Japan-UK Regulation through Chromatin Conference

22-23 August 2022

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Venue

Frank and Katherine May Lecture Theatre

Henry Wellcome Building

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Contents

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Schedule

Monday, 22 August 2022

08:30 - 09:30	Registration and coffee, put up posters
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11:15 - 11:45	Coffee and Tea
11:45 - 13:05	**Session 2: 3D Genome Organisation**
13:05 - 13:30	Lunch
13:30 - 14:30	**Posters session**
14:30 - 16:20	**Session 3: Chromatin in Development**
16:20 - 16:50	Coffee,Tea
16:50 - 18:00	**Session 4: Chromatin and RNA**
19:30 - 22:00	Conference Dinner College Court

Tuesday, 23 August 2022

08:30 - 09:00	Coffee and Tea
09:00 - 10:50	**Session 5: Gene Regulation**
10:50 - 11:20	Coffee and Tea
11:20 - 13:00	**Session 6: 3D Genome Organisation**
13:00 - 13:30	Lunch
13:30 - 14:00	**Posters session**
14:00 - 15:30	**Session 7: Heterochromatin**
15:30 - 16:00	Coffee and Tea
16:00 - 17:20	**Session 8: Chromatin remodelling**
17:20	Closing Remarks and Departure

Programme

Talks Monday, 22 August 2022

09:30 - 09:45 Welcome

09:45 - 11:15 Session 1: Structural Biology of Chromatin
Chair: Thomas Schalch

09:45 - 10:15 **Structure & mechanisms of class I histone deacetylase complexes**
John Schwabe, University of Leicester

10:15 - 10:45 **3D genome structures of single differentiating mouse ES cells reveal a unique reorganisation of chromatin in the formative state**
Ernest Laue, University of Cambridge

10:45 – 11:15 **An acetylation-dependent feed-forward reaction enables long-range chromatin interactions**
Daniel Panne, University of Leicester

11:15 - 11:45 Coffee and Tea

11:45 - 13:05 Session 2: 3D Genome Organisation
Chair: Hiroshi Kimura

11:45 - 12:15 **Spatial multi-omics for understanding gene expression regulated by cell-cell interaction**
Yasuyuki Ohkawa, Kyushu University

12:15 - 12:45 **Chromatin behavior in living cells revealed by single-nucleosome imaging/tacking**
Kazuhiro Maeshima, National Institute of Genetics

12:45 - 13:05 **Visualising chromatin dynamics in living cells at the domain leve**l
Andrew Bowman, University of Warwick

13:05 - 13:30 Lunch

13:30 - 14:30 Poster Session

Talks Monday, 22 August 2022, continued

14:30 - 16:20 Session 3: Chromatin in Development
Chair: Shaun Cowley

14:30 - 15:00 **A critical role for H3K9 methylation in mitotic chromosome bookmarking and compaction**
Amanda Fisher, Imperial College London

15:00 - 15:30 **Quantification of sperm chromatin condensation**
Yuki Okada, The University of Tokyo

15:30 - 16:00 **Canonical PRC1 Is Recruited To Target Genes By JARID2-PRC2 Independently of H3K27me3**
Adrian Bracken, Trinity College Dublin

16:00 - 16:20 **JAZF1-SUZ12 dysregulates PRC2 function and gene expression during cell differentiation**
Manuel Tavares, UCL Cancer Institute

16:20 - 16:50 Coffee and Tea

16:50 - 18:00 Session 4: Chromatin and RNA
Chair: Jun-ichi Nakayama

16:50 - 17:20 **Understanding and reconstructing small RNA-mediated heterochromatin formation**
Yuka Iwasaki, Keio University

17:20 - 17:40 **Investigating the effects of LINE1 promoters' activity in colon cancer**
Cristina Tufarelli, University of Leicester

17:40 - 18:00 **Simultaneous analysis of cell lineage and the regulatory mechanisms by single-cell multi-ChIL-seq**
Takeru Fujii, Kyushu University

19:30 - 22:00 Conference Dinner College Court

Talks Tuesday, 23 August 2022

09:00 - 10:50 Session 5: Gene Regulation
Chair: Yuki Okada

09:00 - 09:30 **Unlocking the differential activities of HDAC1/2 complexes: How many machines does it take to build a road..?**
Shaun Cowley, University of Leicester

