

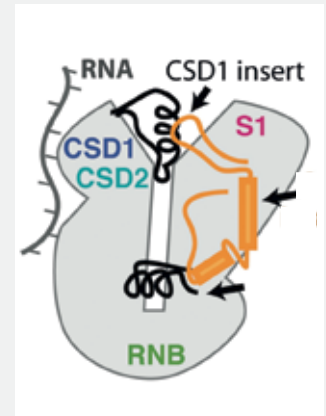
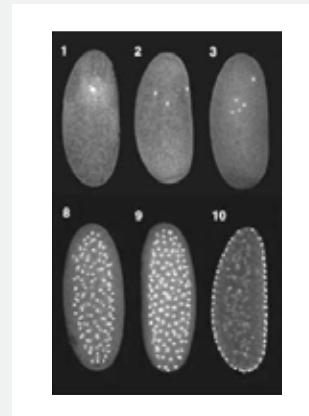
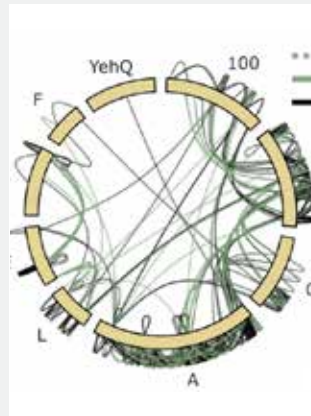
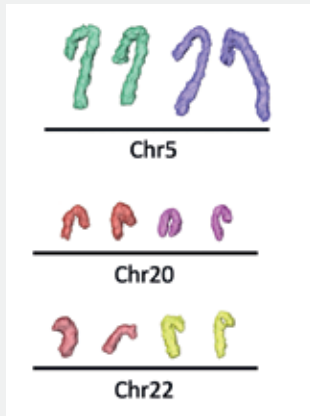
wellcome
centre
cell
biology

2022



THE UNIVERSITY
of EDINBURGH

Content



02	Director's Introduction
04	About the Wellcome Centre for Cell Biology
06	Facilities
08	Robin Allshire
10	A. Jeyaprakash Arulanandam
12	Adrian Bird
14	Dhanya Cheerambathur

16	Atlanta Cook
18	Owen Davies
20	Bill Earnshaw
22	Patrick Heun
24	Georg Kustatscher
26	Adele Marston
28	Dónal O'Carroll

30	Hiro Ohkura
32	Juri Rappsilber
34	Kenneth E Sawin
36	David Tollervey
38	Julie Welburn
40	Marcus D Wilson
42	Emeritus Centre Members

43	International Scientific Advisory Board
44	Public Engagement
46	iCM PhD Programme
47	iCM Summer Internship Programme
48	List of Groups
54	Centre Publications
61	Sustainability at WCB

Director's Introduction

In November 2021, our former Director David Tollervey stepped down after over 10 years of outstanding leadership of the Wellcome Centre for Cell Biology. On behalf of the whole Centre, I would like to express our extreme gratitude to David for all he has done to support and develop our inspiring research community. It is an honour to build on his legacy and that of the inaugural Director, Prof Adrian Bird.

This year, the Centre bounced back following the period of reduced laboratory access and lack of face-to-face interactions that were enforced by the pandemic. It is uplifting to feel the buzz in the laboratories and corridors again. Those impromptu conversations in hallways are so important and often the start of new cross-disciplinary collaborations upon which our Centre thrives.

This brochure presents a brief overview of our world-class discovery science from individual research groups and provides snapshots of our broader research community. This year, we are delighted to welcome two new groups to WCB. Owen Davies is a Wellcome Senior Fellow whose research focuses on the structural biology of meiosis. Georg Kustascher is an MRC Career Development Fellow who investigates how cells regulate protein levels, and how this is disrupted in disease.

I congratulate several WCB groups on their success in attracting major grant funding this year. David Tollervey and Robin Allshire both renewed their Wellcome Principal Research Fellowships for 5 years, Adrian Bird was awarded a Wellcome Investigator Award and Patrick Heun was awarded a BBSRC response-mode grant. Gerard Pieper in the Marston group was awarded a Sir Henry Wellcome Fellowship.

It was also wonderful to see Julie Welburn recognised as an early Career Researcher for her exceptional achievements in life sciences with the award of the Patrick Neill Medal from the Royal Society of Edinburgh. SBS achievement awards were made to Fiona Cullen in the Ohkura group for her long service and to Tania Auchnynnikava in the Allshire group for her contribution to the PhD student experience.

Professor Malcolm Walkinshaw has wound down his research group, but I am delighted that he will maintain his association with WCB as Emeritus Centre Member. We look forward to continuing to benefit from his influence and wisdom.

I congratulate WCB alumni who moved on to prestigious new positions this year and wish them every success in their future careers. Philipp Voigt moved to a new position in the Babraham Institute, Cambridge and Tomasz Turowski (Tollervey group) obtained an independent PI position in Warsaw at the Institute of Biochemistry and Biophysics, Polish Academy of Science. Tania Auchnynnikava (Allshire group) has taken a senior laboratory research scientist position in proteomics at the Francis Crick Institute.

We were also sorry to say goodbye to WCB staff who took retirement this year. As Centre Manager and Administrator of the Wellcome PhD programme for over 10 years, Karen Trail kept WCB running smoothly and offered support to many generations of students. Sarah Keer-Keer, Public Engagement Manager, established a thriving and prominent public engagement vision for WCB. John Connelly, a long-standing member of the Bird group, retired after 28 years. I thank them all for their exceptional service to WCB and wish them all the best in their future endeavours.

We are extremely fortunate to have access to world-class technology platforms supporting our research. I would like to thank the technology platform managers Dave Kelly, Shaun Webb, Christos Spanos, Martin Singleton, and Martin Wear for maintaining exceptionally high standards of service and support for our research through challenging times. I would also like to extend a welcome to Martin Singleton, who joined us this year to replace Maarten Tuijtel as the Cryo-EM Platform Manager.

Despite on-going restrictions for much of the year, the WCB Public Engagement team led by Sarah-Jane Judge has delivered a busy and diverse programme of events. WCB have also been awarded two ScotPEN Wellcome Engagement Awards (SWEA). David Tollervey's SWEA will focus on engagement with Prader-Willi syndrome patients and families. Julie Welburn, Atlanta Cook, Alison Pidoux and Tony Ly (Dundee) will use their SWEA to create fabric with science patterns for public engagement projects. My thanks to all who participated in the design, organisation and delivery of our public engagement programme.

Finally, I would like to finish by thanking and congratulating our entire WCB community for their resilience and excellent work this year. WCB is known for its collaborative ethos and landmark scientific discoveries, both of which are the product of the collective efforts of its many talented and dedicated individuals.



A handwritten signature in black ink, appearing to read 'Adele Marston'.

Adele Marston

About the Wellcome Centre for Cell Biology

The Wellcome Centre for Cell Biology has a mission to discover the fundamental molecular mechanisms that determine cell function in health and disease.

Our vision is to explore and understand how cell states are established and maintained in contexts that include infection, development, aging and disease.

Our culture nurtures ideas, disseminates knowledge and fosters a collaborative environment.

Our collective expertise straddles discipline boundaries, catalyses high quality research and is alert to translation, with ultimate benefits for human health and wellbeing.

Our research themes are intersecting and synergistic:

- Gametogenesis, inheritance and fertility.
- Cell cycle, differentiation and genetic disease.
- Adaptation, gene expression and drug resistance

Our environment is that of a cutting-edge research institute embedded within a globally influential University. The Wellcome Centre for Cell Biology benefits from access to a thriving student population and enjoys strong interdisciplinary links and collaborations with other University departments including engineering, physics, informatics, medicine and chemistry.

Public engagement is integrated into our research vision and reaches into diverse communities, with a particular emphasis on targeting those that have few opportunities for scientific discourse.

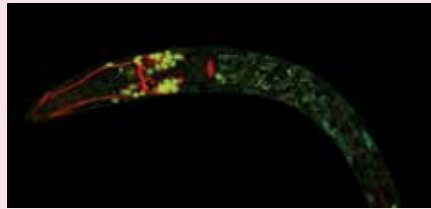
Our history began in 1992 with the vision to expand research in cell biology, developed by Professor Sir Kenneth Murray (Biogen Professor) and the Institute of Cell and Molecular Biology. A seed contribution of £2.5 million from the Darwin Trust leveraged financial support from the Wolfson Foundation, the University of Edinburgh and the Wellcome Trust, allowing construction of the Michael Swann building. The majority of the 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. This was mostly, but not exclusively, through the award of Research Fellowships from the Wellcome Trust. The Michael Swann building was first occupied in January 1996 and the

Wellcome Trust Centre for Cell Biology was founded in October 2001. Professor Adrian Bird served as inaugural Director and successfully renewed Wellcome Centre status in 2006. He was succeeded by Professor David Tollervey in 2011 who led the Centre through a further renewal in 2016. Our current Director, Professor Adele Marston took over in 2021.



Facilities

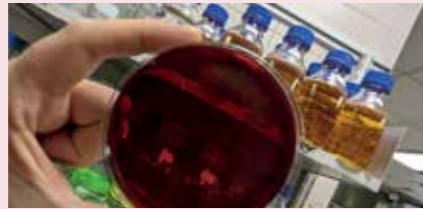
The Centre Optical Instrumentation Laboratory



The Centre Optical Instrumentation Laboratory (COIL) staff provide technical support for a wide range of imaging technologies and image analysis software. As well as user training the facility staff are able to help with experimental design and provide image analysis advice. Bespoke ImageJ plugins for analysis pipelines or to extend the functionality of ImageJ are written on request.

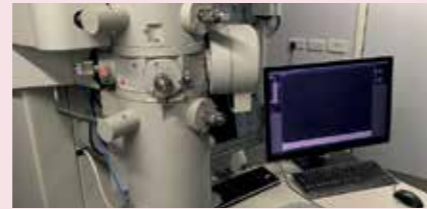
Researchers have access to both laser scanning and spinning disk confocals, a TIRF microscope, several widefield microscopes and a flow cytometer. All microscopes have environmental chambers to maintain temperature and CO₂ for live cell imaging. The equipment is bookable online from a central booking site.

Media Prep



The Media Prep and Wash Up provide the Wellcome Centre with high volumes of Buffers, Growth Media, Agar plates and Fly food. They collect glassware and equipment daily, for washing, sterilization and reuse as well as safely decontaminating lab waste. The team provide many hundreds of litres of Media, Agars and Buffers per week and over 35,000 fly vials per year. Despite the demanding workload the team are keen to promote and improve sustainability and have been working closely with the labs to tackle single use plastic waste and various other environmental issues.

Cryo-Electron Microscopy Platform



The CryoEM facility offers electron microscopy support and training for analysing a variety of biological samples. We are primarily focussed on single-particle approaches but are also interested in electron diffraction and electron-tomography techniques.

Our 200 kV Tecnai F20 microscope has been recently upgraded with a direct electron detector. We have also installed an automated data collection system, complete with online processing pipeline. This allows the microscope to be used both for sample screening prior to submission to external high-end facilities such as eBIC, as well as in-house data collection.

We have equipment for room-temperature and cryogenic sample preparation including a vitrification robot and work closely with the SBS EM facility to accommodate a wide range of sample types.

Edinburgh Protein Production Platform



Rapid solutions to the production of proteins and the biophysical characterisation of their ligands underpins many of the questions in structural, translational and cell biology today.

Located in labs in the Michael Swann Building, The Wellcome Trust, and University of Edinburgh funded Protein Production Facility (EPPF) provides researchers with access to state-of-the-art equipment and excellent end-user core facilities to address these questions.

The facility is operated by a team of three highly skilled experimentalists who not only ensure that the equipment is well maintained, but also provide training, project advice and will help design and implement your experiments to obtain the best possible results from the equipment.

Proteomics Platform



In our Proteomics Facility we use a wide range of techniques to address important biological questions. We are equipped with four state-of-the-art mass spectrometers, which are employed to accurately identify, quantify, provide structural information and demonstrate interactions of proteins even in the most complex biological samples. We are currently moving into large scale high throughput proteomic analyses.

We provide in-person training to the researchers on proteomic applications (experimental design, sample preparation and data analysis) and we offer an annual proteomics course on applications, experimental approaches and data interpretation.

Bioinformatics Core Platform



The bioinformatics core facility supports research by providing data analysis expertise and high-performance compute infrastructure. We collaborate on research projects from inception through to publication, by offering advice on experimental design, managing large amounts of data and performing computational analysis. We have a large focus on high throughput sequencing experiments, including ChIP-seq, HiC, RNA-seq and long read sequencing, and we develop workflows, visualisations and interactive applications for the processing and interrogation of these datasets. The core facility takes a lead role in encouraging researchers to develop their own skills in bioinformatics by offering regular training courses and networking events as well as promoting the tenets of reproducible research.



Robin Allshire

Co-workers: Tatsiana Auchynnikava, Roberta Carloni, Andreas Fellas, Elisabeth Gaberdiel, Nitobe London, Alison Pidoux, Severina Pociunaite, Desislava Staneva, Manu Shukla, Sharon White, Weifang Wu, Imtiyaz Yaseen, Rebecca Yeboah

Antifungal resistance is increasing in prevalence, raising fungal-borne disease frequencies in humans and crops important for human well-being. The survival of fungi in harsh environments involves stress-sensing pathways that reprogram their proteomes. New environmental conditions, including global heating, can push opportunistic fungi to colonise novel niches, thus increasing their potential to become harmful pathogens. Effective antifungal treatments are limited in number precisely because fungi are adept at resisting challenges.

Resistance to fungicides/antifungal compounds can result from genetic mutations, however, it was unknown if resistance might also arise from heritable epigenetic changes mediated by post-translational modifications carried on histones in chromatin. Using the model fission yeast (*Schizosaccharomyces pombe*) fungal system, we discovered that heterochromatin island-mediated 'epimutations' confer resistance following exposure to external insults (Torres-Garcia *et al.* 2020; Figure A). Heterochromatin islands are formed by addition of methyl groups to lysine 9 of histone H3 (H3K9me) over regions of chromatin, resulting in reduced expression of underlying genes (Figure B). For example, epimutation-mediated repression of the *cup1*⁺ gene encoding a mitochondrial LYR protein confers resistance through mitochondrial dysfunction (Figure C, D).

Transient ectopic H3K9me-dependent heterochromatin is normally rapidly erased by the counteracting H3K9 JmjC-domain Epe1 demethylase. Surprisingly, external insults such as antifungal compounds (e.g. caffeine, fluconazole) induce cleavage of Epe1 allowing heterochromatin islands to persist and confer resistance in selected lineages (Figure E). Unlike genetic mutations, such epimutations are unstable - causative heterochromatin islands, associated gene repression and resistance are lost in the absence of antifungal selection. Thus, epigenetic processes promote phenotypic plasticity so that wild-type cells adapt to unfavourable environments without irreversible genetic alterations.

We are exploiting fission yeast to define the mechanisms of epigenetic regulation that govern adaptation to challenging environments. The resulting findings will drive our investigations of processes governing the frequent emergence of antifungal resistance in divergent human (*Cryptococcus neoformans*) and plant (wheat; *Zymoseptoria tritici*) pathogens to identify and understand similarities and differences in the underlying processes.

Key questions:

1. How are heterochromatin-dependent epimutations formed and maintained?
2. What features allow specific loci and individual cells to acquire epimutations and survive insults?
3. Do related epigenetic mechanisms mediate antifungal resistance in divergent pathogenic fungi?

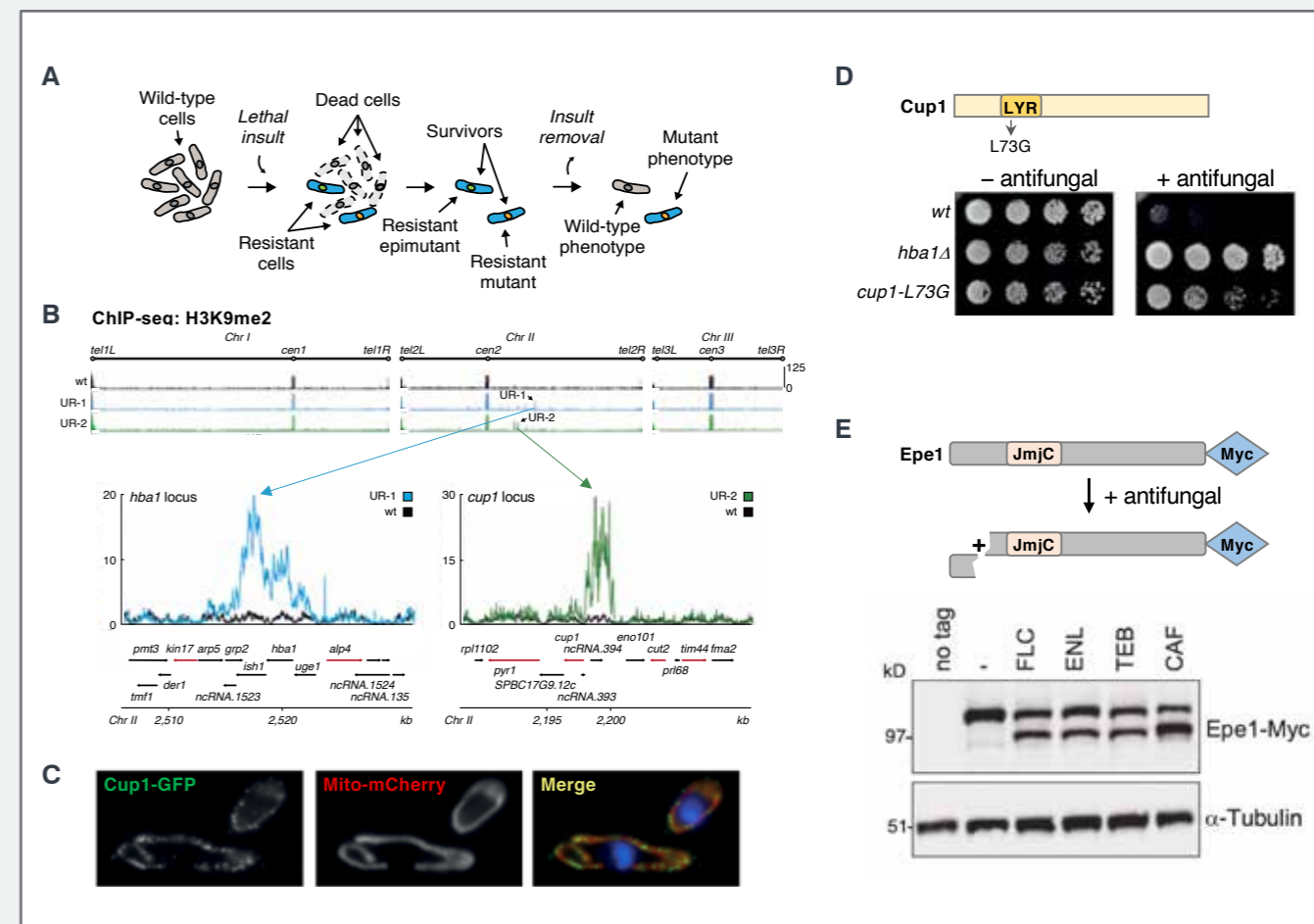
Selected Publications

Fitz-James, M.H., Tong, P., Pidoux, A.L., Ozadam, H., Yang, L., White, S.A., Dekker, J., Allshire, R.C. (2020). Large domains of heterochromatin direct the formation of short mitotic chromosome loops. *Elife* 9, e57212. doi: 10.7554/eLife.57212.

Torres-Garcia, S., Yaseen, I., Shukla, M., Audergon, P.N.C.B., White, S.A., Pidoux, A.L., Allshire, R.C. (2020). Epigenetic gene silencing by heterochromatin primes fungal resistance. *Nature* 585, 453-458. doi: 10.1038/s41586-020-2706-x.

Staneva, D.P., Carloni, R., Auchynnikava, T., Tong, P., Rappsilber, J., Jeyapakash A.A., Matthews K.R., Allshire, R.C. (2021) A systematic analysis of *Trypanosoma brucei* chromatin factors identifies novel protein interaction networks associated with sites of transcription initiation and termination. *Genome Research* 31:2138. doi: 10.1101/gr.275368.121.

Epigenetic mechanisms mediating antifungal resistance



A. Model: Resistant isolates arise in fission yeast after insult exposure. Resistance can be mediated by changes in DNA (resistant mutants) or reversible, heterochromatin-based epimutations (resistant epimutants). Upon withdrawal of insult, epimutants lose heterochromatin islands, gene repression and resistance, reverting to wild-type (sensitive phenotype). In contrast, genetic mutants continue to exhibit the mutant resistant phenotype.

B. Unstable resistant epimutants UR-1 and UR-2 exhibit novel H3K9me-dependent heterochromatin islands compared to wild-type cells (wt). Repression of *hba1*⁺ and *cup1*⁺ genes confer caffeine or antifungal resistance in UR-1 and UR-2, respectively.

C. GFP-tagged Cup1 protein (*cup1*⁺ gene, UR-2) localises to mitochondria.

D. Mutation of a conserved leucine residue (L73G) in the Cup1 LYR domain confers antifungal resistance.

E. Exposure of fission yeast to clinical (FLC, Fluconazole) or agricultural (TEB, Tebuconazole; ENL, Enilconazole) antifungals, or caffeine (CAF) results in cleavage of Epe1 promoting heterochromatin island and resistant epimutation formation (Yaseen, White *et al.*, *BioRxiv* doi.org/10.1101/2021.12.20.473483).



A. Jeyaprakash Arulanandam

Co-workers: Bethan Medina-Pritchard, Maria Alba Abad Fernandez, Carla Chiodi, Pragya Srivastava, Paula Sotelo Parrilla, Lorenza Di Pompeo*, Thomas Davies** and Anjitha Gireesh
(* joint iCM PhD student with Prof Bill Earnshaw; ** joint iCM PhD student with Prof. Kevin Hardwick)

Accurate distribution of chromosomes to the daughter cells during cell division requires selective stabilisation of chromosome-microtubule attachments, capable of supporting chromosome bi-orientation (where sister chromatids are attached to microtubules emanating from opposite spindle poles) and maintaining sister-chromatid cohesion until all sister-chromatids achieve bipolar attachment. Two chromosomal sites work at the heart of these processes: the centromere, defined by the enrichment of CENP-A (a Histone H3 variant) nucleosomes, and the inner centromere, which lies between the two sister-chromatids. The centromere acts as an assembly site for the kinetochore, where microtubules attach. Unlike canonical chromatin, CENP-A nucleosome undergo DNA replication-mediated dilution due to the distribution of existing CENP-A to the newly made DNA strand during each round of the cell cycle. To preserve centromere identity and hence to maintain the microtubule attachment site at the right place, CENP-A levels must be replenished during each cell cycle round. The inner centromere acts as a signalling/regulatory hub, recruiting factors that regulate kinetochore-microtubule attachments and control timely sister-chromatid separation.

We have a good understanding of the mechanisms controlling the assembly and function of the kinetochore. However, structural and molecular bases for the mechanisms underlying the maintenance of centromere identity and the establishment of the centromere-associated regulatory interaction network are just emerging. The overarching goal of our current work is to obtain high-resolution, mechanistic understanding of centromere/inner centromere assembly and their function in ensuring accurate segregation of chromosomes during cell division. This is crucial as defective chromosome segregation often results in aneuploidy, a chromosomal numerical aberration implicated in miscarriages, infertility, birth defects and several human cancers.

Exploiting our experience in integrating structure-function approaches (X-ray crystallography, cryo electron microscopy, Crosslinking/Mass Spectrometry, biochemical/biophysical methods with human cell-line based functional assays) to study chromosome segregation, we currently aim to address three important questions:

1. **How is the inner centromere signalling/regulatory platform established?**
2. **How does the inner centromere recruit enzymatic activities to ensure accurate chromosome segregation?**
3. **How is the centromere identity preserved through generations of cell division?**

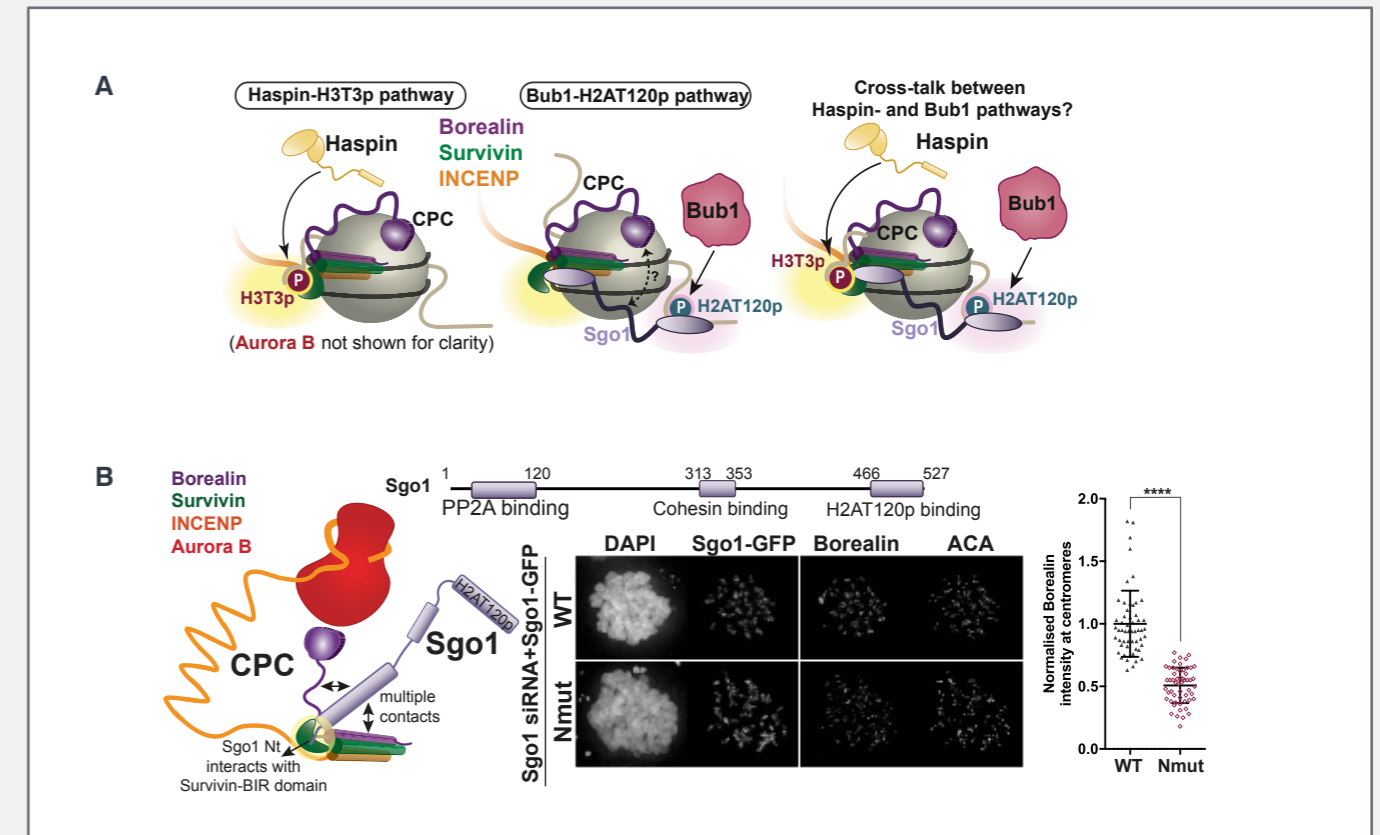
Recently, we discovered that the Chromosomal Passenger Complex (CPC), which is a major centromere associated regulator of chromosome segregation has an intrinsic nucleosome binding activity essential for its chromosome association and function (Abad et al., 2019, J Cell Biol). We have also characterised the molecular basis for how CPC interacts with Sgo1, a key regulator of sister-chromatid cohesion (Abad et al., 2021, bioRxiv).

