas, C.L., Chandra, A., Nejentsev S., Condliffe, A.M., & Okkenhaug, K. (2016) PI3Kdelta and primary immunodeficiencies *Nat Rev Immunol* 16(11): 702-714

3 Okkenhaug, K., Graupera, M. & Vanhaesebroeck, B. (2016) Targeting PI3K in cancer: impact on tumor cells, their protective stroma, angiogenesis, and immunotherapy *Cancer Discov* 2016. 6(10): 1090-1105

Rahul Roychoudhuri

**Group members**

**PhD students:**

Francis Grant

Firas Sadiyah (joined in 2016)

**Postdoctoral researcher:**

Dr Rabab Nasrallah (joined in 2016)

**Visiting researchers in 2016:**

Dr Melanie Stammers (joined in 2016)

Dr Laura Rosenberg

# Decision circuits in the immune system

**T cells coordinate immune function by differentiating into highly specialised cellular lineages. Whereas effector CD4+ and CD8+ T cells promote immune activation and drive clearance of infections and cancer, CD4+ regulatory T cells (Treg), which are dependent upon the transcription factor Foxp3, suppress their function, preventing excessive autoimmune and allergic reactions. The mechanisms by which these powerful cells make such decisions, or 'lineage choices', are not completely clear.**

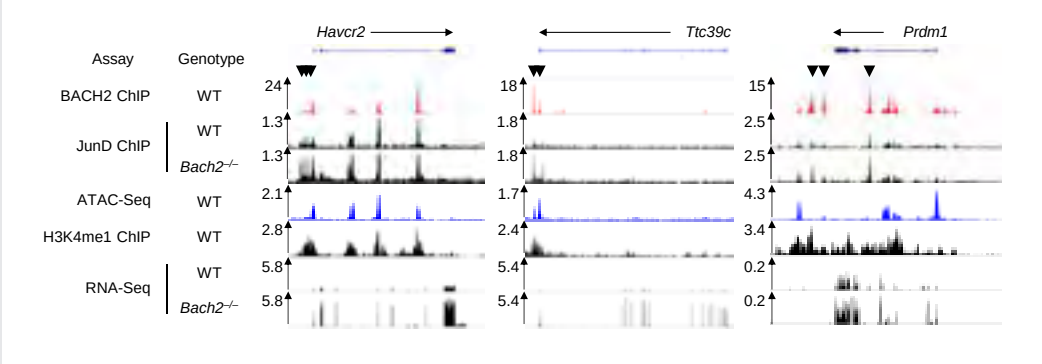
We are interested in understanding the general principles and specific mechanisms driving appropriate lineage choices during T cell differentiation, and the implications of this for immune function in health and disease. We are particularly interested in the following questions:

1. Effector cells and regulatory T cells arise from common precursors yet establish dichotomous functional programmes. What are the transcriptional control circuits that enable these dichotomous functional programmes to be established?

2. Regulatory T cell populations remain stable throughout the adult lifespan and into old age. This remarkable stability is required to prevent otherwise lethal inflammation. What are the mechanisms that enable durable maintenance of the regulatory T cell programme throughout life?

3. The suppressive function of regulatory T cells can prevent appropriate function of the immune system during chronic infections and cancer. What are the external signals driving inappropriate lineage choices in these contexts? Can these processes be modulated?

We have made substantial progress in understanding the mechanisms and external signals underlying lineage choice in T cell differentiation. Highlights from this year's research include establishing a critical role for the transcription factor BACH2 in immunological memory (1), identifying extracellular ionic potassium as a key regulator of T cell activation (2), and identifying post-translational regulatory processes that act upon the transcription factor Hif1a to drive immunosuppressive regulatory T cell differentiation (3).




*Integrative analysis of genome-wide transcription factor binding, chromatin modification and accessibility has enabled us to gain an unprecedented understanding of the mechanisms that guide the differentiation and function of T cells in response to extrinsic signals.*

## Publications

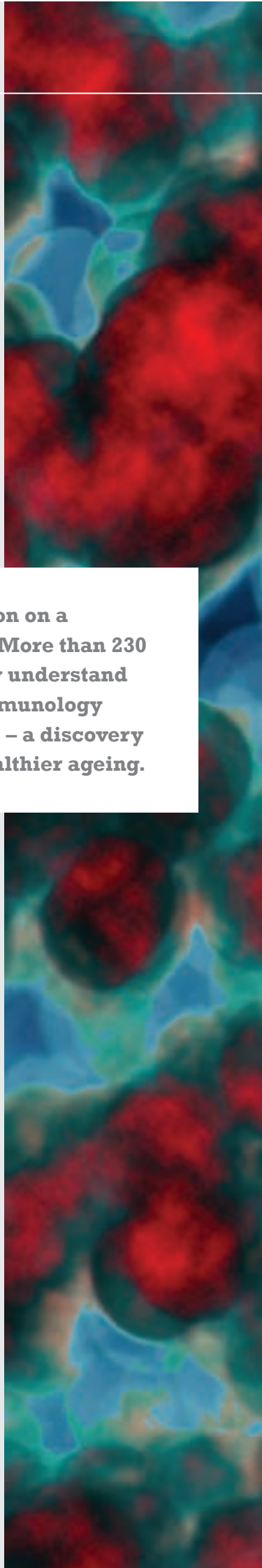
www.babraham.ac.uk/our-research/lymphocyte/rahul-roychoudhuri


1 Roychoudhuri, R. *et al*. (2016) BACH2 regulates CD8(+) T cell differentiation by controlling access of AP-1 factors to enhancers. *Nat Immunol* 17: 851-60

2 Eil, R., Vodnala, S.K. *et al*. (2016). Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature* 537: 539-543

3 Clever, D. *et al*. (2016). Oxygen sensing by T cells establishes an immunologically tolerant metastatic niche. *Cell* 166:1117-31

# Checks and balances in immune system development



**In 1796, a doctor in rural Gloucestershire took pus from a cowpox lesion on a milkmaid's hand to inoculate an eight-year-old boy against smallpox. More than 230 years after Edward Jenner's pioneering vaccination, we still don't fully understand how our immune system works. Now, researchers in the Institute's Immunology programme have uncovered a new layer of regulation in immune cells – a discovery that could have far-reaching implications for vaccines, cancer and healthier ageing.**

Our immune system does a remarkable job of defending us against germs, repelling invaders and keeping us healthy. A key part of this system are our B cells – highly specialised cells capable of responding to myriad disease-causing pathogens. Not only are they hugely diverse in the pathogens they recognise, they can remember the pathogens we have been exposed to in the past, providing us with immunity against future attacks.

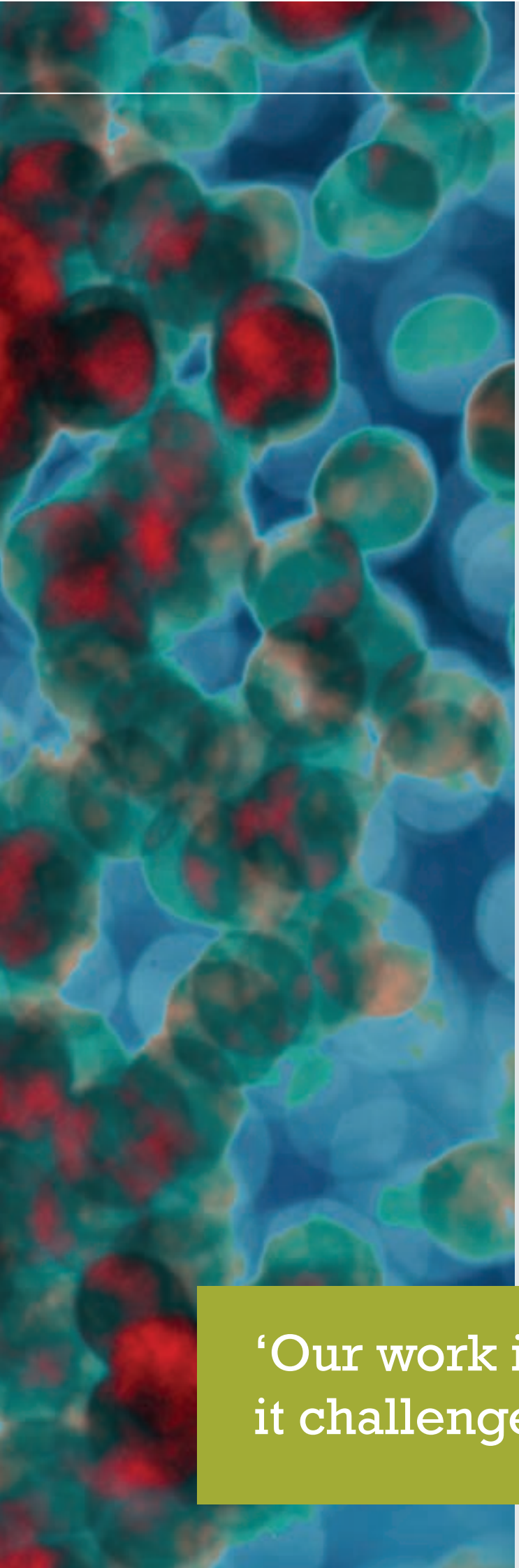
The vast repertoire of responses that these cells possess is due to their unique ability to cut up and rearrange their own DNA. "This variable-diversity-joining recombination only happens in these cells," says Dr Martin Turner of the Immunology research programme, who wants to get to the bottom of when this takes place, and how the mechanism works.

B cells develop in a sequence. During the process, there are several discrete stages where DNA is broken and re-joined, allowing the cells to produce the many different antibodies we need to protect ourselves from many different infections. Rearranging DNA, however, is a risky process.

Breaking DNA at the wrong point in the sequence can cause cells to die, and making mistakes when re-joining DNA can lead to even more sinister consequences. When this happens, the mutated genes can create cancer-causing immune cells, resulting in diseases such as lymphoma and leukaemia. "Immune cell cancers, such as lymphomas and leukaemias, are the payback we get for a functioning immune system," he explains. "The good news is that knowing more about this DNA re-shuffling mechanism, including what happens when it goes wrong, means that in the future we'll be able to treat these types of cancers better."

During their development, B cells switch very rapidly between periods of quiescence (or rest) and proliferation. When they are resting, they cut up and rearrange their DNA, but as soon as this happens there is a rapid burst of cell proliferation. The problem is that DNA works too slowly to explain this switch. "DNA is very stable, it's not a very dynamic on/off switch, yet we know that cells do things very rapidly," he says.

So what is switching cells between quiescence and proliferation – and back again? Received wisdom is that all the necessary information is encoded in the DNA and that you just need to look hard enough to find all the answers. Turner argues that there is another layer of regulation, and is now providing evidence that it exists, and that it has important consequences: "Many people argue that to regulate gene expression, all you need to do is regulate the tempo of what goes on at the DNA level.



# ‘Immune cell cancers are the payback you get for a functioning immune system’

What we’re working on is a bit left field, it challenges the orthodoxy about how things work.”

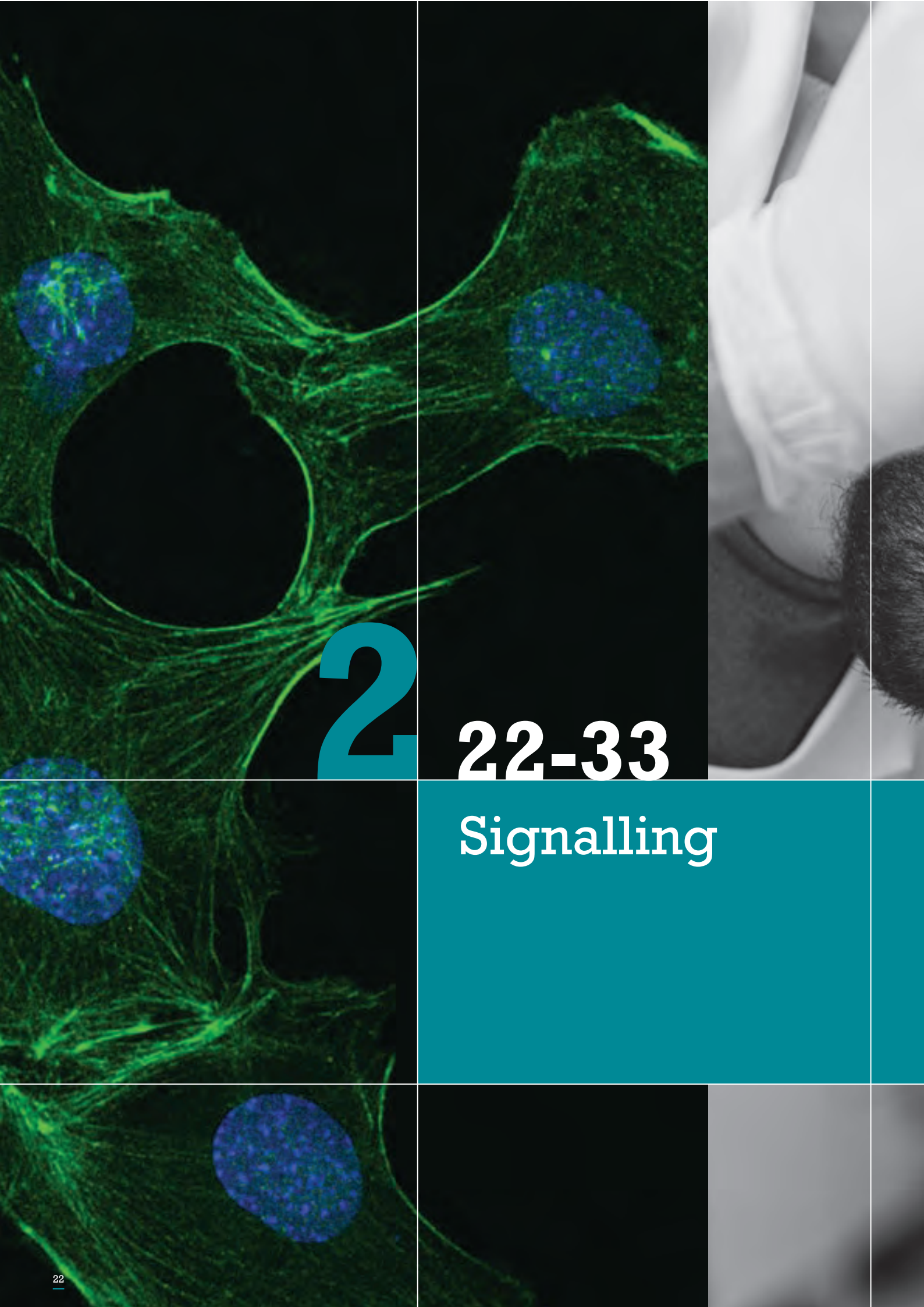
The Institute’s Immunology programme has discovered that the process involves a group of cell cycle proteins known as RNA binding proteins. These act as a switch by affecting the stability of RNA, a single-stranded nucleic acid, and silencing the cell signals promoting proliferation. The same mechanism could also be important in other cell types. According to Turner: “It’s an interesting basic biological question, but there are good reasons why we need to understand the biology of these cells.”

B cells are what enable our immune system to remember past experiences, so knowing how they work is crucial for understanding vaccine responses and immunological memory. Turner’s latest work shows that RNA binding proteins help limit the intensity of the immune response, which ensures that the system isn’t activated inappropriately and that it shuts down as soon as it has fought off a pathogen such as influenza.

“When the ‘flu has been got rid of, you want the immune system to stop because it’s the immune response that makes you feel unwell,” he says. “The same is true of many diseases. In evolutionary terms, there’s been a trade-off between how actively the immune system attacks an infection and the damage that it causes the body. It’s about optimising that balance.”

Knowing that preventing cell proliferation is an active, rather than a passive, process is important in cancer research – and could also be crucial in understanding ageing. We are born with a limited supply of stem cells which we rely on to repair damage, so the body needs a mechanism to ensure these cells are used as sparingly as possible. “The balance between quiescence and proliferation is important, because the pool of stem cells becomes depleted if it’s not kept quiescent,” he concludes. “Maintaining quiescence is fundamental to the ability to rejuvenate. We know that the ability to rejuvenate deteriorates with age, so if we understand the process better, this could make for healthier ageing.”

‘Our work is a bit left field, it challenges orthodoxy’



# 2 22-33 Signalling

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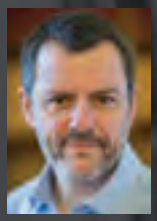
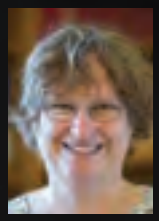


Nicolas Le Novère

Michael Wakelam



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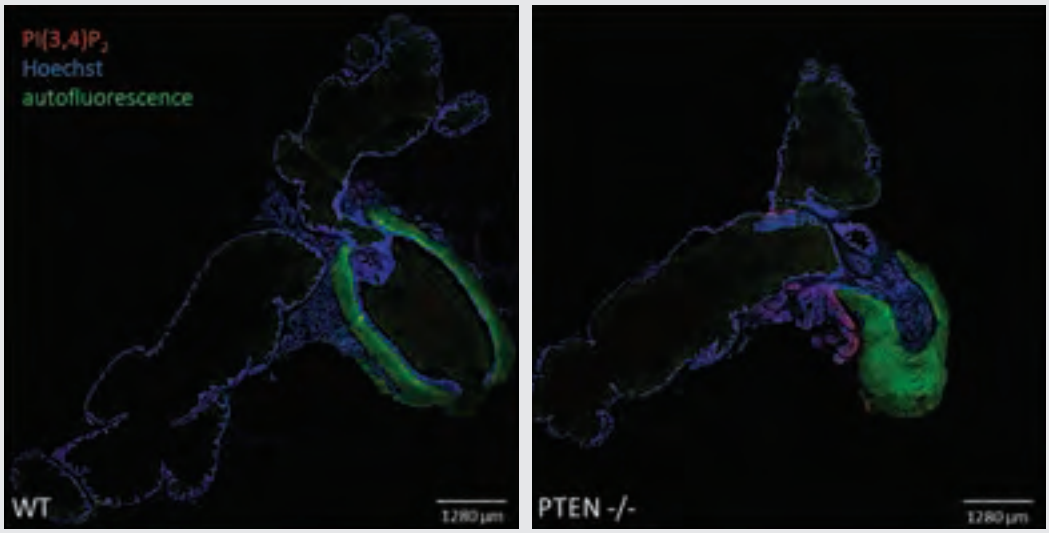
# Intracellular signalling by PI3K enzymes

**Extracellular stimuli, including growth factors, hormones and antigens, regulate the behaviour of target cells by first binding to cell surface receptors and then setting in motion molecular cascades, or signalling pathways, that ultimately connect to the key regulators of activities such as growth, division and movement. We study one of these pathways, the production of two phospholipid signals, called PI(3,4,5)P3 and PI(3,4)P2, by enzymes called phosphoinositide 3-kinases (PI3Ks). This pathway is particularly important in the regulation of metabolism, immunity, ageing and growth and is the most heavily mutated signalling pathway in human cancer.**

We are currently trying to understand how the molecular properties of different PI3K isoforms allow them to be activated by different types of cell surface receptor (1) and control so many different cellular responses. These insights are very useful for both small and large companies with whom we collaborate and who are targeting the PI3K pathway to treat several diseases, including age-related pathologies, inflammation and cancer.

We are also focused on trying to understand the particular significance of PI(3,4)P2 formation. PI(3,4,5)P3 and PI(3,4)P2 are made in the inner leaflet of the plasma membrane, where they recruit and activate several proteins that specifically recognise and bind to these molecules, thus propagating the signal to the cell interior. PI(3,4)P2 has been an enigmatic lipid (2), in that it can apparently be made directly by certain PI3K isoforms but can also be generated by de-phosphorylation of PI(3,4,5)P3 at the 5-position. Further, proteins ('effectors') which bind PI(3,4)P2 specifically have been hard to find, and thus we lack good clues as to its physiological role. We have recently discovered that PTEN, a key regulator of lifespan and a common tumour suppressor (loss of one PTEN allele occurs in approximately 30% of breast cancers, 70% of glioblastomas and 50% of advanced prostate cancers), which hitherto has been assumed to exert its main function by de-phosphorylating PI(3,4,5)P3 at the 3-position, also de-phosphorylates PI(3,4)P2 at the 3-position (manuscript in preparation). Thus, loss of PTEN also causes large elevations in PI(3,4)P2 (see figure) and we are working hard to understand the normal physiological role of PTEN as a PI(3,4)P2-phosphatase and to translate the implications that elevated PI(3,4)P2 might have for tumour progression.

We have also been extending our techniques for measuring PI(3,4,5)P3 and PI(3,4)P2 by mass spectrometry and this had led to several collaborations with other groups to help them analyse the activity of PI3K signalling in their systems (3).