09:30 - 10:00 **Control of signal-responsive enhancer resetting by chromatin remodelling proteins**
Brian Hendrich, University of Cambridge

10:00 - 10:30 **Understanding how CpG islands regulate gene expression**
Rob Klose, University of Oxford

10:30 - 10:50 **Investigating the roles of BET bromodomain proteins in DNA replication and repair**
Eva Petermann, University of Birmingham

10:50 - 11:20 Coffee and Tea

11:20 - 13:00 Session 6: 3D Genome Organisation
Chair: Yuka Iwasaki

11:20 - 11:50 **Dynamics of Histone and RNA Polymerase II Modifications in Living Cells**
Hiroshi Kimura, Tokyo Institute of Technology

11:50 - 12:20 **Replication dynamics identifies the folding principles of the inactive X chromosome**
Ichiro Hiratani, RIKEN

12:20 - 12:40 **Chromatin dynamics during transcription activation in real time**
Kerstin Bystricky, CBI Toulouse

12:40 - 13:00 **Cohesin-independent STAG proteins interact with RNA and localise to R-loops to promote complex loading**
Yang Li, University College London, Cancer Institute

13:00 - 13:30 Lunch

13:30 - 14:00 Poster Session

Talks Tuesday, 23 August 2022, continued

14:00 - 15:30 Session 7: Heterochromatin
Chair: Daniel Panne

14:00 - 14:30 **Epigenetic Regulation, Heterochormatin and Antifungal Resistance**
Robin Allshire, University of Edinburgh

14:30 - 15:00 **Mechanisms regulating Clr4/SUV39H histone methyltransferase activity**
Jun-ichi Nakayama, National Institute for Basic Biology

15:00 - 15:30 **Histone ubiquitination marks antagonize to compartmentalize the genome**
Thomas Schalch, University of Leicester

15:30 - 16:00 Coffee and Tea

16:00 - 17:20 Session 8: Chromatin remodelling
Chair: John Schwabe

16:00 - 16:20 **Proteomic profiling reveals distinct phases to the restoration of chromatin following DNA replication**
Constance Alabert, University of Dundee

16:20 – 16:50 **Epigenetic changes arising from acute depletion of the tumour suppressor ARID1A.**
Tom Owen-Hughes, University of Dundee

16:50 - 17:20 **Structure and mechanism of the SWR1 histone exchange complex**
Dale Wigley, Imperial College London

17:20 - 17:30 Closing Remarks

Posters

Monday, 22. and Tuesday, 23. August 2022

13:30 - 14:30 Poster Session with lunch

Posters will be on display for the entire duration of the conference. Presenters are expected to present their posters in the lunch sessions.

Poster 1: Non-repetitive nucleosome arrays form disrupted, fragile chromatin fibres
Waad AlBawardi, University of Edinburgh

Poster 2: Investigating the role of Raf2 in heterochromatin formation in S. pombe
Ana Arsenijevic, University of Edinburgh

Poster 3: PROTAC mediated degradation of class-I HDACs leads to cell death and profound transcriptional defects in colon cancer cells
India Baker, University of Leicester

Poster 4: Investigating the Role of LSD1 in Early Development
Megan Broderick, University of Leicester

Poster 5: Understanding the RERE Deacetylase Complex role in transcription
Edward Brown, University of Leicester

Poster 6: The context-dependent role of MGA during primordial germ cell development
Erica Calabrese, University of Göttingen

Poster 7: Identifying the acute effects of HDAC1 removal in mouse embryonic stem cells
David English, University of Leicester

Poster 8: Interaction of the MiDAC histone deacetylase complex with chromatin
Louise Fairall, University of Leicester

Poster 9: Structural insights into the 3D genome folding and expression
Leonardo Feletto, University of Leicester

Poster 10: The Role of Cohesin in 3D Genome Architecture
Joshua Graham, University of Leicester

Poster 11: Investigating the role of Ying-Yang 1 (YY1) and its recruitment to chromatin in hormone-sensitive breast cancer
Diana Ivanoiu, Imperial College London

Poster 12: Investigating the role of Sin3a
Samuel Lee, University of Leicester

Poster 13: Import and chaperoning of monomeric histones
Alonso Javier PARDAL, University of Warwick

Poster 14: Dissecting the regulatory mechanisms governing histone lysine 9 methyltransferase Clr4
Panagiotis Patsis, University of Leicester

