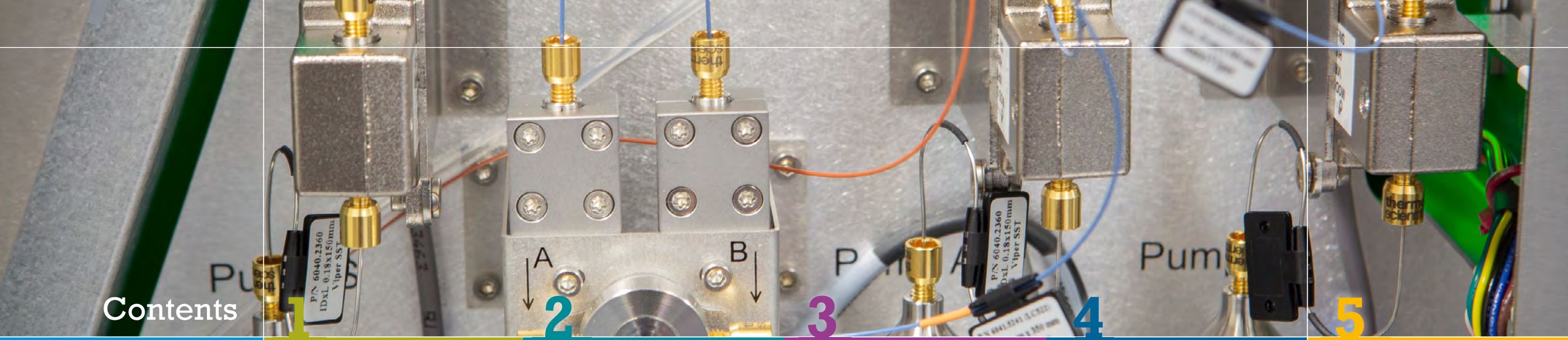

# 2019/20

# Babraham Institute
# Annual Research Report

# Life sciences research for lifelong health

**Whilst we're living longer than ever before, our bodies still decline into old age at around the same point that they always have, a concept called healthspan. This decline brings with it challenges to the individual and to society as our final years are increasingly marred by chronic ill-health.**

**To address these challenges, the Babraham Institute unites wide-ranging expertise in fundamental biology to gain a detailed understanding of lifelong health and ageing. Our research aims to uncover the functioning of the immune system and its decline with age; to investigate how the cells of our body respond and adapt to damage, disease, diet and ageing; and to chart epigenetic changes to gene regulation throughout development and ageing.**




# Contents

# Director's welcome

Dr Simon Cook, Interim Director

2020 was a challenging year for everyone as our lives were turned upside-down by the Covid-19 pandemic. Here at the Babraham Institute we know this only too well as our Director and colleague Michael Wakelam passed away from Covid-19-related complications in March 2020. This was a great trauma for the Institute as Michael was a dear friend to many, and cared passionately about the Institute, its staff and students.

Professor Michael Wakelam, Institute Director 2007-2020

In 2022 we will come together - in person - to celebrate Michael's life, but for now I am reminded of a quote by Abraham Lincoln: "It's not the years in your life that count. It's the life in your years." With great energy Michael packed a lot of life into his years.

Wolf Reik stepped into the role of Acting Director; with more than 30 years of history at Babraham he was the ideal choice to steer the Institute during this difficult time. Whilst we were all delighted when he accepted the role of Director in early 2021 his tenure was sadly brief as he made the difficult decision to grasp an exciting new opportunity. On behalf of all staff and students I want to thank Wolf for his leadership and many contributions to Babraham over the years. We wish him well in his new venture and hope to work closely with him as his association with the Institute evolves.

### Our People

I have stepped into the role of Interim Director at a difficult time, but I remain very optimistic about the future of the Institute for several reasons and at the heart of each of these reasons is my colleagues.

I am immensely proud of the way they 'stepped up' after the loss of Michael; despite very obvious grief there was a determination to support each other and to do our best to progress our science.

The pandemic brought huge challenges to the Institute and the fact that we were able to press ahead with our research – albeit at reduced capacity – and the activities that both support it and provide wider impact from it was down to the hard work and dedication of staff from all parts of the Institute. This shows the care, determination and professionalism of our staff and our progress in knowledge exchange, training, funding and outreach is summarised on pages 8-12.

Following on from three recruits in Immunology in 2018, we were joined by Hayley Sharpe, Rahul Samant and Maria Christophorou in 2019-2020, marking the start of a recruitment phase for our Signalling and Epigenetics research programmes. Learn more about Hayley and Rahul in one of the two Signalling features included in this report.

I am delighted that we will welcome five new group leaders in 2021 and 2022 including Ian McGough (Signalling), Della David (Signalling), Philipp Voigt (Epigenetics), Teresa Rayon and Sophie Trefely (both joint appointments in Signalling and Epigenetics). This represents a recruitment of eleven new group leaders since 2018 and is a strong statement of our aspirations for growth in the future.

### Our Science

Through changes in leadership and global challenges, our research has stood firm and there have been some outstanding science produced across our three programmes, enabled by our pioneering facilities.
To focus on a couple of examples from each, our Immunology research provided important understanding of the differences in immune system response with respect to age in a pre-clinical study of the Oxford/AstraZeneca COVID-19 vaccine (1) and identified a new role for white blood cells in the developing brain (2). The Epigenetics research programme applied the Institute's expertise in single-cell techniques to explore the effects of age on the developmental competence of egg cells in mice (3); and in exciting collaborations contributed to the first comprehensive molecular map of early embryo development in mice (4) and explored the molecular mechanisms that may drive ageing in humans (5). Finally, researchers in our Signalling programme applied their expert knowledge in cell signalling to better understand the mechanism of acquired resistance to anti-cancer drugs (6), possibly influencing treatment models in the clinic, whilst autophagy researchers from across the programme collaborated to show that autophagy is repressed during mitosis and described the mechanisms involved (7) and defined a new molecular signature for non-canonical autophagy (8).

### Our Culture

While the Institute has always had a supportive culture, important and innovative initiatives have strengthened and formalised our commitment to this, recognising that research excellence is only possible when it is inclusive:

- the newly developed Roving Researcher Scheme mitigates the impact of long-term leave on the careers of our excellent young researchers.
- the Research Access Programme, our undergraduate summer placement scheme, is in its second year. It focuses on under-represented groups and addresses a major challenge that students from disadvantaged backgrounds face in obtaining PhD positions due to a lack of relevant research experience compared to students from advantaged backgrounds.
- In December 2019 the Institute became a signatory to the Technician Commitment, a sector-wide initiative signed by ~100 organisations to support their technical workforce. Our technical specialists and their skills, experience and ability to develop and implement methodologies are critical to the Institute being able to perform world-class science. As a signatory, the Institute has developed a bespoke action plan to identify key areas of progress within the Commitment's four core areas of Visibility, Recognition, Career Development and Sustainability.

### Our Impact

The individual reports that follow share the progress made but I'd like to highlight a couple of significant developments and milestones:

- Based on our pioneering research, Enhanc3D Genomics was formed as an Institute spin-out company in January 2020 and has achieved significant success over a very short timescale. Find out more in the Epigenetics feature on pages 50-51.
- A Babraham Research Campus impact report recognised the Institute's vital role in securing the Campus's success, identifying the Institute's discovery research and state-of-the-art scientific facilities as unique features of the Campus (9).
- Our Public Engagement programme was part of the collaborative LifeLab public engagement project that brought an exciting programme of activities to Ely, Peterborough and Cambridge in 2018 and 2019 and the Institute also celebrated 25 years of our annual Schools' Day event in March 2019.

## Recognitions and Key Awards:

- Gavin Kelsey and Simon Cook became the Heads of the Epigenetics and Signalling research programmes respectively
- Claudia Ribeiro de Almeida became a Sir Henry Dale Fellow
- Hayley Sharpe received the 2020 Lister Research Prize Fellowship and became an EMBO Young Investigator in 2019
- Danika Hill from the Linterman lab received the Michelson Prize in 2020, awarded by the Human Vaccine Project
- Stephen Clark from the Reik lab was named as the Research of the Year in the Cambridge Independent Science and Technology Awards 2019
- Michelle Linterman was awarded tenure in October 2019 and received the 2019 Lister Prize.
- The Institute was awarded the Leader in Openness status in 2019 by Understanding Animal Research

Research features:



## Publications

More Institute press releases at www.babraham.ac.uk/news


1. Exploring the effects of age on the immune response to Oxford's COVID-19 vaccine
2. New role for white blood cells in the developing brain
3. Single-cell technique could provide 'egg health' indicators
4. Establishing the molecular blueprint of early embryo development
5. Pinpointing the molecular mechanisms of ageing
6. Tumour cells' drug addiction may be their downfall
7. How the cellular recycling system is put on hold while cells divide
8. Developing our understanding of the fundamentals of autophagy
9. Institute welcomes future vision for the Babraham Research Campus

# Performance in 2019

OUR COLLABORATIVE ACADEMIC PROJECTS UK AND WORLDWIDE

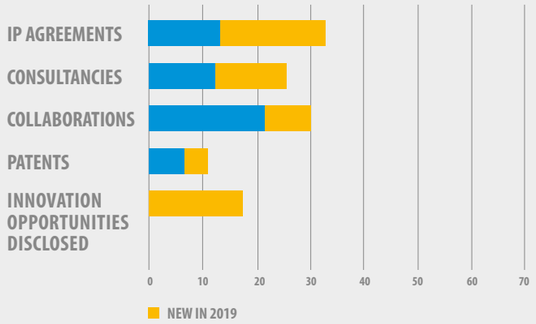
## Working with others in 2019

**64** ACTIVE PROJECTS

**23** COUNTRIES

**131** ORGANISATIONS

## Working with commercial partners




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

**>1000**

BIOINFORMATICS **693**

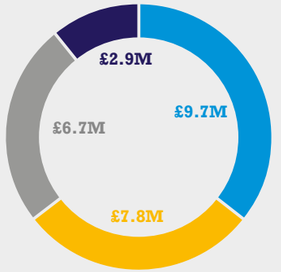
FLOW CYTOMETRY **201**

IMAGING **>100**

ANIMAL FACILITY **34**



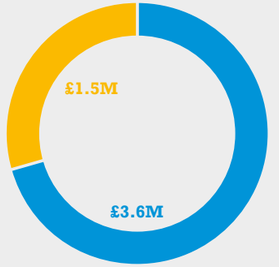
## 2019 income




**£27.1M**

- Core ISP grants & BBSRC non-grant income
- Competitively awarded grant income in 2019
- Income from services provided by the Institute
- BBSRC additional income (including business cases, equipment etc.)

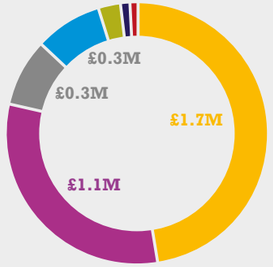
## Value of all grants awarded in 2019




**£5.1M**

- UK funders
- International grants*

## Value of UK grants awarded in 2019




**£3.6M**

- BBSRC
- MRC
- Wellcome Trust
- Lister Institute of Preventive Medicine
- The Dunhill Medical Trust
- The Evelyn Trust
- Phoremost Limited

*International grant sources:
European Commission (EC), European Molecular Biology Organisation (EMBO), SENS Research Foundation

## 2019 successes

**44** PUBLIC ENGAGEMENT EVENTS

INVOLVING

**105** STAFF AND STUDENTS

**7,740** PEOPLE ENGAGED

**77** PUBLICATIONS

**65** RESEARCH PUBLICATIONS

**12** REVIEWS

PROUD SUPPORTER OF THE Technician Commitment

**20** PhDs COMPLETED

# Performance in 2020

OUR COLLABORATIVE ACADEMIC PROJECTS
UK AND WORLDWIDE

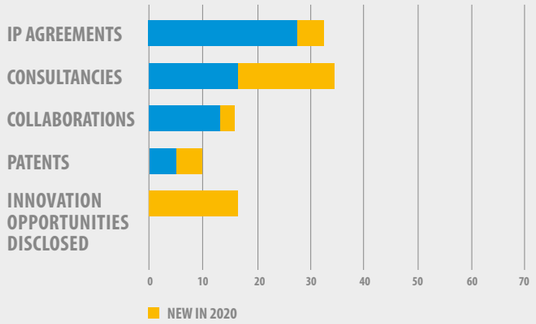
## Working with others in 2020

**63** ACTIVE PROJECTS

**21** COUNTRIES

**96** ORGANISATIONS

## Working with commercial partners




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

**1,323**

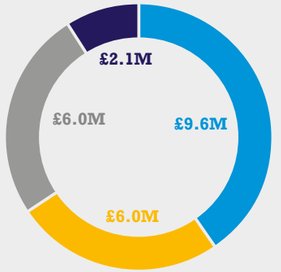
BIOINFORMATICS **1059**

FLOW CYTOMETRY **230**

IMAGING **34**



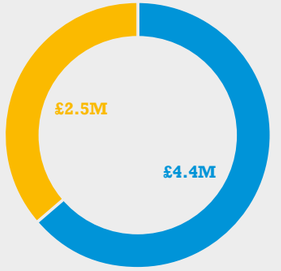
## 2020 income




**£23.6M**

- Core ISP grants & BBSRC non-grant income
- Competitively awarded grant income in 2020
- Income from services provided by the Institute
- BBSRC additional income (including business cases, equipment etc.)

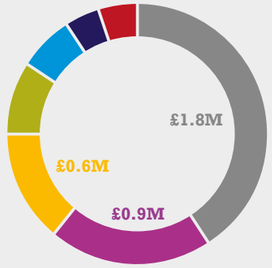
## Value of all grants awarded in 2020




**£6.9M**

- UK funders
- International grants*

## Value of UK grants awarded in 2020




**£4.4M**

- Wellcome Trust
- MRC
- BBSRC
- Cancer Research Technology Ltd
- Lister Institute of Preventive Medicine
- Milner Therapeutics Institute
- UKRI

*International grant sources: European Commission (EC)

## 2020 successes

**180** STUDENTS ENGAGED WITH IN-PERSON SCHOOLS' DAY 2020

**3** ONLINE PUBLIC ENGAGEMENT EVENTS

**83** STAFF AND STUDENTS INVOLVED

**1097** NUMBER OF PEOPLE ENGAGING WITH OUR ONLINE PROGRAMME

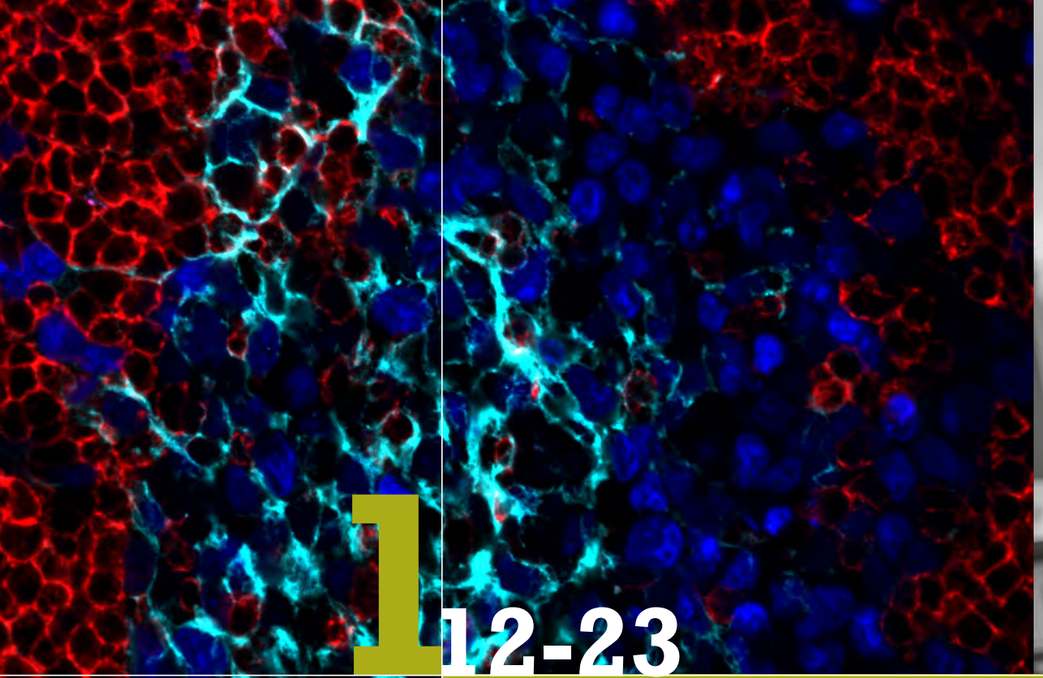
**126** PUBLICATIONS

**109** RESEARCH PUBLICATIONS

**17** REVIEWS

Diversity Access Programme launched hosting undergraduate students for virtual projects

**15** PhDs COMPLETED



1 12-23

# Immunology

The immune system includes cells called lymphocytes, a type of white blood cell, that defend the body from infections including bacteria, viruses and fungi as well as cancer. As we age, the immune system tends to weaken and this contributes to the increased risk of illness during old age. A weakened immune system also means that older people don't always respond fully to vaccinations.

By studying a combination of human samples and mouse models we aim to enhance our understanding of the role of lymphocytes in the immune system. We do this by examining:

- The mechanisms linking ageing to reduced response to vaccinations
- How lymphocytes interact with cells in tissues and organs of the body
- How different molecular signals influence gene activity and ultimately the growth and behaviour of lymphocytes

## Group Leaders

Martin Turner

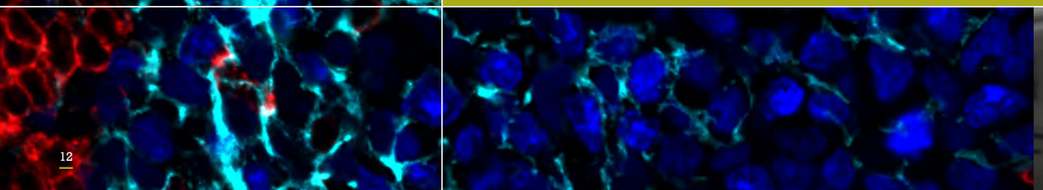
Anne Corcoran

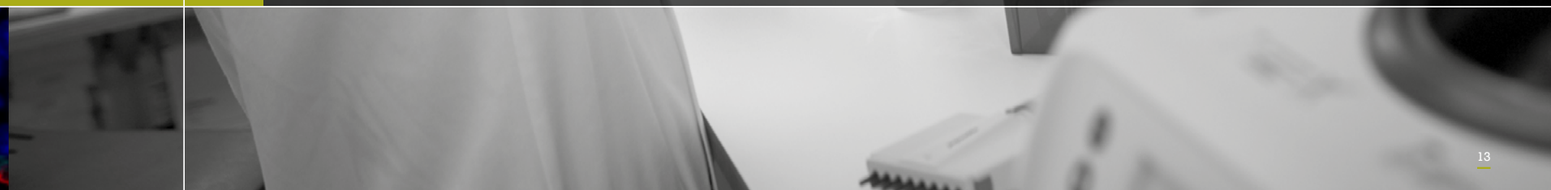
Michelle Linterman

Adrian Liston

Claudia Ribeiro de Almeida

Sarah Ross





**Martin Turner**
**Programme leader**

**Group members**

**Senior research associates:**
Sarah Bell
Elisa Monzón-Casanova

**Postdoctoral researchers:**
Jia Lu
Georg Petkau
Beatriz Sáenz-Narciso
Fiamma Salerno
Alexander Saveliev
Michael Screen

**PhD students:**
Vanessa D'Angeli
Oezge Gizlenci
Fengyuan Hu (Left in 2019)
Twm Mitchell

**Visiting scientist:**
David Turner

**Visiting students:**
Miriam Berry
(Left in 2019)
Francesca Ippolito
(Left in 2019)

# Characterisation of lymphocyte transcriptomes using long-read sequencing

**We study the differentiation of lymphocytes, immune cells that are critical for immune memory (and hence vaccine success) and also tissue homeostasis by limiting inflammation and promoting tissue repair. These cells utilise rapid and dynamic changes in gene expression to mediate their function and our work focuses on understanding the fundamental mechanisms controlling these changes.**

## Current Aims

Most genes produce multiple molecularly distinct messenger RNAs that vary the amount or amino acid sequence of proteins. This is regulated by choosing alternative transcript start- and end-points and splicing of introns. Our recent work has investigated RNA binding proteins that regulate these choices. We are identifying the sequence of full-length mRNAs to discover how they vary between cell types, what controls this variation and how it contributes to cell function.

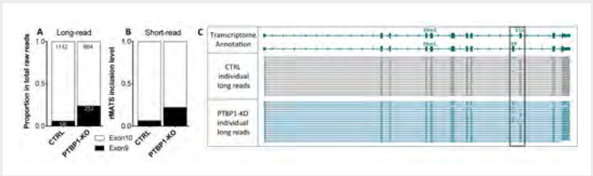
It is essential not only to measure gene expression quantitatively, but to understand how it is regulated qualitatively. We are using emerging technologies for long-read sequencing of full-length transcripts to identify alternative transcript isoforms and their variation in activated lymphocytes.

## Progress in 2019 and 2020

By applying long-read sequencing to B lymphocytes, we detected transcripts from over 9,500 genes with at least five supporting reads, which compares very favourably with the numbers of genes detected using conventional methods on the same samples. We were able to quantitate the effect of the RNA binding protein polypyrimidine tract binding protein-1 (PTBP1) on the alternative splicing of the *Pkm* gene and found the results to be consistent with a short-read sequencing method. Furthermore, the long-read sequencing data allowed unambiguous assignment of reads to specific transcript isoforms arising from a gene with multiple variants, a feat that cannot be accomplished by short-read methods. This technology will give an unprecedented insight into the dynamic regulation of gene expression.

## Selected Impact Activities

- Martin Turner was an invited speaker at the 2019 Keystone Meeting on Transcription and RNA Regulation in Inflammation and Immunity, Lake Tahoe, USA.
- Martin Turner was an invited speaker at the 24th European Haematology Congress in 2019, held in The Netherlands.
- PhD student David Turner presented his work on CRISPR screens for RNA binding proteins in B cell differentiation at the 2020 RNA UK meeting.