Our ongoing and future work will provide unprecedented details of centromere-mediated control of chromosome segregation and allow us to build a comprehensive mechanistic model for error-free chromosome segregation, a process that has been fascinating researchers for more than a century.

Selected Publications

- Abad, M. A*, Gupta, T*, Hadders, M. A., Meppelink, A., Wopken, J. P., Blackburn, E., Zou, J., Buzuk, L., Kelly, D. A., McHugh, T., Rappsilber, J., Lens, S. M. A and Jeyaprakash, A. A. (2021) Molecular Basis for CPC-Sgo1 Interaction: Implications for Centromere Localisation and Function of the CPC. bioRxiv Doi: <https://doi.org/10.1101/2021.08.27.457910> (*equal contribution)
- Medina-Pritchard, B., Lazou, V., Zou, J., Byron, O., Abad, M. A., Rappsilber, J., Heun, P and Jeyaprakash, A. A. (2020) Structural Basis for Centromere Maintenance by Drosophila CENP-A Chaperone Cal1. EMBO J e103234. Doi:10.15252/embj.2019103234
- Abad, M. A., Ruppert, J. G*, Buzuk, L*, Wear, M. A., Zou, J., Webb, K. M., Kelly, D. A., Voigt, P., Rappsilber, J., Earnshaw, W. C and Jeyaprakash, A. A. (2019) Direct Nucleosome Binding of Borealin Secures Chromosome Association and Function of the CPC. J Cell Biol 218, 3912-3925. (*equal contribution)

Structural Biology of Cell Division



A. Overview of proposed pathways responsible for the centromere localization of the Chromosomal Passenger Complex (CPC; Borealin, Survivin, INCENP and Aurora B), a master regulator of chromosome segregation. Two histone phosphorylations, Histone H3 Thr3 (H3T3p) and Histone H2A Thr120 (H2AT20p), mediated by Haspin and Bub1 kinases respectively, recruit CPC to the inner centromere. CPC binds H3T3p directly via Survivin and H2AT20p indirectly via Sgo1.

B. Molecular basis for CPC-Sgo1 interaction: CPC-Sgo1 binding requires physical recognition of Histone H3 like N-terminal tail of Sgo1 by Survivin. Disrupting this interaction perturbs CPC centromere association and leads to chromosome missegregation.



Adrian Bird

Co-workers: Beatrice Alexander-Howden, Megan Brown, Kashyap Chhatbar, Sara Giuliani, Jacky Guy, Matthew Lyst, Baisakhi Mondal, Raphael Pantier, Katie Paton, Christine Struthers

MeCP2 is highly expressed in mature neurons and MeCP2-deficiency causes the profound neurological disorder Rett syndrome (RTT), in which neurons show morphological and electrophysiological defects. We previously showed that the mouse provides a convincing model of this disorder and found, remarkably, that the severe phenotypes are reversed if the protein is restored in adulthood. Thus, MeCP2 is dispensable for neurodevelopment, but essential for maintenance of the mature neuronal state.

We have made significant recent progress in elucidating the molecular mechanism underlying MeCP2 function. We showed previously that DNA binding by MeCP2 depends on 5-methylcytosine in a mCG context. Work by others showed that mCA also bound MeCP2 and this was subsequently narrowed down by our demonstration that the trinucleotide mCAC is the overwhelmingly preferred non-CG DNA binding motif. Coincidentally, CAC is the preferred non-CG target for the DNA methyltransferase DNMT3A and is highly methylated in mature neurons. To determine the biological importance of mCAC binding, we replaced the MeCP2 DNA binding domain with that of the related protein MBD2. The MBD2 domain specifically binds mCG but does not detectably interact with mCAC in vitro or in vivo. The results showed that mice expressing only the domain-swap protein displayed Rett syndrome like phenotypes, indicating that mCAC is an essential MeCP2 target.

Comparative transcriptomics indicates that MeCP2 functions to restrain expression of large numbers of genes in a DNA methylation-dependent manner. Assuming that transcriptional disturbance leads to the neuronal dysfunction that underlies RTT, two extreme hypotheses are: 1) RTT is the aggregate outcome of slightly perturbed expression of very many genes; 2) RTT strongly depends on dysregulation of a few key genes. Our recent work highlights shared dysregulated genes in different mouse models with RTT-like phenotypes, allowing a test the second possibility. Specifically, mice expressing a chimaeric MeCP2 that is unable to bind mCAC and *Mecp2-KO* mice both up-regulate genes causally implicated in autism-related disorders, including *AUTS2*, *CNTN4*, *MEF2C*, *GRIN2A*, raising the possibility that their abnormal expression contributes disproportionately to RTT. Interestingly, these genes are among the most methylated and highly affected by MeCP2 deficiency. Such “convergence” of pathways involved in different intellectual disability syndromes could have therapeutic relevance for neurodevelopmental disorders generally.

A second study published during 2021 involves SALL4 (Figure 1), a multi-zinc-finger protein that plays an important role in development and disease (e.g. SALL4 is highly expressed in many cancers with poor prognosis). We identified this protein in a screen for proteins that might interpret DNA base composition by recognising AT-rich DNA. Zinc finger cluster 4 of SALL4 specifically targets short A/T-rich motifs and recruits a partner corepressor. Inactivation of ZFC4 in embryonic stem cells leads to precocious differentiation and up-regulates AT-rich genes that are normally silenced in embryonic stem cells, thereby destabilising the pluripotent state. Our SALL4 study provides the first evidence that base composition can be read as a biological signal to regulate gene expression.

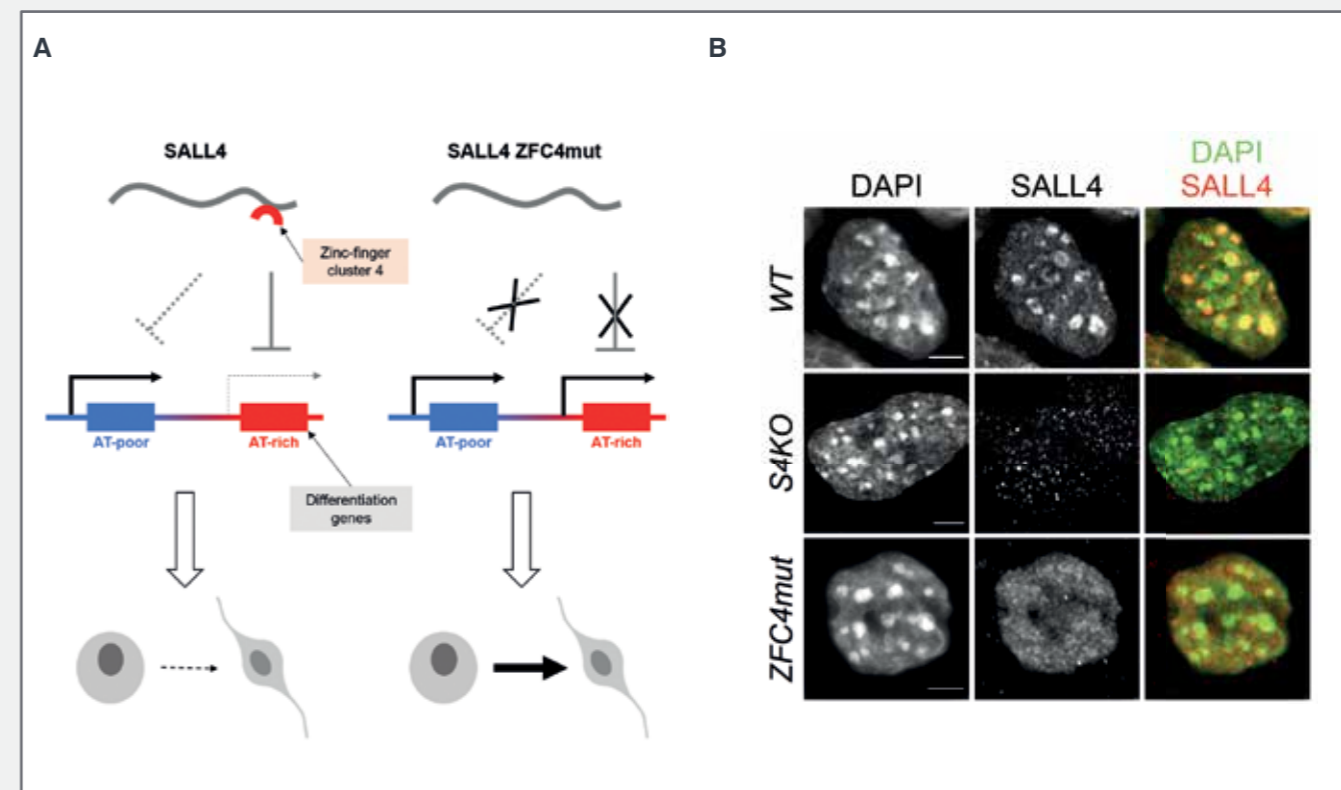
Selected Publications

Pantier R, Chhatbar K, Quante T, Skourti-Stathaki K, Cholewa-Waclaw J, Alston G, Alexander-Howden B, Lee HY, Cook AG, Spruijt CG, Vermeulen M, Selfridge J, Bird A. SALL4 controls cell fate in response to DNA base composition. *Mol Cell*. 2021 Feb 18;81(4):845-858.e8. doi: 10.1016/j.molcel.2020.11.046. Epub 2021 Jan 5. PMID: 33406384; PMCID: PMC7895904.

Tillotson R, Cholewa-Waclaw J, Chhatbar K, Connelly JC, Kirschner SA, Webb S, Koerner MV, Selfridge J, Kelly DA, De Sousa D, Brown K, Lyst MJ, Kriaucionis S, Bird A. Neuronal non-CG methylation is an essential target for MeCP2 function. *Mol Cell*. 2021 Mar 18;81(6):1260-1275.e12. doi: 10.1016/j.molcel.2021.01.011. Epub 2021 Feb 8. PMID: 33561390; PMCID: PMC7980222.

Bird A. The Selfishness of Law-Abiding Genes. *Trends Genet*. 2020 Jan;36(1):8-13. doi: 10.1016/j.tig.2019.10.002. Epub 2019 Oct 29. PMID: 31662191.

Understanding proteins that stabilise cell identity



A. A cartoon showing loss of preferential repression of AT-rich genes by SALL4 when the AT binding domain ZFC4 is mutated, leading to precocious differentiation towards a neuronal fate.

B. Microscopy of mouse embryonic stem cell nuclei showing co-localisation of wildtype SALL4 (WT) with heterochromatic foci containing AT-rich DNA (stained with DAPI). When zinc finger cluster 4 is mutated (ZFC4mut), SALL4 becomes dispersed throughout the nucleus. As a control, we show that staining with the SALL4 antibody is absent when the SALL4 gene is deleted (S4KO).



Dhanya Cheerambathur

Co-workers: Mattie Green, Cameron Finlayson, Lana Buzuk, Vasilis Ouzounidis, Emmanuel Fiagbedzi, Henrique Alves Domingos

The central nervous system is a complex network of neurons and supporting cells that form the information relaying unit of an organism. During neural development, pioneer neurons extend axons in response to guidance cues from other neurons and non-neuronal cells to establish the framework that build the neural circuits. The assembly of this circuit is a highly orchestrated event that involves neurite outgrowth, fasciculation (axon bundling) and synapse formation to generate a functional nervous system. How these organizational features emerge during development is poorly understood

Microtubules are critical for neuron formation and function. As neurons develop, microtubules are organized and sculpted by the cell machinery to form the axons, dendrites and the neural network. Several human neurodevelopmental disorders are linked to mutations in microtubule cytoskeleton-related proteins. Despite the central role of the microtubule, little is known about how the microtubule cytoskeleton contributes to the assembly of the neural circuit. We aim to understand how the microtubule cytoskeleton uses distinct molecular machinery to build and regenerate 3 dimensional neuronal circuits using the simple multicellular organism *C. elegans* as a model.

During my post-doc, I discovered an unexpected role for kinetochore, the chromosome segregation machinery, in developing neurons of *C. elegans*. Our work showed that the evolutionarily conserved 10 subunit KMN (Kn1-Mis12-Ndc80) network, the microtubule coupler within the kinetochore, acts post-mitotically in developing neurons. A similar function for kinetochores proteins has also been described in *Drosophila* and rat hippocampal cultures. KMN proteins are enriched in the dendritic and axonal outgrowth during neurodevelopment. Removal of KMN components post-mitotically from developing neurons resulted in a disorganized nerve ring, a network of 181 axons and synapses, considered as the “brain” of *C. elegans*. We hypothesize that the kinetochore proteins facilitate nerve ring assembly by promoting the proper formation of axon bundles.

Starting from this unique angle, we aim to understand how the microtubule cytoskeleton integrates distinct molecular machinery to build and regenerate 3 dimensional neuronal circuits in *C. elegans*. Our goal is to 1) define the function of the kinetochore proteins in building the nerve ring; 2) build a functional map of microtubule cytoskeleton during nerve ring assembly by addressing the function of non-kinetochore microtubule factors; 3) investigate how kinetochore proteins build and maintain neuronal network by addressing its role in dendritic branching and regeneration.

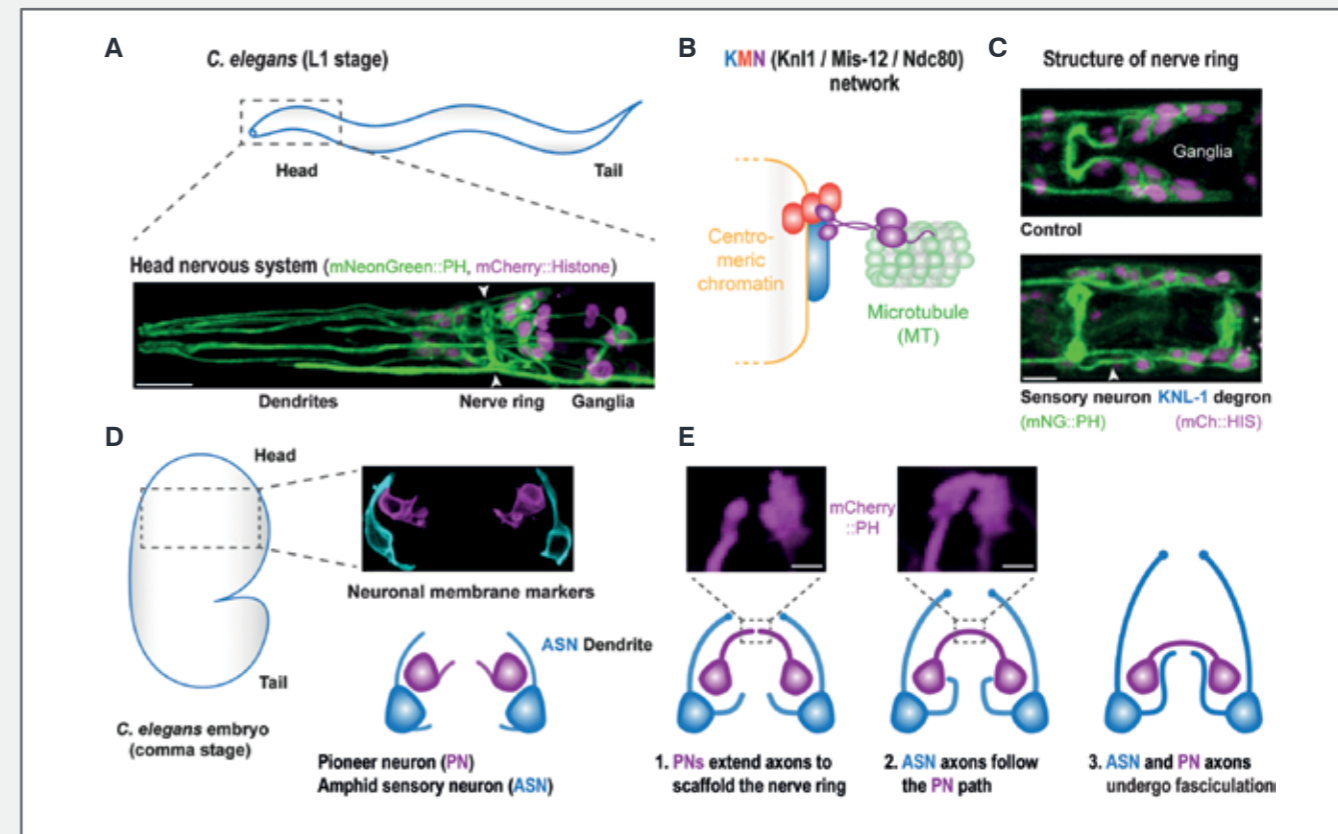
Selected Publications

Cheerambathur, D.K., Prevo, B., Chow, T.-L., Hattersley, N., Wang, S., Zhao, Z., Kim, T., Gerson-Gurwitz, A., Oegema, K., Green, R., et al. (2019). The Kinetochore-Microtubule Coupling Machinery Is Repurposed in Sensory Nervous System Morphogenesis. *Dev. Cell*.

Cheerambathur, D.K., Prevo, B., Hattersley, N., Lewellyn, L., Corbett, K.D., Oegema, K., and Desai, A. (2017). Dephosphorylation of the Ndc80 Tail Stabilizes Kinetochore-Microtubule Attachments via the Ska Complex. *Dev. Cell* 41, 424–437.e424.

Cheerambathur, D.K., Gassmann, R., Cook, B., Oegema, K., and Desai, A. (2013). Crosstalk between microtubule attachment complexes ensures accurate chromosome segregation. *Science* 342, 1239–1242.

Role of microtubule cytoskeleton in building and regenerating the neural connectome



Nerve ring assembly in *C. elegans*

A. The *C. elegans* head nervous system in L1 larvae (PH marks the membrane and histone the cell body). The axon bundle in the nerve ring is between white arrowheads (scale 10 μ m).

B. Schematic of KMN network: Mis-12 interface (red) with the centromere, Ndc80 (purple) binds the microtubule and Kn1 (blue) functions as a scaffold.

C. Structure of *C. elegans* nerve ring in control and after post-mitotic degradation of KNL-1 in the neurons. Axon defasciculation defect (white arrowhead, scale 5 μ m).

D. Fluorescence image and cartoon of the developing nerve ring in *C. elegans* embryo (pioneer neurons (PN) in purple, amphid sensory neurons (ASN) in blue). Note that the ASNs have already extended their dendrites (scale 2.5 μ m).

E. Schematic representing the initial stages of nerve ring formation. Insets show the extension and bridging of bilaterally symmetrical PN axons (scale 1 μ m).



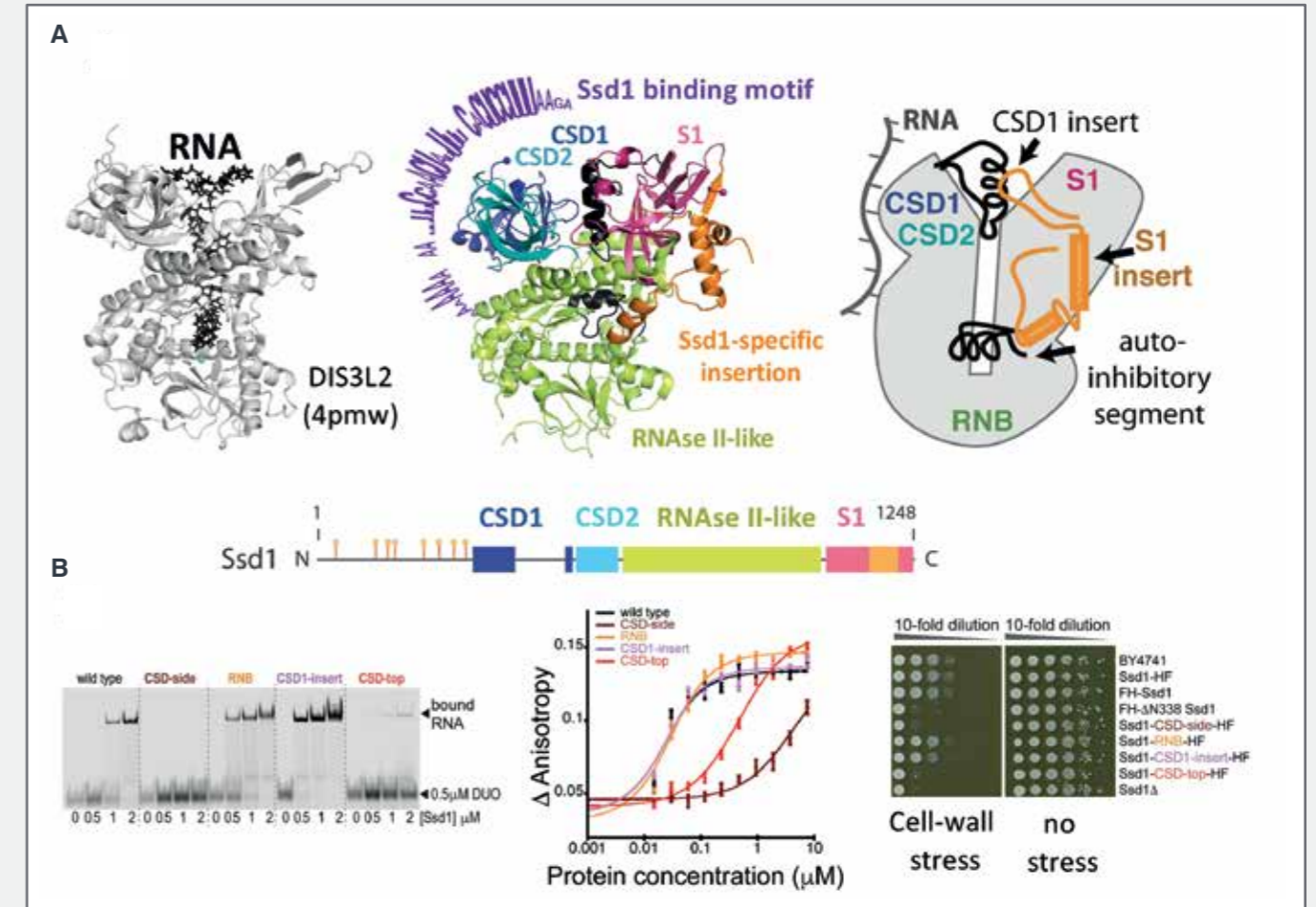
Atlanta Cook

Co-workers: Uma Jayachandran, Ola Kasprowicz, Alexander Will, Mickey Oliver, James Le Cornu (iCM student), Atika Al Haisani, Laura Croenen

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.

Recently, we solved a crystal structure of a yeast RNA binding protein, Ssd1, that is important in cell wall biogenesis. It is thought that Ssd1 functions by repressing translation of cognate transcripts. Using CRAC, we found that Ssd1 binds to specific sequences in the 5'UTRs of a small set of transcripts, several of which encode proteins required for cell wall biogenesis. This suggests that Ssd1 functions by blocking ribosome scanning along 5'UTRs. The structure of Ssd1 shows that it has a classical fold of an RNase II family nuclease. However, RNA degradation activity has been lost by two mechanisms. First, the catalytic residues have been altered during evolution. Second, a channel that, in active enzymes, allows RNA substrates to funnel into the active site has been blocked. We propose that Ssd1 has evolved a new RNA interacting surface.

Structural biology of macromolecular complexes in RNA metabolism and transcriptional silencing



Selected Publications

- Bayne R.A., Jayachandran U., Kasprowicz A., Bresson S., Tollervey D., Wallace E.W.J., Cook A.G. (2021) Yeast Ssd1 is a non-enzymatic member of the RNase II family with an alternative RNA recognition interface. *Nucleic Acids Research*, DOI: 10.1093/nar/gkab615.
- Pantier R., Chhatbar K., Quante T., Skourti-Stathaki K., Cholewa-Waclaw J., Alston G., Alexander-Howden B., Lee H.Y., Cook A.G., C Spruijt C.G., Vermeulen M., Selfridge J., and Bird A. (2021) SALL4 controls cell fate in response to DNA base composition. *Mol Cell* 81:845-858.e8.
- Ballou E.R., Cook A.G. and Wallace E.W.J. (2020) Repeated evolution of inactive pseudonucleases in a fungal branch of the Dis3/RNase II family of nucleases. *Mol. Biol. Evol.* doi:10.1093/molbev/msaa324

A. The structure of Ssd1 (middle) compared with the structure of DIS3L2 (left), where RNA is bound, shows the different RNA binding sites. Domains of Ssd1 are marked in blue (cold shock domain 1, CSD1), cyan (CSD2), green (RNase II-like) and pink (S1). The Ssd1-specific insert is shown in the domain overview (below) and structure in orange. The yellow lollipops are phosphorylation sites. RNA travels down the central channel of DIS3L2 while Ssd1 binds a sequence-specific motif (purple) on the outside of the CSD domains. Two segments of the Ssd1 structure are shown in black – these block the active site funnel. A cartoon overview of the Ssd1-specific structures is shown on the right.

B. Four sets of point mutations were tested for RNA binding by electrophoretic mobility shift assay (left). Mutations to the side and top of the CSDs block binding to RNA. This is further demonstrated by fluorescence anisotropy assays (middle). Phenotypic assays in yeast show that mutations that prevent RNA binding have a cell wall stress phenotype.



