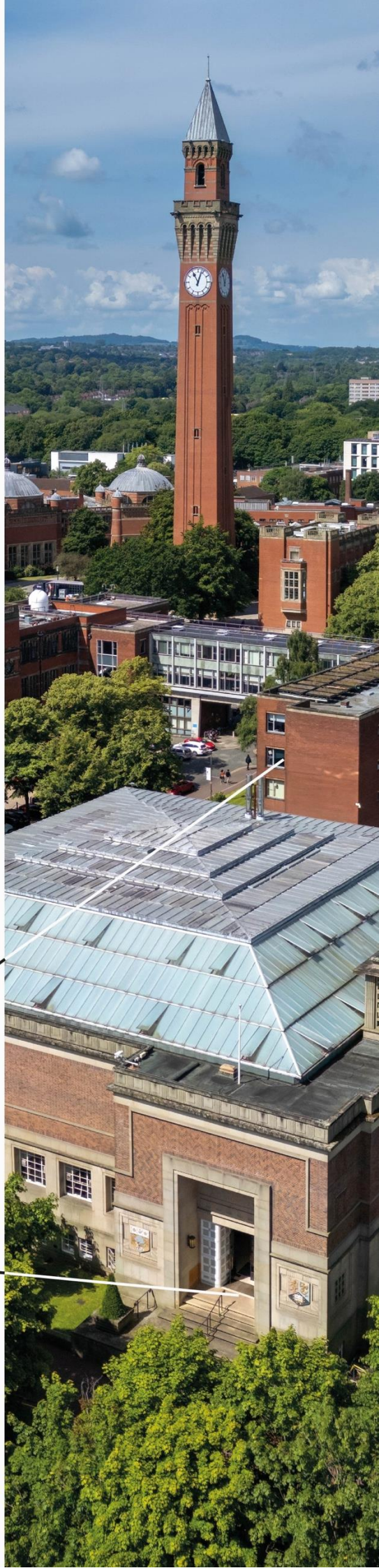
UNIVERSITY OF BIRMINGHAM

BIRMINGHAM CENTRE FOR GENOME BIOLOGY

Birmingham Centre for Genome Biology

Edgbaston Park Hotel,
11th – 12th September 2025

Abstract Book



2025 Conference

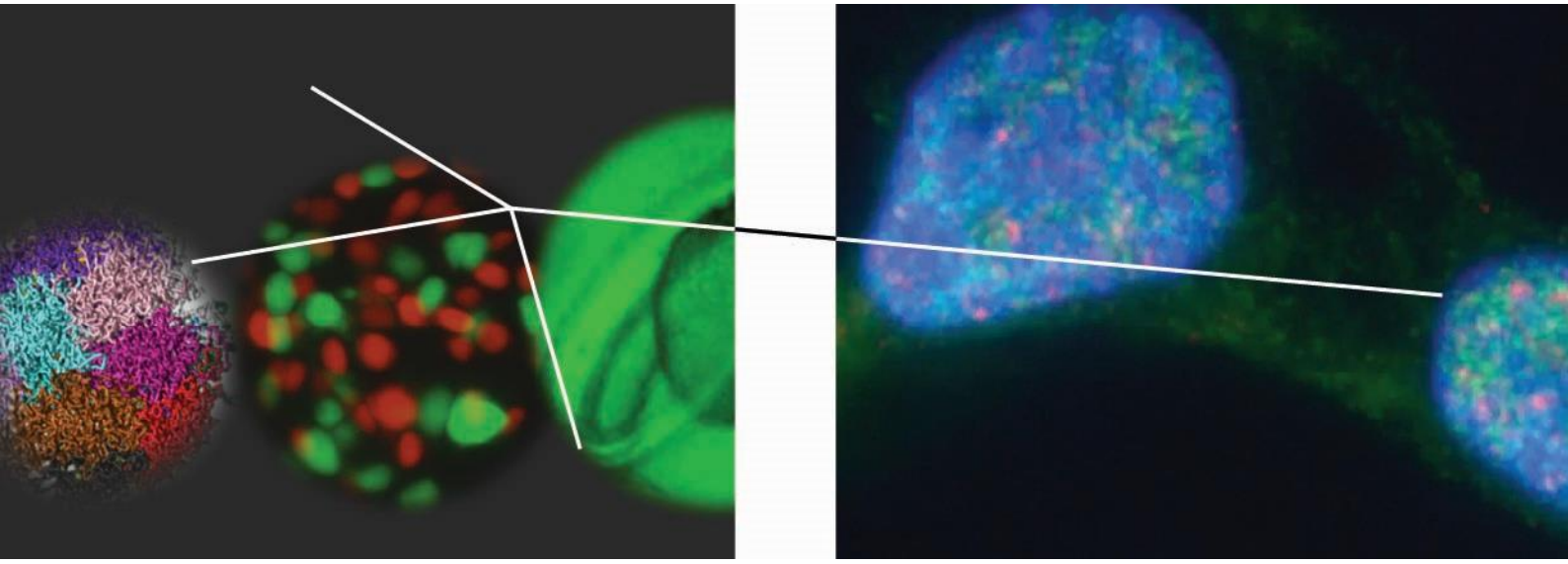
Contents

About the Conference

This conference marks the 10th anniversary of the BCGB and aims to highlight discovery research across the broad field of genome biology. To foster collaboration and knowledge exchange, we have invited world-leading experts from the UK, Europe, and the USA to share insights on key topics, including gene regulation and epigenetics, replication, genome stability, and DNA repair. The program also includes numerous abstract-selected talks, providing opportunities for researchers from the University of Birmingham and the wider Midlands community, including the University of Warwick and the University of Leicester, to present their work and engage in discussions.

About BCGB

The BCGB integrates and cross-fertilises the research activities of a diverse group of University of Birmingham scientists spanning primarily the College of Medicine and Health (CMH) and Life and Environmental Sciences (LES). BCGB is a virtual centre which hosts many internationally competitive research groups working on answering fundamental discovery science questions of genomic function as it relates to regulation of cellular development, differentiation and function, genome maintenance as it relates to genome stability and DNA replication and repair. We aim to understand how genomes function at a deep molecular level by bringing together experts from across our diverse communities **within the University of Birmingham.** Enabling leading research collaborations between specialists in Gene Regulation and Epigenetics in Development and Disease, RNA Biology, DNA Replication and Repair, Cancer Biology, Bacterial Genome Studies, Environmental Toxicology and Computational Biology.



Organising Committee

- Ildem Akerman
- Saverio Brogna
- Teresa Carlomagno
- Clare Davis
- Aga Gambus
- Paloma Garcia
- Hansong Ma
- Jo Morris
- Ferenc Mueller
- Eva Petermann
- Marco Saponaro

Key Information

Conference Registration and Information Desk:

Open on both Thursday 11th and Friday 12th September, 08:30 – 17:00

The desk will be located in the reception area of the Edgbaston Park Hotel.

Conference Social Events:

Bryan Turner's Retirement Party	**Thursday 11th September** 17:00 - 18:00	Gisbert Kapp Building Room NG16
Drinks Reception and Dinner	**Friday 12th September** 18:00 – 23:00	Horton Grange Building Lloyd Suite

Exhibition:

Our sponsors will have an exhibition stand in the Great Hall. They have chosen to sponsor this event because they believe their products and services may interest you. We encourage you to visit each exhibition stand during the breaks.

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Conference Programme

Thursday 11th September

08:30 - 09:30	**REGISTRATION & ARRIVAL REFRESHMENTS**
09:30 - 09:50	**Welcome & Introduction**
SESSION 1: CHROMATIN	
09:50 - 10:30	**Brad Cairns -** Widespread Transient Activation of ERVs in Cleavage-Stage Embryos Involves the Conversion of the Repressor SETDB1 to a Co-Activator by RHOX12L
10:30 - 10:45	**O1 - Karen Lane -** Multi-omic profiling following loss of SWI/SNF chromatin remodelling complexes reveals global changes in cohesin binding and sensitivity to cohesin perturbation
10:45 - 11:00	**O2 - Paul Badenhorst -** Combinatorial histone modifications direct ATP-dependent chromatin remodeling by NURF to promoter proximal nucleosomes
11:00 - 11:20	**REFRESHMENT BREAK**
11:20 - 11:40	**Andrew Bowman -** Dynamic long-range movement of megabase-sized heterochromatin domains in living cells

11:40 - 12:00	**Nitika Taneja -** Mechanisms of Chromatin Re-organization upon Replication Stress
12:00 - 12:10	**Covaris** - Sponsor Talk
12:10 - 12:20	**New England Biolabs** - Sponsor Talk
12:20 - 12:50	**Flash Talks** (2 minutes per talk) **Chongluan Shi -** Investigating the regulation of E3 ubiquitin ligase TRAIP in DNA replication **Gemma Regan-Mochrie -** Histone H4R3C: A new oncohistone that drives genomic instability **Alice Ormrod -** The radiobiological impact of protons in combination with DNA double strand break repair inhibitors in glioblastoma models **Rosa Camarillo Daza -** The where, when and how of DNA repair in quiescent cells **Panagiotis Kotsantis -** RNF169 is implicated in the DNA replication stress response **Elizabeth Anthony and Ruth Densham -** Using a BRCA1/BARD1 gap-suppression separation of function mutant to elucidate mechanisms of therapy responses **Maryam Eftekharifar -** Deciphering cell-type specific inter-chromosomal genome architecture via single-cell Hi-C and machine learning

	Tom Wright - Combining Genome Engineering with Next-Generation Sequencing to Unravel the Functions of the NuRD Complex **Yagiz Ozturk -** Ctdp1, a phosphatase at the intersection of transcription and cell viability **Alexander Lanz -** SUMOylation promotes mitotic DNA damage repair **Adedoyin Adeyemi -** Exploring the link between Transcription and Translation in Fission Yeast **Luis Padilla-Cortes -** Structural characterization of SPEN's IDR and its role the formation of gene silencing supramolecular complexes **Akhil Bowry** - The 7SK small nuclear ribonucleoprotein marries the cell responses to both transcriptional and replication stress by promoting homologous recombination and replication fork reversal
12:50 - 14:20	**LUNCH & POSTER SESSION 1 - ODD POSTER NUMBERS**

SESSION 2: REPLICATION AND DAMAGE	
14:20 - 14:40	**Vincenzo Costanzo -** Unraveling the role of homologous recombination proteins in Vertebrate DNA replication
14:40 - 15:00	**Michiel Vermeulen -** Deciphering gene expression regulation in health and disease using proximity labeling
15:00 - 15:15	**O3 - Alicja Reynolds-Winczura -** CDK-driven phosphorylation of TRAIP is essential for mitotic replisome disassembly and MiDAS
15:15 - 15:30	**O4 - Yara Aghabi -** Cyclin F: A novel BRCA2 interactor with impacts on replication fidelity
15:30 - 15:50	**REFRESHMENT BREAK**
SESSION 3: REPLICATION AND DAMAGE	
15:50 - 16:10	**Massimo Lopes -** Replication fork plasticity in cancer and stem cells
16:10 - 16:30	**Evi Soutouglou -** DNA damage at Lamina Associated Domains triggers nuclear envelope reorganization and chromatin detachment to prevent nuclear envelope blebbing and genome instability

16:30 - 16:45	**O5 - Richard Kelly -** Coordinated Activation of RAS and PI3K Signalling Drives Oncogene-Induced Replication Stress via Hypertranscription
16:45	**CONFERENCE CLOSE**
BRYAN TURNER'S RETIREMENT PARTY - GISBERT KAPP BUILDING - ROOM NG16	
17:00 - 18:00	**Bryan Turner**
18:00 - 19:00	**DRINKS RECEPTION - EDGBASTON PARK HOTEL, LLOYD SUITE LOUNGE**
19:00 - LATE	**DINNER - EDGBASTON PARK HOTEL, LLOYD SUITE**

Friday 12th September

08:30 - 09:00	**REGISTRATION & ARRIVAL REFRESHMENTS**
SESSION 4: CHROMATIN	
09:00 - 09:20	**Tineke Lenstra -** Understanding transcription factor search, one molecule at a time
09:20 - 09:40	**Wendy Bickmore -** Beyond the tail: acetylation of the histone globular domain marks fragile nucleosomes at key regulatory sites
09:40 - 10:00	**O6 - Nicolae Radu Zabet -** Explainable Artificial Intelligence identifies novel mammalian enhancers and their epigenetic code
10:00 - 10:15	**O7 - Colin Hammond -** Exploring the role of heat shock molecular chaperones during histone supply and deposition
10:15 - 10:30	**O8 - Robert Turnbull -** Rapid degradation of six class 1 HDAC complexes reveals minimal functional overlap and unique functions for each complex
10:30 - 10:50	**REFRESHMENT BREAK**
10:50 - 11:10	**Monika Gullerova -** The role of RNA modifications in DNA damage response

11:10 - 11:30	**Thomas Schalch -** The HMD domain of the PAF complex primes Rad6-Bre1 E3 ligase complexes for H2B ubiquitination
11:30 - 11:45	**O9 - Isabel Díez Santos -** Understanding the role of senataxin in the resolution of transcription-replication conflicts
11:45 - 11:55	**Parse Biosciences** - Sponsor talk
11:55 - 12:25	**Flash Talks** (2 minutes per talk) **Nagham Ghadder -** The impact of the SWI/SNF complex subunit ARID1A on the chromatin environment surrounding stalled replication forks **Eduardo De La Vega -** Unbiased detection of replication initiation events in human cells using BrdU incorporation and single-molecule nanopore sequencing **Maria Rita Fabbrizi -** Understanding the cellular DNA damage response in head and neck cancer cells treated with Boron Neutron Capture Therapy **Emily Prowse -** Inhibition of PRMT5-mediated arginine methylation decreases Ewing sarcoma (ES) survival in an EWSR1-ETS-dependent manner by inducing DNA damage **Olga Brieieva -** The lysine methyltransferase SETD1A promotes ssDNA gaps formation under replication stress

	Kristupas Sirvydis - XIST supercomplexes- key actors in X-inactivation during early human embryonic development **Ammarah Tariq -** Structure and Regulation of the H2B E3 Ligase Complex **Paolo Passaretti -** The Structure of the Xenopus laevis Replisome **Rosanna Wilkins -** Upregulation of human RNase H2 counteracts replication stress elicited by oncogenes and chemotherapy drugs **Panagiotis Patsis -** Dissecting the regulatory mechanisms governing the histone 3 lysine 9 methyltransferase Clr4
12:25 - 14:00	**LUNCH & POSTER SESSION 2 - EVEN POSTER NUMBERS**
SESSION 5: TRANSCRIPTION REGULATION	
14:00 - 14:20	**Jorge Ferrer -** A transcription-splicing axis as a therapeutic target for diabetes mellitus
14:20 - 14:40	**Anne Ferguson-Smith** - Mammalian retrotransposon-mediated genetic and epigenetic variation
14:40 - 15:00	**Chris Lord -** Resistance to treatment in HRD breast cancers

15:00 - 15:15	**O10 - Kelly Chiang -** Targeting PRMT5-mediated splicing: A new therapeutic opportunity for triple negative breast cancers
15:15 - 15:30	**O11 - Naomi Eastwood -** The chromatin remodeller ARID1A functions through FOX and AP-2 transcription factors in lobular breast cancers
15:30 - 15:55	**REFRESHMENT BREAK**
SESSION 6: TRANSCRIPTION REGULATION	
15:55 - 16:15	**Alex Stark -** Decoding transcriptional regulation
16:15 - 16:30	**O12 - Nicoletta Bobola -** Ubiquitous transcription factors MEIS actuate lineage-specific transcription to establish cell fate
16:30 - 16:45	**O13 - Michael Tellier -** NELF prevents genomic instability by regulating RNA polymerase II transcription elongation and termination
16:45 - 17:00	**PRIZES & CONFERENCE CLOSE**

Invited speakers

Wendy Bickmore

Director, MRC
Human Genetics Unit

Beyond the tail: acetylation of the histone globular domain marks fragile nucleosomes at key regulatory sites

Acetylation of lysine residues in the tail domain of histone H3 is well characterized, but lysine residues in the histone globular domain are also acetylated and can impact nucleosome stability. I will describe our analysis of acetylated H3 lysine 115 (H3K115ac), a residue on the lateral surface at the nucleosome dyad. In mouse embryonic stem cells, we find that H3K115ac is enriched at the transcription start site of active CpG island promoters, but also at polycomb repressed promoters prior to their subsequent activation during differentiation. By contrast, at enhancers H3K115ac is dynamic, changing in line with gene activation and chromatin accessibility during differentiation. Most strikingly, we show that H3K115ac is enriched on “fragile” nucleosomes within nucleosome depleted regions at promoters, and active enhancers where it coincides with transcription factor binding, and at CTCF bound sites. These unique features suggest that H3K115ac could contribute to nucleosome destabilization and that it might be a valuable marker for identifying functionally important regulatory elements in mammalian genomes.

