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

Len Stephens  Simon Cook  Oliver Florey  Phill Hawkins  Nicholas Ktistakis  Michael Wakelam  Heidi Welch
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, 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 2018

We have defined some of the basic binding preferences between the regulatory and catalytic subunits of the different isoforms of Class I PI3Ks and started to establish how some of them are selectively recruited by growth factor receptors (1).

We have also revealed new mechanisms for how some tumours suppress immune cell function (2).

In addition, we have uncovered a new mechanism to explain how PI3K signalling is upregulated in neutrophils at sites of inflammation (3).

Selected Impact Activities

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

Signalling

Group members

Senior research associates:
Karen Anderson
Sabine Suire

Senior postdoctoral researcher:
Tamara Chessa

Research fellow:
Michael Wilson

Postdoctoral researcher:
Keith Davidson

PhD student:
Piotr Jung

Visiting scientists:
David Barneda
Vishnu Janardan
Francesca Massenzio
(June 2018)
Jemeen Sreedharan
Matthew White

Visiting students:
Danny Collins (Left in 2018)
Piotr Kobialka (Left in 2018)
Clement Pambrun
(Left in 2018)
Marion Trebosc (Left in 2018)
Anna Wulf (Left in 2018)

Publications

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

Signals controlling cell fate and drug resistance

Environmental factors can determine how a cell behaves – these so-called cell fate decisions include whether to divide, change cell type or die. Proteins called protein kinases control cell fate decisions by transmitting information from the outside into the cell. We are interested in how protein kinase pathways function, how they are controlled and how they determine cell fates.

**Current Aims**

Our current work is focused on studying two particular protein kinase families: the extracellular signal-regulated kinases (ERKs, such as ERK1/2) and the related dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs, such as DYRK1B and DYRK2). These protein kinases affect the cell by regulating other specific proteins (substrates), changing their properties (activity, location, binding partners). We want to understand how the ERKs and the DYRKs control cell fate decisions by defining how their activity is controlled, where in the cell they function and by identifying the substrates (proteomics) and the gene expression programmes (genomics) that they control.

**Progress in 2018**

ERK1/2 signalling controls normal cell division but is also implicated in excessive or pathological cell division such as in cancer; consequently chemical inhibitors of ERK1/2 are being developed as anti-cancer drugs. Despite this, ERK1/2 signalling does not function as a simple ‘on-off’ switch. We have found that ERK1/2 signalling operates within a ‘sweet spot’ or ‘Goldilocks zone’ to maintain cell division. If cells activate too much ERK1/2 they will undergo a permanent cell growth arrest called senescence, a form of cellular ageing. This year work in the lab has demonstrated that this response to excessive ERK1/2 signalling even determines whether cancer cells adapt and acquire resistance to ERK1/2 pathway inhibitors. Thus, a hard-wired cellular ageing mechanism also determines whether cancer cell resistance to ERK1/2 pathway inhibitors is reversible or not.

**Selected Impact Activities**

- Two ERK pathway drug discovery collaborations with PhoreMost, separately funded by Innovate UK and Plexxikon.
- Industrial collaborations on ERK pathway inhibitors (with AstraZeneca and Astex Pharmaceuticals) and ubiquitylation (with MISSION Therapeutics).
- Hosted an undergraduate student (Charlotte Wright) for a summer research placement supported by the Biochemical Society.
Signalling

Oliver Florey

Group members
Postdoctoral researchers:
Joanne Durgan
Kirsty Hooper
Nathaniel Hoyle
(Left in 2018)

PhD students:
Katherine Fletcher
Katie Sloan

Understanding autophagy and cellular recycling

Cells need to be able to break down and recycle parts of themselves, a process called autophagy, so they can stay healthy. Disruption of this process is associated with many age-related effects, including cancer and neurodegeneration. Our research explores the molecular mechanisms underlying autophagy and several similar pathways to understand their roles in health and disease.

Current Aims
Our goal is to understand the upstream regulation and downstream consequences of a 'non-canonical' autophagy pathway, which utilises some of the autophagic machinery to target external material eaten by cells, including pathogens and dead cells. This impacts many important processes within the cell. Using novel reagents and strategies developed in our lab, we are now exploring the role of this pathway in the immune system and extending our knowledge of its molecular regulation.