*PTBP1 suppresses inclusion of exon 9 and expression of PKM1. Quantitation of the usage of exons 9 and 10 in Pkm transcripts from PTBP1-sufficient (CTRL) and PTBP1 knockout (PTBP1-KO) follicular B cells is shown.*

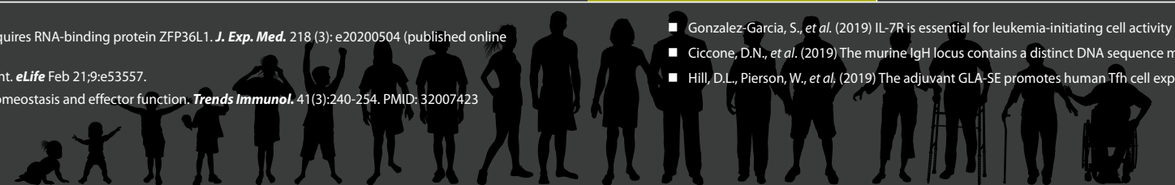
*A) Individual long reads were counted for exon-9 and -10 containing isoforms; numbers are the absolute number of reads for each. B) Inclusion levels for Pkm exons 9 and 10 were quantified by rMATS analysis of Illumina short-read RNA-seq data where Percent of splice-in (PSI) values are scaled to 1. C) Top: annotated mouse Pkm transcript isoforms with exons 9 & 10 boxed in black; bottom: individual long reads for control PTBP1-sufficient (CTRL) and for PTBP1 knockout (PTBP1-KO) cells.*

## Publications


- Saveliev, A., Bell, S.E., Turner, M. (2021) Efficient homing of antibody-secreting cells to the bone marrow requires RNA-binding protein ZFP36L1. ***J. Exp. Med.*** 218 (3): e20200504 (published online December 2020). PMID: 33306108
- Monzón-Casanova, E. *et al.* (2020) Polypyrimidine tract binding proteins are essential for B cell development. ***eLife*** Feb 21;9:e53557.
- Salerno, F., Turner, M. & Wolkers, M.C. (2020) Dynamic post-transcriptional events governing CD8+ T cell homeostasis and effector function. ***Trends Immunol.*** 41(3):240-254. PMID: 32007423


## www.babraham.ac.uk/our-research/immunology/martin-turner





**Anne Corcoran**

**Group members**

**Sequencing Laboratory manager:**
Paula Kokko-Gonzales
Kristina Tabbada (Left in 2019)

**Senior postdoctoral researcher:**
Daniel Bolland (Left in 2019)

**Postdoctoral researchers:**
Sam Rees
Maribel Lara Chica

**PhD students:**
Lina Dobnikar (Left in 2019)
Elise French
Carolyn Rogers
Michiel Thiecke (Left in 2020)

**Visiting scientist:**
Lyobomira Chakalova
(Left in 2020)
Peter Chovanec (Left in 2020)

**Visiting student:**
Irina Ferapontova
Joey Baxter (Left in 2020)

# How we make enough antibodies to fight infection

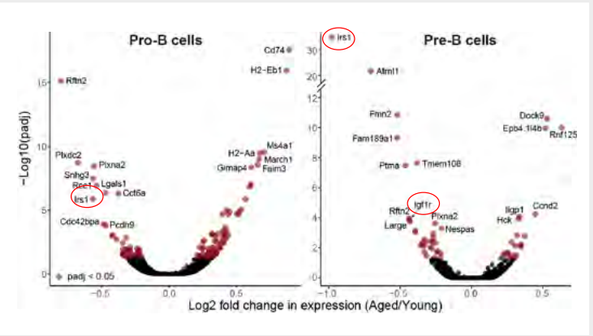
**The immune system creates antibody proteins to help fight diseases. Antibodies are made by white blood cells called B lymphocytes. By mixing and matching genetic information, these cells can produce billions of different antibodies to combat different diseases. We are interested in the mechanisms involved in the development of B lymphocytes and their ability to make antibodies. Reduced ability to produce effective antibodies is one of the reasons the immune system weakens as we age.**

## Current Aims

We aim to understand how the genes that make up antibody proteins come together in so many different combinations, and how epigenetic mechanisms including transcription factor binding and histone modifications affect which genes are more frequently used. We're also looking at how the large-scale 3D folding of these large DNA regions in the nucleus affects antibody production, and at gene expression and regulation in B cells. This will increase our understanding of normal antibody production and help us to understand the events that cause leukaemias and impaired antibody production in ageing.

## Progress in 2019 and 2020

Ageing bone marrow produces fewer B lymphocytes. With other Institute groups we compared gene expression genome-wide in B lymphocytes from young and old mice to reveal genes dysregulated in ageing. We discovered that ageing affects epigenetic mechanisms, including promoters that switch on genes, microRNAs that degrade their RNA, and interactions of promoters with activating enhancer sequences. We found that the insulin-like growth factor receptor signalling pathway is impaired in ageing B cells. This important growth pathway is poorly understood and this work suggests an unappreciated role in B cell development and may help to restore B cell numbers in ageing.




*This 'volcano plot' depicts the changes in gene expression between young and aged pro-B cells that make the antibody heavy chain, and young and aged pre-B cells that make the antibody light chain protein. Red spots or 'sparks' to the left (below 0.0) are genes that are expressed less frequently in ageing, while those to the right are expressed more often in ageing. The genes with lower expression encircled in red are components of the Igf1r signalling pathway.*

## Selected Impact Activities

- Anne Corcoran gave a presentation to the Cambridge Scientific Society: How we make a billion antibodies: genetics and epigenetics, March 2019.
- Immunology video lecture for University of Makerere MSc students, Kampala Uganda, organised by Cambridge for Africa.
- The group hosted a Wellcome Trust Summer Bursary undergraduate student in 2019 and an online undergraduate project in 2020.

## Publications


- Gonzalez-Garcia, S., *et al.* (2019) IL-7R is essential for leukemia-initiating cell activity of T-cell acute lymphoblastic leukemia. ***Blood*** 134 (24):2171-2182
- Ciccone, D.N., *et al.* (2019) The murine IgH locus contains a distinct DNA sequence motif for the chromatin regulatory factor CTCF. ***J. Biol. Chem.*** 294 (37):13580-13592
- Hill, D.L., Pierson, W., *et al.* (2019) The adjuvant GLA-SE promotes human Tfh cell expansion and emergence of public TCRβ clonotypes. ***J. Exp. Med.*** 216 (8):1857-1873


## www.babraham.ac.uk/our-research/immunology/anne-corcoran

Michelle Linterman

**Group members**

**Senior postdoctoral researcher:**
Louise Webb

**Research fellows:**
Alice Denton (Left in 2020)
Danika Hill (Left in 2020)

**Postdoctoral researchers:**
Edward Carr
William Foster
Silvia Innocentin
Alyssa Silva Cayetano
Marisa Stebegg
(Left in 2020)
Ine Vanderleyden
(Left in 2019)

**PhD students:**
Xin Ge
Jia Le Lee

**Research assistants:**
Sigrid Fra-Bido

**Visiting scientists:**
Jelmer Vlasma (Left in 2020)

**Visiting students:**
Rachel Fellows
(Left in 2019)
Jaqueline Siu (Left in 2020)

# Understanding the germinal centre reaction

**Our research focuses on the cellular and molecular mechanisms that underpin a robust germinal centre reaction. The germinal centre forms after antigenic challenge and, because it is the only cellular source of long-lived antibody secreting plasma cells, it is essential for enduring antibody-mediated immunity after infection or immunisation.**

## Current Aims

Research in the Linterman laboratory aims to understand germinal centre function in the context of vaccination and infection. Our current research aims to address the following questions:

1. What causes poor germinal centre responses in ageing?

2. How do ectopic germinal centres form, and what is their function?

3. What are the biological mechanisms that support good germinal centre and vaccine responses?

## Progress in 2019 and 2020

During 2019 and 2020 we made the following contributions to our scientific aims:

1. We demonstrated that aged mice have a reduced immune response to the Oxford/AstraZeneca COVID vaccine which can be boosted by a second dose (Ref. 1. Silva-Cayetano, Foster, Innocentin *et al., Med.* 2020).

2. We discovered that influenza infection remodels the lung to support the CXCR5-dependent recruitment of B cells to this site, enabling the formation of ectopic germinal centres (Denton *et al., J. Exp. Med.* 2019).

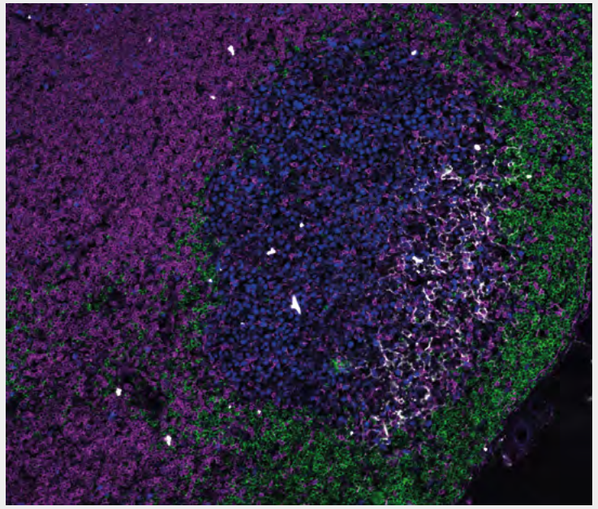


*Image of a germinal centre in a lymph node 14 days after immunisation. Proliferating germinal centre B cells (Ki67, blue), Follicular dendritic cells (white), T cells (pink), B cell follicle (IgD, green).*

3. In a human vaccine trial we discovered that it is possible to boost the T follicular helper cell response in humans, which supports long-lived antibody production after vaccination (Hill, Pierson, *et al., J. Exp. Med.* 2019).

## Selected Impact Activities

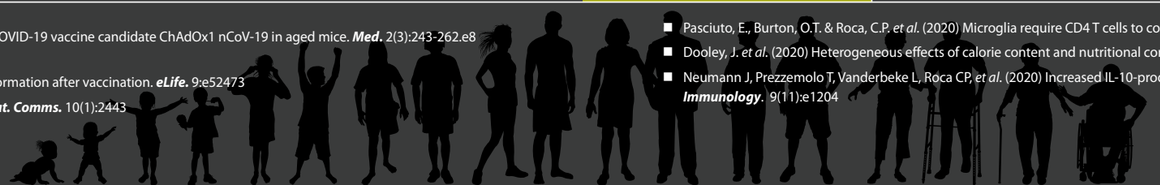
- Invited speaker, Keystone Symposia: B cell-T cell Interactions, USA.
- Hosted project for Babraham Institute 2019 Schools' Day in the laboratory.
- Organised EMBO Young Investigator Immunology meeting, Cambridge, UK.

## Publications


- Silva-Cayetano, A., Foster, W.S., Innocentin, S. *et al.* 2021 A booster dose enhances immunogenicity of the COVID-19 vaccine candidate ChAdOx1 nCoV-19 in aged mice. ***Med.*** 2(3):243-262.e8 [epub December 2020]
- Stebegg, M. & Bignon, A. *et al.* (2020) Rejuvenating conventional dendritic cells and T follicular helper cell formation after vaccination. ***eLife.*** 9:e52473
- Stebegg, M. *et al.* (2019) Heterochronic faecal transplantation boosts gut germinal centres in aged mice. ***Nat. Comms.*** 10(1):2443


www.babraham.ac.uk/our-research/immunology/michelle-linterman @LintermanLab





Adrian Liston

**Group members**

**Senior staff scientist:**
James Dooley

**Senior research scientists:**
Oliver Burton
Carlos Perez Roca
(Left in 2020)

**Postdoctoral research scientists:**
Pascal Bielefeld
Orian Bricard
Vaclav Gergelits
Lubna Kouser
Alena Moudra
Samar Tareen
Carly Whyte (Left in 2020)

**Research fellow:**
Stephanie Lienart

**PhD students:**
Magda Ali
Julian Behr (Left in 2019)
Amy Dashwood
Ntombizodwa Makuyana

**Research assistants:**
David Aaron Posner
(Left in 2020)

**Honorary fellow:**
Meryem Aloulou
Kailash Singh

# T cells in our tissues

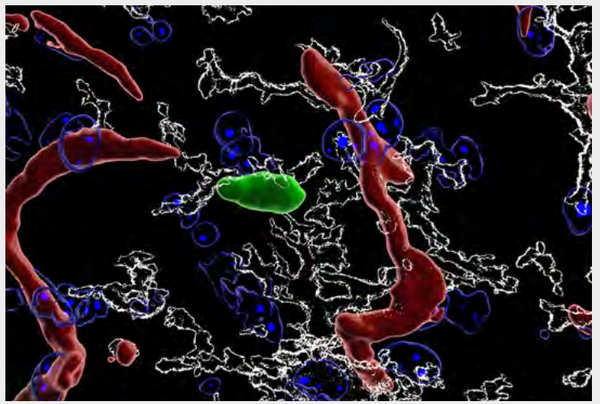
**Lymphocytes are among the best studied cells in the body. Despite this, we know remarkably little about how they operate in the tissues – almost all our knowledge has come from studying blood or lymphoid organs. Our research seeks to understand the genetic programme that is initiated in the tissues, and how we can exploit this programme to maintain health.**

## Current Aims

We aim to understand how T cells, especially the anti-inflammatory regulatory T cells, interact with the tissues. We take a holistic approach to tissue immunology, looking across tissues to determine differences and shared biology. We seek to unravel the genetic and epigenetic programme that allows T cells to enter various tissues. We are actively developing genetic tools that allows us to track and manipulate T cells within the tissues. Finally, we seek to utilise the resident T cell population to enhance the robustness of the tissues to damage, injury and ageing.

## Progress in 2019 and 2020

Over the past two years, we have made enormous strides into understanding the role of T cells in one particular organ – the brain. T cells enter the brain soon after birth, and in both mice and humans the cells undergo a transformation aiding their survival in the brain environment. We have found that this entry of T cells into the brain is linked to a key maturation event during brain development: the differentiation of embryonic microglia into adult microglia. This event is required for microglia to gain the ability to prune neuronal synapses, a rewiring of the brain critical for learning.



*A T cell (green) and a microglial cell (white) collaborate in the brain. Blood vessels are shown in red.*

## Selected Impact Activities

- Flow cytometry is one of the foundational tools for biomedical research, allowing single cell profiling and functional assays. Despite decades of advances in the hardware of flow cytometers, the data processing side remained stagnant. We developed a new algorithm, called AutoSpill, which reduces the error in flow cytometery analysis by 100,000-fold. An industrial partnership allowed integration of this software into FlowJo, with a reach of ~80,000 users.
- To help children understand the COVID lockdown and the importance of vaccination, we produced two children's books, 'Just for Kids! All about Coronavirus' and 'Battle Robots of the Blood', translated into eleven languages and read by more than 5,000 children internationally.
- We developed a new technology allowing us to harness the anti-inflammatory properties of regulatory T cells in neuroinflammation. In animal models, this novel therapeutic can reduce brain damage by 50% following traumatic brain damage, stroke or experimental multiple sclerosis. This work has been patented and is being developed for human application.

## Publications


- Pasciuto, E., Burton, O.T. & Roca, C.P. *et al.* (2020) Microglia require CD4 T cells to complete the fetal to adult transition. ***Cell*** 182(3):625-640
- Dooley, J. *et al.* (2020) Heterogeneous effects of calorie content and nutritional components underlie dietary influence on pancreatic cancer susceptibility. ***Cell Rep.*** 32(2):107880
- Neumann J, Prezzemolo T, Vanderbeke L, Roca CP, *et al.* (2020) Increased IL-10-producing regulatory T cells are characteristic of severe cases of COVID-19. ***Clinical and Translational Immunology***. 9(11):e1204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**Claudia Ribeiro de Almeida**

**Group members**

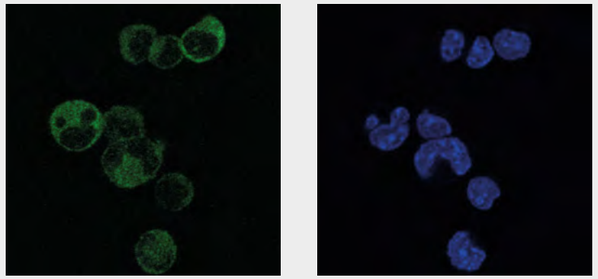
**Postdoctoral research scientist:**
Svetlana Sakhnevych

**PhD student:**
Rachael Kimber

**Visiting scientists:**
Isabel Leitch (Left in 2019)
Gwendoline Muller (Left in 2020)

# Understanding how RNA helicase activity impacts on antibody gene rearrangements

**We are interested in understanding the molecular mechanisms underlying diversification of antibody genes, to gain insight into how B cells can effectively fight infections and the role this plays in age-related immune dysfunction. Emerging evidence suggests important roles for RNA and RNA-binding proteins (RBPs) in these processes, which constitute new opportunities for therapeutic intervention. Our ongoing research focuses on a class of RBPs known as RNA helicases, and how their activity in remodelling RNA and RNA-proteins complexes regulates antibody gene rearrangement.**



*Confocal microscopy images of activated B cells showing cytoplasmic and nuclear staining for AID (green, left); nuclear area is defined using DAPI to stain DNA (blue, right).*

## Current Aims

Our knowledge of how antibody gene rearrangements are regulated at the RNA level is limited, compared to the well-characterised function of transcription factors and chromatin epigenetic modifications. Therefore, much of our current effort goes into profiling protein–RNA interactions that characterise B cell developmental stages undergoing antibody gene rearrangements. These analyses will provide important insight that will help prioritise RNA helicases for subsequent studies. We are also investigating how the RNA helicase DDX1 acts to modulate RNA G-quadruplex structures, which are directly implicated in targeting the DNA mutator enzyme AID to the immunoglobulin heavy-chain locus, to initiate a DNA recombination mechanism that changes the antibody isotype B cells produce.

## Progress in 2019 and 2020

We have implemented state-of-the-art methodologies to capture the RNA-bound proteome, and are now identifying protein–RNA interactions that characterise B cells actively undergoing antibody gene rearrangements. Interestingly, we have found that many RNA-binding proteins (including RNA helicases) interact with RNA in response to DNA damage signalling, initiated by the protein kinase ATM. One example is DDX1 and we are currently investigating its role in B-cell activation in vivo. Possibly related to DDX1's role in antibody isotype switching, we discovered DDX1 is required for B cell differentiation in antibody secreting cells and impacts on the quantity and quality of antibodies produced during an immune response.

## Selected Impact Activities

- Claudia Ribeiro de Almeida participated in the Midlands RNA Salon webinar series with a talk on the role of RNA helicases in modulating RNA structures during antibody gene rearrangements (May 2020).
- Claudia Ribeiro de Almeida presented at the BBSRC Virtual Annual Visit at the Babraham Institute (November 2020).
- The lab hosted Gwendoline Muller as an Erasmus student from Université Grenoble Alpes for a three-month summer research placement carried out remotely.

## Publications


- Ribeiro de Almeida, C. *et al.* (2018). RNA helicase DDX1 converts RNA G-quadruplex structures into R-loops to promote IgH class switch recombination. ***Mol. Cell.*** 70(4):650-662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**Sarah Ross**

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Jonathon Coates (Left in 2020)

**PhD students:**
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Marian Jones Evans

**Research assistant:**
Rebecca England

**Visiting student:**
Lily Tan (Left in 2020)

# Oxygen-dependent control of T cell immunity

**Oxygen is vital for the ability of mammalian cells to survive and function. Oxygen-sensing signalling pathways underpin the ability of cells to adapt to conditions where oxygen is limited. Our research explores the impact of oxygen-dependent signalling on the activity of cytotoxic T cells, which are adaptive immune cells that play an important role in maintaining human health.**

## Current Aims

During immune responses, cytotoxic T cells must attack and destroy infected, diseased or damaged cells in environments where oxygen availability is low.

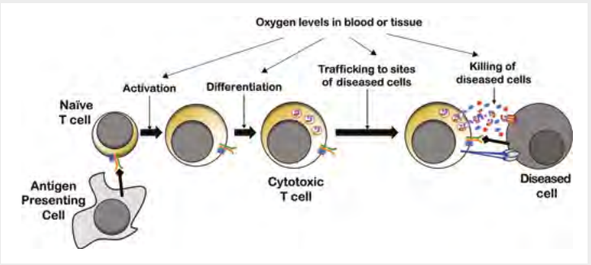
Our goal is to determine how oxygen influences the ability of T cells to respond to the signals in their environment that direct their function. Our current research aims to elucidate the molecules and cellular processes that are regulated by oxygen in T cells and to establish how oxygen availability can determine how T cells become cytotoxic killer cells that can eradicate diseased cells.

## Progress in 2019 and 2020

Oxygen levels control the abundance of many proteins that are critical for the function of cytotoxic T cells. These include metabolic enzymes; adhesion molecules that dictate how T cells migrate to diseased tissues; proteins that T cells use to kill cells; and receptors that determine how active T cells are towards diseased cells. Building on this work, through detailed analysis of cytotoxic T cells exposed to different oxygen environments, we have generated new insights into how oxygen controls the abundance of proteins in T cells. Our findings have important implications for understanding the activation, differentiation and function of T cells during an immune response.

## Selected Impact Activities

- We hosted two teachers in the laboratory as part of the Institute's 'Teachers' Day' initiative (2019).
- Sarah Ross was part of the Internal Management Group that oversaw the ORION public dialogue on genome editing in the UK (2019).
- We hosted two school pupils in the laboratory as part of the Institute's virtual Annual Schools' Day (2020).




*Schematic representation of T cell differentiation and function, indicating where oxygen supply may be playing an important role in influencing the immune response.*

## Publications


- Ross, S.H. & Cantrell, D.A. (2018) Signaling and function of interleukin-2 in T lymphocytes. ***Annu. Rev. Immunol.*** 36:411-433
- Rollings, C.M. *et al.* (2018) Interleukin-2 shapes the cytotoxic T cell proteome and immune environment-sensing programs. ***Sci. Signal.*** 11(526):eaap8112


www.babraham.ac.uk/our-research/immunology/sarah-ross

# The essential element

**Oxygen makes up 21% of the Earth's atmosphere and plays a pivotal role in biological systems. Despite this, huge gaps remain in our understanding of how this essential element regulates cell signalling pathways and affects our immune system – questions that Dr Sarah Ross aims to answer.**

We all need oxygen. We breathe it in some 17,000 times a day. And although we might sometimes feel its lack – sprinting for a bus or travelling to altitude – oxygen levels in our tissues vary widely in health as well during disease.

We are still learning how cells sense and adapt to low oxygen, and how oxygen affects our immune system – knowledge that will be critical for developing new therapies for cancer and other diseases. In 2019, the Nobel Prize for physiology and medicine was won by a trio of scientists who uncovered how cells sense and adapt to low oxygen. At the Institute, Dr Sarah Ross wants to discover how low oxygen – or hypoxia – can prevent our immune system from working effectively.

Ross is studying killer T cells – a critical component of the adaptive immune system – whose job is to hunt down pathogens and protect against cancer by producing cytotoxic compounds. To do their job effectively, they must travel widely and work well in the myriad oxygen climates they encounter.

"Our tissues have different oxygen concentrations and this has implications for immune cells," says Ross. "T cells usually reside in lymph nodes and the spleen, but when they are called on to fight infection or disease they often find themselves in foreign oxygen environments."

Many factors influence oxygen levels in our tissues: some are normal variations that reflect the oxygen needs of particular tissues, while others result from disease or ageing. Bacterial infections can drive up oxygen demand when bacteria and immune cells compete for oxygen. Areas around tumours can also become hypoxic due to uncontrolled cell division in cancer. Ageing, too, affects the picture because diseases that affect the lungs or blood vessels reduce the supply of oxygen.

Teasing out how these low oxygen environments hamper T cells' work is central to Ross's research. Her work focuses on three key areas: the role oxygen plays during an immune response, how oxygen is linked to age-related declines in the immune system and whether or not we can help T cells work better by targeting these oxygen-regulated pathways.

Answering such big physiological questions at a molecular level demands painstaking precision. Ross works in vitro with T cell cultures grown in standard conditions and, while her methods sound simple, they pose huge technical challenges. "Putting cells in different oxygen environments is easy. What's really difficult is controlling the amount of oxygen that each of those cells will sense," she explains.

This is because of the many variables involved. Different culture media will affect oxygen availability, as will biological variation among the cells. And variations in the density of cells in each culture also impact oxygen demand. "Some cultures use more oxygen than others, so we have to be very precise to avoid accidentally creating hypoxia," says Ross.