Andrew Bowman

Associate Professor, Warwick

Dynamic long-range movement of megabase-sized heterochromatin domains in living cells

Chromatin movement underpins essential genomic processes, yet its large-scale dynamics remain poorly understood. While studies tracking individual loci have provided valuable insights, how entire chromatin domains move within the nucleus is less clear. Here, we employed a fluorescent histone pulse-chase strategy combined with replication timing to selectively label late-replicating heterochromatin domains, aligning with Topologically Associating Domains (TADs) and Replication Domains (RDs), in live cells.

Using Lattice-LightSheet microscopy, we tracked the 3D dynamics of these domains for up to an hour with minimal phototoxicity. Mean squared displacement (MSD) analysis revealed a highly mobile sub-population displaying Rapid Translocation Events (RTEs)—bursts of movement followed by periods of relative immobility.

RTEs typically involved megabase-sized chromatin domains moving between the nuclear periphery and interior along defined paths, suggesting movement along stable nuclear structures or channels, with multiple domains translocating together along shared trajectories. Furthermore, domains visibly stretched in the direction of movement before returning to their original shape, suggesting they are under mechanical tension. These findings indicate that, beyond local decompaction and looping, entire chromatin domains can undergo rapid translocations to reposition within the nucleus.

Brad Cairns

Professor and Chair, Oncological Sciences, Spencer Eccles School of Medicine, University of Utah

Chief Academic Officer, Huntsman Cancer Institute, University of Utah

Widespread Transient Activation of ERVs in Cleavage-Stage Embryos Involves the Conversion of the Repressor SETDB1 to a Co-Activator by RHOX12L

The onset of transcription in pre-implantation embryos includes the widespread transient activation of normally silenced endogenous retroviruses (ERVs) and other retrotransposons. In mice, DUX and OBOX transcription factors activate MERVL, but how other retrotransposons are activated remains largely unknown. Here, we identify RHOX12-like (RHOX12L) as a novel homeodomain-containing factor briefly activated by DUX in 2-cell embryos. Independent of specific DNA-binding activity, RHOX12L counteracts the general ERV repressor SETDB1 to transiently activate thousands of IAP/MERVK loci. Our biochemical studies reveal robust interaction of RHOX12L with SETDB1 as well as CBP/P300 and the BAF complex, concomitant with acetylation and chromatin opening at SETDB1-RHOX12L co-bound sites. In keeping, Rhox12l knockout embryos proved defective in IAP/MERVK activation. Furthermore, RHOX12L downregulates the OCT4 pluripotency network in embryonic stem cells (ESCs), potentially by sequestering OCT4 to de-repressed ERVs bearing upstream OCT4 binding sites. Taken together, we provide a mechanism for the strong, transient activation of the TRIM28/SETDB1-bound retrotransposon repertoire in pre-implantation embryos via co-activator tethering by RHOX12L.

Vincenzo Costanzo

Associate Professor at the
Department of Oncology
and Hematology-Oncology,
University of Milan

Unraveling the role of homologous recombination proteins in Vertebrate DNA replication

Homologous recombination (HR) functions in the repair of double-strand breaks (DSBs). However, work from our laboratory and others now indicates that HR safeguards genome stability by also preventing the occurrence and deleterious consequences of DNA lesions during DNA replication. We have previously shown that HR proteins RAD51, BRCA1, BRCA2, and associated factors patrol replication forks, limiting nucleolytic processing by enzymes such as MRE11, and restraining the formation of single-stranded (ss) gaps. More recently, we have uncovered a novel function of RAD51, demonstrating that it binds to abasic sites, protecting them from nuclease-mediated processing. When HR proteins are absent, these preventive roles fail, leading to abasic gap accumulation, fork collapse, and base-substitution in HR-deficient tumors. Our studies also reveal extensive crosstalk between HR, base-excision repair, and translesion synthesis mediated by polymerase zeta and theta, demonstrating that loss of HR reshapes these pathways and amplifies genomic instability. Surprisingly, epigenetically generated abasic sites, particularly those linked to 5-methyl-cytosine metabolism, place additional stress on replication forks and further underscore the need for HR-mediated protection. Viewing HR as a surveillance system rather than solely a repair mechanism provides a new context for understanding the genomic and epigenetic changes that accompany tumor evolution and therapeutic resistance.

Anne Ferguson-Smith

Arthur Balfour Professor of GeneticsPro-Vice-Chancellor for Research, University of Cambridge

Mammalian retrotransposon-mediated genetic and epigenetic variation

Epigenetic variation has been observed genome-wide in inbred strains of mice. Such variation often occurs at young transposable elements, can differ between strains, between individuals and can contribute to phenotypic diversity by influencing the expression of neighbouring genes. We have leveraged natural genetic divergence between mouse strains to investigate the mechanisms governing variable DNA methylation at retrotransposons and the relevance of this for genome regulation, phenotypic differences between individuals within and across generations, and organismal adaptation in populations.

Kazachenka, Bertozzi et al. (2018) PMID: 30500541
Elmer, Hay, Kessler et al. (2020) PMID: 33612119
Bertozzi et al. (2020) PMID: 33239447
Bertozzi et al. (2021) PMID: 33755012
Bertozzi et al. (2021) PMID: 34326545

Francis et al.,
BioRxiv doi: https://doi.org/10.1101/2024.10.24.619615
Bruno et al.,
bioRxiv doi: 10.1101/2025.02.26.640358.

Jorge Ferrer

Group Leader, Coordinator,
Computational Biology
and Health Genomics
Programme, CRG

A conserved regulatory axis that links transcription and splicing in pancreatic beta cells is disrupted in type 2 diabetes

Pancreatic beta cells are equipped with highly complex gene expression programs that allow them to secrete appropriate amounts of insulin at all times. Failure leads to fatal hypoglycemia or hyperglycemia and diabetes mellitus. This specialized function is made possible by a unique gene expression that is guided by combinations of transcription factors. There is also increasing knowledge of splicing factors that control which transcript isoforms are expressed in beta cells. How these two key regulatory layers, transcription and splicing, are interconnected is poorly understood. I will discuss work that uncovers a regulatory axis that coordinates transcription and RNA splicing programs in pancreatic beta cells, and show that defective activity of this axis plays a role in the pathophysiology of human type 2 diabetes. I will further discuss an ongoing strategy to correct molecular defects underlying diabetes by targeting abnormal gene expression programs.

Monika Gullerova

Professor of Molecular Medicine,
University of Oxford

The role of RNA modifications in DNA damage response

DNA integrity is constantly challenged by both endogenous and exogenous damaging agents, resulting in various forms of damage. Failure to repair DNA accurately leads to genomic instability, a hallmark of cancer. Distinct pathways exist to repair different types of DNA damage. Double-strand breaks (DSBs) represent particularly severe form of damage, due to the physical separation of DNA strands. The repair of DSBs requires the activity of RNA Polymerase II (RNAPII) and the generation of Damage-responsive transcripts (DARTs).

We show that the RNA m5C-methyltransferase NSUN2 localises to DSBs in a transcription-dependent manner, where it binds to and methylates DARTs. The depletion of NSUN2 results in an accumulation of nascent primary DARTs around DSBs. Furthermore, we detected an RNA-dependent interaction between NSUN2 and DICER, which was stimulated by DNA damage. NSUN2 activity promoted DICER cleavage of DARTs-associated R-loops, which is required for efficient DNA repair.
We report a previously unrecognised role of the RNA m5C-methyltransferase NSUN2 within the RNA-dependent DNA damage response, highlighting its function as a DICER chaperone for the clearance of non-canonical substrates such as DARTs, thereby contributing to genomic integrity.

Tineke Lenstra

Group leader, Netherlands Cancer Institute

Understanding transcription factor search, one molecule at a time

Transcription in single cells is dynamic, resulting in heterogeneity in gene expression in a population. In our lab, we aim to understand the mechanisms that regulate transcription dynamics in single cells. We use a range of single-molecule imaging techniques to directly observe the stochastic behavior of regulatory factors and the process of transcription inside living cells. An important step in gene regulation is the binding of gene-specific transcription factors to the DNA. In this talk, I will focus on how transcription factors find their target loci in living cells, what regulates their DNA binding kinetics, and how transient DNA binding of transcription factors transcription factors contribute to gene activation.

Massimo Lopes

Coordinator of Master in Cancer Biology, University of Zurich, Switzerland

Replication fork plasticity in cancer and stem cells

Mild replication interference is a consolidated strategy for cancer chemotherapy. Tolerance to mild replication stress (RS) relies on active fork slowing, mediated by transient fork reversal and RECQ1-assisted restart, and modulated by PARP1 and nuclear architectural components via yet-elusive mechanisms. We combined acute protein inactivation with cell biology and single-molecule approaches to investigate the role of Lamin A/C upon mild RS. We found that Lamin A/C dynamically interacts with replication factories throughout the nucleus and, together with its nucleoplasmic partner LAP2□, is required to induce active fork slowing and maintain chromosome stability upon mild genotoxic treatments. Inactivating nucleoplasmic Lamin A/C reduces poly-ADP-ribosylation (PAR) levels at nascent DNA, triggering deregulated RECQ1-mediated restart of reversed forks. Moreover, we found that the heterochromatin mark H3K9me3, previously reported at stalled forks, also accumulates in response to mild RS. H3K9me3 accumulation requires Lamin A/C, which prevents its premature removal by the histone demethylase JMJD1A/KDM3A. H3K9me3 loss per se phenocopies Lamin A/C inactivation, reducing PAR levels and deregulating fork restart by RECQ1. Hence, nucleoplasmic Lamin A/C, H3K9me3 and PARylation levels are crucial, mechanistically linked modulators of fork dynamics upon mild RS, with important implications for chemotherapy response and for Lamin A/C dysfunction in human disease.

Chris Lord

Deputy Head of Division,
Group Leader of the CRUK
Gene Function Laboratory
Professor of Cancer Genomics
in the Breast Cancer Now
Toby Robins Research
Centre at The Institute of
Cancer Research, London

Resistance to treatment in HRD breast cancers

Although PARP inhibitors and platinum salts deliver significant and sustained anti-tumour responses in homologous recombination defective cancers, resistance to these agents, especially in those with metastatic disease, is a growing problem. Pre-clinical work has identified a series of candidate mechanisms of resistance, but as yet the impact of many of these in the clinic is unclear. Using longitudinal profiling of women receiving standard of care PARPi and/or platinum treatment for their metastatic breast cancer, we show that reversion of BRCA1 or BRCA2 is the dominant cause of resistance, being seen in 57%. Although other mechanisms of PARPi resistance such as TP53BP1 mutation are evident in PARPi resistant patients, these are much rarer and often co-occur with BRCA1/2 reversion mutations. I will discuss why reversion mutations, as opposed to compensatory mechanisms such as TP53BP1 defects, are the likely dominant cause of resistance as well as show how vaccinating against neopeptides encoded by BRCA1/2 reversions could be used to target PARPi resistant disease.

Thomas Schalch

Professor of Molecular
and Structural Biology,
University of Leicester

The HMD domain of the PAF complex primes Rad6-Bre1 E3 ligase complexes for H2B ubiquitination

Mono-ubiquitination of histone H2B (H2Bub) facilitates transcription, replication, and DNA repair by modulating chromatin structure. In Schizosaccharomyces pombe, the H2B ubiquitin ligase complex (HULC)—comprising Brl1/Brl2 (Bre1/RNF20/40 homologues), Rhp6 (Rad6), and Shf1—is recruited to RNA polymerase II via the Paf1 complex (PAF1C). While the HMD domain of PAF1C's Rtf1 subunit is known to recruit HULC and stimulate its activity, the mechanistic basis for HMD-mediated activation remains unclear. Here, we combine AlphaFold modelling, crosslinking mass spectrometry, structural, biophysical and mutational analyses to demonstrate that HULC adopts a flexible 1:1:1:1 assembly. We identify HMD domain's RING-binding region (RBR), which directly engages HULC's RING domains, positioning Rhp6 proximal to H2B's target lysine. RBR mutations abolish H2Bub in S. pombe and reduce HULC activity in vitro. Structural conservation of the RBR-HULC interface from yeast to mammals suggests a universal activation mechanism. This work elucidates how PAF1C couples HULC recruitment to catalytic priming, thereby stimulating transcription-associated H2Bub deposition.

Evi Soutoglou

Professor of Genome Stability
(Genome Damage and Stability),
School of Life Sciences,
University of Sussex

DNA damage at Lamina Associated Domains triggers nuclear envelope reorganization and chromatin detachment to prevent nuclear envelope blebbing and genome instability

The nuclear periphery anchors large, transcriptionally silent chromatin domains to the nuclear envelope and plays a central role in maintaining genome stability, acting as a dynamic hub for DNA repair. Nevertheless, how cells process DNA damage within lamina-associated domains (LADs), which reside in this very environment, remains poorly understood, despite their relevance to nuclear architecture, aging, and senescence. Using systems for temporally controlled and spatially precise induction of double-strand breaks (DSBs) within LADs, we show that DNA damage triggers nuclear envelope remodeling, characterized by reduced lamin B1 and LBR levels and ATM-dependent mobilization of the LINC complex components SUN1 and SUN2, which leads to the dynamic detachment of damaged LADs from the nuclear lamina. Persistent tethering of damaged LADs at the nuclear periphery delays the DNA damage response and repair, elevates genomic instability, and drives chromatin extrusion through nuclear blebs - directly linking failed repair to dysregulation of nuclear integrity. These findings reveal a protective mechanism in which ATM-driven LAD dynamics and envelope remodeling might relieve mechanical stress, facilitate repair, and safeguard both genome stability and nuclear architecture.