*Loss of PTEN in mouse prostate drives an elevation in PI(3,4)P2. Low magnification (x20), wide-field fluorescence images were taken of prostate slices from 8-9 week old mice which were either wild-type (WT) or with PTEN deleted specifically in prostate epithelial cells (PTEN -/-). PI(3,4)P2 was detected by an anti-PI(3,4)P2 antibody (red); nuclei are stained with Hoechst dye (blue).*

## Publications

www.babraham.ac.uk/our-research/signalling/len-stephens /phillip-hawkins

1 Houslay, D.M. *et al*. (2016) Coincident signals from GPCRs and receptor tyrosine kinases are uniquely transduced by PI3Kβ in myeloid cells. *Sci Signal* 9(441): ra82
2 Hawkins, P.T. & Stephens, L.R. (2016) Emerging evidence of signalling roles for PI(3,4)P2 in Class I and II PI3K-regulated pathways. *Biochem Soc Trans* 44(1): 307-14
3 Hukelmann, J.L. *et al*. (2016) The cytotoxic T cell proteome and its shaping by the kinase mTOR. *Nat Immunol* 17(1):104-12

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# Signals controlling cell fate decisions

**Cell fate decisions (to divide or not; to differentiate; to die) are critical to maintain health throughout life. External cues activate protein kinase signalling pathways, which drive specific protein phosphorylation events to control these cellular decisions. We are interested in how protein kinase pathways are regulated, identifying protein kinase substrates and understanding how their phosphorylation regulates cell fate choices.**

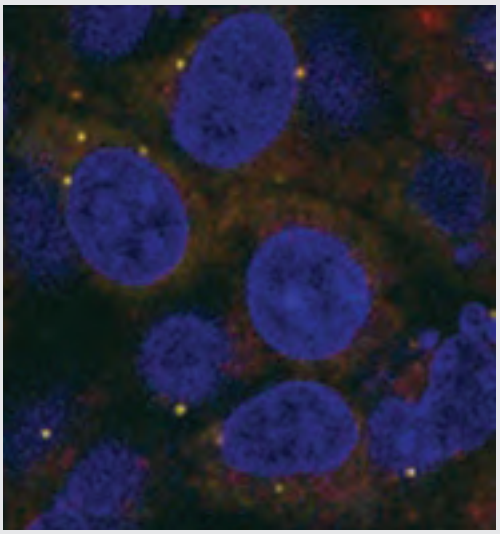
Many current projects in the lab involve the study of two particular protein kinase families: the extracellular signal-regulated kinase (ERKs, such as ERK1/2 and ERK5) and the related dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs, such as DYRK1A and DYRK1B).

ERK1/2 are best known for phosphorylating transcription factors (ETS, FOS, etc.) to drive gene transcription, thereby regulating cell proliferation. Our new data show that both the expression and phosphorylation of the ZFP36 zinc-finger RNA-binding proteins are regulated by ERK1/2 (unpublished). In collaboration with Martin Turner's group we have shown that the ZFP36 proteins have a critical role in imposing quiescence during B cell development and proliferation and in epithelial cells. We used CRISPR/Cas9 gene editing to delete ZFP36L1 to reveal its role in destabilising the mRNAs encoding key cell cycle regulators (cyclins and CDKIs) (3). Prompted by these studies we are studying genome-wide changes in mRNA stability driven by ERK1/2 signalling and investigating how ERK1/2 signalling regulates the ZFP36 proteins.

In contrast to ERK1/2, where hundreds of substrates and binding partners have been identified, few DYRK substrates have been described that provide a context for their biological functions. We previously showed that DYRK1B can phosphorylate cyclin D1 (CCND1) at T286 to promote CCND1 degradation. More recently we have collaborated with Mariona Arbones (IBMB, Barcelona) to show that DYRK1A can also phosphorylate CCND1 at T286 and that this likely accounts for the reduction in CCND1 and premature differentiation of neurons in a mouse model of Downs Syndrome driven by trisomy of *Dyrk1a* (1).

More recently we have uncovered links between ERK1/2 and DYRK1B by showing that DYRK1B is phosphorylated by ERK1/2 at a site that promotes DYRK1B catalytic activity, uncovering new links between two kinases involved in cell fate decisions. We have also shown that DYRK1B mutants described in cancer and metabolic syndrome exhibit normal or reduced intrinsic kinase activity (2). The reduced activity of rare DYRK1B mutants found in cancer may suggest a tumour suppressor role for this kinase.

Finally, in ongoing work, we have used P-SILAC MS screens to identify new DYRK1B and DYRK2 substrates that are involved in mRNA processing/turnover, mTOR signalling and proteostasis/autophagy.



*Processing bodies (sites of mRNA degradation) visualised in HEK293 cells with antibodies to Dcp1a (red) and DDX6 (green). The nucleus is stained with DAPI (blue). Image: Dr Anne Ashford, Cook lab.*

## Publications

www.babraham.ac.uk/our-research/signalling/simon-cook


1 Najas. S. *et al*. (2015) DYRK1A-mediated Cyclin D1 degradation in neural stem cells contributes to the neurogenic cortical defects in Down syndrome. *EBioMedicine* 2:120-134

2 Ashford, A.L. *et al*. (2016) Identification of DYRK1B as a substrate of ERK1/2 and characterization of the kinase activity of DYRK1B mutants from cancer and metabolic syndrome. *Cell Mol Life Sci* 73: 883-900

3 Galloway A. *et al.* (2016) RNA-binding proteins ZFP36L1 and ZFP36L2 promote cell quiescence. *Science* 352(6284): 453-459

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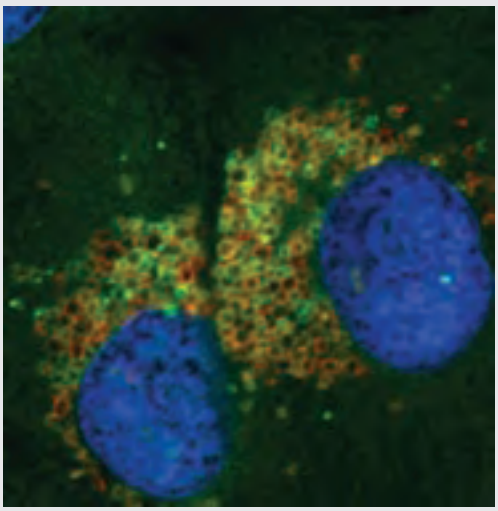
# Autophagy pathways: eating from inside and out

**The ability of cells to engulf and degrade both internal components and external material is essential for maintaining a healthy organism. Disruption of these pathways is associated with many ageing-related pathologies and diseases including cancer and neurodegeneration. Our research explores the molecular mechanisms underlying a range of degradative pathways and their roles in health and disease.**

In times of stress, cells can target components from within their own cytosol for lysosomal degradation via the autophagy pathway. This recycling process is predominantly a survival mechanism, which maintains energy metabolism and clears away damaged organelles or proteins. Considering the importance of autophagy in health and disease, many drugs are being developed or re-purposed for therapeutic activation or inhibition of this pathway.

While autophagy targets intracellular material for recycling, external material can also be engulfed and targeted for degradation. Externally engulfed material can include bacterial or fungal pathogens, dead cell corpses or live cells through cannibalism. We previously discovered that certain components of the autophagy machinery play an additional, essential role during the degradation of external material (1). This non-canonical autophagy pathway is known to be important during innate and adaptive immune responses, although the broader functional implications of non-canonical autophagy remain to be determined through ongoing work.

Recent studies in our lab by Dr Elise Jacquin have revealed, surprisingly, that many drugs implicated in modulating normal autophagy also induce an unexpected, parallel activation of non-canonical autophagy (2). These drugs include FDA-approved therapeutics such as chloroquine (malaria, cancer), lidocaine (anaesthetic) and amiodarone (arrhythmia). This finding highlights a new and unappreciated activity for several commonly used drugs and raises important issues on the interpretation of autophagy-based results obtained using them. Our findings also reinforce the need for further understanding of the functions and implications of non-canonical autophagy, which is a key focus of the lab.



*Cell, drugs and vacuoles: immunofluorescent image of cells treated with betahistine and stained for LC3, (a marker of autophagy; green), the lysosomal membrane protein LAMP1 (red) and DNA (blue).*

Other work in our lab, led by Dr Jo Durgan, has focused on the novel phenomenon of cell cannibalism. Cannibalised cell-in-cell structures have long been observed in a wide range of human cancers, but their role in tumour biology remains elusive. In a recent collaboration with Dr Lucy Collinson at the Crick Institute, we have undertaken an ultrastructural study of cell-in-cell structures using state-of-the-art correlative 3D electron microscopy (3). Through the use of this new technology we have discovered that internalised cells reside in unique membrane compartments, opening an important new line of research regarding the lipid dynamics which operate during cancer cell cannibalism.

## Publications

www.babraham.ac.uk/our-research/signalling/oliver-florey


1 Florey, O. *et al*. (2011) Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. *Nat Cell Biol* 3(11):1335-43

2 Jacquin, E. *et al*. (in press) Pharmacological modulators of autophagy activate a parallel noncanonical pathway driving unconventional LC3 lipidation. *Autophagy*

3 Russell, M.R. *et al*. (2017) 3D correlative light and electron microscopy of cultured cells using serial blockface scanning electron microscopy. *J Cell Sci* 130(1): 278-291

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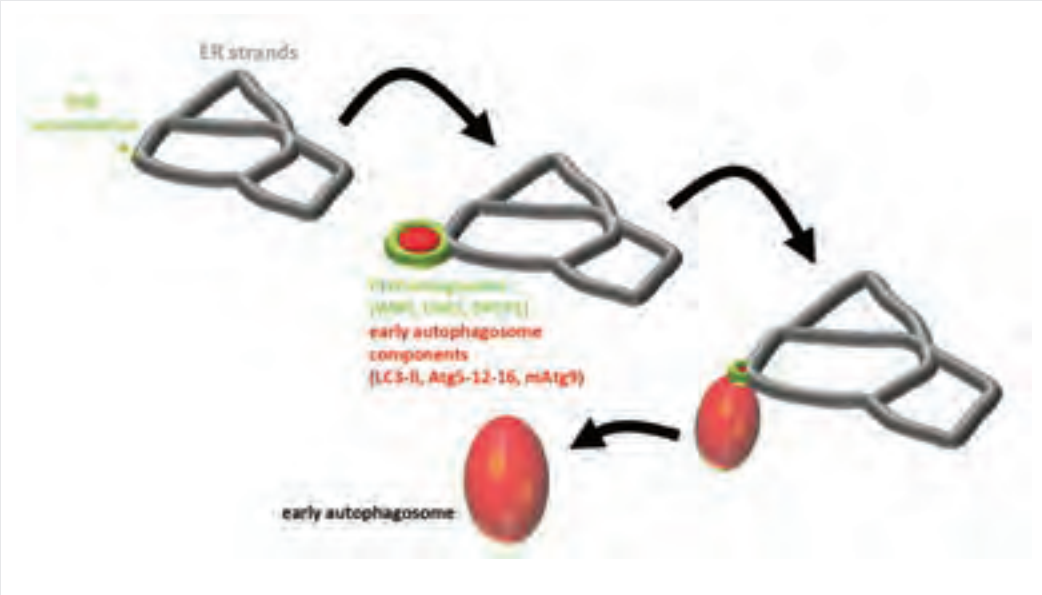
# Autophagy dynamics

**Animal cells maintain elaborate signalling systems to sense nutrient availability and adjust their metabolism accordingly. In extreme cases when nutrients such as amino acids become very scarce, cells activate a self-digesting pathway called autophagy which allows them to generate nutrients from internal sources and survive starvation. Important aims of my groups are to understand how this pathway is triggered and what the molecular details of the autophagic response are.**

We have been able to determine the morphology of the earliest autophagy structures that are formed within a few minutes of the autophagy signal. They are assemblies of the essential regulator of autophagy, the ULK complex of proteins. Using a combination of live imaging and super resolution microscopy techniques we have resolved regular assemblies of this complex on endoplasmic reticulum extensions (2). These regions were also marked by a few vesicles containing another essential autophagy protein, ATG9. Further work using live imaging coupled with Focussed Ion Beam Scanning Electron Microscopy revealed that this region is made up of tubulovesicular elements morphologically (but not functionally) resembling endoplasmic reticulum exit sites. Our working model is that autophagy is initiated when the mTORC1 (mechanistic target of rapamycin complex 1) transmits a signal to ULK complex which interacts with ATG9 vesicles on tubular extensions of the endoplasmic reticulum.

In related work we have generated a stable cell line expressing endogenous RAPTOR (one of the subunits of mTORC1) tagged with GFP (3). These cells have allowed us to determine the dynamics of the mTORC1 complex in one round of amino acid starvation and re-stimulation. In agreement with published work we saw a rapid translocation of the complex to the lysosomal membranes very quickly after amino acid addition. In an elaboration of the standard model, we measured a significant delay between translocation of the complex to lysosomes and phosphorylation of its targets. This suggests the possibility that as mTORC1 transits through the lysosomes it acquires some modification that allows it to maintain its activation state even when it is back in the cytosol.

Our current and future work continues to address questions in autophagy induction (with recent emphasis on selective autophagy of mitochondria) and mTORC1 dynamics.




*Pathway of autophagy via an omegasome intermediate.*

## Publications

www.babraham.ac.uk/our-research/signalling/nicholas-ktistakis

1 Ktistakis, N.T. & Tooze, S.A. (2016). Digesting the expanding mechanisms of autophagy. *Trends Cell Biol* pii: S0962-8924

2 Karanasios, E. *et al*. (2016) Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. *Nature Comm* 7: 12420

3 Manifava, M. *et al*. (2016). Dynamics of mTORC1 activation in response to amino acids. *eLife* 2016;5: e19960

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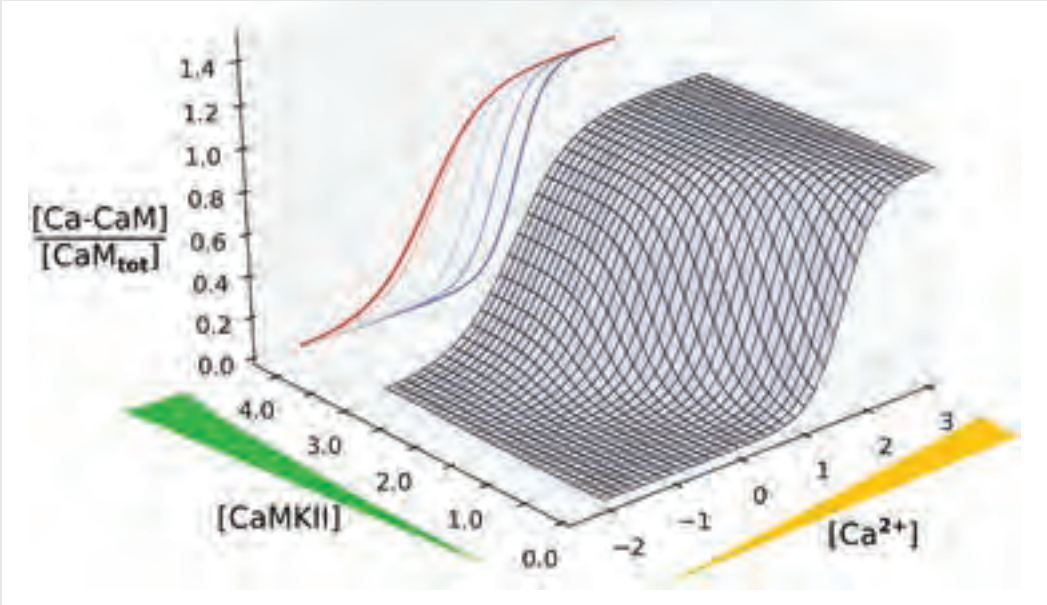
# Modelling biological systems




*Reciprocal effects of increasing concentrations of calcium/calmodulin kinase II on the affinity of calmodulin for calcium. As concentrations of calcium/calmodulin kinase II increases (green triangle), the response to increasing concentrations of calcium (yellow triangle) is shifted to the left, i.e. the affinity of calmodulin for calcium increases.*

**The behaviour of biological systems emerges from the dynamical interactions of their many parts. To understand their functions and the impact of perturbations such as diseases and ageing, we need to adopt a global approach. This relies on bioinformatic methods to analyse large quantitative datasets and mathematical modelling. Our particular interest lies in the interplay between cell signalling, metabolism and epigenetic programming.**

Many of the current projects in the lab involve analysing genome-wide gene expression during cell differentiation or after perturbation of signalling pathways. PIP3 is synthesised by the Class I PI3Ks and regulates complex cell responses, such as growth and migration. We analysed RNA-Seq data in wild-type and oncomutant cells (constitutive PI3Kα, PTEN-KO) responding to epidermal growth factor (EGF) in the presence or absence of a PI3Kα inhibitor (1). We showed that a large number of mRNAs are changed by long-term genetic perturbation of PIP3 signalling as well as acute treatments. Some PIP3-sensitive mRNAs encode PI3K pathway components, forming a transcriptional feedback loop. We have identified the transcription factor binding motifs SRF and PRDM1 as important regulators of PIP3-sensitive mRNAs involved in cell movement.

One of our long-term questions is to understand the regulation of synaptic plasticity by calcium. A key player is the protein calmodulin which regulates target kinases and phosphatases in a calcium-dependent manner. We have developed a new mathematical model of calmodulin that allows for concerted conformational transitions of both calcium binding sites on a given lobe while N-terminal and C-terminal lobes can still be in different conformations (2). We then used this model to show how, depending on the calcium concentration, calmodulin activates CaMII (which increases synaptic weight) or calcineurin (which decreases synaptic weight). We have also studied the effect of Neurogranin, a calmodulin-binding protein expressed in the brain , which binds the low calcium affinity state of calmodulin.

The size of the processes considered in systems biology requires re-use rather than constant re-creation. This is made possible by standard formats, such as SBML and BioPAX. The group develops a set of key tools enabling encoding, enriching and processing pathways and models. An example of such software is the Systems Biology Format Converters (3) which is made up of a common development framework and different modules that can be used as elements in conversion pipelines.

## Publications

1 Kiselev, V.Y. *et al*. (2015) Perturbations of PIP3 signalling trigger a global remodelling of mRNA landscape and reveal a transcriptional feedback loop. *Nucleic Acids Res* 43: 9663-9679

2 Lai, M. *et al*. (2015) Modulation of calmodulin lobes by different targets: an allosteric model with hemiconcerted conformational transitions. *PloS Comput Biol* 11: e1004063

3 Rodriguez, N. *et al*. (2016) The systems biology format converter. *BMC Bioinfo* 17:154

http://lenoverelab.org

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# Why do we need so many distinct lipids?

**Lipids are essential biological molecules required for the integrity and function of cellular membranes that operate as signalling intermediates critical for cells to successfully process, respond and adapt to environmental changes – such as diet, viral infection and as we age. Using lipidomic, molecular, cell biological and bioinformatic methodologies we aim to investigate the regulation and function of these pathways.**

Lipids comprise fatty acids, mono- di- and triacylglycerols, phospholipids, sphingolipids, sterols, prenols, polyketides and saccharolipids. Each lipid class can consist of diverse molecular species differing in acyl carbon chain length and the position and number of double bonds (unsaturation), thereby accounting for the detection and quantified of upwards of 10,000 unique lipid species by liquid chromatography-mass spectrometry (LC-MS).

One of our research challenges is how to integrate data analysis of the LC-MS-identified lipidome into a biological context. Recently we have reported computational methodologies, such as pathway analysis, that can interrogate large lipidomic data sets to uncover the pathways and enzymes modified from analysing the changes in each of the ~10,000 lipid species (1).

Applying LC-MS and pathway analysis methodology we are currently investigating the ageing process in the nematode, *C. elegans* and in the fruit fly, *D. melanogaster*, to better understand the mechanisms that dictate lifespan and healthy ageing in humans.

We are also uncovering how the cellular pathways, and processes they control, are regulated by changes in the lipidome as a consequence of viral infection – rhinovirus and hepatitis B and C. Our long-term objectives are to promote health in later life.

Lipid metabolism is frequently altered in diseases such as cancer. Work from our laboratory has helped discover that cancer cell survival, within the environment of a tumour, is dependent on a re-engineered lipidome brought about through increased rates of *de novo* fatty acid synthesis and desaturation. LC-MS analysis of the lipids in cancer cells has demonstrated that they contain longer, more unsaturated acyl chains, compared to lipids in healthy cells. Our data has identified potential targets to limit tumour growth. Other collaborative work has shown the importance of regulated fatty acid synthesis in macrophage function (3).

Lipids are not only confined to cells, but can also be released from cells to form extracellular vesicles that contain proteins and nucleic acids. Extracellular vesicles (EVs) can interact with local and distant cellular targets, and as a consequence of cell surface receptor activation and/or the delivery of their contents into the target cell, the vesicles can modify the behavior of other cells. Recent work in our laboratory has discovered that exosomes, a type of EV that originates from intracellular membranes – as opposed to EVs that are shed from the plasma membrane – facilitate both the delivery and the potency of signalling lipids to target cells (2).