It's an observation that she believes has important implications for in vitro research well beyond her field. "Even if you're not studying oxygen, changes in cell density could be triggering hypoxia in cell cultures and causing variation in your data. It's across the board – in cancer cells, stem cells and immune cells – and while it's rarely talked about it's fundamental."

Her first couple of years at the Institute have been busy ones: Ross's group has been studying how duration of hypoxia affects T cells, how hypoxia alters the abundance of certain proteins and identifying which molecules are mediating these effects. Ongoing research is expanding this work to develop an understanding how the oxygen levels that a T cell experiences during an immune response defines their fate and function. The capability to do this research has been boosted by the acquisition of equipment to develop a hypoxia and physiological oxygen facility at Babraham, which was funded by the BBSRC.

Since joining the Institute in 2018 she's also been identifying common themes with her colleagues across the Institute. "What we are discovering in immune cells has importance for other cell types too, and we are always finding new connections with the work going on in Signalling and Epigenetics."

Understanding which proteins are being regulated in T cells and how these might be controlling T cell function has important implications, because by working out which molecules are involved, researchers will be able to identify new therapeutic targets against a range of diseases as well as using existing drugs in different ways.

"Hypoxia is relevant for multiple diseases. An obvious one is cancer, because we know it's linked to hypoxia. But it might also play a role in how T cells function in inflammatory diseases like arthritis and Inflammatory Bowel Disease, which is something we need to investigate more," Ross concludes.

**'Learning how oxygen affects our immune system is relevant for multiple diseases'**

# Responding to the Covid crisis

**As well as exposing weaknesses in healthcare systems and supply chains, the coronavirus pandemic has underscored the importance of fundamental research and collective effort. During 2020, scientists rose to the challenge of developing new vaccines and effective treatments for Covid-19. Institute immunologists Dr Michelle Linterman and Professor Adrian Liston describe how their labs responded and the lessons we must learn.**

In the early days of the coronavirus pandemic, as lockdowns loomed, workplaces closed and travel slowed to a trickle, Dr Michelle Linterman was certain of one thing – she wanted to make her group's expertise available to the global vaccines effort.

Among those working on a vaccine against SARS-CoV-2 (the coronavirus that causes Covid-19) was Dr Teresa Lambe at the Jenner Institute in Oxford. "I already knew Tess, so once it became clear they had a vaccine candidate, my first instinct was to ask her what we could do to help," Linterman recalls.

As an immunologist, Linterman's work focuses on how the immune system responds to vaccines. In particular, she wants to understand why older people respond less well to vaccines, something she studies using human vaccination studies and in aged mice. "I thought the most useful thing was for us to offer something that nobody else could contribute quickly – and that was our ability to use aged mice as a pre-clinical test of how this vaccine is likely to work in an ageing immune system," she says.

When Lambe said yes, Linterman set up trials to compare immunological responses to the Oxford/AstraZeneca vaccine in young and aged mice, and discovered that although aged mice responded more poorly than young mice to a single dose, after two doses of the vaccine, the immune responses were very good in both groups.

The study helped both institutes. For the Jenner, it showed two doses of the vaccine would give good protection against infection in all adults. For Babraham, it provided new insights into vaccine responses at a cellular and molecular level, expanded research into new vaccine platforms and led to new collaborations. Most importantly, it illustrated the value of publicly-funded research.

"Because we're funded by the BBSRC – in other words the tax payer – it was incredibly important to use our knowledge and expertise to contribute to vaccine development in the midst of the pandemic," she says.

Fellow immunologist Professor Adrian Liston also stepped up to the mark, using his research to help clinicians make the best treatment choices for Covid-19 patients and his communication skills to provide accurate information to journalists and the public.

"We need to develop good systems for treating emerging viruses before we know much about them, which is something my lab is working on," explains Liston. "We are coming up with treatments that are vaccine agnostic, treatments that will work for most viruses with the potential to become pandemic, regardless of the actual virus."

Liston's group is also interested in systems immunology – exploring what makes people's immune systems so different from each other. This variation has been graphically illustrated during the pandemic, some people experiencing mild symptoms while others died. "Diversity is intrinsically important to the immune system. It's the most genetically-diverse system in the human body, and there are other factors at play, such as age, gender and weight," he explains.

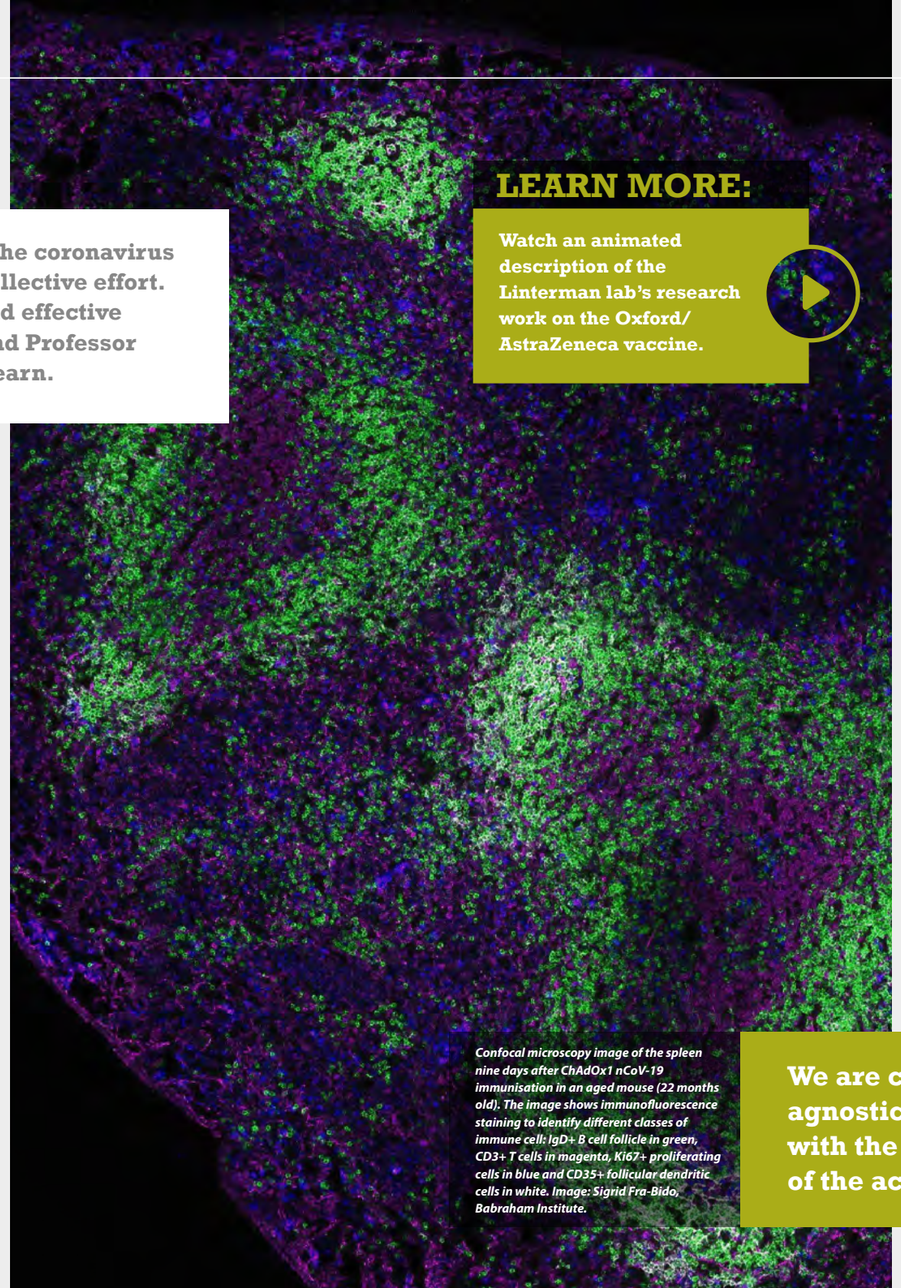
Being so close to events has taught Liston and Linterman many lessons – lessons, they say, that are vital for political leaders to learn. First, zoonoses (diseases spread between animals and humans) with pandemic potential are far from rare events. "They occur every couple of years," says Liston. "We've had coronavirus outbreaks before, like SARS and MERS; they happen like clockwork. In the previous outbreaks we had better luck and better preparation. These are things we must prepare for."

Secondly, we must guard against complacency. "If we pat each other on the back for a job well done, and then slash science budgets, the next outbreak will be as bad as this one," he warns. "We must fund surveillance as well as immunology and virology research, because if you scale down this science it takes a decade or more to rebuild that intellectual capital."

This preparation extends to supporting fundamental research in a broad range of areas. "We need to fund fundamental research because you're never sure which bit of it will save you in the future," says Linterman.

Third, a global approach to research, and funding to support this, is essential, because scientific discoveries are not bounded by borders, adds Linterman: "One of the reasons the Oxford vaccine was developed so fast was because of years of work on Ebola and MERS using the same adenoviral vaccine vector."

As vaccines are rolled out, and countries emerge from lockdown, we might usefully reflect on what we would have done without a vaccine. It's a scenario that frightens Linterman. "There wasn't another exit strategy," she says. "The vaccines are great, far better than we expected. But there are pathogens that we don't have good vaccines for. For me, that's the scary thing. We're lucky the vaccines are so effective – but that doesn't mean the same will be true for the next pandemic."



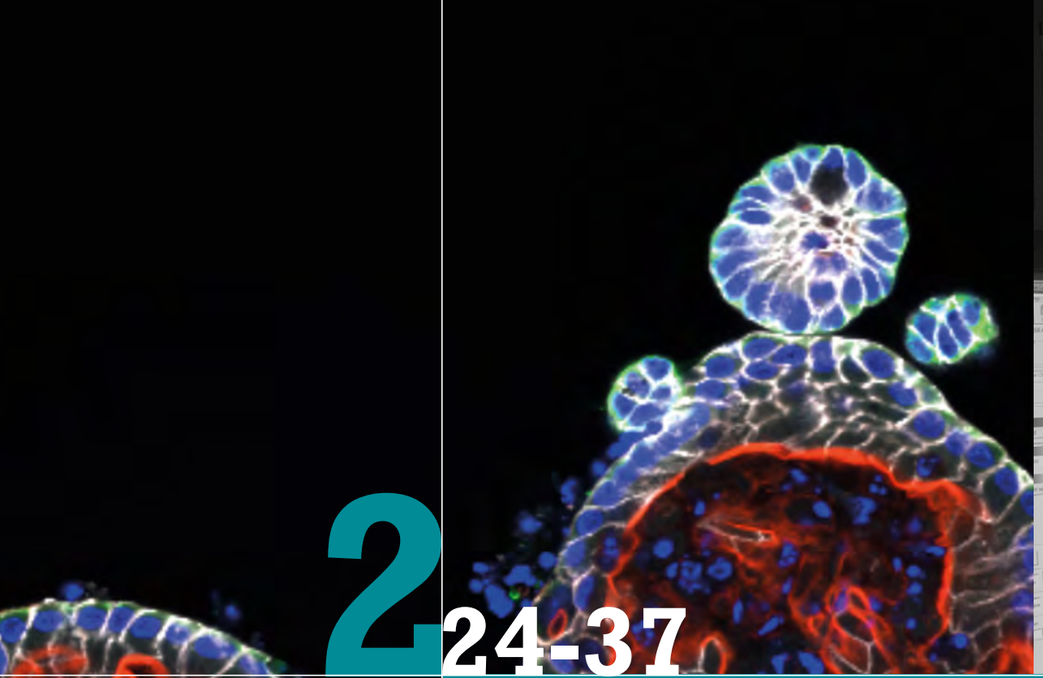
**LEARN MORE:**

**Watch an animated description of the Linterman lab's research work on the Oxford/ AstraZeneca vaccine.**

*Confocal microscopy image of the spleen nine days after ChAdOx1 nCoV-19 immunisation in an aged mouse (22 months old). The image shows immunofluorescence staining to identify different classes of immune cell: IgD+ B cell follicle in green, CD3+ T cells in magenta, Ki67+ proliferating cells in blue and CD35+ follicular dendritic cells in white. Image: Sigrid Fra-Bido, Babraham Institute.*

**It was incredibly important to use our knowledge and expertise to contribute to vaccine development in the midst of the pandemic** – ***Michelle Linterman***

**We are coming up with treatments that are vaccine agnostic, treatments that will work for most viruses with the potential to become pandemic, regardless of the actual virus** – ***Adrian Liston***



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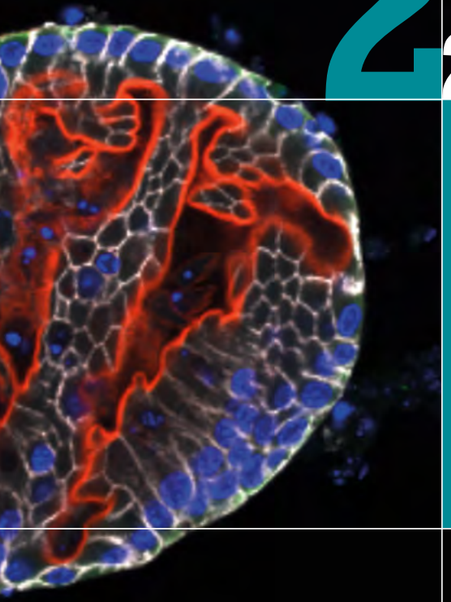
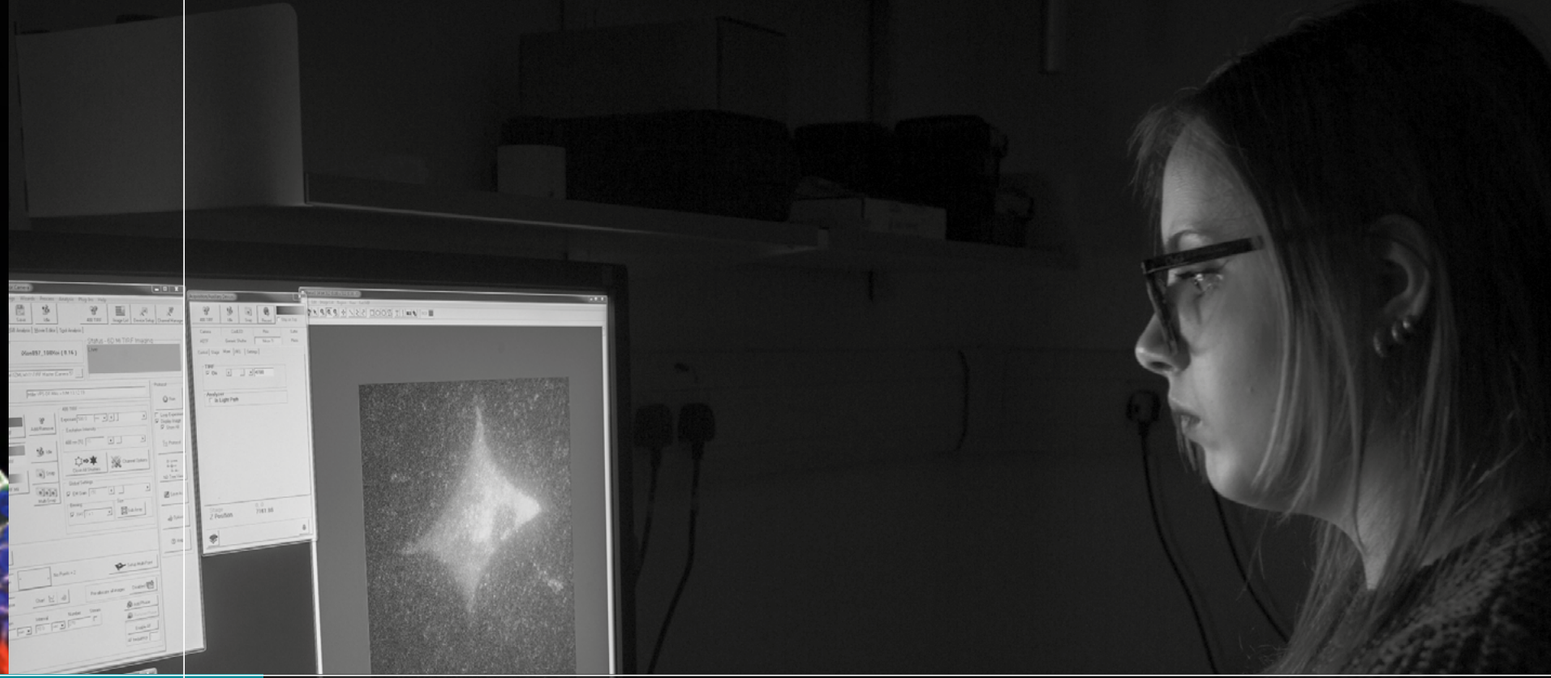


## Signalling

The process of cell signalling consists of several interconnected mechanisms that allow cells to communicate, co-ordinate and respond rapidly to change. By examining these signalling mechanisms and their interactions we seek to understand the effects of signalling on cell growth, survival and behaviour.

Our current focus is to discover the role that signalling has in helping cells to respond and adapt to damage, illness, dietary changes and ageing by investigating:

- How cells called neutrophils detect and respond to infections
- How changes in diet affect metabolism and growth
- The effect of signalling mechanisms on the rate of ageing
- The role of autophagy in recycling cell components following damage or starvation



### Group Leaders


Simon Cook


Oliver Florey


Phill Hawkins


Nicholas Ktistakis


Rahul Samant


Hayley Sharpe


Len Stephens


Heidi Welch

**Simon Cook**
**Programme leader**

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**Senior research scientists:**
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Pamela Lochhead
(Left in 2020)
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Frazer Cook
Laura Weatherdon

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Andrew Kidger (Left in 2020)
Emma Minihane (Left in 2020)
Jack Prescott (Left in 2020)
Matthew Sale
Kate Stuart (Left in 2019)
Maxi Wandmacher
(Left in 2020)

**Visiting students:**
Rachael Huntly (Left in 2020)
Giri Kiritharan (Left in 2019)
Richard Odle (Left in 2020)
Elizabeth Zhabina
(Left in 2019)

# Signalling pathways in health and disease

**Our goal is to better understand how protein kinase signalling pathways maintain health and how this may be deregulated in disease. Some pathways control the cellular recycling process known as autophagy whilst others control whether cells live or die. We also work with biotech and pharma companies to translate our knowledge to support the development of new drugs.**

## Current Aims

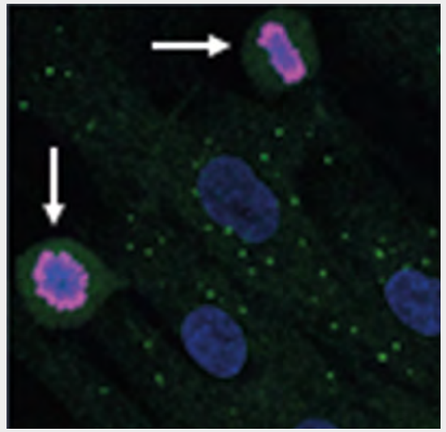
The ERK1/2 and ERK5 signalling pathway controls whether cells survive and divide or whether they senesce (a form of cellular ageing) or die. We are interested in how these different outcomes are controlled and are seeking to identify novel regulators of these pathways. In addition, we are studying autophagy, the cellular recycling process that is activated when nutrients are scarce to keep cells alive. We want to understand how autophagy is controlled so that it is only activated at the right time, to support cell survival, and does not run out of control and kill cells.

## Progress in 2019 and 2020

Ongoing collaborations with AstraZeneca have defined the role of an ERK1/2-regulated protein MCL1 as being critical for the survival of melanoma cells; in so doing we have identified a novel drug combination that effectively kills melanoma tumour cells. We have also shown that autophagy is inhibited during mitosis, the process of cell division into two daughter cells. This repression of autophagy ensures that the non-selective autophagy machinery does not accidentally degrade chromosomes, that would otherwise cause genetic damage to be passed on to daughter cells. We think this temporally distinct repression of autophagy is critical for lifelong health.

## Selected Impact Activities

- Simon Cook and PhD student Richard Odle gave talks at the 2019 mTOR session at the 2019 NCRI conference and the 2019 Keystone Autophagy conference.
- PhD student Richard Odle was part of the team that presented the Signalling Escape Room at the Latitude Festival in 2019.
- The Cook lab has ongoing collaborations with PhoreMost, a biotech company based on the Babraham Research Campus, and AstraZeneca.



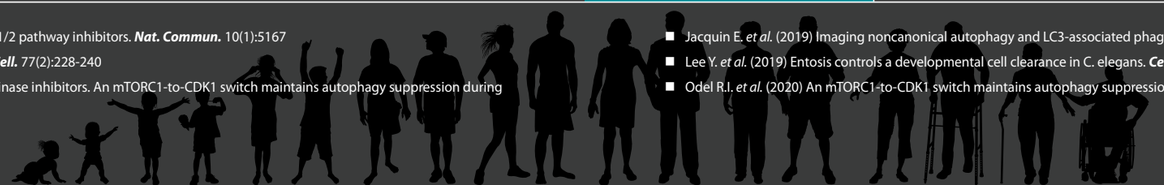
*Immunofluorescent images of autophagy recycling centres (green = ATG13 protein). Individual cells are revealed by staining for DNA in the nuclei (blue). Most cells have active autophagy recycling centres (green dots). However, cells undergoing mitosis (with condensed chromosomes, marked by arrows) lack active autophagy.*

## Publications


- Sale, M.J. *et al.* (2019) Targeting melanoma's MCL1 bias unleashes the apoptotic potential of BRAF and ERK1/2 pathway inhibitors. ***Nat. Commun.*** 10(1):5167
- Odle, R.I. *et al.* (2020) An mTORC1-to-CDK1 switch maintains autophagy suppression during mitosis. ***Mol. Cell.*** 77(2):228-240
- Lochhead, P.A. *et al.* (2020) Paradoxical activation of the protein kinase-transcription factor ERK5 by ERK5 kinase inhibitors. An mTORC1-to-CDK1 switch maintains autophagy suppression during mitosis. ***Nat. Commun.*** 11 (1):1383


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





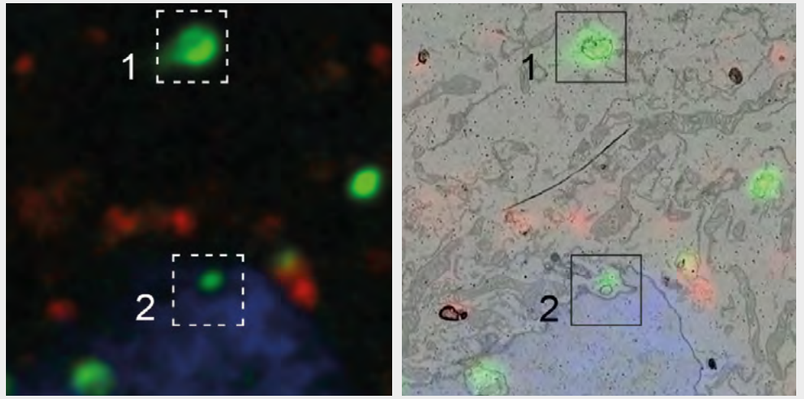
**Oliver Florey**

**Group members**

**Postdoctoral researchers:**
Joanne Durgan
Kirsty Hooper

**PhD student:**
Katie Sloan (Left in 2020)

# Maintaining the cellular waste disposal system




*Immunofluorescent images of autophagy markers (green = LC3, red = LAMP1, blue = DNA) and correlative electron micrographs acquired by FIB-SEM. Boxed areas show two autophagosome structures.*

**Our goal is to better understand lysosome degradation systems in both health and disease. We investigate autophagy and related pathways which we hope can be exploited to reverse the decline in lysosome function that is seen with increased age.**

## Current Aims

We are currently focusing on a 'non-canonical' autophagy pathway, which utilises some of the autophagic machinery to target ATG8 lipidation to endolysosomal membranes. This pathway plays important roles in cellular responses to pathogens, including influenza A virus, and stress. A key open question we are addressing is: what are the exact functions of ATG8 proteins at these membranes? By understanding how the pathway is regulated, we hope to be able to harness it for therapeutic benefit.