Progress in 2018
We have continued to build on previous successes with the publication of several papers from collaborators and our own lab. These results, and the development of a new mouse model by our lab, will extend our understanding of how cellular eating processes are regulated. Based on this success we obtained grant funding from BBSRC-UKRI to extend our work with a focus on the immune system. Experimental evidence from our lab reveals that the 'non-canonical' autophagy pathway plays a key role in how specialised immune cells, called dendritic cells, identify pathogens and communicate their presence to the rest of the immune system, to mount a protective response. This ongoing work will investigate exactly how the non-canonical autophagy pathway regulates the immune system, with a focus on these dendritic cells, and explore how this declines over lifespan. To do this, we will take advantage of our recent work in developing unique models, using both cultured cells and mice, in which the 'non-canonical autophagy' pathway is specifically turned off. We will gain a detailed understanding of this pathway and how aging impacts it, opening up the potential to modify and manipulate the novel 'non-canonical' autophagy pathway for therapeutic benefit.

Selected Impact Activities
- Dr Oliver Florey, with the help of his lab, co-organised the 2018 UK Autophagy Network meeting held in Cambridge.
- Dr Florey presented recent work at a Gordon Research Conference in Italy and the 83rd Harden Conference on Autophagy in the UK.
- Students Katherine Fletcher and Katie Sloan helped deliver the Race Against the Ageing Clock exhibit at the 2018 Royal Society Summer Science Exhibition.

Publications

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

- Fletcher, K. et al. (2018) The WD40 domain of ATG16L1 is required for its non-canonical role in lipidation of LC3 at single membranes. EMBO J. 15:37(4)
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 both cases, autophagy provides a positive response that deals with the stimulus. In the case of nutrient limitation, autophagy generates nutrients from self-digestion whereas in the case of the presence of unwanted components, autophagy 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.

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).

Progress in 2018
We have described a novel pathway of mitophagy in response to a well-used drug molecule. This pathway uses the known machinery of autophagy and some already known specific mitophagy adaptors. However, the dynamics of the response, and the early steps of selective autophagosome formation are distinct from what is known about non-selective autophagy. In other work done in collaboration, we have investigated the mechanism by which synuclein fibres, the key amyloidogenic proteins in Parkinson’s disease, activate autophagy in brain microglial cells. We discovered that these fibres cause lysosomal malfunction, and, because of this, a pathway of lysosomal quality control involving autophagy is activated in order to repair the lysosomal damage. In other work, we examined how the early steps of autophagy are controlled by the kinase TBK1 as it phosphorylates the autophagy regulator syntaxin 17.

Selected Impact Activities
- Board Member, Autophagy Metabolism and Inflammation NIH Center, NM USA.
- With my colleague Oliver Florey we edited a 740-page volume on Autophagy protocols for the series Methods In Molecular Biology, Springer, Humana Press.
- Keynote speaker at two international conferences and invited speaker at several others.

DFCP1
WIP12

Under conditions of stalled autophagy, early intermediate structures known as omegasomes accumulate.

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

Lipids and their role in health and disease

Lipids, also known as cellular fats, are highly dynamic structures with essential structural, metabolic and signalling roles. Our research aims to fully understand the physiological functions of lipids throughout the human lifespan. We use a multidisciplinary approach to identify the cellular signalling pathways and processes that individual lipid species regulate, and to investigate how the enzymes that determine the composition of the lipidome are regulated in response to changes in the environment.

Current Aims
Our present research is focused upon understanding the physiological importance of lipid molecular structures. By making use of cell and molecular biological methods coupled to lipidomics and bioinformatics we are determining the signalling and metabolic pathways that modify cellular lipids and how these are affected by ageing, viral infection and diseases such as cancer. This work utilises cell lines and model systems in mice and C. elegans and allows us to identify potentially novel therapeutic targets to treat such conditions. We are also exploring the regulation of a number of enzymes involved in lipid signalling, fatty acid biosynthesis and metabolism, notably autotaxin, stearoyl CoA desaturase and acetyl CoA synthase.