Owen Davies

Co-workers: Eleanor Casey, Simona DeBilio, Gurusaran Manickam

How is the chromosome number halved during meiosis to create haploid spermatozoa and oocytes that form healthy diploid zygotes upon fertilisation?

Meiosis involves a unique chromosome choreography in which chromosomes search throughout the cell to find their homologous partners, with which they synapse, exchange genetic material by crossing over, and then segregate upon cell division. This is achieved by the combined actions of several molecular machines. Firstly, double-strand breaks are induced across the genome, triggering recombination searches, which result in the formation of recombination intermediates that physically connect matching sequences of homologous chromosome pairs. This process is facilitated by rapid chromosomal movements, in which microtubule forces are transmitted via the LINC complex to chromosome telomere ends that are tethered to the nuclear envelope by the meiotic telomere complex. Once established, the discrete physical connections of recombination are converted into continuous synapsis between homologous chromosomes by assembly of the synaptonemal complex, a supramolecular protein structure that 'zips' together homologous chromosome pairs along their entire length. The assembled synaptonemal complex then facilitates the resolution of recombination intermediates, with the formation of crossovers in which diversity is enhanced by the exchange of genetic material between homologous chromosome partners that subsequently segregate into daughter cells.

Our research aims to uncover the structural basis of how the synaptonemal complex, recombination machinery, meiotic telomere complex and meiotic LINC complex perform their critical functions in meiosis, and how they operate together as an integrated molecular machine. Our main research questions are:

1. What is the structure, function and assembly mechanism of the synaptonemal complex?
2. How is meiotic recombination regulated within the synaptonemal complex?
3. How are meiotic chromosome telomere-ends anchored to the nuclear envelope?
4. How are cytoskeletal forces transmitted to chromosomes by the meiotic LINC complex?

We adopt a structural biology approach in which we integrate solution biophysics, high-resolution structure determination by X-ray crystallography and Cryo-EM, with EM-based imaging of macromolecular assemblies formed by recombinant proteins and within heterologous cellular systems. We translate our structural findings to a functional understanding of meiosis through the structure-directed design of separation-of-function mutations that are tested *in vivo*, in mouse and lower organism systems, by our collaborators.

Ultimately, we aim to achieve a complete molecular understanding of how the integrated machineries of the synaptonemal complex, recombination, telomere complex and LINC complex perform the chromosome choreography of meiosis.

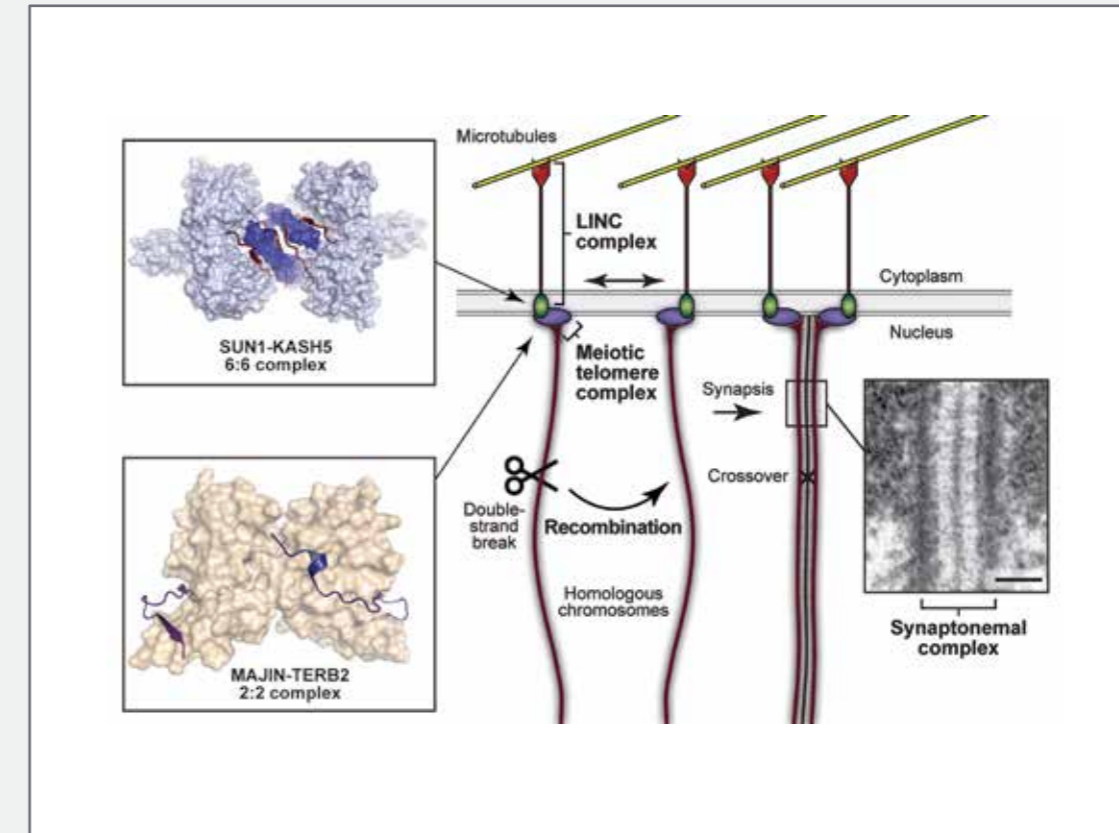
Selected Publications

Dunce, J.M., Salmon, L.J., and Davies, O.R. (2021). Structural basis of meiotic chromosome synaptic elongation through hierarchical fibrous assembly of SYCE2-TEX12. *Nat Struct Mol Biol* 28, 681-693.

Gurusaran, M., and Davies, O.R. (2021). A molecular mechanism for LINC complex branching by structurally diverse SUN-KASH 6:6 assemblies. *eLife* 10.

Sanchez-Saez, F., Gomez, H.L., Dunne, O.M., Gallego-Paramo, C., Felipe-Medina, N., Sanchez-Martin, M., Llano, E., Pendas, A.M., and Davies, O.R. (2020). Meiotic chromosome synapsis depends on multivalent SYCE1-SIX6OS1 interactions that are disrupted in cases of human infertility. *Science advances* 6.

Structural biology of meiosis



Schematic of recombination and chromosome synapsis in meiosis, highlighting the meiotic LINC complex formed of SUN1-KASH5 (Gurusaran and Davies, 2021), the meiotic telomere complex containing MAJIN-TERB2 (Dunce et al, 2018), and the synaptonemal complex.



Bill Earnshaw

Co-workers: Blandine Arleri, Mar Carmena, Fernanda Cisneros-Soberanis, Lorenza Di Pompeo, Moonmoon Deb, Natalia Kochanova, Emma Peat, Elisa Pesenti, Bram Prevo, Nina Pucekova, Caitlin Reid, Lucy Remnant, Itaru Samejima, Kumiko Samejima

Over the past year, much of our research focused on structural dynamics in chromatin during the transition of cells from G₂ phase into mitosis, the role of SMC proteins in mitotic chromosome formation and structure and the structure and assembly of the chromosome periphery.

One highlight was the publication of a study that has been ongoing for several years in which Itaru examined the changes in protein association with chromatin during synchronous mitotic entry. This study used Chromatin Enrichment for Proteomics (ChEP), a method developed by Georg Kustatscher when he was a postdoc with Juri Rappsilber, and was a collaboration between the three labs. We discovered that the earliest events of prophase appear to primarily involve changes in RNA processing in nuclei as well as changes in interactions with the nuclear envelope and pores. All of these events begin before chromatin condensation is visible, and the study was only made possible by using the chemical-genetic system for synchronous mitotic entry developed by Kumiko.

We are currently writing up the results of our long-running study of interactions between cohesin and condensin during mitotic chromosome formation. This is a truly interdisciplinary collaboration with the groups of Job Dekker, Leonid Mirny and Anton Goloborodko. We do the genetics, cell biology and imaging. They do Hi-C and polymer modelling, respectively. We have discovered that cohesin has a significant effect on mitotic chromosome structure that has been previously overlooked and gained surprising new insights into the organisation of the chromatin fiber in chromosomes. Kumiko has made many genomic knock-in cell lines, performed the cell synchrony and carried out extensive light microscopy analysis. Fernanda and Nina have been performing serial block face scanning electron microscopy with our collaborators Ian Prior and Alison Beckett in Liverpool. Itaru has been doing ChEP and Moonmoon has been doing ChIP - both to quantitate the amounts of condensin and cohesin on the chromosomes during mitotic entry.

In other ongoing work, Lucy and Fernanda are studying the enigmatic protein Ki-67 and the RNA/protein-rich mitotic chromosome periphery compartment (MCPC), Lorenza is performing a structure/function analysis on CENP-V, Caitlin is studying the role of topo II β in chromosome formation, Natalia is using proteomics to look at protein conformations and interactions during mitotic entry, and Bram is developing new ways to image chromosomes.

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

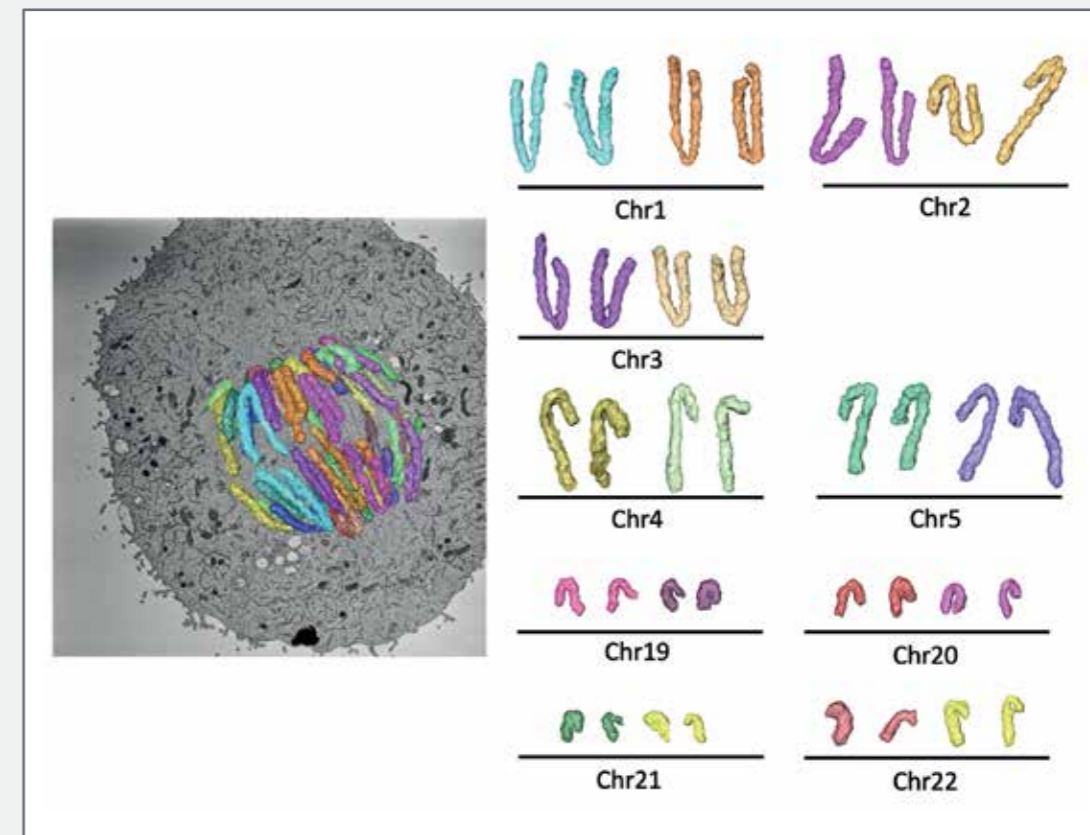
Selected Publications

Samejima, I., C. Spanos, K. Samejima, J. Rappsilber, G. Kustatscher & W.C. Earnshaw. (2022). Mapping the invisible chromatin transactions of prophase chromosome remodeling. *MOL. CELL* 82:696-708; PMID: 35090599; PMCID: 8823707; DOI: 10.1016/j.molcel.2021.12.039.

Paulson JR, Hudson DF, Cisneros-Soberanis F, Earnshaw WC. (2021). Mitotic chromosomes. *SEMIN CELL DEV BIOL.* 117:7-29. PMID: 33836947; PMCID:8406421; DOI:10.1016/j.semcdb.2021.03.014.

Pesenti, E., M. Liskovych, K. Okazaki, A. Mallozzi, C. Reid, M.A. Abad, A.A. Jeyaprakash, N. Kouprina, V. Larionov, H. Masumoto, W.C. Earnshaw. (2020) Analysis of Complex DNA Rearrangements During Early Stages of HAC Formation. *ACS SYNTH BIOL.* 9:3267-3287. PMID: 33289546; PMCID:7754191; DOI: 10.1021/acssynbio.0c00326.

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



Three-dimensional reconstruction of an anaphase human RPE1 cell. Left, projection of the three-dimensional reconstruction superimposed on an orthoslice from the electron microscopy map. Corresponding sister chromatids have the same colours. Right, partial karyotype with individual sister chromatids (identified by size and centromere position) extracted from the map and displayed next to their sisters. Sample preparation and modeling in AMIRA by Fernanda Cisneros-Soberanis. Serial block face scanning electron microscopy by Alison Beckett and Ian Prior, University of Liverpool.



Patrick Heun

Co-workers: Mathilde Fabe, Meena Krishnan, Alessandro Stirpe, George Yankson, Hwei Ling Tan

Our lab is interested in the organisation, establishment, and maintenance of specialised chromatin states. Epigenetic transmission of centromere identity through many cell generations is required for proper centromere function and when perturbed can lead to genome instability and cellular malfunction. We use *Drosophila* and human tissue culture cells as model organisms to address the following questions:

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. Using fast acting transcriptional inhibitors in combination with a newly developed CENP-A loading system, we demonstrate that centromere transcription is required for loading of new dCENP-A by removing placeholder nucleosomes and promoting dCENP-A transition from chromatin association to nucleosome incorporation (Bobkov et al., 2020). Unlike placeholder nucleosomes, previously deposited CENP-A is specifically retained by Spt6 both in human and *Drosophila* cells, identifying Spt6 as a CENP-A maintenance factor that ensures the stability of epigenetic centromere identity (Figure 1). We are currently investigating the molecular mechanism how some histones like CENP-A are maintained while others like H3.3 placeholders are evicted to preserve epigenetic centromere identity.

How is the centromeric chromatin fiber organised?

To map centromere proteins on the linear centromeric chromatin fiber, we have recently developed a novel approach where proteins-of-interest fused to Biotin ligases or DNA methyltransferases leave a “footprint” on the underlying nucleosomes through proximity-labelling. With this methodology we have described novel localization patterns of a subset of centromere proteins at human centromeres (Kyriacou and Heun, 2018). We are extending this approach to proteins localising to all layers of the centromere using different technologies like stretched chromatin fibers and long read-DNA sequencing.

How does Su(var)2-10/PIAS contribute to heterochromatin organisation?

The *Su(var)2-10* gene has been originally identified in position-effect-variegation (PEV) assays designed to uncover proteins involved in heterochromatin formation. Cloning of the gene revealed its homology to the protein family SUMO E3-ligase PIAS (Protein Inhibitor of activated STAT), but how sumoylation promotes heterochromatin formation remains unknown. While PIAS does not localise to pericentric heterochromatin in somatic cells, it is enriched next to centromeres in early fly embryogenesis, suggesting a role in heterochromatin establishment (Figure 2). We are specifically depleting PIAS at this point of development to shed light on the link between PIAS’ SUMO targets and chromatin organisation.

Selected Publications

Bobkov, G.O.M., Huang, A., van den Berg, S.J.W., Mitra, S., Anselm, E., Lazou, V., Schunter, S., Feederle, R., Imhof, A., Lusser, A., et al. (2020). Spt6 is a maintenance factor for centromeric CENP-A. *Nature communications* 11, 2919.

Logsdon, G.A., Gambogi, C.W., Liskovych, M.A., Barrey, E.J., Larionov, V., Miga, K.H., Heun, P., and Black, B.E. (2019). Human Artificial Chromosomes that Bypass Centromeric DNA. *Cell* 178, 624-639 e619. PMC6657561

Roure, V., Medina-Pritchard, B., Lazou, V., Rago, L., Anselm, E., Venegas, D., Jeyaprakash, A.A., and Heun, P. (2019). Reconstituting *Drosophila* Centromere Identity in Human Cells. *Cell Rep* 29, 464-479 e465.

Establishment and maintenance of chromatin identity

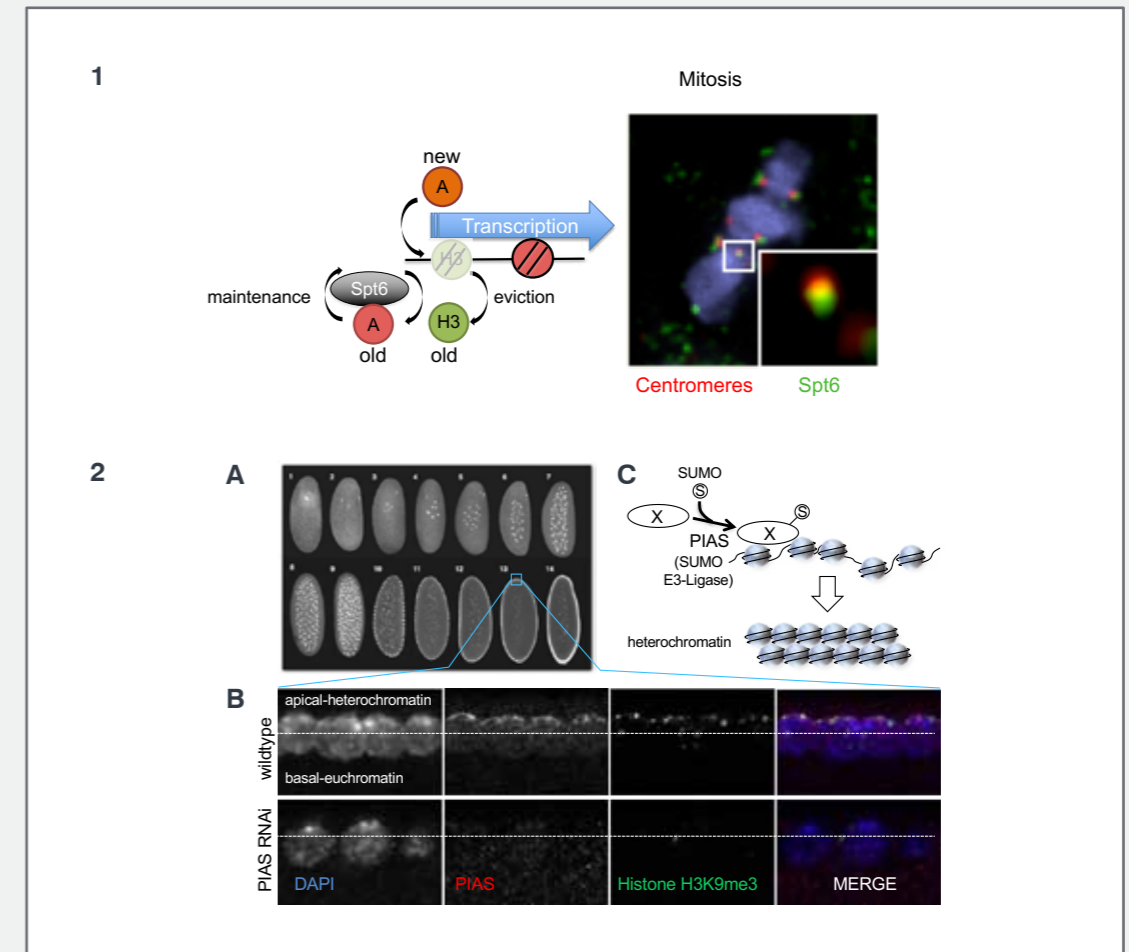


Figure 1. Model for the role of transcription at centromeres: Transcription remodels centromere chromatin and evicts H3-nucleosomes (green) to allow new CENP-A (orange) loading. Evicted old CENP-A (red) is maintained by the transcription elongation factor Spt6.

Figure 2. The SUMO E3 Ligase PIAS is required for heterochromatin formation. **A.** Embryonic cycle 1-14 (image William Sullivan) **B.** Fixed cycle 13 embryos showing α PIAS and heterochromatin marker α H3K9me3 in apical heterochromatin in wildtype and PIAS RNAi. **C.** Proposed role of PIAS in chromosome organisation.



Georg Kustatscher

Co-workers: Van Kelly, Savvas Kourtis, Emmanuel Fiagbedzi

There is a major discordance between mRNA and protein expression levels in human cells. Why is this so and what mechanisms are behind it? Our aim is to understand the principles, mechanisms and regulators that shape the proteome at the level of translation and protein degradation. Despite their importance for cancer and other diseases, these regulatory processes remain poorly understood, leaving an enormous potential for therapeutic intervention unfulfilled. We aim to address three key questions:

1. What is the role of translation and degradation rates in regulating protein levels, for example when buffering the impact of chromosome abnormalities in glioblastoma stem cells.
2. Which proteins regulate translation and degradation rates, e.g. can we reveal regulatory networks between E3 ubiquitin ligases and their targets.
3. Which unconventional translation products exist in healthy and in cancer cells and what are their biological functions.

From a technological perspective we plan to address these questions using a combination of proteomics and computational approaches and, where necessary, RNA sequencing. We are currently at the beginning of these projects and focus on the development of the necessary proteomics techniques that will allow us to carry out these investigations. The Centre has recently obtained a Sciex tripleTOF mass spectrometer, which is suitable for high-throughput (HT) proteomics, a rapidly emerging mass spectrometry approach for the robust quantitation of proteomes in a matter of minutes. HT proteomics differs from conventional proteomics on every level of the experimental workflow: the chromatography, the mass spectrometer and the data processing. To harvest the power of HT proteomics for the analysis of proteome dynamics we are developing DIA-pulse-SILAC, a method that will allow the rapid quantitation of protein synthesis and degradation rates by mass spectrometry.

An important aspect of HT proteomics, and indeed all proteomics experiments, is the statistical analysis and interpretation of the data. This is a second area of focus for our group. For example, we recently collaborated with the Earnshaw group to create an interactive proteomic map of chromatin transactions during mitotic entry (<https://mitoChEP.bio.ed.ac.uk>). We are also working together with the Ralser lab (Charité, Berlin) to predict the potential function of uncharacterised yeast proteins based on the proteomic characterisation of thousands of yeast knock-out strains.

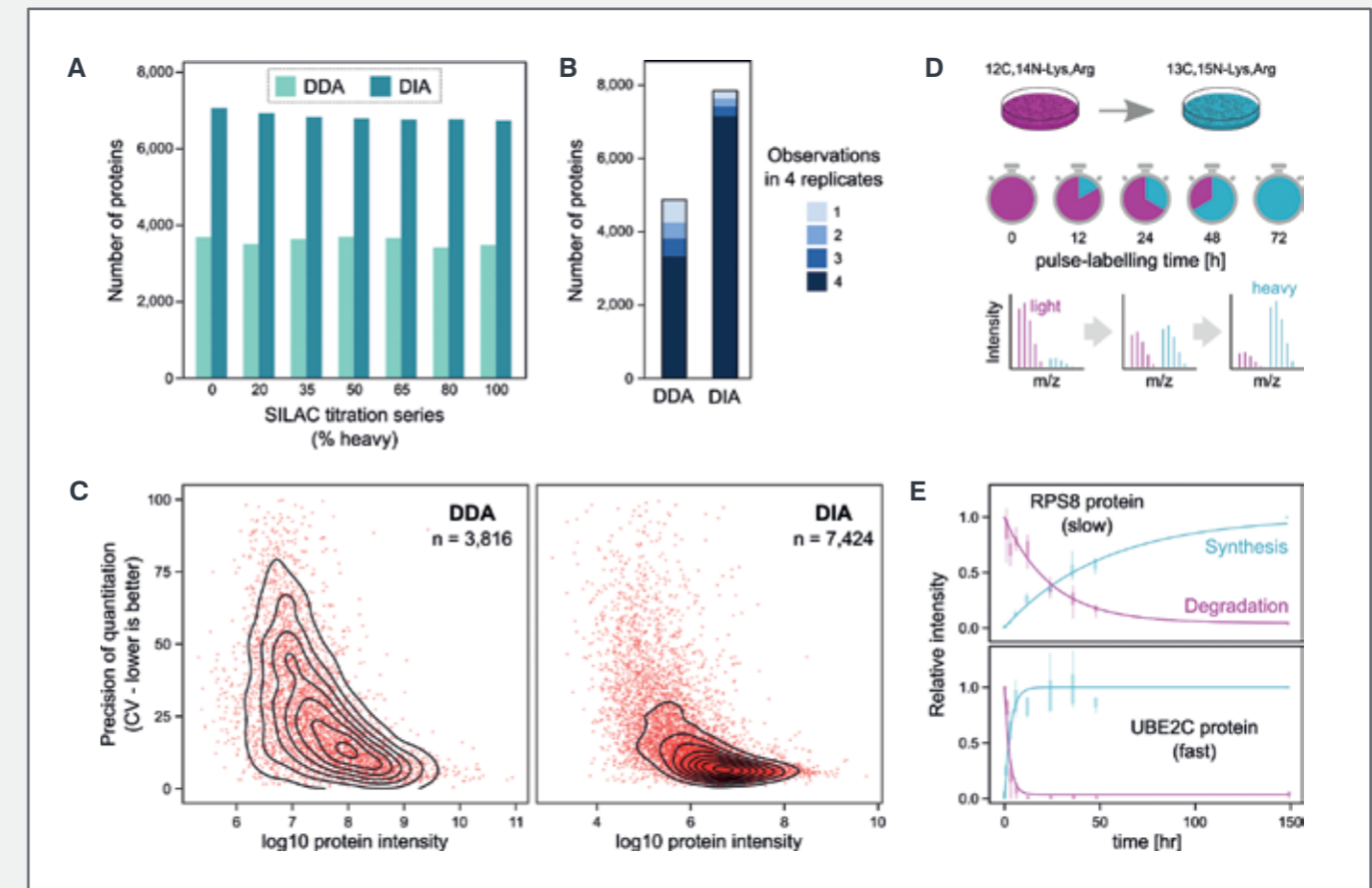
Selected Publications

Samejima, I., Spanos, C., Samejima, K., Rappsilber, J., Kustatscher, G.*, and Earnshaw, W.C.* (2022). Mapping the invisible chromatin transactions of prophase chromosome remodeling. *Mol. Cell* 82, 696–708.e4. * co-corresponding authors

Kelly, V., Al-Rawi, A., Lewis, D., Kustatscher, G., and Ly, T. (2022). Low Cell Number Proteomic Analysis Using In-Cell Protease Digests Reveals a Robust Signature for Cell Cycle State Classification. *Mol. Cell. Proteomics* 21, 100169.