*Pathways analysis of LC-MS generated lipidomic data identifies rhinovirus-induced changes in bronchial epithelial cells.*

## Publications

www.babraham.ac.uk/our-research/signalling/michael-wakelam


1 Nguyen, A., Rudge, S.A., Zhang, Q. & Wakelam, M.J.O. (2016) Using lipidomic analysis to determine signaling and metabolic changes in cells. *Curr Opin Biotechnol* 43: 96-103

2 Jethwa, S.A. *et al*. (2016) Exosomes bind autotaxin and act as a physiological delivery mechanism to stimulate LPA receptor signalling in cells. *J Cell Sci* 129: 3948-3957

3 Cader, M.Z. *et al*. (2016) C13orf31 (FAMIN) is a central regulator of immunometabolic function. *Nature Immunol* 17:1046-56

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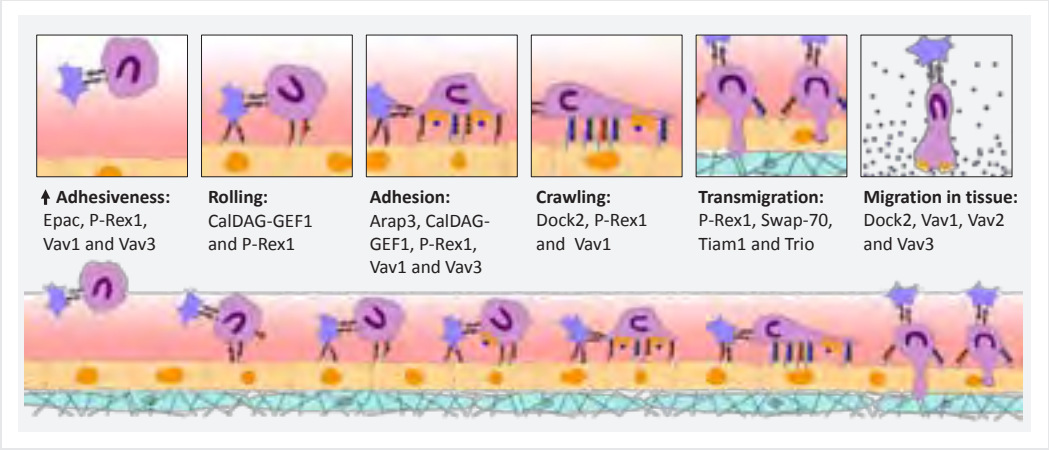
# Rac-GEF signalling




*GEFs and GAPs control the recruitment of white blood cells from the blood stream into inflamed and infected tissues. GEFs (activators) and GAPs (inhibitors) of small G proteins present in white blood cells such as neutrophils (purple), in blood platelets (blue), and in the endothelial cells that line the blood vessel wall (beige) control specific stages of the recruitment of white blood cells from the blood stream into inflamed and infected tissues. Figure adapted from ref.1.*

**Rac proteins are key controllers of cell adhesion and migration. Our lab studies Rac-GEFs, activators of Rac. Our recent research identified new roles of Rac-GEFs in the immune system and in cancer. We have also discovered a new mechanism to control Rac-GEF activity.**

Several years ago, we discovered a family of Rac-GEFs called P-Rex and described that P-Rex1 controls the recruitment of neutrophils, a type of white blood cell, from the blood stream into inflamed and infected tissues. This was important because neutrophil recruitment is essential for our immunity against bacterial and fungal infections. In recent work, we investigated the mechanisms through which P-Rex1 controls neutrophil recruitment. This new study showed, surprisingly, that the presence of P-Rex1 and other Rac-GEFs in blood platelets dictates whether neutrophils can be recruited into tissues. The presence of these Rac-GEFs in platelets promotes the cell–cell interactions that enable neutrophils to adhere to the blood vessel wall prior to their transmigration into the tissue (1).

In a different recent project, we discovered a new mechanism to regulate P-Rex. We showed that the adaptor protein Norbin can directly stimulate the Rac-GEF activity of P-Rex1 and can pull P-Rex1 from the cytosol to the plasma membrane, which is where P-Rex1 needs to be localised in order to activate Rac (2). We are currently investigating further the consequences of the interaction between P-Rex1 and Norbin for various cell functions.

We have also contributed to recent work by others which explored the role of P-Rex2 in melanoma. Our contribution showed that the isolated catalytic core of the P-Rex2 protein (the DH-PH domain tandem) is constitutively active, suggesting that the full-length P-Rex2 protein is controlled by intramolecular inhibition, as is typical for Rac-GEFs. Our collaborators showed that several truncation mutations of P-Rex2 which are observed in human melanoma also result in increased Rac-GEF activity. These P-Rex2 mutations can increase tumour proliferation provided the oncogene N-Ras is also active in the tumour cells (3).

## Publications

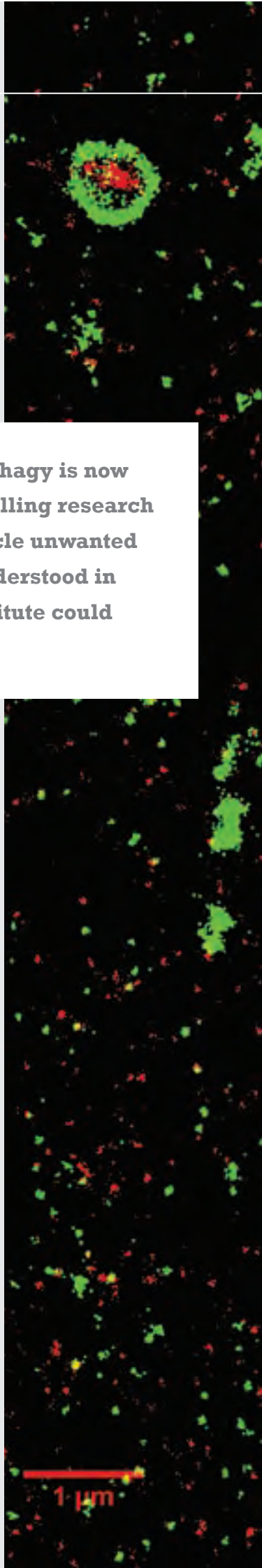
www.babraham.ac.uk/our-research/signalling/heidi-welch


1 Baker, M.J. *et al.* (2016) Small GTPases and their GEFs and GAPs in neutrophil recruitment. *Curr Opin Hematol* 23: 44-54

2 Pan, D. *et al.* (2016) Norbin stimulates the catalytic activity and plasma membrane localization of the guanine-nucleotide exchange factor P-Rex1. *J Biol Chem* 291: 6359-6375

3 Deribe, Y.L. *et al.* (2016) Truncating PREX2 mutations activate its GEF activity and alter gene expression regulation in NRAS-mutant melanoma. *Proc Natl Acad Sci USA* 113: E1296-1305

# The quiet pathway




**For many years regarded as merely a cell biological process, autophagy is now implicated in many diseases. Thanks to progress made in the Signalling research programme this year autophagy – the mechanism cells use to recycle unwanted or damaged components to create molecules they need – is now understood in greater detail than ever before. We find out how research at the Institute could harness autophagy to help us age more healthily.**

The more we learn about autophagy, the more fascinating, important and complex it becomes. Literally the process of 'self-eating', autophagy is the cell's way of recycling itself to survive short-term starvation, as well as cleaning itself of unwanted and potentially harmful material. Although most of us know little about it, autophagy is vital from the moment we are born until we die.

"It's a quiet pathway, but it's super important," says Dr Nicholas Ktistakis, group leader in the Signalling research programme. "It is an ancient pathway – all cells have it – but normally it works in the background."

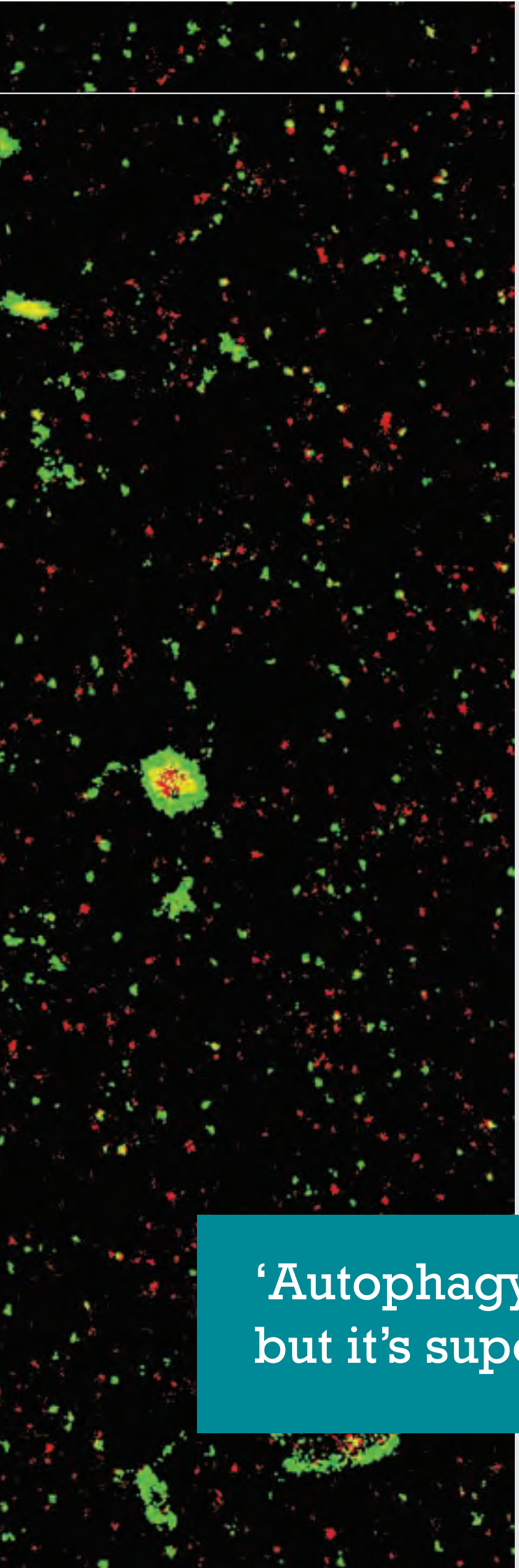
Despite its usually unsung role, the better we understand autophagy, the more we discover about its links with health and disease. As newborns, it is what tides us over the period immediately after birth when our cells are our only fuel. Boosting autophagy seems strongly linked with longevity. There is evidence that by cleaning our cells of potentially damaging material, autophagy could be involved in protecting against neurodegenerative diseases such as Alzheimer's and Parkinson's. We now know that cancer cells use autophagy to fuel their uncontrolled growth. And it is even linked to the health benefits of fasting, including the 5:2 diet.

First described in 1963, Ktistakis has found references to autophagy dating back to 1860. During the past 50 years we have learned that many steps, and more than 30 genes, are involved in autophagy, and that two protein complexes – mTOR and ULK – are pivotal to the process. We have also discovered that it can be turned on and off with remarkable rapidity, and that switching on autophagy by inactivating mTOR can increase lifespan in model organisms by an astonishing 30%.

Autophagy works by forming small membrane-bound sacs or autophagosomes to bag up material for clean up or for fuel. It's likely that these two types of autophagy – the selective clean-up variety and the general nutrient-generating type – share the same machinery but rely on different signals.

Understanding these early signals is a key focus for Ktistakis' group and other researchers at the Institute. "A lot of our work is trying to figure out the signal the cell uses to start the process. It happens very quickly – within 15 minutes of detecting a drop in nutrients – and must stop very fast when conditions improve, because you don't want to be digesting yourself for any longer than necessary," Ktistakis explains.

"We want to understand it at the molecular level – what happens when the signal arrives, how quickly mTOR is switched off and on, and what happens when ULK is activated and leads to formation of the autophagosome. I'm interested in this early part of the pathway – in identifying how dynamic it is and which are the important players controlling how it happens."



# ‘When autophagy is more active, it makes cells healthier’

But studying such a complex, finely-tuned and rapidly reactive system is a huge technical challenge – one recently solved by the expertise in biological chemistry and imaging technologies in the Institute’s core facilities and collaborating teams. With so many proteins involved, many of which are protein complexes rather than single proteins, developing ways to tag them with fluorescent markers in order to observe autophagy as it happens is a tall order.

And it’s not the only challenge. Studying events so early in the pathway, before the autophagosome is visible, means Ktistakis had to develop new ways of seeing. By teaming up with scientists at the Francis Crick Institute in London and the Zeiss Microscopy Labs in Munich, the Babraham group has successfully combined live imaging with other forms of microscopy. These new techniques reveal how the first autophagy structure forms and the protein and membrane associations that lead to it developing into a fully-fledged autophagosome.

According to Ktistakis: “We now know more about where the autophagosomes form, and how the autophagy machinery uses the cell’s membranes to generate these tiny sacs. We still don’t know how these regions are selected, but we are keen to find out, because it will give us the final level of understanding.”

Understanding is important, but it’s the impact this knowledge could have on our lives that matters. Once we understand the process fully, it could enable us to find ways of harnessing autophagy to tackle neurodegenerative diseases and cancers, helping us age more healthily.

“When autophagy is more active, it is likely to make cells healthier, so knowing more about the process increases our ability to find ways to manipulate or boost it for future therapeutic benefit. The idea ultimately is that if we understand autophagy enough we can change it in a way that benefits the cell and the organism,” Ktistakis concludes. “We think this is probably a very good idea.”

‘Autophagy is a quiet pathway, but it’s super important’

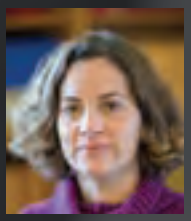
# 3

# 34-43

# Epigenetics

## Group leaders

Wolf
Reik



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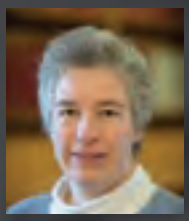

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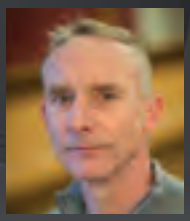

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# Epigenetic reprogramming in development and ageing

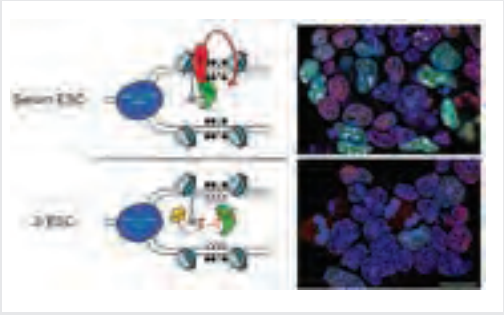
**Our lab is interested in epigenetic mechanisms in mammalian development and ageing. Epigenetic memory arises during development concomitantly with cell fate decisions, and is needed to confer a memory of cell state. This in part is brought about by epigenetic mechanisms such as DNA or chromatin modifications with an inherent memory. Our research interests are particularly on understanding how such epigenetic memory is erased in the germ identity.**

Consistency in cell identity is important. For example, liver cells when they divide need to produce more liver cells, otherwise cancer or other diseases may be the result. One of the epigenetic modifications we study is DNA methylation – a major biological DNA modification. Maintenance of this memory of cell identity involves a protein called UHRF1 which when DNA is replicated prior to cell division reads the methylation pattern of the genome and copies it over to the newly synthesised DNA. Hence epigenetic information present in the mother cell is retained in the daughters. This can help with maintaining appropriate gene expression patterns since promoter or enhancer methylation is associated with altered transcription of linked genes.

However, this epigenetic memory of somatic cells needs to be erased in primordial germ cells (PGCs, the precursors of egg and sperm) and in early embryos. This is so that 'pluripotent' cells in the embryo can develop into the many different cell types required in the new organism. This global erasure process is of great interest not only because it is probably important for pluripotent stem cells (including for induced pluripotent stem cells, iPSCs), but also because it removes epigenetic memories which would otherwise be passed down generations from grandparents to parents to children, which is called transgenerational epigenetic inheritance.

We discovered that the main mechanism of global epigenetic erasure is by downregulation of UHRF1 (1). As a result, DNA methylation is no longer copied from mother to daughter cells and this results in global loss of the epigenetic mark. Our research this year has also shown that the dynamics of specification and epigenetic reprogramming show species-specific differences, in particular markedly slower reprogramming kinetics in the human germline (2).

Following erasure of epigenetic information in early embryos, cells then begin to diversify in the run-up to gastrulation where the main cell lineages of the body emerge (mesoderm, endoderm, ectoderm). This diversification involves transcriptional programmes and presumably also epigenomes. The decisions to change cell fate are likely to be made by individual cells. Hence together with the Kelsey lab and collaborators at the EBI and the Sanger Institute we have invented methods by which both the methylome and transcriptome can be read from single cells (3). This has revealed epigenetic heterogeneity in the early embryo at an unprecedented scale. In ongoing work we aim to understand how this heterogeneity is created, and how it relates to transcriptional heterogeneity and cell fate decisions. Changes in epigenetic and transcriptional heterogeneity may also underlie the ageing process and this is something we are interested in investigating in future work.



*In most cells, DNA methylation is copied faithfully from mother to daughter cells. This process is mediated by a number of key enzymes, including DNMT1 (red) and UHRF1 (green). In cells undergoing global epigenetic erasure, such as mouse embryonic stem cells grown in serum (serum ESC) versus a 2i culture system (2i ESC), this process is impaired and as a result, DNA methylation is no longer copied from mother to daughter cells resulting in global loss of the epigenetic mark.*

## Publications

www.babraham.ac.uk/our-research/epigenetics/wolf-reik

1. von Meyenn, F., Iurlaro, M., Habibi, E. *et al*. 2016 Impairment of DNA methylation maintenance is the main cause of global demethylation in naive embryonic stem cells. *Mol Cell* 62(6): 848-61
2. von Meyenn, F. *et al*. 2016 Comparative principles of DNA methylation reprogramming during human and mouse in vitro primordial germ cell specification. *Dev Cell* 39(1):104-115
3. Angermueller, C., Clark, S.J., Lee, H.J., Macaulay, I.C. *et al*. 2016 Parallel single-cell sequencing links transcriptional and epigenetic heterogeneity. *Nat Methods* 13(3): 229-32

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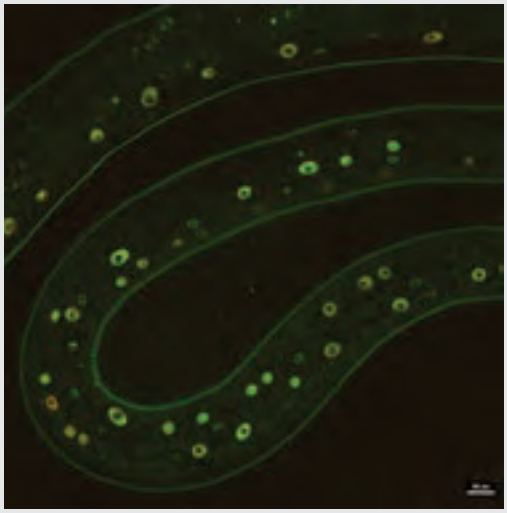
# When does ageing begin and why does it vary so much among individuals?

**Our aim is to understand why among different individuals – even among identical twins – there is so much variability in life expectancy. We also aim to identify the first events that trigger the ageing process, as we think that strategies that aim at promoting healthy ageing should reverse or delay these early molecular events. To gain molecular insights into these complex questions we use a simple model organism, the nematode *Caenorhabditis elegans*.**

We have previously shown that inter-individual variability in the expression of stress response genes in *C. elegans* has consequences for genetic and environmental phenotypic robustness as well as for lifespan. Our main interest is to uncover the causes that explain the extensive and unaccounted for variability in robustness and lifespan. The variability across individuals must include an important non-genetic component because the laboratory strains of this nematode are genetically homogeneous. We have found that intergenerational non-genetic inheritance is a key source of heterogeneity for many life-history traits and we are currently investigating how epigenetic mechanisms influence the probabilistic nature of lifespan.

We are also interested in developing tools to understand and exploit inter-individual variability in the expression of genes that influence robustness. We have found that stress inducible genes are highly variable across animals and we have taken advantage of this variability to infer the architecture of the stress-related gene networks during early ageing. In particular, this approach has revealed that there is a tight link between transcriptional and post-transcriptional mechanisms that control stress-responsive genes. The tight crosstalk between these two regulation steps ensures precision in gene expression. We are interested in understanding what triggers the loss of such robustness-ensuring mechanisms during early ageing.

Besides the network of genes that control cellular stress responses, most gene networks lose robustness during the ageing process. For example, in young animals, there is widespread redundancy in metabolic networks. However, we think that the loss of gene expression and reduction in metabolic fluxes during ageing may change network structure and render new sub-networks essential. We use systems biology tools based on flux balance analysis and network theory to unravel the first 'causal' events that start the ageing process. We are also interested in understanding how these first events influence long-term health via epigenetic memory. The loss of robustness may create novel age-related risk factors that should be targeted for successful interventions aiming at prolonging life.