Progress in 2018
During 2018 we have continued to build upon our lipidomics expertise through our role in maintaining and developing the LIPID MAPS platform. We completed and published the determination of novel therapeutic targets to treat rhinovirus infection of human bronchial epithelial cells by integrating our novel pathway analysis of lipidomics data. We made further use of this to define lipid enzyme changes in the liver and adipose of ageing mice and to identify which enzymes respond to dietary restriction and its reversal. Our ongoing studies into the importance of autotaxin and stearoyl CoA reductase demonstrated the importance of both in hepatitis C infection of liver cells.

Optimal subnetwork analysis comparing the lipidomes of uninfected and rhinovirus virus infected primary human bronchial epithelial cells. This method identified infection-related changes in the activities of a number of lipid metabolism and signalling pathways, providing a number of potential anti-rhinoviral targets.

Selected Impact Activities
- Invited speaker at ASBMB, San Diego, USA; ESOF, Toulouse, France; International Mass Spectrometry Conference, Florence, Italy.
- Biochemical Society Morton Prize Lecture, Birmingham.
- Lab hosted a 12 month undergraduate sandwich student plus gap year and school student projects.

Publications

Cell signalling through Rac-GEFs

Rac is a protein that enables cells to attach and move through their surroundings. We study how Rac is controlled, in particular 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 cancer. In addition, we have made progress in understanding how Rac-GEFs are controlled.

Current Aims
We previously discovered a family of Rac-GEF proteins that we called P-Rex. We described how P-Rex1 allows white blood cells to be drawn to the site of an infection and how it helps to fight disease (see figure and refs 1 and 2). Our current aims are to investigate the functions of other types of Rac-GEFs in the immune system, to study the roles of P-Rex GEFs in metabolism, to evaluate the importance of their catalytic activity, and to develop new methods for monitoring GEF activity. 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 2018
We have been evaluating the importance of a protein called Norbin – a regulator of P-Rex1 which we identified (Pan et al., 2016) – in controlling defence against infections. This work has begun to uncover surprising and important roles with implications for lifelong health. We have also made progress in identifying novel roles for Rac-GEFs in the immune system and in the maintenance of healthy blood glucose levels, and we have helped our collaborators at the Garvan Institute in Sydney develop a new method for monitoring the activity of proteins such as Rac (3).

Signalling pathways of neutrophil Rac-GEFs. Many different Rac-GEFs are found within white blood cells such as neutrophils, which serve to protect us against bacterial infections. These different Rac-GEFs, which include P-Rex1 and Vav1, are all required in order to couple the various subtypes of Rac protein to a vast array of signals from the blood stream or tissues. These diverse Rac-GEF signalling pathways then lead to neutrophil responses such as adhesion and migration, and the killing of bacteria by neutrophils through various means. Figure adapted from (1) Pantarelli, C. & Welch, H.C. (2018).

Selected Impact Activities
- Members of the Welch lab gave three talks and two flash poster presentations at the ‘Small GTPases’ Biochemical Society meeting in Cambridge, July 2018.
- Heidi Welch was keynote speaker at the Cell Migration Retreat for Swiss PhD students, Bern, Switzerland in September 2018.
- Elizabeth Hampson from the Welch lab travelled to a secondary school in the Netherlands to help run a workshop on the ‘Ethics of Animal Research’ in November 2018.
Modelling biological systems

Complex behaviours of biological systems arise from the ever-changing interactions of their many parts. To understand this behaviour and the effect of various changes including ageing and disease, we need to consider these systems as a whole. This is only possible using computers to analyse large amounts of data, and simulate mathematical models reproducing the systems. Our particular interest lies in examining links between cell signalling, metabolism and epigenetics.

Current Aims
We are pursuing the use of mathematical modelling approaches to address fundamental questions relating to ageing. These range from highly focused models of specific interventions, such as the use of antibody therapy to treat rheumatoid arthritis, to more expansive projects modelling the entire ageing process of more simple organisms.

Progress in 2018
In collaboration with Dr Nicholas Ktistakis, we have built and analysed mathematical models of the autophagosome formation to gain insight into the mechanisms that regulate this process.

As part of the WormJam community we established a model of the entire metabolism of the nematode worm *Caenorhabditis elegans*. We used this model to study the metabolic changes that occur during ageing in this organism, and developed new methods which will be applicable to future ageing studies.

