The expansion of research in cell biology was planned in 1992 as a result of the vision of Professor Sir Kenneth Murray, who was at the time Biogen Professor at the Institute of Cell and Molecular Biology. A seed contribution of £2.5 million from the Darwin Trust was followed by financial commitments from The Wolfson Foundation, the University and the Wellcome Trust, allowing construction of the Michael Swann Building. The majority of research space was earmarked for Wellcome Trust-funded research. Recruitment, based on research excellence at all levels in the area of cell biology, began in earnest in 1993, mostly but not exclusively, through the award of Research Fellowships from the Wellcome Trust. The Swann Building was first occupied by new arrivals in January 1996 and became “The Wellcome Trust Centre for Cell Biology” from October 2001. Core funding for the Centre from the Wellcome Trust was renewed in 2006 and 2011.
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Director’s Report

The Wellcome Centre for Cell Biology (WCB) is one of fifteen UK-based, Wellcome-funded research centres. The seventeen research groups that currently comprise the WCB, and our associated research facilities, occupy the Michael Swann Building on the King’s Buildings Campus of the University of Edinburgh. Constructed in the mid 1990s, the Swann Building was designed as a centre for research in molecular cell biology.

Cell biology is a broad discipline that encompasses the study of all that it takes to make a cell – from molecules to biological pathways and complex structures. Astonishingly, our bodies are made up of some 50 trillion human cells, every one of which contains an entire copy of the genome and all the machinery needed to duplicate itself. The many biochemical reactions taking place in those cells form pathways that are highly organised; physically, in space and in time. The key goal of the WCB is to gain new insights into cell structure and function at levels from molecular interactions to complex systems.

Research in the Centre is particularly strong in the broad field of cellular epigenetic mechanisms. The term epigenetics was coined by Conrad Waddington, the former Professor of Genetics in Edinburgh, as “the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being”. This original definition embraces all systems controlling gene expression in eukaryotes and encompasses key topics of our research. By bringing together the major themes of nuclear organisation, genome packaging and transmission, chromatin states and RNA biology, we aim to chart key interconnections between these processes and identify their mechanisms, regulation and role(s) in human disease.

This brochure presents a very brief overview of our research and highlights some of the exciting results obtained during the past year. I am delighted that the WCB has again made excellent progress with every group reporting innovative, world-class research, as reflected in our many notable publications. Centre staff have also delivered an outstanding programme of public engagement; my thanks go to everyone who gave up their time and brought their enthusiasm to share our work with the broader community in Edinburgh.

I would like to take this opportunity to congratulate WCB staff for some notable successes during the past year. In particular, A. Jeyaprakash Arulanandam, Atlanta Cook, and Julie Wellburn, who joined us as junior Research Career Development Fellows, have now been awarded highly competitive Wellcome Senior Research Fellowships. Their success in achieving this key step in the progression of their scientific careers is both a tribute to their insight and dedication and a positive indication of the support and mentoring provided by their WCB colleagues. I am also very pleased to welcome the newest member of WCB, Tony Ly, who joined us in September 2017. Tony obtained a prestigious Sir Henry Dale Fellowship from the Royal Society and Wellcome to establish his WCB research group, which focuses on the use of mass spectrometry to follow proteomic changes during the cell cycle. We also congratulate Dhanya Cheerambathur and Marcus Wilson, who are recent recipients of Sir Henry Dale Fellowships. Dhanya, who will shortly join us from the University of California, San Diego, aims to understand the specialised microtubule architecture of neuronal cells during nervous system development. Marcus will be moving to Edinburgh from the Francis Crick
Institute, to determine the mechanisms that underpin establishment and maintenance of DNA methylation, a key epigenetic mark, and its functional consequences. The recruitment and progression of these outstanding younger researchers bodes well for the long-term success of WCB. More senior WCB researchers have also been honoured recently, with the award of the Charles Rudolphe Brupbacher Prize to Sir Adrian Bird, the Mendel Medal to David Tollervey and the RNA Society Lifetime Achievement Award to Jean Beggs.

Let me end by reiterating my deepest thanks to all of our talented and dedicated researchers and support staff and congratulating them again on their excellent work over the year. Their efforts underpin all of the continuing successes of the WCB.

David Tollervey
Epigenetic inheritance: establishment and transmission of specialized chromatin domains

Chromosomal DNA is wrapped around nucleosomes containing core histones (H3/H4/H2A/H2B). However, at centromeres a specific histone H3 variant, CENP-A, replaces histone H3 to form specialized CENP-A nucleosomes. CENP-A chromatin is critical for assembly of the chromosome segregation machinery – kinetochores – at these specific chromosomal locations and is flanked by histone H3 lysine 9 methylated heterochromatin.

Our goal is to decipher conserved mechanisms that establish, maintain and regulate the assembly of heterochromatin and CENP-A chromatin domains. Heterochromatin is required for the establishment of CENP-A chromatin on centromere DNA. One objective is to provide further insight into mechanisms that promote heterochromatin formation on pericentromeric repeats. Heterochromatin might also silence genes throughout the genome, we therefore also investigate how heterochromatin formation is regulated and whether such mechanisms influence phenotype. We endeavour to determine how heterochromatin, spatial nuclear organisation and non-coding RNAPII transcription combine to mediate CENP-A incorporation at centromeres.

Our main questions are:
1. How do DNA, RNA and chromatin signatures instigate the assembly of specialized chromatin domains?
2. How does chromatin architecture and subnuclear compartmentalization affect specialized chromatin domains?
3. How does heterochromatin influence gene expression?

Long-read sequencing allowed the de novo assembly of the genomes of two fission yeast species that are evolutionarily distinct from Schizosaccharomyces pombe. Our assemblies are contiguous across all three centromeres, and other heterochromatin regions, of both species and permits comparison of centromere organization between these divergent species (Figure a). Centromeres from all three species retain an overall structural resemblance, however, no sequence similarity is detected between repetitive elements and central regions of even the closest two species, apart from the presence of similarly ordered tRNA genes (Figure b). Interspecies functional tests reveal that non-homologous S. octosporus centromere DNA is recognized and promotes centromere assembly in S. pombe. We surmise that conserved processes recognize features associated with non-conserved sequences allowing preservation of centromere identity and location over evolutionary time. Principal Component Analysis detect a distinct pattern of all possible nucleotide pentamers enriched in CENP-A-associated DNA from all three species (Figure c). Conserved processes, such as transcription, may promote recognition of these centromeric DNAs and the replacement of histone H3 chromatin with CENP-A chromatin. Consistent with this, other analyses indicate that H3 nucleosomes turnover at a high rate on centromere DNA and de novo CENP-A assembly requires H2A.Z deposition by the Swr1C chromatin remodeling complex.
**Selected Publications:**