## Progress in 2019 and 2020

We have continued to make progress in our understanding of the non-canonical autophagy pathway by revealing molecular signatures that are associated with it, and the development of tools to specifically manipulate it. Ongoing commercial collaborations have revealed a link between activation of the pathway and maintenance of the lysosomal system, which may underlie the functional role of non-canonical autophagy.

## Selected Impact Activities

- Dr Oliver Florey presented recent work at both Keystone and EMBO conferences.
- Jo Durgan has been invited to speak at many institutes and universities on Green Lab initiatives.
- The Florey lab has ongoing collaborations with Casma Therapeutics, a biotech company based in the US.

## Publications


- Jacquin E. *et al.* (2019) Imaging noncanonical autophagy and LC3-associated phagocytosis in cultured cells. ***Methods Mol. Biol.*** 1880:295-303
- Lee Y. *et al.* (2019) Entosis controls a developmental cell clearance in C. elegans. ***Cell Rep.*** 26(12):3212-3220
- Odel R.I. *et al.* (2020) An mTORC1-to-CDK1 switch maintains autophagy suppression during mitosis. ***Mol. Cell.*** 77(2):228-240


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

Phill Hawkins

Len Stephens

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**Senior postdoctoral researcher:**
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**Research fellow:**
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Piotr Jung

**Senior technician :**
Keith Davidson

**PhD students:**
Arqum Anwar
Danny Collins
Beth Cragoe

*Continued on page 33*

# The regulation of cell signalling by PI3Ks

**Cells communicate and respond to their environment through signalling pathways. These are molecular pathways that allow changes in the levels of hormones, growth factors or nutrients to be sensed by cell surface receptor proteins and then translated into defined changes in cell behaviour. One such signalling pathway involves the production of a chemical signal inside cells called PI(3,4,5)P3, which is a particular type of membrane phospholipid that is made by enzymes called phosphoinositide 3-kinases (PI3Ks). This pathway plays a major role in the regulation of growth, metabolism, and immunity, and changes to this pathway are seen during ageing and in several human diseases.**

## Current Aims

Our current work is aimed at:

1. Understanding how the PI3K signalling pathway allows certain immune cells (neutrophils and macrophages) to combat foreign invaders and how this capability declines with age.

2. Defining how different, closely related PI3K enzymes are used selectively to regulate cell growth and metabolism in response to changes in nutrient supply and growth factors. This work supports the pharmaceutical industry's attempts to target this pathway therapeutically.

3. Discovering new molecular mechanisms that drive activation of the PI3K pathway.

4. Discovering how the cell compartmentalises the synthesis of PI(3,4,5)P3 and related phospholipids from other, non-signalling molecules.

## Progress in 2019 and 2020

Work in the group during this period has led to the following developments:

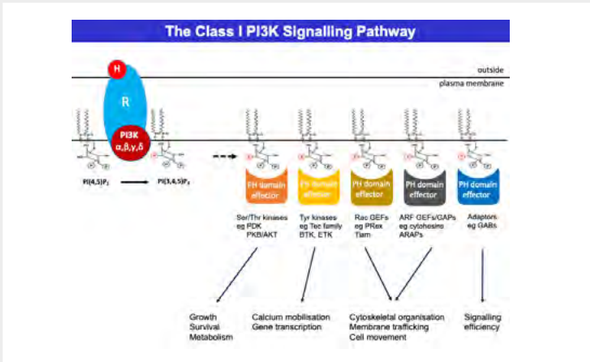
1. We have identified some of the key players in 'priming' the PI3K pathway during a neutrophil's response to pro-inflammatory stimuli (ref. 1).

2. We have made progress in understanding how the hydrophobic chain composition of relevant phospholipids regulates their synthesis and function (ref. 2).

3. We have identified a key molecular mechanism by which PI3K is regulated by cell surface receptors in neutrophils (ref. 3).

## Selected Impact Activities

- We have collaborated with the pharmaceutical industry through joint grants and serving on scientific advisory boards.
- We have collaborated with academic groups in the USA, Ireland, Canada, Switzerland Germany and the UK and presented our work at five international conferences.
- We have trained five overseas students (from Germany, France, Spain and the Netherlands).




*An overview of the PI3K signalling pathway. Hormones (H) bind to cell surface receptors (R) to activate one of four PI3K isoforms (α, β, γ, δ), which then convert a phospholipid called $PI(4,5)P_2$ into one called $PI(3,4,5)P_3$ (by attaching a phosphate group from ATP onto the 3-position of its inositol ring). $PI(3,4,5)P_3$ then diffuses through the membrane and selectively binds to a conserved 'PH domain' that is present in 20-30 'effector' proteins (some examples are given). This interaction alters the location and activity of these effector proteins and thus passes the message from the hormone onto the proteins regulating cell growth, metabolism, movement etc.*

## Publications

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


- Suire, S. *et al.* (2019) TNF-α and GM-CSF1 priming augments the role of SOS1/2 in driving activation of Ras, PI3K-γ, and neutrophil proinflammatory responses. ***J. Leukoc. Biol.*** 106:815-822
- Barneda, D. *et al.* (2019) How is the acyl chain composition of phosphoinositides created and does it matter? ***Biochem. Soc. Trans***. 47:1291-1305
- Rynkiewicz, N. *et al.* (2020) Gβγ is a direct regulator of endogenous p101/p110γ and p84/p110γ PI3Kγ complexes in mouse neutrophils. ***Sci. Signal.*** 13:eaaz4003




Nicholas Ktistakis

**Group members**

**Senior postdoctoral researcher:**
Maria Manifava

**Research assistants:**
Bonnie Man
Peri Tate (Left 2019)

**Visiting students:**
Qashif Ahmed
Angela Braho
Emilia Hubbard (Left in 2019)
Katerinai Kafka (Left in 2019)
Nikolaos Kontopoulos (Left in 2020)
Theodora Maniati (Left in 2020)
Felipe Renna
Milene Ortiz Silva
Filianna Tanti (Left in 2019)
Charalampos Toramanidis

**Visiting scientists:**
Luisa Giudici (Left in 2019)
Varvara Kandia

# Dynamics of autophagy in animal cells

**Autophagy is a conserved pathway among all eukaryotes that senses either nutrient levels or damaged organelles and proteins in the cytosol. In the case of starvation, autophagy generates nutrients from self-digestion whereas the presence of damaged proteins or organelles triggers autophagy to eliminates them via delivery to the lysosomes. Autophagy is mediated by double membrane vesicles termed autophagosomes that engulf either random cytoplasmic material for nutrient generation or specific cargo for elimination.**


*Modelling of selective autophagy on large targets. A series of translocations of the autophagic machinery generates piece by piece formation of autophagic structures that eventually cover all the targeted area. Such a mechanism will generate oscillatory behaviours during live imaging.*

## Current Aims

Our work aims to understand how autophagy is induced in mammalian cells, and the specific dynamics of the membrane re-arrangements required for the appearance of autophagosomes. Although we initially focused specifically on non-selective autophagy, we are now working on various pathways of selective autophagy, such as mitophagy (mitochondrial autophagy) and aggrephagy (autophagy of protein aggregates). In addition to work on tissue culture cells, we are now working on iPSC-derived neuronal cells trying to understand how autophagy modulates neurodegeneration.

## Progress in 2019 and 2020

We have modelled the process of autophagy and mitophagy using an extensive collection of live imaging data and discovered a possible explanation for why the process of mitophagy involves the sequential translocation of autophagy components to the targeted mitochondrion in an oscillatory fashion. Our proposal is that creating autophagosomes on large structures, such as a bacterium or a mitochondrion, requires piece by piece formation of pre-autophagosomal membranes that are 'stitched' together to cover the entire structure (shown in the figure).

We are currently working on aspects of autophagy induction prior to the omegasome step, on mitochondrial autophagy in fibroblasts from mitochondrial disease patients and on the characterisation of two novel autophagy inducers isolated in our lab.

## Selected Impact Activities

- Invited speaker at the University of Michigan Protein Folding Diseases seminar series on the dynamics of autophagy, November 2020.
- Invited speaker for the Pollard Lecture at MitOX 2020 hosted by the University of Oxford in December 2020.
- Invited speaker at the Molecular Mechanisms of Autophagy in Diseases conference, St Petersburg, Russia, October 2020.

## Publications

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


- Dalle Pezze, P. *et al.* (2020). ATG13 dynamics in nonselective autophagy and mitophagy: insights from live imaging studies and mathematical modeling. ***Autophagy*** 17(5):1131-1141
- Kishi-Itakura, C. , Ktistakis, N. T. & Buss, F. (2020). Ultrastructural insights into pathogen clearance by autophagy. ***Traffic*** 21(4):310-323
- Dong, X. *et al.* (2021). Sorting nexin 5 mediates virus-induced autophagy and immunity. ***Nature*** 589(7842):456-461 [Epub 16 Dec 2020]

**Rahul Samant**

**Group members**

**Postdoc research scientist:**
Harvey Johnston

**PhD student:**
Yasmeen Al-Mufti

**Research assistant:**
Estelle Wu

# Why misfolded proteins accumulate with age

**Cellular accumulation of misfolded proteins is a hallmark of ageing. In young cells, the proteostasis network limits toxicity by activating one or more systems for misfolded protein clearance. We focus on how these clearance systems are integrated within the network to maintain proteome health during youth, and how loss of this integration contributes to cellular senescence, another ageing hallmark with strong links to chronic inflammation and organismal frailty.**

## Current Aims

We use two evolutionarily distant cell types, budding yeast and primary human fibroblasts, to identify common, conserved lines of communication between different clearance systems of the proteostasis network, and investigate how these are re-wired during replicative ageing (yeast) and senescence (mammals). Our lab employs multi-disciplinary approaches such as super-resolution imaging, flow cytometry, and mass spectrometry-based proteomics to measure proteostasis capacity and senescence phenotypes as quantitatively and robustly as possible. As proteostasis modulators hold therapeutic promise in ageing-associated pathologies—with renewed interest in 'senolytics' specifically targeting senescent cells—we hope to drive fundamental discoveries that have a direct impact on promoting lifelong health.

## Progress in 2019 and 2020

Our lab was established in October 2019, and we have grown to four people. Harvey Johnston has been developing a proteomic method that will considerably simplify and accelerate sample preparation for mass spectrometry, as well as setting up a high-performance liquid chromatography system that will allow more in-depth proteome coverage in these experiments. Estelle Wu and Yasmeen Al-Mufti have been building up a library of primary human fibroblasts at different stages along the senescence process. Estelle has also been using CRISPR-Cas9 to tag key members of the proteostasis network in these fibroblasts, so that we can track misfolded protein accumulation and clearance using super-resolution microscopy. We are also excited about working with the Imaging and Flow Cytometry facilities to quantify and isolate live senescent cell populations for downstream functional assays, a technique that would open up many new possibilities in senescence research.


*We use a multi-disciplinary approach (left panel), using high-resolution imaging, flow cytometry, and mass spectrometry-based proteomics, to probe the relationship between loss of proteostasis and onset of senescence—two of the hallmarks of ageing (middle—adapted from Lopéz-Otín et al., Cell, 153(6), 2013 and replicated with permission from Elsevier). Our current focus is on the interplay between different protein clearance systems in young vs. senescent cells (right).*

## Selected Impact Activities

- We have initiated collaborative projects focusing different aspects of proteostasis and senescence with academic groups in the UK, Germany, and Canada, as well as industrial partners on the Babraham Research Campus (Methuselah Health UK and PhoreMost Ltd.).
- Rahul Samant gave invited presentations at national (UK Chaperone Club Meeting 2020) and international (EMBO Workshop on Proteostasis; Methuselah Health Conference on 'Why we age') meetings.
- Harvey Johnston, as chair of the London Proteomics Discussion Group, helped transform these in-person meetings into a successful webinar series during the pandemic—which now regularly attracts speakers and audiences from across the world.

## Publications


- Johnston, H.E., & Samant, R.S. (2020) Alternative systems for misfolded protein clearance: life beyond the proteasome. ***FEBS J.*** doi: 10.1111/febs.15617
- Samant, R.S., Masto, V.M. & Frydman, J. (2019) Dosage compensation plans: protein aggregation provides additional insurance against aneuploidy. ***Genes Dev.*** 33(15-16):1027-1030
- Samant, R.S., & Frydman, J. (2019) Methods for measuring misfolded protein clearance in the budding yeast Saccharomyces cerevisiae. ***Methods Enzymol.*** 619:27-45


www.babraham.ac.uk/our-research/signalling/rahul-samant

@SamantLab

**Hayley Sharpe**

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Kasia Wojdyla

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Tiffany Lai
Lauren Maggs

**Research assistant:**
Oisharja Rahman

**Visiting students:**
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Iain Hay
Katherine Young

# Cell signalling through tyrosine phosphatases

**The reversible phosphorylation of protein tyrosine residues enables cells to dynamically respond to changes in their environment and is regulated by the antagonistic actions of kinases and phosphatases. We focus on the understudied phosphatases to understand how they signal, their roles in health and disease and how they are regulated, particularly by reactive oxygen species, which are implicated in the ageing process.**

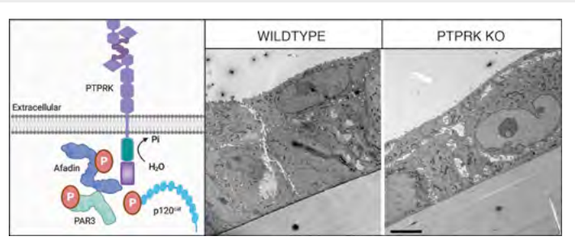
## Current Aims

Our overarching aim is to understand mechanisms of tyrosine phosphatase signalling in order to understand their fundamental functions but also to reveal new approaches to targeting them in disease, to overcome their undruggable reputation. Our current work is focused on a family of receptor tyrosine phosphatases that are present on the cell surface and form homophilic interactions at points of cell–cell contact. The receptor PTPRK is a tumour suppressor and had been suggested to regulate cell adhesion. To gain insight into its signalling we are working to identify its direct substrates and to understand its cellular function using proteomic approaches, structural studies, mouse models and gene expression profiling.

## Progress in 2019 and 2020

We have defined high confidence substrates for PTPRK, using unbiased interactomes and phosphoproteomics. This revealed a key role for PTPRK in the regulation of cell–cell adhesion, and we found that deleting PTPRK leads to changes in cell morphology that we plan to further investigate (research described in ref. 1).

We have also found that a related receptor, PTPRU, is in fact a pseudophosphatase. Using structural studies we found conformational features that explain its lack of enzyme activity. Curiously, despite being inactive, PTPRU binds to PTPRK substrates. This led us to propose that this inactive receptor competes for substrates with its active paralogues and could even form a signalling scaffold (ref. 2). Pseudophosphatases are poorly studied but play an increasingly appreciated role in cellular signalling events (reviewed in ref. 3).




*The adhesive receptor protein tyrosine phosphatase PTPRK is expressed in epithelial cells at sites of cell–cell contact. We have identified key substrates linked to cell adhesion (left). Transmission electron microscopy images reveal that deleting PTPRK from mammary epithelial cells leads to disrupted cell junctions and adhesions, as well as decreased cell height, reminiscent of an epithelial to mesenchymal transition (right).*

## Selected Impact Activities

- Industrial collaboration underway with AstraZeneca (through a Collaborative Training Partnership).
- Hosted a Wellcome-funded summer student.
- Hayley Sharpe was profiled in the Journal of Cell Science in November 2020 as a cell scientist to watch.

## Publications


- Fearnley, G.W. *et al.* (2019) The homophilic receptor PTPRK selectively dephosphorylates multiple junctional regulators to promote cell-cell adhesion. ***eLife*** pii: e44597
- Hay, I.M. *et al.* (2020) The receptor PTPRU is a redox sensitive pseudophosphatase. ***Nat. Commun.*** 11:3219
- Reiterer, V. *et al.* (2020) The dead phosphatases society: a review of the emerging roles of pseudophosphatases. ***FEBS J.*** 287(19):4198–4220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@Sharpe_lab

**Heidi Welch**

**Group members**

**Senior postdoctoral researcher:**
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Elizabeth Hampson
Polly Machin
Chiara Pantarelli (Left in 2019)
Elpida Tsonou (Left in 2020)

**Research assistant:**
Laraine Crossland

**Visiting students:**
Harriet Banks (Left in 2020)
Abhi Gowda (Left in 2019)
Borjan Venovski

# Cell signalling through Rac-GEFs

**Rac is a protein that enables cells to attach and to move. We study how Rac is controlled by other proteins called GEFs that switch Rac on. Our recent research has identified new roles for Rac-GEFs in the immune system and in metabolism. In addition, we have made progress in understanding how Rac-GEFs are controlled and how they carry out many different roles within the same cell.**

## Current Aims

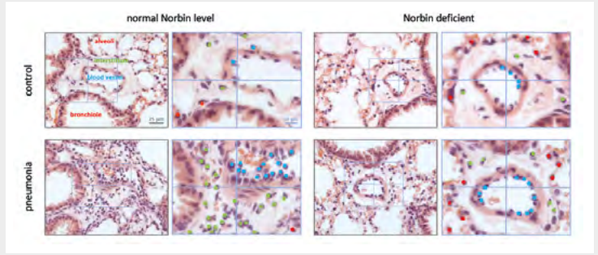
We previously discovered a family of Rac-GEF proteins we called P-Rex. We described how P-Rex1 allows white blood cells to fight disease, and we found a new protein, Norbin, that controls P-Rex1. Recently, we found unexpectedly that Norbin suppresses the immune system, and our current aim is to uncover the underlying mechanisms. We also work towards a better understanding of the importance of P-Rex GEFs and their catalytic activities in metabolism, and of the catalytic activities and roles of other Rac-GEFs in the immune system. This knowledge will be valuable for understanding the basic biology of these proteins, how they contribute to maintaining lifelong health, and what diseases can arise when they do not work properly.

## Progress in 2019 and 2020

We showed that Norbin suppresses immune defence against infections through surprising and important roles in neutrophils, a type of white blood cell, with implications for lifelong health. We have also identified new roles for Rac-GEFs in the immune system and in the maintenance of healthy blood glucose levels, and this work is ongoing. We found new cellular roles for P-Rex1 in nerve cells. Finally, we contributed to a study by the Mitchell lab in Melbourne, Australia, which identified that P-Rex1 is important for the initiation and metastasis of mammary tumours in mice (ref. 1).

## Selected Impact Activities

- Ongoing collaborations with Bioscience Metabolism, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM), AstraZeneca, Cambridge, UK and with Vernalis (R&D) Ltd, Cambridge, UK.




*Mice that lack Norbin protein in their neutrophils and macrophages (types of white blood cells), have tenfold increased immunity against bacterial pneumonia infections. This is dependent on neutrophils rather than macrophages. These images show neutrophils labelled with coloured dots depending on where they are in the lung, demonstrating than neutrophils which lack Norbin get to the site of infection in a different manner to normal neutrophils. Norbin-deficient neutrophils are also better at killing bacteria than normal neutrophils. This study identified Norbin as a suppressor of the immune response to bacterial infections, which was surprising as the protein was previously only known for its roles in nerve cells.*

*Continued from page 28*

**Phill Hawkins**
**Len Stephens**

**Group members continued:**

**Visiting students:**
Sarah Perrenot
(Left in 2019)
Paula Samso Ferre
(Left in 2019)

## Publications


- Srijakotre, N. *et al.* (2020) PtdIns (3,4,5)P3-dependent Rac exchanger 1 (P-Rex1) promotes mammary tumor initiation and metastasis. ***Proc. Natl. Acad. Sci.*** USA 117(45):28056-28067


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

# Back to basics

**Setting up a new group is exciting and daunting. Two group leaders who joined the Signalling programme in 2019 – Dr Hayley Sharpe and Dr Rahul Samant – talk about their research and the supportive, collaborative and open environment that they say marks out the Institute.**

Lots of ingredients go into building a successful new research group. Great ideas, a productive team and the right environment are all part of the mix.

A great illustration of the Institute's ethos was a colleague's reaction to news that Sharpe had been selected as an EMBO Young Investigator. "I was at my computer, saw the news and leapt up. One of the Principle Investigators happened to be passing and just walked in and gave me a big hug," Sharpe remembers. "Everyone is so supportive – and everyone's made a real effort to make us feel very welcome."

Sharpe's group works on a family of enzymes that – for the past two decades – has been largely ignored but which Sharpe believes is ripe for a renaissance.

These Cinderella enzymes are receptor tyrosine phosphatases, which play a vital role in intercellular communication and in the 1990s were seen as promising therapeutic targets. "There was huge interest in them 20 years ago and lots of work was done," Sharpe explains. "But they proved very hard to drug, so the pharmaceutical industry fell out of love with these enzymes and abandoned them," Sharpe explains.