Nakamura, K.*, Kustatscher, G.*, Alabert, C., Hödl, M., Forne, I., Völker-Albert, M., Satpathy, S., Beyer, T.E., Mailand, N., Choudhary, C., et al. (2021). Proteome dynamics at broken replication forks reveal a distinct ATM-directed repair response suppressing DNA double-strand break ubiquitination. *Mol. Cell* 81, 1084–1099.e6. *joint first authors

Proteome dynamics: The role of synthesis and degradation in regulating protein levels



Development of DIA-pulse-SILAC for the rapid and precise measurement of protein synthesis and degradation.

A. SILAC titration series created by mixing defined ratios of light and heavy extracts from RPE1 cells. In a direct comparison our new DIA-SILAC workflow (DIA; data-independent acquisition) quantifies considerably more proteins than the traditional data-dependent acquisition (DDA) of SILAC samples. The DIA workflow also quantifies the same set of proteins more consistently across replicates

B. The DIA workflow quantifies the same set of proteins more consistently across replicates.

C. The DIA workflow quantifies proteins more precisely than the DDA workflow.

D. Schematic of a pulse-SILAC experiment.

E. DIA-pulse-SILAC was used to quantify synthesis and degradation rates in RPE1 cells. Shown are two representative example proteins with slow and fast turnover rates, respectively.



Adele Marston

Co-workers: Eleanor Casey, Kirsty Dewars, Chuanli Huang, Dilara Kocakaplan, Lori Koch, Melanie Lim, Lucia Massari, Bettina Mihalas, Anuradha Mukherjee, Meg Peyton-Jones, Gerard Pieper, Ola Pompa, Hollie Rowlands, Julia Van Bockstaele, Aparna Vinod, Menglu (Lily) Wang

Specialization of chromosome segregation mechanisms in meiosis

Meiosis generates gametes with half the parental genome through two consecutive chromosome segregation events, meiosis I and meiosis II. Meiotic errors are prevalent in humans, accounting for frequent miscarriages, birth defects and infertility. Our vision is to elucidate the molecular basis of the adaptations that sort chromosomes into gametes during meiosis. We use budding and fission yeast as general discovery tools, and *Xenopus* and mouse oocytes to uncover meiotic mechanisms in vertebrates. Using patient-donated oocytes and ovarian tissue, we address the relevance of our findings for human fertility.

Structural and functional organisation of meiotic chromosomes

During meiosis, chromosomes undergo extensive remodelling for transmission into gametes. Chromosomes are broken and reciprocally exchanged in prophase, specifically cohered at centromeres during meiosis I and permanently separated at meiosis II. The cohesin complex is a major definer of chromosome structure, establishing intra and inter-sister chromatid linkages and providing the context for spatial control of homolog interactions. Cohesin defines a specialized chromosomal domain, called the pericentromere, surrounding each budding yeast centromere. We discovered that cohesin extrudes a chromatin loop on either side of the centromere until halted by convergent genes at pericentromere borders. Our recent work revealed that cohesin acetylation prevents extrusion through pericentromere borders and demonstrated that this boundary formation is critical for meiotic chromosome segregation. Therefore, we determined how chromosome loops are positioned to functionally structure the genome. A key ongoing focus is to understand how pericentromere structure influences its role as a signalling platform that safeguards chromosome segregation, both in the model yeast system and in human oocytes. In a new initiative, we are also using phospho-proteomics in synchronised yeast to uncover the cell cycle controls that allow two successive meiotic divisions.

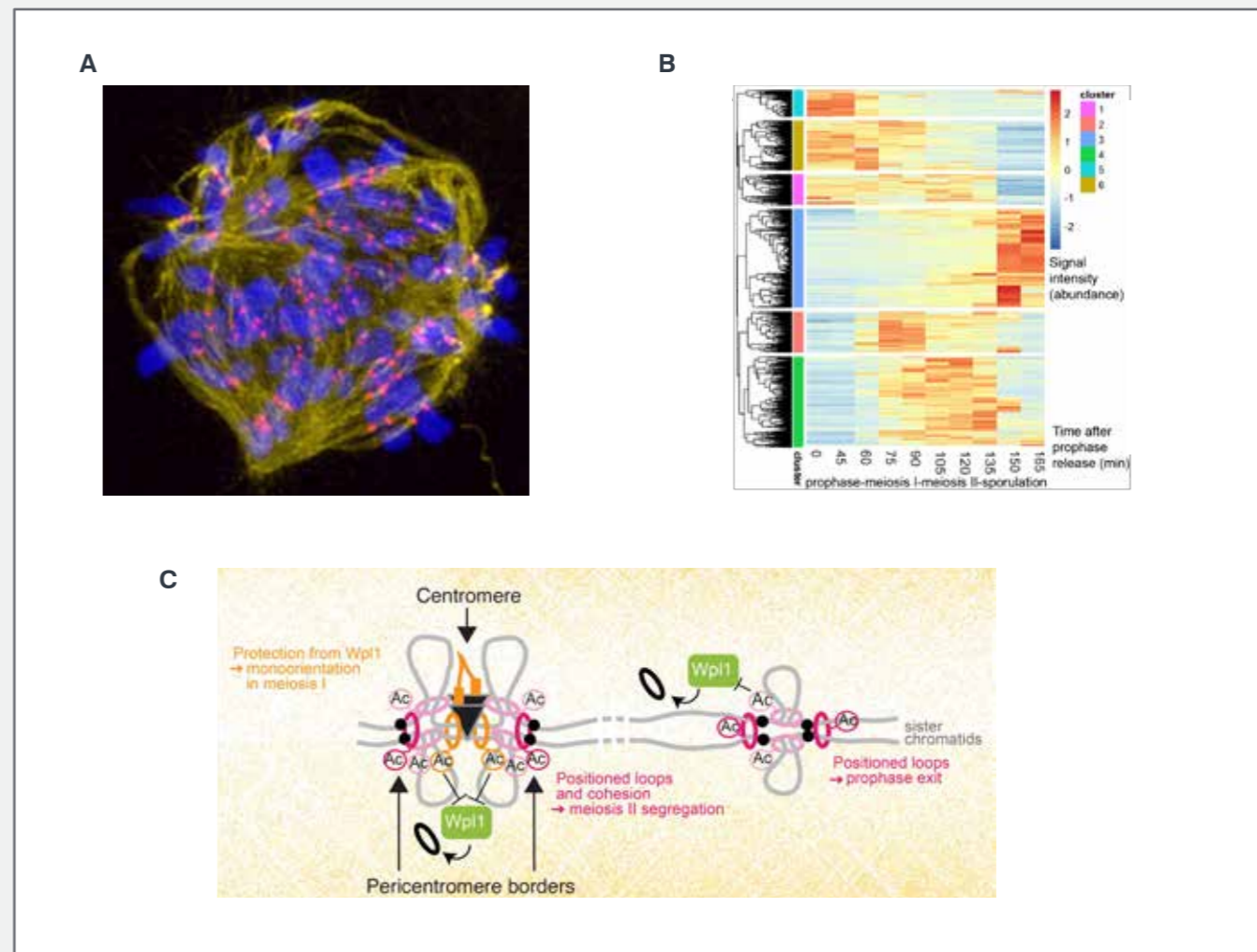
Specialization of meiotic kinetochores

Kinetochores link centromeric nucleosomes to microtubules for chromosome segregation. Our goal is to understand how the kinetochore is adapted to perform its meiosis-specific functions in suppression of meiotic recombination, directing the co-segregation of sister chromatids during meiosis I, and maintaining linkages between sister chromatids until meiosis II. We defined the proteomic landscape of yeast kinetochores and centromeric chromatin during meiosis, revealing extensive remodelling during prophase and meiosis I. We are now addressing the mechanism of kinetochore remodelling, as well as its functional importance. In many organisms, sister kinetochores are fused in meiosis I, while a lack of fusion in human oocytes may account for susceptibility to segregation errors and fertility problems. Ongoing work in *Xenopus*, mouse and human oocytes aims to test this hypothesis.

Selected Publications

- Barton R†, Massari LF†, Robertson D and Marston AL (2022). Eco1-dependent cohesin acetylation anchors chromatin loops and cohesion to define functional meiotic chromosome domains. *eLife*. 11, e74447. †Equal contribution
- Su XB, Wang M, Schaffner C, Nerusheva OO, Clift D, Spanos C, Kelly DA, Tatham M, Wallek A, Wu Y, Rappsilber J, Jayaprakash AA, Storchova Z, Hay RT and Marston AL. (2021) SUMOylation stabilizes sister kinetochore biorientation to allow timely anaphase. *J Cell Biol*. 220, e202005130.
- Borek WE, Vincenten N, Duro E, Makrantonis V, Spanos C, Sarangapani KK, de Lima Alves F, Kelly DA, Asbury CL, Rappsilber J and Marston AL. (2021) The proteomic landscape of centromeric chromatin reveals an essential role for the Ctf19CCAN complex in meiotic kinetochore assembly. *Current Biology* 31, 283-296.

Orienting Chromosomes during Mitosis and Meiosis



- A.** Mitosis 1 in the human embryo after inhibition of the CENP-E motor protein. The spindle is stained in yellow, centromeres in red and chromosomes in blue.
- B.** Phosphoproteome changes during budding yeast meiosis. Heatmap shows hierarchical clustering of changes in phospho-site intensity across a time course (meiotic prophase until sporulation).
- C.** Model showing how anchoring of chromatin loops and sister chromatid cohesion by Eco1-dependent acetylation of cohesin structures meiotic chromosomes for their segregation.



Dónal O'Carroll

Co-workers: Pedro Moreira, Ansgar Zoch, Yuka Kabayama, Azzurra DePace, Theresa Schöpp, Madeleine Heep, David MacLeod, Christopher Mapperly, Tamoghna Chowdhury, Martina Schito

The O'Carroll laboratory has a longstanding interest in mechanisms that regulate gene and transposon expression. Using the germline and haematopoiesis as model systems, our research explores the importance and molecular mechanisms of regulatory RNA pathways in dynamic developmental and physiological contexts. We currently focus on the PIWI-interacting RNA (piRNA) and RNA modification pathways.

The piRNA pathway

In mammals, the acquisition of the germline from the soma provides the germline with an essential challenge, the necessity to erase and reset genomic methylation. This is one of the most drastic epigenetic events in mammalian life. *De novo* genome methylation re-encodes the epigenome, imprinting and transposable element (TE) silencing. In the male germline piRNA-directed DNA methylation silences young active TEs. Antisense TE-derived piRNAs generated from intricate biogenesis pathways act to guide the nuclear PIWI protein MIWI2 to instruct TE DNA methylation. piRNAs are proposed to tether MIWI2 to the young transcriptionally active TE loci that escape the first phase of genome methylation by base pairing to nascent transcripts. The recruitment of MIWI2 sets in motion TE silencing and methylation through the recruitment of unknown effector molecules. Recently we have found the first such effectors of nuclear MIWI2 function. Both SPOCD1 and TEX15 interact with MIWI2 and are essential for piRNA-directed DNA methylation (*Nature* 2020 and *Nature Communications* 2020). SPOCD1 interacts with the *de novo* methylation machinery, whereas the molecular function of TEX15 remains unknown. Our future goal is to fully understand the mechanism by which MIWI2 instructs TE methylation and epigenetic silencing (Figure 1).

RNA modification

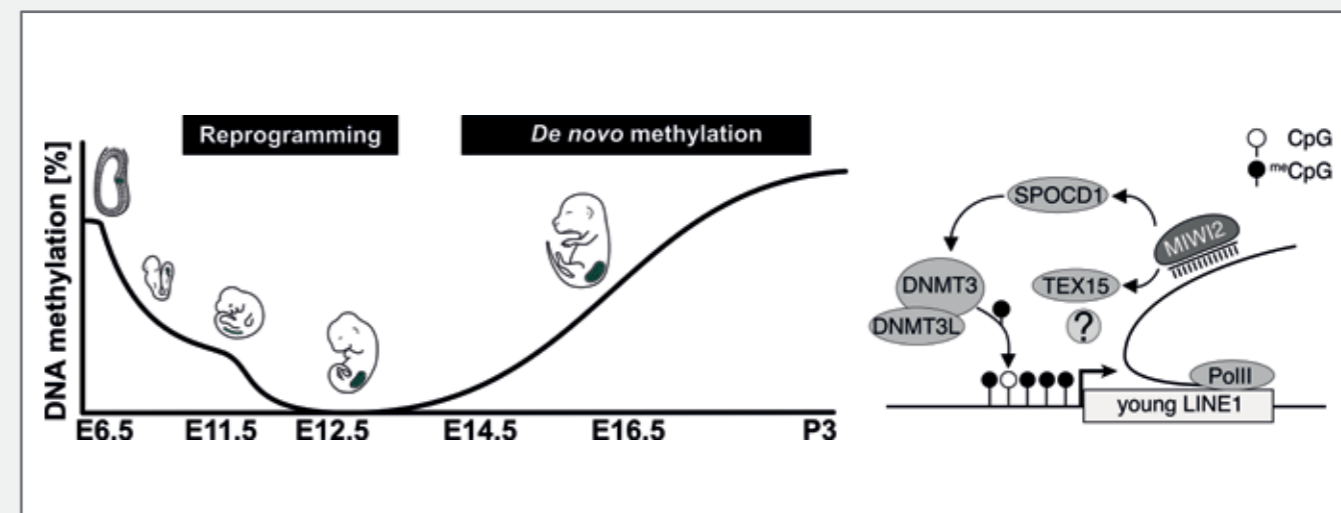
The role of RNA modification in the post-transcriptional regulation of gene expression is only beginning to be understood. The O'Carroll laboratory focuses on understanding the function of N6-methyladenosine (m⁶A) and 3' terminal uridylation mRNA modifications, both of which can promote RNA degradation. TUT4 and TUT7 mediate 3' uridylation of mRNAs with short poly(A) tails that primes these transcripts for degradation, whereas the binding of YTHDF2 to m⁶A-modified mRNA promotes transcript decay. We have shown critical functions for these pathways in the metabolism of the maternal transcriptome and oocyte competence. We have also demonstrated that YTHDF2 is overexpressed in multiple sub-types of acute myeloid leukaemia (AML) and its deletion selectively compromises cancer stem cells, without grossly perturbing normal haematopoiesis (*Cell Stem Cell* 2019). We are currently exploring basic questions regarding the mechanism, regulation and redundancy of the YTH domain family of m⁶A readers.

Selected Publications

- Zoch, A., Auchynnikava, T., Berrens, R. V., Kabayama, Y., Schöpp, T., Heep, M., Vasiliauskaitė, L., Pérez-Rico, Y. A., Cook, A. G., Shkumatava, A., Rappsilber, J., Allshire, R. C., & O'Carroll, D. (2020). SPOCD1 is an essential executor of piRNA-directed *de novo* DNA methylation. *Nature*, 10.1038/s41586-020-2557-5. <https://doi.org/10.1038/s41586-020-2557-5>
- Schöpp, T., Zoch, A., Berrens, R. V., Auchynnikava, T., Kabayama, Y., Vasiliauskaitė, L., Rappsilber, J., Allshire, R. C., & O'Carroll, D. (2020). TEX15 is an essential executor of MIWI2-directed transposon DNA methylation and silencing. *Nature Communications*, 11(1), 3739. <https://doi.org/10.1038/s41467-020-17372-5>
- Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, Mapperley C, Lawson H, Wotherspoon DA, Sepulveda C, Vukovic M, Allen L, Sarapuu A, Tavosanis A, Guitart AV, Villacreces A, Much C, Choe J, Azar A, van de Lagemaat LN, Vernimmen D, Nehme A, Mazurier F, Somervaille TCP, Gregory RI, O'Carroll D*, Kranc KR*. Targeting the RNA m(6)A Reader YTHDF2 Selectively Compromises Cancer Stem Cells in Acute Myeloid Leukemia. *Cell Stem Cell*. 2019 Apr 24. pii:S1934-5909(19)30120-1. doi: 10.1016/j.stem.2019.03.021.

* co-corresponding authors

Regulation of gene expression by non-coding RNA and RNA modification



Genomic DNA methylation is erased (reprogramming) and reset (*de novo* methylation) during germ cell development. Our current model of MIWI2-piRNA directed TE methylation based on functionally defined interactions is presented.



Hiro Ohkura

Co-workers: Aleksandra Ciszek, Fiona Cullen, Rayane Kaade, Jule Nieken, Gera Pavlova, Emma Peat, Charlotte Repton, Emiliya Taskova, Elsa Tungadi, Xiang Wan

Accurate segregation of chromosomal DNA is essential for life. An error in this process 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. Chromosome segregation in oocytes shares many similarities with those in somatic divisions, but also has notable differences. Distinct features of oocytes potentially hinder accurate chromosome segregation. They include (1) lack of centrosomes, the major microtubule nucleation centres in mitosis, (2) exceptionally large cell volume, and (3) cell cycle arrests at two stages. Oocytes are likely to have specific molecular mechanisms which mitigate negative impacts of these features, but little is known about how oocytes set up the chromosome segregation machinery. Defining the oocyte-specific mechanisms would be crucial to understand error-prone chromosome segregation in human oocytes. Furthermore, it may provide an insight into whether and how cancer cells might gain resistance to anti-mitotic drugs by activating these pathways.

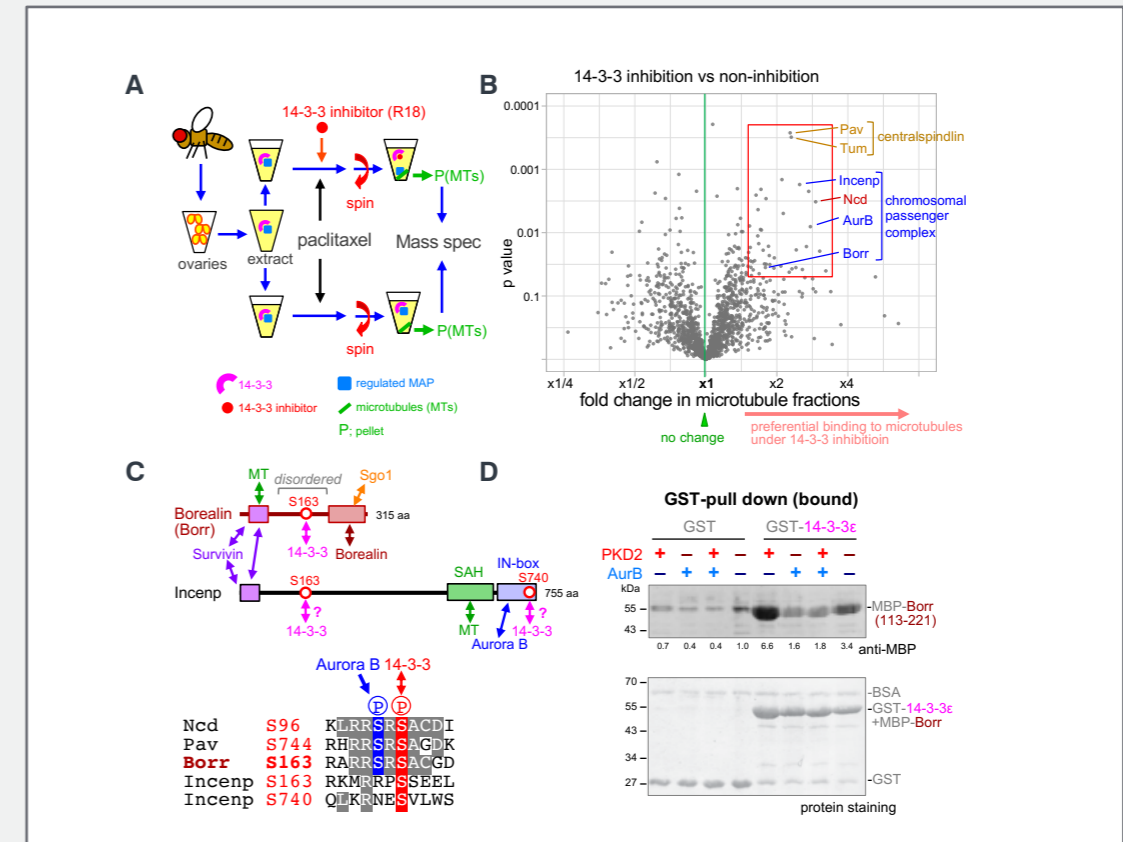
To understand the molecular pathways which set up the chromosome segregation machinery in oocytes, we take advantage of *Drosophila* oocytes as a "discovery platform" because of their similarity to mammalian oocytes and suitability for an approach combining Genetics, microscopy and biochemistry. In *Drosophila* 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 genes/proteins and regulations specifically important for chromosome organisation and/or spindle formation in oocytes.

Global regulation of spindle-associated proteins is crucial in oocytes due to the absence of centrosomes and their very large cytoplasmic volume, but little is known about how this is achieved beyond involvement of the Ran-importin pathway. We previously uncovered a novel regulatory mechanism in *Drosophila* oocytes, in which the phospho-docking protein 14-3-3 suppresses microtubule binding of Kinesin-14/Ncd away from chromosomes. To systematically identify microtubule-associated proteins regulated by 14-3-3 from *Drosophila* oocytes, proteins from ovary extract were co-sedimented with microtubules in the presence or absence of a 14-3-3 inhibitor. Through quantitative mass-spectrometry, we identified proteins or complexes whose ability to bind microtubules is suppressed by 14-3-3, including the chromosomal passenger complex (CPC), the centralspindlin complex and Kinesin-14/Ncd. We showed that 14-3-3 binds to the disordered region of Borealin, and this binding is regulated differentially by two phosphorylations on Borealin. Mutations at these two phospho-sites compromised normal Borealin localisation and centromere bi-orientation in oocytes, showing that phospho-regulation of 14-3-3 binding is important for Borealin localisation and function.

Selected Publications

- Repton, C., Cullen, C. F., Costa, M. F. A., Spanos, C., Rappsilber, J., Ohkura, H. (2022) The phospho-docking protein 14-3-3 regulates microtubule-associated proteins in oocytes including the chromosomal passenger Borealin. *bioRxiv*. doi: 10.1101/2021.12.16.472885
- Barbosa, P., Zhaunova, L., Debilio, S., Steccanella, V., Kelly, V., Ly, T., and Ohkura H. (2021) SCF-Fbxo42 promotes synaptonemal complex assembly by downregulating PP2A-B56. *J. Cell Biol.* 220, e202009167.
- Costa, M. F. A., and Ohkura, H. (2019) The molecular architecture of the meiotic spindle is remodeled during metaphase arrest in oocytes. *J. Cell Biol.* 218, 2854-2864.

The meiotic spindle and chromosomes in oocytes



A. Identification of 14-3-3 regulated microtubule-associated proteins from *Drosophila* oocytes. Microtubules and their associated proteins were purified from *Drosophila* ovaries in the presence and absence of a 14-3-3 inhibitor, and analysed by label-free quantitative mass-spectrometry.

B. Volcano plot showing the fold changes of the amounts of each protein detected in microtubule fraction in the presence of the 14-3-3 inhibitor in comparison to its absence. The red box contains 47 proteins that significantly increased their microtubule binding under 14-3-3 inhibition.

C. Upper panel: Diagram of domain organisation of Borealin and Incenp proteins, with three predicted 14-3-3 binding sites (red circles). Lower panel: the sequence surrounding S163 of Borealin has high similarity to the 14-3-3 binding sites of Ncd/Kinesin-14 and Pav/Kinesin-6.

D. An additional phosphorylation by Aurora B prevents PKD2-phosphorylated MBP-Borealin(113-221) from interacting with GST-14-3-3ε. Borealin(113-221) was incubated with human PKD2 kinase alone, human Aurora B kinase alone, both kinases or without kinases, and tested for pull down using GST or GST-14-3-3ε. Repton et al (2022).



Juri Rappsilber

Co-workers: Colin Combe, Christos Spanos, Juan Zou

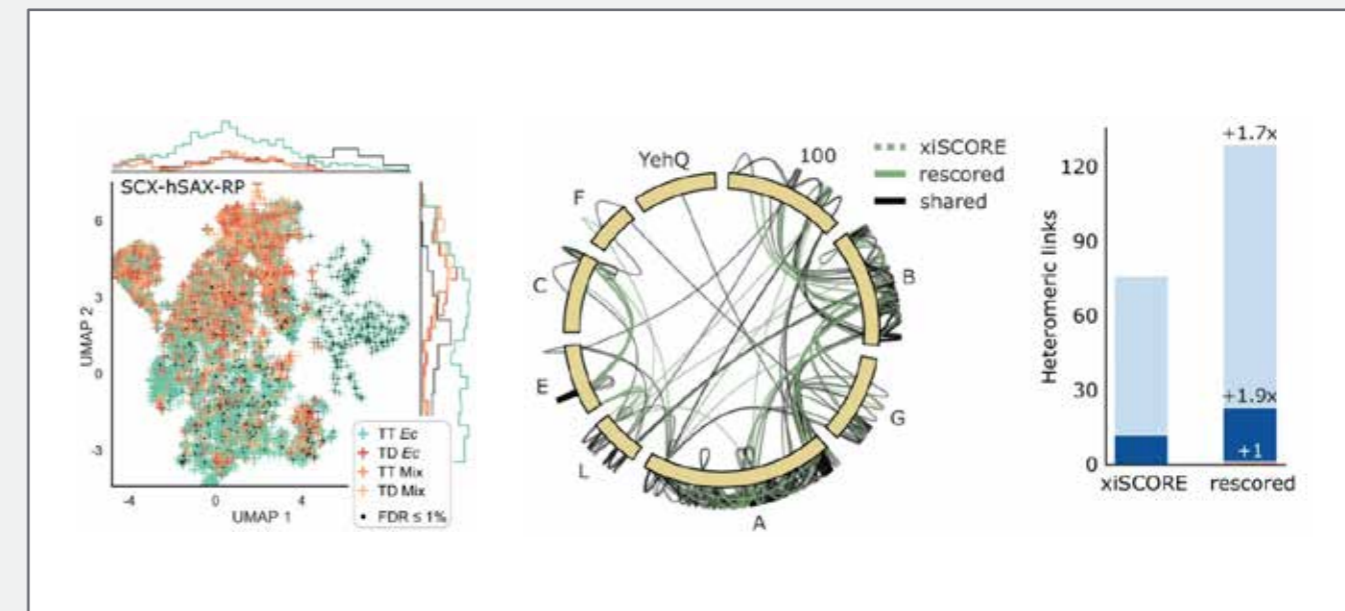
Protein-protein interactions (PPIs) govern most cellular pathways and processes, and multiple technologies have emerged to systematically map them. Assessing the error of interaction networks has been a challenge hitherto, however. This has been true also for protein-protein interactions detected by crosslinking mass spectrometry. Crosslinking mass spectrometry is currently widening its scope from structural analyses of purified multi-protein complexes towards systems-wide analyses of PPIs, to systematically reveal contact surfaces of the proteins. Using a carefully controlled large-scale analysis of *Escherichia coli* cell lysate, we demonstrated in 2021 that false-discovery rates (FDR) for PPIs identified by crosslinking mass spectrometry can be reliably estimated. The two key aspects for reliable error assessment in crosslinking are: (1) the separate handling of links that fall within proteins from those that fall between proteins, as they differ fundamentally in the size of the associated search spaces and consequently also in their random match behaviour (noise) and (2) assessing the error at the information level of interest (usually residue pairs or protein pairs, as opposed to the frequently used peptide-spectra matches). Applying these principles to our data using an open source tool that we made available, xiFDR, yielded an interaction network comprising 590 PPIs at 1% decoy-based PPI-FDR for *E. coli*. The structural information included in this network localises the binding site of the hitherto uncharacterised protein YacL to near the DNA exit tunnel on the RNA polymerase.