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**Figure.**

- **a.** Centromere organisation is conserved, sequence is not. CENP-A chromatin (Purple) and H3K9me2 heterochromatin (Orange) domains mapped by ChIP-seq to *S. octosporus* and *S. cryophilus* centromere 3.
- **b.** tDNA order is conserved but intervening sequence is not. Pairwise dotplot comparison of *S. octosporus* and *S. cryophilus* outer repeat regions encoding DVAIR tDNAs.
- **c.** PCA analysis of pentamer frequencies in 12 kb sliding windows (4.5 kb intervals) across the entire genomes of *S. pombe*, *S. octosporus* and *S. cryophilus* (Grey) reveals that a statistically significant distinct pattern of pentamers resides in CENP-A chromatin regions.
Structural Biology of Cell Division

Cell division is an essential biological process that ensures genome integrity by equally and identically distributing chromosomes to the daughter cells. Errors in cell division often result in daughter cells with inappropriate chromosome numbers, a condition associated with cancers and birth defects. Key events that determine the accuracy of cell division include centromere specification, kinetochore assembly, physical attachment of kinetochores to spindle microtubules and successful completion of cytokinesis. These cellular events are regulated by a number of mitotic molecular assemblies (including the Chromosomal Passenger Complex (CPC), KMN (Knl1-Mis12-Ndc80) network, the Ska complex, Spindle Assembly Checkpoint and the Anaphase Promoting Complex) involving an extensive network of protein-protein interactions.

Although much is known about the basic mechanisms of cell division, structural level mechanistic details of pathways regulating error free chromosome segregation are still emerging. In particular, a high-resolution understanding of centromere inheritance and how kinetochores employ dynamic protein interaction to harness the forces generated by spindle microtubules to drive chromosome segregation is yet to be obtained. To address these important questions requires an approach that integrates structural and functional methods capable of dissecting and probing individual roles of protein interactions mediated at varying timescale. We use molecular biology and biochemical approaches to characterize protein interactions in vitro, X-ray crystallography, Cross-linking/Mass spectrometry, Small Angle X-ray Scattering (SAXS) and Electron Microscopy for structural analysis and a combination of in vitro and cell-based in vivo functional assays using structure-guided mutations for functional characterization.

The specific questions that we aim to address currently are i) What is the molecular basis for the establishment and maintenance of CENP-A nucleosomes at centromeres? ii) How do the outer kinetochore microtubule binding components cooperate to facilitate spindle driven chromosome segregation? and iii) How CPC, a key player required for eliminating incorrect kinetochore-microtubule attachment is targeted to the kinetochore? We address these question by characterizing protein complexes involved in centromere maintenance (Mis18 and Mis18-associated), physically coupling chromosomes to kinetochores (the Ska complexes and other outer kinetochore microtubule binding factors) and error-correction (CPC and its centromere/kinetochore receptors). The structural and functional insights from these studies will also provide new avenues for targeting specific protein-interactions to fight mitosis related human health disorders.
Figure. a. CENP-A deposition model. b. Oligomerisation of s.pombe Mis18 is critical for its centromere association and centromere maintenance (Subramanian et al., 2016) c. Human Mis18 complex forms a hetero-octameric structure and the timing of the assembly is controlled by Cdk1 phosphorylation on Mis18BP1 to achieve cell-cylic controlled CENP-A deposition (Spiller et al., 2017).

Selected Publications:


Transcription and RNA splicing are at the centre of gene expression in eukaryotes, controlling gene expression levels and removing introns from primary transcripts. The mechanisms and machineries involved in both transcription and RNA splicing are highly conserved throughout eukaryotes, and the budding yeast *Saccharomyces cerevisiae* makes an excellent model system, permitting the application of genetic approaches in parallel with molecular studies. Our current focus is on links between RNA splicing and other metabolic processes, especially transcription and chromatin. Our approaches include: quantitative RT-PCR, ChIP-seq, RNA-seq, biochemical analyses and molecular genetics.

In Barrass et al. (2015) we describe the use of metabolic labelling with 4-thio-uracil for very short times to isolate newly synthesised precursor RNAs and perform RNA-seq. In this way we compared the relative speed of splicing of different pre-mRNAs, observing that, on average, ribosomal protein gene transcripts are spliced faster than most other intron-containing transcripts. Moreover, splicing is faster for introns with secondary structures that are predicted to be less stable. In Wallace and Beggs (2017) we compared data from several sources, finding that ribosomal transcripts are also spliced more efficiently (i.e. more pre-mRNA gets spliced) and more co-transcriptionality (more splicing happens while the RNA is still associated with polymerase) compared to other intron-containing transcripts.

To investigate how speed of transcription elongation affects splicing, we measured the efficiency, the co-transcriptionality and the fidelity (accuracy of correct splice site use) of splicing in yeast strains with wild-type (WT) RNA polymerase II (Pol II), or with mutant Pol II that elongates faster or slower. We show that slow Pol II elongation increases both co-transcriptional splicing and splicing efficiency and that faster elongation reduces co-transcriptional splicing and splicing efficiency in budding yeast, suggesting that splicing is more efficient when co-transcriptional. Moreover, we demonstrate that altering the Pol II elongation rate in either direction compromises splicing fidelity (e.g. Figure 1). These effects are notably stronger for the highly expressed ribosomal protein coding transcripts, which are spliced with much higher fidelity than other intron-containing transcripts (Figure 2). We propose that transcription by RNA polymerase II is tuned to optimize the efficiency and accuracy of ribosomal protein gene expression, while allowing flexibility in splice site choice with the non-ribosomal protein transcripts.
Selected Publications:

Figure 1. Sashimi plots with examples of splicing errors and how they are affected by faster or slower rates of transcription. Numbers are sequence reads across splice junctions.

Figure 2. Ribosomal protein transcripts are spliced with higher fidelity. Upper: Diagram illustrating how Splicing Error Frequency (SEF) was measured, using an alternative upstream 3’SS event as an example. Below: Distribution of the SEF in RP (pink) and non-RP (grey) intron-containing transcripts in the WT, slow and fast Pol II strains. (Aslanzadeh et al., 2018).
MeCP2 and Rett syndrome

In 2017 we significantly advanced our understanding of the function of the chromosomal protein MeCP2. This protein, which we discovered in 1992, has been the subject of intense study since the finding that mutations within it cause the profound neurological disorder Rett syndrome. Despite these efforts, its precise role has remained uncertain, with several independent hypotheses in circulation. We originally proposed that a primary role is to target sites of DNA methylation and the evidence in favour of this from several research groups has strengthened significantly during the year. A prominent cluster of Rett-causing missense mutations co-localises with the methyl-CpG binding domain and causes its inactivation. We showed that in addition to the canonical binding site mCG, MeCP2 also targets the trinucleotide mCAC, which is abundant in neurons. In addition to methylated DNA, MeCP2 has been linked with numerous protein partners. Of particular interest, a discrete region that binds to the TBL1/R1 subunits of the NCoR and SMRT corepressor complexes coincides with a cluster of Rett syndrome mutations. Using X-ray crystallography in a collaboration with Atlanta Cook, we found that the four amino acids mutated in Rett syndrome make intimate contact with the TBL1/R1 surface. This finding makes it highly likely that loss of this specific interaction is a root cause of the disorder.