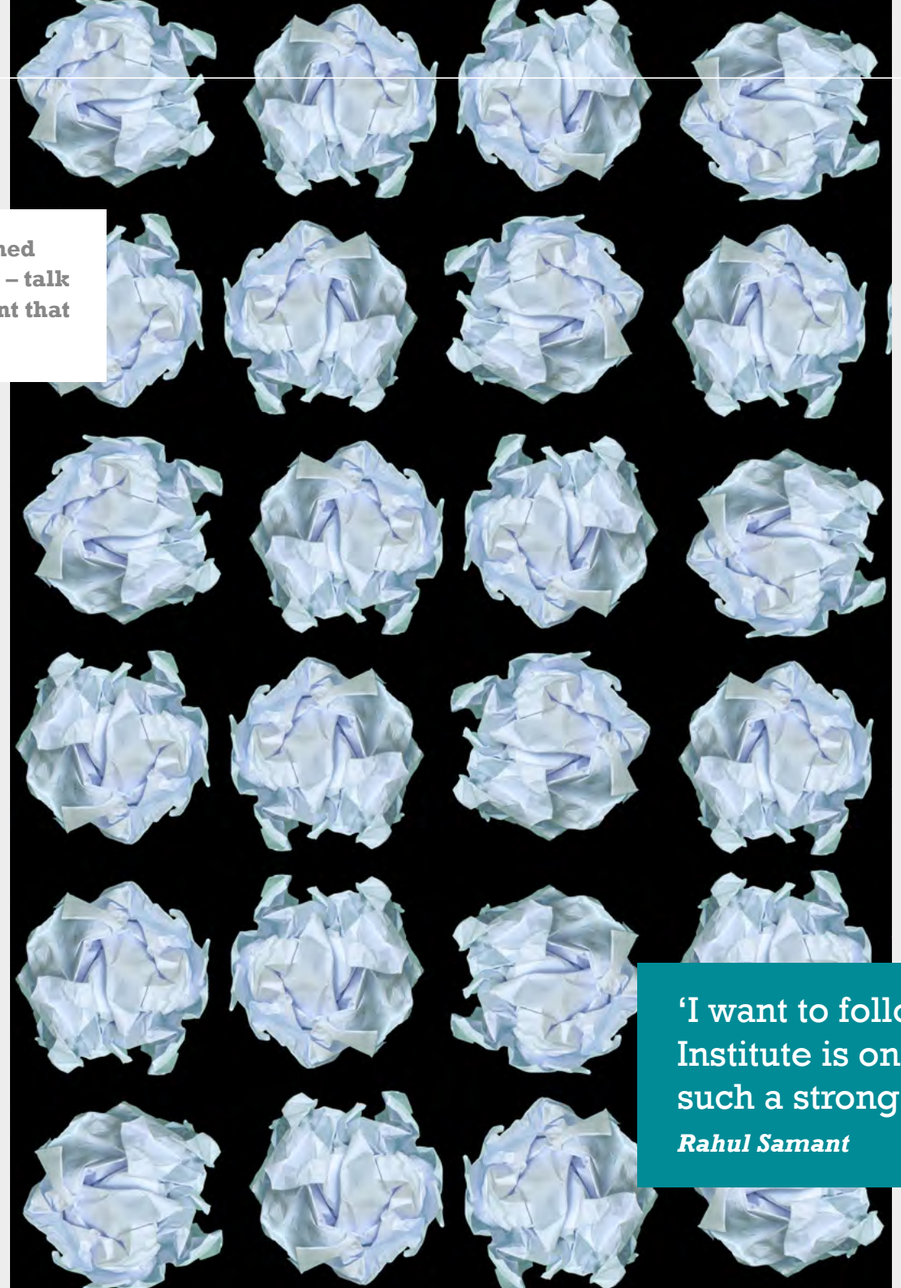
Recent advances, however, have rekindled research interest. Big data, CRISPR and other new tools point to tyrosine phosphatases being important in many diseases – including some cancers and diabetes-related macular degeneration – as well as spinal cord injuries and skin ageing. "When you're setting up a lab you want to go into an area where you can work for the next 30 years, hence the appeal of these enzymes," she says.

In a neglected field, developing new therapies means going back to basics, which is part of Sharpe's approach. She is working at a molecular level to discover how these enzymes help build up the layers of our skin and other tissues. She's also using mouse models to understand their role in disease, aiming to translate new knowledge into future new therapies.

Basic biology is also what drives Dr Rahul Samant. Reflecting on his impressions as a new appointment at the Institute Samant says that what struck him most about the Institute was its openness.

"The environment here opens up broader scientific thinking; conversations are like relaxed brainstorming, and having people to bounce ideas off is important for me," he says. "I do science because I want to know how things work. I want to be able to follow where the science leads me. And the Institute is one of the few research centres that has such a strong focus on fundamental, mechanistic biology."

As a cell biologist, Samant is fascinated by misfolded proteins and the way our cells prevent them from building up. Our cells are complex machines with many moving parts; to work smoothly, proteins must be the right size, shape, and in the right place. It only takes one misfolded protein to trigger a chain reaction that can lead to disease, so our cells invest heavily in sophisticated quality control systems to deal with misfolded proteins before they cause damage.



**'The Institute is so supportive and everyone's made us feel very welcome'**
***Hayley Sharpe***

This quality control machinery declines as we age. As a result, most age-related diseases, including Alzheimer's disease, Parkinson's and cancer, are related to misfolded proteins. "There is good evidence that these quality control machines get deregulated during many ageing-related diseases," he says. "So I'm trying to study these machines in great detail: how they work normally and how they get deregulated during these diseases."

Despite being crucial for healthy ageing, this cellular clear-up process is shrouded in mystery. Text books tell us the major misfolded protein clearance route involves attachment of a ubiquitin tag, which serves as a fast-track protein disposal signal. But, says Samant, we have known for the past 15 years that this is too simple to be true.

"It's very context dependent, and only now are we developing the tools to look at all the different type of ubiquitin tags in sufficient detail," he explains. "I'm interested in using this increased resolution to go back to these basic questions that we've assumed to be true, but which the data now shows to be vague and hand-wavy."

To study the process, Samant combines tools he honed during his time as a research associate at Stanford University with the state-of-the-art proteomics facility at the Institute. He also collaborates with the Institute's world-leading experts in autophagy – another crucial cellular process for clearing up misfolded proteins.

It's work that could reshape our understanding healthy ageing and disease, identifying new therapeutic targets and allowing us to treat neurodegenerative diseases and cancer much earlier. But only, Samant concludes, if we go back to basics. "We don't yet understand how ageing affects the prevalence of misfolded proteins – and understanding these processes at a fundamental level is really important before we can start addressing the disease aspects."

**'I want to follow where the science leads. The Institute is one of the few research centres with such a strong focus on fundamental biology'**
***Rahul Samant***

# A remarkable partnership

**Great science depends on teamwork, yet genuine partnerships are rare, especially those which sustain success over decades. Dr Len Stephens and Dr Phill Hawkins, both group leaders in the Institute's Signalling programme, have worked together for more than 30 years. Here, they reflect on their research, their relationship – and their distinctly different approaches to fishing.**

In 1980, Fred Sanger and Walter Gilbert won the Nobel Prize in chemistry for work on nucleic acid sequences; Polish workers set up the trade union Solidarity; and the Rubik's Cube made its debut. It's also the year that Phill Hawkins and Len Stephens met for the first time, with Phill a PhD student and Len a final year undergraduate at Birmingham University.

Hawkins remembers their meeting clearly. "I was working late in the lab and Len came by looking for my supervisor, Bob Michell," he recalls. "When I told him it was late, and Bob had gone home, Len asked if he could come in and watch me do an experiment. We chatted, found out we both fished, and Len invited me to go fishing with him in Cannon Hill Park."

After both worked as postdocs at SmithKline Beckman (now GSK) their paths diverged briefly, but in 1990 when an opportunity arose for Hawkins to join Stephens in Robin Irvine's lab at the Institute of Animal Physiology at Babraham, they jumped at the chance.

Irvine's lab focused on cell signalling pathways involving a sugar-like molecule called inositol. Lew Cantley's lab in Boston had just discovered a new enzyme called phosphoinositide 3OH-kinase, or PI3K for short, that made a new family of inositol-containing molecules in cells in response to growth factors. There was much to discover and while the science was challenging, there was a sense in the lab that they were hunting down something significant.

"At the beginning it was about correctly identifying the molecules that were appearing in cells and working out how they were made," Stephens explains. "Our instincts told us they'd turn out to be a key piece of biology that controlled how cells behave, and that spurred us on to try and understand what was going on."

We now know that cells monitor and respond to their environment by controlling the activity of a few key proteins, which in turn regulate a cascade of downstream events. Together, they orchestrate the complex behaviour that happens in cells, from cell growth and division to cell survival and movement. When these signalling networks go wrong, they can lead to a range of diseases, including cancers, chronic inflammation and immunodeficiencies, so understanding the basic biology has opened up new treatments for these diseases.

"We thought our research would be a fundamental bit of cell biology, but at the time we didn't know where it would lead," says Hawkins. "There wasn't a single eureka moment, many small step changes along the way allowed us to piece together the basics of what was going on. Then other labs discovered that mutations in PI3Ks are often associated with cancer. That's when it became a much wider impact story, and attracted the attention of other researchers and pharmaceutical companies."

Since then, Stephens and Hawkins have worked closely with industry, collaborating with Onyx Pharmaceuticals, UCB, AstraZeneca, GSK and Pfizer. By 2013, the pharmaceutical industry had invested £350 million in PI3K research and had more than 20

**LEARN MORE:**

**Watch 'PI3K signalling: from basic biology to new cancer therapies', an animated description of cell signalling and how a deeper understanging of this process is helping to improve human health.**

**Both of us wanted to find out something fundamental about how the world works, and how cells work – *Phill Hawkins***

drugs targeting PI3Ks in clinical trials. In 2014 the first – Idelalisib – was licensed for treating chronic lymphocytic leukaemia.

Reflecting on their partnership, both agree that a natural friendship, shared values, and contrasting – but complementary – personalities have underpinned its longevity and success. Hawkins is more emotional, he says, his moods tracking the highs and lows in the lab, whereas Stephens is steadier and a battler, with a bold ambition for their science.

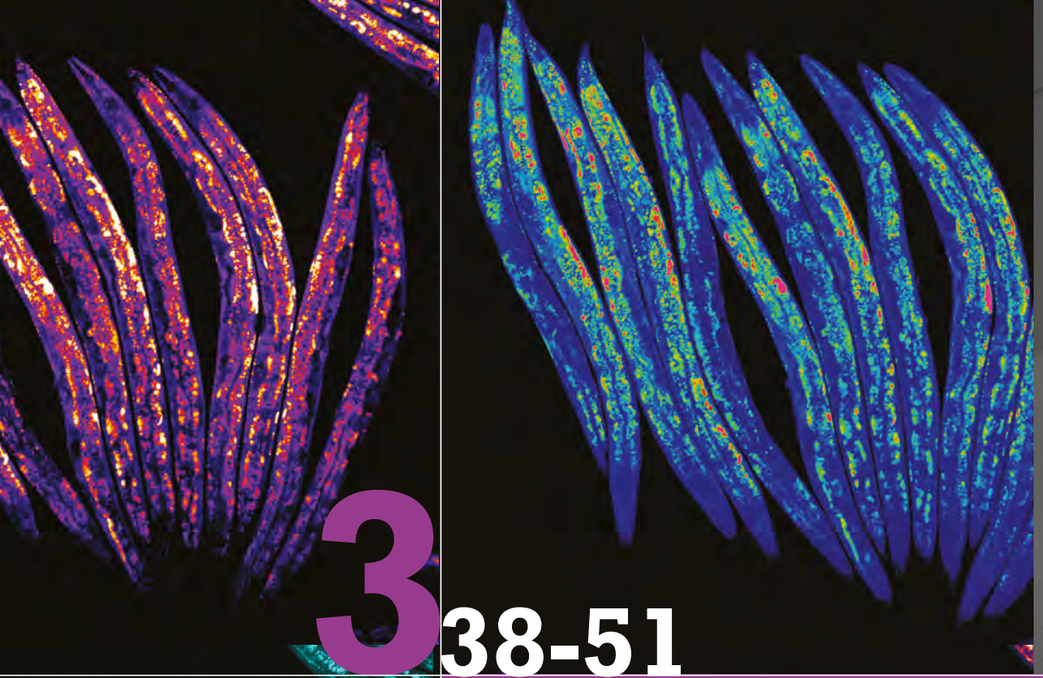
According to Stephens, having similar values is key. "The rest flows from there," he says. "Are we similar in all respects? No, and that's important. We have different strengths and weaknesses. It's about your motives, the things that inspire you, the things you respect in others, what you find joy in. And sometimes it's about understanding – at a deep level – what can hack someone off."

Both are eminent scientists and fellows of the Royal Society, but say that what matters most is a shared scientific curiosity, and a happy working environment. "Neither of us wanted success for its own sake or accolades," says Hawkins. "We wanted to find out something fundamental about how the world works, and how cells work."

And then there's the fishing, which seems to encapsulate what they share, as well as their differences. Like their research and friendship, their fishing goes back to Birmingham. "When he invited me to Canon Hill Park, I saw fishing in a totally new light," laughs Hawkins. "It's a science for Len: what type of line to use, which home-made floats – he even made catapults with different types of elastic to deliver a certain number of maggots to a precise spot in the water! It's fishing on a whole other level."

Stephens agrees. "As far as I'm concerned it's incredibly similar to doing experiments. I love trying to figure out what the fish are doing under the water, and doing experiments to work out how to catch them," he concludes. "So yes, I can get very intense about going fishing."

**All our instincts told us PI3Ks would turn out to be a key piece of biology that controlled how cells behave, and that spurred us on to try and understand what was going on – *Len Stephens***





# 3

38-51

# Epigenetics

Inside cells, genetic information stored in DNA is packaged by proteins into a structure called chromatin. Epigenetics is the study of chemical modifications to DNA and to chromatin and the effects that these modifications have on genome function. Epigenetic marks are involved in the creation of different types of cells from stem cells and epigenetic changes over time are associated with ageing. Epigenetic marks also provide a form of cellular memory, recording certain information about past events and potentially carrying it between parent and child.

Our work in this area aims to enhance our understanding of how epigenetics shapes human development and affects healthy ageing by examining:

- How stem cells develop into different types of cells
- How epigenetic differences influence cell diversity
- The impacts of diet on epigenetics, health and ageing
- The inheritance of epigenetic memory between generations
- How life events affect biological ageing through an epigenetic clock
- New approaches and technologies to drive further progress

## Group leaders

Gavin Kelsey

Olivia Casanueva

Maria Christophorou

Jon Houseley

Wolf Reik

Peter Rugg-Gunn

Stefan Schoenfelder

Martin Howard

Honorary group leader

**Gavin Kelsey**
**Programme leader**

**Group members**

**Honorary fellows:**
Courtney Hanna
Vicente Perez Garcia

**Postdoctoral researchers:**
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Juan Castillo Fernandez
Hannah Demond
Antonio Galvao
Elena Ivanova
Anastasios Mastrokolias

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Bess Chau

**PhD students:**
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Leach McHugh

**Visiting scientists:**
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Laura Saucedo-Cuevas
(Left in 2019)
Claire Senner (Left in 2019)
Shinichi Tomizawa (Left in 2019)

**Visiting student:**
Caroline Faessler (Left in 2020)
Rebecca Koeck (Left in 2019)
Maribel Montufar-Martinez
(Left in 2019)
Benjamin Planells (Left in 2019)
Edyta Walewska (Left in 2019)
Karolina Wolodko (Left in 2020)

# Epigenetic legacies from eggs

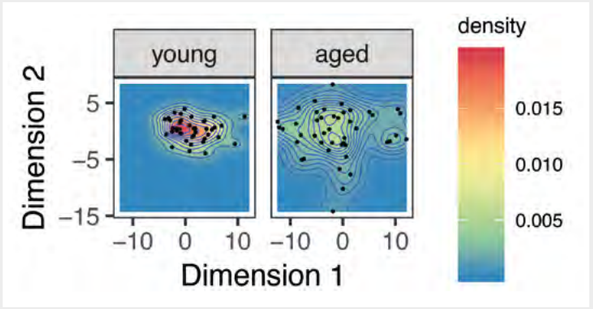
**As well as genetic information, the egg and sperm contribute epigenetic annotations that can influence how genes act after fertilisation. We examine how epigenetic patterns are set up during egg development and the effects of epigenetic marks on gene activity in the embryo. Our goal is to understand whether, through epigenetics, factors such as a mother's age or diet have consequences for the health of a child.**

## Current Aims

A major aim is to understand how repressive chromatin marks in oocytes lead to long-term silencing – imprinting – of genes from the mother, particularly in cells that form the placenta. We are seeking to understand the genetic elements that carry this epigenetic memory between generations, how they control these 'imprinted' genes, and how important the genes are for development and function of the placenta and consequently growth of the fetus. To investigate these questions, we develop methods to profile epigenetic information in small numbers of cells or even single cells, as well as using gene-editing methods.

## Progress in 2019 and 2020

A highlight has been the discovery that a newly-described form of imprinting is controlled by genomic parasites – endogenous retroviral elements. Such elements can be co-opted during evolution as novel gene control modules. They can act in a highly tissue-specific way, and are controlled by the epigenetic machinery,




*Oocytes (egg cells) from reproductively old female mice have more variable gene expression than those from younger females. This is depicted as a 2D distribution of the distances between single oocytes as based on their similarity in gene expression. From Castillo-Fernandez, J. et al. (2020).*

suggesting they could be influenced by environmental or nutritional factors (ref. 2). This discovery also provides clues about the conservation of this form of imprinting.

We also provided the first genome-wide assessment of DNA methylation and gene expression in eggs from aged female mice (ref. 3). Eggs from older females had less active and less consistency in gene expression. Methylation in general correlated well between eggs from younger and older mice, providing reassurance that age does not affect key sites of DNA methylation in the genome, but there were gene-specific changes coupled to gene transcription differences.

## Selected Impact Activities

- Group representation for the 'Race Against the Ageing Clock' exhibit at the Cambridge Science Festival, March 2019.
- Plenary speaker at the 18th International Conference on Pre-implantation Genetic Diagnosis Geneva, April 2019.
- Speaker at Royal Society of Medicine continuing professional development workshop on 'Epigenetics', June 2019.

**Olivia Casanueva**

**Group members**

**Senior research assistant:**
Sharlene Murdoch
(Left in 2019)

**Postdoctoral research scientists:**
Laetitia Chauve
Celia Raimondi (Left in 2020)
Pun Suriyalaksh (Left in 2020)
Boo Virk (Left in 2019)

**PhD students:**
Janna Hastings (Left in 2019)
Abraham Mains (Left in 2019)

**Research assistants:**
Aina Bellver Sanchis
(Left in 2020)
Andriana Botan (Left in 2019)
Francesca Hodge
(Left in 2020)
Sheikh Mukhtar (Left in 2020

**Visiting students:**
Fatemeh Masoudzadeh
(Left in 2020)

# Using systems biology tools in C. elegans to extend life

**It is estimated that more than 20% of the global population will be over 60 by 2050, the highest percentage of older persons in recorded history (World Health Organization factsheet on Ageing and Health, 2018). Over the last two decades studies have drawn a remarkable link between metabolism, stress and longevity and this links needs exploration in the face of current societal challenges. We use the nematode C. elegans to study this important question.**

## Current Aims

The specific problem that we look at in my lab is the interplay between stress and metabolism during early ageing. To tackle this question, we have optimised new cutting-edge computational tools that bring new perspectives and insights into the problem. We have also used more traditional methods to uncover a novel longevity pathway. This is a neuronal thermostat that senses and help animals adapt to warming temperatures by modulating fat desaturation across tissues.

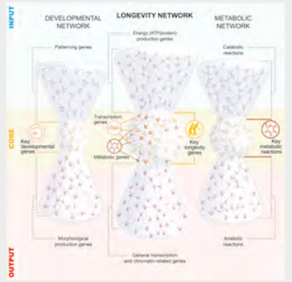
## Progress in 2019 and 2020

My group used two very different and complementary approaches to explore key concepts that characterise longevity pathways. In one approach, we used classic genetics and lipidomics to discover that neurons can sense environmental temperature and send neurohormonal signals to help the body cope with a warming environment. We find that the neuronal thermostat regulates the membrane lipid composition and in doing so, extend lifespan. A similar neuro-hormonal axis may be key to healthy ageing in humans.

In a second approach, we took a highly systemic view of the germline longevity pathway and applied computational tools to understand how information flows within a network of gene interactions. This 'bird's-eye view' of a longevity pathway has a high predictive value for identifying the key genes that contribute to long life.

## Selected Impact Activities

- The lab showcased the power of genetics using C. elegans as a model organism at the Cambridge Science Festival in 2019 as part of the 'Race Against the Ageing Clock' exhibit.
- The lab hosted students as part of the Institute's Schools' Day events in 2019 and 2020.
- We also presented our work at the 17th Animal Science Meeting (2019), co-organised by the Royal Society of Biology and the Animals in Science Regulation Unit.




*Network inference methods allowed us to obtain a 'bird's-eye view' of long-lived sterile worms. The hour-glass shape of information flow in the longevity network (at the centre) is similar to other well-described networks (developmental and metabolic networks). In the longevity network, the information from the input module—enriched in genes required for the production of energy—feeds information to a core module. The core module, in turn, feeds back information to an output module that is enriched in genes that modulate basal transcription and chromatin remodelling. What is very striking is that the hour-glass shape is predictive of functionality: the core module is enriched in genes that are key to long life. This finding fast-tracks ageing research in model organisms and can potentially be applied to humans.*

## Publications


- Sendžikaitė, G. *et al.* (2019) A DNMT3A PWWP mutation leads to methylation of bivalent chromatin and growth retardation in mice. ***Nat. Commun.*** 10:1884
- Hanna, C. W. *et al.* (2019) Non-canonical imprinting in extra-embryonic tissues is driven by endogenous retroviral insertions. ***Genome Biol.*** 20:225
- Castillo-Fernandez, J. *et al.* (2020) Increased transcriptome variation and localised DNA methylation changes in oocytes from aged mice revealed by parallel single-cell analysis. ***Aging Cell*** 19: e13278


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

@LabGavin

## Publications


- Özbey, N. *et al.* (2020). Tyramine acts downstream of neuronal XBP-1s to coordinate inter-tissue UPRER activation and behavior in C. elegans. ***Dev. Cell*** 55(6):754-770.e6
- Okkenhaug, H. *et al.* (2020) Worm-align and Worm_CP, two open-source pipelines for straightening and quantification of fluorescence image data obtained from Caenorhabditis elegans ***J. Vis. Exp.*** (159):10.3791/61136
- Chauve, L. & Le Pen, J. *et al.* (2020) High-throughput quantitative RT-PCR in single and bulk C. elegans samples using nanofluidic technology. ***J. Vis. Exp.*** (159):10.3791/61132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**Maria Christophorou**

**Group members**

**Postdoctoral researcher:**
Johanna Grinat

**PhD students:**
Daniel Moore
Robert Walmsley

# Signalling to stem cell chromatin

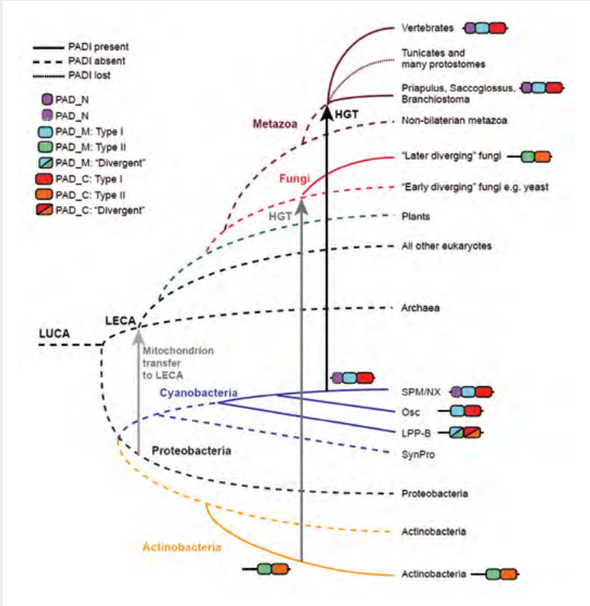
**Changes in the cellular environment, such as stresses and developmental cues, affect the epigenetic and transcriptional state of cells, thereby influencing cell identity. We aim to understand how environmental signals are translated into epigenetic changes through studying the biochemical regulation of epigenetic factors. We focus on one particular type of biochemical mechanism, the conversion of arginine residues to non-coded citrullines, or protein citrullination.**

## Current Aims

The citrullinating enzyme PADI4 modulates transcription and chromatin compaction and has well-established roles in innate immunity and the development of autoimmunity.
We discovered that PADI4 also has a role in stem cells, regulating the establishment of pluripotency during cell reprogramming and early embryo development. Our current research aims to identify the mechanism through which PADI4 influences cell fate during embryo development, in adult tissues, and during the generation of induced pluripotent stem (iPS) cells. In parallel, we study the biochemical mechanisms that determine PADI4 activation and how citrullination, in turn, modulates the function of epigenetic regulators.