While this allowed to control the error in our data, we were left with limitations in the number of identified crosslinks. The incomplete and noisy information in the mass spectra of crosslinked peptides severely limits the numbers of protein-protein interactions that can be confidently identified. We therefore leveraged chromatographic retention time information to aid the identification of crosslinked peptides from mass spectra. Our Siamese machine learning model xiRT achieved highly accurate retention time predictions of crosslinked peptides in a multi-dimensional separation of crosslinked *E. coli* lysate. Importantly, supplementing the search engine score with retention time features led to a substantial increase in protein-protein interactions without affecting confidence. This approach is not limited to cell lysates and multi-dimensional separation but also improved considerably the analysis of crosslinked multiprotein complexes with a single chromatographic dimension, as we could demonstrate for an analysis of the Fanconi anemia complex (see figure). Retention times are a powerful complement to mass spectrometric information to increase the sensitivity of crosslinking mass spectrometry analyses.

Selected Publications

- O'Reilly, F.J., Xue, L., Graziadei, A., Sinn, L., Lenz, S., Tegunov, D., Blötz, C., Singh, N., Hagen, W.J.H., Cramer, P., Stülke, J., Mahamid, J., Rappsilber, J. (2020). In-cell architecture of an actively transcribing-translating expressome. *Science* 369, 554–557.
- Giese, S.H., Sinn, L.R., Wegner, F., and Rappsilber, J. (2021). Retention time prediction using neural networks increases identifications in crosslinking mass spectrometry. *Nat. Commun.* 12, 3237.
- Lenz, S., Sinn, L.R., O'Reilly, F.J., Fischer, L., Wegner, F., and Rappsilber, J. (2021). Reliable identification of protein-protein interactions by crosslinking mass spectrometry. *Nat. Commun.* 12, 3564.

Cellular Tomography



Retention time data complements substantially the currently exclusively used mass spectrometric evidence for the identification of crosslinks between proteins.

Left: The combined retention information of crosslinked peptides from three different chromatography modes suffices to effectively separate plausible identifications (green) from modelled noise (all other colours) in a crosslink analysis of *E. coli* lysate.

Middle: Crosslink network from the Fanconi anemia complex analysis, shown in the circular view. Unique residue pairs from xiSCORE (gray), after rescoring (green), and shared (black) between these analyses are depicted (1% residue-pair FDR). Proteins associated to the Fanconi anemia core complex are indicated with their gene name suffix. The *E. coli* protein YehQ represents a match from the entrapment database.

Right: Quantitative assessment of residue-pairs with and without rescoring, and including calculated distances in the model (all, light blue; $\leq 35 \text{ \AA}$, blue; $> 35 \text{ \AA}$ red), showing a gain in 70% on identified crosslinks between proteins.



Kenneth E. Sawin

Co-workers: Gaurav Barve, Tanya Dudnakova, Ye Dee Tay, James Le Cornu, Beste Bayrak, Ankita Gupta, Adam Kovac, Domenico Modaffari, Monique Scott

We are interested in two general areas related to cellular organisation: 1) regulation of cell polarity, under both normal and stress conditions, and 2) the molecular mechanisms underlying microtubule nucleation. In both areas we use fission yeast *Schizosaccharomyces pombe* as a model single-celled eukaryote. We combine classical and molecular genetic analysis with live-cell fluorescence microscopy, biochemistry, proteomics/phosphoproteomics, and structural biology methods.

Cell polarity in fission yeast is regulated by 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, through the microtubule-associated protein Tea1 and its interactors. We have shown how the Tea1/microtubule system coordinates polarity regulation by a conventional Cdc42 guanine-nucleotide exchange factor, Scd1, with regulation by an unconventional exchange factor, Gef1. Our work has also led to the discovery of new cell-polarity regulators outside of the Cdc42- and Tea1/microtubule-based systems, and a new understanding of how the conserved NDR kinase Orb6 regulates cell polarity. A major current focus is on how the stress-activated kinase Sty1 (homolog of human p38 MAP kinase) regulates cell polarity; we are addressing this through large-scale phosphoproteomics and genetics approaches.

Microtubule nucleation depends on the γ -tubulin complex, a large multi-protein complex enriched at microtubule organising centres such as the centrosome. Many aspects of γ -tubulin complex regulation remain a mystery. We discovered the fission yeast proteins Mto1 and Mto2, which form an oligomeric "Mto1/2 complex". The Mto1/2 complex targets the γ -tubulin complex to different sites in the cell and also activates γ -tubulin complex. Mutations in the human homolog of Mto1 lead to the brain disease microcephaly. Our current work involves understanding the mechanism of γ -tubulin complex activation by the Mto1/2 complex, using yeast genetics, microscopy, and biochemistry approaches. In recent work we have reconstituted multi-protein complex-dependent microtubule nucleation *in vitro* using purified proteins, and we have characterised elements of functional nucleation complexes through cross-linking mass spectrometry as well as X-ray crystallography. We have also developed new methods to interrogate protein-protein interactions in complex "solid-phase" subcellular structures *in vivo*, and we have used these to investigate how Mto1/2 complex is localised to nuclear pores.

In all of our work we adopt and develop new tools and techniques as necessary to address the biological questions of interest.

Selected Publications

Ashraf, S., Tay, Y.D., Kelly, D.A., and Sawin, K.E. (2021). Microtubule-independent movement of the fission yeast nucleus. *J Cell Sci.* Feb 23:jcs.253021 PMID: 33602740

Leong, S.L., Lynch, E.M., Zou, J., Tay, Y.D., Borek, W.E., Tuijtel, M.W., Rappsilber, J., and Sawin, K.E. (2019). Reconstitution of Microtubule Nucleation In Vitro Reveals Novel Roles for Mzt1. *Curr Biol* 29, 2199-2207 e2110. PMID: 31287970

Tay, Y.D., Leda, M., Spanos, C., Rappsilber, J., Goryachev, A.B., and Sawin, K.E. (2019). Fission Yeast NDR/LATS Kinase Orb6 Regulates Exocytosis via Phosphorylation of the Exocyst Complex. *Cell Rep* 26, 1654-1667 e1657. PMID: 30726745

Cell polarity and cytoskeletal organisation

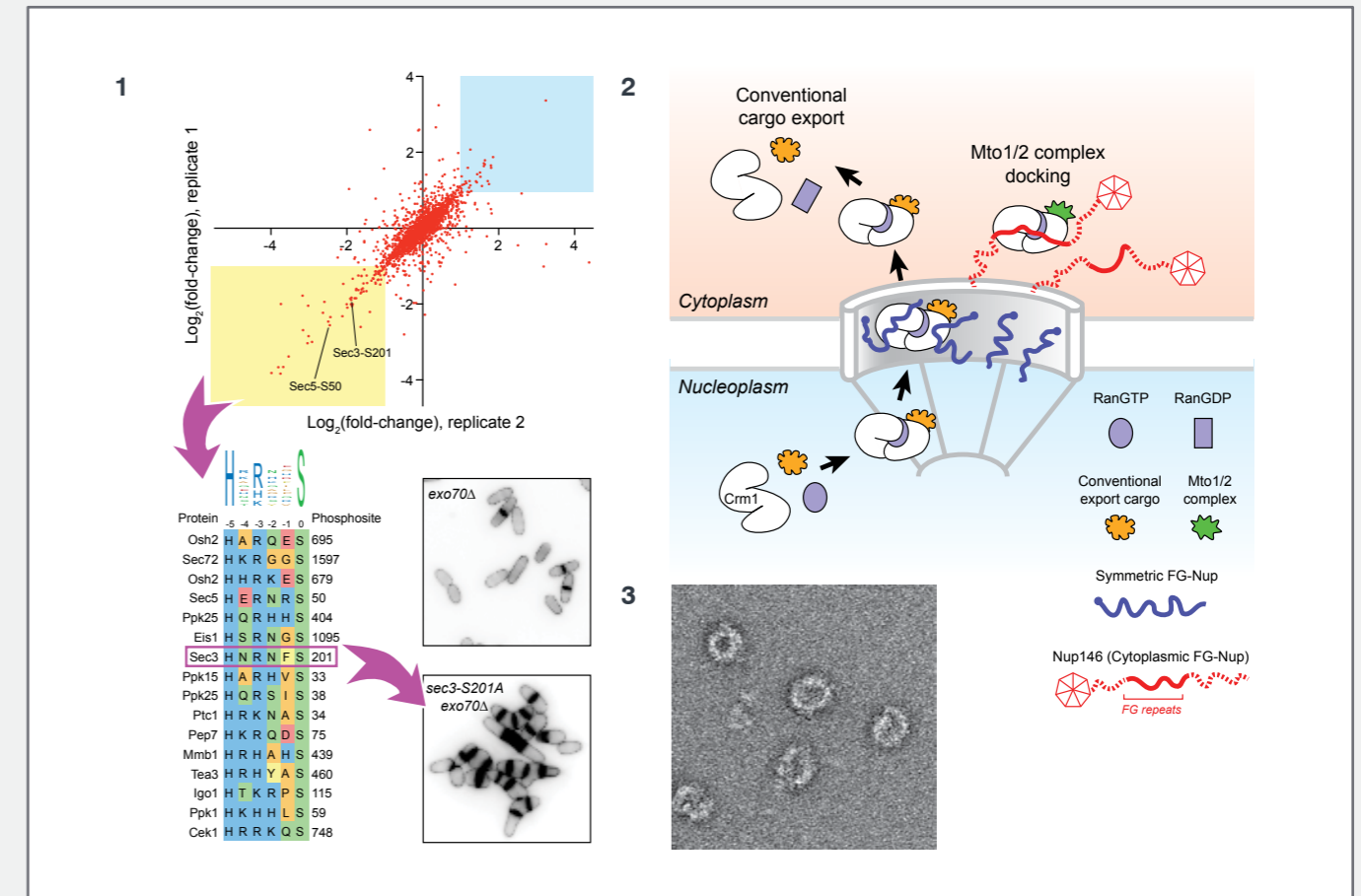


Figure 1. Global phosphoproteomics after inhibition of NDR kinase Orb6 *in vivo*. Many phosphorylation sites with decreased phosphorylation after Orb6 inhibition match the NDR consensus. Phosphosite mutation of Sec3 (component of exocyst complex) impairs exocytosis and cell separation after cytokinesis.

Figure 2. Model for docking Mto1/2 microtubule nucleation complex at the nuclear pore. Mto1 mimics a nuclear export cargo but uses this for docking at the pore, not for nuclear export.

Figure 3. Negative-stain electron microscopy of reconstituted fission yeast γ -tubulin ring complex.



David Tollervey

Co-workers: Katie Bexley, Stefan Bresson, Aziz El Hage, Aleksandra Helwak, Michaela Kompauerova, Alexandra Lehmann, Laura Milligan, Elisabeth Petfalski, Nic Robertson, Emanuela Sani, Vadim Shchepachev, Tomasz Turowski

RNA-protein interactions have important functions at all steps in gene expression, including transcription, RNA processing and mRNA translation. RNA defects underpin many genetic diseases, while responses to environmental stress are frequently mediated by altered RNA-protein interactions. Over the past year we have made good progress in understanding stress responses in yeast and have started to apply these insights in human cells. We have also applied our techniques to understand the molecular basis of RNA-linked disease, and this report will focus on these advances.

Coronavirus infection involves a complex pathway of coding and non-coding RNA synthesis. To better understand the biology of viral gene expression and replication we generated constructs for studying RNA interactions by viral proteins (doi: 10.12688/wellcomeopenres.16322.1). We are also following host and viral RNA metabolism and RNA-protein interactions over detailed time courses during infection (Fig. 1). This is giving insights into the timing and regulation of viral RNA replication and virus-host interactions.

In the 1980s, we discovered that eukaryotic cells contain large numbers of small nucleolar RNAs (snoRNAs). More recently, we applied CLASH, a proximity ligation technique developed in the group, to systematically map snoRNA interactions with rRNAs and mRNAs in yeast and human cells (10.12688/wellcomeopenres.14735.2; bioRxiv 2021.07.22.451324). We are now characterising the basis of the neurological disease Prader-Willi Syndrome, which can be caused by loss of a single family of brain-enriched snoRNAs called SNORD116. We have created a PWS model system based on pre-neuronal cells that lack SNORD116, and are determining the role of this snoRNA in neuronal differentiation. From our preliminary data, we already know that lack of SNORD116 expression leads to substantial alterations in gene expression and accelerates the differentiation process. Now we aim to determine the molecular mechanisms.

We recently identified the molecular defects underlying another genetic disease Cartilage Hair Dysplasia (CHD), characterised by reduced stature and immunodeficiency. This can be caused by mutations in RMRP, another nucleolar ncRNA. RMRP provides the core of an RNA-protein complex with RNA cleavage activity, RNase MRP, which we characterized in the 1990s. Mutations in the mouse RMRP gene impaired T cell activation, which must occur during immune response, and delayed pre-ribosomal RNA (pre-rRNA) processing. Recapitulation of the major disease-linked mutation in human cells (Robertson et al. 2022) induced a defect in pre-rRNA processing, leading to reduced accumulation of the large ribosomal subunit (Fig. 2). A similar pre-rRNA processing defect was seen in patient-derived fibroblasts, establishing CHD as a disease of ribosome synthesis or "ribosomopathy".

Together, these analyses increased our understanding of important, disease-related pathways in RNA biology.

Selected Publications

- Robertson, N., Shchepachev, V., Wright, D., Turowski, T.W., Spanos, C., Helwak, A., Zamoyska, R., and Tollervey, D. (2022). A disease-linked lncRNA mutation in RNase MRP inhibits ribosome synthesis. *Nat Commun* 13, 649.
- Bresson, S., Shchepachev, V., Spanos, C., Turowski, T., Rappsilber, J., and Tollervey, D. (2020). Stress-induced translation inhibition through rapid displacement of scanning initiation factors. *Mol Cell* 80, 470-484.
- Turowski, T.W., Petfalski, E., Goddard, B.D., French, S.L., Helwak, A., and Tollervey, D. (2020). Nascent Transcript Folding Plays a Major Role in Determining RNA Polymerase Elongation Rates. *Mol Cell* 79, 488-503.e411.

RNA Processing and Quality Control

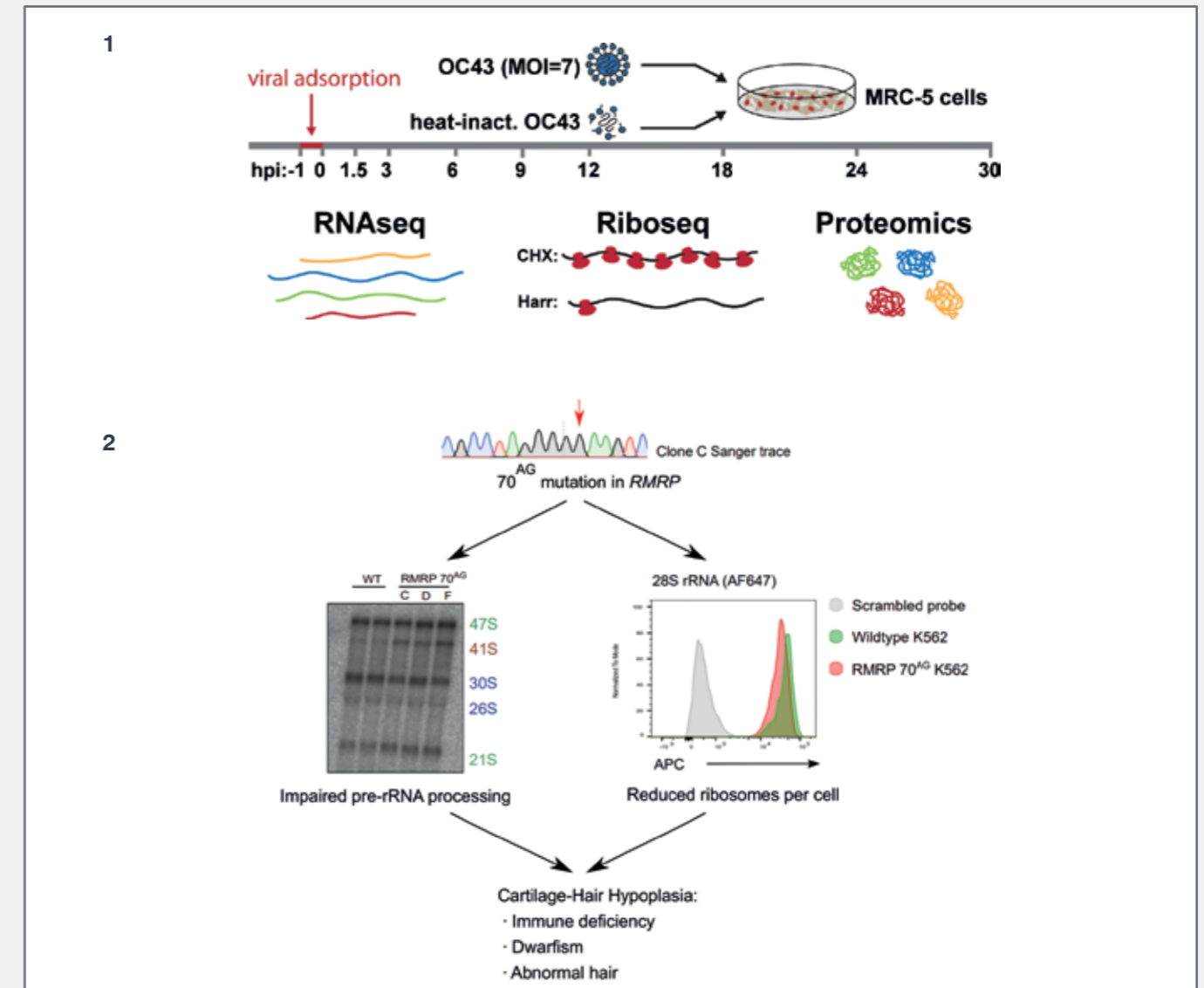


Figure 1. Analyses of the Coronavirus infection time-course

Figure 2. Recapitulation of disease-linked mutations in the human lncRNA, RMRP



Julie Welburn

Co-workers: Thomas Attard, Ben Craske, Agata Gluszek, Haonan (Leo) Liu, Toni McHugh, Calum Paterson

Research

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 (730 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. Using a non-biased approach, we recently found the C-terminal kinetochore-targeting region of CENP-E interacts with BubR1, amongst other proteins in nocodazole-arrested mitotic cell extracts. We have defined the molecular determinants that specify the interaction between BubR1 and CENP-E. The basic C-terminal helix of BubR1 is necessary but not sufficient for CENP-E interaction, while a minimal key acidic patch on the kinetochore-targeting domain of CENP-E, is also essential. We then demonstrated that BubR1 is required for the recruitment of CENP-E to kinetochores to facilitate chromosome alignment. In collaboration with the Gruneberg lab, University of Oxford, we showed this BubR1-CENP-E axis is critical to align chromosomes that have failed to congress through other pathways and recapitulates the major known function of CENP-E. Overall, our current studies define the molecular basis and the function for CENP-E recruitment to BubR1 at kinetochores during mammalian mitosis. Our future work now focused on what is the basis and function for the 2 distinct recruitment pathways of CENP-E to kinetochores and defining the activation mechanism of human CENP-E to ensure faithful mitosis.

Mitotic motors and microtubule dynamics

Our lab has made new discoveries on the mechanism of mitotic microtubule depolymerases for two families recently published: the Kinesin-8 and the Kinesin-13 motors. Our current work now addresses how MCAK and Kinesin-8 motors cooperate to control microtubule length in mitosis.

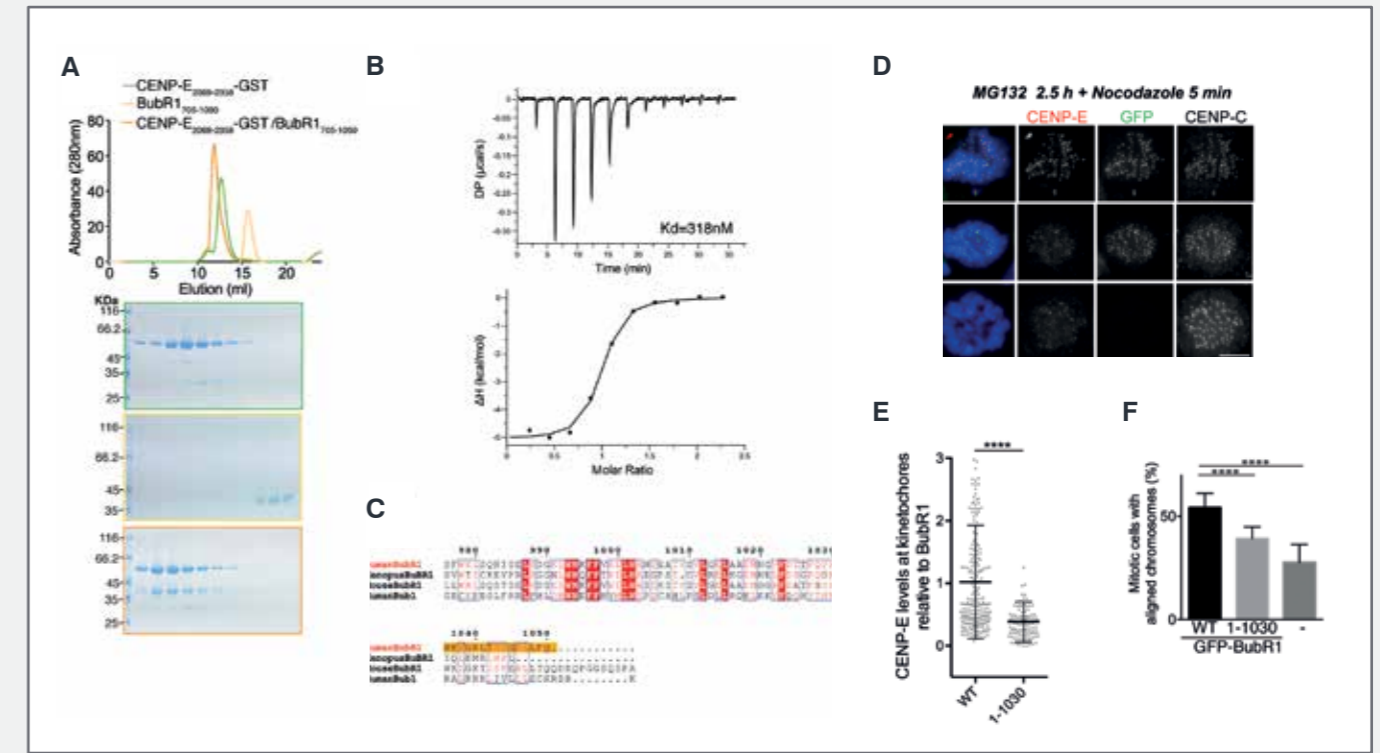
Selected Publications

Legal T., Hayward D., Gluszek-Kustusz A., Blackburn EA., Spanos C., Rappsilber J., Gruneberg U., Welburn JPI. The C-terminal helix of BubR1 is essential for CENP-E-dependent chromosome alignment. *JCS* (2020). 133:16. PMID: 32665320.

McHugh T., Zou J., Volkov V., Bertin A., Rappsilber J., Dogterom M., Welburn JPI. The depolymerase activity of MCAK shows graded response to Aurora B phosphorylation through allosteric regulation. *JCS*. (2019). PMID: 30578316

McHugh T., Gluszek-Kustusz A., Welburn JPI. Kinesin-8 motor Kif18b tethering to microtubule ends is a requirement for spindle positioning. *JCB*. (2018). 217:7. 2403-2416. PMID: 29661912

Mechanisms of chromosome alignment in cell division



A. Top, SEC analysis and elution profile for CENP-E₂₀₆₉₋₂₃₅₈-GST (green), BubR1₇₀₅₋₁₀₅₀ (yellow) and CENP-E₂₀₆₉₋₂₃₅₈-GST/BubR1₇₀₅₋₁₀₅₀ (orange). Bottom, Coomassie-stained gels showing elution profiles for the corresponding protein complexes.

B. Thermodynamics of BubR1₇₀₅₋₁₀₅₀/CENP-E₂₀₉₁₋₂₃₅₈-GST interaction determined by isothermal titration calorimetry. The y-axis indicates kcal/mol of injectant. The dissociation constant (K_d) between BubR1₇₀₅₋₁₀₅₀ and CENP-E₂₀₉₁₋₂₃₅₈-GST was determined to be 318 ± 90 nM.

C. Sequence alignment of the C terminus of human BubR1 with mouse and *Xenopus* BubR1, and human Bub1. Boxed red and blue are the conserved and similar amino acids across all 4 proteins, respectively. Amino acids in red are those with conserved properties in at least 3 sequences. The sequence necessary for BubR1 binding to CENP-E₂₀₅₅₋₂₆₀₈ is highlighted in orange.

D. Representative immunofluorescence images of HeLa cells treated with BubR1 siRNA and induced to express GFP-BubR1 WT and GFP-BubR1₁₋₁₀₃₀, stained with CENP-E, CENP-C and Hoechst after treatment with MG132 for 2.5 hrs. Scale bar: 10 μ m.

E. Scatter plot showing CENP-E intensity relative to GFP-BuR1 at individual kinetochores plotted as grey circles, with mean and standard deviation represented by black lines.