These results add to the weight of evidence supporting the “bridge hypothesis”, whereby MeCP2 recruits the corepressor complexes based on the density of methylated sites in the genome. Re-analysis of chromatin immunoprecipitation data confirms the predictions of this model and, further, provides evidence that transcriptional inhibition at gene loci is proportional to the density of methylated binding sites. Since the great majority of Rett syndrome mutations inactivate either the DNA binding domain or the corepressor interaction domain, we speculated that these domains alone, which amount to only 32% of the full-length protein, would be sufficient to fulfil key functions of MeCP2. The results strongly support this hypothesis, as the MeCP2 “minigene” prevents Rett-like phenotypes in mice. Interestingly, injection of the minigene in an adeno-associated virus vector rescued mice that lacked endogenous MeCP2. This raises the possibility that the truncated MeCP2 might be used therapeutically for gene therapy. With our collaborator Stuart Cobb, also at Edinburgh University, we are exploring this possibility further.
**Selected Publications:**


**Figure 1. Functional domains of MeCP2.**

a. Molecular structure of the Methyl-CpG Binding Domain (MBD) of MeCP2 bound to DNA. Grey balls denote the methyl groups on cytosine that are essential for DNA binding.

b. A close-up of the molecular interaction between the NCoR Interaction Domain (NID) and the NCoR subunit TBLR1. The yellow backbone corresponds to the NID, with key amino acids (single letter code) denoted by orange numbers. The TBLR1 backbone is in blue, with important amino acids highlighted by black numbers. Stabilizing contacts between the two proteins are shown as dotted green lines.

c. Top: MeCP2 shown as a grey bar with the MBD (blue), NID (pink), Enhanced Green Fluorescent Protein (EGFP) tag and Nuclear Localisation Signal (NLS) all highlighted. Radically truncated versions of MeCP2 that were introduced into mice to test their ability to prevent or reverse Rett-like phenotypes. Modified forms were missing the N-terminal domain (∆N), N- and C-terminal domains (∆NC) or N- and C-termini plus the region between the MBD and the NID (∆NIC). Each of these proteins retained wildtype or near-wildtype function suggesting that domains other than the MBD and the NID are dispensable.
The expression of individual genes is controlled at the levels of mRNA transcription and also post-transcriptionally, by processes such as splicing, localization, modification or editing, and degradation. To gain a mechanistic understanding of these processes it is important to understand the interactions between the individual players, including both protein and nucleic acid components, at the molecular level. We have used structural approaches to tackle mechanistic questions about how protein-RNA interactions can control RNA maturation and RNA editing and how transcriptional repressors are recruited to methylated DNA. By combining structural studies with biochemical, biophysical and cell-based functional assays we can gain powerful insights into these molecular processes.

Previously, we focused on proteins that control RNA metabolism during eukaryotic ribosome biogenesis and RNA turnover. By solving the structure of yeast Tsr1, an essential ribosome biogenesis factor, we were able to model it into low-resolution maps of immature 40S ribosomal. This gave a key insight into how this protein controls the timing of events during maturation of the ribosomal small subunit.

We also solved several structures of an essential vertebrate protein complex that is thought to be an RNA chaperone on many types of transcript including rRNA. This complex, made up of nuclear factors 90 and 45 (NF90/NF45) specifically recognizes stretches of dsRNA. We have shown that NF90 has an evolutionary relationship to proteins involved in adenosine-to-inosine editing. In future work we hope to better understand how the full-length form of this protein recognizes dsRNA and how this may impact on RNA editing in cells.

More recently, in collaboration with Adrian Bird’s laboratory, we gained new insights into the molecular basis for a genetic autism spectrum disorder known as Rett syndrome (RTT) (Figure 1). Mutations to the methylated DNA binding protein MeCP2 cause RTT and fall in to two clusters, one in the DNA binding domain and one in a region that recruits the transcriptional co-repressor complex called NCoR/SMRT. We mapped the MeCP2 binding to the C-terminal WD40 domain TBLR1, a tetrameric core protein of NCoR/SMRT. The crystal structure of a complex between TBLR1 and a fragment of MeCP2 reveals that the only residues that make extensive contacts to TBLR1 are exactly those mutated in RTT. This suggests that a functional interaction between this region of MeCP2 and TBLR1 is required for the development of a healthy brain.
Figure 1. a. MeCP2 provides a molecular bridge between methylated CpG sequences in DNA and a large multicomponent co-repressor complex called NCoR/SMRT. This complex contains HDAC3 which deacetylates histones to maintain transcriptional repression.

b. The NCoR interaction domain (NID) of MeCP2 contains a cluster of amino acids that are known to be mutated in RTT syndrome. Some common mutations are shown under the wild-type sequence. This segment of MeCP2 was used in co-crystallisation studies with the C-terminal domain of TBLR1 but only the darker region was visible.

c. An overview of the MeCP2-TBLR1 complex and a zoomed-in view showing that the key interactions are made by residues commonly mutated in RTT.

Selected Publications:


The role of non-histone proteins in chromosome structure and function during mitosis

Our research focuses on three main aims.

1. Making mitotic chromosomes: How do mitotic chromosomes condense, and what is the role of histones and non-histone proteins in shaping them?

2. Segregating the chromosomes: How are centromere specification and kinetochore assembly controlled epigenetically?

3. Controlling the process: How does the chromosomal passenger complex (CPC) regulate chromosome segregation?

This year, a collaboration exploiting Hi-C technology (with Job Dekker at U. Mass. Medical School in Worcester) with mathematical modelling (with Leonid Mirny at M.I.T.) allowed us to propose a new model for the pathway of mitotic chromosome formation. Our lab did all the cell biology and imaging. The resulting model integrates all previous major models of chromosome structure, showing that chromosomes are built of nested dynamic loops emanating from a spiral scaffold structure composed of condensin II. The key advance enabling this study was development of a chemical biology protocol yielding an almost perfectly synchronous entry of DT40 cells into mitosis. We also completed a study showing that rapid depletion of condensin leads to novel and much more dramatic phenotypes than seen previously. Earlier this year, we published a study strongly suggesting that histone posttranslational modifications may be a key factor driving mitotic chromatin compaction.

Studies of chromosome segregation focused on using human synthetic artificial chromosomes to probe the role of epigenetics and mitotic transcription in centromere stability and function. We expanded our approach to removing histone marks from centromeres, showing that we can target multiple competing activities simultaneously to perform what are essentially in situ epistasis studies. This led to a hypothesis that centromeric H3K9ac defends centromeres against invasion by surrounding heterochromatin. We also began work on a new generation of synthetic human chromosomes containing separate heterochromatin and centromeric arrays that will allow us to systematically probe interactions between these two centromeric domains.