Alex Stark

Senior Group Leader, Medical
University of Vienna

Decoding transcriptional regulation

In higher eukaryotes, genes are expressed dynamically in complex spatial and temporal patterns, which are progressively refined to set up body plans and define specific cell-types. The information about when and where each gene is to be expressed is encoded in the sequences of promoter-, enhancer- and silencer regions and realized by transcription factor and cofactor proteins.

I am presenting our work towards understanding the how this regulatory information is sequence-encoded and how cells utilize this information with transcription factor and cofactor proteins. We characterize regulatory sequences by functional screens in cell lines and by a genome-scale candidate testing approach in developing Drosophila embryos. We then use deep-learning approaches to model the sequence-to-function relationship for enhancers and build synthetic enhancers de novo. We also employ functional screens, mutagenesis, and biochemistry to dissect functions and mechanisms of transcription regulation in flies and mammals.

Nitika Taneja

Assistant Professor, Department of Molecular Genetics, Erasmus University Medical Center

Mechanisms of Chromatin Reorganization under Replication Stress

The mammalian genome is intricately organized within chromatin, with distinct roles for heterochromatin and euchromatin regions in cellular processes. Despite its gene silencing role, the accumulation of heterochromatin in cancer cells suggests additional functions beyond transcriptomic changes. Recently, we demonstrated that cells experiencing replication stress accumulate de novo heterochromatin marks at stressed replication forks, leading to transient compaction crucial for replication fork stability1. However, the broader impact of this compaction on genome organization remains unclear.

High-throughput chromosome conformation capture (Hi-C) techniques have extensively explored genome organization globally. Yet, studying the organization of newly replicated DNA, particularly during replication stress, presents challenges. To address this, we developed Rep-Hi-C, enabling investigation of 3D organization of newly replicated DNA and capturing replication stress-induced genomic interactions. We find replication stress triggers local and spatial chromatin reorganization, enclosing stressed replicating regions within distinct chromatin loops. Using ForkDeg-Seq, a method we developed for genome-wide mapping of fork degradation sites, we show that these chromatin loops act as protective structures, safeguarding stressed replicated regions from degradation and maintaining genome stability. This study provides high-resolution insights into the three-dimensional spatial organization of replication forks under replication stress, emphasizing their critical role in preserving genome stability.

Michiel Vermeulen

Member of the management board, Interdisciplinary Research Platform

Professor, Molecular Biology, Radboud University

Oral Presenter Abstracts

O1 - Multi-omic profiling following loss of SWI/SNF chromatin remodeling complexes reveals global changes in cohesin binding and sensitivity to cohesin perturbation

Dr Karen Lane

SWI/SNF is a family of multi-subunit chromatin remodelling complexes which play an important role in maintaining genome stability, with over 20% of all cancers having damaging mutations in a SWI/SNF subunit. To model this, we generated and profiled a panel of SWI/SNF perturbations in the widely used cell line, RPE1, by creating knockouts of both shared and complex-specific subunits, as well as using PROTACs to acutely degrade various subunits. Comparative transcriptomic and proteomic analyses after SWI/SNF loss revealed that both acute and chronic loss led to significant changes in cohesin dynamics on chromatin. These changes were also observed following inhibition of catalytic activity, implicating SWI/SNF remodelling function in cohesin stabilisation on chromatin. Mapping of CTCF on DNA revealed that a proportion of CTCF-bound sites are lost in SWI/SNF knockouts, while another subset localises to novel sites on the genome, suggesting a change in 3D chromatin structure in the absence of SWI/SNF. Strikingly, these alterations lead to significant sensitivity of SWI/SNF deficient cells to inhibition or depletion of cohesin subunits. This multi-omic approach to studying SWI/SNF perturbation has uncovered novel functions for SWI/SNF in cohesin maintenance on DNA, with potential therapeutic implications for SWI/SNF mutant cancers.

O2 - Combinatorial histone modifications direct ATP-dependent chromatin remodeling by NURF to promoter proximal nucleosomes

Dr Paul Badenhorst

NURF is a conserved ISWI-containing ATP-dependent chromatin remodeling complex that slides nucleosomes to control transcription and genome organization. Recognition of histone modifications (HPTMs) by reader domains has been proposed to focus remodeler action at discrete genome targets, either by controlling recruitment or through local allosteric regulation of core enzymatic activities. To distinguish mechanisms by which HPTMs influence NURF, we defined full HPTM-binding specificity of NURF by screening novel combinations of histone-reader interactions. We observe the NURF-selective subunit (BPTF/NURF301) recognises multiple H3 and H4 tail modifications via C-terminal PHD2 and bromodomains. Modified H3 recognition requires a new binding-pocket on PHD2 for H3K9AcS10p that cooperates with the known H3K4me3-binding hydrophobic-cage to enable high-affinity binding to triply-modified H3K4me3K9AcS10p. This combinatorial HPTM recognition discriminates and stabilises NURF recruitment to +1 nucleosomes of active genes, maintaining nucleosome position to control transcription. Our data establish direct, causal links between HPTM recognition, remodeler recruitment and consequent activity.

O3 - CDK-driven phosphorylation of TRAIP is essential for mitotic replisome disassembly and MiDAS

Dr Alicja Reynolds-Winczura

TRAIP is an E3 ubiquitin ligase that maintains genome stability by resolving replication stress and DNA replication-transcription conflicts in S-phase and promotes replisome disassembly. S-phase disassembly is orchestrated by Cul2^LRR1-dependent ubiquitylation of the CMG helicase subunit MCM7. However, when S-phase is perturbed, resulting in regions of under replicated DNA and/or stalled replication forks, replication machinery may be retained on the DNA until mitosis. Mitotic removal of replisomes is driven by TRAIP.

A key question is how TRAIP activity is precisely regulated to avoid premature unloading of active forks during S-phase (potentially catastrophic event), while ensuring proper disassembly in mitosis.

To investigate TRAIP regulation, we employed Xenopus laevis egg extract and in vitro biochemical approaches. We demonstrate that TRAIP is phosphorylated by cyclin-dependent kinases (CDKs) exclusively during mitosis, and that this modification is essential for TRAIP-dependent replisome disassembly. Phosphorylated TRAIP exhibits enhanced auto-ubiquitylation and greater activity toward replisomes isolated from mitotic chromatin. Furthermore, in human cells, TRAIP phosphorylation is required for effective mitotic DNA synthesis (MiDAS), reinforcing its role in maintaining genome stability under replicative stress. Overall, our study shows that CDK-dependent phosphorylation of TRAIP is a critical regulatory mechanism that controls the timing of replisome disassembly in S-phase and mitosis.

O4 - Cyclin F: A novel BRCA2 interactor with impacts on replication fidelity

Ms Yara Aghabi

Cyclin F is the component of the Skp-Cullin-Fbox (SCFcyclin F) E3 ubiquitin ligase complex that directs substrate specificity. The complex acts to ubiquitylate and target its substrates, such as EXO1 and RRM2, for proteasomal degradation. Here, we demonstrate that cells lacking cyclin F exhibit replication defects, including poor fork protection against nucleolytic degradation and ssDNA gap accumulation, both through inadequate RAD51 function. Surprisingly, we show that the interaction of cyclin F with the SCF complex and the complex itself is dispensable for this activity. Instead, we identify that an interaction between cyclin F and a conserved region of BRCA2 is needed for normal replication kinetics. In this talk, I will characterise the role of cyclin F in replication and explore its interaction with BRCA2.

O5 - Coordinated Activation of RAS and PI3K Signalling Drives Oncogene-Induced Replication Stress via Hypertranscription

Dr Richard Kelly

Oncogene-induced replication stress is a key driver of genomic instability in cancer. We previously demonstrated that overexpression of HRASG12V induces global RNA hypertranscription, leading to replication stress and DNA damage. To further dissect RAS-driven replication stress, we established inducible cell lines expressing either KRASG12V or the downstream effector BRAFV600E. Our findings reveal that KRASG12V induces only a modest hypertranscription and replication stress, while BRAFV600E has minimal impact on transcriptional output or replication fork speeds. In HRASG12V cells, hypertranscription was characterised by robust upregulation of rRNA, tRNA, and E2F/MYC/FOXM1 transcriptional programs, along with increased S-phase entry. Our analysis suggests that RNA polymerase activation, rather than cell cycle changes, drives replication stress. Notably, HRASG12V—unlike KRASG12V or BRAFV600E—strongly activates AKT signalling downstream of PI3K. Chemical inhibition of the PI3K-AKT-mTOR signalling significantly attenuated HRASG12V-induced replication stress. Co-expression of BRAFV600E and PIK3CAE545K reduces replication fork speed, indicating a cooperative role between RAS and PI3K pathways. These findings suggest that RAS and PI3K signalling converge to drive replication stress through transcriptional amplification. Consistent with this, cancer datasets show that co-occurring mutations in BRAF/KRAS and PI3K are linked to higher replication stress signatures and aneuploidy.

O6 - Explainable Artificial Intelligence identifies novel mammalian enhancers and their epigenetic code

Dr Nicolae Radu Zabet

Enhancers are non-coding regulatory elements in the genome that control gene expression. Majority of mutations associated with complex diseases (e.g., cancer) are often located in enhancers underlying their role in many human diseases. While both experimental and computational efforts have been made for their annotation, we still lack an accurate enhancer map in many human cells, tissues and disease contexts.

Here, we generated a rule-based explainable artificial intelligence (XAI) model for the enhancer prediction in several human and mouse cell lines which displays high accuracy. Most importantly, our models are explainable and provides if/then set of rules that classify the enhancer and non-enhancer regions. Interestingly, seven epigenetic marks are sufficient to annotate enhancers without losing accuracy. We have trained models in both human (H9) and mouse (E14) cells and, to generate accurate enhancer maps in additional cell lines. Our AI models classified many novel regions as putative enhancers, and these putative enhancers display the same epigenetic patterns as experimentally validated enhancers. We have further validated these enhancers by both global epigenetic perturbations and directed enhancer epigenetic rewriting.

Overall, we have deciphered the epigenetic code of mammalian enhancers and were able to annotate enhancers in multiple human and mouse cell lines.

O7 - Exploring the role of heat shock molecular chaperones during histone supply and deposition

Dr Colin Hammond

Mislocalization of CENP-A to non-centromeric regions contributes to chromosomal instability (CIN), a hallmark of cancer. However, pathways that promote or prevent CENP-A mislocalization remain poorly defined. Here, we performed a genome-wide RNAi screen for regulators of CENP-A localization which identified DNAJC9, a J-domain protein implicated in histone H3–H4 protein folding, as a factor restricting CENP-A mislocalization. Cells lacking DNAJC9 exhibit mislocalization of CENP-A throughout the genome, and CIN phenotypes. Global interactome analysis showed that DNAJC9 depletion promotes the interaction of CENP-A with the DNA replication-associated histone chaperone MCM2. CENP-A mislocalization upon DNAJC9 depletion was dependent on MCM2, defining MCM2 as a driver of CENP-A deposition at ectopic sites when H3–H4 supply chains are disrupted. Cells depleted for histone H3.3, also exhibit CENP-A mislocalization. In summary, we have defined novel factors that prevent mislocalization of CENP-A and demonstrated that the integrity of H3–H4 supply chains is regulated by histone chaperones such as DNAJC9 restrict CENP-A mislocalization and CIN. Progress on current work aimed at exploring how HSP70 enzymes contribute to the supply and deposition of histones on chromatin will also be presented.

O8 - Rapid degradation of six class 1 HDAC complexes reveals minimal functional overlap and unique functions for each complex

Dr Robert Turnbull

The class 1 HDACs 1, 2 and 3 form seven families of distinct large multiprotein complexes that regulate gene expression via deacetylation of lysines in histone tails. The degree of redundancy and functional overlap between complexes and their primary gene targets, remains unknown. We used CRISPR/Cas9 to create six cell lines in which individual HDAC complexes were tagged with FKBP12F36V enabling rapid (<1 hr), PROTAC-mediated, degradation. RNA sequencing at 6 h reveals that together, the 4 major complexes (CoREST, NuRD, NCoR/SMRT and SIN3A) perturbed >50% of expressed genes. More than 60% of these are specific to an individual complex. Of genes regulated by more than one complex, approaching 50% are reciprocally regulated such that HDAC complexes act as antagonistic regulators. Homer analysis strongly suggests that the complexes are reliant on different transcription factors. This is the first study to identify the primary targets of individual HDAC complexes and directly compare the effects of rapid degradation on gene regulation in the same biological system.

O9 - Understanding the role of senataxin in the resolution of transcription-replication conflicts

Dr Isabel Díez Santos

Transcription and replication are essential cellular processes that compete for the same DNA template, requiring precise coordination to avoid conflicts that threaten genome stability. The mechanisms to resolve transcription-replication conflicts remain elusive, although it is proposed that senataxin, a helicase enzyme, plays a pivotal role by displacing RNA polymerase from the DNA.

To investigate the role of senataxin in mitigating transcription conflicts, we used budding and fission yeast models and DNAscent, a single-molecule method to detect replication fork dynamics. In a budding yeast senataxin mutant that does not interact with the replisome (sen1-3), preliminary results show replication pauses at locations associated with high RNA polymerase II occupancy. In fission yeast, which has non-essential senataxin homologs (sen1 and dbl8), sen1-3 mutants have a higher recombination rate than WT cells at a highly transcribed gene. These findings suggest that senataxin may help resolve transcription-replication conflicts at specific genomic loci. Single-molecule methods are therefore essential to find these rare events. Future work will map replication fork pausing across the genome in fission yeast senataxin mutants.

O10 - Targeting PRMT5-mediated splicing: A new therapeutic opportunity for triple negative breast cancers

Dr Kelly Chiang

One of the major clinical challenges in the treatment of triple-negative breast cancer is the lack of molecular targets. Consequently, the prognosis for patients of this cancer subtype remains poor, with a high proportion of patients suffering from relapse and metastasis.

The major symmetric arginine methyltransferase, PRMT5, has been shown to be important for many fundamental cellular processes including splicing. We have previously shown that PRMT5 is critical for the survival and function of breast cancer stem cells (BCSCs). One of the hallmarks of BCSCs is chemoresistance which drives relapse and metastasis. More recently we have described a role for PRMT5 in driving chemoresistance in ER+ and Her2+ breast cancers, particularly within the breast cancer stem cell. Mechanistically, this is through PRMT5-regulated splicing of DNA repair genes.

Here we assess whether inhibiting PRMT5 in triple-negative breast cancer stem cells can identify new vulnerabilities in DNA repair caused by aberrant splicing, which could be targeted through combination therapies with PRMT5 inhibitors.
Alternatively, discovery of aberrant splicing events which enhance chemosensitivity could be exploited through antisense oligonucleotide therapy.
Further insight into these splicing vulnerabilities and how they are regulated by PRMT5 could reveal new therapeutic opportunities for this clinically challenging disease.