F. Graph showing percentage of cells with at least 1 misaligned chromosome for BubR1-depleted cells induced to express GFP-BubR1, BubR1₁₋₁₀₃₀ or without induction. Error bars represent standard deviation. **** indicating $P < 0.0001$.



Marcus D Wilson

Co-workers: Hayden Burdett, Gillian Clifford, Alakta Das, Gauri Deák, Dhananjay Kumar, Hannah Wapenaar, James Watson

We are interested in understanding how epigenetic marks are placed, read and interpreted on chromatin. Chromatin becomes decorated with a variety of chemical tags or epigenetic marks to control the myriad of DNA-related processes in the cell. Epigenetic modifications are initially deposited by writer enzymes. These are then read and interpreted in a co-operative manner by effector proteins. Epigenetic marks can also be removed by eraser proteins resetting the system (Figure 1 A). We look at this process in the test tube by creating modified chromatin using chemical biology and biochemical methods. We then use our defined modified chromatin to study individual nucleosome-chromatin protein complexes using single-particle cryo-electron microscopy (cryo-EM), Biochemical, Biophysical and Cell Biology approaches. We are particularly interested in understanding how DNA damage repair and DNA methylation pathways are orchestrated by epigenetically-modified nucleosomes.

How is DNA Methylation guided by chromatin?

DNA methylation is a common epigenetic mark that is often associated with turning off genes and compacting DNA. Other epigenetic marks have the power to regulate DNA methylation, controlling when and where DNA methylation is placed on DNA, but we do not understand how this works. We are rebuilding the DNA methylation machinery within chromatin to help us answer this question.

DNA methylation is a highly regulated process, so by looking at the structure of the methylation machinery and the modified nucleosomes we hope to understand how methylation is targeted at specific times and to specific sites on DNA, hopefully helping us to understand how this process can become faulty leading to disease.

How do post-translational modifications foster DNA repair?

DNA is under constant attack, which can cause unwanted genetic mutations and cancer. Luckily our cells have a host of DNA repair proteins, which help to fix most of the damage. These highly efficient repair proteins are recruited to sites of damage by recognition of DNA damage-specific marks on chromatin. We are hoping to understand how DNA damage is signalled on chromatin and how this leads to correct repair.

Our recent work has shown that multiple DNA repair proteins interact multivalently with the nucleosome, commonly interacting with a conserved region called the acidic patch (Figure 1B). Through our in vitro studies we showed that the negatively charged surface of the nucleosome acidic patch is essential for binding of the listed DNA damage proteins. Mapping of the interaction regions and mutation has shown these are mediated by electrostatic interactions, typically through a highly conserved arginine anchor.

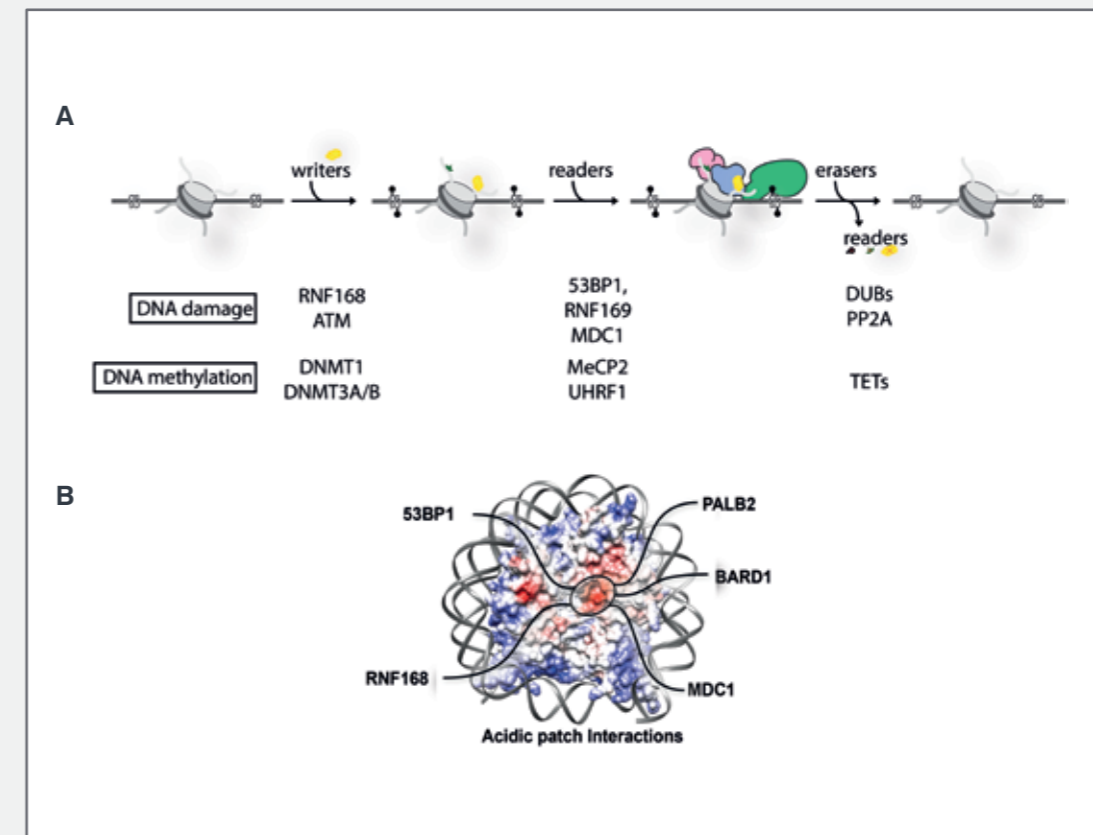
Selected Publications

Becker, J.R., Clifford, G., Bonnet, C., Groth, A., Wilson, M.D., and Chapman, J.R. (2021). BARD1 reads H2A lysine 15 ubiquitination to direct homologous recombination. *Nature* 596, 433-437.

Belotserkovskaya, R., Raga Gil, E., Lawrence, N., Butler, R., Clifford, G., Wilson, M.D., and Jackson, S.P. (2020). PALB2 chromatin recruitment restores homologous recombination in BRCA1-deficient cells depleted of 53BP1. *Nat Commun* 11, 819.

Wilson, M.D., Renault, L., Maskell, D.P., Ghoneim, M., Pye, V.E., Nans, A., Rueda, D.S., Cherepanov, P., and Costa, A. (2019). Retroviral integration into nucleosomes through DNA looping and sliding along the histone octamer. *Nat Commun* 10, 4189.

Investigating the reading writing and erasing of epigenetic marks



A. Schematic of the cycle of epigenetic modifications in the cell. All modifications focus on the nucleosome hub, which can be modified on both the histone proteins and wrapped DNA. Example Reader, writer and eraser proteins DNA damage repair and DNA methylation pathways are labelled.

B. Multivalent interactions in the DNA damage response focus on the acidic patch of the nucleosome.

Emeritus Centre Members



Emeritus Professor of Molecular Biology and Emeritus Member of the Centre.

Prior to closing in June 2019 when Jean retired, the Beggs lab used biochemistry, cell biology, genetics and quantitative systems biology approaches to investigate RNA processing pathways in budding yeast, with a focus on pre-mRNA splicing and links between RNA splicing, transcription and chromatin modifications.

Three recent publications:

Maudlin, I.E. and J.D. Beggs (2019) Spt5 modulates cotranscriptional spliceosome assembly in *Saccharomyces cerevisiae*. *RNA* 25:1298–1310. doi: 10.1261/rna.070425.119.

Aslanzadeh, V. and Beggs, J.D. (2020) Revisiting the window of opportunity for cotranscriptional splicing in budding yeast. *RNA* 26:1081–1085. doi:10.1261/rna.075895.120

Maudlin, I.E. and Beggs, J.D. (2021) Conditional depletion of transcriptional kinases Ctk1 and Bur1 and effects on co-transcriptional spliceosome assembly and pre-mRNA splicing. *RNA Biol.* 18:782-793. doi: 10.1080/15476286.2021.1991673.



Emeritus Professor of Structural Biochemistry and Emeritus Member of the Centre

Malcolm Walkinshaw's interest is in mechanisms of molecular recognition and in the regulation of protein-ligand and protein-DNA interactions. Structural and enzymatic studies of allosteric proteins in the glycolytic pathway have been used to develop biologically active drug-like molecules that are active against trypanosome parasites. Similar approaches have been used to develop small molecule ligands that inhibit cyclophilin isoforms and these are currently being investigated for their potential antiviral and anti-cancer activities.

Three recent publications:

McNae IW, Kinkead J, Malik D, Yen LH, Walker MK, Swain C, et al. Fast acting allosteric phosphofructokinase inhibitors block trypanosome glycolysis and cure acute African trypanosomiasis in mice. *Nat Commun.* 2021;12:1052.

Zhong W, Li K, Cai Q, Guo J, Yuan M, Wong YH, et al. Pyruvate kinase from *Plasmodium falciparum*: Structural and kinetic insights into the allosteric mechanism. *Biochem Biophys Res Commun.* 2020;532:370-6.

Pinto Torres JE, Yuan M, Goossens J, Versees W, Caljon G, Michels PA, et al. Structural and kinetic characterization of *Trypanosoma congolense* pyruvate kinase. *Mol Biochem Parasitol.* 2020;236:111263.

International Scientific Advisory Board

Margaret Fuller

Department of Developmental Biology
and Department of Genetics
Stanford University School of Medicine
291 Campus Drive, Li Ka Shing Building
Stanford, CA 94305-5101
USA

Iain Mattaj

Fondazione Human Technopole
Palazzo Italia
Via Cristina Belgioioso, 171
20157 Milan, Italy

Michael Rout

The Rockefeller University
1230 York Avenue
New York, NY 10021
USA

Andrea Brand

Wellcome Trust/Cancer Research UK Gurdon Institute,
Henry Wellcome Building of Cancer and Developmental
Biology,
Tennis Court Road,
Cambridge CB2 1QN,
UK

Elena Conti

Max-Planck-Institut für Biochemie
Structural Cell Biology Department
Am Klopferspitz 18
D-82152 Martinsried
Germany

Public Engagement

Whilst 2021 was not the 'business as usual' year we had hoped it might be, the Public Engagement Team were able to build on our successes from the previous year, continuing to provide a diverse range of socially distanced and virtual activities for audiences old and new.

With the invaluable help of researchers from across the centre, we hosted or contributed to over 35 events, engaging with approximately 1000 audience members. These included running a mix of established activities such as micro-art marbling and cell biscuit making, alongside newer offerings such as the hybrid science show/workshop 'Marvellous Microscope Tricks'. Post out kits, video calls and WCB research images enabled school and family audiences to experiment along with researchers, whilst building a knowledge of the science behind the centre's impressive range of microscopes.

In the second half of the year, as restrictions eased off, we were lucky enough to be able to get out of the centre and deliver some face-to-face sessions. In September, we were invited to showcase our RNA World exhibition as part of the Glasgow Science Festival. The glass art, including some 2021 updates such as an additional RNA virus tile (SARS-CoV-2 of course!) were a big hit with visitors at Glasgow Botanic Gardens. (Figure 1.)

The relationship between the centre and Engage Nepal with Science continued to grow this year as we supported the charity (led by Arulanandam lab researcher Alba Abad) in a successful bid to secure British Council funding for their "Connecting the Climate" project. The centre was able to introduce many of our local schools to the project, who were then twinned with schools in Nepal to undertake parallel environmental experiments. We were even lucky enough to be able to tag along to one of their school visit in Shetland where we ran our ever popular 'Life Through a Lens' workshop. (Figure 2)

After winning a School of Biology Public Engagement Seed Funding award, Gerard Pieper (Marston lab) piloted a new event aimed at engaging IVF patients in fertility research. The session, hosted by the ASCUS team at Summerhall, allowed participants to look at oocytes under microscopes, get hands on with pipettes and other scientific equipment and make their own DNA jewellery, all whilst chatting to our researchers. Artist Emily Fong was there to capture some moments from this session, in her own beautiful and unique style (Figure 3)

2021 proved to be a very successful year for the centre in terms of securing public engagement funds. We supported Young Carers Edinburgh in winning one of the University's community grants, allowing them to run their outdoor summer camp sessions 'Nurture in Nature'. We were also delighted to secure ScotPen Wellcome Public Engagement Awards in both the competitive rounds in 2021.

The Tollervey lab's Prader Willi Superheroes project brings researchers together with PWS families to co-develop an exhibition about the strengths and challenges of both living with, and conducting research connected to, the condition. This project is supported by current centre Artist in Residence, Neil Bratchpiece, who continued to work on the whole centre comic book throughout 2021 (Figure 4).

"The Fabric of Life", a collaborative project between Julie Welburn, Atlanta Cook, Alison Pidoux and Tony Ly, involves the creation of fabrics with research themed patterns, which will go on to be used in public engagement sessions with a range of community groups.

Both these major projects will be sure to keep the Public Engagement Team busy in 2022, and as restrictions further ease we look forwards to taking more of our activities out into the 'real' world and to welcoming community groups back to visit the centre.



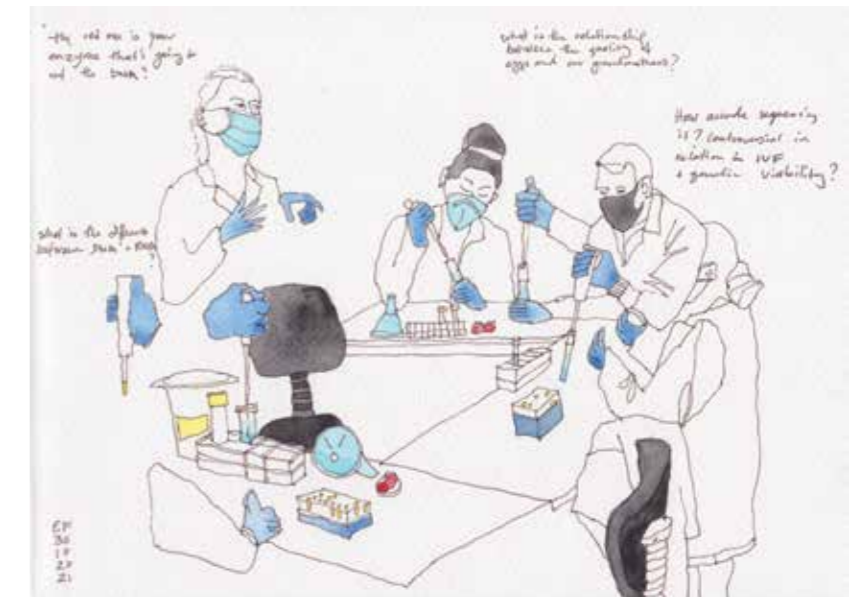
1



2



3



4

iCM PhD Programme

Wellcome Four Year PhD Programme in Integrative Cell Mechanisms

Training a new generation of cross-disciplinary Molecular Cell Biologists

The Wellcome Four Year PhD Programme in Integrative Cell Mechanisms (iCM) is closely associated with the Wellcome Centre for Cell Biology and trains the next generation of cell and molecular biologists in the application of quantitative methods to understand the inner workings of distinct cell types in different settings.

Data generated by biological research requires increasingly complex analysis as technological advances in sequencing, mass spectrometry/proteomics, super-resolution microscopy, synthetic and structural biology produce increasingly large, complex datasets. Innovations in computer sciences and informatics are transforming data acquisition and analysis and breakthroughs in physics, chemistry and engineering are allowing the development of devices, molecules and instruments that drive the biological data revolution. Exploiting technological advances to transform our understanding of cellular mechanisms will require scientists who have been trained across the distinct disciplines of natural sciences, engineering, informatics and mathematics.

To address this training need, iCM PhD projects are cross-disciplinary involving two primary supervisors with complementary expertise. Supervisor partnerships pair quantitative scientists with cell biologists ensuring that students develop pioneering cross-disciplinary collaborative projects to uncover cellular mechanisms relevant to health and disease.

Applications are encouraged from students with a variety of backgrounds across the biological and physical sciences, including Biochemistry, Biomedical Science, Cell Biology, Chemistry, Computational Data Sciences, Engineering, Genetics, Mathematics, Molecular Biology and Physics. Students are trained to adapt, broaden and apply their skill set to the understanding of cellular mechanisms of biomedical importance.

The first two cohorts of iCM students are currently being trained in WCB and other labs associated with the iCM PhD programme. The next cohort has been recruited and will be joining us in October 2022

Want to apply?

Applications for students starting autumn 2023 will open in October 2022 find out how to apply at the iCM PhD programme website: www.wcb.ed.ac.uk/how-apply

iCM Summer Internship Programme

Summer Internship Programme in Integrative Cell Mechanisms

Training a new generation of cross-disciplinary Molecular Cell Biologists

The Summer Internship Programme in Integrative Cell Mechanisms (iCM) is closely associated with the Wellcome Centre for Cell Biology. Our 8 week programme provides undergraduates who are interested in a career in science with an immersive experience embedded in a research group associated with the Integrated Cell Mechanisms (iCM) Wellcome Trust PhD programme. It provides lab-based experience and training for budding cell and molecular biologists in the application of quantitative methods to understand the inner workings of cells and will help prepare participants to be successful in a variety of PhD programmes.

We are committed to fostering a diverse and inclusive research environment. The programme is open to students of any nationality who are registered at a UK University and are about to enter their final year of study. Applications are encouraged from individuals from a wide range of backgrounds who have studied a variety of subjects including Biochemistry, Biomedical Science, Cell Biology, Chemistry, Computational Data Sciences, Engineering, Genetics, Mathematics, Molecular Biology and Physics.

Applications from members of groups typically under-represented in STEM are particularly encouraged and around half of our Scholarships are allocated to give students from disadvantaged and underrepresented backgrounds the opportunity to carry out research and experience all that a supportive research environment has to offer.

To facilitate this students receive a generous stipend equivalent to the real Living Wage, free accommodation or assistance with accommodation, and travel expenses.

We hope to provide the opportunity for bright students studying relevant subjects to learn and experience biological research and all it has to offer irrespective of their background.

Applications for Summer 2023 internships will open in December 2022 and will be posted at:

www.wcb.ed.ac.uk/icm-summer-internship-program

List of Groups

Robin Allshire

Wellcome Principal Research Fellow

Tatsiana Auchynnikava	Wellcome Research Associate (Francis Crick Institute, London from 11/04/22)
Roberta Carloni	MRC Research Associate
Andreas Fellas*	iCM Graduate Student
Elisabeth Gaberdiel**	Wellcome iCM Graduate Student
Nitobe London	Wellcome Research Associate
Alison Pidoux	Wellcome Research Associate
Severina Pociunaite	Darwin Trust Graduate Student
Desislava Staneva	MRC Research Associate
Manu Shukla	Wellcome Research Associate
Sharon White	Wellcome Research Associate
Weifang Wu	Wellcome Research Associate
Imtiyaz Yaseen	Wellcome Research Associate (Ramalingaswami Fellow, CSIR-Indian Institute of Integrative Medicine, Jammu from 28/03/22)
Rebecca Yeboah	Darwin Trust Graduate Student

* joint iCM Graduate Student with Peter Swain

** joint iCM Graduate Student with Meriem El Karoui

A. Jeyaprakash Arulanandam

Wellcome Senior Research Fellow

Maria Alba Abad	
Fernandaz	Wellcome Research Associate
Carla Chiodi	Wellcome Research Associate
Thomas Davies*	iCM PhD Student
Lorenza Di Pompeo**	iCM Graduate Student
Anjitha Gireesh	Darwin Trust Graduate Student
Bethan Medina-Pritchard	Wellcome Research Assistant
Paula Sotelo Parrilla	Principal's Career Development Graduate Student
Pragya Srivastava	Darwin Trust Graduate Student

* joint iCM PhD student with Kevin Hardwick

** joint iCM PhD student with Bill Earnshaw

Adrian Bird

Wellcome Investigator in Science

Beatrice	
Alexander-Howden	Wellcome Research Assistant
Megan Brown	Research Assistant/Lab Manager
Kashyap Chhatbar	ERC Bioinformatician
Sarah Giuliani	ERC Visiting Student
Jacky Guy	Wellcome Research Associate
Matthew Lyst	SIDB Research Associate
Baisakhi Mondal	ERC Research Associate
Raphael Pantier	ERC Research Associate
Katie Paton	BBSRC/SBRC Postgraduate Student
Christine Struthers	Personal Assistant
James Watson*	Wellcome iCM Graduate Student

* joint iCM PhD student with Marcus Wilson

Dhanya Cheerambathur

Sir Henry Dale Fellow

Lana Buzuk	Wellcome Research Technician
Henrique Alves Domingos	Darwin Trust Graduate Student
Mattie Green	Wellcome Research Assistant
Cameron Finlayson	Wellcome Research Technician
Vasilis Ouzounidis	Darwin Trust Graduate Student
Emmanuel Fiagbedzi*	Wellcome iCM Graduate Student

* joint iCM PhD student with Georg Kustatscher

Atlanta Cook

Wellcome Senior Research Fellow

Laura Croenen	Cunningham Trust Graduate Student
James Le Cornu*	iCM Graduate Student
Atika Al Haisani	Graduate Student
Uma Jayachandran	Wellcome Research Assistant
Ola Kasprowicz	Wellcome Research Associate
David McCleod**	Wellcome iCM Graduate Student
Mickey Oliver	Wellcome Research Associate
Alexander Will	Darwin Trust Graduate Student

* joint iCM PhD student with Ken Sawin

** joint iCM PhD student with Donal O'Carroll

Owen Davies

Wellcome Senior Research Fellow

Eleanor Casey*	Wellcome iCM Graduate Student
Simona DeBilio	Wellcome Research Assistant
Gurusaran Manickam	Wellcome Research Associate

* joint iCM PhD student with Adele Marston

Bill Earnshaw

Wellcome Principal Research Fellow

Blandine Arleri	Erasmus intern
Mar Carmena	Wellcome Research Associate
Fernanda Cisneros	Lab Manager/Wellcome Research Associate
Lorenza Di Pompeo*	iCM Graduate Student
Moonmoon Deb	Royal Society Newton Fellow
Natalia Kochanova	Wellcome Research Associate
Emma Peat	Part-time Technician
Elisa Pesenti	Lab Manager/Wellcome Research Associate
Bram Prevo	Sir Henry Wellcome, Postdoctoral Fellow
Nina Pucekova	Technician
Caitlin Reid	SBS Ph.D. Student/Technician
Lucy Remnant	Wellcome Research Associate
Kumiko Samejima	Wellcome Research Associate
Itaru Samejima	Wellcome Research Associate

* joint iCM PhD student with A. Jeyaprakash Arulanandam

Patrick Heun

Wellcome Senior Research Fellow

Mathilde Fabe	Darwin Trust Graduate Student
Meena Krishnan	Darwin Trust Graduate Student
Alessandro Stirpe	Wellcome Research Associate
Hwei Ling Tan	EASTBIO/BBSRC Graduate Student
George Yankson	Darwin Trust Graduate Student

Georg Kustatscher

MRC Career Development Fellow

Van Kelly	Postdoctoral Fellow
Savvas Kourtis	PhD Student
Emmanuel Fiagbedzi*	Wellcome iCM Graduate Student

* joint iCM PhD student Dhayna Cheerambathur

Adele Marston

Director. Wellcome Investigator in Science

Eleanor Casey*	Wellcome iCM Graduate Student
Kirsty Dewars	MSci placement Student
Chuanli Huang	PhD Student
Dilara Kocakaplan	Darwin Trust Graduate Student
Lori Koch	Wellcome Research Associate
Melanie Lim	Carnegie Trust Graduate Student
Lucia Massari	BBSRC Research Associate
Bettina Mihalas	Wellcome Research Associate
Anuradha Mukherjee	Darwin Trust Graduate Student
Meg Peyton-Jones	EASTBIO/BBSRC Graduate Student
Gerard Pieper	Sir Henry Wellcome Postdoctoral Fellow
Ola Pompa	Darwin Trust Graduate Student
Hollie Rowlands	Wellcome Research Associate
Julia Van Bockstaele	Université de Paris MSc Student
Aparna Vinod	Darwin Trust Graduate Student
Menglu (Lily) Wang	Wellcome Research Associate

* joint iCM PhD student with Owen Davies

List of Groups

Dónal O'Carroll

Wellcome Investigator in Science

Tamoghna Chowdhury	Darwin Trust Graduate Student
Azzurra DePace	Wellcome Graduate Student
Madeleine Heep	Wellcome Graduate Student
Yuka Kabayama	Wellcome Research Associate
David MacLeod*	Wellcome iCM Graduate student
Christopher Mapperly	Wellcome Tissue Repair Graduate Student
Pedro Moreira	Lab Manager
Martina Schito	Research Assistant
Theresa Schöpp	Wellcome Graduate Student
Ansgar Zoch	Wellcome Research Associate

* joint iCM PhD student with Atlanta Cook

Hiro Ohkura

Wellcome Investigator in Science

Aleksandra Ciszek	Wellcome Research Assistant
Fiona Cullen	Wellcome Research Associate
Rayane Kaade	Darwin Trust Graduate Student
Jule Nieken	Wellcome Research Associate
Gera Pavlova	Wellcome Research Associate
Emma Peat	Wellcome Technician
Charlotte Repton	Wellcome Research Associate
Emiliya Taskova	BBSRC Research Technician/Graduate Student
Elsa Tungadi	Nagoya-Edinburgh joint degree PhD Student
Xiang Wan	Darwin Trust Graduate Student

Juri Rappsilber

Wellcome Senior Research Fellow

Colin Combe	Wellcome Research Associate
Christos Spanos	Wellcome Research Associate
Juan Zou	Wellcome Proteomics Data Analysis Manager

Kenneth E Sawin

Wellcome Investigator in Science

Beste Bayrak	Darwin Trust Graduate Student
Gaurav Barve	Wellcome Research Associate
Tanya Dudnakova	Wellcome Research Associate
Ye Dee Tay	Wellcome Research Associate
James Le Cornu*	iCM Graduate Student
Ankita Gupta	Darwin Trust Graduate Student
Adam Kovac	EASTBIO PhD Student
Alexandra Lehmann	MRC Graduate Student
Domenico Modaffari**	Wellcome iCM Graduate Student
Monique Scott	Darwin Trust Graduate Student

* joint iCM PhD student with Atlanta Cook

**joint iCM PhD student with Edward Wallace

David Tollervey

Wellcome Principal Research Fellow

Stefan Bresson	Wellcome Research Associate
Katie Bexley*	Wellcome iCM Graduate Student
Aziz El Hage	Wellcome Research Associate
Aleksandra Helwak	Wellcome Research Associate
Michaela Kompauerova	EASTBIO BBSRC Graduate Student
Alexandra Lehmann	MRC Graduate Student
Laura Milligan	Wellcome Research Associate
Elisabeth Petfalski	Wellcome Research Associate
Nic Robertson	ECAT Fellow
Emanuela Sani	Wellcome Research Associate
Vadim Shchepachev	SNSF Research Fellow
Tomasz Turowski	Polish Academy Research Fellow

* joint iCM PhD student with Diego Oyarzun

Julie Welburn

Wellcome Senior Research Fellow

Thomas Attard	MRC Precision Medicine Student
Ben Craske	BBSRC DTP Graduate Student
Agata Gluszek	Wellcome Research Associate
Haonan (Leo) Liu	Darwin Trust Graduate Student
Toni McHugh	Wellcome Research Associate
Calum Paterson	Wellcome Research Assistant

Marcus D Wilson

Sir Henry Dale Fellow

Hayden Burdett	MRC Research Associate
Gillian Clifford	Research Assistant
Alakta Das	Darwin Trust Graduate Student
Gauri Deák	EASTBIO/BBSRC Graduate Student
Dhananjay Kumar	Darwin Trust Graduate Student
Hannah Wapenaar	Wellcome Research Associate
James Watson*	Wellcome iCM Graduate Student

* joint iCM PhD student with Adrian Bird

Administration/Support Staff

Greg Anderson	Centre Laboratory Manager
Elizabeth Blackburn	EPPF Research Associate
Hywel Dunn-Davies	Bioinformatics Developer
Maria Fanourgiaki	Science Communicator
Carolyn Fleming	Centre Administrative Assistant
Sarah-Jane Judge	Centre Public Engagement Manager
David Kelly	Centre Optical Instrumentation Laboratory Manager
Toni McHugh	Centre Imaging Facility Assistant
Colin McLaren	Computing Support
Karen May	Centre Manager/Wellcome 4yr PhD Programme Administrator
Daniel Robertson	Bioinformatics Support Officer
Christine Struthers	PA to Adrian Bird/Centre Administrator
Martin Singleton	Cryo-EM Technologist
Shaun Webb	Centre Bioinformatics Core Facility Manager

Technical support

Scott Macrae

Media Prep and Wash-up

Denise Affleck
Andrew Kerr
Margaret Martin
Donna Pratt



Centre Publications 2020–2022

Abad, M.A*. T*. Gupta, M.A. Hadders, A. Meppelink, J.P. Wopken, E. Blackburn, J. Zou, L. Buzuk, D.A. Kelly, T. McHugh, J. Rappsilber, S.M.A. Lens and A.A. Jeyaprasath (2021) "Molecular basis for CPC-Sgo1 interaction: implications for centromere localisation and function of the CPC". bioRxiv Doi: <https://doi.org/10.1101/2021.08.27.457910>

Agostini, F., L. Sinn, D. Petras, C.J. Schipp, V. Kubyshkin, A.A. Berger, P.C. Dorrestein, J. Rappsilber, N. Budisa, and B. Kocsch (2021). "Multiomics analysis provides insight into the laboratory evolution of *Escherichia coli* toward the metabolic usage of fluorinated indoles". ACS Cent Sci. 7(1):81-92. doi:10.1021/acscentsci.0c00679.PMID: 33532571.