Our work on the CPC revealed that interactions between heterochromatin protein HP1 and the CPC play a key role in targeting and activation of this important mitotic kinase complex during mitotic entry. Other studies probed the role of nucleoporin Seh1 in targeting regulators of Tor kinase signaling to mitotic chromosomes and examined the mitotic phenotypes resulting from treatment of cells with p53-activating inhibitors of the enzyme DHODH in one study and inhibitors of telomerase in a second.

Our work is supported by a Wellcome Principal Research Fellowship and by the Centre for Mammalian Synthetic Biology.
**Selected Publications:**


**Figure.** Simulation of nested loops on a prometaphase chromosome formed by the action of condensin I and II superimposed on a micrograph showing the synchronous entry of a DT40 CDK1as cell line into mitosis. Simulation by Anton Goloborodko. Micrograph by Kumiko Samejima.
Our lab is interested in the epigenetic inheritance and organization of centromeres. Epigenetic transmission of centromere identity through many cell generations is required for proper genome regulation and when perturbed can lead to genome instability and cellular malfunction. *We use the fruit fly Drosophila melanogaster* and human cells as a model organism to address the following questions:

**How is the epigenetic identity of centromeres propagated?**

Centromeres are found at the primary constriction of chromosomes in mitosis where they remain connected before cell division. This structure is essential for an equal distribution of chromosomes to the daughter cells.

The centromere specific histone H3-variant CENP-A\textsuperscript{cenH3} is essential for kinetochore formation and centromere function. We have recently established a biosynthetic approach to target dCENP-A\textsuperscript{cenH3} to specific non-centromeric sequences such as the Lac Operator and follow the formation of functional neocentromeres. Using this approach we were able to directly demonstrate that a dCENP-A\textsuperscript{cenH3} -LacI fusion is sufficient to induce centromere formation as well as self-propagation and inheritance of the epigenetic centromere mark (Figure 1a and b); Using the LacO/LacI tethering system, we are interested in dissecting the function of centromere factors in *Drosophila* and human cells for propagation of CENP-A\textsuperscript{cenH3}. This approach has been successfully introduced into a heterologous system comprised of human centromere factors expressed in *Drosophila* Schneider S2 cells (Logsdon et al., 2015). We are currently trying to reconstitute the loading and self-propagation of either human or *Drosophila* CENP-A at the ectopic LacO locus (Figure 1c).

**What is the role of transcription at the centromere?**

Loading of CENP-A at the centromere occurs outside of S-phase and requires the removal of H3 placeholder" nucleosomes. Transcription at centromeres has been linked to the deposition of new CENP-A, although the molecular mechanism is not understood. Interestingly, transcription is able to evict nucleosomes, which can be recycled by the histone chaperone Spt6. We find that centromeric localization of Spt6, RNAPII and centromere-associated transcripts temporally coincides with dCENP-A loading from mitosis to G1. Using fast acting transcriptional inhibitors in combination with a newly developed CENP-A loading system, we demonstrate that centromeric transcription is required for dCENP-A loading by evicting placeholder nucleosomes and promoting dCENP-A transition from chromatin association to nucleosome incorporation. In contrast, loss of parental CENP-A in Spt6 depleted cells underlines the importance of CENP-A maintenance during transcription. Thus, co-operated actions of transcription and Spt6-mediated nucleosome recycling are essential for the stability of the epigenetic centromere mark dCENP-A.
Selected Publications:


Figure 1. Heterologous system to dissect the epigenetic inheritance of centromere histone CENP-A.

Figure 2. Model for the role of transcription at centromeres: Transcription remodels centromere chromatin and evicts H3-nucleosomes (green) to allow new CENP-A (red) loading. Evicted old CENP-A is recycled by transcription elongation factor Spt6.
Our research aim is to characterise the proteomic changes that accompany and control cell state transitions during cell growth and division in human cells. To achieve this aim, we use a combination of state of the art techniques, including fluorescence-activated cell sorting (FACS) and quantitative mass spectrometry (MS)-based proteomics.

We will focus initially on cell state transitions that occur during the mitotic cell division cycle. This cycle can be separated into four major phases (G1, S, G2, and M) that are largely defined by two major processes: DNA replication (S phase) and chromosome segregation (Mitosis). Cells can also enter a reversible quiescent state, called G0.

Mitosis can be further resolved into discrete subphases based on changes in cellular architecture that can be visualized by light microscopy, or by immunostaining for specific molecular signaling events, including phosphorylation of histone H3 and degradation of cyclin proteins (e.g. cyclin A and cyclin B). Unlike in mitosis, it is less clear if gap phases are similarly structured as a linear sequence of state transitions. Evidence in support of a linear progression model during gap phases is the proposed existence of an ‘antephase’ during G2. Antephase is a short time window late in G2 that precedes nuclear envelope breakdown and chromatin condensation that is characterized by an increased sensitivity towards DNA damage. On the other hand, recent studies suggest gap phases of the cell cycle are characterized by bifurcations in cell fate trajectories, leading to heterogeneous, temporally aligned cell states.

Comprehensive, molecular definitions of cell state and identity can be obtained using quantitative mass spectrometry-based proteomics (Ly et al. eLife 2014). Recent developments in mass spectrometry (MS) enable the high throughput identification and quantitation of thousands of proteins in a single analysis. Multidimensional analysis of the proteome is now possible. Static and dynamic parameters of proteins can be measured, including protein copy numbers, post translational modifications, protein-protein interactions, and protein half-life.

We developed a method combining FACS and MS to measure protein changes during mitosis proteome-wide in an asynchronous culture of human leukemia cells (Fig. 1A).

Using this method, we aim to dissect cell state transitions in the mitotic cell division cycle using quantitative, multidimensional proteomics as comprehensive readouts of cellular state (Fig. 1B).
Selected Publications:


**Figure 1.**

*a.* Four mitotic subpopulations are isolated from an asynchronous population of human cells by FACS and intracellular immunostaining of mitotic markers. 

*b.* FACS-isolated mitotic subpopulations are then subjected to proteome-wide analysis to compare differences in protein abundance, protein-protein interactions, and post translational modifications (PTMs).
Orienting Chromosomes during Mitosis and Meiosis

Our goal is to understand the molecular mechanisms that ensure the accurate transmission of chromosomes to daughter cells during cell division. Errors in chromosome segregation generate cells with the wrong number of chromosomes, known as aneuploidy. Aneuploid somatic cells, arising as a result of errors in mitosis, are associated with cancer. Aneuploid gametes are generated from erroneous meiosis and are causative for miscarriages, infertility and birth defects. We aim to uncover conserved and fundamental mechanisms in both mitosis and meiosis by employing yeast cells and frog oocytes as models, together with a wide range of cell biological and biochemical methodologies.