*This strain of C. elegans worm carries a functional single-copy insertion of Heat Shock Factor 1 (HSF-1) fused to GFP. HSF-1 is a highly conserved transcription factor that coordinates stress-induced transcription of molecular chaperones. This C. elegans strain also carries the gene his-58 fused to a red fluorescent protein as a nuclear marker. In C. elegans, HSF-1 is ubiquitous and nuclear and forms transient intra-nuclear granules upon heat shock. This picture shows mostly epidermal nuclei (the black area in the centre is the nucleolus). These worms have been heat shocked and imaged one hour later, when HSF-1 granules have disappeared in the epidermal nuclei.*

## Publications

www.babraham.ac.uk/our-research/epigenetics/olivia-casanueva


1 Li, C. & Casanueva, O. (2016) Epigenetic inheritance of proteostasis and ageing. *Essays Biochem* 60(2): 191-202

2 Casanueva, M.O., Burga, A. & Lehner, B. (2012) Fitness trade-offs and environmentally induced mutation buffering in isogenic C. elegans. *Science* 335(6064): 82-5

3 Burga, A., Casanueva, M.O. & Lehner, B. (2011) Predicting mutation outcome from early stochastic variation in genetic interaction partners. *Nature* 480(7376): 250-3

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# Trophoblast stem cells and placentation

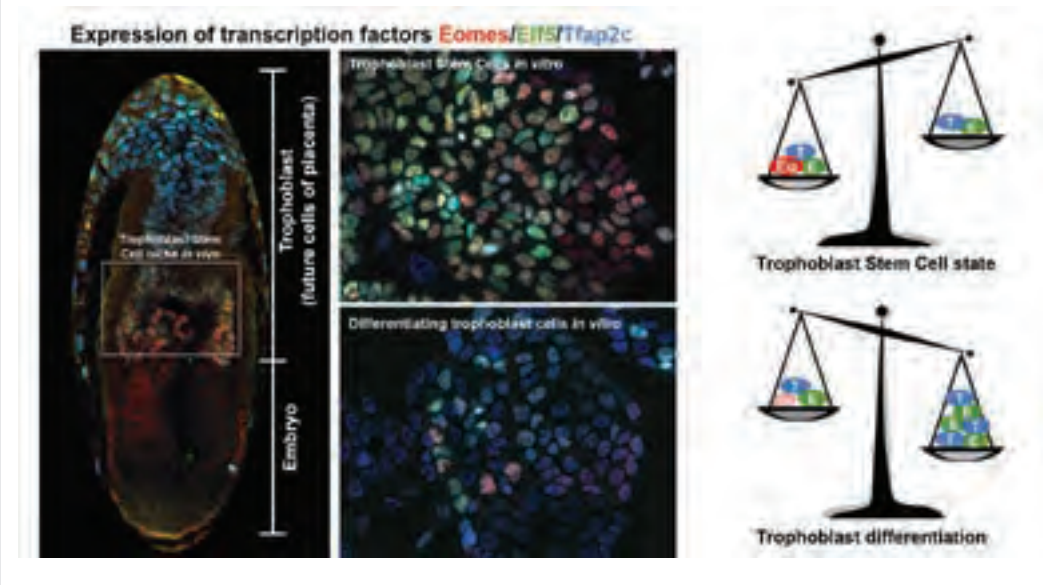
**Formation of a functional placenta is critical for normal embryonic development and lifelong health. Trophoblast stem cells represent the earliest building block of this organ as they recapitulate the potential to differentiate into all cell types of the mature placenta. Our work focuses on the transcriptional and epigenetic regulation of trophoblast stem cells during the process of placental development.**

Pregnancy complications are often rooted in the earliest steps of the placentation process when trophoblast cells need to expand and differentiate in a coordinated manner to ensure an adequate nutrient supply for the growing embryo throughout gestation.

In work aimed at understanding how the intricate balance between self-renewal and differentiation is controlled in trophoblast stem cells, we have identified a cell surface protein whose expression is sensitively regulated by the epigenetic repressive modification DNA methylation and that can serve to distinguish very early trophoblast sub-populations (1). In collaboration with the lab of Wolf Reik (page 36) we have also found that DNA methylation marks inherited from the oocyte are critical for trophoblast differentiation and embryonic survival. This demonstrates that the foundations for normal placentation are already laid down in the egg's epigenome long before fertilisation (2).

Moreover, focusing on the transcriptional regulation of trophoblast stem cell behaviour, we discovered an abundance-dependent role of the three interacting transcription factors Eomes, Elf5, and Tfap2c. All three need to be jointly present to make and maintain a trophoblast stem cell. However, if their relative levels shift towards higher amounts of Elf5 and Tfap2c and a proportional decrease of Eomes, this causes a change in their genomic binding profile. As a consequence, the gene network controlled by these transcription factors is rewired and triggers trophoblast stem cells to differentiate (3). This is the first example of a stoichiometry-sensitive network of interacting transcription factors that is operational in trophoblast stem cells where they act as a pivot between self-renewal and differentiation (see figure).

In ongoing work we are investigating how these sensitive epigenetic and transcriptional control mechanisms are affected by maternal age and by factors influencing uterine health.




*Expression of the three transcription factors Eomes, Elf5 and Tfap2c in the trophoblast stem cell compartment of an early mouse conceptus in vivo (left-hand panel) and in cultured trophoblast stem cells in vitro (middle panel). All three transcription factors are expressed in the trophoblast stem cell niche and are necessary to maintain the self-renewal potential of these placenta-specific stem cells. If the relative abundance changes through loss of Eomes or proportional increase of Elf5 and Tfap2c, trophoblast stem cells start to differentiate into more mature placental cell types. These stoichiometry-sensitive relationships between the three factors are depicted in the model (right-hand panel).*

## Publications

www.babraham.ac.uk/our-research/epigenetics/myriam-hemberger


1 Murray, A., Sienerth, A., & Hemberger, M. (2016). Plet1 is an epigenetically regulated cell surface protein that provides essential cues to direct trophoblast stem cell differentiation. *Sci Rep* 6: 25112

2 Branco, M.R. *et al*. (2016). Maternal DNA methylation regulates early trophoblast development. *Dev Cell* 36: 152-163

3 Latos P.A. *et al*. (2015). Elf5-centered transcription factor hub controls trophoblast stem cell self-renewal and differentiation through stoichiometry-sensitive shifts in target gene networks. *Genes Dev* 29: 2435-2448

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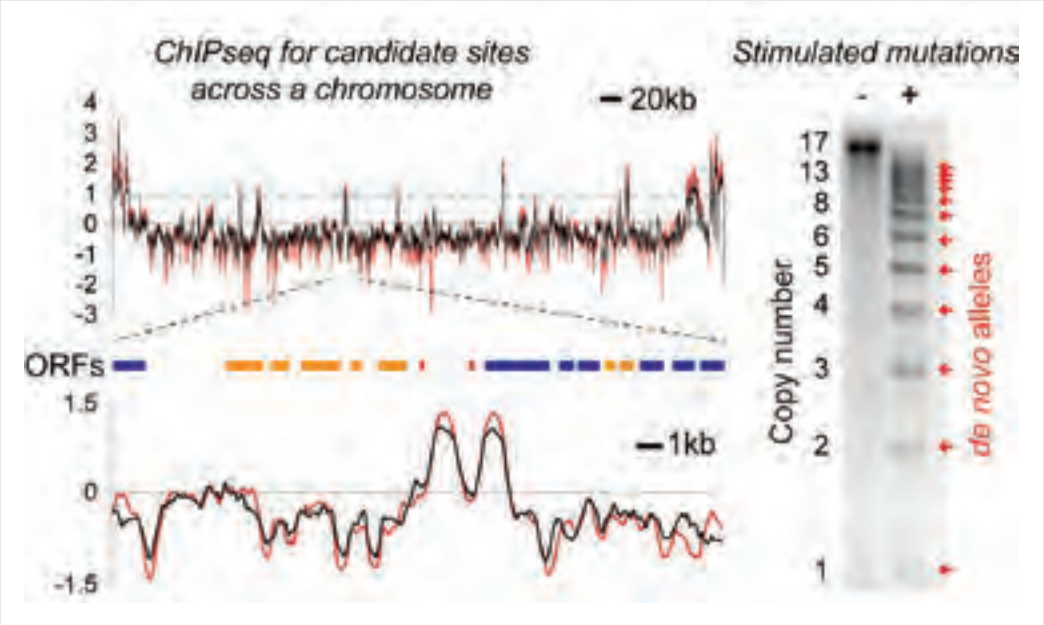
# How cells adapt to their environment




*Using high-throughput sequencing combined with classical DNA analysis techniques to reveal environmental stimulation of genetic change.*

**We study the ways in which cells adapt to their environment at the genetic and epigenetic level, particularly adaptation to challenging and toxic environments. Our research aims to discover and control mechanisms by which pathogenic organisms and cancer cells gain drug resistance, and to understand the genetic and epigenetic changes that occur in our cells throughout life.**

We have previously demonstrated that yeast cells can control the genetic makeup of their ribosomal DNA in response to the current environment, and shown that this process can be actively inhibited by drugs (1). This year we were awarded a major Wellcome Trust grant to ask whether similar mechanisms allow organisms to alter protein-coding genes in response to challenging environments. We have identified a yeast gene that shows this behaviour, and demonstrated that adaptation of yeast to environmental copper can occur through genetic changes stimulated by the cell in response to copper exposure. This is a dramatic departure from the normal assumption that genetic adaptation occurs through natural selection of random mutations (due for publication in 2017). We have used this model to identify and patent druggable pathways that block such stimulated genetic changes, and are extending this work to mammalian cells. We have also collaborated with other groups studying mechanisms that drive genetic change, particularly retrotransposition (3).

Our other major research focus is the effect of the environment on ageing cells. Remarkably, using a yeast model we have found that even very old cells showing many signs of physiological impairment can be highly competitive if put in the correct environment. This shows that ageing (at least in yeast) does not represent a simple irreversible decline in fitness, but a much more complex process that impacts fitness depending on the environment (2).

## Publications

www.babraham.ac.uk/our-research/epigenetics/jon-houseley


1 Jack, C.V. *et al*. (2015). Regulation of ribosomal DNA amplification by the TOR pathway. *PNAS* 112: 9674-9679

2 Frenk, S., Pizza, G., Walker, R.V. & Houseley, J. (2017). Ageing yeast gain a competitive advantage on non-optimal carbon sources. *Aging Cell*, doi:10.1111/acel.12582

3 de la Rica, L. *et al*. (2016). TET-dependent regulation of retrotransposable elements in mouse embryonic stem cells. *Genome Biol* 17: 234

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# Epigenetic marks from egg to embryo

**As well as DNA sequence, the egg and sperm contribute epigenetic information at the time of fertilisation. We explore how epigenetic marks are established during egg development and how they persist and guide gene activity in the embryo, as we seek to understand whether these marks are altered by maternal age, diet or assisted reproduction with long-term consequences on health.**

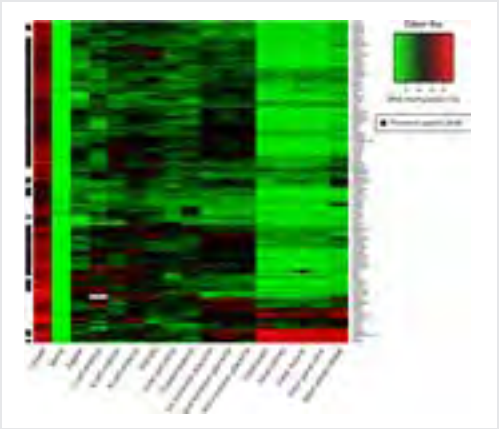
During gamete development, the egg and sperm acquire quite distinct epigenetic landscapes – referring to how the DNA sequence is modified by chemical tags such as methyl groups, and how the DNA molecule is organised into chromatin (1). Epigenetic differences between egg and sperm are the basis for genomic imprinting, a process that programmes some genes to be expressed only from the copy passed on from the mother or father. Around two hundred imprinted genes are known, but it is likely that these represent the tip of the iceberg and that epigenetic marks in the egg and sperm have more pervasive effects in offspring.

During work to examine the extent of imprinting in human placenta, we discovered a large number of imprinted genes that unusually showed variation in the extent of imprinting between individuals (2); imprinting is generally very robust.

To account for this unexpected finding, we assume that when the first lineages are determined in the embryo – before implantation and after loss of most gamete-derived DNA methylation – each cell has a distinct methylation level. Then, in the cells that give rise to the embryo proper, this heterogeneity is over-written when the genome reacquires DNA methylation around the time of implantation. But it seems that in the cells destined for extra-embryonic lineages – ultimately the placenta – the methylation variation is 'frozen' because it is not fully over-written. This highlights the placenta as an organ in which epigenetic information is most variable, and perhaps most vulnerable to factors such as maternal age, physiology or the procedures associated with assisted reproductive technologies.

We are now using single-cell profiling of DNA methylation and gene transcription to evaluate the degree of heterogeneity between cells in pre-implantation embryos and whether such differences influence whether cells adopt embryonic or extra-embryonic cell fates (3).

Current work is also improving methods to profile chromatin marks in very small numbers of cells, so that we can follow chromatin states during egg development and into the embryo to enable us to explore the full extent of transmission of epigenetic information and its consequences for the next generation.



*Methylation status throughout early human development of newly identified placenta-specific imprinted loci. The colour-coding indicates how loci fully methylated (red) in oocytes but unmethylated (green) in sperm retain intermediate, imprinted methylation in preimplantation embryos and placental tissues, but lose imprinted methylation in fetal tissues. Reproduced from ref. 2.*

## Publications

www.babraham.ac.uk/our-research/epigenetics/gavin-kelsey


1 Stewart, K.R., Veselovska, L. & Kelsey, G. (2016) Establishment and function of DNA methylation in the germline. *Epigenomics* 8:1399-1413

2 Hanna, C.W. *et al*. (2016) Pervasive polymorphic imprinted methylation in the human placenta. *Genome Res* 26: 756-767

3 Angermueller, C. *et al*. (2016) Parallel single-cell sequencing links transcriptional and epigenetic heterogeneity. *Nat Methods* 13: 229-232

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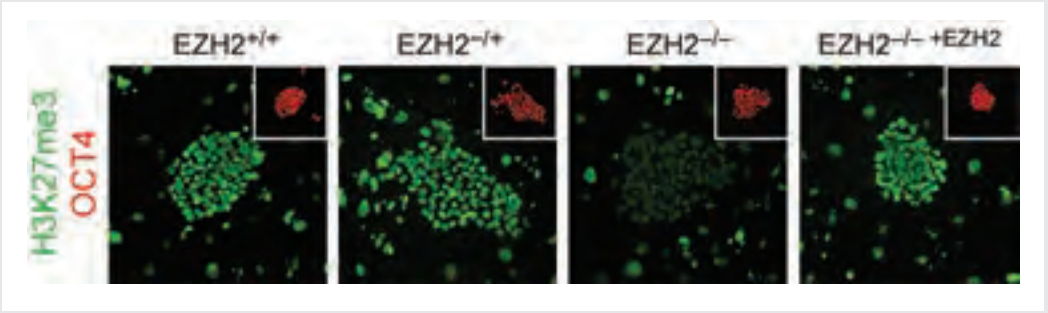
# Epigenome regulation of human development




*Immunofluorescent microscopy images of individual human embryonic stem cell colonies. Deletion of the Polycomb-group protein EZH2 in human embryonic stem cells leads to the loss of H3K27me3 signal (green). OCT4 expression in inset (red) indicates undifferentiated embryonic stem cells within the field of view. Image: Adam Collinson and Peter Rugg-Gunn.*

**How DNA is packaged in cells, and how the DNA is decorated with biochemical switches, is central to the epigenetic control of gene activity. Our main interests are in understanding how epigenetic processes are established during human development and stem cell differentiation. This is important for long-term health and for driving stem cells to become desired cell types in regenerative medicine.**

We have uncovered important new mechanisms that drive changes in the epigenome during the early stages of development and stem cell differentiation. For example, we found that pluripotency transcription factors provide a direct connection between cell state and chromatin organisation through modulation of heterochromatin regions in embryonic stem cells (1). We are now investigating how changes in heterochromatin organisation might affect centromere function and chromosome stability in pluripotent cells and during cell reprogramming.

We have also discovered a human-specific, X-chromosome pre-inactivation state, which is defined by the co-expression of two opposing long, non-coding RNAs, XIST and XACT, and this pattern is tightly linked to pluripotent state in human embryos and stem cell lines (2). As XACT exists in humans but not in mice, this works exemplifies that mechanisms of epigenetic regulation can vary substantially between species.

We have discovered new insights into the epigenetic changes and functions during the transition between naïve and primed human pluripotent states (Collier *et al*., *Cell Stem Cell*, in press; von Meyenn *et al*., *Developmental Cell* 2016). Focusing on the regulation of early lineage decisions, we have characterised the first EZH2-deficient human pluripotent stem cells and found there is a broad conservation of Polycomb-group protein function in controlling cell fate decisions and transcriptional programs during early human development (3; see image). We also uncovered unexpected human-specific differences that result in a more severe self-renewal and proliferation phenotype than that of Polycomb Repressive Complex 2-deficient mouse ESCs. Together, these studies provide new concepts and new technologies for understanding how epigenetic processes impact developmental and stem cell regulation, particularly in humans.

We have also begun to explore the policy implications of our work, particularly in relation to gene editing in human stem cells and embryos, and contributed to discussions of whether to extend the current 14-day rule for working with human embryos (e.g. to access tissues undergoing lineage-decisions during gastrulation). We have taken part in several round-table discussions, workshops and science festivals on these topics, and helped to draft various position statements with a view to informing policy decisions.

## Publications

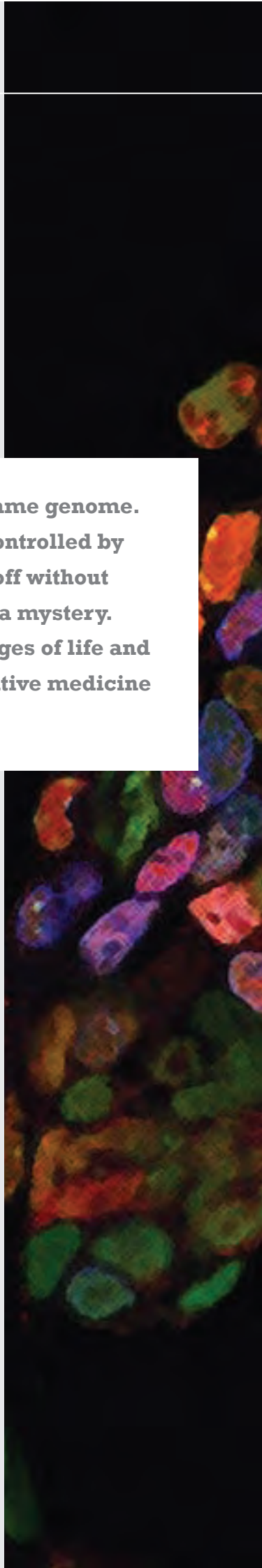
www.babraham.ac.uk/our-research/epigenetics/peter-rugg-gunn

1 Novo, C. *et al*. (2016) The pluripotency factor Nanog regulates pericentromeric heterochromatin organization in mouse embryonic stem cells. *Genes Dev* 30:1101-1115

2 Vallot, C. *et al*. (2017) XACT noncoding RNA competes with XIST in the control of X chromosome activity during human early development. *Cell Stem Cell* 20: 102-111

3 Collinson, A. *et al*. (2016) Deletion of the Polycomb: group protein EZH2 leads to compromised self-renewal and differentiation defects in human embryonic stem cells. *Cell Rep* 17: 2700-2714

# Unlocking the secrets of early development



**Every cell type in our body results from a different reading of the same genome. Over the past 30 years, scientists have learned that our genes are controlled by epigenetics – a combination of processes that switch genes on and off without altering the DNA sequence itself. But much of epigenetics remains a mystery. The Institute's Epigenetics programme is exploring the earliest stages of life and how understanding this could help reprogramme cells for regenerative medicine applications in the future.**

From neurones and hepatocytes to lymphocytes and erythrocytes, our bodies contain more than 200 different types of cell. Thanks to them, we can perform the myriad functions necessary to survive, thrive and reproduce. But how does this extraordinary diversity develop from one cell type – the zygote – when most of our cells contain the same genetic information?

It's a puzzle Professor Wolf Reik, head of the Epigenetics research programme, has been working on for the past 30 years. "Every cell in the body contains the same DNA, so there must be something else that interprets it in a different way," he explains. "This 'something else' is actually several things – transcription factors, proteins on the DNA that switch genes on and off – and it's also epigenetics."