Alcón P., S. Shakeel, Z.A. Chen, J. Rappsilber, K.J. Patel and L.A. Passmore (2020). "FANCD2-FANCD1 is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair". Nat Struct Mol Biol. Feb 17. doi: 10.1038/s41594-020-0380-1. PMID: 32066963.

Ambrose, A.J., N.T. Pham, J. Sivinski, L. Guimarães, N. Mollasalehi, P. Jimenez, M.A. Abad, A.A. Jeyaprasath, S. Shave, L. Costa-Lotufu, J.J. La Clair, M. Auer and Chapman (2020). "A two-step resin based approach to reveal survivin-selective fluorescent probes". RSC Chemical Biology. DOI:10.1039/d0cb00122h.

Ashraf, S., Y.D. Tay, D.A. Kelly and K.E. Sawin (2021). "Microtubule-independent movement of the fission yeast nucleus". J Cell Sci. Feb 23:jcs.253021.

Bai X., M. Melesse, C.G. Sorensen Turpin D.E. Sloan, C.Y. Chen, W.C. Wang, P.Y. Lee, J.R. Simmons B. Nebenfurhr, D. Mitchell, L.R. Klebanow, N. Mattson, E. Ketzig, B.C. Chen, D. Cheerambathur, J.N. Bembenek (2020). "Aurora B functions at the apical surface of specialized cytokinesis during morphogenesis in *C. elegans*". Development. 147(1) pii: dev181099. doi: 10.1242/dev.181099.

Ballou E.R., Cook A.G. and E.W.J. Wallace (2020). "Repeated evolution of inactive pseudonucleases in a fungal branch of the Dis3/RNase II family of nucleases". Mol. Biol. Evol. doi:10.1093/molbev/msaa324.

Banchenko, S., F. Krupp, C. Gotthold, J. Burger, A. Graziadei, F.J. O'Reilly, L. Sinn, O. Ruda, J. Rappsilber et al (2021). "Structural insights into Cullin4-RING ubiquitin ligase remodelling by Vpr from simian immunodeficiency viruses." PLoS Pathog 17(8): e1009775.

Barbosa, P., L. Zhaunova, S. DeBilio, V. Steccanella, V. Kelly, T. Ly and H. Ohkura (2021). "SCF-Fbxo42 promotes synaptonemal complex assembly by downregulating PP2A-B56". J. Cell Biol. 220, e202009167.

Bartont, R., L.F. Massari, D. Robertson and A.L. Marston (2022). Eco1-dependent cohesin acetylation anchors chromatin loops and cohesion to define functional meiotic chromosome domains. eLife. 11, e74447. †Equal contribution

Bayne, R.A., U. Jayachandran, A. Kasproicz, S. Bresson, D. Tollervey, W. Wallace and A.G. Cook, (2020). "Yeast Ssd1 is a non-enzymatic member of the RNase II family with an alternative RNA recognition interface". <https://www.biorxiv.org/content/10.1101/2020.10.22.350314v1>

Becker, J.R., C. Bonnet, G. Clifford, A. Groth, M.D. Wilson, and J.R. Chapman (2020). "BARD1 links histone H2A Lysine-15 ubiquitination to initiation of BRCA1-dependent homologous recombination". Nature 596, 433-437.

Bel Borja, L., F. Soubigou, S. Taylor, C. Bringas, J. Budrewicz, P. Lara-Gonzalez, C. Sorensen-Turpin, J.N. Bembenek, D.K. Cheerambathur and F. Pelisch (2020). "PP2A:B56 regulates meiotic chromosome segregation in *C. elegans* oocytes". Elife. Dec 23;9:e65307.

Belotserkovskaya, R., E. Raga Gil, N. Lawrence, R. Butler, G. Clifford, M.D. Wilson and S.P. Jackson (2020). "PALB2 chromatin recruitment restores homologous recombination in BRCA1-deficient cells depleted of 53BP1." Nat Commun 11(1): 819.

Belsom, A. and J. Rappsilber (2020). "Anatomy of a crosslinker". Curr Opin Chem Biol. 60:39-46. doi: 10.1016/j.cbpa.2020.07.008. PMID: 32829152.

Bhagwat, N.R., S.N. Owens, M. Ito, J.V. Boinapalli, P. Poa, A. Ditzel, S. Kopparapu, M. Mahalawat, O.R. Davies, S.R. Collins, et al. (2021). "SUMO is a pervasive regulator of meiosis". eLife 10.

Bird, A. (2020). "The selfishness of law-abiding genes". Trends Genet 36, 8-13. 10.1016/j.tig.2019.10.002 PMID: 31662191

Bird, A. (2020). "DNA methylation: mega-year inheritance with the help of Darwin". Curr Biol 30, R302-R328 <https://doi.org/10.1016/j.cub.2020.02.034> PMID: 32259507

Bloomer, R.H., C.E. Hutchison, I. Bäurle, J. Walker, X. Fang, P. Perera, C.N. Velanis, S. Gümüs C. Spanos, J. Rappsilber, X. Feng, J. Goodrich and C. Dean (2020). "The Arabidopsis epigenetic regulator ICU11 as an accessory protein of Polycomb Repressive Complex 2". Proc Natl Acad Sci U S A. 14;117(28):16660-16666. doi: 10.1073/pnas.1920621117. PMID: 32601198.

Bobkov, G.O.M., A. Huang, S.J.W. van den Berg, S. Mitra, E. Anselm, V. Lazou, S. Schunter, R. Federle, A. Imhof, A. Lusser, E.T. Jansen and P. Heun (2020). "Spt6 is a maintenance factor for centromeric CENP-A". Nature communications 11, 2919.

Borek, W.E., Vincenten, N., Duro, E., Makrantonis, V., Spanos, C., Sarangapani, K.K., de Lima Alves, F., Kelly, D.A., Asbury, C.L., Rappsilber, J. and A.L. Marston (2021). "The proteomic landscape of centromeric chromatin reveals an essential role for the Ctf19CCAN complex in meiotic kinetochore assembly". Current Biology 31, 283-296.

Borg, M., Y. Jacob, D. Susaki, C. LeBlanc, D. Buendía, E. Axelsson, T. Kawashima, P. Voigt, L. Boavida, J. Becker, T. Higashiyama, R. Martienssen and F. Berger (2020). "Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin". Nat. Cell Biol. 22, 621–629.

Boteva L., R.S. Nozawa, C. Naughton, K. Samejima, W.C. Earnshaw and N. Gilbert (2020). "Common fragile sites are characterised by faulty condensin loading after replication stress". Cell Reports 32: 108177. PMID: 32966795.

Bresson, S., N. Robertson, E. Sani, T.W. Turowski, V. Shchepachev, M. Kompauerova, C. Spanos, A. Helwak, and D. Tollervey (2020). "Integrative vectors for regulated expression of SARS-CoV-2 proteins implicated in RNA metabolism". Wellcome Open Res. <https://www.biorxiv.org/content/10.1101/2020.07.20.211623v1.full> Data deposited Springer Nature figshare repository: <https://doi.org/10.6084/m9.figshare.13013492>

Bresson, S., V. Shchepachev, C. Spanos, T.W. Turowski, J. Rappsilber and D. Tollervey (2020). "Stress-induced translation inhibition through rapid displacement of scanning initiation factors". Mol Cell. 5;80(3):470-484.e8. doi:10.1016/j.molcel.2020.09.021. PMID: 33053322.

Bryan, E., M. Warburton, K.M. Webb, K.A. McLaughlin, C. Spanos, C. Ambrosi, V. Major, T. Baubec, J. Rappsilber and P. Voigt (2021). "Nucleosomal asymmetry shapes histone mark binding and promotes poisoning at bivalent domains". bioRxiv <https://doi.org/10.1101/2021.02.08.430127>.

Cerritelli, S., J. Iranzo, S. Sharma, A. Chabes, R. Crouch, D. Tollervey, D. and A. El Hage (2020). "High density of unrepaired genomic ribonucleotides leads to Topoisomerase 1-mediated severe growth defects in absence of ribonucleotide reductase". Nucleic Acids Res. 48: 4274-4297. PMID: PMC7192613.

Chang, C.H., H.Y. Chang, J. Rappsilber and Y. Ishihara, (2020). "Isolation of acetylated and unmodified protein N-terminal peptides by strong cation exchange chromatographic separation of trypsin-digested peptides". Mol Cell Proteomics. 24;20:100003. doi: 10.1074/mcp.TIR120.002148. 33517145.

Chhatbar, K., Cholewa-Waclaw, J., Shah, R. Bird, A. and G. Sanguinetti (2020). "Quantitative analysis questions the role of MeCP2 as a global regulator of alternative splicing". PLOS Genetics 16(10): e1009087 <https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1009087> PMID: 33048927

Ciesla, M., T.W. Turowski, M. Nowotny, D. Tollervey, and M. Boguta (2020). "The expression of Rpb10, a small subunit common to RNA polymerases, is modulated by the R3H domain-containing Rbs1 protein and the Upf1 helicase". Nucleic Acids Res., 48, 12252-12268. PMID: PMC7708074.

Codino, A., T. Turowski, L.N. van de Lagemaat, I. Ivanova, A. Tavosanis, C. Much, T. Auchynnikava, L. Vasiliauskaitė, M. Morgan, J. Rappsilber, R.C. Allshire, K.R. Kranc, D. Tollervey and D. O'Carroll (2021). "NANOS2 is a sequence-specific mRNA-binding protein that promotes transcript degradation in spermatogonial stem cells". iScience 24, 102762. doi: 10.1016/j.isci.2021.102762.

Connelly, J.C., J. Cholewa-Waclaw, S. Webb, V. Steccanella, B. Waclaw and A. Bird (2020). "Absence of MeCP2 binding to non-methylated GT-rich sequences in vivo". Nucleic Acids Res. 48(7): 3542-3553 <https://doi.org/10.1093/nar/gkaa102>.

Consolati, T., J. Locke, J. Roostalu, Z.A. Chen, J. Gannon, J. Asthana, W.M. Lim F. Martino, M.A. Cvetkovic, J. Rappsilber, A. Costa and T. Surrey (2020). "Microtubule nucleation properties of single human γ TuRCs explained by their cryo-EM structure". DevCell. 8;53(5):603-617.e8. doi: 10.1016/j.devcel.2020.04.019. PMID: 32433913. PMC7280788

Coscia, F., A.Taler-Verčič, V.T. Chang, L. Sinn, F.J. O'Reilly, T. Izoré, M. Renko, I. Berger, J. Rappsilber, D. Turk and J. Löwe (2020) "The structure of human thyroglobulin". Nature. Feb;578(7796):627-630. doi: 10.1038/s41586-020-1995-4. Epub 2020 Feb 5. PubMed PMID: 32025030.

Craske B. and J.P.I. Welburn (2020). "Leaving no-one behind: how CENP-E facilitates chromosome alignment". Essays in Biochemistry. PMID: 32347304.

Cuijpers S.A.G., E. Willemstein, J. Ruppert, D.M. van Elstrand, W.C. Earnshaw and A.C.O. Vertegaal. (2020). "Chromokinesin KIF4A teams up with Stathmin 1 to regulate abscission in a SUMO-dependent manner". J. Cell Sci. 133: jcs248591: PMID: 32591481.

Dau, T., G. Bartolomucci and J. Rappsilber (2020). "Proteomics using protease alternatives to trypsin benefits from sequential digestion with trypsin" (2020). Anal Chem. 21;92(14):9523-9527. doi: 10.1021/acs.analchem.0c00478. PMID: 32628831.

Delan-Forino, C. and D. Tollervey (2020). "Mapping exosome-substrate interactions in vivo by UV cross-linking." Methods Mol Biol 2062: 105-126. PMID: 31768974.

Delan-Forino, C., C. Spanos, J. Rappsilber, and D. Tollervey (2020). "Substrate specificity of the TRAMP nuclear surveillance complexes". *Nature Comms.*, **11**: 3122. doi: 10.1038/s41467-020-16965-4. PMID: PMC7305330.

Del Toro N., A. Shrivastava, E. Ragueneau, B. Meldal, C. Combe, E. Barrera, L. Perfetto, K. How, P. Ratan, G. Shirodkar, O. Lu, B. Mészáros, X. Watkins, S. Pundir, L. Licata, M. Iannuccelli, M. Pellegrini, M.J. Martin, S. Panni, M. Duesbury, S.D. Vallet, J. Rappsilber et al (2022). "The IntAct database: efficient access to fine-grained molecular interaction data". *Nucleic Acids Res.* 2022 Jan7;50(D1):D648-D653. doi: 10.1093/nar/gkab1006. PMID: 34761267; PMID: PMC8728211.

Dixon, C. R., P. Malik, J.I. de Las Heras, N. Saiz-Ros, F. de Lima Alves, M. Tingey, E. Gaunt, A.C. Richardson, D.A. Kelly, M.W. Goldberg, G.J. Towers, W. Yang, J. Rappsilber, P. Digard, E.C. Schirmer (2021). "STING nuclear partners contribute to innate immune signaling responses." *iScience* **24**(9): 103055.

Dunn-Davies, H., T. Dudnakova, J.-L. Langhendries, N. Watkins, D.L.J. Lafontaine, and D. Tollervey (2022) Systematic mapping of small nucleolar RNA targets in human cells. *bioRxiv* 2021.07.22.451324; doi: <https://doi.org/10.1101/2021.07.22.451324>

Dong, J., C. LeBlanc, A. Poulet, B. Mermaz, G. Villarino, K.M. Webb, V. Joly, J. Mendez, P. Voigt and Y. Jacob (2020). "H3.1K27me1 maintains transcriptional silencing and genome stability by preventing GCN5-mediated histone acetylation". *bioRxiv* <https://doi.org/10.1101/2020.07.17.209098>.

Dunce, J.M., L.J. Salmon and O.R. Davies (2021). "Structural basis of meiotic chromosome synaptic elongation through hierarchical fibrous assembly of SYCE2-TEX12". *Nat Struct Mol Biol* **28**, 681-693.

Fajardo, J. E., R. Shrestha, N. Gil, A. Belsom, S.N. Crivelli, C. Czaplewski, K. Fidelis, S. Grudin, M. Karasikov, A.S. Karczynska, A. Kryshchovych, A. Leitner, A. Liwo, E.A. Lubecka, B. Monastyrskyy, G. Pages, J. Rappsilber et al (2020). "Assessment of chemical-crosslink-assisted protein structure modeling in CASP13." *Proteins* **88**(3): 540.

Fang, X., Z. Wu, O. Raitskin, K.M. Webb, P. Voigt, T. Lu, M. Howard and C. Dean, (2020). "The 3' processing of antisense RNAs physically links to chromatin-based transcriptional control". *Proc Natl Acad Sci U S A.* **117**, 15316-15321.

Fitz-James, M.H., Tong, P., Pidoux, A.L., Ozadam, H., Yang, L., White, S.A., Dekker, J. and R.C. Allshire (2020). "Large domains of heterochromatin direct the formation of short mitotic chromosome loops". *Elife* **9**, e57212. doi: 10.7554/eLife.57212.

Ford, E., C.E. Currie, D.M. Taylor, M. Erent, A.L. Marston, G.M. Hartshorne and A.D. McAinsh (2020). "The first mitotic division of the human embryo is highly error-prone". *bioRxiv* 208744. Galander, S. and A.L. Marston (2020). "Meiosis I kinase regulators: conserved orchestrators of reductional chromosome segregation". *Bioessays*, **42**, e2000018.

Giese, S. H., L.R. Sinn, F. Wegner and J. Rappsilber (2021). "Retention time prediction using neural networks increases identifications in crosslinking mass spectrometry." *Nat Commun* **12**(1): 3237.

Graziadei A. and J. Rappsilber (2021). "Leveraging crosslinking mass spectrometry in structural and cell biology". *Structure*. 2022 Jan 6;30(1):37-54. doi:10.1016/j.str.2021.11.007. Epub 2021 Dec 10. PMID: 34895473.

Guillaume, C., B.-I Valérie, M-G. Nathalie, R. Nicolas, W. Ludivine, M. Xavier, C. Christophe, K. Joanna, M. Agnès, S.-S Véronique, D. Tollervey, D.L.J. Lafontaine, Denis, C. Branlant and M. Rederstorff (2020). "Synergistic defects in pre-rRNA processing from mutations in the U3 snoRNA and U3-specific protein Rrp9". *Nucleic Acids Res.* **48**: 3848-3868. PMID: PMC7144924.

Guitart, P. Giles, M. O'Shea, D. Vernimmen, N.M. Morton, N.P. Rodrigues, B. Göttgens, C.J. Schofield, A. Lengeling, D. O'Carroll, K.R. Kranc (2021). "JMJD6 promotes self-renewal and regenerative capacity of hematopoietic stem cells". *Blood Adv.* 2021 Feb 9;5(3):889-899. doi: 10.1182/bloodadvances.2020002702. PMID: 33560400; PMID: PMC7876897.

Gurusaran, M., and O.R. Davies (2021). "A molecular mechanism for LINC complex branching by structurally diverse SUN-KASH 6:6 assemblies". *eLife* **10**.

Gutierrez-Escribano, P., S. Horneño, J. Madariaga-Marcos, R. Solé-Soler, F.J. O'Reilly, K. Morris, C. Aicart-Ramos, R. Aramayo, A. Montoya, H. Kramer, J. Rappsilber, J. Torres-Rosell, F. Moreno-Herrero and L. Aragon (2020). "Purified Smc5/6 complex exhibits DNA substrate recognition and compaction. *Mol Cell.* 2020 Dec 17;80(6):1039-1054.e6. doi: 10.1016/j.molcel.2020.11.012. Epub 2020 Dec 9. PMID:33301732; PMID: PMC7758880.

Hammond, C. M., H. Bao, I.A. Hendriks, M. Carraro, A. Garcia-Nieto, L. Liu, N. Reveron-Gomez, C. Spanos, L. Chen, J. Rappsilber et al (2021). "DNAJC9 integrates heat shock molecular chaperones into the histone chaperone network." *Mol Cell* **81**(12): 2533-2548 e2539.

Higashi, T.L., P. Eickhoff, J.S. Sousa, J. Locke, A. Nans, H.R. Flynn, A.P. Snijders, G. Papageorgiou, N. O'Reilly, Z.A. Chen, F.J. O'Reilly, J. Rappsilber, A. Costa and F.A. Uhlmann (2020). "Structure-based mechanism for DNA entry into the cohesin ring" (2020). *Mol Cell.*17;79(6):917-933.e9. doi: 10.1016/j.molcel.2020.07.013. PMID: 32755595.

Hsieh Y.Y.P, V. Makranton, D. Robertson A.L. Marston and A.W. Murray (2020). "Evolutionary repair: changes in multiple functional modules allow meiotic cohesin to support mitosis". *PLoS Biol.* **18**, e3000635.

Kelly, V., A. Al-Rawi, D. Lewis, G. Kustatscher and T. Ly (2022). "Low cell number proteomic analysis using in-cell protease digests reveals a robust signature for cell cycle state classification". *Mol. Cell. Proteomics* **21**, 100169.

Kind, P.C. and A. Bird (2021). "CDKL5 deficiency disorder: a pathophysiology of neural maintenance." *J Clin Invest* **131**(21).

Koch, N. G., P. Goettig, J. Rappsilber, and N. Budisa, N. (2021). "Engineering Pyrrolysyl-tRNA Synthetase for the Incorporation of Non-Canonical Amino Acids with Smaller Side Chains." *Int J Mol Sci* **22**(20).

Kolbowski, L., A. Belsom, and J. Rappsilber (2020). "Ultraviolet photodissociation of tryptic peptide backbones at 213 nm". *J Am Soc Mass Spectrom.* 3;31(6):1282-1290. doi: 10.1021/jasms.0c00106. PMID: 32352297;

Kyriulis, F.L., Semchonok, D.A., Skalidis, I., Tüting, C., Hamdi, F., O'Reilly, F.J., Rappsilber, J., and P.L. Kastriitis (2021). "Integrative structure of a 10-megadalton eukaryotic pyruvate dehydrogenase complex from native cell extracts". *Cell Rep.* **9**;34(6):108727. doi: 10.1016/j.celrep.2021.108727. PMID: 33567276.

Kuhl, L-M., Makranton, M., Recknagel, S., Vaze, A.N., Marston, A.L. and G. Vader (2020). "A dCas9/CRISPR-based targeting system identifies a central role for Ctf19 in kinetochore-derived suppression of meiotic recombination". *Genetics*, **216**, 395-408.

Lawson H, L.N. van de Lagemaat, M. Barile, A. Tivosanis, J. Durko, A. Villacreces, A. Bellani, C. Mapperley, E. Georges, C. Martins-Costa, C. Sepulveda, L. Allen, J. Campos, K.J. Campbell, D. O'Carroll, B. Göttgens, S. Cory, N.P. Rodrigues, A.V. Guitart and K.R. Kranc (2021). "CITED2 coordinates key hematopoietic regulatory pathways to maintain the HSC pool in both steady-state hematopoiesis and transplantation". *Stem Cell Reports.* Nov 9;16(11):2784-2797. doi: 10.1016/j.stemcr.2021.10.001. Epub 2021 Oct 28. PMID: 34715054; PMID: PMC8581166.

Lau, C.K. F.J. O'Reilly, B. Santhanam, S.E. Lacey, J. Rappsilber and A.P. Carter (2021). "Cryo-EM reveals the complex architecture of dynactin's shoulder region and pointed end." *EMBO J* **40**(8): e106164.

Lawson, H., C. Sepulveda, L.N. van de Lagemaat, J. Durko, M. Barile, A. Tivosanis, E. Georges, A. Shmakova, P. Timms, R.N. Carter, L. Allen, J. Campos, M. Vukovic, A.V.

Lee, B.G, F. Merkel, M. Allegritti, M. Hassler, C. Cawood, L. Lecomete, F.J. O'Reilly, L.R. Sinn, P. Gutierrez-Escribano, M. Kschonsak, S. Bravo, T. Nakane, J. Rappsilber L. Aragon, M. Beck, J. Löwe, and C.H. Haering (2020). "Cryo-EM structures of holo condensin reveal a subunit flip-flop mechanism". *Nat Struct Mol Biol.* **27**(8):743-751. doi: 10.1038/s41594-020-0457-x. PMID: 32661420.

Legal, T., D. Hayward, A. Gluszek-Kustusz, E.A. Blackburn, C. Spanos, J. Rappsilber, U. Gruneberg and J.P.I. Welburn (2020). "The C-terminal helix of BubR1 is essential for CENP-E-dependent chromosome alignment". *J Cell Sci.* **25**;133(16):jcs246025. doi:10.1242/jcs.246025. PMID: 32665320.

Leitner, A., A.M.J.J. Bonvin, C.H. Borchers, R.J. Chalkley, J. Chamot-Rooke, C.W. Combe, J. Cox, M.Q. Dong, L. Fischer, M. Götz, F.C. Gozzo, A.J.R. Heck, M.R. Hoopmann, L. Huang, U. Ishihama, A.R. Jones, N. Kalisman, O. Kohlbacher, K. Mechtler K, R.L. Moritz, E. Netz, P. Novak P, E. Petrotchenko, A. Sali, R.A. Scheltema, C. Schmidt, D. Schriemer, A. Sinz, F. Sobott, F. Stengel, K. Thalassinou, H. Urlaub, R. Viner, J.A. Vizcaino, M.R. Wilkins and J. Rappsilber (2020). "Toward increased reliability, transparency, and accessibility in cross-linking mass spectrometry". *Structure*. **3**;28(11):1259-1268. doi:10.1016/j.str.2020.09.011. PMID: 33065067.