Poster 15: Histone demethylase KDM4A can modulate the efficiency of post-stress recovery and the efficacy of Pol I inhibitors
Xiyuan Qi, Queen's University Belfast

Poster 16: Investigations into the structure and chromatin interactions of the NuRD complex.
Liam Regan, University of Leicester

Poster 17: Investigating the Role of the Sin3A/HDAC1 Complex in DNA Replication and Mitosis
Khadija Sabat, University of Leicester

Poster 18: Investigating the specialized role of MiDAC- member of the Class 1 histone deacetylase complex family
Kristupas Sirvydis, University of Leicester

Poster 19: Defining the functional components of constitutive heterochromatin through genetic interaction screening
Anna Townley, University of Cambridge

Poster 20: An unexpected histone chaperone function for the MIER1 histone deacetylase complex
Siyu Wang, University of Leicester

Speaker Abstracts

Structure & mechanisms of class I histone deacetylase complexes

John Schwabe

Institute for Structural and Chemical Biology, Department of Molecular and Cell Biology, University of Leicester. UK.

In higher eukaryotes there are seven essential, non-redundant histone deacetylase complexes that regulate chromatin accessibility across the genome. These class I HDAC complexes share a common catalytic subunit, yet contain very different accessory proteins, have diverse oligomeric states and distinct molecular mechanisms. Despite their importance in gene regulation, very little is known about their mechanisms of action or even the specificity of their chromatin substrates.

We are taking a variety of structural and functional approaches to understand these complexes. In this talk I will address questions of substrate-specificity of the different complexes against both peptide and nucleosome substrates. I will also present a very recent study of the MIER1 HDAC complex that reveals an unexpected histone chaperone function that fits well with its role as a transcriptional repressor.

References:

Wang, S., Fairall,L., Pham, K., Ragan, T.J., Vashi D., Collins, M.O., Dominguez, C. and Schwabe, J.W.R. (2022) An unexpected histone chaperone function for the MIER1 histone deacetylase complex. BioRxiv.

Wang, Z., Whedon, S., Wu, M., Wang, S., Brown, E., Anmangandla, A., Regan, L., Lee, K., Du, J., Hong, J., Fairall, L., Kay, T., Lin, H., Zhao, Y., *Schwabe, J.W.R. & *Cole, P. (2022) Histone H2B Deacylation Selectivity: Exploring Chromatin's Dark Matter with an Engineered Sortase. Journal of the American Chemical Society 144, 3360. Wang, Z.A., Millard, C.J., Lin, C-L., Gurnett, J., Wu, M., Lee, K., Fairall, L., *Schwabe, J.W.R., *Cole, P.A. (2020) Diverse Nucleosome Site-Selectivity Among Histone Deacetylase Complexes. eLife 9, e57663.

Smalley, J., Adams, G., Millard, C., Song, Y., *Schwabe, J.W.R., *Cowley, S., *Hodgkinson, J. (2020) PROTAC mediated degradation of Class-I histone deacetylase enzymes in corepressor complexes. Chemical Communications doi: 10.1039/d0cc01485k. *Co-corresponding authors

3D genome structures of single differentiating mouse ES cells reveal a unique reorganisation of chromatin in the formative state

David Lando[1#], Xiaoyan Ma[1#], Yang Cao[1,5], Timothy Stevens[2], Wayne Boucher[1], Dominic Hall[1], Aleksandra Jartseva[1], Nicola Reynolds[3], Bertille Montibus[3,6], Ramy Ragheb[3], Andreas Lackner[4], Martin Leeb[4], Brian Hendrich[1,3] &* ***Ernest Laue*** *[1,3*]*

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2 MRC Laboratory of Molecular Biology, Cambridge
3 Wellcome-MRC Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge
4 Max Perutz Laboratories Vienna, University of Vienna
# These authors contributed equally to this work; * To whom correspondence should be addressed.