## Progress in 2020

Our work towards understanding PADI4 activation led us to the unexpected discovery that the PADI enzymes, which were widely thought to be vertebrate-specific, are also present in some bacteria and fungi and have a highly unusual phylogenetic distribution. Through studies of phylogeny, protein structure, sequence evolution and protein biochemistry, we demonstrated that animal PADIs were introduced into animals through horizontal gene transfer from cyanobacteria. This work has implications for how we think about the organismal roles of PADIs, why they were retained and multiplied during animal evolution and how aberrant citrullination may promote the development of autoimmune diseases.




*The distribution of PADI genes across the tree of life and proposed model of PADI evolution*




- Christophorou, M.A. *et al.* (2014). Citrullination regulates pluripotency and histone H1 binding to chromatin. ***Nature*** 507(7490):104-8
- Casanova, V. *et al.* (2020). Citrullination alters the antiviral and immunomodulatory activities of the human cathelicidin LL-37 during rhinovirus infection. ***Front. Immunol.*** 11:85
- Wiese, M. *et al.* (2019). Citrullination of HP1γ chromodomain affects association with chromatin. ***Epigenetics Chromatin.*** 12(1):21




**Jon Houseley**

**Group members**

**Senior research associate:**
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**Postdoctoral researchers:**
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Ryan Hull (Left in 2019)
Alex Whale

**PhD students:**
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Neesha Kara
Andre Zylstra (Left in 2020)

**Research assistant:**
Michelle King (Left in 2020)

**Visiting students:**
Ania Daniels Uribarri
Sumaera Rathore
(Left in 2019)
Fabiola Vacca (Left in 2019)

# How cells interact with their environment

**We study how cells adapt to their environment at the genetic and epigenetic level, particularly how they adjust to challenging and toxic environments. This contributes to our understanding of how our cells change in response to environmental pressures and as a consequence of ageing. Our work aims to discover ways of improving health throughout life and to find better approaches to chemotherapy.**

## Current Aims

1. To determine how conflicts between replication and transcription cause mutations in patterns specific for the current environment, leading to both chromosomal changes and formation of extrachromosomal DNA.

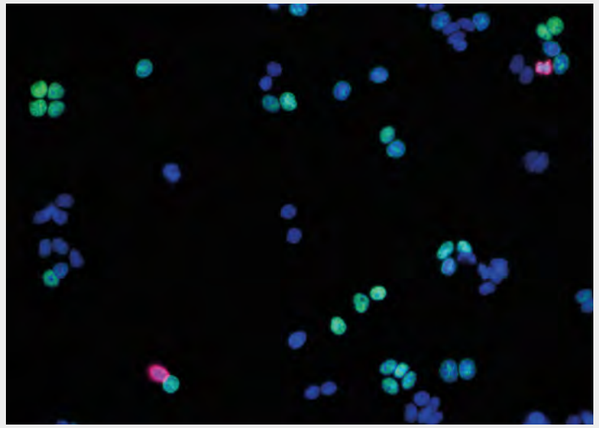
2. To elucidate the contribution of DNA replication errors to the acquisition of novel mutations in cancer cells that underlie acquisition of drug resistance.

3. To understand how epigenetic marks contribute to gene expression changes during ageing, and the extent to which these age-linked changes are controlled by signalling of nutrient availability.

## Progress in 2019 and 2020

We have continued our work on extrachromosomal circular DNA – unstable DNA species that often host oncogenes and drug resistance genes in cancer cells. Circular DNA is implicated in cellular ageing, and we are investigating the accumulation of circular DNA during ageing and the impact of circular DNA on ageing phenotypes. This work is revealing how circular DNA can accelerate adaptation to challenging environments, promote drug resistance and mediate progressive changes in cell physiology.

In 2020 we saw major advances in our work on mechanisms of adaptive genome change, as we learn more about how replication defects caused by environmental change can form new adaptive mutations. The resulting publications will emerge next year, but some of the underlying methods, for example to improve genome-wide analysis of small, defined cell populations, have been released.



*HT-29 cells (a colorectal cancer cell line), stained for DNA replication (green, EdU incorporation) and mitosis marker phopho-H3S10 (red). Image acquired in the Institute's Imaging facility by Prasanna Channathodiyil.*

## Selected Impact Activities

- We contributed a research spotlight article on epigenetics in ageing for The Physiological Society policy document 'Growing older, better'.
- We have a new collaboration in place with AstraZeneca to recruit a PhD student who will study the acquisition of drug resistance during chemotherapy.
- Our work was featured in an article on extrachromosomal circular DNA in Chemical and Engineering News: The curious DNA circles that make treating cancer so hard.




- Hull, R.M. *et al.* (2019). Transcription-induced formation of extrachromosomal DNA during yeast ageing. ***PLoS Biol.*** 17:e3000471
- Hull, R. M. & Houseley, J. (2020). The adaptive potential of circular DNA accumulation in ageing cells. ***Curr. Genet.*** 66:889-94
- Prada-Luengo, I. *et al.* (2020). Replicative aging is associated with loss of genetic heterogeneity from extrachromosomal circular DNA in Saccharomyces cerevisiae. ***Nucleic Acids Res.*** 48:7883-98

**Wolf Reik**

**Group members**

**Laboratory manager:**
Annalisa Mupo

**Senior research scientists:**
Stephen Clark
Wendy Dean (Left in 2019)
Debbie Drage
Mariya Rostovskaya
Fatima Santos

**Research fellow:**
Irina Abnizova
Melanie Eckersley-Maslin (Left in 2020)
Irene Hernando Herraez
Aled Parry
Carine Stapel
Jasmin Stowers

**Postdoctoral researchers:**
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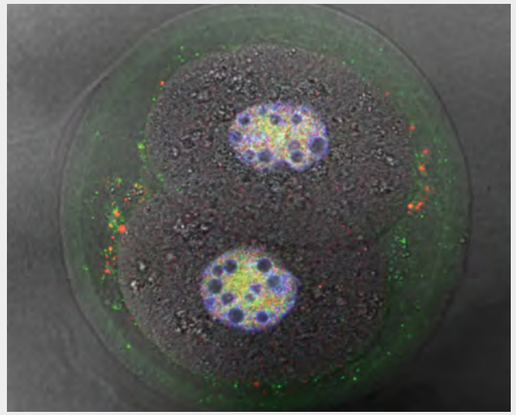
Ricard Argelaguet
Julia Spindel
Chris Todd

**PhD students:**
Diljeet Gill
Oana Kubinyecz
Georgia Lea
Juliette Pearce (Left in 2020)
Brendan Terry

**Research assistant:**
Laura Benson

# Single-cell multi-omics landscape of development and ageing



*Mouse two-cell embryo stained for DNA-blue, Dppa2-green and Smarca5-red. Credit: Oana Kubinyecz, Reik lab.*

**Selected Impact Activities**

- We were awarded a Wellcome Collaborative Award and an ERC Advanced Grant.
- Melanie Eckersley-Maslin won the 2020 Metcalf prize and was appointed group leader at Peter MaCallum Cancer Centre Melbourne.
- Wolf Reik was named Highly Cited Researcher in 2020 (Clarivate Analytics).

**We are interested in epigenetic gene regulation in mammalian development and ageing. Epigenetic marks (such as DNA or histone modifications) act in concert with cell signalling and transcription factors to regulate cell fate. We are particularly interested in the epigenetic rules that govern cell fate decisions in early development, and how cell fate degrades during ageing. Our research uses single-cell sequencing methods to investigate cell fate decision at the level of individual cells.**

## Current Aims

A primary interest of the group is the regulation of zygotic genome activation, the sudden springing to life of transcription of the embryonic genome shortly after fertilisation. Our work also looks to identify DNA binding proteins which 'prime' enhancers or promoters for gene activation at later stages in development. In terms of our expertise in epigenetic clocks, we are working on a protocol by which human fibroblasts are reprogrammed towards induced pluripotent stem cells (iPSCs) but then released from reprogramming to adopt their original cell identity again whilst becoming more youthful in the process.

## Progress in 2019 and 2020

We have carried out a screen for regulators of zygotic genome activation in the mouse. The screen was undertaken in embryonic stem cells by CRISPR activation with a single cell transcriptional read-out. We identified several proteins such as Dppa2, Smarca5, and Patz1 as putative ZGA regulators. We are now testing the roles of these proteins in mouse embryos in vivo. We also found that the two DNA binding proteins Dppa2 and Dppa4 are important for priming of promoters of bivalent developmental genes. Bivalent genes are marked by both active and repressive histone modifications which keeps them poised for future activation. Dppa2 and Dppa4 were found to be important for targeting bivalency to promoters of developmental genes and thus for the later activation of these genes.

**Group members**

**Visiting scientists:**
Romina Durigon (Left in 2019)
Daniel Ives (Left in 2019)
Ferdinand von Meyenn
Nelly Olova

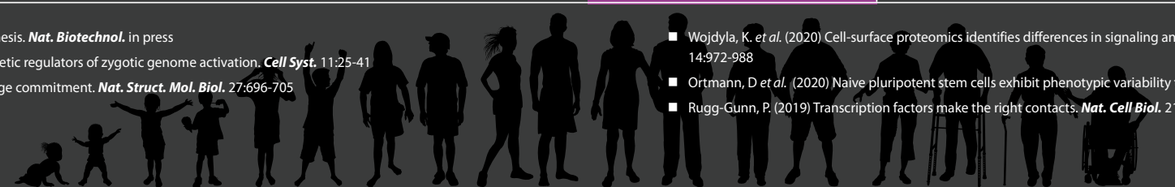
**Visiting students:**
Daniel Elias Martin Herranz
Adriana Fonseca (Left in 2019)
Lori Kregar (Left in 2020)
Tim Lohoff
Hendrik Vogt

## Publications

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


- Lohoff, T. *et al.* (2021) Highly multiplexed spatially resolved gene expression profiling of mouse organogenesis. ***Nat. Biotechnol.*** in press
- Alda-Catalinas, C. *et al.* (2020) A single-cell transcriptomics CRISPR-activation screen identifies new epigenetic regulators of zygotic genome activation. ***Cell Syst.*** 11:25-41
- Eckersley-Maslin, M. *et al.* (2020) Epigenetic priming by Dppa2 and 4 in pluripotency facilitates multi-lineage commitment. ***Nat. Struct. Mol. Biol.*** 27:696-705



**Peter Rugg-Gunn**

**Group members**

**Senior researcher:**
Clara Novo (Left in 2019)

**Postdoctoral researchers:**
Stephen Bevan
Claudia Semprich
Yang Wang

**PhD students:**
Adam Bendall
Mandy Collier (Left in 2019)
Charlene Fabian
Andrew Malcolm
Kate Maskalenka

**Visiting scientist:**
Jazmine Murray (Left in 2020)

**Visiting student:**
Monica Della Rosa (Left in 2019)

# Epigenetic regulation of human development

**How DNA is packaged in cells and the use of biochemical switches in the genome are key aspects of the epigenetic control of gene activity. We are interested in understanding how epigenetic processes are established during human development and during the differentiation of stem cells to form various cell types. This is important for understanding health and for finding ways to use stem cells in regenerative biology.**

## Current Aims

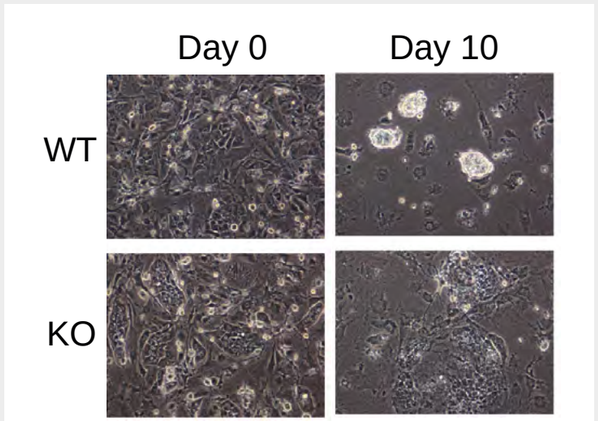
We aim to understand the epigenetic and gene regulatory mechanisms that operate in unspecialised pluripotent stem cells and in cells transitioning towards more specialist cell types. We examine how these mechanisms are established in development, how they control cell state changes more generally, and how their alteration can be helpful to reprogramme mature cell types back into an unspecialised form. Our work also investigates how certain epigenetic marks can anticipate future decisions made by stem cells as they specialise. Applying this information will allow us to more precisely control cell fate decisions and to better understand the processes that shape human development.

## Progress in 2019 and 2020

It is increasingly important to understand how pluripotency, the ability to specialise into all other cell types, is established during human development and in stem cell lines. Towards this goal, we have completed a genome-wide genetic screen that has newly identified many genes and pathways that are involved in establishing pluripotency in human cells. We found that the identified factors have important roles in controlling gene activity and epigenetic modifications to DNA. Investigating these processes will help to define the molecular mechanisms that control early development and will provide new insights into stem cell properties such as cell identity, differentiation and reprogramming.

**Selected Impact Activities**

- Working with the ORION Open Science consortium, we participated in a series of events to better understand public attitudes in different European countries towards genome editing technologies.
- We co-authored a strategy report by the Regenerative Biology and Stem Cell Working Group commissioned by the BBSRC.
- We collaborated with Lonza and Pfizer to leverage our understanding of gene regulatory control to improve manufacturing processes.




*Microscopy images showing human stem cells before (Day 0) and after (Day 10) reprogramming into a 'naïve' pluripotent state. The image shows the dramatic effect observed when a key epigenetic protein that we have identified is removed from the cells. The normal, wild-type cells (WT) are shown in the upper row, where 'naïve' pluripotent cells can be seen at Day 10 as a tightly compacted ball of cells. In contrast, cells lacking the protein (KO) shown in the lower row are unable to generate naïve pluripotent cells, revealing that the identified epigenetic protein has a critical function in establishing naïve pluripotency in humans.*

## Publications

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


- Wojdyla, K. *et al.* (2020) Cell-surface proteomics identifies differences in signaling and adhesion protein expression between naive and primed human pluripotent stem cells. ***Stem Cell Rep.*** 14:972-988
- Ortmann, D *et al.* (2020) Naive pluripotent stem cells exhibit phenotypic variability that Is driven by genetic variation. ***Cell Stem Cell*** 27:470-481
- Rugg-Gunn, P. (2019) Transcription factors make the right contacts. ***Nat. Cell Biol.*** 21:1173-1174

**Stefan Schoenfelder**

**Group members**

**Postdoctoral researcher:**
Yang Cao

# 3D genome organisation and effect on genome function

**The three-dimensional organisation of our genome is tightly linked to its function. Gene regulatory elements such as enhancers control spatiotemporal gene expression programmes in development by engaging in contacts with their target genes, sometimes over large genomic distances. Our research investigates how the three-dimensional organisation of the genome enables specific enhancers to control gene expression during stem cell renewal and differentiation.**

## Current Aims

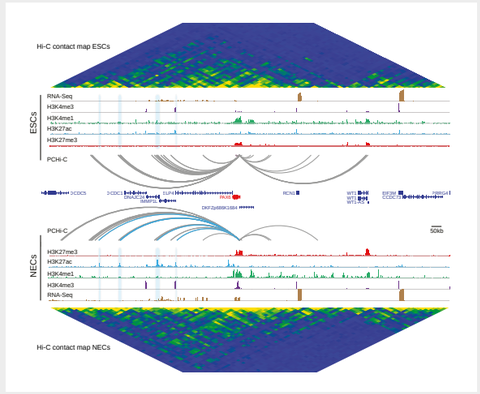
Human induced pluripotent stem cells (iPSCs) hold great potential for cell-based applications in regenerative medicine and disease modelling. However, individual iPS cell lines differ markedly in their ability to differentiate into bespoke cell types for applications in biomedicine. Non-coding genetic variants are emerging as key contributors to this functional heterogeneity between iPSCs, but their role is poorly understood. We combine functional genomics approaches with human genetics to understand how regulatory variants in the non-coding genome affect cell fate decisions during stem cell differentiation.

## Progress in 2019 and 2020

We have mapped the gene regulatory landscape across a panel of iPSC lines by profiling chromatin accessibility, 3D genome organisation and long-range enhancer-promoter contacts genome wide. This lays the foundation for our ongoing functional dissection of the gene regulatory networks in human pluripotent stem cells, using a combination of massively parallel reporter gene assays and CRISPR (epi)genome editing. Our aim is to identify non-coding genetic variants that contribute to functional heterogeneity between human pluripotent stem cell lines, and to understand how they exert their function in the context of the three-dimensional organisation of the pluripotent genome.

## Selected Impact Activities

- Incorporation of a start-up company 'Enhanc3D Genomics' (as co-founder) commercialising Institute research.
- Talk at Masters' student course 'Genomic Medicine: Epigenetics and Epigenomics'.
- Participation in the Institute's Schools' Day 2020.




*Developmental control of enhancer-promoter contacts during early human cell lineage specification: Transcriptional upregulation of PAX6 during the conversion of human embryonic stem cells (ESCs) into neuroectodermal cells (NECs) is accompanied by the formation of enhancer-promoter contacts in NECs (mapped by Promoter Capture Hi-C; PCHi-C), which involves both novel enhancer-promoter contacts and the emergence of the enhancer associated mark acetylation at lysine 27 of histone H3 (H3K27ac) at genomic regions that already interacted with PAX6 in ESCs (modified from Freire-Pritchett et al., eLife 2017).*

## Publications


- Schoenfelder, S.& Fraser, P. (2019) Long-range enhancer-promoter contacts in gene expression control. ***Nat. Rev. Genet.*** 20:437-455
- Olan, I. *et al.* (2020) Transcription-dependent cohesin repositioning rewires chromatin loops in cellular senescence. ***Nat. Commun.*** 11:6049
- Thiecke, M.J. *et al.* (2020) Cohesin-dependent and -independent mechanisms mediate chromosomal contacts between promoters and enhancers. ***Cell Rep. 32***:107929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**Martin Howard**
**Honorary group leader**

**Group members**

**Visiting postdoctoral researcher:**
Govind Menon

# Probing epigenetics through mathematics

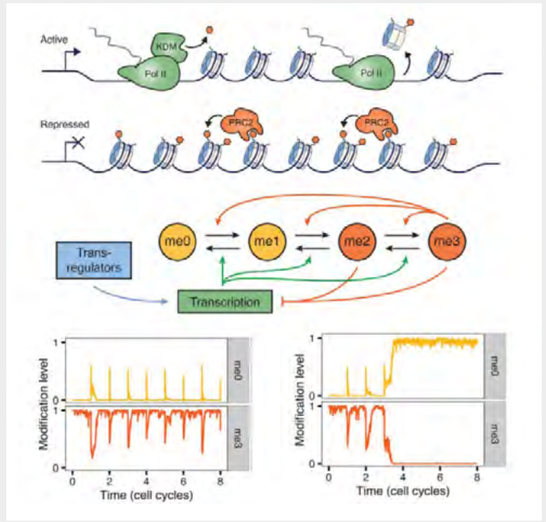
**Our research explores how epigenetic memory is stably stored and propagated through the cell cycle. We take a theoretical approach using mathematical modelling in combination with experiments from collaborators, in an iterative cycle. This interdisciplinary approach helps to speedily unlock underlying mechanisms in systems which are otherwise too complex to dissect with experiments alone.**

## Current Aims

My lab at the John Innes Centre is currently investigating epigenetics in the context of the stable gene silencing provided by the Polycomb system, vital to developmental transitions and environmental response. In particular, we are trying to understand how memory of the Polycomb silenced state is propagated despite the perturbation of DNA replication. We are also investigating the dynamics of DNA methylation and how it is stably propagated. The group also has a long-standing interest in spatiotemporal protein dynamics and patterning processes, which we are currently studying through the phenomenon of crossover interference in meiotic crossover positioning.

## Progress in 2019 and 2020

We uncovered a new state of Polycomb silencing (ref. 3) present after the apparatus that initialises the silenced state has disassembled, and whose epigenetic stability is modulated through Single Nucleotide Polymorphisms (SNPs). The system we studied is epigenetically silenced by prolonged cold: we therefore investigated how the cold is perceived, which led us to a novel mechanism [ref. 2] where cold slows growth and thereby allows the concentration of a stable protein to rise. Finally, we investigated the consequences of rapid cell cycles in the early Drosophila embryo [ref. 1], finding that rapid cycling may play a crucial role in keeping chromatin naïve. Collaborations with group leaders at the Babraham Institute are also now being developed, focusing particularly around the concept of bivalency (with the Reik lab).




*Modelling Polycomb epigenetic dynamics. Top: schematic of chromatin state in active (upper) and silenced (lower) state. Middle: key ingredients of a mathematical model for Polycomb dynamics. Bottom: simulation of silenced state (high H3K27me3) periodically perturbed by DNA replication (left) and of silenced state switching to an active state (right).*

Professor Howard is a senior group leader at the John Innes Centre and joined the Institute in 2020 as an honorary group leader as part of an affiliate programme that develops exciting research collaborations in areas of complementary expertise.

## Publications


- Reinig, J. *et al.* (2020) A theoretical model of Polycomb/Trithorax action unites stable epigenetic memory and dynamic regulation. ***Nat. Commun.*** 11:1-16
- Zhao, Y., Antoniou-Kourounioti, R.L. *et al.* (2020) Temperature-dependent growth contributes to long-term cold sensing. ***Nature*** 583:825-829
- Qüesta, J.I., Antoniou-Kourounioti, R.L. *et al.* (2020) Noncoding SNPs influence a distinct phase of Polycomb silencing to destabilize long-term epigenetic memory at Arabidopsis FLC. ***Genes Dev***. 34:446-461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# How yeast is reshaping ideas on ageing

**Healthy ageing is one of society's most pressing concerns, but basic questions like why we age remain a mystery. Dr Jon Houseley, a group leader in the Institute's Epigenetics programme, studies the ways in which yeast cells adapt to new environments. As well as uncovering new connections between adaptation and ageing, his research is challenging our ideas about ageing itself.**

Dr Jon Houseley is a heretic. Along with others in the field of ageing research, he is questioning long-established orthodoxies of ageing. For decades, ageing has been seen as inevitable, a paradigm that has so far failed to help us answer the most basic questions about the nature of ageing.

If you rewind to the 1920s, however, alternative theories of ageing existed. Rather than simply a slow, inexorable process akin to a once pristine car rusting and breaking down, some argued that ageing had evolved for some purpose. In the absence of evidence, they lost the argument. Today, Houseley and others think they may have been right.

"I've been in the field long enough to say that you should be wary of any theories about ageing – including mine," says Houseley. "The problem is that 60 years on, it's really hard to know what this conserved underlying mechanism that's breaking down in ageing actually is. We need fresh ideas, even if they are heretical."