O11 - The chromatin remodeller ARID1A functions through FOX and AP-2 transcription factors in lobular breast cancers

Miss Naomi Eastwood

Invasive Lobular Carcinoma (ILC) is known to have a higher prevalence of mutations in the SWI/SNF chromatin remodelling subunit ARID1A. SWI/SNF mutations are common in cancers and are highly context specific. This study aimed to investigate the context-specific role of ARID1A in ILC to better understand the epigenetic regulation of key transcriptional pathways in this understudied cancer context. Knockdown (KD) of ARID1A in ILC cell lines significantly reduces cell viability, increases apoptosis, and decreases cell migration. RNA-seq analysis found that ARID1A KD results in transcriptional changes in a subset of oestrogen response genes but is insufficient to completely disrupt oestrogen response in lobular cells. Chromatin accessibility profiling found ARID1A to be particularly important for maintaining chromatin opening at sites containing Forkhead box (FOX) and AP-2 transcription factor (TF) motifs. ChIP-seq data confirmed the presence of ARID1A binding along with its partner protein BRG1/SMARCA4 at genomic regions containing these transcription factor motifs and demonstrated co-occupancy with FOX TFs. Examination of the transcriptional effects of these factors further establishes functional similarities of ARID1A with FOX and AP-2 TFs. This data suggests a mechanism whereby ARID1A controls gene expression programs in lobular cancers in a FOX and AP-2 dependent manner.

O12 - Ubiquitous transcription factors MEIS actuate lineage-specific transcription to establish cell fate

Dr Nicoletta Bobola

Control of gene expression is generally mediated by combinations of transcription factors (TFs). This cooperative action allows the integration of multiple biological signals at regulatory elements, resulting in highly specific spatial and temporal gene expression patterns. It remains unclear whether combinatorial binding is also required to assemble TFs with distinct biochemical functions, that collaborate to effectively recruit and activate RNA polymerase II. MEIS homeodomain proteins belong to the three amino acid loop extension (TALE) superclass, which includes largely ubiquitous, evolutionary ancient TFs characterized by an atypical homeodomain. Using a cardiac differentiation model, we find that MEIS TFs act as actuators, fully activating the transcriptional programs selected by lineage-restricted TFs. The combinatorial binding of MEIS with lineage-enriched TFs, GATA and HOX, provides selectivity and directs MEIS to function at cardiac-specific enhancers. At these enhancers, MEIS promotes accumulation of the methyltransferase KMT2D to initiate lineage-specific enhancer commissioning. The dynamics of MEIS combinatorial binding, regulated by the changing levels of its partners, drive cells through progressive stages of differentiation. Our results uncover tissue-specific transcriptional activation as the result of ubiquitous actuator TFs harnessing general transcriptional activator at tissue-specific enhancers, to which they are guided by binding with lineage- and domain-specific TFs.

O13 - NELF prevents genomic instability by regulating RNA polymerase II transcription elongation and termination

Dr Michael Tellier

Regulation of RNA polymerase II (Pol II) transcription is tightly coupled to cellular proliferation, yet how transcriptional programmes are reconfigured in cancer remains unclear. Here, we show that NELFCD, encoding a component of the negative elongation factor complex, is upregulated in colorectal tumours. To investigate the direct role of NELF-C in cancer-associated transcription, we used an auxin-inducible degradation system combined with nascent transcript sequencing in a colorectal cancer cell line. Acute depletion of NELF-C increases Pol II transcription elongation rate, impairs recruitment of the mRNA cleavage and polyadenylation complex recruitment at the 3’end of genes, and induces widespread transcriptional readthrough. These defects result in Pol II transcribing into DNA replication zones, triggering transcription-replication conflicts and DNA damage that drive cells into a quiescent state. Our findings uncover a key role for NELF-C in regulating transcription elongation and termination to prevent genomic instability. Our data also suggest that targeting NELF could represent a therapeutic strategy for limiting proliferation of colorectal cancer cells

Poster Presenter Abstracts

P1 - Investigating the regulation of E3 ubiquitin ligase TRAIP in DNA replication

Ms Chongluan Shi

Accurate DNA replication is essential for maintaining genome integrity and stability. TRAIP is an E3 ubiquitin ligase that plays a critical role in this process by ubiquitylating replisome components, facilitating their removal from DNA. In human cells, TRAIP is needed to resolve replication–transcription conflicts during S phase and deal with DNA replication stress. In Xenopus laevis egg extract, TRAIP is essential for removal of replisome when replisome encounter interstrand crosslink (ICL), and ICL repair through the NEIL3 pathway or Fanconi anaemia (FA) pathways. TRAIP also ubiquitin replisomes which retained on DNA until mitosis leading to its removal. Due to this function, TRAIP is essential for processing of under-replicated DNA in mitosis (MiDAS). Pathogenic mutations in TRAIP, such as R18C and R185X, are associated with primordial dwarfism, highlighting its importance in human development. However, the regulation of multiple functions of TRAIP in S phase and mitosis in human cells remains not understood. Our aim is to investigate how patient-derived TRAIP variants impact its function in genome maintenance.

Using the 100,000 Genomes Project (100KGP) and The Cancer Genome Atlas (TCGA), we identified potentially pathogenic TRAIP variants using the SIFT and PolyPhen predictive tools. Five variants were selected and introduced into an engineered HCT116 cell line where endogenous TRAIP can be conditionally depleted using an auxin inducible degron system.

Previous work in our lab demonstrated that TRAIP is essential for cell viability and cell cycle progression. TRAIP depletion leads to inhibition of proliferation and G2/M arrest and re-expression of wild-type TRAIP, but not RING domain mutant (C7A), rescues these phenotypes.

We assessed the ability of patient-derived TRAIP variants to rescue these defects. Colony formation assays revealed varied effects on cell proliferation and viability, while all variants were able to rescue the G2/M arrest to levels comparable to wild-type TRAIP. We will present the progress of our work in analysing the effects of chosen mutations on different TRAIP functions.

P2 - The role of protein interactions in DNA double strand break in chromatin

Mrs Mona Elmokadem

DNA double strand breaks are the most crucial type of DNA damage to the cell that is if left unrepaired it leads to mutations and chromosomal arrangements that further lead to the predisposition of cancer. DSB are repaired via two common pathways, the HR and the NHEJ pathway. NHEJ pathway is error prone pathway that however is mostly used to repair DSB by the cells.

Previous Work done by our lab found that a mixture of all somatic linker histones was able to inhibit ligation step in the NHEJ pathway. Further studies showed that only one linker histone was able to stimulate it by binding to the LX complex. Depletion of the linker histone variant led to the accumulation of DSB and delay in cell cycle progression in response to ionizing radiation.

Co immunoprecipitation and mass spectrometry studies showed that the linker histone variant has wide and complex proteomic interactions. We aim to study the role of these protein partners in the DSB repair and genome stability maintenance by addressing the phenotypes associated with them and post translational modifications.

P3 - Genomic profiling of oncohistone H4R3C and its links to genome instability

Miss Jemma Holland

Histones are encoded by multiple genes; it has long been assumed mutations to a single allele would not impact cellular function. Cancer associated histone mutations, also termed ‘oncohistones’, have challenged this theory, providing insight into the transformative effect of a single mutation.

These mutations are particularly prominent at residues that undergo post translational modifications (PTMs). One such putative oncohistone residue is H4R3C. This is interesting as H4R3 is targeted for methylation by protein arginine methyltransferases (PRMTs) 1 and 5, catalysing asymmetric and symmetric methylation respectively. Here, H4R3me2a by PRMT1 is thought to drive gene activation, whilst H4R3me2s by PRMT5 is a repressive epigenetic mark. Since little is understood about the role of arginine methylation of histones in cancer, we are using the H4R3C oncohistone as a biological tool to distinguish between methylation dependent and non-methylation dependent transcriptional events.

Moreover, H4R3C expression leads to genome instability, however we do not know if this is enriched at particular loci. Here we investigate the effects of H4R3C expression on global transcription and in that of individual genes using RNA-seq. Using ChIP-Seq, we also show for the first time H4R3C localisation in the genome and if this correlates with markers of DNA damage.

P4 - Unravelling mechanistic insights between X-Chromosome inactivation dysregulation and female-biased mitochondrial dysfunction underlying autoimmunity

Dr Alkisti Manousaki

X-chromosome inactivation (XCI) is a key mechanism that balances X-linked gene dosage. Incomplete silencing has been implicated in female-biased autoimmune diseases such as Systemic Lupus Erythematosus (SLE) and Long Covid, where immune regulators like TLR7 are overexpressed. These diseases also feature mitochondrial dysfunction, yet the link between XCI dysregulation and impaired metabolism remains unclear.

We propose that XCI erosion drives upregulation of X-linked genes, affecting mitochondrial gene expression and function. To investigate this, we first analysed publicly available transcriptomic datasets from autoimmune diseases. We identified consistent upregulation of X-linked mitochondrial transcripts, with nearly 20% showing increased levels in lupus and other XCI-disrupted conditions. Using an integrated pipeline combining 3D RNA/DNA FISH, super-resolution microscopy (SR-SIM), and mitochondrial imaging, we next examined female patient-derived lupus and lymphoma cell lines. Preliminary findings reveal fragmented mitochondrial networks and disrupted ultrastructure, accompanied by upregulation of immune-related and mitochondrial X-linked genes, consistent with other disease states such as Long Covid.

Future work will correlate gene expression with cellular ultrastructural changes to explore causality and define molecular signatures of autoimmune disease.
This study offers mechanistic insight into metabolic dysfunction in autoimmunity and its link to increased female susceptibility, with the potential to inform novel therapeutic targets.

P5 - Histone H4R3C: A new oncohistone that drives genomic instability

Dr Gemma Regan-mochrie

Recently described oncohistones, or histone mutations found in cancer, provide a unique tool to explore the role of specific histone post translational modifications when they occur at modified residues. We are interested in histone modifications by protein arginine methyltransferases (PRMTs) and how these modifications affect protein recruitment, the DNA damage and stress responses, and replication. We focus on the methylation of Histone H4 at H4R3. This site can be modified by both PRMT1 and PRMT5 which are responsible for asymmetric and symmetric demethylation respectively. Here we explore the role of H4R3 methylation using the cancer associated mutation H4R3C as a tool to both better understand the fundamental function of modifications at this site, as well as how this mutation contributes to oncogenesis. We find that this mutation, similarly to other oncohistones, has dominant negative effects. When expressed at a lower level than endogenous H4, H4R3C leads to spontaneous DNA damage and increased susceptibility to DNA damaging agents. We compare this amino acid mutation to more conservative changes to dissect the biochemical properties that are necessary to cause the observed spontaneous DNA damage. Finally, we begin to explore a possible role for this modification in R-loop resolution.

P6 - The impact of the SWI/SNF complex subunit ARID1A on the chromatin environment surrounding stalled replication forks

Dr. Nagham Ghaddar

The BAF complex is one of the three SWI/SNF chromatin remodelling complexes. ARID1A is a defining subunit of BAF, and it is commonly mutated in cancer. BAF plays a role in fundamental biological processes such as transcription and DNA repair. Growing evidence shows that ARID1A loss is associated with increased replication stress and sensitivity to ATR inhibitors. During replication stress, the replication forks (RFs) are remodelled to allow proper repair and restart; therefore, maintaining genome stability. All these processes occur in a crowded chromatin environment that also regulates the choice of DNA repair.

For that reason, we sought to investigate the role of BAF at stressed RFs by monitoring chromatin states at acute stalled and/or restarted RFs in ARID1A proficient or deficient cells. In parallel, we performed a subcellular proteomic analysis to compare protein dynamics on chromatin in the presence or absence of ARID1A under conditions of replication stress. Interestingly, we found that features of heterochromatin are associated with ARID1A loss at stressed replication forks and these are maintained following release from acute fork stalling. Our findings suggest that ARID1A aids in promoting a chromatin environment that allows replication fork restart.

P7 - Covaris truCOVER Library Prep: A Streamlined WGS Workflow for Enhanced NGS Library Performance and Data Quality

Dr Erik Söderbäck

As whole-genome sequencing (WGS) transitions from research to clinical use, optimization of workflows for precision, scalability, and efficiency is essential. Key challenges such as inconsistency in DNA fragmentation, suboptimal library conversion rates, and cumbersome optimization for sample input variation, needs to be addressed for high quality data. Covaris new truCOVER WGS NGS library preparation kit provides a solution. Here, DNA from any source, including blood, saliva, and FFPE, can be used in a single-vessel standardized workflow in which Covaris' Adaptive Focused Acoustics® (AFA®) technology is optimized to achieve precise library fragment sizes optimized for sequencing. The PCR-free solution allows DNA input from 50ng to 500ng, while the use of the truCOVER Amp module allows input down to 100pg DNA.

The truCOVER solution integrates AFA-based DNA shearing, end-repair and adapter ligation, and a post-library size selection that results in reproducible and controlled insert sizes. It consistently delivers highest performance based on any evaluation parameters (e.g. fragmentation bias, conversion rates, median insert sizes, and variant detection accuracy) across diverse sample types, and eliminates the need for post-library QC steps like qPCR. This approach reduces workflow complexity, shortens turnaround time, enhances overall performance, and allows for better economy compared to competitor workflows.

P8 - An expanded repertoire of germline variants in known cancer predisposition genes are predicted to be pathogenic by machine learning in childhood and TYA cancers

Dr David Barnes

An urgent unmet need in screening children, teenagers and young adults for germline variants predisposing to cancer is the uncertain status of variants of unknown significance (VUS). We have previously identified participants in the National Genomics Research Library (NGRL) with a cancer aged under 25 (cohort = 1481) as carriers of known pathogenic or likely pathogenic (P/LP) variants (defined by ClinVar), in 249 genes included in Genomics England's cancer panels. We also identified variant carriers in 30,680 controls in NGRL and tested variants for enrichment in the cancer cases. We have now used the machine learning tool, AlphaMissense, to identify carriers of variants predicted to be LP in the same cancer predisposition genes. 41 participants with cancer (2.8%) carried one of 38 significantly enriched ClinVar P/LPs in 16 genes and 104 participants (7.0%) carried one of 96 significantly enriched variants in 33 genes, predicted to be LP by AlphaMissense. Of the 38 ClinVar P/LPs, 33 (87%) were also predicted to be LP by AlphaMissense. The 67 variants predicted to be LP by AlphaMissense alone include 43 VUS from ClinVar and 24 variants not present in ClinVar. This approach identifies a significant number of additional possible cancer predisposition variants.

P9 - The radiobiological impact of protons in combination with DNA double strand break repair inhibitors in glioblastoma models

Dr Alice Ormrod

Glioblastoma multiforme (GBM) has dismal survival prospects (~14.6 months) due to limited efficacy of conventional X-ray radiotherapy and chemotherapy. Whilst proton beam radiotherapy is superior to X ray due to delivering maximum energy targeted to the solid tumour with a low entrance dose sparing healthy tissues, the optimal use of protons in GBM treatment has yet to be realised.