Pluripotency is the ability of an embryonic stem (ES) cell to differentiate into all the different cell lineages of a mature organism. Naïve and primed pluripotent ES cells in vitro correspond to the pre- and post-implantation populations in the embryo, and they are developmentally linked to each other via progression through a 'formative' transition state where naïve ES cells mature in response to inductive cues prior to becoming specified towards certain cell fates. Studying the formative state is thus crucial for understanding the mechanisms of multi-lineage decision-making. We have found that in the formative state there is a profound reorganisation of 3D genome structure, where genes whose expression levels are changing become located in multi-way chromatin hubs and highly intermingled regions of the genome. Our results suggest that the reorganisation of 3D genome structure is driven by changes in DNA methylation and Polycomb-mediated heterochromatin formation, which is accompanied by a global increase in transcriptional bursting, leading to a reconfiguration of enhancer-promoter interactions – consistent with changes in the epigenome priming promoters for lineage specific gene expression.

An acetylation-dependent feed-forward reaction enables long-range chromatin interactions

Daniel Panne[1], *Ziad Ibrahim*[1], *Tao Wang*[2], *Olivier Destaing*[2], *Nicola Salvi*[3], *Naghmeh Hoghoughi*[2], *Clovis Chabert*[2], *Alexandra Rusu*[1], *Jinjun Gao4, Leonardo Feletto*[1], *Nicolas Reynoird*[2], *Thomas Schalch*[1], *Yingming Zhao*[4], *Martin Blackledge3, Saadi Khochbin*[2]

1 Leicester Institute of Structural and Chemical Biology, Department of Molecular and Cell Biology, University of Leicester
2 CNRS UMR 5309, INSERM U1209, Université Grenoble Alpes, Institute for Advanced Biosciences, Grenoble, France.
3 Institut de Biologie Structurale, CNRS, CEA, UGA, Grenoble, France
4 Ben May Department of Cancer Research, The University of Chicago, Chicago [2]

Chromatin modification has long been linked to large-scale genome organization and compartmentalization. Acetylation alters chromatin structure and enables binding of Bromodomain-containing proteins. BRD4-NUT is an oncogenic fusion protein of BRD4 that binds to the acetylase p300 and enables formation of long-range intra- and interchromosomal interactions. We here report insights into how ‘reading’ and ‘writing’ of chromatin acetylation by BDR4-NUT/p300 drives large-scale chromatin organization and how dysregulation leads to disease.

We found that NUT contains an acidic Activation Domain (AD) that interacts with the p300 TAZ2 domain. Structural studies show that NUT contains a 5 helical amphipathic AD that binds the TAZ2 domain. We systematically probed which elements of BRD4-NUT and p300 drive large scale chromatin condensation in cells. Our data indicate that the TAZ2 domain has an auto-inhibitory function that is relieved upon ligand-binding. We found that condensation depends on a feed-forward mechanism that involves allosteric activation of p300 by binding of NUT to the p300 TAZ2 domain, nucleation of the condensation reaction through BRD4 and p300 BD-acetyl-lysine binding and that BD multivalency is required. Mutations found in cancer that interfere with autoinhibition by TAZ2 allosterically activate p300 and result in similar condensation reactions.

As the NUT interaction with TAZ2 is similar to that seen of other transcription factor ADs, we predict that p300 activation is a general mechanism in TF-mediated signal transmission. We will discuss how activation of p300 and a self-organizing positive feedback mechanism establishes an acetylation-dependent read-write mechanism and how this reaction drives larger-scale chromatin architecture.

Spatial multi-omics for understanding gene expression regulated by cell-cell interaction

Yasuyuki Ohkawa

Medical Institute of Bioregulation, Kyushu Univeristy

Skeletal muscle stem cells exist in the stem cell niche in adults and interact with various cells to form specific chromatin structures for maintaining phenotypes with specific gene expression. In aging, the dysregulation of cell-cell interaction in the stem cell niche leads to a decrease in skeletal muscle stem cells caused by abnormal gene expression. To understand the aging of skeletal muscle tissue, we have focused on spatial omics technology. Spatial omics technology is the analytical methodology that enables comprehensive and quantitative measurement of the gene expression to protein expression with the location of the cells in the tissue. However, the current technologies are still insufficient in terms of resolution and data depth at the single-cell level. Therefore, we developed a unique spatial omics technology with high depth and single-cell resolution. In this presentation, I will introduce the spatial multi-omics analysis we have developed by combining sequential immunostaining and sequential single-molecule RNA-FISH techniques and discuss its potential.