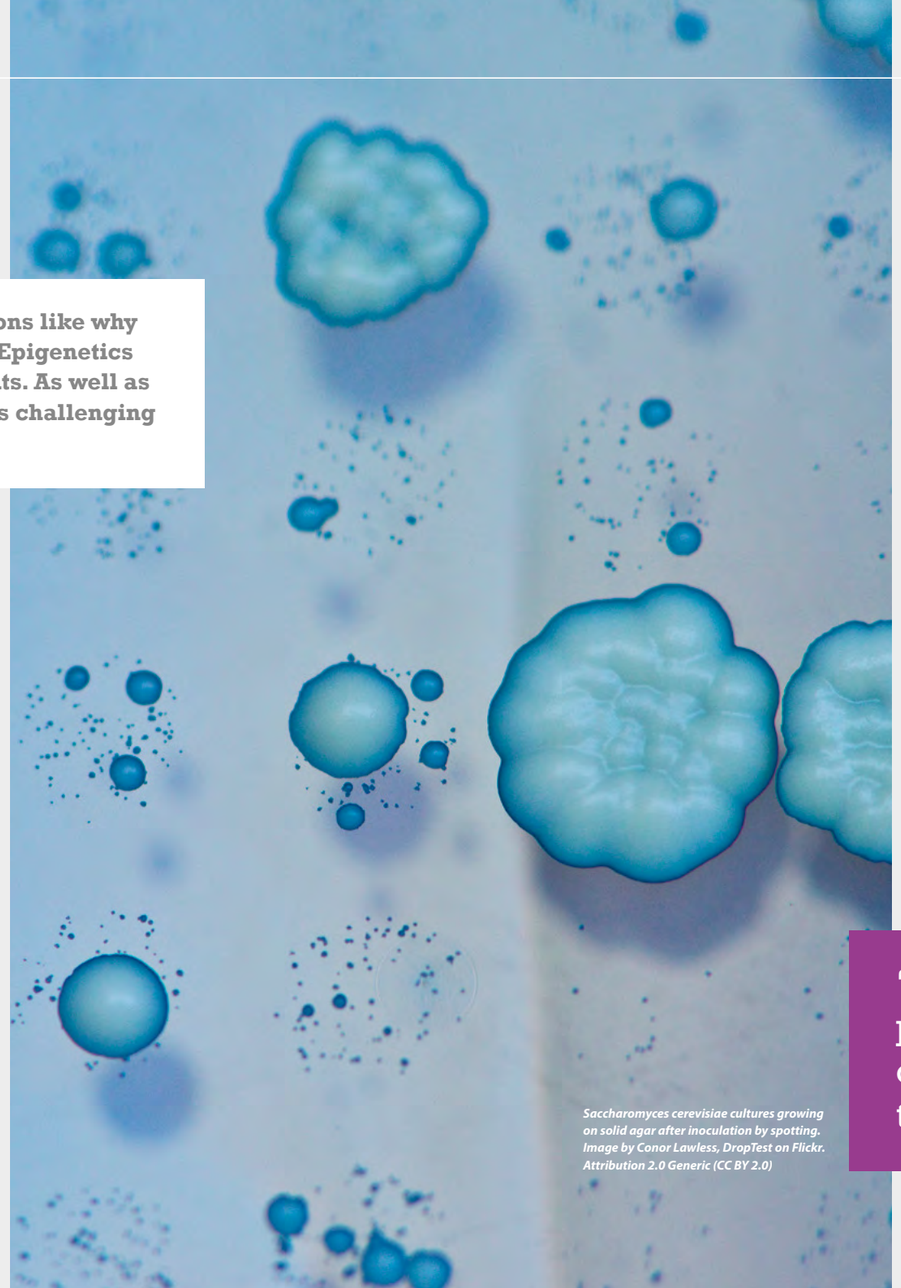
Ageing is hard to study. So much happens in a slow and concerted way in myriad systems that coming up with cause and effect is mind-bogglingly hard. Many genes and processes are involved, all of which are interconnected, so identifying the underlying 'thing' has so far proved impossible. It's even possible that ageing is not, in fact, one single thing.

"Ageing is really difficult to get your hands on, which is why we've started to revisit these old debates. But rather than argue about it intellectually, we have rolled up our sleeves and done experiments with yeast," Houseley explains. "Using yeast, we are asking whether there are situations in which being old is an advantage – and it turns out that there are."

One of Houseley's central aims is understanding how organisms adapt to challenging – and changing – environments. "It sounds like a weird fit with our focus on ageing," he admits. "But a major angle we have on ageing is that it could be a process that exists in basic eukaryotes like yeast to help them cope with stress."

His studies provide persuasive evidence to support this idea. One focused on CUP1, a gene in yeast that allows cells to survive in high-copper environments. Some yeast cells make extra copies of the CUP1 gene, allowing them to survive and outcompete cells with fewer copies of the gene.

Surprisingly, the study revealed that extra copies of CUP1 result from an active process rather than random mutations. "We think of genomes as long-term, stable repositories of information but they change – and not only over evolutionary timescales," says Houseley. "We know genome changes are hallmarks of cancer, but we're discovering them in healthy cells too. Some parts of the genome are very prone to change, suggesting that organisms have more control over their genomes than once thought."



*Saccharomyces cerevisiae cultures growing on solid agar after inoculation by spotting. Image by Conor Lawless, DropTest on Flickr. Attribution 2.0 Generic (CC BY 2.0)*

But this raises a problem: cancer aside, organisms care about the genomes they pass on to future generations and are careful to minimise mutation rates in their genes. This is where ageing can be useful, because genome changes can be restricted to old cells that only reproduce rarely, and to the strange non-chromosomal DNA elements that accumulate in old cells.

The Houseley lab examined how yeast forms circular non-chromosomal DNA elements in response to copper and how this differs between young and old yeast cells. The results revealed that older yeast cells hoard circular DNA containing extra copies of genes like CUP1. "It's like a selfish insurance policy," he says. "But while these extra genes might provide an evolutionary advantage, they might also come at the cost of ageing because these extra bits of DNA could hamper normal essential cell pathways."

Understanding more about DNA circles, and how cells can change particular parts of their genomes in response to particular environments has important implications for both cancer and ageing. Cancers use DNA circles to carry cancer-causing oncogenes and to adapt in the face of chemotherapy, enabling them to develop resistance to anti-cancer drugs.

Houseley is at pains to stress that evidence for ageing being adaptive in yeast does not mean it's also adaptive in higher eukaryotes. It does, however, explain how it evolved and may explain why ageing still occur in higher organisms despite bringing no benefit.

As well as setting us on a new path to better understanding ageing, his research is also relevant to efforts to stop cancers from becoming drug resistant and could pave the way to healthier ageing. "Ageing is the biggest risk factor in a vast number of human disorders, but to come up with ways of dealing with ageing, we first need to understand it," he concludes. "At the moment, we don't understand how ageing works in any organism, so knowing how it works in one – even yeast – would be a major advance."

'Some parts of the genome are very prone to change, suggesting that organisms have more control over their genomes than once thought.'

# Promoter Capture Hi-C: from academic tool to £1.5M startup

**Fundamental research is vital for science and society. Many medical and technological revolutions are rooted in basic research, yet those roots can be hard to trace. Today, spinouts are key to turning academic bioscience into healthcare treatments. Dr Stefan Schoenfelder, a group leader in the Institute's Epigenetics programme and co-founder of Enhanc3D Genomics, discusses taking a tool developed for fundamental research and building a business around it.**

Setting up a new biotech spinout is a demanding business, full of funding rounds, legal structures and recruitment decisions. Doing so at the start of a global pandemic, however, adds a new set of challenges. Founded in January 2020, Enhanc3D Genomics secured its first round of funding the following month and by March 2021 had closed a second round of funding worth £1.5 million.

Being involved in a biotech spinout is somewhat surprising for Schoenfelder, who has worked at the Institute for 15 years, because it was never part of his career plan. "If you'd asked me five years ago, I'd have said that I wanted to focus solely on academic research," he explains. "I've always hoped my research would help patients, but never saw myself as part of the commercial world. Now, I believe that I can play a more active role in making that transition from basic science to clinical application."

The first fully sequenced human genome was published in 2003, with hope that this 'operator's manual' for the human body would revolutionise our ability to treat, prevent and cure disease.

During the past two decades, however, research has revealed that our DNA contains intricacy and complexity unimagined in 2003. Together with our genes, the non-coding sequences of DNA between the genes – once dismissed as 'junk DNA' – are just as important. And as well as its linear sequence, the 3D structure of DNA as it is folded within the nuclei of our cells is crucial, because it brings genes into close proximity to their regulatory elements – a key step in gene expression control.

According to Schoenfelder: "These regulatory elements function like molecular switches to control which genes are active, and thus produce proteins, in which cells. This process of gene expression control is vital to allow cells – which all contain the same genes – to specialise to carry out different tasks, and to help them respond to changes." Identifying which regulatory elements act on which genes has been an epic challenge in genome biology, but thanks to an ingenious modification to the Hi-C technique called Promoter Capture Hi-C, developed by Schoenfelder and his colleagues at the Institute, this can now be done for all human genes in a single experiment.

Crucially, studies deciphering the 3D folding of the genome at high resolution also reveal how small mistakes in regulatory elements interfere with normal control of genes, leading to a greater chance of developing specific diseases.

**LEARN MORE:**

**Find out more about the 3D organisation of the genome and how regulatory elements control gene expression.**

The best example of how Promoter Capture Hi-C is helping researchers understand the genetic basis of important human diseases comes from so-called Genome Wide Association Studies (GWAS): hugely powerful studies sequencing the whole genome of people with and without particular diseases to spot the differences involved in causing the disease. By applying Promoter Capture Hi-C, research by the Institute linked the tiny changes in non-coding DNA to target candidate genes implicated in rheumatoid arthritis, type 1 diabetes and Crohn's disease.

"The vast majority of genetic variants associated with disease are located in non-coding parts of our genome," says Schoenfelder. "That means if we want to understand how most diseases arise – and find new targets for therapy – we need to be looking in the non-coding genome."

Having pioneered Promoter Capture Hi-C as a tool for doing fundamental research into gene regulation, Stefan and his colleagues soon realised it could be invaluable in understanding diseases and finding new ways to treat them, and that the best way to translate their fundamental research into clinical applications was through a spinout.

The company's vision is ambitious. "Our vision is to create 3D genome maps of all the cell types in the human body. If we succeed, it will be a unique database that will allow us to establish causal links between disease-associated regions and disease genes in almost every disease. Then, we will partner with experts in different disease areas to find new therapeutic targets," Schoenfelder says.

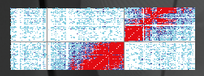
Reflecting on a momentous year, he is enthusiastic about the spinout process and Enhanc3D Genomic's potential: "The prospect of creating a legacy that uses fundamental research to create a business and help patients is hugely exciting."

"Covid-19 has shown the world what small, agile biotech firms can achieve. The most amazing research stories have come out of companies like Moderna and BioNTech," he concludes. "I hope more people now see there's lots of interesting research going on in biotech. The Institute and the many companies on the Campus create the ideal environment to foster these interactions. We need to get out of our ivory towers a bit more!"

**'To understand how most diseases arise – and find new targets for therapy – we need to be looking in the non-coding genome'**

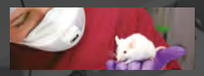
# 4 52-63

## Facilities

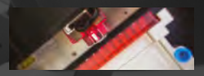


Bioinformatics

Biological Chemistry



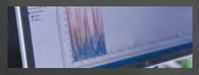
Biological Support Unit



Flow Cytometry

Gene Targeting

Imaging



Lipidomics

Mass Spectrometry

Sequencing

**Simon Andrews**
**Facility head**

**Facility members**

**Biological statistician:**
Anne Segonds-Pichon

**Bioinformaticians:**
Laura Biggins
Christel Krueger
Felix Krueger
Louise Matheson
Steven Wingett
(Left in 2020)

**Lipid Maps web developer:**
Caroline Gaud

**Training developer:**
Jo Montgomery

# Bioinformatics

**The Bioinformatics facility exists to support the Institute's research groups in the analysis, processing and organisation of their research data. We do this in a number of ways including providing a large compute cluster and an associated suite of software tools. We train scientists in the latest computational techniques and tools and we provide a consultancy service where we can either advise researchers or perform analysis on their behalf.**

## Capabilities

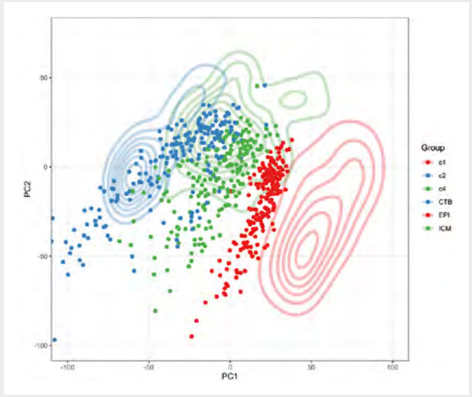
- An 800 node compute cluster with an extensive collection of bioinformatics software and pipelines.
- An extensive modular portfolio of bioinformatics training courses targeted at biologists.
- A range of custom software, including software focused on next generation sequencing, data visualisation and quality control.
- Experience in the processing, management and analysis of large biological data sets.

## Progress in 2019 and 2020

We have continued to work on a wide range of projects covering all of the major research areas at the Institute. We have developed new capabilities in the analysis of single cell data and in data from the Institute's nanopore sequencer. These techniques allow for a more complete analysis of gene activity in biological systems. We have developed our existing suite of software to support new epigenetics techniques such as slamSeq and singleShot and have worked on integrating results from more complex experimental designs covering a variety of different experimental techniques. Our training courses expanded to encompass python, R package development, source code management and machine learning.

## Selected Impact Activities

- We undertook a major programme of training for AstraZeneca to add to our existing programme for other commercial and academic groups around the world. Our courses can all now be taught remotely using cloud based computational infrastructure.
- We created a series of short online talks 'Bitesize Bioinformatics' to present new and interesting bioinformatics developments to both the Institute and external organisations.
- After a successful in-person event in 2019 we ran the first virtual Cambridge Bioinformatics Hackathon in 2020 attracting over 60 bioinformaticians (from Cambridge and the UK but also internationally) to develop new software and skills.




*A comparison of a published single cell dataset with data from Peter Rugg-Gunn's research group, showing that both datasets contain similar cell subtypes.*

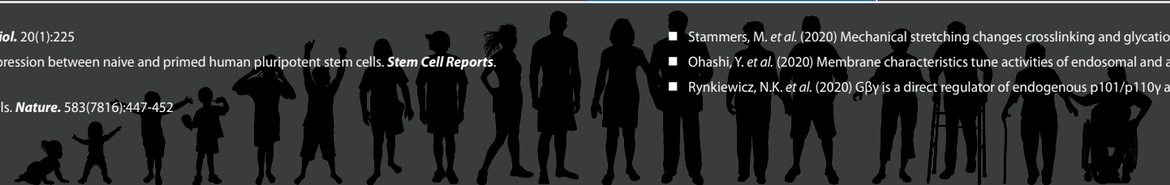
## Publications


- Argelaguet, R. *et al.* (2019) Multi-omics profiling of mouse gastrulation at single-cell resolution. ***Genome Biol.*** 20(1):225
- Wojdyla, K. *et al* (2020) Cell-surface proteomics identifies differences in signaling and adhesion protein expression between naive and primed human pluripotent stem cells. ***Stem Cell Reports***. 14(5):972-988
- Nasrallah, R. et al. (2020) A distal enhancer at risk locus 11q13.5 promotes suppression of colitis by Treg cells. ***Nature.*** 583(7816):447-452


www.bioinformatics.babraham.ac.uk

@babraham_bioinf





**Jonathan Clark**
**Facility head**

**Facility members**

**Postdoctoral research scientist:**
Izabella Niewczas

# Biological Chemistry

**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 solving biological problems along with the capability to implement our suggestions using chemical and analytical tools.**

**In addition to our collaborations with the research groups we are investigating the chemical changes which occur in connective tissues as we age.**

## Capabilities

- Chemical synthesis of standards and reagents which are not commercially available.
- Analysis of biological molecules by mass spectroscopy.
- Development of new reagents and analytical methods.
- Help and advice on any aspect of the application of chemistry/biochemistry to the exploration of biological problems.

## Progress in 2019 and 2020

During 2020 we have continued to supported groups throughout the Institute on a wide range of varied projects. These have ranged from synthetic chemical projects to make compounds which are not commercially available through to developing new analytical methods to analyse lipids in cell extracts. In addition to these activities we have also continued to run routine lipid analysis for a number of groups, both within the Institute and externally.

2020 saw the publication of key results from our research describing how the chemistry of collagen changes dynamically during tendon stretching and the implications for this when considering changes in tendon with age.

## Selected Impact Activities

- Through 2020 we have provided phosphatidyl inositide analysis on samples for commercial and external academic groups.
- We collaborate with external academic groups studying the ageing of connective tissue.

## Publications


- Stammers, M. *et al.* (2020) Mechanical stretching changes crosslinking and glycation levels in the collagen of mouse tail tendon. ***J. Biol. Chem.*** 295(31):10572-10580
- Ohashi, Y. *et al.* (2020) Membrane characteristics tune activities of endosomal and autophagic human VPS34 complexes. ***eLife.*** Jun 30;9:e58281
- Rynkiewicz, N.K. *et al.* (2020) Gβγ is a direct regulator of endogenous p101/p110γ and p84/p110γ PI3Kγ complexes in mouse neutrophils. ***Sci. Signal.*** 13(656):eaaz4003


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

**Paul Symonds**
**Co-Facility Head**

**Marc Wiltshire**
**Co-Facility Head**

**Facility members:**

6 Managers
8 Supervisors
4 Deputy Supervisors
30 Experienced Animal Technicians
4 Trainee/Apprentice Animal Technicians
4 Support Services Technicians
1 Technical Services Technician
1 Veterinary Services Manager

# Biological Support Unit

**The use of animals in research continues to be key in helping to understand biology and disease. 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. Our professionally qualified animal technicians provide expert technical support to researchers by undertaking regulated procedures, maintaining the animal health barrier and undertaking animal husbandry.**

## Capabilities

- The BSU is made up of four bio-science units, each performing a unique role in the provision of flexible services to meet the dynamic requirements of biological research. Our highly trained animal technicians and service technicians perform daily animal husbandry duties and provide essential services to the facility.
- Our animal technicians hold Home Office Personal Licences enabling us to provide technical support for researchers. We have a commitment to uphold the highest standards of animal welfare in all aspects of our work.
- Our Central Services unit utilises robotic cage-washing technology and automated sterilisation processes to provide equipment and consumables to the bio-science units.

## Progress in 2019 and 2020

- The BSU has formed a successful partnership with Avidity Science and become a recognised 'Centre of Research Excellence'. This partnership allows the BSU input and access to the latest technology and R&D discussions. The facility is currently trialling a new drinking valve prior to global launch.
- Working in collaboration with Agenda Life Sciences, the first three apprentice animal technicians all completed the programme and passed their Institute of Animal Technology (IAT) Level 2 qualifications. All three are now continuing their careers in animal care within the commercial sector. Two further apprentice candidates are now undertaking their training within the facility. The facility continues to provide advice to industry on best practice with regards to foundation training, evidence gathering and record keeping.

*Image courtesy of Datesand.*

## Selected Impact Activities

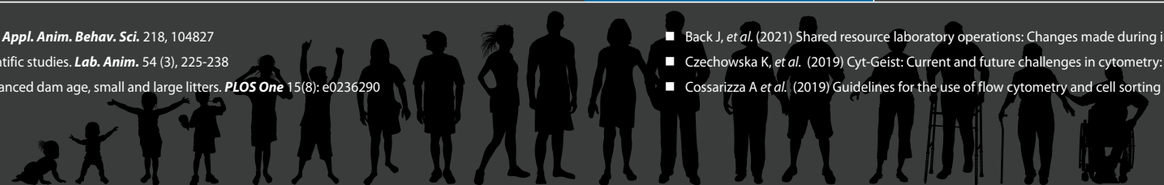
- The CellPad, a new cellulose hanging environmental enrichment for mouse cages, designed by Luke Mercer (BSU employee), was made commercially available in 2020 by Datesand, a developer and supplier of animal welfare and environmental enrichment products. The device is designed to provide environmental enrichment for mice kept in research facilities, while offering several addition benefits such as being low cost, compostable and easily sterilised and stored.
- Having contributed to the IAT syllabus review in 2020, the IAT have now advertised the release of the updated syllabus which will be rolled out in full.
- The BSU co-heads were keynote speakers at the Avidity Science Biomedical Symposium Virtual Event in October 2020. The BSU presented a virtual tour of the facility to a global industry audience, highlighting the unique design features and approaches to animal technology used on the Babraham Research Campus.

## Publications

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


- Brajon, S. *et al.* (2019). Social environment as a cause of litter loss in laboratory mouse: a behavioural study. ***Appl. Anim. Behav. Sci.*** 218, 104827
- Wilkinson, M.J. *et al.* (2019) Progressing the care, husbandry and management of ageing mice used in scientific studies. ***Lab. Anim.*** 54 (3), 225-238
- Morello, G.M. *et al.* (2020) High laboratory mouse pre-weaning mortality associated with litter overlap, advanced dam age, small and large litters. ***PLOS One*** 15(8): e0236290



**Rachael Walker**
**Facility head**

**Facility members**

**Deputy manager:**
Rebecca Roberts

**Flow cytometry specialists:**
Attila Bebes (Left in 2020)
Christopher Hall

**Flow cytometry technicians:**
Isobel Darling
Aleksandra Lazowska-Addy

# Flow Cytometry

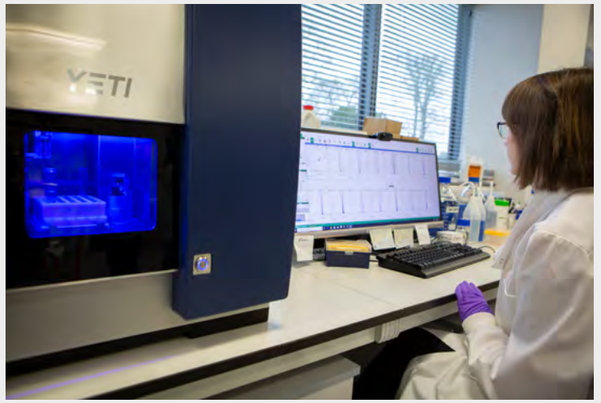
**Flow cytometry is a single cell technology that allows cells to be identified, counted, analysed, and sorted on the basis of specific features including the expression of proteins labelled with fluorescent antibodies. The Flow Cytometry facility provides a number of services to support the work of scientists from both the Institute and external companies. These include an expert cell sorting service, analyser training, help with experimental design, troubleshooting and data analysis.**

## Capabilities

- Cell sorting service: The facility provides an expert cell sorting service for Institute and external users. A range of sorters in biosafety cabinets allows for a wide range of cell types and experimental designs to be accommodated. Europe's first Thermo Fisher Bigfoot Spectral Sorter was installed in the facility in August 2020 for beta development.
- State-of-the-art analysers: BD LSRFortessa, Propel Labs YETI, and the Cytek Aurora spectral analyser allow high parameter analysis.
- Image cytometry: The Merck Millipore Imagestream MkII allows quantitative flow cytometry data to be produced with images of each cell.
- Training: The facility delivers modular training courses alongside practical training to enable scientists to use the analysers independently.

## Progress in 2019 and 2020

In 2019, the facility expanded with the acquisition of two BD Jazz sorters. These high-speed sorters have increased capacity and capability for cell sorts such as large CRISPR screens. A Cytek Aurora Spectral analyser was purchased through a BBSRC Alert-18 grant in 2019, expanding the core's multicolour capabilities. In August 2020, through a collaboration with Propel Labs (now part of Thermo Fisher) a Bigfoot 60 parameter high-speed Spectral Sorter was installed in the facility. The interactive flow cytometry training programme is in its sixth year and has supported nearly 1000 scientists. During 2020, the face-to-face training was adapted into an online virtual format.