GBM stem-like cells (GSCs) reside in a hypoxic niche. With unlimited proliferative capability and increased radioresistance GSCs and hypoxic cells can drive GBM. Therefore radiosensitising strategies are required to effectively target this population and increase survival for GBM patients.

We show inhibition of key DNA damage response (DDR) pathway proteins combined with low-LET photons and protons significantly reduces GBM cell survival compared to radiation alone in both bulk and stem-like conditions. In hypoxic conditions some GBM cell lines gain further radioresistance to proton and photon radiation treatment. This altered response, which can be partially overcome with DDR inhibition, has been observed in both monolayer and 3D spheroid models. We have investigated molecular mechanisms that may underpin the altered GBM cellular hypoxic response to irradiation and have identified possible targets for combination therapy, offering an exciting novel therapeutic strategy for GBM patients.

P10 - Unbiased detection of replication initiation events in human cells using BrdU incorporation and single-molecule nanopore sequencing

Dr Eduardo De La Vega

The identification of replication initiation sites in mammalian cells has been challenging and limited by the resolution of population-based methods. Here, we present methods for detection of replication initiation events in human cells using BrdU incorporation combined with single-molecule nanopore sequencing, and for Cas9-targeted enrichment sequencing (nCATS) at specific genomic loci. Increases in BrdU incorporation allow us to measure DNA replication dynamics, including replication initiation, fork direction, and termination, on individual single molecules at a high resolution, throughout S-phase and genome-wide. Whilst targeted enrichment can resolve local replication architecture and identify common and rare initiation events at defined genomic loci, that are missed by population-level methods. We also show implementation of subtle adjustments in BrdU addition regimes, prior to single molecule sequencing, could aid an improved detection of DNA replication dynamics in mammalian cell lines.

P11 - Investigating the impact of NELF-C on transcriptional dynamics and nuclear features

Miss Abby Gardner

Promoter proximal pausing of RNA polymerase II (RNAPII) is a key step in metazoan transcription. An essential factor in this process is negative elongation factor (NELF) protein complex. NELF is made up of 4 subunits (NELF-A, NELF-B, NELF-C/D & NELF-E), each of which have been shown to have unique functions. For example, NELF-C degradation has previously been shown to cause cell cycle arrest & readthrough of a subset of genes past traditional transcription termination sites (Nakayama et al., 2024). Here super resolution fluorescence microscopy has been used to gain a detailed view of nuclear changes upon NELF-C degradation including RNAPII dynamics, nuclear organisation (splicing speckles & nucleoli), epigenetic features etc. As wells as nascent transcripts of readthrough genes to visualise their localisation within the nucleus upon NELF-C degradation.

NAKAYAMA, C., DAIGAKU, Y., AOI, Y., FANG, Q., KIMURA, H., SHILATIFARD, A., TELLIER, M. & NOJIMA, T. 2024. NELF coordinates Pol II transcription termination and DNA replication initiation. bioRxiv.

P12 - Benzamide-Derived PROTACs induce Class I HDAC degradation in HCT116 cells

Miss AlAnood Waslullah AlHjajji

Class I histone deacetylases (HDAC1/2/3) are critical epigenetic regulators that modulate chromatin architecture and gene expression via deacetylation of histone and non-histone proteins. These enzymes function within multiprotein co-repressor complexes and are essential for cell proliferation and survival, making them attractive targets for cancer therapy. While traditional HDAC inhibitors lack isoform selectivity and can lead to off-target effects, proteolysis-targeting chimeras (PROTACs) offer a precise strategy for HDAC degradation.

This study investigates the efficacy of benzamide-derived PROTACs (JPS004, JPS062, and JPS06) in HCT116 colorectal cancer cells. Each PROTAC links an HDAC-binding moiety to a VHL E3 ligase ligand via a 12-carbon alkyl linker. JPS065 emerged as the most potent and selective, inducing robust degradation of HDAC1 and HDAC3, while exerting minimal impact on HDAC2, as confirmed by Western blotting.

Treatment with JPS065 significantly increased histone acetylation levels (H2BK5ac and H3K27ac), disrupted co-repressor gene networks (e.g., RCOR1, CHD4), and promoted apoptosis without major alterations in cell cycle distribution. RNA-seq profiling revealed over 2,600 differentially expressed genes (1,382 upregulated; 1,238 downregulated), including marked repression of DNA replication and cell cycle genes (e.g., MCM2-10, CDC6, CDK2). Notably, JPS065 uniquely modulated 680 genes, reflecting a distinct transcriptional footprint. HDAC1 and HDAC3 mRNA levels remained largely unchanged, indicating post-transcriptional degradation.

Compared to JPS004 and the HDAC inhibitor CI994, JPS065 induced stronger epigenetic and transcriptional effects while JPS062 showed minimal activity. These findings highlight JPS065’s superior ability to selectively degrade HDAC1 and HDAC3, reprogram chromatin states, and induce apoptosis in colorectal cancer cells through a mechanistically distinct pathway.

In conclusion, JPS065 represents a promising next-generation HDAC-targeting therapeutic, offering enhanced specificity and biological precision compared to conventional inhibitors, and holds potential for advancing epigenetic cancer therapies.
JPS065 demonstrated superior efficacy in degrading HDAC1-3, inducing apoptosis, and enhancing histone acetylation in HCT116 colorectal cancer cells, outperforming both CI-994 and an earlier PROTAC, JPS004. In contrast, JPS062 showed reduced activity, suggesting that the 4-pyridine ring may hinder degradation efficiency. Transcriptomic analysis revealed that treatment with these PROTACs downregulated genes linked to cell cycle and DNA replication. Additionally, differential expression of zinc finger protein genes indicated their involvement in regulating proliferation, apoptosis, and stress responses.

In conclusion, this study presents novel HDAC-targeting PROTACs that effectively induce apoptosis and modulate gene expression in cancer cells, offering promising tools for targeted therapy and mechanistic studies in oncology.

P13 - The where, when and how of DNA repair in quiescent cells

Dr Rosa Camarillo Daza

During the cell cycle, DNA is exposed to various sources of damage that can compromise genome integrity and promote cancer development. In response, cells typically activate checkpoints that delay cell cycle progression until damage is repaired. However, increasing evidence shows that low levels of replication-associated DNA damage may not halt the cycle. Instead, such damage can be transmitted to daughter cells during mitosis. In G1, these inherited lesions are sequestered into 53BP1 nuclear bodies (NBs), and cells often enter a p53–p21-dependent quiescent state. Although this process prevents immediate propagation of damaged DNA, the mechanisms by which these lesions are repaired in daughter cells remain poorly understood.

Using immunofluorescence and live-cell imaging, we study the fate of 53BP1-NBs in quiescent cells. Contrary to previous assumptions, we find that these structures can be resolved during G1 and not solely during S-phase. Inhibition of G1-specific repair pathways, such as non-homologous end joining (NHEJ), delays NB dissolution, suggesting repair during G1. Furthermore, live-cell imaging indicates that NB resolution dynamics vary depending on the cell cycle phase. Ongoing experiments aim to identify the genomic loci associated with 53BP1-NBs in quiescent cells and to determine which DNA repair pathways are engaged to resolve these persistent lesions.

P14 - Understanding the cellular DNA damage response in head and neck cancer cells treated with Boron Neutron Capture Therapy

Dr Maria Rita Fabbrizi

Boron Neutron Capture Therapy (BNCT) is a precision targeted radiotherapy technique for cancer, involving delivering boron compounds into cancer cells, and then subjecting to thermal neutron beams, causing the release of densely ionising (high-LET) helium and lithium ions. These high-LET ions drive the formation of highly complex DNA damage (CDD) which isn't efficiently repaired by cancer cells and therefore causes significantly enhanced biological effectiveness compared to conventional X-rays irradiation.

Despite this, the cellular DNA damage response to BNCT and the optimal compounds stimulating boron uptake are currently unclear. We have access to the only high flux accelerator-driven neutron source in the UK for BNCT. We are currently investigating the effects of BNCT on relatively radioresistant head and neck squamous cell carcinoma (HNSCC) cells treated with increasing concentrations of several boron-containing compounds. Preliminary results show drastically reduced survival in HNSCC treated with boron-containing compounds after neutron irradiation compared to control cells treated with water. Additionally, significant DNA damage persistence in boron-treated HNSCC cells irradiated with neutrons is observed, coupled with increased micronuclei formation. This investigation will lead to a more comprehensive understanding of the cellular response mechanisms in HNSCC following high-LET BNCT treatment.

P15 - Using in vitro stem cell models to study the biology of fusion genes during embryonic haematopoiesis

Dr Rachel Bayley

Paediatric blood cancers are the most common cancer in children, and the precise cause is often unknown. Fusion genes are a predominant feature and can result in the production of oncogenic fusion proteins. Fusion events are thought to occur in utero, however, are insufficient to induce cancer development until another genetic abnormality occurs after birth. Oncogenic fusions can also be detected in cord blood in the absence of disease, adding further complexity to their biology. We hypothesise that fusion genes contribute to physiological embryonic haematopoiesis and after development they can become oncogenic under certain conditions. To investigate this, we have studied murine embryonic haematopoiesis using foetal liver cells and embryonic stem cell derived hematopoietic progenitors. Analysis of publicly available RNAseq data indicated that fusion events could be detected in stem and progenitor cells at E14 and persist into adulthood.

We have also developed a lentiviral system to allow for inducible expression of the NPM-ALK fusion associated with anaplastic large cell lymphoma in haematopoietic cells. Overall, these in vitro models will allow us to study the biology of fusion genes at different stages of embryonic haematopoiesis to gain further insight into how they contribute to blood cancers in children.

P16 - Randomised control generation for Hi-C analysis by data distribution extrapolation.

Mr Raul Sanchez Pastor

3D chromatin conformation is essential for understanding gene transcription modulation. The Transcriptional condensates model states that DNA is organised in high transcriptional compartments (hubs), composed of topologically associating domains (TADs). TADS may form through liquid-liquid phase separation (LLPS) and be guided by loop conformation, triggered by the aggregation of distal loci with shared regulatory proteins, and thus similar transcription start site TSS promoter regions. Aggregate Peak Analysis (APA), based on Hi-C data, facilitates the identification of such structures (e.g. loops, TADs and chromatin compartments). However, the complex and structured nature of Hi-C data challenges the generation of randomised controls, limiting the accuracy of biological interpretations.

This study hypothesised that control datasets with a standard normal distribution that overlap with target gene subsets can improve APA resolution. This project aims to generate suitable controls that comprise the inherent data patterns, integrating transcriptional activity (Hi-C) and chromatin states (and CAGE-seq) datasets from HCT-116 cells. Statistical overlap detection, followed by machine learning approaches such as random forest classifiers, will be used to assess cluster similarity and structural correspondence. This approach aims to enhance Hi-C accuracy, its biological interpretation and ultimately the understanding of the underlying 3D chromatin mechanisms involved in transcriptional regulation.

P17 - RNF169 is implicated in the DNA replication stress response

Dr Panagiotis Kotsantis

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Perturbation of DNA replication fork progression causes replication stress, a major driver of genomic instability and cancer. The replication stress response activates repair pathways to facilitate replication restart, including fork reversal; a process mediated by DNA damage proteins like SMARCAL1. Reversed forks are vulnerable to nucleolytic degradation unless stabilised by RAD51 recombinase. Fork stability is important in cancer biology, as it contributes to chemoresistance in certain cancers. A key source of replication stress is R-loop-mediated fork stalling. R-loops occur during transcription, and although they support essential cellular processes, their persistence can trigger replication stress. Recently, R-loops were shown to protect stalled forks from resection.

To explore replication stress regulation, we conducted two unbiased proteomic screens and identified the E3 ubiquitin ligase RNF169 as a novel R-loop-proximal protein that interacts with SMARCAL1 and is involved in the replication stress response. RNF169 loss causes SMARCAL1-dependent replication fork slowing, suggesting that RNF169 regulates SMARCAL1 activity. Additionally, RNF169 loss causes replication fork resection, increased sensitivity to hydroxyurea, and elevated micronuclei formation, indicative of genomic instability. Moreover, RNF169 loss increases R-loop levels.

Our findings suggest that RNF169 facilitates the replication stress response, potentially by regulating SMARCAL1 function and modulating R-loop dynamics.

P18 - Inhibition of PRMT5-mediated arginine methylation decreases Ewing sarcoma (ES) survival in an EWSR1-ETS-dependent manner by inducing DNA damage

Dr Emily Prowse

PRMT5 is an arginine methyltransferase with important roles in splicing and the DNA-damage response (DDR), processes which are deregulated by the EWSR1-ETS fusion protein that defines ES. PRMT5 function is reduced in cells lacking MTAP, a deletion found in various cancers. Second-generation PRMT5 inhibitors are in clinical trials for adult cancers, where PRMT5 is selectively inhibited in MTAP null tumor cells, thus providing a wider therapeutic index than first-generation non-selective PRMT5 inhibitors. Here, we aimed to determine the effectiveness of first and second generation PRMT5 inhibitors (GSK591 and AZD3470, respectively) in treating ES, elucidate the interaction between EWSR1-ETS and PRMT5, and to understand how PRMT5 inhibition influences the DDR. We determined that PRMT5 inhibitors reduce cell viability and that AZD3470 specifically targets MTAP null ES cells. In addition, we found sensitivity to AZD3470 and GSK591 depends on EWSR1-ETS. We observed increased 53BP1 foci in MTAP null cells when exposed to AZD3470 compared to the MTAP WT cells, indicating defective DDR. Together, our data suggests that ES depends on the interaction between EWSR1-ETS and PRMT5 for survival. We plan to determine the mechanism by which PRMT5 inhibition induces defects in the DDR and how PRMT5 regulates EWSR1-ETS.

P19 - The Structure of the core SMRT Complex

Dr Edward Brown

Class I Histone Deacetylases (HDACs) are key regulators of gene transcription, functioning within a network of protein factors that read, write, and erase histone modifications - known as the histone code. One such modification is lysine acetylation, which is associated with active transcription.

HDACs contribute to transcriptional repression by removing acetyl groups from lysine residues on histone tails. This acts as a signalling mechanism for other proteins and restores the positive charge of lysine, strengthening histone-DNA interactions, promoting chromatin compaction.

Class I HDACs function within seven distinct multi-protein complexes, which are essential for their activity. Six of these contain HDAC1/2, while HDAC3 is uniquely found in the SMRT/NCoR complex.

The core SMRT/NCoR complex includes the N-terminal region of SMRT bound to HDAC3, TBL1, and GPS2. Its functions vary depending on interaction partners at the SMRT C-terminus, such as roles in nuclear receptor repression and X-chromosome inactivation. Previous work revealed a higher-order inositol phosphate bound at the HDAC3–SMRT interface, acting as an essential activator.

While structures of subcomponents have been studied, the complete architecture of the core SMRT complex remains unresolved. Here, we present the structure of the core SMRT complex and investigate the role of a potential inhibitory peptide.