Chromatin behavior in living cells revealed by single-nucleosome imaging/tacking

Kazuhiro Maeshima

National Institute of Genetics and Sokendai, Mishima, Shizuoka, Japan

Dynamic chromatin behavior plays a critical role in various genome functions (1). However, it remains unclear how chromatin behavior changes during the cell cycle. In interphase, the nucleus enlarges, and genomic DNA doubles. It was previously reported that chromatin movements varied during interphase when measured using a minute or longer time-scale. However, using single-nucleosome imaging/tracking (2), we unveil that local chromatin motion on a second time-scale remained steady throughout G1, S and G2 phases in live human cells (3). This motion mode appeared to change beyond this time-scale. A defined genomic region also behaved similarly during interphase. Combined with Brownian dynamics modeling, our results suggest that this steady-state chromatin motion was mainly driven by thermal fluctuations. Steady-state motion temporarily increased following a DNA damage response. Our findings support the viscoelastic properties of chromatin. We propose that the observed steady-state chromatin motion allows cells to conduct housekeeping functions, such as transcription and DNA replication, under similar environments during interphase (3).

References:

1, Maeshima, K., Iida, S., Tamura, S. (2021) Cold Spring Harbor Perspectives in Biology. a040675.

2, Ide, S., Tamura, S., Maeshima, K. (2022) BioEssays. 44, 2200043.

3, Iida, S. et al. (2022) Science Advances. 8, eabn5626

Visualising chromatin dynamics in living cells at the domain level

Filipe Fernandes-Duarte, Alonso J. Pardal, ***Andrew J. Bowman***

Division of Biomedical Sciences, Warwick Medical School, University of Warwick,

Chromatin movement is implicit in all genomic processes, yet it is only beginning to be understood. At the mesoscale, chromatin is organised into large loop domains thought to be formed by the actions of the architectural factors cohesin and CTCF. Whilst visualisation of individual genetic loci in living cells has greatly expanded our knowledge of how spatial dynamics relate to function, how chromosomes move at the domain level is relatively unexplored. Here I will introduce a chromatin pulse-labelling approach we developed to visualise chromatin specifically at the domain level and describe how we have leveraged Lattice-LightSheet microscopy to analyse domain-level dynamics over extended time periods. We found that whilst time and ensemble averaged MSD values were comparable to those reported previously for individual loci, the exponents of the domain trajectories (relating to the level of confinement) split into two discrete populations, revealing heterogeneity in domain movement. Performing the same experiments under conditions of DNA damage significantly shifted the exponent, albeit slightly, but had little effect on the diffusion coefficient. Visualising dynamics for extended periods of up to one hour revealed rare but significant translocation events. In these events, domains could exceed three microns of translocation and displayed elastic behaviour, often retracing trajectories and displaying extrusions/stretching long their vector of travel. We discuss our observations and the implications they have for the topological dynamics of chromatin.

A critical role for H3K9 methylation in mitotic chromosome bookmarking and compaction

Dounia Djeghloul[1], Sherry Cheriyamkunnel[1], Andrew Dimond[1], Karen E. Brown[1], Holger Kramer[1], Bhavik Patel[1], Thomas Jenuwein[2], Thomas Montavon[2], Matthias Merkenschalger[1] and ***Amanda G Fisher[1]****.*

1. Epigenetic Memory and Lymphocyte Development Groups, MRC London Institute of Medical Sciences, Imperial College London
2. Max Planck Institute of Immunobiology and Epigenetics, Freiburg.

As cells divide, epigenetic information is conveyed from mother to daughter cells. This ensures that cellular identity is retained through cell proliferation. To better understand the contributions of DNA-binding proteins and chromatin modifications to this 'mitotic memory', we analysed the repertoire of proteins retained on native mitotic chromosomes using liquid chromatography-tandem mass spectroscopy using native chromosomes purified by flow cytometry.

Surprisingly we found that DNA methylation machinery and complexes that catalyse repressive chromatin modifications (including PRC1, PRC2 and Suv39h1/h2), were enriched on mitotic chromosomes in addition to numerous well-characterised bookmarking factors (such as Esrrb, Sox2 and Tead4). In cells lacking Suv39h1/h2, and therefore lack H3K9me3, efficient binding of Esrrb and Tead4 to mitotic chromosomes was eroded, and chromosome compaction increased. These data suggest that transcription factor retention during mitosis is critically dependent upon chromatin and chromosome structure. We will discuss the mechanisms that likely underlie this dependency.