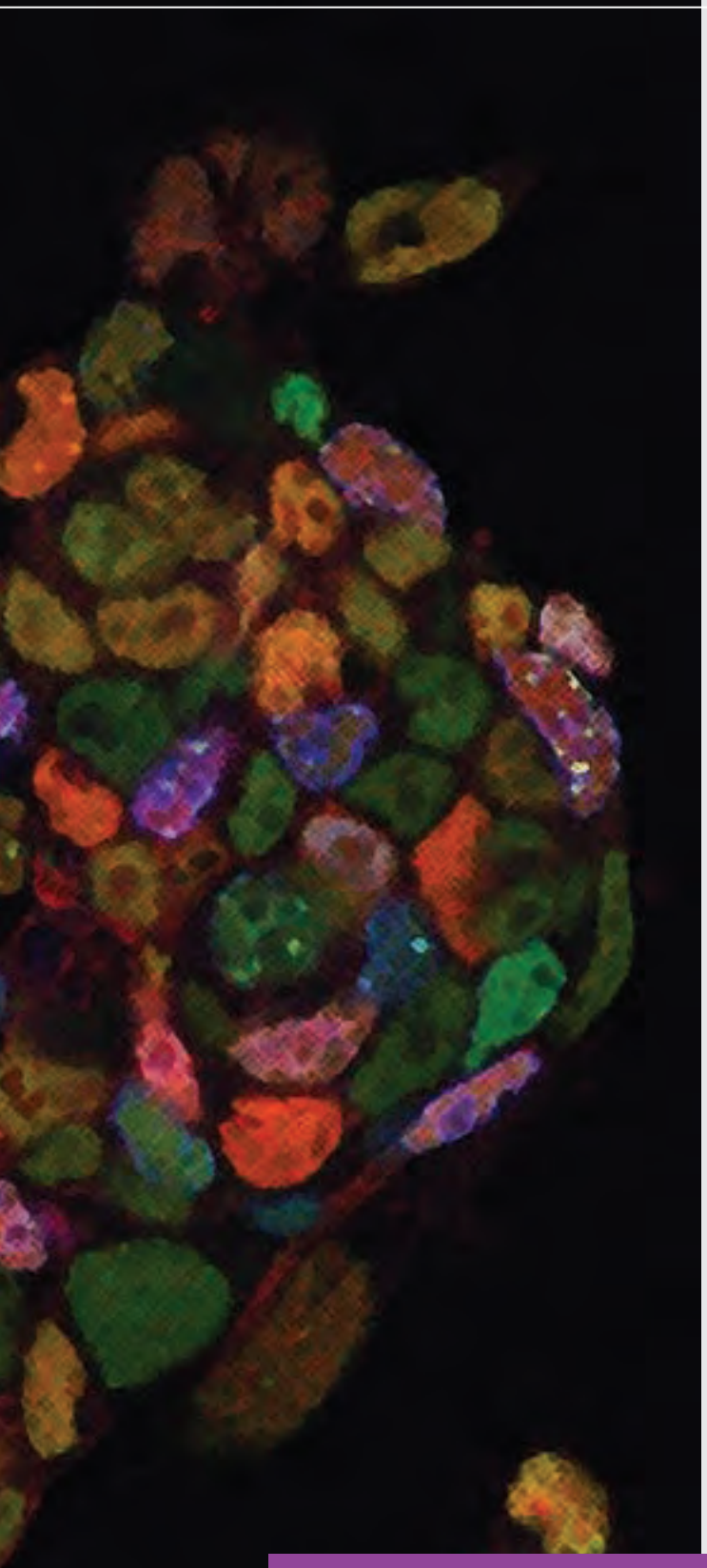
Literally meaning 'on top of our genes', epigenetics refers to an extra layer of control achieved by annotating our DNA with a series of tags, such as the simple methyl group of one carbon and three hydrogen atoms. It's thought that each cell type has a different set of tags – a different epigenome. Akin to highlighting recipes in a cookbook that together form a specific menu you want to create, each epigenome enables a specific cell type to develop by ensuring that only genes relevant to that cell type are switched on.

As well as enabling cell differentiation, the system must also be heritable, so that as we grow and repair ourselves, our cells and organs retain their individual identities. "There is a memory in the system, and that's important," says Reik. "As a liver cell divides, it needs to remember that it's a liver cell, and epigenetics helps with that."

Equally importantly, the system must be able to forget, so that when we produce eggs and sperm, the newly developing embryo can start the development process afresh. "This is vital because after fertilisation, new embryos must undergo this process of diversification again, building new cell types and new organs. Without erasing the cells' memory, the whole thing might become confused and lead to developmental problems and abnormalities," he says.

Reik's group is particularly interested in the epigenomes of stem cells, extraordinary cells with the capacity to develop into any type of cell in the body, and which are present in early embryos. By studying these cells, they are discovering how epigenetic information affects the way that important organs – such as the placenta, heart and brain – function throughout our lives. "It's a hugely exciting area for us now," he says. "In early development, once you get away from all the cells looking the same, you have to make different cells to produce a body with all its constituent parts. How this happens is a huge, unresolved question."

To answer that question, the researchers at the Institute have developed pioneering new single cell technologies able to read



# ‘It’s hugely exciting to see such progress in epigenetics’

the epigenome of an individual cell, without which it would be impossible to tease out how stem cells decide whether to become a blood cell or a brain cell, for example. They also want to answer two other key questions: how the global erasure process works, and how epigenetics affects ageing, both of which have major implications for maintaining health and managing disease.

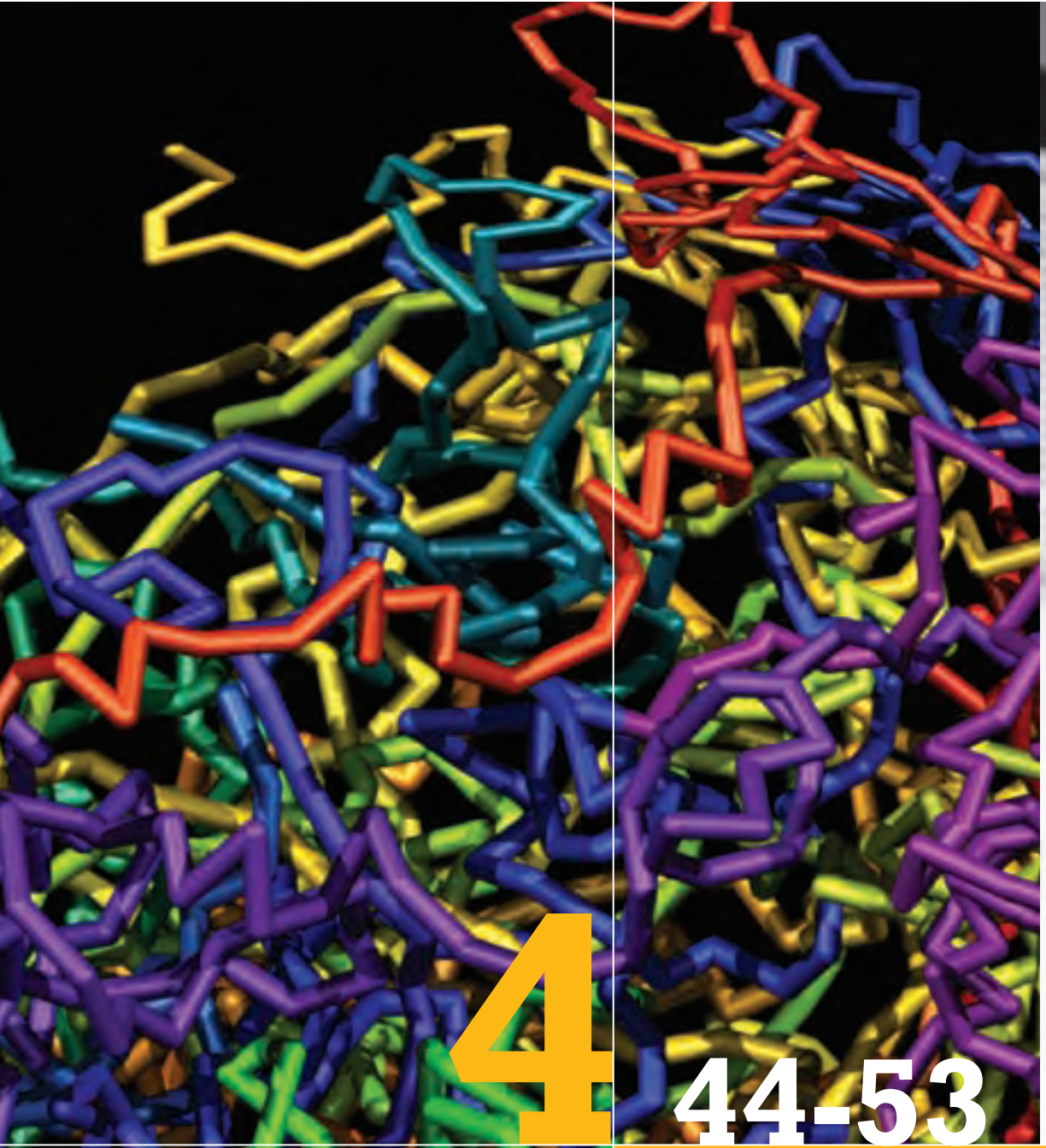
For many years, regenerative medicine – using our own cells to repair or replace damaged or diseased cells – has offered a promising way of treating devastating diseases such as Parkinson’s and multiple sclerosis. It’s becoming clearer that it is epigenetics that could be the key to unlocking the potential of regenerative medicine.

So-called embryonic stem cells and induced pluripotent stem cells are the holy grail for regenerative medicine, but many questions remain, including how to ensure that the memory of cells used in therapies have been wiped clean. “For us, epigenetic memory is a problem that lurks in these cells,” says Reik. “They are derived from skin and other cells but if they retain some epigenetic memory of where they come from, this could affect how well they perform in patients.”

As well as discovering how cells erase their epigenetic memories, the Institute’s researchers are also investigating links between ageing and epigenetics. It’s now thought that together with our chronological age, we have an epigenetic age influenced by our diet and environment. According to Reik: “We are interested in how the epigenetic clock ticks and how it affects the ageing process. We hope that by manipulating the ticking rate, we can have an effect on ageing.”

“As someone who has been in this field since before it was called epigenetics, it’s hugely exciting to see such progress. Instead of phenomena that couldn’t be explained, we now have molecules, mechanisms and the possibility of manipulating them in animal models and – potentially – in patients in the future.”

‘Epigenetics could be the key to unlocking the potential of regenerative medicine’



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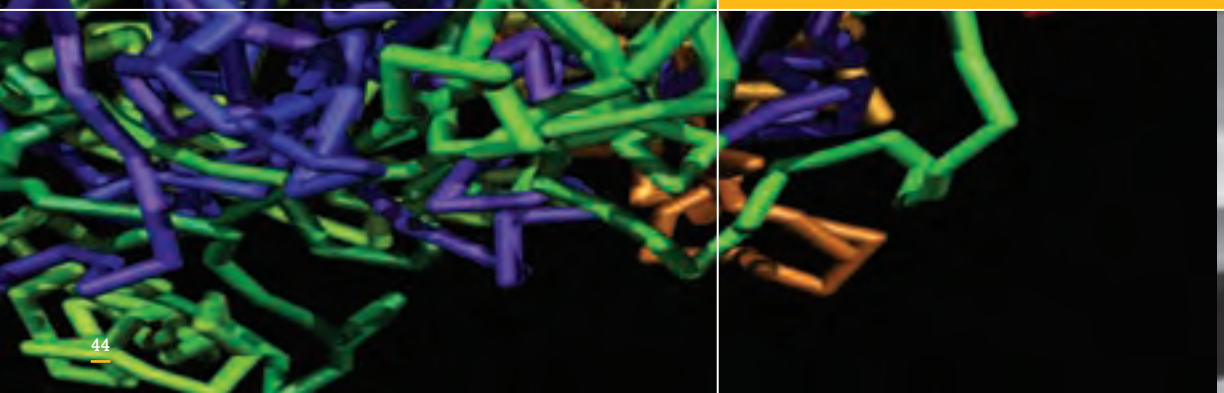
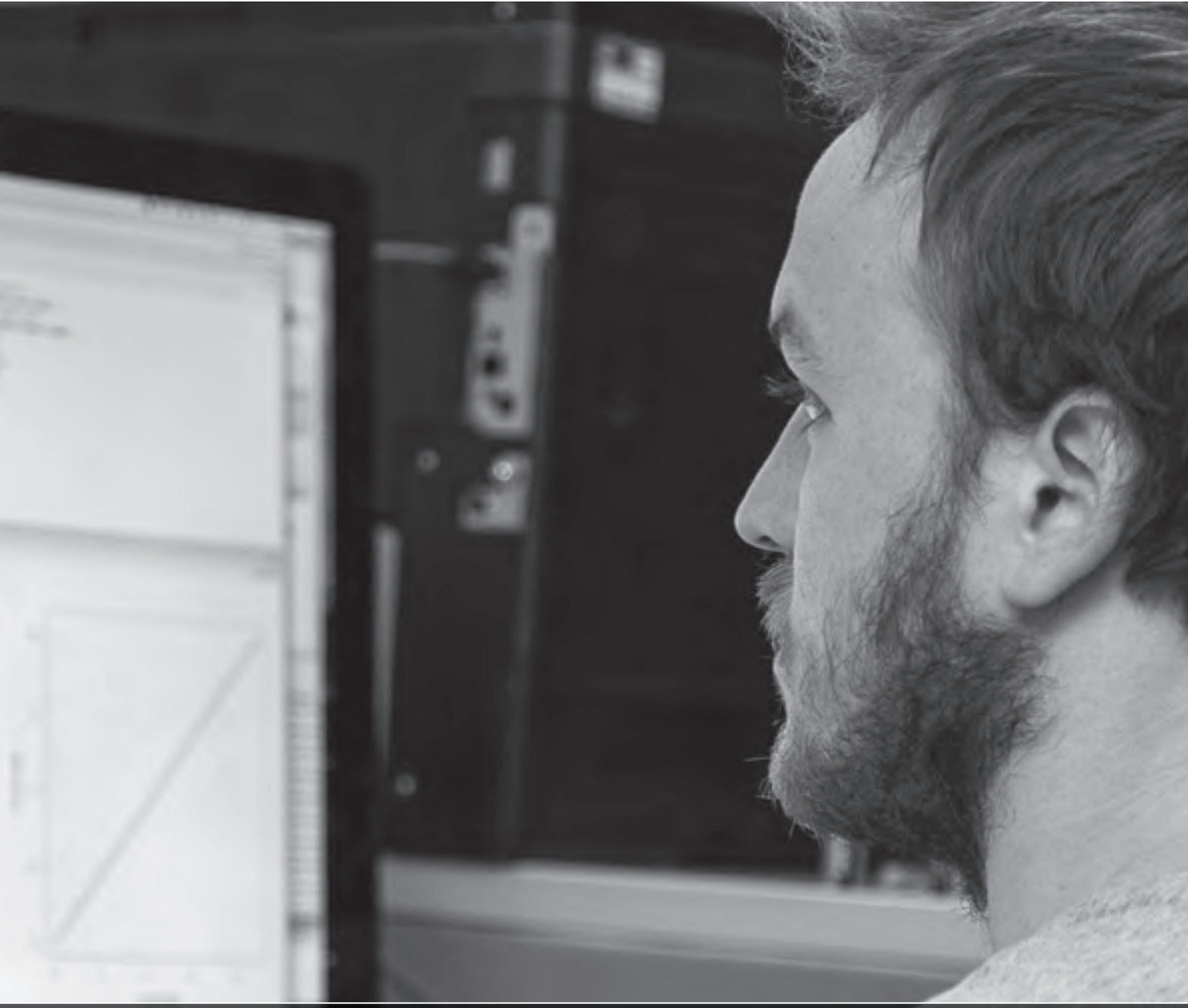
# Nuclear Dynamics





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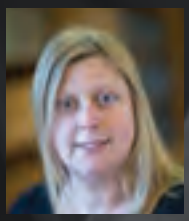
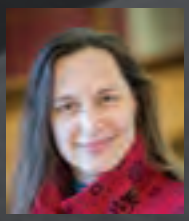
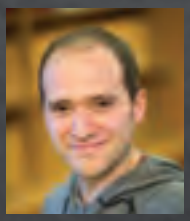


Sarah Elderkin



Karen Lipkow



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# How your genome works in health and disease

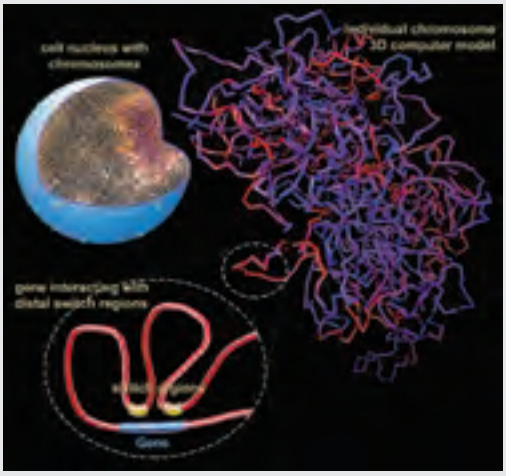
**Our two-meter long genome is packed into a nucleus 10 microns across. Amazing? Yes, but truly astonishing is that our genomes are exquisitely folded and organised in 3D space inside the nucleus. Changes in the 3D shape control gene expression, allowing cells to develop normally and respond to the environment, and when it goes wrong, impacting thousands of genetic diseases.**

The information encoded in our genes occupies less than 2% of our genome sequence. The rest, referred to as non-coding DNA, was originally thought to be useless and often described as 'junk DNA'. Our research shows that this junk DNA actually harbours critical regulatory switches that turn genes on and off. The way these switches achieve gene control is by folding of the DNA so that the distal switch regions directly contact the gene or genes they control. Our genomes contain over a million such hidden switch regions that fold the genome into highly complex 3D shapes that are specific to each particular cell type. This allows our relatively small number of genes to be expressed in thousands of different complex patterns that are needed for normal development, response to signals such as diet and the environment, and result in the different cell types and behaviours of a highly complex human organism.

We have developed powerful techniques that allow us to identify which switch regions contact each of the 22,000 genes in our genome. Knowing 'who contacts who' in these highly complex 3D networks is essential to understand how the genes in our genomes are controlled. This information is not only essential to understand normal genome control, but is also essential to understand what goes wrong in disease and to pinpoint key genes that are potentially causative.

It turns out that small changes in the DNA sequence of these switch regions is what makes us different from other mammals, including the great apes whose genomes are 98% identical to ours. These small changes to the DNA of the switch regions cause our genes to be expressed at different times or at different amounts ensuring that we develop, look and behave differently compared to our evolutionary cousins.

Individual humans also have natural variation in these switch regions, which is part of what makes each of us unique. However, these changes can also lead, in some cases, to susceptibility to genetic disease. We are mapping 3D genome structures in many cell types as well as using advanced techniques to create computer models of the entire genome in 3D to study and understand the dynamics of this incredible and complex machine.




*Top left: Cut-away nucleus showing highly folded DNA of chromosomes. Right: a 3D computer model of a single chromosome based on single-cell Hi-C experiments. A single chromosome can have thousands of genes and tens of thousands of gene-regulatory switch regions that are often located considerable distance away on the linear genome sequence. Bottom left: Folding of the chromosome, which allows the correct switch regions to interact with the genes they regulate is essential for normal gene expression. Small, naturally occurring changes to the DNA sequence in a switch region can lead to mis-regulation of the target gene and lead to disease susceptibility.*

## Publications

www.babraham.ac.uk/our-research/nuclear-dynamics/peter-fraser

1 Javierre, B.M. *et al*. (2016) Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. *Cell* 167:1369-1384
2 Stunnenberg, H.G. *et al*. (2016) The International Human Epigenome Consortium: A blueprint for scientific collaboration and discovery. *Cell* 167:1145-1149
3 Nagano, T. *et al*. (2016) Cell cycle dynamics of chromosomal organisation at single-cell resolution. *bioRxiv* doi: https://doi.org/10.1101/094466

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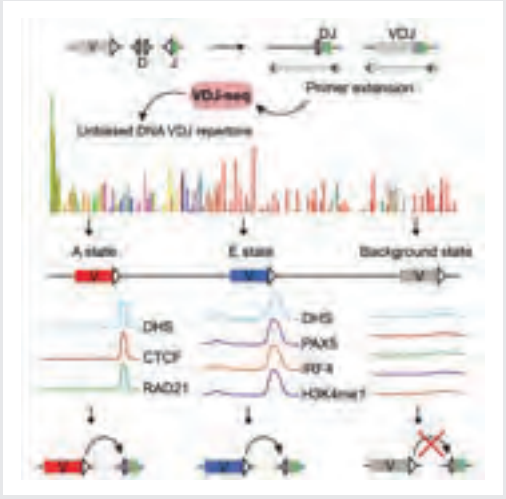
# What makes antibodies recombine?

