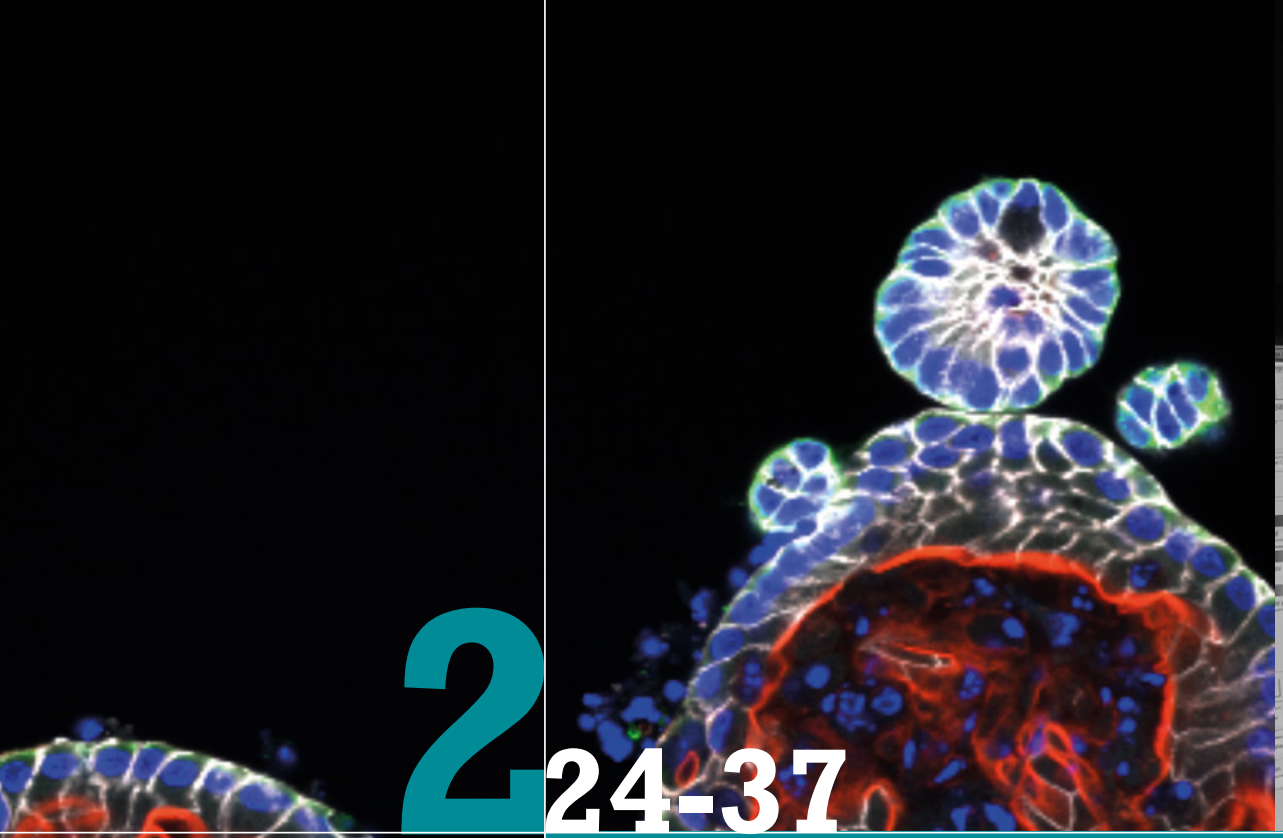
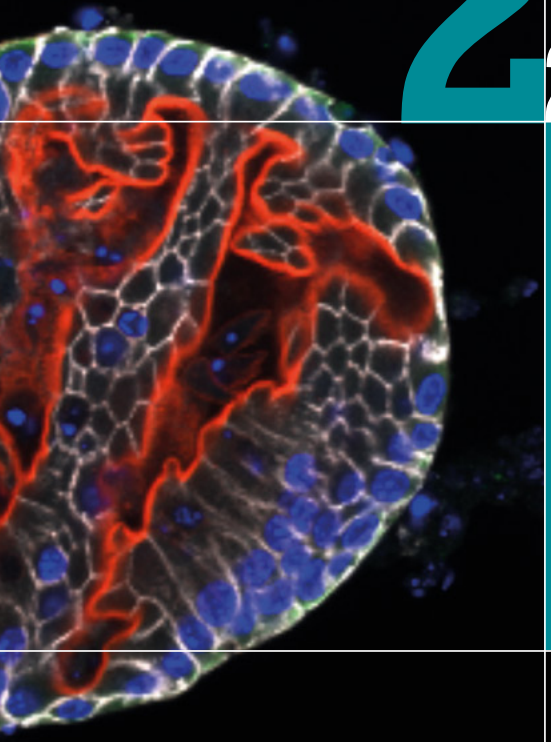
2019/20