Lenz, S., L.R. Sinn, F.J. O'Reilly, L. Fischer, F. Wegner and J. Rappsilber (2021). "Reliable identification of protein-protein interactions by crosslinking mass spectrometry." *Nat Commun* **12**(1): 3564.

Mapperley C, L.N. van de Lagemaat, H. Lawson, A. Tivosanis, J. Paris, J. Campos, D. Wotherspoon, J. Durko, A. Sarapuu, J. Choe, I. Ivanova, D.S. Krause, A. von Kriegsheim C. Much, M. Morgan, R.I. Gregory, A.J. Mead, D. O'Carroll* and K.R. Kranc* (2021). "The mRNA m6A reader YTHDF2 suppresses proinflammatory pathways and sustains hematopoietic stem cell function". *J Exp Med.* 2021 Mar 1;218(3):e20200829. doi: 10.1084/jem.20200829. PMID: 33156926; PMID: PMC7653684. * co-corresponding authors

Mali, G.R., Ali, F.A., Lau, C.K., Begum, F., Boulanger, J., Howe, J.D., Chen, Z.A., Rappsilber, J., Skehel, M. and A.P. Carter (2021). "Shulin packages axonemal outer dynein arms for ciliary targeting. *Science.* 2021 Feb 26;371(6532):910-916. doi: 10.1126/science.abe0526. PMID: 33632841.

Marston, A.L. (2021) A SUMOylation wave to anchor the genome. *J Cell Biol.* **220**, e202110031.

Martins*, N.M.C., F. Cisneros-Soberanis*, E. Pesenti*, N.Y. Kochanova, W.-H. Shang, T. Hori, T. Nagase, H. Kimura, V. Larionov, H. Masumoto, T. Fukagawa and W.C. Earnshaw. (2020). "H3K9me3 maintenance on a human artificial chromosome is required for segregation but not centromere epigenetic memory". *J.Cell Sci.* **133**: jcs242610: PMID: 32576667.

Meldal, B.H.M., L. Perfetto, C. Combe, T. Lubiana, J.V. Ferreira Cavalcante, H. Bye-A-Jee, A. Waagmeester, N. Del-Toro, A. Shrivastava, E. Barrera, E. Wong, B. Mlecnik, G. Bindea, K. Panneerselvam, E. Willighagen, R. Rappsilber, et al. (2022). "Complex Portal 2022: new curation frontiers." *Nucleic Acids Res* **50**(D1): D578-D586.

Medina-Pritchard, B., V. Lazou, J. Zou, O. Byron, M.A. Abad, J. Rappsilber, P. Heun and A.A. Jeyaprakash, (2020). "Structural basis for centromere maintenance by Drosophila CENP-A chaperone CAL1". *EMBO J* **39**, Doi: <https://doi.org/10.15252/embj.2019103234>

Morton, C.R., N.J. Rzechorzek, J.D. Maman, M. Kuramochi, H. Sekiguchi, R. Rambo, Y.C. Sasaki, O.R. Davies and L. Pellegrini (2021). "Structural basis for the coiled-coil architecture of human CtIP". *Open Biol* **11**, 210060.

Muller, F. and J. Rappsilber (2020). "A protocol for studying structural dynamics of proteins by quantitative crosslinking mass spectrometry and data-independent acquisition." *J Proteomics* **218**: 103721. PMID: 32109607.

Murawska, M., T. Schauer, A. Matsuda, M.D. Wilson, T. Pysik, F. Wojcik, T.W. Muir, Y. Hiraoka, T. Straub and A.G. Ladurner (2020). "The chaperone FACT and histone H2B ubiquitination maintain *S. pombe* genome architecture through genic and subtelomeric functions". *Mol Cell* **77**(3): 501-513 e507.

Nakamura, K., G. Kustatscher, C. Alabert, M. Hödl, I. Forne, M. Völker-Albert, S. Satpathy, T.E. Beyer, N. Mailand, C. Choudhary, A. Imhof, J. Rappsilber, and A. Groth (2021). "Proteome dynamics at broken replication forks reveal a distinct ATM-directed repair response suppressing DNA double-strand break ubiquitination". *Mol Cell* **4**;81(5):1084-1099.e6. doi: 10.1016/j.molcel.2020.12.025. PMID: 33450211.

Nehlig A., C. Seiler, Y. Steblyanko, F. Dingli, G. Arras, D. Loew, J. Welburn, C. Prigent, M. Barisic, C. Nahmias (2020). "Aurora kinase A-dependent ATIP3-Kif2a-Dda3 complex maintains metaphase spindle length". *Cellular and Molecular Life Sciences*. PMID: 32789689.

O'Reilly, F.J. L. Xue, A. Graziadei, L. Sinn, S. Lenz, D. Tegunov, C. Blötz, N. Singh, W.J.H. Hagen, P. Cramer, J. Stülke J, Mahamid and J. Rappsilber (2020). "In-cell architecture of an actively transcribing-translating expressome". *Science* **31**;369(6503):554-557. doi: 10.1126/science.abb3758. PMID: 32732422.

Otake, K.J. Ohzeki, N. Shono, K. Kugou, K. Okazaki, T. Nagase, H. Yamakawa, N. Kouprina, V. Larionov, H. Kimura, W.C. Earnshaw and H. Masumoto. (2020). "CENP-B creates alternative epigenetic chromatin states permissive for CENP-A or heterochromatin assembly". *J.Cell Sci.* **133**: jcs243303: PMID: 32661090.

Oveisi, M., M. Shukla, N. Seymen, M. Ohno, Y. Taniguchi, S. Nahata, R. Loos, R. G.J. Mufti, R.C. Allshire, S. Dimitrov, M.M. Karimi. (2021) "iNucs: Inter-Nucleosome Interactions". *Bioinformatics* **37**, 4562. doi: 10.1093/bioinformatics/btab698

Paldi, F., B. Alver, D. Robertson, S.A. Schalbetter, A. Kerr, D. Kelly, J. Baxter, M.J. Neale and A.L. Marston (2020). Convergent genes shape budding yeast pericentromeres. *Nature*, **582**, 119-123.

Pantier, R., K. Chhatbar, T. Quante, K. Skourti-Stathaki, J. Cholewa-Waclaw, G. Alston, B. Alexander-Howden, H.Y. Lee, A.G. Cook, C.G. Spruijt, M. Vermeulen, J. Selfridge and A. Bird (2021). "SALL4 controls cell fate in response to DNA base composition". *Mol Cell* **81**(4): 845-858 e848 <https://doi.org/10.1016/j.molcel.2020.11.046> PMID: 33406384

Paulson, J.R., D.F. Hudson, F. Cisneros-Soberanis and W.C. Earnshaw (2021). "Mitotic chromosomes". *Semin Cell Dev Biol* **117**:7-29. PMID: 33836947; PMC8406421; DOI: 10.1016/j.semcd.2021.03.014.

Pei, H.H., T. Hilal, Z.A. Chen, Y.H. Huang, Y. Gao, N. Said, B. Loll, J. Rappsilber, G.A. Belogurov, I. Artsimovitch and M.C. Wahl (2020). "The δ subunit and NTPase HelD institute a two-pronged mechanism for RNA polymerase recycling". *Nat Commun.* **18**;11(1):6418. doi: 10.1038/s41467-020-20159-3. PMID: 33339827.

Pesenti, E., Liskovych, M., Okazaki, K., Mallozzi, A., Reid, C., Abad, A. A., Jeyaprakash, A. A., Kouprina, N., Larionov, V., Masumoto, H., Earnshaw, W. C. (2020). "Analysis of complex DNA rearrangements during early stages of HAC formation". *ACS Synth. Biol.* **9**, 12, 3267-3287 PMID: 33289546.

Petrov, N., H.-S. Lee, M. Liskovych, M.-P. Teulade-Fichou, H. Masumoto, W.C. Earnshaw, Y. Pommier, V. Larionov and N. Kouprina. (2021). "Terpyridine platinum compounds induce telomere dysfunction and chromosome instability in cancer cells". *Oncotarget* **12**:1444-1456. PMID: 34316326; PMC8310675; DOI: 10.18632/oncotarget.28020.

Prior, C., O.R. Davies, D. Bruce and E. Pohl (2020). "Obtaining tertiary protein structures by the ab initio interpretation of small angle X-ray scattering data". *J Chem Theory Comput* **16**, 1985-2001.

Remnant L., N.Y. Kochanova, C. Reid, F. Cisneros-Soberanis and W.C. Earnshaw (2021). "The intrinsically disorderly story of Ki-67". *Open Biol.* **11**:210120. PMID: 34375547; PMC8354752; DOI: 10.1098/rsob.210120.

Repton, C., C.F. Cullen, M.F.A. Costa, C.Spanos, J. Rappsilber, and H. Ohkura (2022). "The phospho-docking protein 14-3-3 regulates microtubule-associated proteins in oocytes including the chromosomal passenger Borealin". *bioRxiv*. doi: 10.1101/2021.12.16.472885

Roberston, N., V. Shchepachev, D. Wright, T.W. Turowski, C. Spanos, A. Helwak, R. Zamoyska and D. Tollervey (2021) A disease-linked lncRNA mutation in RNase MRP inhibits ribosome synthesis. *Nature Comms.* **13**, 649. doi: 10.1038/s41467-022-28295-8.

Ross, J., Z. Mclver, T. Lambert, C. Piergentili, J.E. Bird, K.J. Gallagher, F.L. Cruickshank, P. James, E. Zarazua-Arvizu, L.E. Horsfall, K.J. Waldron and M.D. Wilson et al. (2022). Pore dynamics and asymmetric cargo loading in an encapsulin nanocompartment. *Sci Adv* **8**, eabj4461.

Ryl, P., S.J., M. Bohlke-Schneider, S. Lenz, L. Fischer, L. Budzinski, M. Stuiver, M.M.L. Mendes, L. Sinn, F.J. O'Reilly and J. Rappsilber (2020). "In Situ Structural Restraints from Cross-Linking Mass Spectrometry in Human Mitochondria." *J Proteome Res* **19**(1): 327-336.

Samejima, I., C. Spanos, K. Samejima, J. Rappsilber, G. Kustatscher and W.C. Earnshaw, (2022). "Mapping the invisible chromatin transactions of prophase chromosome remodeling". *Mol. Cell* **82**, 696–708.e4.

Sanchez-Saez, F., H.L. Gomez, O.M. Dunne, C. Gallego-Paramo, N. Felipe-Medina, M. Sanchez-Martin, E. Llano, A.M. Pendas and O.R. Davies (2020). "Meiotic chromosome synapsis depends on multivalent SYCE1-SIX6OS1 interactions that are disrupted in cases of human infertility". *Science advances* **6**.

Sanchez Rodriguez, F., A.J. Simpkin, O.R. Davies, R.M. Keegan and D.J. Rigden (2020). "Helical ensembles outperform ideal helices in molecular replacement". *Acta Crystallogr D Struct Biol* **76**, 962-970.

Sandhu, S., I.F. Sou, J.E. Hunter, L. Salmon, C.L. Wilson, N.D. Perkins, N. Hunter, O.R. Davies, and U.L. McClurg (2021). "Centrosome dysfunction associated with somatic expression of the synaptonemal complex protein TEX12". *Commun Biol* **4**, 1371.

Scacchetti, A., A. Reim, T. Schauer, Z. Apostolou, A. Campos Sparr, S. Krause, P. Heun, M. Wierer and P.B. Becker (2020). "Drosophila SWR1 and NuA4 complexes are defined by DOMINO isoforms". *Elife* **9** PMC7239659.

Schöpp, T., A. Zoch, R.V. Berrens, T. Auchynnikava, T., Kabayama, Y., Vasiliauskaitė, L., Rappsilber, J., Allshire, R.C., O'Carroll, D. (2020). "TEX15 is an essential executor of MIWI2-directed transposon DNA methylation and silencing". *Nat. Commun.* **11**, 3739. doi: 10.1038/s41467-020-17372-5.

Sheahan, T.W., V. Major, K.M. Webb, E. Bryan and P. Voigt (2020). "The TAZ2 domain of CBP/p300 directs acetylation towards H3K27 within chromatin". *bioRxiv* <https://doi.org/10.1101/2020.07.21.214338>.

Singh, P.P., M. Shukla, S.A. White, M. Lafos, P. Tong, T. Auchynnikava, C. Spanos, J. Rappsilber, A.L. Pidoux and R.C. Allshire (2020). "Hap2-Ino80-facilitated transcription promotes de novo establishment of CENP-A chromatin". *Genes Dev* **34**, 226–238.

Sobral, A.F., F.Y. Chan, M.J. Norman, D.S. Osorio, A.B. Dias, V. Ferreira, D.J. Barbosa, D. Cheerambathur, R. Gassmann, J.M. Belmonte, A.X. Carvalho. (2021) "Plastin and spectrin cooperate to stabilize the actomyosin cortex during cytokinesis". *Current Biology*. **31**(24):5415-5428.

Staneva, D.P., R. Carloni, T. Auchynnikava, P. Tong, J. Rappsilber, A.A. Jeyaprakash, K.R. Matthews, and R.C. Allshire (2021) "A systematic analysis of Trypanosoma brucei chromatin factors identifies novel protein interaction networks associated with sites of transcription initiation and termination". *Genome Research* **31**, 2138. doi: 10.1101/gr.275368.121.

Su, X.B., M. Wang, C. Schaffner, O.O. Nerusheva, D. Clift, C. Spanos, D.A. Kelly, M. Tatham, A. Wallek, Y. Wu, J. Rappsilber, A.A. Jeyaprakash, Z. Storchova, R.T. Hay and A.L. Marston (2021). "SUMOylation stabilizes sister kinetochore biorientation to allow timely anaphase." *J Cell Biol* **220**(7).

Thamkachy, R., B. Medina-Pritchard, S.O. Park, C.G. Chiodi, J. Zou, M. de la Torre-Barranco, K. Shimanaka, M.A. Abad, G. Páramo, R. Feederle, E. Ruksenaite, P. Heun, O.R. Davies, J. Rappsilber, D. Schneidman-Duhovny, U-S. Cho. A.A. and Jeyaprakash (2021) Structural Basis for Mis18 Complex Assembly: Implications for Centromere Maintenance. *bioRxiv* Doi: <https://www.biorxiv.org/content/10.1101/2021.11.08.466737v1>

Thomas, C., B. Wetherall, L.D. Levasseur, R.J. Harris, S.T. Kerridge, J.M.G. Higgins, O.R. Davies, and S. Madgwick (2021). "A prometaphase mechanism of securin destruction is essential for meiotic progression in mouse oocytes". *Nature communications* **12**, 4322.

Thomas, J.M.H., R.M. Keegan, D.J. Rigden and O.R. Davies (2020). "Extending the scope of coiled-coil crystal structure solution by AMPLE through improved ab initio modelling". *Acta Crystallogr D Struct Biol* **76**, 272-284.

Tillotson, R., J. Cholewa-Waclaw, K. Chhatbar, J. Connelly, S.A. Kirschner, S. Webb, M.V. Koerner, J. Selfridge, D. Kelly, D. De Sousa, K. Brown, M.J. Lyst, S. Kriaucionis and A. Bird (2021) "Neuronal non-CG methylation is an essential target for MeCP2 function". *Mol Cell* **81**, 1-16. [https://www.cell.com/molecular-cell/pdfExtended/S1097-2765\(21\)00011-3](https://www.cell.com/molecular-cell/pdfExtended/S1097-2765(21)00011-3) PMID: 33561390

Torres-Garcia, S., I. Yaseen, M. Shukla, P.N.C.B. Audergon, S.A. White, A.L. Pidoux and R.C. Allshire (2020a) "Epigenetic gene silencing by heterochromatin primes fungal resistance". *Nature* **585**, 453-458. doi: 10.1038/s41586-020-2706-x.

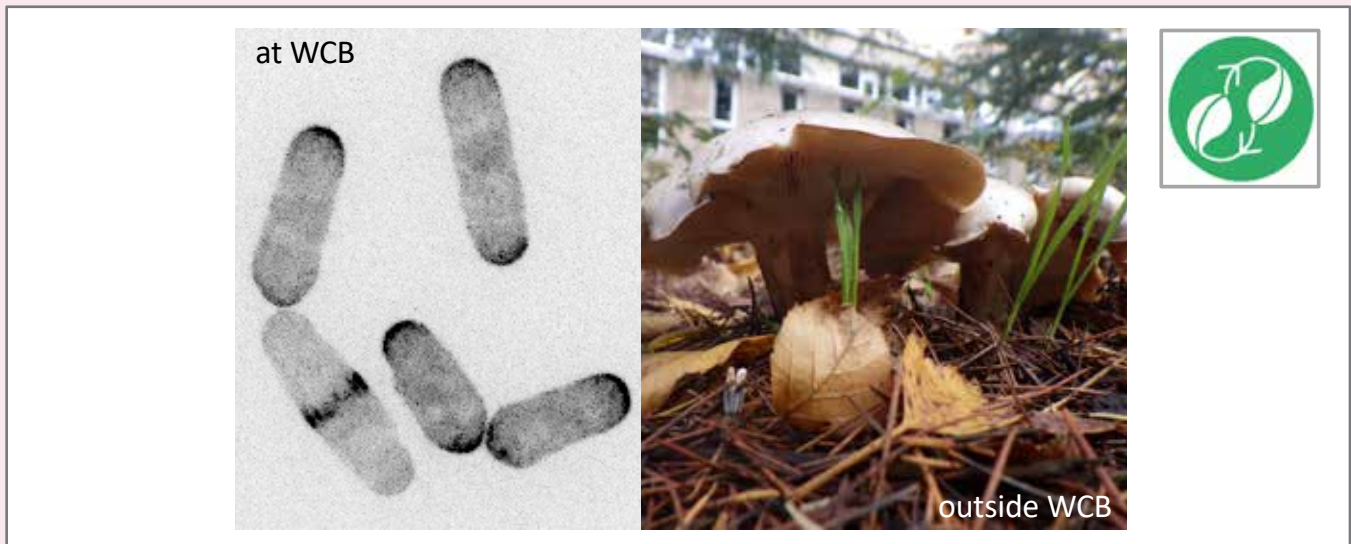
Torres-Garcia, S., L. Di Pompeo, L. Eivers, B. Gaborieau, S.A. White, A.L. Pidoux, P. Kanigowska, I. Yaseen, Y. Cai and R.C. Allshire. (2020b) "SpEDIT: A fast and efficient CRISPR/Cas9 method for fission yeast". *Wellcome Open Res.* **5**, 274. doi: 10.12688/wellcomeopenres.16405.1.

Tremel, S., Y. Ohashi, D.R. Morado, J. Bertram, O. Perisic, L.T.L. Brandt, M.K. von Wrisberg, Z.A. Chen, S.L. Maslen, O. Kovtun, M. Skehel, J. Rappsilber, J.et al. (2021). "Structural basis for VPS34 kinase activation by Rab1 and Rab5 on membranes." *Nat Commun* **12**(1): 1564.

- Tsunematsu T., R. Arakaki, H. Kawai, J. Ruppert, K. Tsuneyama, N. Ishimaru, W.C. Earnshaw, M. Pagano and Y. Kudo. (2020) "APC/CCdh1 is required for the termination of chromosomal passenger complex activity upon mitotic exit". *J. Cell Sci.* **133**: jcs251314. PMID: 32934012.
- Turowski, T.W. and D. Tollervey (2020) "Extended ncRNAs interfere with promoter nucleosome dynamics". *Trends Genet.* **36**: 637-639. PMID: 32546405.
- Turowski, T.W., E. Petfalski, B.D. Goddard, S.L. French, A. Helwak, and D. Tollervey (2020) "Nascent transcript folding plays a major role in determining RNA polymerase elongation rates". *Mol. Cell*, **79**, 488-503. PMID: PMC7427326
- Ueki, Y., M. Hadders, M.B. Weisser, P.P. Sotelo, I. Nasha, I. Gupta, E.T. Hertz, T. Kruse, G. Montoya, A.A. Jeyaprakash, A. Kettenbach, S.M.A. Lens and J. Nilsson (2020) "A highly conserved binding pocket on PP2A-B56 is required for shugoshin binding and cohesion protection". *bioRxiv*. Doi: <https://doi.org/10.1101/2020.12.18.423432>
- Vacante, F., J. Rodor, M.K. Lalwani, A.D. Mahmoud, M. Bennett, A.L. De Pace, E. Miller, K. Van Kuijk, J. de Bruijn, M. Gijbels, T.C. Williams, M.B. Clark, J.P. Scanlon, A.C. Doran, R. Montgomery, D.E. Newby, M. Giacca, D. O'Carroll, P.W.F. Hadoke, L. Denby, J.C. Sluimer and A.H. Baker (2021) "CARMN loss regulates smooth muscle cells and accelerates atherosclerosis in mice. *Circ Res.* Apr 30;128(9):1258-1275. doi: 10.1161/CIRCRESAHA.120.318688. Epub 2021 Feb 24. PMID: 33622045; PMID: PMC7610708.
- Velanis, C.N., P. Perera, B. Thomson, E. de Leau, S.C. Liang, B. Hartwig, A. Förderer, H. Thornton, P. Arede, J. Chen, K.M. Webb, S. Gümüs, G. De Jaeger, C.A. Page, C.N. Hancock C. Spanos, J. Rappsilber, P. Voigt, F. Turck, F. Wellmer, and J. Goodrich (2020) "The domesticated transposase ALP2 mediates formation of a novel Polycomb protein complex by direct interaction with MSI1, a core subunit of Polycomb Repressive Complex 2 (PRC2)". *PLoS Genet.* **28**;16(5):e1008681. doi: 10.1371/journal.pgen.1008681. PMID: 32463832.
- Verzini, S., M. Shah, F.X. Theillet, Belsom, J. Bieschke, E.E. Wanker, J. Rappsilber, A. Binolfi and P. Selenko (2020) "Megadalton-sized dityrosine aggregates of α -synuclein retain high degrees of structural disorder and internal dynamics". *J Mol Biol.* **4**;432(24):166689. doi: 10.1016/j.jmb.2020.10.023. PMID: 33211011.
- Vidakovic, A.T., R. Mitter, G. Kelly, M. Neumann, M. Harreman, M. Rodriguez-Martinez, J. Weems, S. Boeing, V. Encheva, L. Gaul, L. Milligan, D. Tollervey, R.C. Conaway, J.W. Conaway, A.P. Snijders, A. Stewart and J.Q. Svejstrup (2020) "A single RNA polymerase II ubiquitylation site coordinates transcription with the DNA damage response". *Cell* **180**: 1245-1261. PMID: PMC7103762.
- Villaseñor, R., R. Pfaendler, C. Ambrosi, S. Butz, S. Giuliani, E. Bryan, T.W. Sheahan, A.L. Gable, N. Schmolka, M. Manzo, J. Wirz, C. Feller, C. von Mering, R. Aebersold, P. Voigt and T. Baubec, T. (2020) "ChromID identifies the protein interactome at chromatin marks". *Nat. Biotechnol.* **38**, 728-736.
- Visintin, R. and A.L. Marston (2021) Angelika Amon (1967-2020): Breakthrough scientist, extraordinary mentor, and loyal friend. *J Cell Biol.* **220**, e202012031.
- Welburn, J.P.I. and C. Friel (2020). The Kinesin-13 family: specialist destroyers. Book Chapter.
- Wimbish, R.T., K.F. DeLuca, J.E. Mick, J. Himes, I.J. Sanchez, A. A. Jeyaprakash and J.G. DeLuca (2020). "Coordination of NDC80 and Ska Complexes at the Kinetochore-Microtubule Interface in Human Cells". *Mol Biol Cell* Doi: <https://doi.org/10.1091/mbc.E20-05-0286>
- Zhang, J., M. Gurusaran, Y. Fujiwara, K. Zhang, M. Echbarthi, E.Vorontsov, R. Guo, D.F. Pendlebury, I. Alam, G. Livera, M. Emmanuelle, P.J. Wang, J. Nandakumar, O.R. Davies and H. Shibuya (2020). "The BRCA2-MEILB2-BRME1 complex governs meiotic recombination and impairs the mitotic BRCA2-RAD51 function in cancer cells". *Nature communications* **11**, 2055.
- Zhu, S., N.R. Choudhury, S. Rooney, N.T. Pham, J. Koszela, D. Kelly, C. Spanos, J. Rappsilber, M. Auer and G. Michlewski (2021). "RNA pull-down confocal nanoscanning (RP-CONA) detects quercetin as pri-miR-7/HuR interaction inhibitor that decreases alpha-synuclein levels." *Nucleic Acids Res* **49**(11): 6456-6473.
- Zoch, A., Auchynnikava, T., Berrens, R.V., Kabayama, Y., Schöpp, T., Heep, M., Vasiliaskaitė, L., Pérez-Rico, Y.A., Cook, A.G., Shkumatava, A., Rappsilber, J., Allshire, R.C. and D. O'Carroll (2020). "SPOCD1 is an essential executor of piRNA-directed de novo DNA methylation" *Nature.* **584**, 635-639. doi: 10.1038/s41586-020-2557-5.

Sustainability at WCB

Sustainability means living and doing what we enjoy in a way that is indefinite over Earth's lifetime. Our science supports life by contributing to medicine and it celebrates processes of life themselves. However, science is also extremely resource intensive, which means that it significantly and permanently affects the environment. At WCB we care that life that we appreciate in our studies will thrive and stimulate future generations of scientists. Therefore, we aim to minimize the effect of our work on the environment by cutting out unnecessary waste of resources. We do that by promoting efficient lab practices, reducing single use plastic and by minimizing energy and water consumption.



Single cell fungi (image by Tay, the Sawin lab) used as a model organism to study life processes at WCB and a wild multicellular fungus (image by Agata, the Welburn lab) growing graciously at WCB footsteps.



THE UNIVERSITY *of* EDINBURGH

Wellcome Centre for Cell Biology
School of Biological Sciences
The University of Edinburgh
Michael Swann Building
Max Born Crescent
Edinburgh EH9 3BF
Scotland, UK

Telephone +44 (0)131 650 7005
Fax +44 (0)131 650 4968

Website www.wcb.ed.ac.uk

Design: Graphic Design Service, LTW, ISG, The University of Edinburgh www.ed.ac.uk/is/graphic-design

Cover image: Illustrates the gain and loss of epigenetically regulated phenotypes in a cell population by Sito Torres-Garcia (Allshire Lab, now Cancer Research UK Cambridge Institute)

The University of Edinburgh is a charitable body, registered in Scotland, with registration number SC005336.