A central theme of current work in our laboratory is non-canonical roles of the kinetochore. The kinetochore is a complex molecular machine that assembles on the centromere and is best known for its role in coupling chromosomes to microtubules, thereby mediating the movement of chromosomes. Our work has uncovered key regulatory and structural functions of the kinetochore that impinge on various aspects of chromosome segregation:

1. **Chromosome organisation** We showed that the kinetochore targets cohesin loading to the centromere through a dedicated pathway that enriches cohesin throughout the surrounding chromosomal region (the pericentromere). We uncovered the mechanism of this targeted cohesin loading (Figure 1) and demonstrated its importance for chromosomal organisation in this region (Figure 2).

2. **Cell cycle regulation** Shugoshin is a pericentromeric adaptor protein that performs multiple distinct functions in chromosome segregation during mitosis and meiosis. We showed that shugoshin delocalizes from the pericentromere to indicate that chromosomes are properly attached to microtubules, called biorientation. Our recent work has uncovered a regulatory pathway that inactivates shugoshin, to allow cell cycle progression once biorientation has been achieved.

3. **Adaptations for meiosis** Meiosis is a modified cell division that produces gametes through two consecutive rounds of chromosome segregation. During the first meiotic division, uniquely, the maternal and paternal chromosomes or homologs are segregated. This requires several adaptations to the way in which chromosomes are segregating including the way in which chromosomes attach to the microtubules that will pull them apart, and the way in which linkages between them are lost. Our recent work has revealed how a master meiosis I-specific regulator establishes these modifications, essentially converting mitosis into meiosis. In addition, we have uncovered aspects of kinetochore assembly and function that are critical for meiosis, but not mitosis. Our future focus is to gain a thorough understanding of how kinetochores are adapted for meiosis both in yeast and vertebrates.
Figure 1. Targeted cohesin loading at the centromere. Schematics depict mechanism of kinetochore-driven cohesin loading (top left) and phosphorylation sites in the Ctf19 protein that are required for targeted cohesin loading (top right). Cohesin ChIP-Seq performed in wild type cells and cells lacking Ctf19 phosphorylation sites (ctf19-9A) is shown below.

Figure 2. Targeted cohesin loading at the centromere ensures robust centromeric cohesion. Image shows metaphase-arrested cells with a single centromere and spindle pole bodies labeled with GFP and tdTomato, respectively. Schematic and measurements of inter-centromere distance are shown to the right.

Selected Publications:
The meiotic spindle and chromosomes in oocytes

Accurate segregation of chromosomal DNA is essential for life. A failure or error in this process during somatic divisions could result in cell death or aneuploidy. Furthermore, chromosome segregation in oocytes is error-prone in humans, and mis-segregation is a major cause of infertility, miscarriages and birth defects. The chromosome segregation machinery in oocytes shares many similarities with these in somatic divisions, but also has notable differences. In spite of its importance for human health, little is known about the molecular pathways which set up the chromosome segregation machinery in oocytes. Defining these molecular pathways is crucial to understand error-prone chromosome segregation in human oocytes. Furthermore, evidence indicates that these apparent oocyte-specific pathways also operate in mitosis, although less prominently, to ensure the accuracy of chromosome segregation. Therefore uncovering the molecular basis of these pathways is also important to understand how somatic cells avoid chromosome instability, a contributing factor for cancer development.

To understand the molecular pathways which set up the chromosome segregation machinery in oocytes, we take advantage of *Drosophila* oocytes as a "discovery platform" because of their similarity to mammalian oocytes and suitability for a genetics-led mechanistic analysis. In *Drosophila* oocytes, as in human oocytes, meiotic chromosomes form a compact cluster called the karyosome within the nucleus. Later, meiotic chromosomes assemble a bipolar spindle without centrosomes in the large volume of the cytoplasm, and establish bipolar attachment. We have identified a number of genes defective in chromosome organisation and/or spindle formation in oocytes.

From studying the karyosome, we found the histone demethylase Kdm5/Lid plays an important role forming the karyosome and stable synaptonemal complex, independently of its catalytic activity. In addition, we uncovered a novel regulatory loop which controls interaction between the nuclear pore and chromatin in oocytes and somatic cells.

For bipolar spindle formation, we found that the phospho-docking 14-3-3 protein is crucial for spatial regulation of a spindle protein. It suppresses microtubule binding of the kinesin-14 Ncd in the large cytoplasm of oocytes, and this suppression is locally removed by the Aurora B kinase that acts as a chromosomal signal. We also showed that Sentin-EB1 actively prevents microtubule plus ends from forming stable kinetochore attachments during spindle formation to facilitate bipolar attachment of homologous chromosomes in *Drosophila* oocytes.
Selected Publications:


Figure. a. Meiotic spindles from control and 14-3-3ε RNAi oocytes.
b. The non-motor region of the kinesin-14 Ncd has two phosphorylation sites (S94, S96) and interacts with microtubules and the phospho-docking protein 14-3-3.
c. Two phosphorylations on Ncd differentially regulate its microtubule binding in the presence of 14-3-3. S96 phosphorylation (by PDK2) promotes 14-3-3 binding which inhibits microtubule interaction. Further phosphorylation at S94 by Aurora B releases Ncd from 14-3-3 to allow microtubule binding.
d. A model of spatial regulation of Ncd in oocytes. The phospho-docking 14-3-3 protein suppresses microtubule binding of the kinesin-14 Ncd in the large cytoplasm of oocytes, and the Aurora B kinase acting as a chromosomal signal locally removes this suppression. All mages are reproduced from Beaven et al (2017).
3D proteomics

Genes are not randomly distributed in the genome. In humans, 10% of protein–coding genes are transcribed from bidirectional promoters and many more are organised in larger clusters. Intriguingly, neighbouring genes are frequently coexpressed but rarely functionally related. We could show recently that coexpression of bidirectional gene pairs, and closeby genes in general, is buffered at the protein level (Kustatscher et al., 2017). Taking into account the 3D architecture of the genome, we found that co-regulation of spatially close, functionally unrelated genes is pervasive at the transcriptome level, but does not extend to the proteome. Non-functional mRNA coexpression in human cells appears to arise from stochastic chromatin fluctuations and direct regulatory interference between spatially close genes. Protein-level buffering likely reflects a lack of coordination of post-transcriptional regulation of functionally unrelated genes. Grouping human genes together along the genome sequence, or through long-range chromosome folding, is associated with reduced expression noise. Our results support the hypothesis that the selection for noise reduction is a major driver of the evolution of genome organisation. The large presence of non-functional coexpression of genes at the transcript but not protein level suggests that proteomics data should surpass transcriptomics data when screening for functional links between genes. We decided to follow up on this by collating protein expression datasets and mining them for functional protein associations with machine-learning.