Babraham Institute
Annual Research Report
Signalling





2 24-37 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

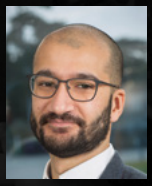
Group Leaders

Simon Cook

Oliver Florey

Phill Hawkins

Nicholas Ktistakis



Rahul Samant

Hayley Sharpe

Len Stephens

Heidi Welch

Simon Cook
Programme leader

Group members

Senior research associate:
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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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Emma Minihane (Left in 2020)
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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

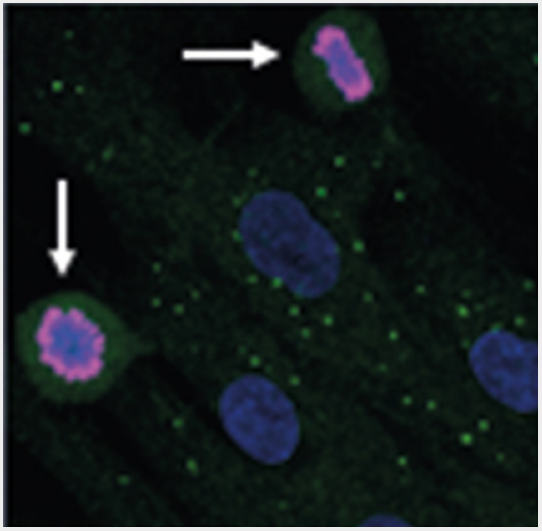
The ERK1/2 and ERK5 signalling pathway controls whether cells survive and divide or whether they senesce (a form of cellular ageing) or die. We are interested in how these different outcomes are controlled and are seeking to identify novel regulators of these pathways. In addition, we are studying autophagy, 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

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

- 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

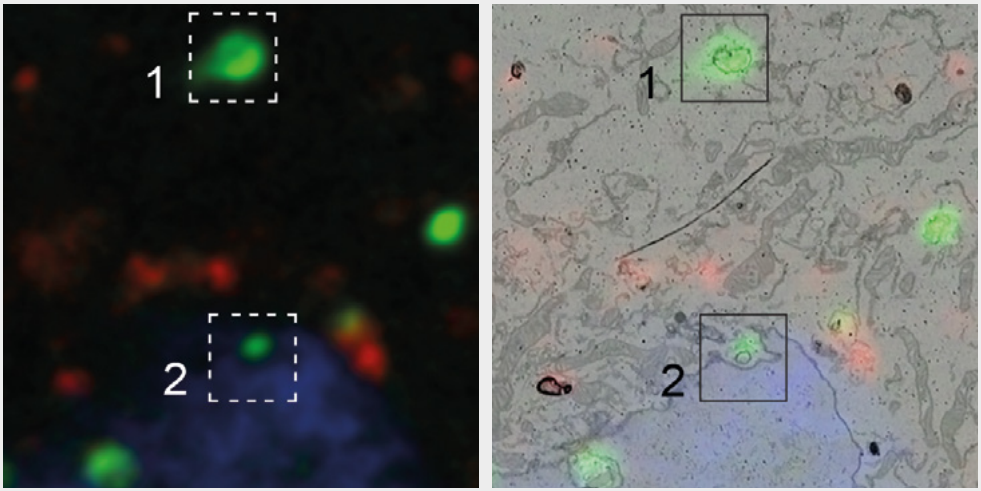
Oliver Florey

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

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

- 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

The regulation of cell signalling by PI3Ks

Phill Hawkins

Len Stephens

Group members

Senior research associates:
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Continued on page 33