**We are studying the genetic and epigenetic mechanisms that regulate development of B lymphocytes (white blood cells) in the bone marrow. We are particularly interested in how B cells make antibodies (immunoglobulins), the proteins used by the immune system to neutralise foreign antigens. We focus on mechanisms in the nucleus where immunoglobulin recombination occurs. This process is the cutting and pasting together of hundreds of antibody genes to generate the millions of different antibodies we need to fight infections. Defects in recombination contribute to immunodeficiency diseases and impaired immune response in the elderly.**

Our aim is to understand epigenetic mechanisms that underpin immunoglobulin recombination. These include activation of immunoglobulin genes by histone modifications, binding of transcription factors, non-coding RNA transcription and 3D organisation of the enormous immunoglobulin gene loci within the nucleus.

We are using a combination of mouse models, human samples, single cell visualisation and next generation sequencing approaches. We have developed a DNA-based non-biased high-resolution sequencing assay called VDJ-seq that can sequence hundreds of thousands of immunoglobulin gene segments. This has provided the first accurate picture of which immunoglobulin genes are used efficiently to generate the immunoglobulin repertoire and which are not.

We have integrated this data with analyses of histone modification and binding of key transcription factors and have used computational approaches to identify the most important drivers of efficient recombination (see image).




*The VDJ-seq assay enables us to accurately measure how frequently individual antibody gene segments are used. This has revealed two chromatin states, A and E, that define the factors associated with efficiently recombining genes.*

This research has identified two new evolutionarily conserved chromatin signatures that characterise the 3′ end of actively recombining immunoglobulin V genes, while inactive V genes are devoid of these marks (1). These findings reveal a new local layer of regulation at V genes, in addition to large-scale 3D looping mechanisms that bring immunoglobulin genes together for recombination over enormous distances.

We are now applying this knowledge to ageing B cells. Older mice and humans exhibit defective use of antibody genes, leading to a restricted antibody repertoire, and increased susceptibility to infection. We are currently quantifying this defect in mice and exploring the underlying mechanisms to determine which are defective, with the ultimate aim of restoring B cell function. We have also filed a patent on VDJ-seq and are adapting the assay for analysis of patient antibody repertoires to reveal signatures of immune function.

## Publications

www.babraham.ac.uk/our-research/nuclear-dynamics/anne-corcoran


1 Bolland, D.J. & Koohy, H. *et al*. (2016) Two mutually exclusive local chromatin states drive efficient V(D)J recombination. *Cell Reports* 15: 2475-87

2 Galloway, A. *et al*. (2016) RNA-binding proteins ZFP36L1 and ZFP36L2 promote cell quiescence. *Science* 352(6284): 453-459

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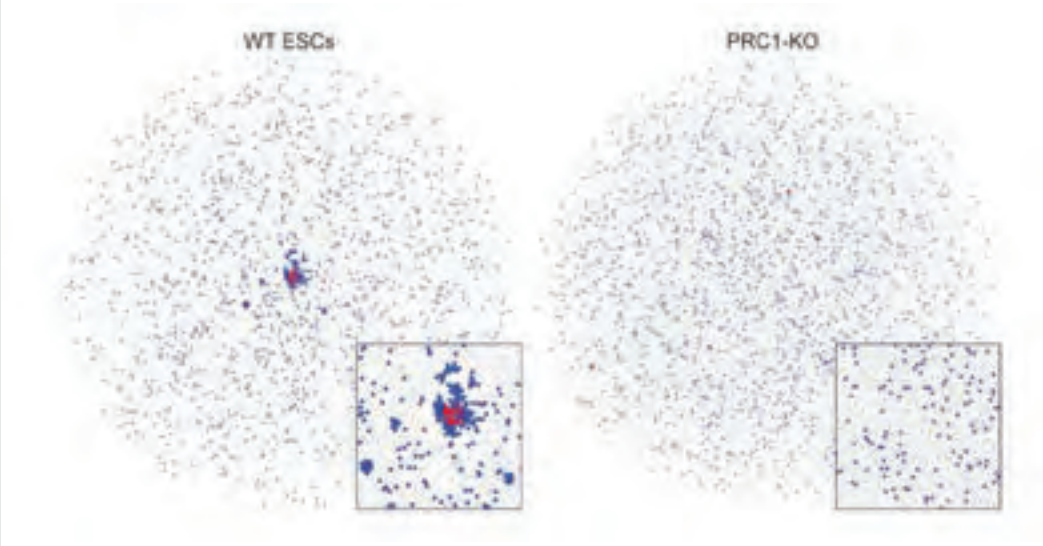
# Holding genes in a silencing grip




*Promoter to promoter contact network for all 22,425 captured promoters in wild-type mouse ESCs and PRC1 conditional knockout ESCs. PRC1 regulated genes are shown in blue, Hox clusters in red, all other promoters in grey. Inset; left: zoom in on the Polycomb-spatial network and five smaller networks. Right: networks completely disrupted in PRC1 knockout ESCs.*

**The precise regulation of gene expression is essential for maintaining cell identity throughout life. Stem cells have a particularly thorny problem when it comes to gene expression control. They must maintain a gene expression profile which keeps them in a primitive stem cell state, with the potential to rapidly activate key genes allowing them to differentiate into any cell type.**

Embryonic stem cells (ESCs) appear in the very early developing embryo, and eventually differentiate into all of the various cell lineages that are required for the adult organism. They have the potential to divide and produce two new stem cells, or to differentiate into any tissue type. They do this by maintaining a gene expression pattern that keeps them stem-cell-like, while at the same time maintaining the ability to rapidly activate key genes which control which tissue type they will differentiate into. These key genes are developmental transcription factors that control body plan specification, morphogenesis and organogenesis.

These genes are kept in a poised state by the Polycomb repressive complex. Polycomb complexes are known to bind to hundreds of genes in embryonic stem cells to keep them silent. It's also known that Polycomb complexes form foci in cell nuclei. Our work has shown that these foci contain clusters of Polycomb regulated genes and suggests that the spatial clustering of the genes in the nucleus is essential to keep them silent.

By analysing the spatial network of all genes in mouse embryonic stem cells we found that the strongest interactions are between a subset of approximately 120 genes that are controlled by the Polycomb repressive complex 1 (PRC1) (1). This network comprises promoters of PRC1-bound transcription factor genes that are essential in early development, including the four Hox gene clusters which regulate anterior-posterior axis patterning of bilaterian embryos, and other transcription factor genes required for cell fate specification. Mis-regulation of Polycomb function or disruption of the spatial network results in altered stem cell self-renewal, inappropriate activation of developmental pathways, altered cell proliferation and embryo death. Understanding how Polycomb-mediated genome organisation contributes to control of these key developmental genes is important in normal development, health and disease.

## Publications

www.babraham.ac.uk/our-research/nuclear-dynamics/sarah-elderkin

1 Schoenfelder, S. *et al*. (2015) Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. *Nat Genet* 47: 1179-1186

2 Tavares, L. *et al*. (2012) RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. *Cell* 148: 664-678

3 Novo, C. *et al*. (2016) The pluripotency factor Nanog regulates pericentromeric heterochromatin organization in mouse embryonic stem cells. *Genes & Dev* 30: 1101-1115

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# Mechanisms driving 3D genome architecture

**Chromosomes, which contain our genes, are usually not the familiar X-shape structure, but disentangled and highly mobile. Still, order exists, as active regions co-localise in 3D. Rapid progress has been made in detecting these structures, but the mechanisms responsible remain unclear. We study the physical and molecular mechanisms that drive organisation, by integrating computational modelling, bioinformatics, molecular biology and microscopy.**

The aims of my lab are to identify the biophysical and molecular mechanisms that underlie genome organisation. How the genome is arranged in detail in 3D is of great importance. The genome reshapes in many different situations: during cellular development, in response to external signals and according to genetic activity. It can be seen to be different in individual cell types and can be observed to be misfolded in a growing number of diseases, such as the rare premature ageing disease progeria, and in more common diseases, such as glioblastomas.

We use several model systems, such as the single-celled yeast *Saccharomyces cerevisiae*, to measure and simulate genome dynamics. The starting point of our analysis is the bioinformatic determination of chromatin states: groups of genes that are defined by their protein binding pattern. The analysis of yeast grown at two temperature conditions showed widespread and state-specific movements of proteins.

We have recently demonstrated by modelling how the unequal distribution of proteins on the DNA molecule can have a profound effect on genome organisation. Depending on their occupancy levels, segments of the DNA molecule show different dynamic behaviour and this enables known biological function, e.g. relocating active genes to the nuclear periphery. We have verified our simulation results using Hi-C and quantitative microscopy.

We will now apply our insights and similar methods to study how genome dynamics affects the DNA recombination and hence the antibody repertoire in the immune system.

*Computational model of the budding yeast nucleus with chromatin states (colours) assigned to gene segments. The chromosomes' centromeres are attached to the spindle pole body with microtubuli. Image credit: Zahra Fahmi.*

## Publications

www.babraham.ac.uk/our-research/nuclear-dynamics/karen-lipkow


1 Sewitz, S.A., Fahmi, Z. & Lipkow, K. (in press): Higher order assembly: Folding the chromosome. *Curr Op Struct Biol* 42. Folding and binding, Clarke, J. & Pappu, R.V. (Ed.)

2 Sewitz, S. & Lipkow K. (2016) Systems biology approaches for understanding genome architecture. in: *Methods Mol Biol* 1431:109-26, Chromosome Architecture: Methods and Protocols, Leake, M.C. (Ed.)

3 Schmidt, H.G. *et al*. (2014) An integrated model of transcription factor diffusion shows the importance of intersegmental transfer and quaternary protein structure for target site finding. *PLOS ONE* 9: e108575

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# Genome regulation in three dimensions

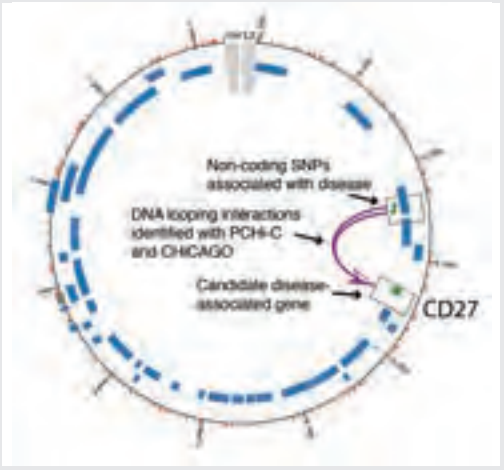
**Gene expression is tightly controlled by millions of non-coding DNA regulatory elements such as enhancers. Much of gene regulation takes place from a distance, mediated by looping contacts between DNA elements and their target genes. Charting these contacts and deciphering their logic is crucial for understanding gene control in development, ageing and disease.**

Our group studies the basic principles of gene regulation and their implications for ageing, development and disease. Our focus is on the computational analyses of high-throughput genomics data using advanced tools, some of which we develop ourselves.

Much of our work capitalised on the Promoter Capture Hi-C (PCHi-C) technique developed in the Fraser group (page 46). This method makes it possible to map DNA loops involving gene promoters globally and at high resolution. We devised CHiCAGO, a statistical pipeline for high-confidence signal detection in PCHi-C data (1), which has enabled robust PCHi-C analyses in multiple organisms and tissues. In a multi-centre study with the Fraser group and external partners in Cambridge and beyond, we used PCHi-C to link enhancers with their target genes in multiple human primary blood cell types (2). We mapped nearly 700,000 promoter-associated contacts, charting gene regulatory circuitries in these cells and characterising the principles of their organisation. Enhancers harbour genetic variants associated with disease. Therefore, detailed enhancer-promoter maps also allowed us to link many of these variants with their candidate target genes, predicting over 2,500 gene associations with more than 30 common diseases. This information will be instrumental for clinical scientists to advance the understanding of disease mechanisms and enable novel treatments.

DNA regulatory elements participate in processes beyond gene expression control, such as V(D)J recombination of immunoglobulins and T cell receptors. In collaboration with the Corcoran group (page 47), we identified the design principles of regulatory elements associated with DNA segments used in V(D)J recombination (3). Defects in these elements (either genetic or epigenetic) may exclude some segments from recombination, leading to reduced immune system potency.

The activity of regulatory elements is potentially altered in ageing through a combination of cell-intrinsic and extracellular factors. In an integrated project with all other groups within the Nuclear Dynamics programme, we are currently investigating age-related changes in the regulatory landscape of immune cells in ageing mice. These analyses will further our understanding of the molecular hallmarks of ageing and point to possible ways of maintaining immune system health throughout the lifespan.




*A circularised representation of a fragment of human chromosome 12, with arcs showing DNA looping interactions between the CD27 promoter and distant SNPs (green circles) associated with multiple sclerosis (MS). Genes are shown as blue rectangles, with CD27 highlighted in green. The image has been modified from a screenshot generated with CHiCP browser (www.chicp.org, Schofield et al., Bioinformatics 2016).*

## Publications

www.babraham.ac.uk/our-research/nuclear-dynamics/mikhail-spivakov

1 Cairns, J. *et al*. (2016). CHiCAGO: Robust detection of DNA looping interactions in Capture Hi-C data. *Genome Biol* 17:127

2 Javierre, B.M. *et al*. (2016). Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. *Cell* 147:1369–1384

3 Bolland, D.J. & Koohy, H. *et al*. (2016) Two mutually exclusive local chromatin states drive efficient V(D)J recombination. *Cell Reports* 15: 2475-87

Patrick
Varga-Weisz

**Group members**

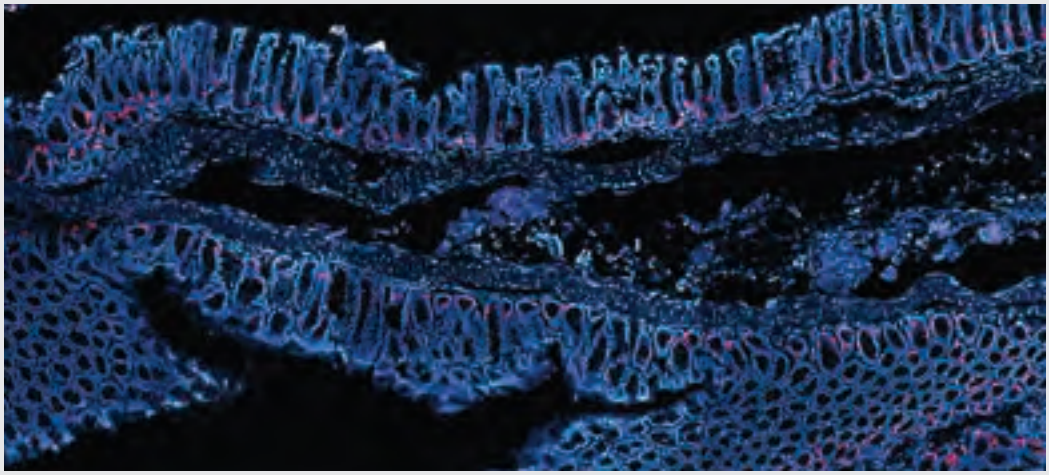
**PhD students:**
Rachel Fellows
Keith Porter (left in 2016)
Elena Stoyanova
(joined in 2016)

**Postdoctoral researchers:**
Dr Juri Kazakevych
(joined in 2016)
Dr Anke Liebert
Dr Claudia Stellato

**Fellow:**
Dr Payal Jain (left in 2016)

**Visiting researchers in 2016:**
Szabina Balázsi
Hector Blackburn
Marina Celestine
Renan Correa
Zoltán Hajnády
Hélène Perée
Calvin Rodrigues
Mariangela Spagnuolo
Marco Vinolo

# Chromatin regulation of the intestine



*The intestinal epithelium (shown here from the colon) is constantly being renewed and is therefore full of proliferating cells (red cells). This renewal is necessary for its barrier function but uncontrolled proliferation may lead to cancer.*

**The intestinal epithelium is essential for nutrient uptake, integrating commensal microbiota, while warding off pathogenic microbes. We study how changes in chromatin, such as histone modifications, are involved in the regulation of gene expression in this tissue by diet and the microbiota and how this relates to immunity. Furthermore, we explore how chromatin remodelling factors coordinate these processes.**

We combine mouse genetics, gut organoid culture and cell culture, genome-wide next generation-based mapping (chromatin immunoprecipitation sequencing, ChIPseq), transcriptomics, proteomics and *in vitro* biochemistry to obtain mechanistic and biologically relevant information. Our research currently addresses three main foci:

**Histone modifications, link to microbiota and intestinal homeostasis**
Histone lysine acylations, such as histone crotonylation, have recently emerged as important components of genome regulation that link to metabolism. We have identified histone crotonylation in the intestinal epithelium and its links to gene regulation. Importantly, its levels are affected by the presence of the microbiota and diet and we are exploring how this modification is affected by gut content and how it mechanistically impacts gene regulation. We identified novel regulators of this modification, which potentially link this modification to cancer (Denizot *et al*., submitted). This work will contribute to our understanding of how the microbiota control gene expression through histone modifications.

**Chromatin remodelling in intestinal homeostasis and innate immunity**
Chromatin remodelling factors are key regulators of gene expression. To what extent such factors can coordinate gene expression of specific biological processes and by which mechanism are important questions. We found that deletion of a chromatin remodelling factor, Smarcad1, in the intestinal epithelium renders mice resistant in an experimental microbiota-driven colitis. Deletion of this factor is linked to changes in gene expression, especially up-regulation of genes linked to innate immunity, even in the absence of the colitis challenge (e.g. in organoid culture). Our working hypothesis is that this factor controls genes linked to the innate immunity response and their up-regulation primes mice to the colitis challenge. We are exploring how this factor affects chromatin structure in the intestinal epithelium and how this is linked to immunity.

**Ageing and chromatin changes in pro-B cells**
Weaken immunity in the elderly is partly because the aged B cells cannot generate high quality antibodies compared to the young. Thus, the success of vaccination is compromised in the elderly. Within the Nuclear Dynamics programme we are involved in exploring how ageing affects chromatin structure and, thereby, gene expression in mouse pro-B cells where the first steps of antibody generation occurs. We found that changes in gene expression in aged pro-B cells is mirrored by changes in histone modification H3K4me3.

## Publications

www.babraham.ac.uk/our-research/nuclear-dynamics/patrick-varga-weisz

1 Sun, H. *et al*. (2015) ACF chromatin remodeling complex mediates stress-induced depressive-like behavior through nucleosome repositioning and transcriptional regulation. *Nat Medicine* 21 (10): 1146-1153

2 Petrini, E. *et al*. (2015) A novel phosphate-starvation response in fission yeast requires the endocytic function of Myosin I. *J Cell Sci* 128 (20): 3707-3713

3 Varga-Weisz, P. (2014) Chromatin remodeling: a collaborative effort. *Nat Struct Mol Biol* 21(1):14-6

# Welcome to the 3D genome

**When the first draft of the human genome was published in 2001, it was described as a treasure trove of information. But using that information to understand disease demands going far beyond the DNA code. Now, researchers at the Institute are pioneering a new method of mapping our genome's complex regulatory interactions that could open up new ways to treat genetic diseases and understand ageing.**

If we think about our genome at all, it's probably in a linear, two dimensional way: as a string of bead-like genes threaded along a necklace of DNA, a code to be cracked or a blueprint to be read. It turns out, however, that understanding what our genes do – and how tiny changes in our genome can cause genetic diseases – demands different ways of thinking.

The story begins, says Dr Peter Fraser, head of the Nuclear Dynamics research programme, by focusing less on genes – which occupy only 2% of our genome – and putting more thought into the remaining 98%. "This non-coding space between the genes, sequences of DNA that don't encode for particular proteins, was once viewed as an evolutionary wasteland of junk DNA," he says.

In fact, these regions are crucial because they regulate our genes by switching them on and off. "There are at least 1 million regulatory elements in the genome. Each of our 22,000 genes uses an average of five of them. But the problem has been understanding how a regulatory element so far away from the gene it controls actually exerts its influence," Fraser explains.

To understand this, we must add new dimensions to our thinking. "We need to think in 3D because that's how it is inside the cell," he says. "DNA is wrapped around proteins and how that bundle is folded in the nucleus is really important."

Time matters too, because the folding is dynamic, not fixed, and as the folding patterns change, different genes make contact with regulatory elements in distant regions of DNA. But while this new way of thinking offers a better way of explaining how genes are regulated, how can we unravel such a complex web of changing connections?

For the past 15 years, Fraser and his team have been studying how DNA folds to bring genes and their regulatory elements together. Now, they have developed a pioneering method of mapping these myriad connections, and produced the first 'pages' of an atlas they hope will eventually cover every cell type in the human body.