P20 - Endogenous Purification and Structural Characterization of the NuRD Corepressor Complex Using CRISPR Knock-In in HEK293F Cells

Dr Oksana Gonchar

The Nucleosome Remodeling and Deacetylase (NuRD) complex is a multifunctional chromatin-modifying assembly that plays a critical role in gene expression in particular transcriptional regulation, maintaining genome integrity and development. In this study, we focus on studying the functional and structural analysis of the endogenous NuRD complex in human cells. Using CRISPR/Cas9 system, we introduced FKBP-FLAG tags at the C-terminus of the endogenous MTA2 protein in human cells (HCT116 and HEK293F). This strategy allowed homozygous integration of the tags. The FKBP tag allows rapid and efficient protein degradation for functional studies while FLAG tag allows selective affinity purification of NuRD complex.

A series of immunoprecipitation experiments and mass spectrometry analysis confirmed the co-purification of core subunits including MTA2, HDAC1, CHD4, and RBBP4. Although buffer optimisation and enzymatic treatments did not significantly enhance the yield, they preserved complex integrity, which is crucial for downstream structural work. The project is at the stage of extensive optimization of sucrose gradient (GraFIX). The purified endogenous NuRD complex will be subsequently subjected to cryo-electron microscopy (cryo-EM) analysis with the aim of determining its high-resolution structure. This work lays the foundation for understanding the architecture of the native NuRD complex and its mechanistic roles in chromatin remodelling.

P21 - Using a BRCA1/BARD1 gap-suppression separation of function mutant to elucidate mechanisms of therapy responses

Dr Elizabeth Anthony and Ruth Densham

Elizabeth J. Anthony, Ruth M. Densham, Ann Liza Piberger, Hannah Mackay, George Ronson, Isobel Bonning, Jo R. Morris.

Inheritance of mutations in the breast cancer type 1 susceptibility BRCA1 gene confers a high risk of developing breast and ovarian cancer. BRCA1/2-mutated cancers are regularly treated with poly (ADP-ribose) polymerase inhibitors (PARPi), but novel therapeutic approaches are necessary to overcome PARPi resistance. BRCA1/2-mutated cancers are complex and typically exhibit deficient DNA double-strand break repair by homologous recombination (HR) and are subjected to replication stress such as poor fork protection and accumulate post-replicative single-stranded DNA (ssDNA) gaps.

To enhance our understanding of specific contributions of replication gaps to therapy sensitivity, we have identified a separation-of-function mutation in BARD1 that exhibits post-replicative ssDNA gaps, but has no detected defect in HR-mediated repair of DSBs. Using these cells, we hope to understand the contribution of gaps to therapy responses. In common with full BRCA1 mutants, we find these cells exhibit reduced fitness after loss of USP1 which can sensitise our cells to PARPi. We will present data that suggests chromatin regulation is key to BRCA1-BARD1-mediated gap suppression.

P22 - The lysine methyltransferase SETD1A promotes ssDNA gaps formation under replication stress

Dr Olga Brieieva

An inability to resolve replication stress represents a major driver of genome instability. It can lead to the accumulation of single-stranded DNA (ssDNA) gaps, which can be deleterious if left unrepaired and may contribute to therapeutic sensitivity. Despite increasing understanding of the molecular pathways that mitigate replication stress, the role of epigenetic regulation in these processes remains poorly characterized.

Previously, we showed that the lysine methyltransferase SETD1A is required for maintaining replication fork stability and DNA repair. Here, we examine whether SETD1A is also involved in the formation/processing of ssDNA gaps. We determined that such gaps induced by Olaparib and Hydroxyurea were abolished following depletion of SETD1A from both BRCA1- and ATM-deficient cells. Mechanistically, we found that loss of SETD1A compromised the recruitment of gap-promoting factor PRIMPOL to chromatin. Furthermore, we observed that gap formation in SETD1A-deficient cells was restored after inhibition of the lysine demethylase KDM1A. This may contribute to the fact that cells lacking SETD1A alongside BRCA1 or ATM are resistant to PARP inhibition, as previously reported by our group.

Together, these results demonstrate that SETD1A and KDM1A regulate ssDNA gap formation, highlighting the potential of targeting epigenetic factors for improving the therapeutic efficacy of anti-cancer agents.

P23 - Understanding the role of PRMT5 in the DNA damage response in MTAP-deficient lung cancer cells

Miss Ciara Ward

Accurate DNA damage repair (DDR) is essential for maintaining genome stability and the prevention of diseases such as cancer. The protein methyltransferase PRMT5 is - associated with DDR and splicing with multiple studies indicating a role in double strand break (DSB) repair and the replication stress (RS) response. MTAP deletion, a common event occurring in ~12% lung cancer due to its proximal location to the commonly deleted tumour suppressor gene CDKN2A, has been identified as providing tumour cell sensitivity to MTA cooperative PRMT5 inhibitors. This is because MTAP deletion leads to the accumulation of the metabolite MTA that is an endogenous PRMT5 inhibitor. Since MTAP null cells are viable even with compromised PRMT5 activity, this genetic background provides us with a model to identify critical PRMT5 targets and activities involved in DDR or RS. Here, we have found, through a combination of cell titre glo assays, clonogenic assays and immunofluorescence, that loss of MTAP increases spontaneous DNA damage and confers sensitivity to DNA damage signalling inhibitors and Polθ inhibition. Our data thus identifies a novel role for PRMT5 in the back-up theta-mediated end joining pathway.

P24 - XIST supercomplexes- key actors in X-inactivation during early human embryonic development

DR. Kristupas Sirvydis

The formation of heterochromatin, the silenced part of the genome, is essential for normal embryonic development and cell type-specific phenotypes. The dysregulation of epigenetic gene-silencing mechanisms is a hallmark of various diseases, including cancer. However, the requirements for the transmission of heterochromatin and maintenance of heritable epigenetic memory remain poorly understood. The inactivation of the X chromosome in female mammals triggered by the RNA XIST provides a model system to study these fundamental gene-regulatory mechanisms. Here we integrate human stem cell models, quantitative super-resolution structured illumination microscopy and gene expression profiling to investigate epigenetic and transcriptional changes involved in heterochromatin formation during the inactivation of one X chromosome. We identify the formation and function of XIST supercomplexes (SMACs) consisting of XIST RNA and numerous epigenetic factors, including histone deacetylases. We show how changes in the composition of SMACs drive the propagation of gene silencing and higher-order chromosome changes.

P25 - Deciphering cell-type specific inter-chromosomal genome architecture via single-cell Hi-C and machine learning

Mrs Maryam Eftekharifar

The 3D organisation of the genome is critical for gene regulation and cell fate. While intra-chromosomal interactions are well-studied, inter-chromosomal contacts are an over-looked layer of nuclear organisation that may encode cell identity. We aimed to characterise inter-chromosomal structural features distinguishing mouse embryonic stem cells (ESC), neural progenitors (NPC) and mature neural cell types, thereby contributing to a deeper understanding of how 3D genome architecture relates to cellular identity. We analysed the allele-specific whole-genome 3D reconstruction of single cells from over 1,000 cells using single-cell Hi-C data, and computed chromosome territory features such as radial positioning and pairwise distances. After rigorous quality control and processing, we applied machine learning and statistical approaches to analyse how chromosome territories rearrange in neural development.

We will present our results on the discriminatory power of the 3D positioning of chromosome territories in neural development, and identify the most important features that discriminate between these cell types, and compare these to known regulatory networks such as the polycomb network in ESCs including the Hox gene clusters.

Keywords: Single-cell Hi-C, 3D Genome, Inter-chromosomal interactions, Machine Learning, Cell Fate

P26 - Identifying inter-chromosomal transcription regulatory networks from the 3D genome

Dr Csilla Varnai

The 3D organization of the genome plays an important role in how the genome functions, on different length scales. Chromatin loops can bring together regulatory elements and their target genes, and form topologically associating domains (TADs) at the sub-megabase scale.

Transcriptionally active and inactive parts of the genome segregate into nuclear compartments. The conformation of the 3D genome can be studied using genome-wide chromosome conformation capture techniques (Hi-C) which allow the analysis of chromatin contacts genome-wide. To date, intra-chromosomal contacts have been analysed extensively and their analysis has been used to first describe nuclear compartments and TADs. However, inter-chromosomal contacts have largely been ignored because of their sparsity and the noise in Hi-C experiments.

Single-cell Hi-C allows whole-genome 3D reconstruction of chromatin, and the resulting 3D models enable us to bioinformatically increase data with reduced noise. I will present our recent work on mouse embryonic stem cells. By mapping transcription regulator binding data from ChIP-seq experiments onto the 3D models from individual F1 hybrid mouse ES cells, we construct the transcription regulatory networks of these transcription regulators. These include known as well as novel networks, highlighting a complex, well-orchestrated interplay between the 3D genome and gene regulation.

P27 - The role of UPF1 in transcription and translation in eukaryotic cells

Mrs Sahar Shahidkalhori

In my project, I will examine the role of the RNA helicase UPF1, which is widely recognised as the main player in the cellular process called the nonsense-mediated mRNA decay (NMD) pathway in the cytoplasm. This process degrades some, but not all, mRNAs that contain a premature termination codon (PTC). Although the primary function of this RNA helicase remains unclear, research so far has focused on UPF1's role in the cytoplasm and its influence on NMD, RNA translation, and protein quality control(Hwang et al., 2021). Nonetheless, the full extent of UPF1's role is still uncertain. As UPF1 moves between the cytoplasm and the nucleus, my project will explore its function within the nucleus, its association with nascent RNA, and the impact it has on transcription and translation through interaction with Pol II and the various stages of phosphorylation of the carboxyl-terminal domain (CTD). My research will be conducted using Drosophila melanogaster as a model. In my poster, I will demonstrate the association of UPF1 with highly transcribed genes by NGS data analysis from Drosophila melanogaster.

References

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P28 - Structure and Regulation of the H2B E3 Ligase Complex

Dr Ammarah Tariq

Bre1-like H2B E3 ligase complexes ubiquitinate histone H2B during transcription, which is required for downstream events including histone H3 methylation, chromatin remodelling, and mRNA export. Dysregulation of this histone modification has been linked to tumorigenesis, impaired cell differentiation, and developmental disorders. Despite its biological significance, the structural and mechanistic details of H2B E3 ligase function remain poorly understood. Although the role of the polymerase-associated factor 1 (Paf1) complex (Paf1C) in facilitating H2B K123 ubiquitination has been recognized for some time, the specific mechanism by which Paf1C contributes to this process has not been fully elucidated. Here, we combine cross-linking mass spectrometry (XL-MS), small-angle X-ray scattering (SAXS), cryo-electron microscopy (cryo-EM), in vitro and in vivo ubiquitination assays to characterise the architecture and regulatory features of the H2B ubiquitin ligase complex. The insights gained from this research will contribute to a deeper understanding of how ubiquitination affects chromatin dynamics and gene expression.

P29 - Combining Genome Engineering with Next-Generation Sequencing to Unravel the Functions of the NuRD Complex

Mr Tom Wright

Class I histone deacetylases (HDACs) are critical regulators of gene expression, catalysing the removal of acetyl groups from lysine residues present on histone tails. This activity is typically associated with transcriptional repression due to increased chromatin compaction, although more recent data suggests HDACs can also influence the activation of certain genes. HDACs function as the catalytic core of several transcriptional regulatory complexes, including the Nucleosome Remodelling and Deacetylase (NuRD) complex. Within NuRD, MTA proteins play a central scaffolding role, coordinating the assembly and positioning of other subunits. The incorporation of one of three MTA paralogs gives rise to different NuRD architectures with distinct stoichiometries and genomic targets.

My research investigates the unique and overlapping roles of MTA family members in NuRD-mediated gene regulation. Using CRISPR-Cas9, I have engineered HCT116 cell lines with endogenous, in-frame FKBP12F36V degron tags fused to MTA proteins, allowing rapid degradation via the dTAG system. By combining this system with next-generation sequencing methods, including RNA-Seq, ChIP-Seq and CUT&Tag, I aim to dissect the transcriptional, chromatin accessibility, and histone modification changes resulting from loss of individual MTA paralogs, and to understand the contribution of each paralog towards maintaining regular gene expression profiles.

P30 - A single cell RNA-seq analysis into Immunoglobulin expression and potential function in AML myeloblasts

Ms Eve Day

Until recently, immunoglobulin (Ig) proteins were considered to be produced exclusively by B lymphocytes. This view was challenged when Ig expression was detected in non-B lymphocytes, first in haematopoietic stem cells from umbilical chord blood and then in acute myeloid leukaemia (AML) cells.

AML is a cancer of myeloid (bone marrow derived) cells, marked by the rapid proliferation and build-up of irregular blood cells leading to disruption of normal haematopoiesis. Myeloid cells are distinct from the lymphoid lineage and thus assumed not to express Ig. However, mounting evidence suggests that a range of Ig genes and proteins may be expressed in some AMLs, with one study linking elevated levels to poorer clinical outcomes, implying a possible pathogenic role.

To date, no study has explored Ig expression using single-cell RNA transcriptome AML datasets. This project aims to explore the extent of Ig gene expression through analysis of a range of bone-marrow derived single cell datasets. If expression can be demonstrated this should provide insights into the role of Ig and perhaps aid as a novel biomarker for risk classifications or targeted therapies. Further, identifying genes co-expressed with Ig in AML myeloblasts may help reveal functional relevance and inform future research directions.

P31 - X Inactivation In Lymphocytes and Systemic Lupus Erythematosus

Ms Gina Rose

Women account for 80% of autoimmune disease cases worldwide. One key reason is the X chromosome, which harbours many immune-related genes. To balance gene dosage between males and females, X chromosome inactivation (XCI) occurs, producing a transcriptionally silenced X chromosome (Xi). This inactive X is characterized by heterochromatin markers, CpG methylation, and XIST-mediated silencing complexes.

Lymphocytes have dynamic XCI. During activation, silencing is partially lost and later re-established, losing heterochromatin and increasing transcription from the Xi. However, the molecular organization of effector proteins and associated 3D chromatin changes during this partial reactivation remain poorly understood.

Patients with systemic lupus erythematosus (SLE), XIST and associated silencing marks become delocalized from the Xi, leading to partial reactivation of X-linked genes.

I am working to characterize XIST silencing complexes in lymphocytes using super-resolution microscopy and compare the spatial organization and expression of key XCI components and immune-related genes in both healthy and SLE lymphocytes, to provide insights into the dynamics of XCI in immune function and autoimmune disease.

P32 - The Structure of the Xenopus laevis Replisome

Dr Paolo Passaretti

Cell division is essential for life and requires precise duplication of genetic material to maintain genome stability and protect against diseases such as cancer, genetic disorders, and premature ageing. Over the past decade, cryogenic electron microscopy (cryo-EM) has transformed our understanding of DNA replication by revealing detailed structures of the replisome, particularly highlighting the function of the replicative helicase during origin licensing, initiation, and termination. Most of these structural studies have focused on budding yeast and used in vitro assembled complexes from purified proteins. To expand this understanding in a more complex system, we developed an alternative approach using Xenopus laevis egg extract—a unique higher eukaryotic cell-free system that contains all the factors required for DNA replication. This ex vivo method enables the isolation of native replisomes under near-physiological conditions for structural analysis by cryo-EM. Using this system, we determined the structure of dimeric DONSON in complex with double CMG during replication initiation, and we are now working to capture replisomes at additional stages of DNA replication to further understand their dynamic assembly and regulation.