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:

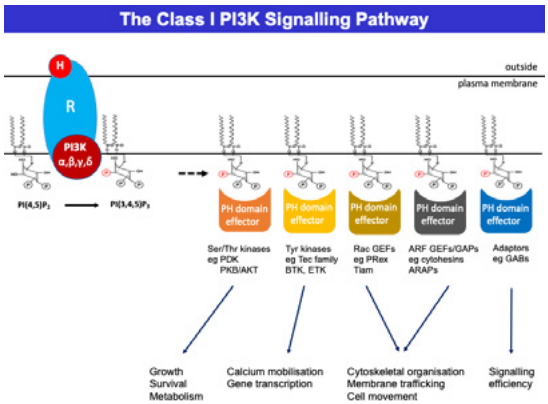
1. We have identified some of the key players in 'priming' the PI3K pathway during a neutrophil's response to pro-inflammatory stimuli (ref. 1).

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:
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Peri Tate (Left 2019)

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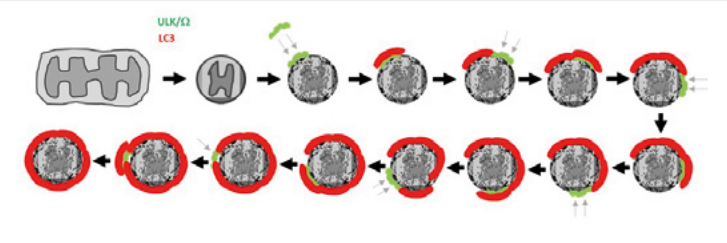
Angela Braho
Emilia Hubbard (Left in 2019)
Katerinai Kafka (Left in 2019)
Nikolaos Kontopoulos (Left in 2020)
Theodora Maniati (Left in 2020)
Felipe Renna
Milene Ortiz Silva
Filianna Tanti (Left in 2019)
Charalampos Toramanidis

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

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

@SamantLab

- 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

Hayley Sharpe

Group members

Postdoc research scientists:
Gareth Fearnley (Left in 2020)
Katie Mulholland
Kasia Wojdyla

PhD students:
Roksana Dutkiewicz
Ian Hay
Tiffany Lai
Lauren Maggs

Research assistant:
Oisharja Rahman

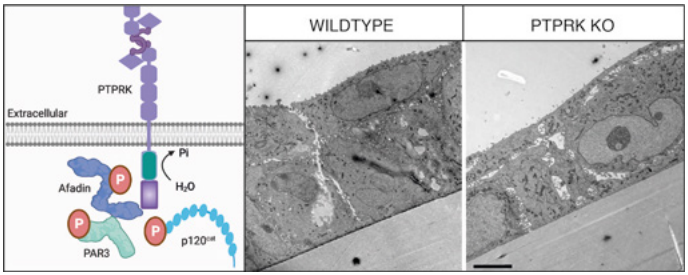
Visiting students:
Oliver Cottrell (Left in 2019)
Iain Hay
Katherine Young

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.



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).

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).

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

www.babraham.ac.uk/our-research/signalling/hayley-sharpe

@Sharpe_lab

- 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

Heidi Welch

Group members

Senior postdoctoral researcher:
Kirsti Hornigold (Left in 2020)

PhD students:
Stephen Chetwynd
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

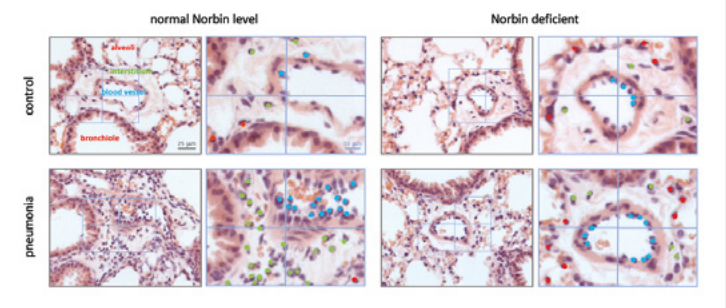
We previously discovered a family of Rac-GEF proteins we called P-Rex. We described how P-Rex1 allows white blood cells to fight disease, and we found a new protein, Norbin, that controls P-Rex1. Recently, we found unexpectedly that Norbin suppresses the immune system, and our current aim is to uncover 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.