The annotation of protein function is a longstanding challenge of cell biology that suffers from the sheer magnitude of the task. We therefore developed ProteomeHD, which documents the response of 10,323 human proteins to 294 biological perturbations, measured by isotope-labelling mass spectrometry. Using this data matrix and robust machine learning we create a co-regulation map of the cell that reflects functional associations between human proteins and that outperforms predictions done by STRING based on the NCBI GEO repository currently holding mRNA expression profiling data from more than one million human samples. Our map identifies a functional context for many uncharacterized proteins, including microproteins that are difficult to study with traditional methods. Co-regulation also captures relationships between proteins which do not physically interact or co-localize. For example, co-regulation of the peroxisomal membrane protein PEX11 with mitochondrial respiration factors led us to discover a novel organelle interface between peroxisomes and mitochondria in mammalian cells. The co-regulation map can be explored at www.proteomeHD.net.

Our lab is also continuing its development of cross-linking/mass spectrometry as a tool to investigate in cells structures of proteins and their complexes.
**Selected Publications:**


**Figure.** Housekeeping genes are clustered in the human genome, which minimizes stochastic silencing but leads to partial co-expression of thousands of functionally unrelated genes. This non-functional mRNA co-expression is buffered at the protein level.

- Genes that are spatially proximal in sequence or 3D structure of the human genome are often co-regulated at the mRNA abundance level.
- The co-expression of spatially proximal, functionally unrelated genes is buffered at the protein level.
- The co-expression of neighbouring genes at the mRNA level is driven by chromatin fluctuations and direct regulatory interference.
- Regulatory interference is likely buffered by a neutral mechanism.
Microtubule nucleation, cytoskeletal organisation, and cell polarity

Our laboratory is interested in two main areas related to cellular and cytoskeletal organisation: 1) the molecular mechanisms underlying microtubule nucleation; and 2) the regulation of cell polarity, in a systems context, under both normal and stress conditions. In both areas we use the fission yeast *Schizosaccharomyces pombe* as a model eukaryotic organism. We combine classical and molecular genetic analysis with live-cell fluorescence microscopy, biochemistry, proteomics and computational modeling.

Microtubule nucleation in all eukaryotic cells depends on the $\gamma$-tubulin complex ($\gamma$-TuC), a multi-protein complex enriched at microtubule organizing centres such as the centrosome. The spatial and temporal regulation of the $\gamma$-TuC remains largely a mystery. We discovered the fission yeast proteins Mto1 and Mto2, which form an oligomeric complex (the Mto1/2 complex) that targets the $\gamma$-TuC to different sites in the cell and also activates $\gamma$-TuC during the cell cycle. Mutations in the human homolog of Mto1 lead to the brain disease microcephaly. Our current work involves understanding the mechanism of $\gamma$-TuC activation by the Mto1/2 complex, through genetic approaches in yeast, and through expression, purification and characterization of recombinant multi-protein complexes in insect cells, *in vitro* functional reconstitution, and structural biology analysis, including X-ray crystallography. We are also using new methods to investigate how the Mto1/2 complex is localized to different subcellular structures.

Regulation of cell polarity in fission yeast is particularly interesting because it involves multiple internal cues that cooperate and compete with each other. The Rho-family GTPase Cdc42 and its associated regulators and effectors control the actin cytoskeleton and exocytosis. Microtubules provide an additional level of control in regulating site-selection for polarised growth, through the microtubule plus-tip-associated protein Tea1, the membrane protein Mod5, and their interactors. We are currently studying how the Cdc42 system and the microtubule-based system “talk to each other” under different environmental stimuli and under stress, using a combination of mutational analysis, proteomics, FRAP microscopy, and mathematical modeling. This work has led to the discovery of new cell-polarity regulators, outside of the Cdc42- and microtubule-based systems.

An important component of our work involves developing new tools in genetics, microscopy, and proteomics. This includes a robust platform for differential proteomics in fission yeast, using Stable Isotope Labeling by Amino Acids in Culture (SILAC), which we are applying to global analysis of protein phosphorylation in cell polarity, and new methods for interrogating protein-protein interactions in complex “solid-phase” organelles.
**Selected Publications:**


**Figure 1.** Stress-activated MAP kinase (SAPK) pathway in fission yeast.

**Figure 2.** The Sty1 SAPK pathway is required for cell depolarisation after actin depolymerisation by latrunculin A (LatA). Arrows show Cdc42-GTP dispersal from cell tips. In sty1Δ cells, actin is depolymerised, but Cdc42-GTP remains polarised.

**Figure 3.** Model for activation of the γ-tubulin complex by Mto1/2 complex, together with crystals of an Mto1 domain.
Mutations in widely expressed nuclear envelope (NE) proteins cause many distinct diseases with tissue-specific pathologies including muscular dystrophies, lipodystrophies, neuropathy, dermopathy, and premature-aging syndromes. This raised the question: how could mutations in the same ubiquitous protein cause distinct diseases affecting different tissues? Hypothesizing that tissue-specific partners mediate the tissue-specific pathologies, we identified candidate partners with proteomics. The NE connects on the inside to chromatin and genome organisation is disrupted in patient cells. If our hypothesis is correct, it follows that these tissue-specific NETs might direct tissue-specific patterns of genome organisation with consequences for gene expression and we have found this to be the case.

We found three muscle-specific NETs that re-position genes to the NE that are needed early in myogenesis, but subsequently become inhibitory and must be tightly shut down. Their combined knockdown blocks myogenesis. Thus, NE gene recruitment enables tighter regulatory control. Importantly, we found mutations in these muscle NETs in unlinked Emery-Dreifuss muscular dystrophy patients, further arguing the importance of this novel regulatory mechanism. We have found similar effects with a fat-specific NET in adipogenesis and found that mice lacking this protein have difficulty producing fat, become insensitive to insulin, have metabolic dysfunction and a general lipodystrophy phenotype (Fig. 1).

It appears that NE connections can also influence gene activities in the nuclear interior as during lymphocyte activation we found that released genes that were flanked by unchanging NE-associated regions remained within <0.8 µm from the NE, presumably because the flanking contacts restrict their diffusion and thus promote their association in chromosome compartments in what we call the "constrained diffusion" hypothesis. We showed that several genes and an enhancer up to 14 Mb away from one another are all released upon lymphocyte activation and associate in A2 sub-compartments. This type of regulation could contribute temporal control to lymphocyte activation.

Other lines of investigation include: 1) Studying the structure of intermediate filament lamins with the Rappsilber lab. 2) Investigating NET effects on nuclear size changes in several cancer types and screening for small molecules targeting this with the Auer and Tyers labs. Nuclear size changes mark increased disease severity and this is also tissue-specific. 3) Testing how another NET contributes to signaling of innate immune responses. 4) Investigating how herpesviruses escape through the NE, finding that vesicle fusion proteins in the NE are needed for efficient virus nuclear egress.
Selected Publications:


**Figure 1.** Phenotype of a mouse with knockout (KO) of fat-specific NET Tmem120A. a. KO and wild-type (WT) mice are similar on a healthy diet, but KO mice fail to gain weight like WT mice on a high-fat diet. b. KO mice cannot clear glucose after insulin injection. c. Respiratory exchange rate (RER) is similar between WT and KO mice on regular diet, but KO mice show a clear metabolic defect on high-fat diet.
Nuclear RNA Processing and Surveillance

We aim to understand the nuclear pathways that synthesise and process newly transcribed RNAs, the assembly of RNA-protein complexes and the surveillance activities that monitor their fidelity.