P33 - Ctdp1, a phosphatase at the intersection of transcription and cell viability

Mr Yagiz Ozturk

Transcription is a tightly regulated process essential for gene expression, and RNA Polymerase II (Pol II) plays a central role by transcribing protein-coding genes. Its largest subunit contains a C-terminal domain (CTD) with YSPTSPS heptapeptide repeats that undergo dynamic serine 2 and serine 5 phosphorylation during the transcription cycle. CTD phosphorylation is regulated by C-terminal domain phosphatase 1 (CTDP1/FCP1), which is also involved in cell cycle control and DNA damage response.

In this study, we investigated CTDP1 function in mouse embryonic stem cells (mESCs) using the dTAG system for rapid protein degradation (<1 hour). Acute CTDP1 loss caused a marked increase in total Pol II levels and hyperphosphorylation at Ser2 and Ser5. Western blot analysis showed ~40% reduction in H2BK5ac, while H3K27ac remained unchanged. RNA-seq revealed widespread transcriptional dysregulation. 5EU and EdU staining demonstrated reduced nascent RNA and replicating DNA levels, respectively, indicating impaired transcription and replication. Cell viability dropped by 50% after 24 hours and 80% after 48 hours of CTDP1 depletion.

We next aim to investigate CTDP1-associated chromatin and protein complexes using ChIP-seq and Co-IP approaches.

P34 - Uncovering the molecular stoichiometries of transcriptional silencing in X-inactivation

Miss Anna Devari

Nuclear organization is a key regulator of gene expression. Chromatin is organized into specialized domains containing either transcriptionally active or repressed genes. These compartments are formed by local concentrations of epigenetic factors, non-coding RNAs, and architectural proteins. The three-dimensional (3D) organization of chromatin is dynamically remodelled during embryonic development. X chromosome inactivation (XCI) is an essential developmental process that equalizes X-linked gene dosage between XX females and XY males by silencing one X chromosome.

Central to XCI is the long non-coding RNA Xist, which initiates gene silencing by recruiting effector proteins to the X chromosome. Our work has revealed that Xist achieves its regulatory role through the formation of nanosized supramolecular complexes (SMACs). SPEN is a major transcriptional repressor that recruits HDAC3 to halt transcription and initiate XCI. However, how local changes in the 3D molecular stoichiometries of transcriptional activators and repressors are orchestrated to convert an active chromatin compartment into an inactive one remains unclear. Here, we use super-resolution structured illumination and single-molecule microscopy, coupled with quantitative image analysis in stem cell models, to explore these spatial molecular relationships. We find that increasing the molecular density of SPEN progressively evicts RNA Polymerase II from X1linked genes, resulting in transcriptional silencing. This process occurs concomitantly with changes in chromosomal ultrastructure and compaction.

P35 - Profiling molecular changes driven by dietary substances in healthy and cancer colon epithelial cells

Dr Cristina Tufarelli

To provide information with implications for cancer preventive measures, we are gaining insights into the mechanisms through which substances in the diet can influence inflammatory responses and cancer risk by profiling molecular changes occurring in healthy and cancer colon epithelial cells in response to exposure to resveratrol - a naturally occurring polyphenol found in grapes, berries and nuts - and fructose. The two have opposite effects in animal experiments, whereby resveratrol ameliorates colitis [1] and can protect against tumorigenesis [2,3], while a high-fructose diet worsens colitis, increases the expression of inflammatory cytokines, and enhances the risk of colorectal tumour formation [4].

We have profiled in two-dimensional (2D) monocultures the changes occurring in response to exposure over time (1, 24, and 48 hrs) both at transcriptional (RNAseq) and cytokine production (Luminex on culture media) levels. Despite the lack of tissue complexity, we believe 2D cell culture systems represent a fast and economically viable method to gain valuable insights into the cellular responses to dietary substances that allow us to build novel hypotheses that can be tested in the future in more complex systems, e.g., organoids and/or patient-derived explants.

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2. doi: 10.1126/science.275.5297.218.
3. doi: 10.1126/scitranslmed.aaa7619.
4. doi: 10.1093/carcin/bgab007.

P36 - Upregulation of human RNase H2 counteracts replication stress elicited by oncogenes and chemotherapy drugs

Dr Rosanna Wilkins

RNase H2 is a heterotrimeric endoribonuclease that resolves genome-embedded ribonucleotides and RNA:DNA hybrids, both implicated in DNA replication stress and cancer. Targeting replication stress as a cancer therapeutic requires mechanistic understanding of how cancer cells tolerate increased RNA:DNA hybrids. Individual RNase H2 subunit protein and/or mRNA levels are elevated in certain cancers; however, the underlying mechanisms and consequences of RNase H2 upregulation remain unclear. We demonstrate that RNase H2 subunit protein levels are upregulated in response to oncogene- and chemotherapy-induced replication stress in human cancer and non-cancer cell lines.
Utilising inducible RNASEH2B overexpression systems to model increased active RNase H2 heterotrimer, we report that in response to chemotherapy drugs, camptothecin and hydroxyurea, RNASEH2B overexpression mitigates further increases in RNA:DNA hybrid levels and alleviates replication fork stalling, however, has little impact on cell survival. Instead, RNASEH2B overexpression has subtle effects on genomic instability and innate immune signalling.

In the context of oncogenic HRASG12V, RNase H2 upregulation is required to limit HRASG12V-induced RNA:DNA hybrid accumulation, replication fork stalling and cell death. Our findings provide new insight into RNase H2 functions, highlighting that RNase H2 subunit upregulation may be important indicators of the replication stress response to chemotherapy drugs and oncogenes in cancer.

P37 - SUMOylation promotes mitotic DNA damage repair

Dr Alexander Lanz

When DNA is damaged during mitosis the lesion can be preserved for repair in G1 or repaired during mitosis. These mechanisms are important for genome stability and present a cancer treatment opportunity. Mitotic cells are thought not to employ classical non-homologous end joining or homologous recombination repair pathways, instead repair of mitotic DNA double-strand breaks involves stabilising the broken ends by protein ‘tethers’ using CIP2A and TOPBP1 (Leimbacher et al., 2019; Adam et al., 2021), and Polθ-mediated repair (Gelot et al., 2024).

Here we present evidence that the SUMO conjugation pathway is critical to the repair of DNA breaks occurring in mitosis. Our data indicate that SUMO is a regulator of proteins that have not previously been implicated in the mitotic DNA-damage response, as needed prior to TOPBP1 filament assembly. We also identify the mechanisms of damage signalling to the SUMO conjugation machinery. The presentation will discuss the implications for SUMO E1 inhibitors, currently in clinical trials, for solid tumours.

P38 - Determining the dynamic arrangements of repair factors during homology dependent DNA repair by super-resolution microscopy

Dr. Stefan Koestler

Homology dependent DNA repair is an essential process that is orchestrated by repair factors whose interactions change over time. Their interactions have either been derived from genetic and molecular biology studies, or by high resolution, e.g. cry-electron tomography, studies of isolated or reconstituted protein complexes. However, deciphering the in-situ spatial arrangement of repair factors and their temporal rearrangement is crucial to understand the mechanism of the repair process. Standard diffraction-limited microscopy only produces images of repair foci, where signals of different proteins cannot be resolved and appear as overlapping blobs. Currently, in-situ studies of these structures, e.g. by super-resolution microscopy, are rare, due to the difficulties that the higher nuclear volume, as compared to the cytoplasm, poses. Here, we apply super-resolution expansion microscopy with markers for the different repair stages at different time-points after induction of DNA-damage by irradiation to determine the changing arrangements of repair factors.

P39 - The 7SK small nuclear ribonucleoprotein marries the cell responses to both transcriptional and replication stress by promoting homologous recombination and replication fork reversal

Dr Akhil Bowry

Replication stress in cells can lead to transcriptional stress, either through direct interference or by blocking transcriptional progression. The 7SK small nuclear ribonucleoprotein complex is a key regulator of transcription and plays an important role in responding to transcriptional stress. Individual components of the 7SK-snRNP—HEXIM1, LARP7, and MEPCE—have also been implicated in processes relevant to replication stress, including transcription-replication conflicts and homologous recombination at DNA double-strand breaks.

We investigated the roles of 7SK-snRNP components in the cellular response to replication stress, focusing on DNA damage and repair mechanisms. We report that HEXIM1 and LARP7 promote replication fork slowing in response to agents that induce both replication and transcription stress. Our data suggest that this fork slowing is primarily mediated through RAD51-dependent replication fork reversal and recruitment rather than being driven by transcription-replication conflicts. Importantly, our data also supports a role for HEXIM1 and LARP7 in promoting cell survival in response to replication stress-inducing agents.

Together, these findings support a model in which 7SK-snRNP regulation of RNA polymerase II activity enables effective RAD51 activation and function during transcription stress, thus coordinating cellular responses to both transcriptional and replication stress.

P40 - Exploring the link between Transcription and Translation in Fission Yeast

Miss Adedoyin Adeyemi

Eukaryotic gene expression is currently understood to be highly compartmentalised, with transcription and mRNA processing occurring in the nucleus, while translation takes place in the cytoplasm 1. However, emerging evidence challenges this view. For example, efficient nonsense-mediated mRNA decay (NMD), a translation-coupled mechanism, that degrades unproductive mRNAs containing a premature termination codon (PTC) within their open reading frame, relies on splicing, which takes place in the nucleus. This link between nuclear and cytoplasmic events is commonly explained by the Exon Junction Complex (EJC) model.

Contrary to this model, data from our laboratory show that splicing-dependent NMD does not require the EJC in Schizosaccharomyces pombe. Based on evidence that functional ribosomes are present in the nucleus, we propose an alternative hypothesis in which either a direct interaction between the spliceosome and a ribosome translocating on the pre-mRNA, or the translocating ribosome influencing transcription, is required for correct pre-mRNA processing.

Consistent with the hypothesis that ribosomes translate nascent RNA, we observed that inhibition of translation results in a dramatic reduction in the transcription of several genes in S. pombe.

Reference(s)
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P41 - Uncovering the roles of the peptidyl prolyl isomerase PIN1 in the replication stress response

Miss Poppy Conway-Thomas

Prevention of age-related diseases like cancer is reliant on the sustained integrity of the genome, determined by the continued fidelity of DNA replication. While DNA replication contains inbuilt mechanisms to safeguard fidelity, endogenous and exogenous stressors, in addition to aberrations in pathways that deal with replicative stress, can lead to the stalling of replication forks in a process known as replicative stress. Replication stress can result in increased mutational burdens, DNA breaks and chromosomal aberrations, driving tumorigenesis or cell death.

Proline isomerization, whereby prolines are isomerized between their cis and transforms by catalytic enzymes known as PPIases, is a key post translational modification that can alter the stability and function of proteins, including in the replication stress and DNA damage response. PIN1 is PPIase that binds to phosphorylated serine/threonine proline motifs (pS/T-P) to catalyse their isomerization, altering the function of key proteins involved in the replicative stress response. PIN1 is overexpressed across many cancers, correlating with poorer survival rates. Resultantly, PIN1 remains an attractive therapeutic target. However, its role and regulation during the replication stress response remains incompletely understood. Here, we aim to identify novel roles of PIN1, and novel regulation mechanisms of PIN1, during the replication stress response.

P42 - Comparative transcriptomics of Daphnia and Biomedical models for the evolutionary conservation of a gene network for fatty liver disease

Miss Shaleen Glasgow

Comparative evolutionary biology demonstrates that disease-associated genes are evolutionarily conserved across metazoans. The peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that regulates hepatic lipid metabolic processes in vertebrates. These metabolic processes can become perturbed by environmental pollutants, leading to fatty liver diseases and carcinoma. Rodents are primary models for human chemical safety testing due to our shared mammalian biology.

However, the importance of mammalian systems is being challenged by ethical and scientific considerations. Comparative transcriptomics facilitates our understanding of evolutionarily shared responses to toxicants and broadens the utility of alternative models in human health. The molecular networks governing liver fatty acid metabolism may be evolutionarily conserved in invertebrates and possibly predictive of adverse health in humans. Here, we show that the microcrustacean Daphnia possesses sequence orthologs of human genes for 70% of a PPARα signalling pathway.

Moreover, Daphnia signals human pathways regulated by PPARα when exposed to a PPARα agonist. Additionally, we use comparative single cell and nuclei transcriptomics of Daphnia and Zebrafish to investigate the tissue-specific responses to the PPARα agonist and identify homologous liver-like tissues in Daphnia. Through conserved molecular biology, this research proposes Daphnia as an alternative model for chemical safety testing of liver toxicity.

P43 - Dissecting the regulatory mechanisms governing the histone 3 lysine 9 methyltransferase Clr4

Dr Panagiotis Patsis

The formation of heterochromatin plays a crucial role in the regulation of genome repair, replication, and transcription. The lysine methyltransferases of the SUV39H family, which are evolutionarily conserved from fission yeast to humans, catalyse the methylation of histone H3 lysine 9 (H3K9me2/3), a hallmark of heterochromatin. In fission yeast, Clr4 is a highly conserved SUV39H methyltransferase that is solely responsible for the methylation of H3K9. Recent work from our laboratory and others has revealed that ubiquitination of H3K14 stimulates the methyltransferase activity of Clr4 towards H3K9. In this project, Nuclear Magnetic Resonance (NMR) and biochemical approaches will be used to study the regulation of Clr4. The techniques developed in this project will be used as a structural toolbox for investigating the regulation of Clr4 and of other SUV39 enzymes, to broaden our understanding into the mechanisms that underline heterochromatin formation.

P44 - Understanding oncogene-induced replication stress in tumorigenesis using HPV disease model

Dr. Christy Susan Varghese

Genomic instability is a hallmark of cancer. Whether genomic instability is a cause or consequence of cancer remains unknown. One proposed mechanism of cancer initiation is oncogene-induced replication stress (Oi-RS). We used oncogenic human papillomavirus type 18 infected human foreskin keratinocytes (HPV18+ HFK) passaged either short or long term as our model to study Oi-RS, as it recapitulates cancer initiation. Using DNA fiber assay, we have observed that HPV18+ HFK displayed accelerated fork progression with oscillating fork speeds from early to late passage cells. HPV-induced fork acceleration is found to be PRIMPOL and p53 dependent. DNA damage response foci such as γH2AX and 53BP1 were increased in HPV18+ HFK. Chromosome segregation errors such as acentric chromosome fragments and chromatin bridges were observed in HPV18+ HFK. Bulk and single cell genome sequencing revealed that aneuploidies and copy number alterations (CNAs) emerge over time in HPV18+ HFK with some emergent CNAs like HPV-positive cancer genomes. Late passage HPV18+ HFK formed tumours in chick embryo xenograft model.

In summary, we have shown that HPV-induced Oi-RS and chromosomal instability promote clonal selection and display cancer-like rates of genomic instability in late passage HPV18+ HFK leading to transformation.