Publications

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

- 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

Continued from page 28

Phill Hawkins
Len Stephens

Group members continued:

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

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.

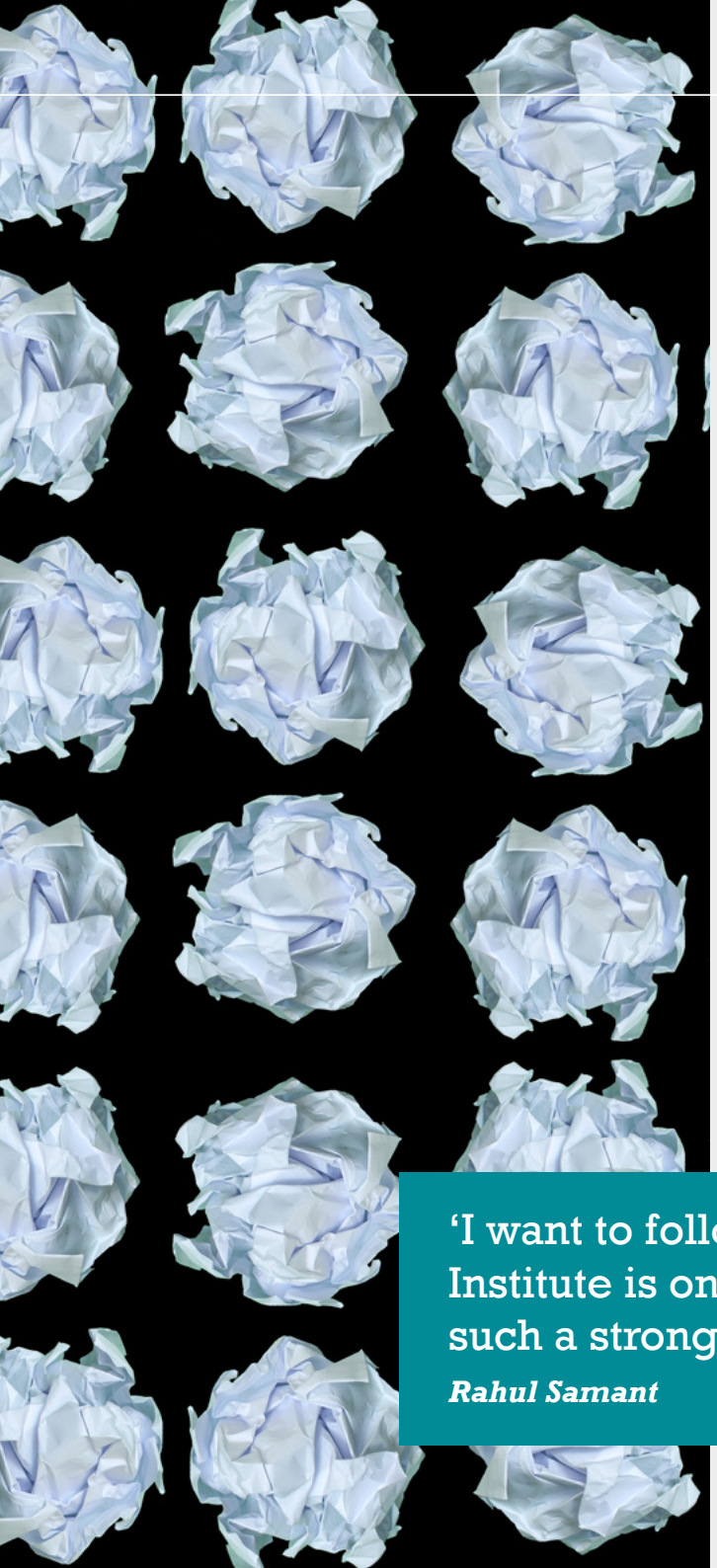
Recent advances, however, have rekindled research interest. Big data, CRISPR and other new tools point to tyrosine phosphatases being important in many diseases – including some cancers and diabetes-related macular degeneration – as well as spinal cord injuries and skin ageing. "When you're setting up 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***

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