But why is this atlas so important in the quest to understand common genetic diseases – and find new ways to treat them? The answer, he says, is because the atlas allows us to make sense of a vast database of hundreds of thousands of so-called SNPs (pronounced 'snips') – single nucleotide polymorphisms – the tiny errors in DNA that are related to genetic diseases such as Crohn's disease and rheumatoid arthritis.

This database of SNPs has been built up over decades of research using genome-wide association studies – trials comparing the genomes of healthy volunteers with those suffering from a genetic disease.

Sometimes these variations occur in genes, making them malfunction and resulting in genetic diseases. More commonly, however, the error lies in non-coding DNA. Hundreds of thousands of these mistakes have been discovered, but until the atlas, it's been impossible to explain how such a tiny change, so far from any known relevant gene, could affect a disease.

# ‘Our 3D maps can identify new potential disease-causing genes’

According to Fraser: “What we’ve found is that these SNPs are in regulatory elements, so by being able to map these onto specific genes, we can identify new potential disease-causing genes.”

Precisely how powerful this mapping could be in combating genetic diseases can be seen from results that Fraser’s team published last year. By mapping the 750,000 connections between regulatory elements and the genes they control in 17 types of white blood cell they were able to identify 2,600 potential disease genes. Only 25% of these disease-associated genes had previously been identified – 75% were new, including genes involved in a range of autoimmune diseases from type 1 diabetes to celiac disease.

As well as helping us understand the genetic basis of disease, the discovery of these potential disease genes also provides the pharmaceutical industry with new targets for novel drugs – or new ways to use (or repurpose) existing drugs.

“The 25% of disease genes we already know about have been very hard fought – one at a time over decades of research. So what we’ve done has really burst the dam,” he says.

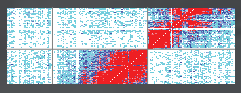
As well as white blood cells, the team has mapped the genetic connections in red blood cells and is moving on to map muscle and pancreatic cells. Fraser hopes that these findings will deliver candidate genes relevant to diseases such as certain types of anaemia, type 2 diabetes and muscular dystrophy.

With more than 200 different cell types in the human body, the atlas will be huge. And its impact on our understanding of disease and healthy ageing could be even more significant.

“It’s a big leap,” Fraser says. “There are SNPs in these databases associated with healthier ageing. By mapping those, we may be able to identify more genes involved in ageing. There’s lots of work to be done and lots of genetic diseases we know nothing about. This could potentially crack quite a few.”

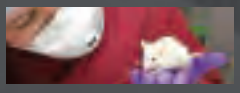
‘There are lots of genetic diseases we know nothing about; this could crack quite a few.’

# 5 54-65 Facilities

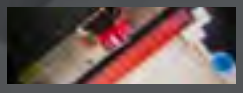


Bioinformatics

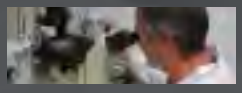
Biological Chemistry



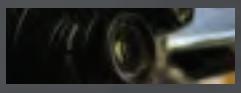
Biological Support Unit



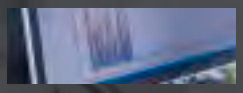
Flow Cytometry



Gene Targeting



Imaging



Lipidomics

Mass Spectrometry

Sequencing

Simon Andrews
Facility head

**Facility members**

**Bioinformaticians:**
Felix Krueger
Laura Biggins
Christel Krueger (maternity cover)
Stephen Wingett

**Training developer:**
Bhupinder Virk

**Biological statistician:**
Anne Segonds-Pichon

# Bioinformatics

**The bioinformatics facility provides the infrastructure needed for the analysis of biological data. Its friendly and approachable staff provide support and training in data analysis, statistics and data management to both internal and external groups. The group also develops novel tools where required, and administers the Institute's compute cluster.**

In 2016 the group greatly expanded the range and availability of its bioinformatics training courses. We ran over 40 courses during the year, training over 400 individuals. The group's suite of training courses was expanded with new courses in epigenetics, statistics, programming and figure design and this will be added to further in coming years. The group also expanded their set of online video tutorials, adding new videos for their HiCUP, Fastq Screen and SeqMonk software packages.

The group invested significant time into new areas of science. They developed new software to work efficiently with allele-specific sequencing data (1) and adapted existing software to provide workflows for the analysis of single-cell data (2). Meeting these requirements will serve the analytics demands created by the Institute's research as it grows in each of these areas. The year also saw the completion of a long-term project looking at immunological recombination with Anne Corcoran's group (page 47) which led to the development of the Link-On software package (3).

The computational requirements of the Institute's science continue to increase and 2016 saw the compute cluster expand from 512 cores to 768 cores to accommodate the higher demands of the Institute's science. We also now officially offer access to the compute cluster as a service to companies on the Babraham Research Campus and two companies have already signed up to have their staff access the cluster.

## Publications

www.bioinformatics.babraham.ac.uk


1 Krueger, F. & Andrews, S.R. (2016) SNPsplit: Allele-specific splitting of alignments between genomes with known SNP genotypes. *F1000Res* 5:1479. doi: 10.12688/f1000research.9037.1

2. von Meyenn, F. *et al*. (2016) Comparative principles of DNA methylation reprogramming during human and mouse in vitro primordial germ cell specification. *Dev Cell* 39(1):104-115

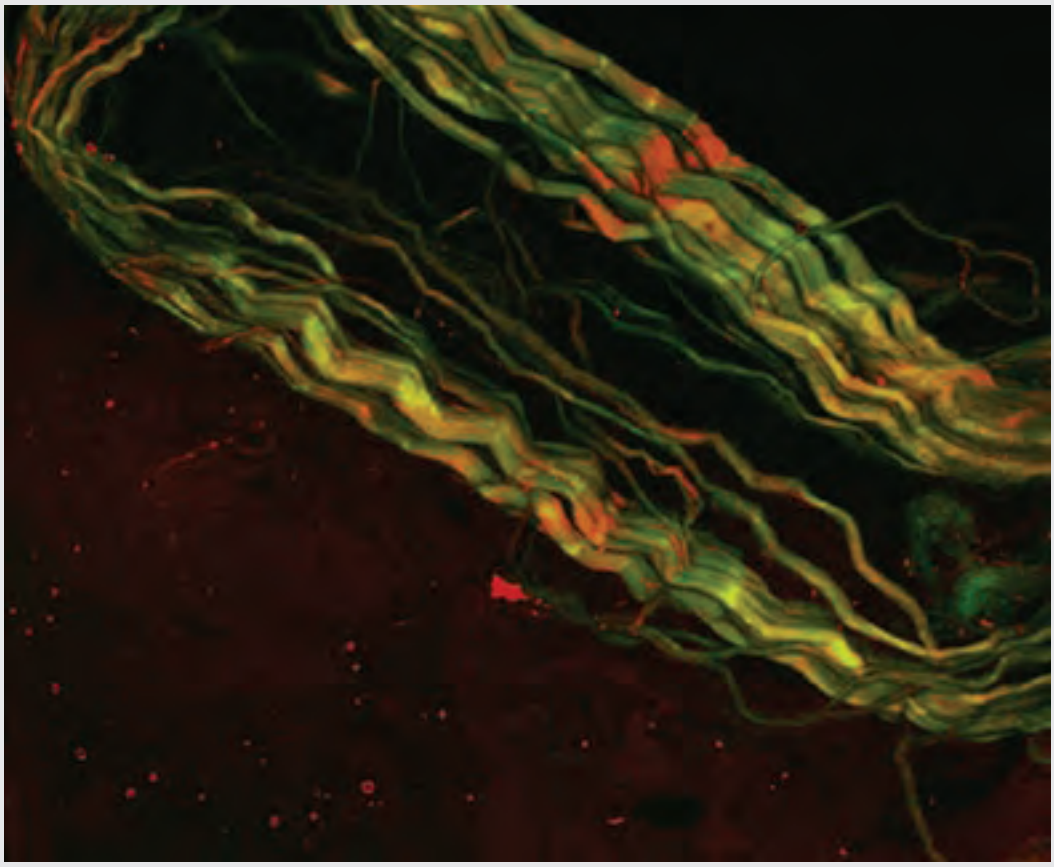
3. Bolland, D.J. & Koohy, H. *et al*. (2016) Two mutually exclusive local chromatin states drive efficient V(D)J recombination. *Cell Reports* 15: 2475-87. doi: 10.1016/j.celrep.2016.05.020

Jonathan Clark
Facility head

**Facility members**

**Postdoctoral researcher:**
Dr Izabella Niewczas

# Biological Chemistry



*Confocal image of labelled mouse tail tendon x10. The green channel is from a fluorescent probe that we designed to specifically label reactive groups that are likely to be involved in the formation of collagen crosslinks. The red channel is from a fluorescently labelled lectin that was incubated with the collagen which specifically binds to galactose bound through hydroxlysines in the collagen structure.*

**The Biological Chemistry group provides support for scientists working at the interface between chemistry and biology. We bring an understanding of chemistry and its application to solve biological problems using chemical and analytical tools. In addition to our collaborative work with researchers we are also investigating the chemical changes which occur in connective tissue with age.**

Core to the functioning of the Biological Chemistry group is our detailed knowledge of chemistry, both synthetic and analytical. Multiple projects are carried out in any year making small molecules for use as standards in analytical methods, or as probes with biological or reporter functions. An example of a more challenging project is the development of a new synthetic route for the stereospecific isotopic labelling of inositol for work studying inositol signalling pathways.

In addition to the synthetic work occurring in the facility, we provide support in the development of new methods for the analysis of lipids by mass spectroscopy for groups in the Institute's Signalling programme. An example of a current project is the development of mass spectroscopy methods to study the incorporation of labelled inositol into cell lipids.

Within the group, in addition to the support research described above, we are looking at the changes that occur in the chemistry of connective tissues as we age, primarily the changes which occur in collagen and elastin. The clearest changes are found in the degree and type of crosslinking which has a direct effect on the physical properties of tissues. Understanding how and why these changes occur may ultimately enable strategies to be developed to improve resilience, elasticity and healing of tissues as we age. This project is still in its early stages.

## Publications

www.babraham.ac.uk/science-services/biological-chemistry

1. Manifava, M. *et al*. (2016). Dynamics of mTORC1 activation in response to amino acids. *eLife* 5: e19960

Tim Pearce
Facility head

**Facility staffing:**

2 Deputy facility heads
5 Unit managers
10 Supervisors
29 Animal technicians
5 Service technicians
3 Apprentices
2 Veterinarians

# Biological Support Unit

**The Biological Support Unit provides state-of-the-art housing and care for pathogen-free rodents used in both academic and private company research programmes. The facility's animal technicians provide expert technical support to researchers by undertaking regulated procedures, maintaining the animal health barrier and husbandry.**

**Progress and key highlights:**

- Since the BSU opened in 2009 staffing has grown from 50 members of staff to 55.
- When the facility opened, 85% of the staff held a Home Office licence; this is now at 98%.
- All technicians are trained to Level 2 of the Institute of Animal Technology (IAT) qualification which is the national standard. In 2009 22% held the IAT Level 3 or the higher National Diploma. Today, 40% are trained to this level.
- In early 2015 we moved the whole facility to a newly imported database (MCMS from the Sanger Institute) within a period of three months. This meant that 36,000 individual mouse records were moved without disrupting ongoing research or the facility.
- Due to the shortage of animal technicians nationally and the expensive and competitive area the facility is sited in, we have proactively improved recruitment with various initiatives. These include accommodation provision – we provide two hostel houses able to accommodate up to seven members of staff, subsidised travel for weekend work, and fast-track promotion. We are currently hosting three apprenticeship positions and all staff benefit from training programmes with clear career pathways, regular CPD opportunities and staff seminars.
- The use of cameras to provide virtual tours of the facility continues to support our commitment to openness around the use of animals in research. In 2016, 173 people received virtual tours.

**Aims:**

- All mouse biopsies to be taken at pre-weaning utilising a Centralised Genotyping Service giving greater efficiency of colony management.
- A new committee has been formed to push forward and further refine the requirement to consider and apply the 3Rs principles of Replacement, Reduction and Refinement.
- Through efficiencies and LEAN management complemented by a greater overall staff skill set, we aim to maximise scientific use per cage.
- We will continue to present information about the facility and careers in animal technology at conferences, schools and outreach events and careers fairs.
- We will continue to evolve our recruitment initiatives to attract highly skilled technicians to fit the ever-changing technical role. We are intending to produce a 'day in the life of an animal technician' video to further promote the industry and career prospects.

www.babraham.ac.uk/science-services/biological-support-unit

# Flow Cytometry

Rachael Walker
Facility head

**Facility members:**

Arthur Davis
Dr Rebecca Roberts
Lynzi Waugh

**The Flow Cytometry facility provides a world-class core facility to meet the scientific research goals of the Institute. It houses five state-of-the-art analysers, an image cytometer and three cell sorters. The facility uses its expertise to help scientists from both the Institute and campus companies with experimental design, training, troubleshooting experiments and data analysis.**

The facility is run by four experienced staff members totalling over 30 years of flow cytometry experience between them. It is led by Dr Rachael Walker who is an International Society for Advancement of Cytometry Shared Resource Laboratory Emerging Leader.

In 2016, the facility saw the addition of its third Becton Dickinson LSRFortessa instrument to meet growing demand for flow cytometry use and an increase in complexity of the experiments being run. This five laser, 20 parameter instrument has helped to strengthen the multiparameter instruments already operated in the facility. The three Fortessa analysers, as well as the BD LSRII, are regularly used by members of the Institute with unallocated capacity being used by companies co-located with the Institute on the Babraham Research Campus.

In February 2016, Propel Labs placed a YETI five laser, 30 parameter high-throughput analyser in the facility which allowed the Institute to become an early adopter of this system. The facility is working in collaboration with Propel to test the instrument for use in a core facility and the availability of this instrument has allowed Institute researchers to increase the sophistication of their flow cytometry experiments and utilise a wider range of the available fluorochromes in their experiments.

In 2016, the cell sorting service provided by the facility carried out nearly 2,000 hours of sorting for both the Institute, campus companies and external companies. This sorting was carried out on the three high-end cell sorters housed in the facility.

The Institute's highly-rated modular flow cytometry training courses entered their second year in 2016, with all nine courses being fully subscribed and attended by over 125 delegates.

## Publications

www.babraham.ac.uk/science-services/flow-cytometry

1. Barsky, L.W. *et al*. (2016) International Society for Advancement of Cytometry (ISAC) flow cytometry shared resource laboratory (SRL) best practices. *Cytometry* A 89(11): 1017-1030

Dominik Spensberger
Facility head

# Gene targeting and genome editing

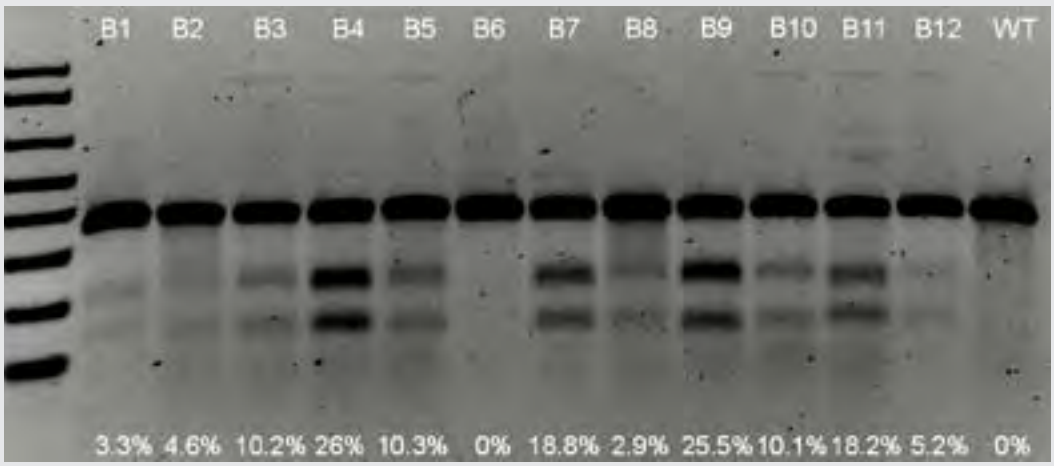
**The Gene Targeting facility undertakes the creation of designed genomic modification by manipulation of any locus in the genome using homologous recombination gene targeting and CRISPR/Cas9 genome technology to generate genetically altered mouse models, embryonic stem (ES) cells and somatic cell lines. The facility provides assistance with design concept, the generation of targeting tools, locus targeting, screening and evaluating correctly targeted clones, ES cell injection to generate chimeras and CRISPR/Cas9 injection to generate desired genome modification *in vivo*.**

The facility's aims are to improve the reliability of the technology and to reduce the animal cost of research involving genetically modified animal models. The research activity of the facility is focused on the development or improvement of current and novel methodologies for the generation of genetically modified models.

The facility has successfully generated several new genetically altered animal models for internal and external research groups using ES cell gene targeting in C57BL/6 and 129 mice strains. We have also successfully generated new lines of genetically altered animal models using EUCOMM ES cell targeted clones.

Due to recent new developments in the field of genome manipulation we have adopted and implemented Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas) technology for several new projects. The technology was tested *in vitro* and modified or enhanced to adopt its applications for our genome editing projects. The CRISPR/Cas9 technology is now well established for all ES cell targeting projects. For *in vivo* CRISPR/Cas9 implementation we tested direct injection into zygotes using different reagents to fully optimised the system. We are currently in a position to directly inject mouse zygotes to generate deletions, point mutations, small insertion and knockouts. Using CRISPR/Cas9 technology we can further reduce animal usage as the technique can be performed in different genetic backgrounds which minimises or eliminates the requirement to backcross mutations between different strains.

We are currently testing new emerging technologies using other endonuclease systems to enhance our portfolio of genome editing tools to generate novel genome modifications that can be offered as a service to the wider scientific community.




*High injections delivery and insertion/deletion (indel) formation in zygotes using CRISPR/Cas9 optimised technology. Indel formation in blastocysts was measured using a T7 endonuclease 1 assay. B1 to B12 are single injected blastocysts samples and WT is a sample from non-injected blastocysts. The efficiency of indel formation after injection is higher than 90%.*

www.babraham.ac.uk/science-services/gene-targeting

Simon Walker
Facility head

**Facility members**

**Deputy manager:**
Dr Hanneke Okkenhaug

# Imaging


**The Imaging facility provides a range of services to support the Institute's research. These include access to state-of-the-art light microscopy technology, training and support for a variety of different imaging modalities, help and advice with experiment design and an advanced image processing and analysis service.**

The Imaging facility was established in 2004 and has grown from a single room containing two systems to now occupy seven rooms with more than twelve systems. The facility aims to provide an all-encompassing resource for researchers wanting to use light microscopy. The systems available are state-of-the-art commercial technologies which make the Institute one of the best resourced light microscopy facilities in the UK. Imaging modalities available include wide-field, confocal, multi-photon, laser micro-dissection, high content and super resolution. These provide an array of tools which are used independently or in combination to address a wide range of biological questions. The facility also provides an image analysis suite with training available on all software packages and an image analysis service for more challenging requirements. Over the last five years, around a quarter of Institute publications have acknowledged use of the imaging resources available, demonstrating the return on the investments that have been made in developing the facility.

This year we have been directly involved in a number of high-profile publications, including work looking at the nascent autophagosome (1). This work combined live cell imaging with our super resolution capabilities to describe the temporal and spatial distribution of some of the components in the early autophagy pathway. Some of the methods used will be published in a methods chapter (2). Among other highlights we have also contributed to work looking at the distribution of PI3K lipid signals in neutrophils (3), which builds on our extensive experience of imaging fluorescence reporters in live neutrophils.

In addition to our work with Institute groups, we also provide access for an increasing number of commercial users. Many companies based on the Babraham Research Campus use the facility as well as several large companies based off campus. This growing demand has been driven by the advanced equipment we have available and recognition of our ability to provide expert advice and support.

## Publications

www.babraham.ac.uk/science-services/imaging

1. Karanasios, E. *et al*. (2016) Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. *Nature Comm* 7: 12420
2. Walker, S.A., Karanasios, E. & Ktistakis, N.T. (in press) Correlative live cell and super resolution imaging of autophagosome formation. *Methods Enzymol*
3. Norton, L. *et al*. (2016) Localizing the lipid products of PI3Kγ in neutrophils. *Adv Biol Reg* 60: 36-45

Michael Wakelam
Facility head

**Facility members**

Dr Jonathan Clark,
Biological Chemistry facility

Research fellow to be appointed in 2017

**Research assistant:**
Dorottya Horkai

# Lipidomics

**The Lipidomics facility provides the capability to identify and semi-quantify a vast range of neutral, phospho- and sphingolipids. The facility houses a GC-MS/MS, three triple quad MS machines, one with a solexion attachment and an Orbitrap MS. Each machine has a hyphenated GC, HPLC or UPLC chromatography system and an Advion Nanomate front end is also available.**

The facility's lipidomic analysis has shown that physiologically important changes in lipids are frequently not at the level of lipid class, but rather in a subset of individual lipid molecular species. Integrating this information with pathway analysis allows us to suggest pathways and enzymes responsible. The facility also has the rare capacity to determine changes in phosphoinositides. Making use of a methodology devised by Jonathan Clark, Head of the Biological Chemistry facility (page 57), we can determine changes in the key second messenger lipid PtdIns3,4,5P3.