Over the past year, we discovered novel functional links between chromatin structure, transcription and RNA metabolism in baker’s yeast *Saccharomyces cerevisiae*. Reversible phosphorylation of the C-terminal domain of RNA polymerase II (RNAPII) provides a flow of information from transcribing RNAPII to the RNA processing and surveillance machinery, acting on the nascent transcript. We recently reported that the catalytic RNAPII subunit is ubiquitinated close to the DNA entry path (Ref. 1). This provides a reverse flow of information linking events on the nascent transcript back to transcribing RNAPII. In particular, we proposed that splicing-associated transcriptional pausing is enforced by RNAPII ubiquitination (Figure 1). This promotes co-transcriptional splicing of the nascent pre-mRNA, which is the norm in both yeast and humans.

We also discovered links between the nascent transcript and major chromatin modifications; methylation of histone H3 at lysine 4 (H3K4) and lysine 36 (H3K36), catalysed by the Set1 and Set2 methyltransferases, respectively. We reported that both Set1 and Set2 bind nascent RNA transcripts (Ref 2). Interactions between Set1 and RNA are predominately mediated by RRM2 and deletion of this region reduced the chromatin association of Set1, accompanied by reduced levels of H3K4 tri-methylation and increased di-methylation on protein coding genes. Notably, a class of non-coding RNAs (ncRNAs), termed CUTs, failed to bind Set1 and their genes showed high levels of H3K4 mono-methylation rather than tri-methylation that characterises protein coding genes. H3K4 mono-methylation is also a feature of human enhancers, which are transcribed into ncRNAs, termed eRNAs. Both yeast CUTs and human eRNAs are highly unstable, due to rapid degradation by the exosome complex, which is potentially linked to their histone modification status.

Long-standing observations by the group indicated that the activity of nuclear RNA degradation by the exosome nuclease complex is responsive to nutrient availability. We have now discovered that alterations in the targeting of nuclear surveillance pathways function, together with transcriptional changes, to rapidly remodel gene expression following nutrient shift - acting both positively and negatively (Ref. 3). This identified nuclear RNA surveillance as an actively regulated step in gene expression. It seems likely that changes in nuclear RNA degradation pathways will play important roles in other situations that require large scale reprogramming of gene expression, such as developmental steps in metazoans.
Figure 1. Many RNA processing factors associate with the RNA polymerase, allowing information to be transmitted from the transcribing polymerase to the processing machinery on the nascent transcript. However, it was unclear whether or how information might be transmitted to the polymerase. We recently reported (Ref. 1) that ubiquitination close to the active site of RNAPII occurs in response to RNA processing events, including pre-mRNA splicing, and is linked to transcriptional pausing. This is released by de-ubiquitination catalysed by a Bre5-Ubp3 complex that associates with the nascent transcript following successful completion of splicing.

Selected Publications:


The overarching goal of research in our lab is to elucidate how histone modifications regulate gene expression. We are keen to understand how different histone modifiers and readers interact to establish complex regulatory systems that control development and affect disease states. We are taking a multidisciplinary approach to tackle these questions, combining biochemistry with proteomic, genomic, cell-biological, imaging-based, and systems biology-inspired techniques.

We focus on the interplay between Polycomb and Trithorax group proteins at bivalent domains and poised enhancers, in order to clarify how these complexes regulate expression of developmental genes in embryonic stem (ES) cells. Bivalent domains harbour a distinctive histone modification signature featuring both the active histone H3 lysine 4 trimethylation (H3K4me3) mark and the repressive H3K27me3 mark (Figure 1A). They are presumed to maintain developmental genes in a poised state, allowing for timely expression upon differentiation while maintaining repression in ES cells. Bivalent nucleosomes adopt a previously unknown asymmetric conformation, carrying the active and repressive mark on opposite copies of histone H3 (Voigt et al., Cell, 2012). However, it remains unclear how bivalent domains function to poise genes for expression in ES cells and whether they are essential for proper ES cell differentiation and development.

To address these questions mechanistically, we performed pulldown experiments with ES cell nuclear extract and recombinant asymmetric, bivalent nucleosomes. We found that bivalent nucleosomes are unable to recruit binding proteins for H3K4me3, despite presence of the mark. In contrast, bivalent nucleosomes retain binding of H3K27me3 binders. Moreover, we further uncovered binding proteins for bivalent nucleosomes that are not recruited by either mark on its own, representing factors that might specifically read the asymmetric bivalent configuration. Our findings suggest a model by which bivalent nucleosomes mediate poising by preventing binding of activating factors while being bound by Polycomb complexes and bivalency-specific binding proteins.

To explore the function of bivalent domains and associated genetic elements from a systems biology-inspired perspective, we are establishing a reporter gene system that allows us to quantify the impact of bivalent domains on gene activation and repression kinetics in order to understand how this chromatin signature acts to fine-tune gene expression during development. We are testing the hypothesis that bivalent domains integrate repressive and activating signals in a dynamic fashion to fine-tune expression during development (Figure 1B).
Figure 1. **a.** Interactions between active and repressive factors control the establishment and function of bivalent domains in embryonic stem cells. **b.** By maintaining a plastic state, bivalent domains may ensure proper timing of expression during development.

**Selected Publications:**


Drug Discovery and Molecular Recognition in Biological Systems

Organisms from bacteria to mammals have a remarkably well conserved glycolytic (and gluconeogenic) pathways that use the same ten enzymatic steps to convert glucose to pyruvate. Though the active sites have been conserved throughout 2 billion years of evolution, there is an interesting divergence in allosteric regulatory mechanisms. We have studied the enzymatic mechanisms of allosteric enzymes from mammals, trypanosomatid parasites and pathogenic bacteria including Mycobacterium tuberculosis.

By trapping the enzymes in different conformational states it is possible to show how small molecule effector molecules including amino acids, AMP and ADP can enhance or inhibit enzyme activities. For example, the enzyme FBPase from the Leishmania parasite can be regulated by a simple feedback mechanism with increasing concentration of AMP (1) that traps the tetramer in an inactive twisted form (Figure A). Pyruvate kinase from Mycobacterium tuberculosis can act like a logical ‘OR-gate’ (3) in which two metabolites (AMP and glucose-6-P) bind to different allosteric pockets and synergistically activate the tetramer (Figure B). More sophisticated still is the regulation of the M2 isoform of human pyruvate kinase (M2PYK) where we have shown that selected amino acids (Ala, Phe, Trp) and reactive oxidation species (ROS) are potent inhibitors while other metabolites and amino acids (His and Ser) are activators. M2PYK interprets these multiple input signals that display the nutritional and stress state of the cell providing an appropriate output response to rebalance cellular metabolism (Figure C). This competition at a single allosteric site between activators and inhibitors provides a novel regulatory mechanism by which M2PYK activity is finely tuned by the relative (but not absolute) concentrations of activator and inhibitor amino acids which we call ‘allostatic regulation’.