## Selected Impact Activities

- The Flow Cytometry facility hosted the 2019 flowcytometryUK one day meeting in November 2019, bringing together over 125 delegates from all over the UK and Europe.
- In September 2019, Attilla Bebes visited several flow cytometry core facilities in Belgium and the Netherlands, including two in EU-LIFE institutes, to share best practice.
- Rachael Walker organised and co-hosted three virtual flowcytometryUK facility meetings in May, June and July 2020 to establish safe working practices in the COVID-19 era. Each meeting was attended by over 150 delegates from over a dozen countries.

## Publications

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

@babraham_flow


- Back J, *et al.* (2021) Shared resource laboratory operations: Changes made during initial global COVID-19 lockdown of 2020. ***Cytometry A.*** 99(1):22-32 (E-published Nov 2020)
- Czechowska K, *et al.* (2019) Cyt-Geist: Current and future challenges in cytometry: Reports of the CYTO 2019 conference workshops ***Cytometry A.*** 95(12):1236-127
- Cossarizza A *et al.* (2019) Guidelines for the use of flow cytometry and cell sorting in immunological studies (2nd edition). ***Eur. J. Immunol.*** 49(10):1457-1973

**Asif Nakhuda**
**Facility head**

# Gene Targeting Facility

**The main purpose of the facility is to produce new mouse strains for use in the Institute's research, aiding in the design, generation, screening and evaluation of genetic modifications. The facility also provides expertise and guidance on the use of CRISPR/Cas9, including on the design and production of CRISPR reagents such as sgRNA, Cas9 protein and long single-stranded DNA (lssDNA).**

## Capabilities

- Generation of mouse models: ranging from single nucleotide polymorphisms (SNPs), domain deletions, multiplex gene knockouts and large knock-ins such as Mini-Turbo.
- Guidance in CRISPR/Cas9-based technologies for producing transgenic cell lines and mice.
- High-throughput production of single-guide RNA with efficiency validated in embryos.
- Production of CRISPR/Cas9 reagents such Cas9 protein and lssDNA.
- Designing strategies for Gibson assembly and recombineering in bacterial artificial chromosomes (BACs) for complex constructs.
- Advice on reagents based on mechanisms of DNA repair, such as single-strand template repair, microhomology mediated end-joining and homologous recombination.
- High-throughput screening of gene-edited cell lines using Next Generation Sequencing.

## Progress in 2019 and 2020

Since re-establishing the facility in 2019, the facility has supported the Institute's researchers in achieving desired genome modifications in cell lines and mice. The facility specialises in using Cas9-sgRNA ribonucleoprotein combined with electroporation to target various cell types, and we share our methodologies with groups across the Institute to help optimise their gene editing experiments. Also, for generating mouse models, the facility has optimised electroporation and microinjection protocols for embryos. During 2020, the facility has made three new mouse lines and multiple loss-of-gene-function embryos. These new lines were made without using embryonic stem cells, meaning that mouse models can be delivered in a shorter time frame and overall using fewer mice in the process. We have also developed protocols to make in-house Cas9 protein and other Cas9 variants.

### Selected Impact Activities

- Created the first mini-turbo gene-tagged mouse using pronuclear injection.
- Created a mouse with two SNPs on the same gene, where the SNPs are in different exons.
- Generated PCV-Cas9, which is a Cas9 variant that tethers ssDNA to increase the efficiency of homology-directed repair.

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

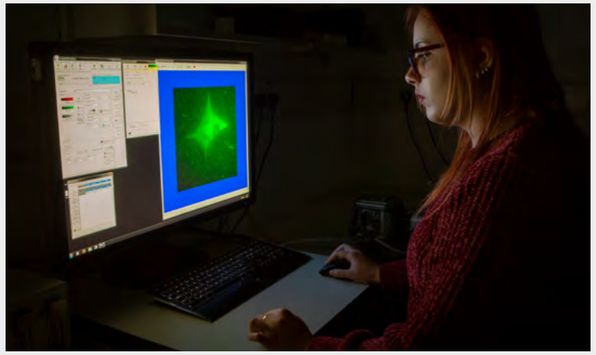
**Simon Walker**
**Facility head**

**Facility members**

**Deputy manager:**
Hanneke Okkenhaug

**SEM specialist:**
Chieko Itakura

# Imaging



**The Imaging facility provides supported access to advanced light microscopy and electron microscopy resources. Our aim is to deliver a comprehensive solution to meet the imaging demands of our user base: whether it's looking at dynamic processes in living cells, imaging cellular ultrastructure in 3D, or providing bespoke image analysis solutions, we aim to cover it all.**

## Capabilities

- High resolution fluorescence imaging: We have advanced wide-field microscopes, point scanning and spinning disk confocal systems and a super resolution imaging system, all of which can be used with both fixed and live samples.
- High content imaging: We have an InCell 6000 system which can image cells at high resolution in up to 1536-well format. We provide bespoke analysis solutions for high content imaging data.
- Focused ion beam-scanning electron microscope (FIB SEM): Our dual beam Zeiss CrossBeam 550 can acquire nanometer resolution volumetric EM data and is also equipped with a STEM detector to provide TEM-like images.
- Image analysis: We provide access to advanced commercial image analysis software and can provide tailor-made image analysis solutions for demanding applications.

## Progress in 2019 and 2020

The biggest challenge of 2019 was the full integration of our Zeiss 550 CrossBeam electron microscope into the facility. The acquisition of this microscope means we can now offer a full electron microscopy service to our users, including sample fixation and labelling, ultrathin sectioning and 3D image acquisition. Combining these capabilities with our fluorescence imaging technologies facilitates correlative imaging workflows, where samples are imaged on one of our fluorescence microscopes and then re-imaged using the electron microscope.

2020 required adapting to new working conditions brought about by Covid-19. The facility was quick to install remote training and user support systems, which have proven to be very effective at maintaining our day-to-day operations. The Imaging facility was awarded funding from BBSRC to support a major equipment upgrade, ensuring that the facility's light microscopy capabilities remain at the cutting edge.

### Selected Impact Activities

- The facility continues to provide imaging solutions for a significant number of commercial organisations, and despite the challenges, researchers from five additional companies were trained during 2020.
- The facility showcased its resources at the Babraham Research Campus as part of the virtual 2020 Campus Science Week event.
- During extended periods of lockdown, our staff have continued to work productively from home, including helping external collaborators with their image analysis requirements.

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

## Publications


- Okkenhaug, H. *et al.* (2020) Worm-align and Worm_CP, two open-source pipelines for straightening and quantification of fluorescence image data obtained from Caenorhabditis elegans. ***J. Vis. Exp.*** (159):10.3791/61136
- Dalle Pezze P. *et al.* (2020), ATG13 Dynamics in nonselective autophagy and mitophagy: Insights from live imaging studies and mathematical modeling. ***Autophagy*** 1, 1
- Odle, R.I. *et al.* (2020) An mTORC1-to-CDK1 switch maintains autophagy suppression during mitosis. ***Mol. Cell.*** 77(2): 228-240.e7

**Andrea Lopez**
**Facility head**

**Facility members**

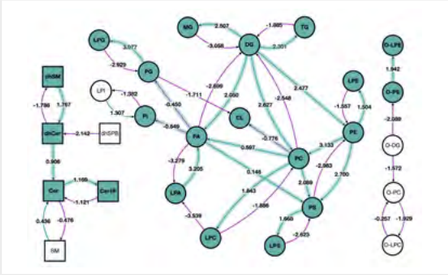
**Postdoctoral research scientist:**
Bebiana Da Costa Sousa

**Research assistant:**
Diane Taylor

**Summer placement student:**
Ethan Vicars

# Lipidomics

**The Lipidomics facility undertakes the detection and identification of lipid profiles, with the aim of providing a detailed understanding of lipid roles in cellular structure, signalling lipid/metabolic pathways, and the regulation in health and disease. Nine chromatographic methods developed in our lab allow the study of a wide range of lipid molecular species including neutral, phospho-and sphingolipids, and fatty acyl metabolites such as Coenzyme A and carnitine.**



*Automated lipid biosynthetic pathways (BioPAN) obtained using the lipid profile of young and aged mice. BioiPAN predicts the genes that activate or suppress enzymes involved in lipid metabolism calculating Z-scores. Active reaction Z > 1.9 in green.*

## Capabilities

- The facility uses liquid chromatography hyphenated to high resolution / high accuracy mass spectrometry for untargeted lipidomics (Orbitrap technology).
- Targeted lipidomics is performed by liquid chromatography hyphenated to triple quadrupole mass spectrometers (SCIEX 6500).
- Shotgun throughput analysis is performed via an Advion NanoMate coupled to a high resolution/high mass accuracy mass spectrometer.
- Automated semi-quantitation of the lipid levels compared to control samples prior to normalisation of the data to the weight/DNA or protein content.
- Hydrophobic extraction of the lipids present in cell lines and tissues from mouse, worm and human samples.
- Bioinformatic pathway analysis of lipidomics data utilising BioPAN (a free software tool availble on the LIPID MAPS website), which suggests active or suppressed enzymes modified by treatment or physiological state (as shown in the figure).
- Automated statistical analysis of the relative and/or absolute quantitation of the lipid levels of a 'before' sample and an 'after' sample, and then comparison of the data sets to see what has changed with significant biological relevance ($p < 0.05$).

## Progress in 2019 and 2020

We have continued to develop two quantitative methods for the identification of short and long chain CoA within a range of biological systems. Our work during 2019 and 2020 included internal collaborations with the Florey, Hawkins/ Stephens and Casanueva labs. After the sad loss of our Head of facility the facility team continued to progress on established collaborative projects with King's College London and Imperial College London analysing 40,400 lipid molecular species. In addition to these, the facility expanded its emphasis on collaborative projects, with new scientific partnerships across the UK. We also participated in an international interlaboratory methodology comparison exercise for the absolute quantitation of ceramides in human plasma. The team was delighted to successfully recruit a research associate in early 2020, with effective induction, training, and team integration achieved during the UK lockdown.

## Selected Impact Activities

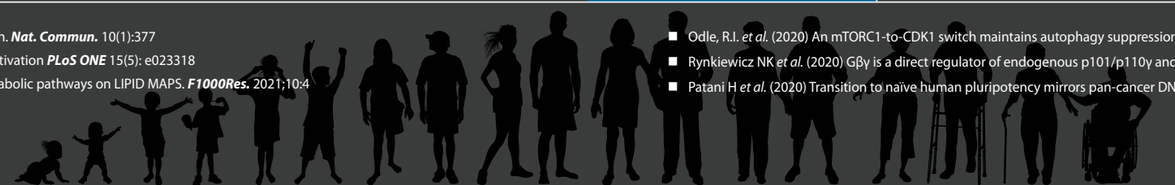
- Active external collaborations with research groups from KCL-Wolfson Centre for Age-Related Diseases; MRC Mitochondrial Biology Unit; ICL Faculty of Medicine; Nuffield Department of Medicine, Oxford University; Centre for Immunobiology, Institute of Infection, Immunity and Inflammation, University of Glasgow.
- Increased engagement with the LIPID MAPS consortium and the British Mass Spectrometry Society (BMSS): The facility hosted a BMSS-funded summer studentship in 2020.
- BioPAN (ref. 3) was set up as a free access software on the LIPID MAPS® Lipidomics Gateway website tool with support from the Bioinformatics facility.

## Publications

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


- Zhuang X. *et al.* (2019) The circadian clock components BMAL1 and REV-ERBα regulate flavivirus replication. ***Nat. Commun.*** 10(1):377
- Marshall, J.D. *et al.* (2020) THP-1 macrophage cholesterol efflux is impaired by palmitoleate through Akt activation ***PLoS ONE*** 15(5): e023318
- Gaud C, C Sousa B, Nguyen A, *et al.*. (2021) BioPAN: a web-based tool to explore mammalian lipidome metabolic pathways on LIPID MAPS. ***F1000Res.*** 2021;10:4



**David Oxley**
**Facility head**

**Facility members**

**Senior research assistant:**
Judith Webster

**Postdoctoral researcher:**
Kranthikumar Yadav G

# Mass Spectrometry

**The facility's expertise is in analytical biochemistry, and we use (bio)chemical methods in combination with mass spectrometry to study a range of biomolecules, especially proteins. Working in collaboration with scientists from across all three Institute research programmes and beyond, we use these methods to identify, quantify and obtain structural information on proteins that are involved in important biological processes, in order to help us understand how they function.**

## Capabilities

- The facility has three high-resolution tandem mass spectrometers (Orbitrap Eclipse, Q-Exactive Plus and Q-Exactive), each interfaced to nanoLC systems.
- We can undertake a full range of high-sensitivity mass spectrometric protein analyses including:
  - quantitative proteome analysis (label-free, SILAC, isobaric tagging);
  - identification/quantitation of proteins in purified complexes;
  - identification, localisation and quantitation of post-translational modifications;
  - detailed structural characterisation of individual proteins;
  - targeted protein quantitation.
- Quantitation of DNA modifications, particularly cytosine modifications 5mC, 5hmC, 5fC and 5caC.

## Progress in 2019 and 2020

Following a successful bid for funding to BBSRC, our ageing Orbitrap Velos was replaced with a state-of-the-art Orbitrap Eclipse. This new instrument, which was installed at the end of 2019, has far superior performance in terms of scan speed, sensitivity and resolution, and has some additional capabilities (including FAIMS interface, high mass-range, and UVPD fragmentation). This enhanced performance will enable us to analyse complex, low abundance samples in much greater detail, which is essential in trying to identify critical regulators of important biological processes, and to understand how they function.

During the Covid-19 pandemic in 2020, access to the mass spectrometry facility was restricted, however many projects were able to be progressed. Significant optimisation of the Eclipse instrument was carried out and the first large experiments have been completed.

## Selected Impact Activities

- Provided mass spectrometry analyses for external companies and academics.
- Facility staff joined the Covid-19 Mass Spectrometry Coalition (https://covid19-msc.org), which is an international group whose aim is to use mass spectrometry skills/ best practice to increase knowledge of the Covid-19 virus mechanisms and drive therapeutic/vaccine development.
- Showcased the Mass Spectrometry facility at various campus events such as Campus Science Day and Babraham Campus Science Week (virtual event, 2020).

## Publications

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


- Odle, R.I. *et al.* (2020) An mTORC1-to-CDK1 switch maintains autophagy suppression during mitosis. ***Mol Cell*** 77(2):228-240.e7
- Rynkiewicz NK *et al.* (2020) Gβγ is a direct regulator of endogenous p101/p110γ and p84/p110γ PI3Kγ complexes in mouse neutrophils. ***Sci. Signal.*** 13(656):eaaz4003
- Patani H *et al.* (2020) Transition to naïve human pluripotency mirrors pan-cancer DNA hypermethylation. ***Nat. Commun.*** 11(1):3671

**Paula Kokko-Gonzales**
**Facility head**

**Facility members**

**Sequencing specialist:**
Amelia Edwards
(left in 2020)

**Research assistant:**
Nicole Forrester
(left in 2020)

# Sequencing

**Sequencing large amounts of DNA from many samples, a process called high-throughput sequencing, has the potential to further our understanding of mechanisms for gene regulation. It can also help to enhance our knowledge of DNA organisation and structure. The Sequencing facility provides researchers with access to cutting-edge sequencing technology to advance their research.**

## Capabilities

- Sequencing service using a range of sequencing instruments (NextSeq500, HiSeq2500 and MiSeq) that enables researchers to select the sequencing depth and read length needed for their project.
- Quality control services for RNA to improve success in library preparation and for DNA libraries to ensure optimal sequencing quality and yield.
- Library preparation services using the automated liquid handling technology of the Hamilton NGS Star. Currently automated protocols include the SmartSeq v2 and NEB Next Ultra II RNA-seq library preparation protocols.
- Single cell library preparation using the 10x Genomics Chromium Controller.

## Progress in 2019 and 2020

During 2019 and 2020 the Sequencing facility continued to improve automated RNA-seq library preparation services to make this powerful investigative tool available to a wider range of researchers. Library preparation using the Hamilton NGS Star liquid handling system with on-deck thermal cycling provides an integrated sample-to-library solution with enhanced reproducibility and throughput.

Introduction of the 10x Genomics Chromium Controller single cell partitioning and barcoding system allowed researchers to study gene expression, copy number variation and chromatin accessibility as well as to profile the immune system repertoire at an unprecedented level of resolution.

## Selected Impact Activities

- Nicole Forrester attended the Research Institute Technician Symposium (RITS2019), The Crick Institute, London, November 2019.
- Facility participated in a public engagement event presenting to Chesterton Community College students, June 2019.
- The facility showcased its resources as part of the virtual 2020 Babraham Research Campus Science Week event.


## Publications

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


- Hull, R. M. *et al.* (2019). Transcription-induced formation of extrachromosomal DNA during yeast ageing. ***PLoS Biol.*** 17 (12):e30000471
- Hill, D. L. *et al.* (2019). The adjuvant GLA-SE promotes human Tfh cell expansion and emergence of public TCRβ clonotypes. ***J. Exp Med.*** 216 (8):1857-1873
- Argelaguet, R. *et al.* (2019). Multi-omics profiling of mouse gastrulation at single-cell resolution. ***Nature*** 576:487-491

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## Impact

**Our mission is to uncover knowledge that translates into healthier lifespans, securing health throughout life. The Institute is active in working with organisations and communities to ensure that our research has impacts for society and the economy. These interactions reach from the local area to raise public awareness of our research to working with global pharmaceutical companies where our research informs drug discovery and treatment regimes. Our research also feeds into policy development to ensure that the latest healthcare, technologies and policies incorporate the latest science.**

# Translating our science

The Knowledge Exchange and Commercialisation (KEC) team works to maximise the impact of Institute science, by ensuring that our knowledge and skills are accessible to a wide range of professionals beyond the scientists in our own field, and to ensure that our research makes an impact on society outside of academia.

Knowledge exchange harnesses the formidable know-how and cutting-edge technical skills of Institute staff to aid professionals in academia, industry, the wider scientific community and beyond. We arrange training courses, fund scientific exchanges and provide opportunities for Institute staff to interact with professionals in other sectors. This encompasses the provision of expert advice to policy makers, as well as collaborations and consultancies for biotech and pharmaceutical companies to address specific problems and foster new ideas.

Commercialisation provides a revenue source for the Institute, but more importantly is a pathway that translates our science into products useful in sectors such as healthcare. Examples from the last two years include Enhanc3D Genomics Ltd, a new company spun-out from Institute research aimed at discovering the function of disease-associated genetic polymorphisms, and the award of European Research Council (ERC) Proof of Concept funding in 2020 to Professor Adrian Liston's team for developing a pioneering anti-inflammatory platform technology to treat CNS injuries and disorders.

Individual scientists and labs do not have the knowledge, skills or resources to span discovery science, product development, clinical trials and all the other steps necessary for capitalising on an exciting bioscience discovery, so partnering with industry, clinicians, learned societies and charitable organisations helps deliver real-world applications from our science. The Institute actively promotes collaborations between Institute scientists and pharma companies, particularly with those on the Babraham Research Campus, to develop ideas and undertake early stage translational research, while also licensing discoveries and forming spin-out companies when clear pathways to market are available.

Our on-going commitment to furthering academic-industry connections on the campus promotes the commercialisation of life science research and the life science knowledge base, fosters collaboration, and enables the formation of entrepreneur driven businesses. More widely, by facilitating the exchange of our knowledge and skills, and the translation of our science, KEC helps to deliver wider economic and societal benefits arising from our science and our scientific community.

# Opening up our research

**Our Vision**

The Institute is a fully open and transparent research organisation, where public engagement is embedded throughout our research ethos. Our engagement programme maximises the impact of our research by advancing understanding, supporting innovation and addressing societal challenges. Through the programme we aim to build trust, confidence, value and dialogue between our researchers and public groups and we strive to be inspirational, highlighting the role that our research and fundamental bioscience has in our everyday lives.

**2019 and 2020 Highlights**

Throughout 2019 and 2020 public engagement remained a key part of Institute work with 105 staff and students taking part in 2019, and a further 83 in 2020, to facilitate 47 events across the two years. The work of these teams, from all parts of the Institute, allowed discussions around our research to be had with over 9,000 people who joined from Cambridge, the wider UK and beyond.

2019 was a year for reaching out. We started the year off by hosting our 25th annual Schools' Day – an event where staff and students guide secondary and sixth-form students through hands-on lab projects. Over the years this celebration of curiosity and discovery has reached nearly 3,000 students from across the UK offering them the chance to experience the work of our research programmes and facilities first-hand. We also launched our fantastic cell signalling-themed escape room experience and showcased it at a number of events including the Latitude music festival. In September, working with a number of organisations across Cambridge, we returned with 'LifeLab'. Supported by the European Commission, this project was an international celebration of science in public and saw our research showcased across the region. We ran pop-up events simultaneously in Cambridge, Peterborough, and Ely; bringing science where people were not expecting to see it and allowing more people than ever to engage with our research. Public dialogue events were also held as part of the ORION Open Science project to better understand how citizens from across Europe view genome editing. Work from this and the wider project has gone on to produce a framework for better incorporating open science practices into our engagement work as well as that of the wider Institute.

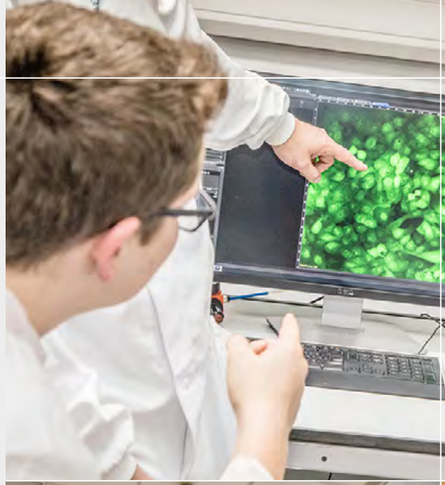
2020 brought many challenges and our programme adapted accordingly. Members of our Epigenetics research programme developed a new, online escape room experience and we also launched our Science Spotlight series of online talks. Both these initiatives allowed audiences to participate remotely and even allowed people, who are often located too far from our in-person events, to engage with the Institute's science.

**2021 Forward Look**

We will be continuing our work to reach out to audiences who have been traditionally excluded from engagement programmes with new partnerships forming in many of the rural areas of Cambridgeshire and East Anglia. We're looking forward to reintroducing in-person events into our programme as this becomes more possible. Our aim is to offer opportunities for rich discussion around the Institute's work whilst also maintaining many of our online events which have proven to make our science accessible to many more people.

www.babraham.ac.uk/about-us/impact/public

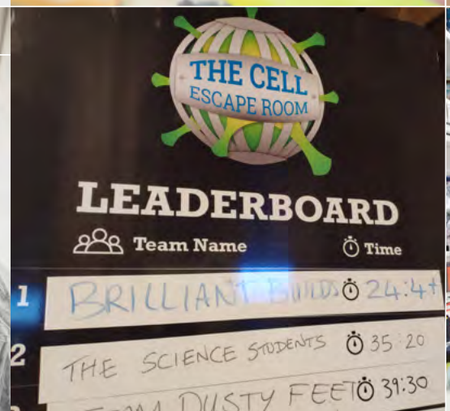
**In 2019 and 2020:**
**47 engagement events involving 188 Institute members reaching over 9,000 people**









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