The facility works closely with Institute researchers to provide lipidomics analysis and, increasingly, metabolomics. In addition, the facility has collaborated with colleagues in Cambridge and elsewhere providing a unique analytical capability. Samples that can be analysed include cultured cells, human and rodent tissue samples and serum samples. This work has generated significant publications and data to support successful applications for response mode funding. In addition, we have been working with industrial colleagues to determine both changes in lipids and also in flux through pathways. This has been successful in generating data suggesting potential therapeutic targets.

The facility takes advantage of a range of bioinformatics tools and additionally benefits from our award of Wellcome Trust funding in 2016, together with colleagues at Cardiff University and the University of California at San Diego, which is facilitating the migration of the LIPID MAPS website and databasing capability to the UK.

Members of the facility are always happy to discuss potential projects and provide advice on experimental design so that the lipids can be accurately analysed. For ease of working, the facility receives frozen samples and performs the extraction, using appropriate methodologies, prior to addition of standards and mass spectrometry. Whilst we are able to perform shotgun lipidomics, we take the view that far more accurate mass spectrometric data can be generated through a prior chromatographic step. We will always discuss methodology with colleagues and collaborators before analysis is performed.

## Publications

www.babraham.ac.uk/science-services/lipidomics

1. Cader, M.Z. *et al.* (2016) C13orf31 (FAMIN) is a central regulator of immunometabolic function. *Nature Immunol* 17: 1046-56
2. Peck, B. & Schug, Z.T. *et al.* (2016) Inhibition of fatty acid desaturation is detrimental to cancer cell survival in metabolically compromised environments. *Cancer Metab* 4: 6
3. Nguyen, A. *et al.* (2016) Using lipidomics analysis to determine signalling and metabolic changes in cells. *Curr Opin Biotechnol* 43: 96-103

# Mass Spectrometry

David Oxley
Facility head

**Facility members**

**Senior research assistant:**
Judith Webster

**Postdoctoral researcher:**
Dr Katarzyna Wojdyla
(joined in 2016)

**The primary role of the Mass Spectrometry facility is to apply state-of-the-art techniques in mass spectrometry and develop new methods to address important biological questions within the remit of the Institute. This is done in collaboration with our colleagues across all four of the Institute's research programmes.**

Mass spectrometry is a very powerful analytical technology that can be used to study almost any type of organic molecule. The Mass Spectrometry facility is equipped with several very sensitive, high-resolution mass spectrometers, which allow us to detect, quantify and obtain structural information on extremely small amounts of biological molecules, even in highly complex samples. This is important because biological samples may only be available in small amounts, and the molecules that take part in the biological processes we study are often present in cells at very low levels in complex mixtures of many thousands of different molecules.

Our mass spectrometers are used mainly for the analysis of proteins and DNA, but we can also analyse other types of molecules e.g. carbohydrates and metabolites. The most common types of protein analyses are: (1) protein identification (typically members of a protein complex), (2) identification and localisation of the sites of post-translational modifications (PTMs) (e.g. phosphorylation, glycosylation and acetylation), (3) targeted analyses for the quantitation of specific proteins or PTMs in a complex background, such as a total cell lysate. DNA analyses are based on a method developed in the facility for the ultra-high sensitivity quantitation of cytosine modifications in DNA (mC, hmC, fC, caC).

In 2016 we have been involved in more than twenty projects with nine Institute research groups, including: a large-scale quantitative proteomic analysis of PI3-kinase signalling in a mouse model of prostate cancer, a phosphorylation analysis of TORC1 complex in autophagy, and the development of a method for the quantitation of uracil in genomic DNA.

## Publications

www.babraham.ac.uk/science-services/mass-spectrometry

1. Hore, T.A. *et al.* (2016) Retinol and ascorbate drive erasure of epigenetic memory and enhance reprogramming to naïve pluripotency by complementary mechanisms. *Proc Natl Acad Sci USA* 113(43):12202-12207
2. Jethwa, S.A. *et al.* (2016) Exosomes bind to autotaxin and act as a physiological delivery mechanism to stimulate LPA receptor signalling in cells. *J Cell Sci* 129(20): 3948-3957
3. Pan, D. *et al.* (2016) Norbin stimulates the catalytic activity and plasma membrane localization of the guanine-nucleotide exchange factor P-Rex1. *J Biol Chem* 291(12): 6359-75

Kristina Tabbada
Facility head

**Facility members**

Clare Murnane

# Next Generation Sequencing

**High-throughput sequencing has the potential to further our understanding of the mechanisms behind epigenetic, transcriptional and post-transcriptional gene regulation as well as enhance our knowledge of nuclear and chromosomal function and structure. The Next Generation Sequencing (NGS) facility aims to provide researchers in these fields with access to state-of-the-art technology that will generate the sequence data to advance their research.**

The facility utilises Illumina sequencers, the most widely-adopted NGS technology, to produce highly accurate sequencing results in a time- and cost-effective manner. This includes the HiSeq 2500, a high-throughput sequencing instrument which maximises data yield by running as many as 16 samples or sample pools at the same time. At the other end of the scale, the MiSeq personal sequencer runs a single sample or sample pool and provides maximum flexibility with a wide range of read lengths and short turnover times. The NextSeq 500, newly introduced in the NGS facility in 2016, utilises a novel two-dye sequencing technology to achieve the fast turnaround and flexibility of a personal sequencer without reducing the depth of sequencing.

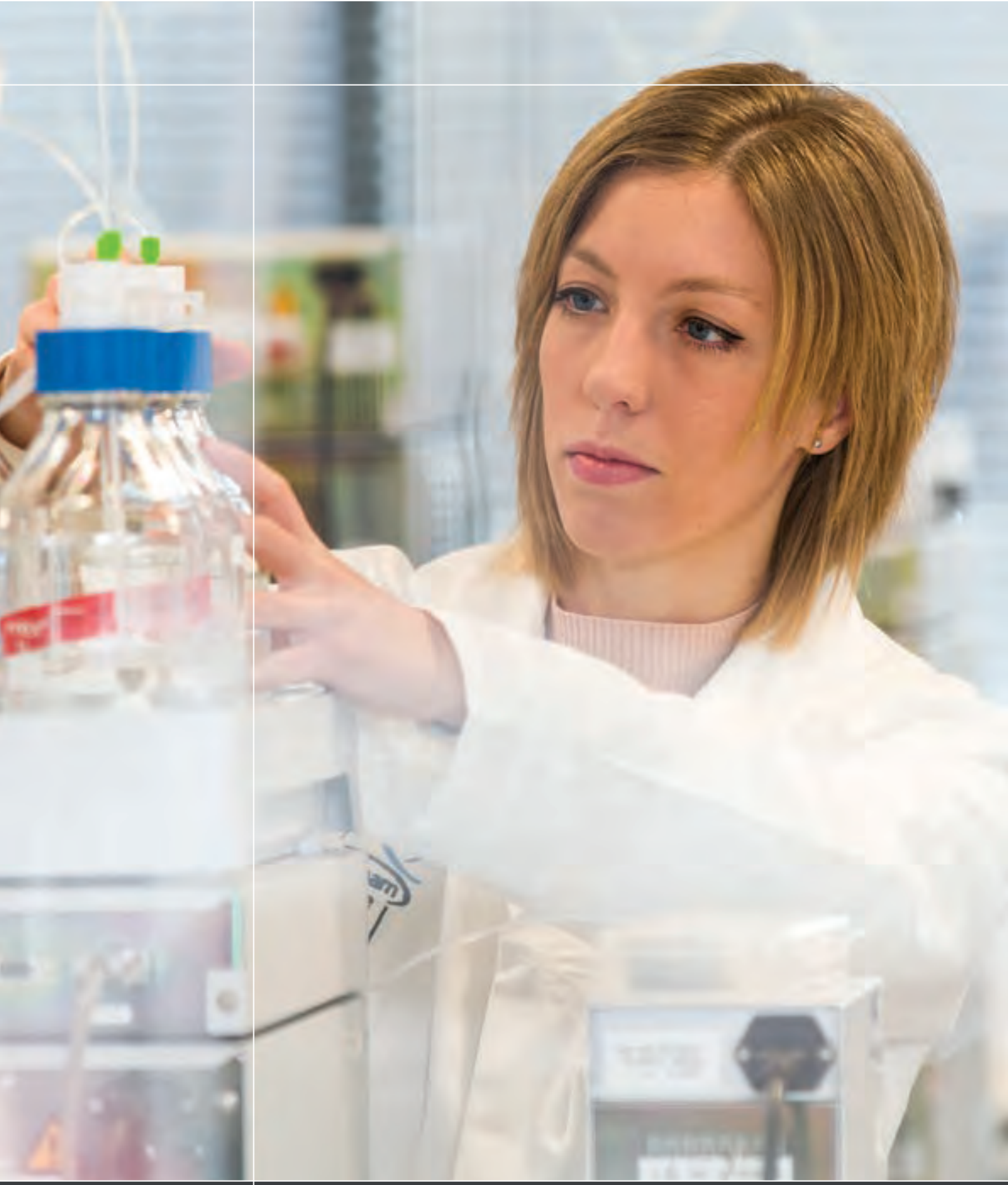
Researchers using the facility can select the sequencing platform and run type that provides the sequencing depth, read length and turnaround time that best suits their project. All run types are available in both paired-end and single-read analysis formats.

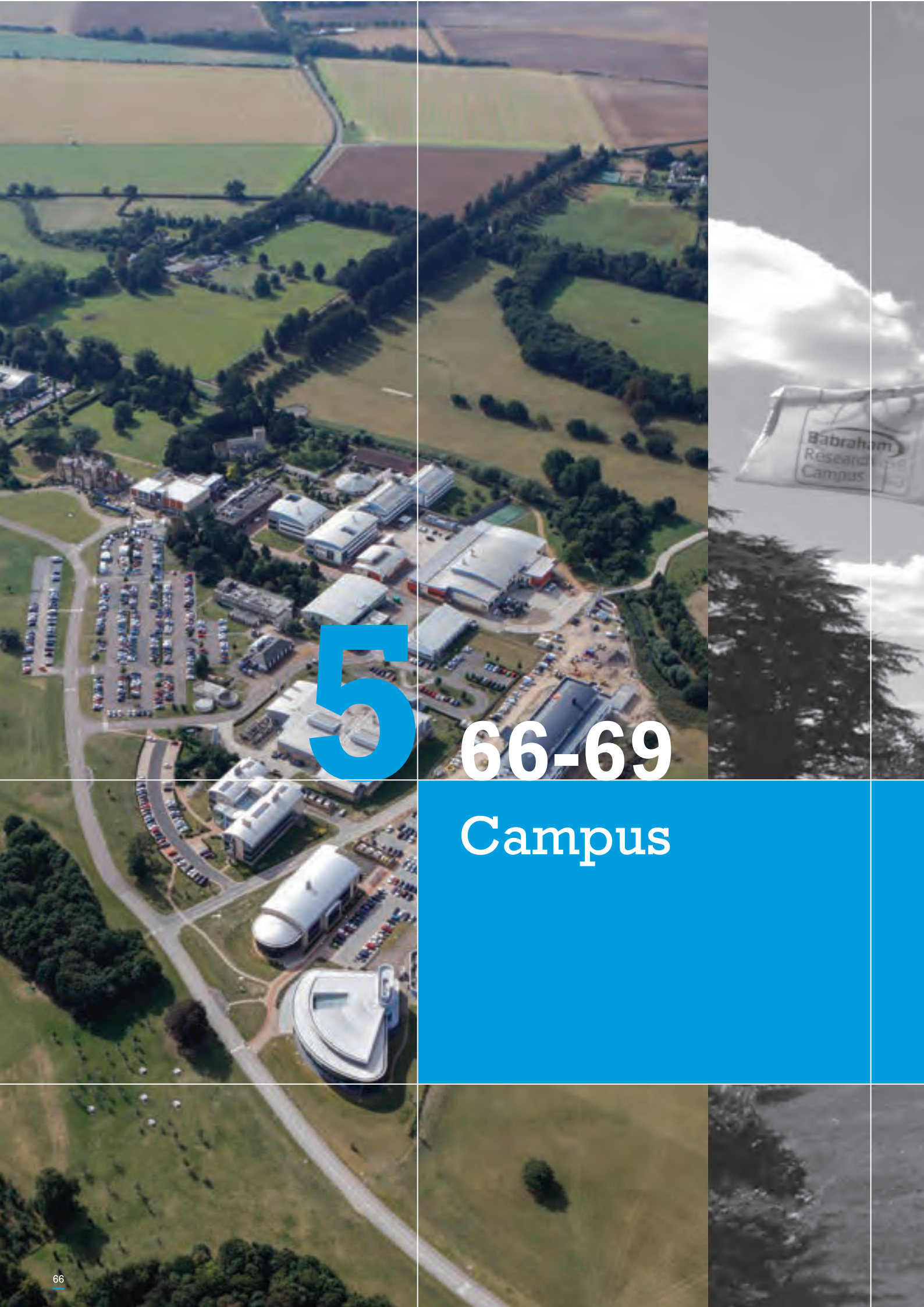
The facility provides sequencing support including library quality control on the Agilent Bioanalyzer 2100. In 2017, the facility is also aiming to expand its services to include library preparation as well as qPCR quality control, to offer scientists a beginning-to-end solution for their sequencing requirements.


## Publications

www.babraham.ac.uk/science-services/sequencing-facility

1. Bolland, D.J. & Koohy, H. *et al*. (2016) Two mutually exclusive local chromatin states drive efficient V(D)J recombination. *Cell Reports* 15: 2475-87
2. Schoenfelder, S. *et al*. (2015) Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. *Nat Genet* 47:1179-1186
3. Schoenfelder, S., Furlan-Magaril, M., Mifsud, B. *et al*. (2015). The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. *Genome Res* 25: 582-597






# 5

# 66-69

# Campus

**Babraham Research Campus companies play an important role in our strategy to maximise the impact of the Institute's research.**

# Collaborating with our campus community

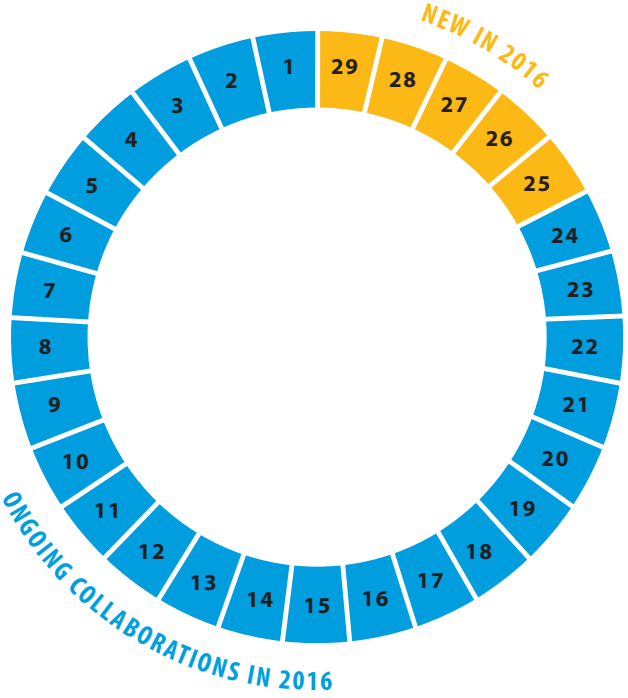
Our academic and commercial partnerships are part of the conduit by which our knowledge and discoveries are passed forward to ultimately benefit society via improvements in clinical diagnostics and healthcare. The thriving Babraham Research Campus, operated and developed by Babraham Bioscience Technologies with significant funding support from BBSRC, promotes innovation across the academic and commercial sectors and contributes significantly to the bioscience community in Cambridge, the UK and internationally. The success of the Institute directly translates into the success of the campus, which has evolved through the commitment of Institute staff and scientists over the last twenty years to become recognised as a world-leading bioincubator campus.

The co-location of bioscience companies and the Institute offers industry scientists access to the leading edge of fundamental research and world-class facilities. Campus companies play an important role in our strategy to maximise the impact of the Institute's research and are a focus of the Institute's knowledge exchange activities. 45% of the life science companies located on the Babraham Research Campus have a collaborative project with the Institute, through research consultancies, PhD studentships, research collaborations, commercial agreements and scientific facility use.

**115**
**COLLABORATIONS**

**WITH**

**29**
**CAMPUS COMPANIES**



Rahul Roychoudhuri

## Immunology researchers working with Cancer Research Technology

The Babraham Institute's basic science benefits in a very real sense from its proximity to a number of industry-leading biotechnology companies on the Babraham Research Campus. One of many examples of such interactions is a new collaboration forged between the researchers in the Immunology programme and the campus company Cancer Research Technology (CRT). CRT is the commercialisation arm of Cancer Research UK and has an in-house drug discovery unit, CRT Discovery Laboratories, which translates cutting-edge science into innovative new therapies for cancer patients.

Following on from an earlier campus collaboration grant from the Institute's Knowledge Exchange and Commercialisation programme, group leaders Rahul Roychoudhuri and Klaus Okkenhaug were awarded a Cancer Research UK Small Molecule Drug Discovery Project for three years to perform high-throughput screening of new small molecule immune-stimulatory drugs for immune-based therapy of cancer. This project is a collaboration with Stuart Farrow and Laura Rosenberg at CRT.

By designing new cell-based functional reporter assays for the function of immunosuppressive molecular pathways in T cells, Institute scientists hope to leverage the expertise and resources of CRT to identify new small molecules that can suppress such pathways. This approach will utilise high-throughput small molecule screens using compound libraries held by CRT and the Institute for Cancer Research (ICR). The research will be led by new Institute scientist Teresa will join the Roychoudhuri group as a postdoctoral research scientist in 2017.

Rachael Walker

## The Flow Cytometry Core Facility and Kymab Ltd

Kymab Ltd, a company based on the Babraham Research Campus, have been using the Institute's Flow Cytometry facility for the past four years and this relationship has developed into a strong collaboration. Having identified flow cytometry and cell sorting techniques as central to their work into drug and vaccine discovery, Kymab purchased a BD FACSAria Fusion sorter in July 2015. Having had experience of the expertise with the Flow Cytometry facility, they chose to place this high-end instrument into the facility to be run by Institute specialists. With priority bookings given to Kymab, any spare capacity is available for use to support the Institute's science.

This collaboration has ensured that the Kymab-owned instrument is a well maintained, well used piece of core equipment. The benefits of this arrangement are seen by both parties as Institute researchers have access to a high-end cell sorter and Kymab have access to the expertise in the facility as well as access to the facility's other sorters in case of any required maintenance on Kymab's Fusion sorter. Not only does the collaboration cover cell sorting, but wider advice on experimental design, data analysis and other aspects of flow cytometry is also provided.

This close collaboration highlights the value of academic–commercial partnerships made possible by the co-location of a world-class Institute with leading biotechnology and life science companies.

Simon Cook

## Simon Cook working with PhoreMost Ltd

The Institute's Signalling programme studies the signalling pathways that regulate how cells develop and respond to their environment to maintain lifelong health. Many of these pathways are also de-regulated in diseases making them attractive drug targets. For example, the KRAS-regulated RAF-MEK-ERK signalling pathway is very important in cancer; 20% of all cancers harbour mutations in KRAS but no anti-KRAS drugs exist. Whilst RAF and MEK inhibitors have been approved for clinical use, tumours quickly adapt and acquire resistance. New approaches are required to develop new distinct RAF, MEK or ERK inhibitors and to target mutant KRAS, a protein that some consider undruggable.

Simon Cook, a group leader in the Signalling programme, has studied the ERK pathway for 20 years. He uses inhibitors that target the pathway as research tools but recognised that they might also be relevant as future anti-cancer drugs. This led him to contact PhoreMost Ltd, a company co-situated on the Babraham Research Campus. PhoreMost is a new-model drug discovery company that seeks to inhibit the most intractable drug targets using its novel SiteSeeker® technology, a live-cell phenotypic assay system that can rapidly identify unexpected or 'cryptic' druggable sites in specific disease-driving targets and pathways that cannot be readily seen using conventional non-cell based analytical methods.

Using a campus collaboration grant from the Institute's Knowledge Exchange and Commercialisation programme, Rebecca Gilley in Simon's group and Grahame McKenzie at PhoreMost have established cell-based assays for the KRAS-RAF-MEK-ERK pathway that are compatible with PhoreMost's screening technology. This has prompted a successful joint application to Innovate UK and interest in a three-way collaboration with a drug discovery company based in the USA.

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