Apart from the basic biochemical, structural and evolutionary insights gained from these studies, the allosteric binding pockets all provide excellent targets for species-specific inhibitors. We have already identified Tb phosphofructokinase inhibitors that cure mouse models of African Trypanosomiasis. High throughput screens of bacterial targets have allowed us to identify a series of hit compounds currently being modified in collaboration with the European Lead Factory. The link between cancer and glycolysis and more recently as a regulator of immune response make glycolytic enzymes interesting and relatively unexplored therapeutic targets. We are currently screening human PFK isoforms for new families of allosteric inhibitors as anticancer therapeutics.
Figure 1. The diversity of allosteric regulatory mechanisms in glycolysis.

a. Leishmania mexicana fructose bisphosphatase is regulated in by its allosteric inhibitor AMP that keeps the tetramer in an inactive conformation.

b. Mycobacterium tuberculosis pyruvate kinase acts like an ‘logical OR’ gate and is activated synergistically by two allosteric activators that bind at two distinct sites.

c. Human M2 pyruvate kinase is allosterically regulated by amino acids and fructose 2,6-bisphosphate. Blue solid arrows show the transformation of M2PYK between active tetramer (green) and inactive tetramer (orange). Green dotted arrows show that FBP and serine stabilise M2PYK in the active tetrameric form while phenylalanine, tryptophan, valine and alanine inhibit M2PYK.

Selected Publications:


To maintain their genomic integrity, eukaryotic cells must replicate their DNA faithfully and distribute it equally to the daughter cells. Mitotic defects lead to aneuploidy and cancer. This indicates that the mitotic mechanisms that are in place to allow faithful division have been compromised. The segregation of chromosomes is mediated by polarized and highly dynamic filaments, termed microtubules. Microtubules depend on motor proteins to assemble into a spindle and segregate chromosomes. These motors play key roles in cytoskeletal organization during cell division but also in cell migration, polarity, and axonal and cytoplasmic transport. However, the reductionist approach to studying these motors in isolation is not sufficient to understand their function in the cellular context. It remains unclear how the activities of individual motors and their interacting regulatory networks cooperate to generate physiological cellular function such as chromosome segregation. We aim to define how kinesin motors are modulated by their cargos to provide a specific output, and how the coordinated activities of kinesin motors are greater than the sum of their individual activities in vitro and in human cells.

**Kinetochores and motors.** CENP-E is a huge motor (312 kD), recruited to unattached kinetochores. CENP-E moves kinetochores along microtubules to facilitate chromosome alignment. How CENP-E associates with the kinetochore, how human CENP-E is activated to walk on microtubules and how CENP-E motor ensembles are coordinated to move chromosomes is currently not known. We are addressing these questions.

**Mitotic motors and microtubule dynamics.** Our lab has made new discoveries on the mechanism of mitotic microtubule depolymerases. While Kinesin-13 motors are major microtubule depolymerases, the role of Kinesin-8 motors in microtubule depolymerization remains controversial. We have shown using gene knockout that the Kinesin-8 Kif18b controls microtubule length to center the mitotic spindle at metaphase (Fig 1A, B). Using *in vitro* reconstitution, we reveal that Kif18b is a highly processive plus end-directed motor that uses a C-terminal non-motor microtubule-binding region to accumulate at growing microtubule plus ends (Fig 1C). This region is regulated by phosphorylation to spatially control Kif18b accumulation at plus ends and is essential for Kif18b-dependent spindle positioning and regulation of microtubule length (Fig 1D). Finally we demonstrate that Kif18b shortens microtubules by increasing the catastrophe rate of dynamic microtubules. Overall, our work reveals that Kif18b utilizes its motile properties to reach microtubule ends where it regulates astral microtubule length to ensure spindle centering.
Figure 1. Kif18b is a processive plus end-directed microtubule motor that controls astral microtubule length and spindle positioning. a. Representative immunofluorescence images of control and Kif18b-KO HeLa cells, acquired using antibodies against Ndc80 and β-tubulin. b. Box and whisker plot showing quantification of the spindle displacement from the center of the cell during metaphase. Each point represents the displacement of 1 spindle over at least 30 minutes. c. Kif18b is a plus end directed motor with a run length of >7µm. Motors walk processively towards the plus end of the microtubules, pausing at the microtubule tips before dissociation. d. Schematic model of Kif18b walking towards the plus end of a microtubule. Kif18b is phosphorylated close to centrosomes and chromosomes. As Kif18b reaches the cortex, the C terminus is dephosphorylated by cytoplasmic or cortical phosphatases, which allows Kif18b to accumulate at the ends of astral microtubules to destabilize them. Kif18b and MCAK could cooperate at microtubule plus ends to destabilize microtubules.

Selected Publications:
Public Engagement

In 2017, ninety-two of our researchers engaged with over 2200 members of the public, our support for Midlothian Science Festival (MSF) engaged a further 9500 members of the public: 11,833 people in total. The time that each engagement lasted was high, 51 minutes on average.

Our most successful new project in 2017, was Tattoo my Science, a project designed for adults and teenagers at festivals. We ran this at the Meadows Festival with 395 people and received some of the most positive feedback we have ever had. We also ran the event with families in Midlothian (it worked equally well) and presented it at Engage 2017 to engagement professionals. In a survey to follow up after Engage, 94% of respondents said they would or probably would use tattoos in their own engagement projects. Figs 1, 2 & 3

We completed many new presentations of projects that have been run successfully in previous years: two phases of Pupils in Labs with local senior pupils (in total 120 pupils spent the day with us) Figs 4 & 5, two days of Life Through a Lens at the Botanic gardens held together with the Epigenetic Voyage Figs 6 & 7, and 4 sessions of Life Through a Lens at primary schools.

New for 2017, we:

• Supported two of our researchers in developing and running their own fantastic engagement project DNA journey, which was presented to over 300 people, Fig 8.

• Produced nine days of Public Engagement using Glass training, reaching 15 researchers, including many outside of our centre: two of these researchers went on to hold an exhibition of their work,

• Produced a workshop that uses story-telling for children age 3-7,

• Produced a day in a local shopping centre engaging the shoppers (a different demographic to other venues).

We are currently focussed on our Cell Block Science project, a collaboration with St. Andrews University where we are delivering public engagement in HMP Edinburgh prison, Fig 9 shows a photo of a page from a prisoner’s lab book.
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