P45 - Role of the BPTF subunit of the NURF chromatin remodeling enzyme in melanoma

Miss Yuton Liang

NURF is an ISWI-class ATP-dependent chromatin remodeler that uses the energy from ATP hydrolysis to reposition nucleosomes, altering DNA accessibility transcription. BPTF, the largest and only specific subunit of the NURF complex, utilises domains including PHD fingers that are proposed to interact with specific HPTMs, targeting NURF nucleosome remodelling to genomic regions that include gene promoters. BPTF is found frequently overexpressed in cancers including melanoma where it is linked to abnormal oncogenic, signalling suggesting that BPTF/NURF is a potential target for therapeutic cancer intervention. However, development of BPTF/NURF as an oncotarget is hindered by the absence of comprehensive genome-wide maps of BPTF in cancer cells and to availability of specific inhibitors.

In this study, we addressed these knowledge gaps by developing and validating highly specific anti-BPTF antibodies and using CUT&Tag to determine the whole genome distribution of BPTF/NURF in A375 melanoma cells. We also investigated effects of two inhibitors on BPTF chromatin-targeting, testing effects of the H3S10p writer inhibitor SB747651A and the PHD binding pocket inhibitor H6. Our research established an effective method for analysing BPTF and laid the groundwork for studying the role of BPTF inhibition at the transcriptional level and its potential impact across a broader range of cancers.

P46 - Illustrating the role of lysine-specific demethylase 1 in early mammalian development using an intrinsic protein degradation system

Miss Leona Iwrin

The tapestry of histone modifications woven upon nucleosomes controls a realm of gene expression, cell growth, differentiation and epigenetic states. However, the biological significance of these epigenetic modifications in early post-implantation development has been difficult to decipher for many years. Lysine-specific demethylase 1 (LSD1) is a key epigenetic driver of transcriptional gene repression with a role in early embryonic development as seen previously in mouse knockout embryos with lethality at embryonic day 6.5.

Previously, The Cowley Lab characterised the effects of the genetic ablation of LSD1 at a differentiated epiblast-like state. While these results were informative, they were limited by the length of time taken to observe the effects of protein loss after gene knockout techniques. To surpass these limitations, here we were able to abolish LSD1 protein within mouse embryonic stem cells during differentiation into epiblast like-cells. The transcriptomic consequences that followed the degradation of LSD1 during epiblast-like differentiation were investigated by RNA-sequencing.

Epiblast-like cells were successfully formed by differentiation with expected reductions in pluripotency related factors alongside an increased expression of epiblast-like genes at both 24- and 48-hour post-differentiation. In the absence of LSD1, at 24 hours, 186 genes were upregulated with a conservative number of 59 genes downregulated. By 48 hours, the number of differentially expressed genes had increased with a bias towards upregulation with 128 genes downregulated and 252 genes upregulated significantly.

Differentiation into epiblast-like cells was able to occur in the absence of LSD1 at both 24- and 48-hours of differentiation. By just 24 hours an upregulation in smooth muscle associated genes was observed alongside an expected downregulation of chromatin organisation associated genes. However, by 48-hours, genes relating to spermatogenesis are both up- and down-regulated with findings of male fertility associated genes observed in this post-implantation embryonic model of epiblast-like cells.

P47 - Investigating the role of HDAC3 in human embryonic development

Miss Alice Victory

In humans, little is known about peri-implantation, due to difficulties in acquiring material and establishing model systems recapitulating pre- and post-implantation cell states. Embryo derived hPSCs become ‘primed’ in culture, representative of cells post-implantation, possessing an inactive X chromosome. These cells are reprogrammed to a ‘naive’ cell state, recapitulating pre-implantation cells, with 2 active X chromosomes. Recently established culturing conditions allowed for the study of pre- and post-implantation, and transitions between these states.

Naive to primed pluripotency transition involves major genome remodeling and the formation of heterochromatin through histone and DNA methylation. Histone deacetylases (HDACs) facilitate this process, removing acetyl groups from histone tails, allowing for further silencing modifications. In mice, histone deacetylase 3 (HDAC3) is responsible for the initiation of X chromosome inactivation, forming facultative heterochromatin. HDAC3 also has functions independent of deacetylase activity, interacting with nuclear envelope proteins tethering facultative heterochromatin to the nuclear periphery. Deletion of HDACs has been shown to be embryonic lethal, however their individual roles in early human embryonic development remain unclear.

Super-resolution microscopy reveals that hPSCs form supramolecular complexes (SMACs) on the inactive X chromosome, including HDAC3. HDAC3 depletion results in X-linked gene reactivation, increased histone deacetylation and disruption of SMACs.

P48 - Structural characterization of SPEN’s IDR and its role the formation of gene silencing supramolecular complexes

Dr. Luis Padilla-Cortes

X-Chromosome Inactivation (XCI) is a process that ensures gene dosage compensation in eutherian female mammals. The process is initiated and mediated by a lncRNA, XIST, which is transcribed from, and coats the chromosome to be silenced. It had been previously shown that XCI is driven by the formation of supramolecular complexes (SMACs) of different XCI effector proteins around XIST1. SMACs generate local gradients of effector proteins to mediate gene silencing of ~700 genes across an entire chromosome. One important transcription silencer present in SMACs is SPEN, a 402 KDa protein that binds to XIST through its RNA binding motifs (RRMs) on the N-terminal and recruits the histone deacetylation complexes, crucial for gene silencing, through its SPOC domain on the C-terminal. In between the RRMs and SPOC, SPEN contains a 2900 amino acid-long intrinsically disordered region (IDR) that has been shown to play a role in the assembly of SPEN into SMACs. Deletion of the IDR, results in abolishment of the silencing function of SPEN1. Here we address the structural characterization of SPEN’s IDR and its role in the formation of SMACs. Using a combination of computational approaches such as MD simulations and molecular biology techniques such as Y2H assays, we have identified specific regions within SPEN’s IDR, candidates to play a role in SPEN’s self-assembly. We will further characterize the selected region through biophysical methods and understand the role, in vivo, of those specific deletions using FLIM/FRET and Super Resolution Microscopy.

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P49 - Investigation of KRAS mutation effects on transcription and genomic integrity

Mr Thomas Strange

KRAS is a commonly mutated member of the RAS protein family, acting as a key oncogenic driver in cancer. Previous studies have demonstrated that mutation of KRAS glycine 12 induces changes in transcription, DNA damage, and genomic stability. In this study, hTERT-RPE-1 cells with KRAS G12 mutation to valine (G12V), aspartic acid (G12D), and cysteine (G12C) were compared to investigate the effects of each mutation on transcription, DNA damage, and R-loops. G12V and G12D mutations promote an increase in RNA polymerase II phosphorylation, R-loops, and γH2AX foci count.

However, more differentially expressed genes are down-regulated in the RNA-seq data such as those involved in cell adhesion. This work highlights that KRAS G12 mutations have different effects on transcription, the DNA damage response, and genomic integrity. As G12D induced the most significant changes in transcription, DNA damage, and R-loops, we are now investigating how a specific KRAS G12D inhibitor affects these cellular processes. Preliminary experiments show that inhibiting KRAS G12D increases nascent transcription and promotes an initial rise in γH2AX foci count within 3 hours before decreasing after a 6-hour treatment. Our results suggest that KRAS G12D inhibition could affect cell viability through transcriptional changes and DNA damage induction.

P50 - The role of BAHD1 in heterochromatin formation and maintenance during early embryonic development

Miss Eve Dixon

The nucleus is a highly compartmentalized organelle and this spatial organization reflects gene-regulatory environments. Chromatin exists in two distinct forms: transcriptionally active, euchromatin and silenced, compacted heterochromatin. Formation of heterochromatic compartments initiates in the embryo is essential for correct development, while if not tightly regulated, can lead to cancer, disease, and even lethality. Here we study the role of the key transcriptional repressor BAHD1 to the process of heterochromatin formation in early embryonic development using differentiating embryonic stem cells as a model system. We have generated a female mESC cell line in which the endogenous BAHD1 protein can be rapidly degraded at will during a time course of differentiation. Using this system, we identify that BAHD1 recruits histone deacetylase 1 (HDAC1) on the inactivating X chromosome (Xi) via its interaction with MIER1, and that this interaction is linked to the subsequent accumulation of Polycomb complexes on the Xi. By using high- and super-resolution microscopy, we identify an emerging role of HDAC1, acting within the BAHD1 complex in X-inactivation through its ability to promote higher order chromosome changes and maintenance of gene silencing.

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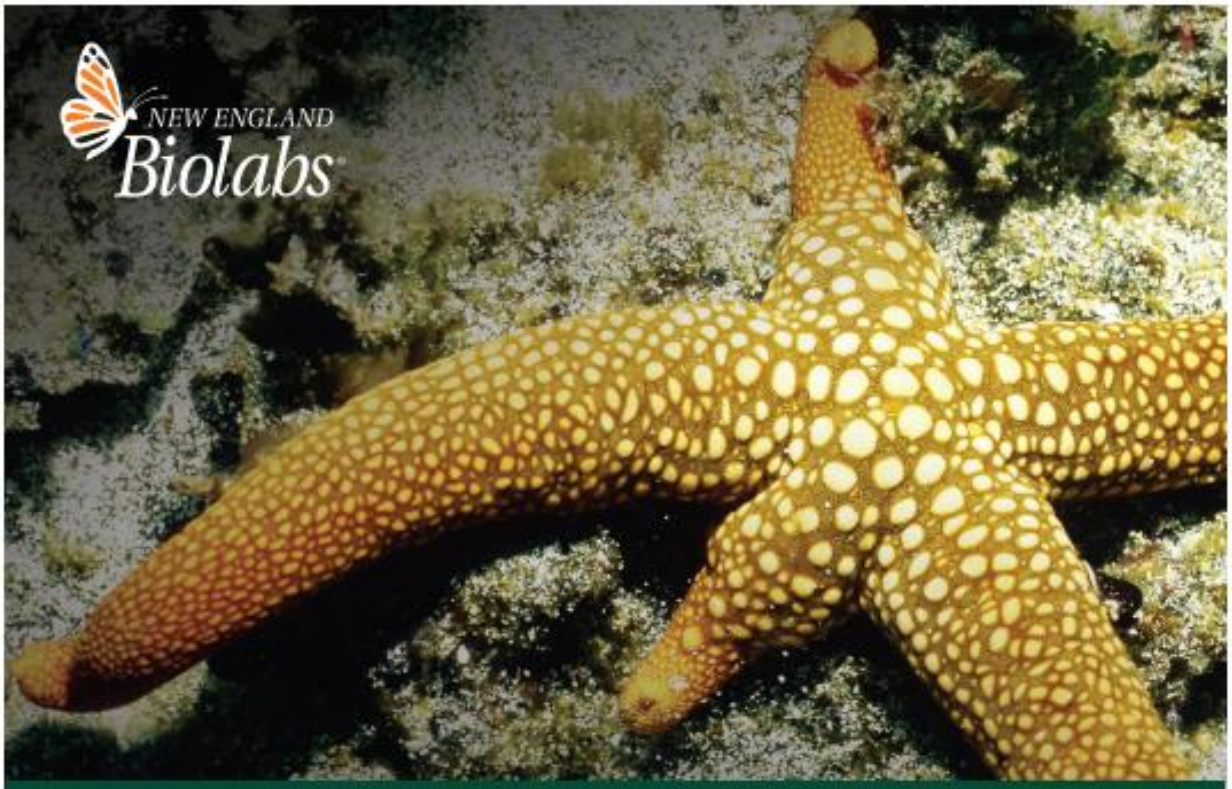

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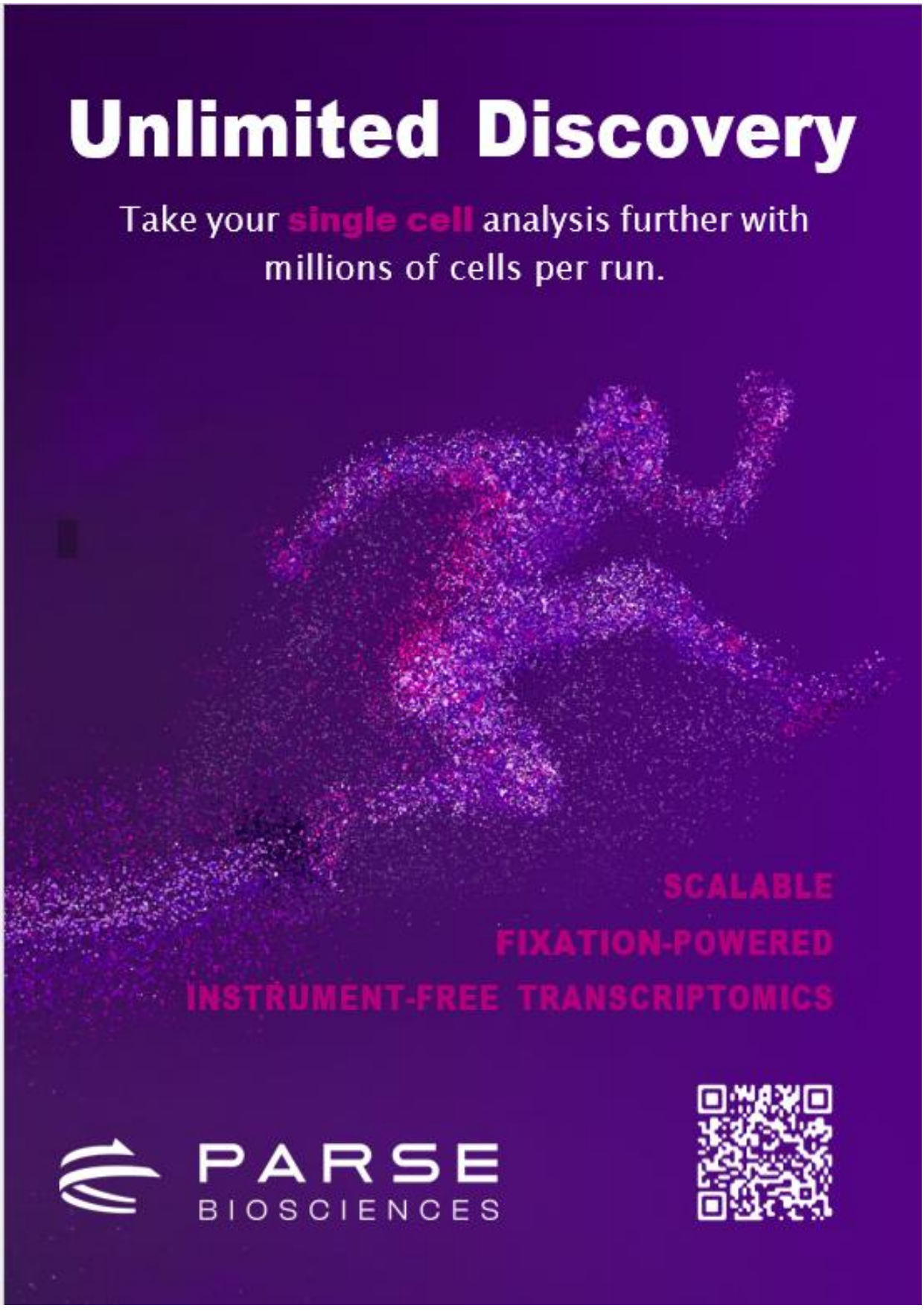
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