Selected Impact Activities
- Participated in the 2018 Computational Modelling in Biology Network meeting in Boston, USA.
- Contributed to the creation of a model of the entire metabolism of *C. elegans* which is available for use by the research community.
Welcome to the lipidome

Once neglected as too dull to study and too sticky to work with, lipids are at last stepping out of the shadows. Institute Director Michael Wakelam and lipidomics facility manager Andrea Lopez-Clavijo explain the challenges of working with these cellular Cinderellas and share their excitement of research in a field that’s finally giving up its secrets.

For Professor Michael Wakelam, there’s never been a better time to be studying lipids. On becoming the Institute’s Director in 2007, he joined a thriving lipid research community. Things were very different, however, at the beginning of his career. “When I got my first lectureship in 1985 I worried that all the good stuff had been discovered,” he remembers. “Now, I wonder how I can cram it all in before I retire. I wish I was just starting out again, because the things we can do are awesome.”

Lipids are essential components of all our cells, but were for many years neglected by many scientists because they were difficult to study and seen as less exciting than genes or proteins. “They aren’t easy to work with,” Wakelam says. “They’re not water soluble and some lipids stick to plastic. It can be painstaking work and until recently they were incredibly difficult to analyse and quantify.”

Today, all that has changed. The Institute has played a central role in lipid research since the 1950s, but it’s been the development of bioinformatics and mass-spectrometry over the past 20 years, together with the decision in 2016 that the Institute would co-host LIPID-MAPS, the world’s largest lipid database, which has opened up the field and fueled Wakelam’s excitement.

We now know that as well as making up membranes and storing energy, lipids play a vital role in cellular signalling pathways. “They don’t just hold things together and store energy. They’re actually incredibly dynamic and regulate almost every function of the cell,” he says. We have also discovered that the lipidome – which describes the total lipid landscape of our cells – is astonishingly complex and diverse. Thanks to new mass-spectrometry techniques, many of which were pioneered at the Institute, some 20,000 different lipid species from 30 distinct classes have so far been identified.

Structurally, lipids are proving fascinating too. Small differences in lipid structure and saturation have major impacts on cell membranes: whether they are thick or thin, straight or curved, rigid or flexible all depend on membranes’ lipid makeup, with far-reaching implications for how immune cells work, how cancers spread and how viruses are able to infect our cells.

Lipidomics has exploded thanks to advances in mass-spectrometry. “It’s allowed us to recognise an astonishing structural diversity in lipid molecules,” says senior research fellow and expert analytical chemist Dr Andrea Lopez-Clavijo. “Without mass-spectrometry, we wouldn’t have been able to determine what lipids were there and in what quantities. And without the bioinformatics capability to understand this data, all this would be pointless.”
But gaining a clearer view of the lipidome is only the beginning, Lopez-Clavijo explains: “Lipidomics is about finding connections between the data and its biological relevance. Making sense of our experiments is all about what the numbers tell us about the biology.”

Now that we can start asking more interesting questions, the data is revealing some remarkable things. In 2018 Institute researchers published an important paper using lipidomics to unpick the common cold. The cold virus hijacks the cellular machinery in order to replicate and release virus, so membranes must be modified for this to occur; the implications, however, had been largely ignored. “How does catching a cold affect our lipid molecules during the first few hours of infection? It surprised me that no-one had asked this question,” says Wakelam.

By culturing human bronchial epithelial cells, infecting them with the cold virus and then using mass-spectrometry and bioinformatics to examine changes in hundreds of lipids over the course of seven hours, they found that the infection caused changes in almost 500 lipids, a discovery that has allowed the team to identify pathways that could be new anti-viral drug targets.

More importantly, it shows that lipidomics has the potential to uncover new treatments for other previously hard to treat diseases from cancer to hepatitis. Recent studies with colleagues at Oxford, for example, revealed that the unsaturation of lipids affects the ability of hepatitis C virus to infect liver cells.

“Modifying the membranes modifies the viruses’ ability to get into cells. Everywhere we look we find this – it’s a completely understudied area,” Wakelam concludes. “There are many more discoveries to be made with the help of lipidomics – and it has huge potential to change what we know about our own cells.” For a former cellular Cinderella, the future for lipid research looks bright.