Quantification of sperm chromatin condensation

Yoshinori Makino, Masashi Hada, ***Yuki Okada***

Institute for Quantitative Biosciences, The university of Tokyo
Corresponding author: Yuki Okada (ytokada@iqb.u-tokyo.ac.jp)

Background: Sperm chromatin condensation is an essential event for sperm in the acquisition of resistance to DNA damage. The most critical step is histone-protamine exchange, while a little (2-10 %) amounts of histones are still retained in the chromatin. We have previously demonstrated the genomic localization of sperm-retained histones using mouse sperm. Since the amount of retained histones differs markedly between mice (~2 %) and humans (~10 %), we next examined human sperm to aim i) determination of the localization of sperm-retained histones for a comparison between mice and humans, ii) quantification of human sperm chromatin condensation in both bulk and single-cell levels for future clinical applications.

Results: CUT&Tag analyses demonstrated that modified histone H3s are localized at certain gene promoters and (peri)centromeric regions depending on the types of modifications. Interestingly, the characteristics are similar to those of mouse sperm, suggesting that the modification-specific histone retention is a common feature in mammals. Next, ATAC-seq was applied to quantify the sperm chromatin state. In the bulk sperm analyses, ATAC-seq results could divide 39 patient-derived samples into 3 groups depending on the extent of chromatin condensation. Single sperm ATAC-seq further demonstrated the heterogeneity of chromatin state of each sperm derived from one patient.

Conclusions Human sperm chromatin has similar characteristics to mouse sperm with respect to histone retention. In addition, ATAC-seq was effective in quantifying sperm chromatin status, suggesting that the importance of heterogeneous chromatin features in each sperm should be carefully considered.

Canonical PRC1 Is Recruited To Target Genes By JARID2-PRC2 Independently of H3K27me3.

Cheng Wang, Eleanor Glancy, Ellen Tuck, Evan Healy, ***Adrian P. Bracken****.*

Smurfit Institute of Genetics, Trinity College Dublin, Dublin , Ireland.

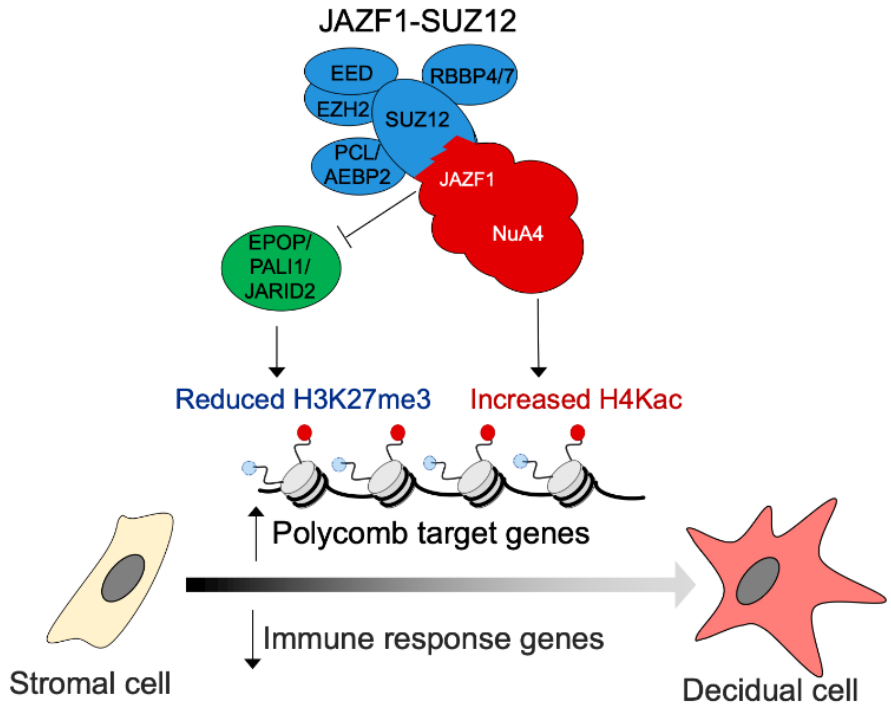
Polycomb repressive complex 2 (PRC2) mediates H3K27me3 deposition, which is believed to direct recruitment of canonical PRC1 (cPRC1) to promote stable repression of developmental genes. While PRC2 forms two major subcomplexes, PRC2.1 and PRC2.2, their specific roles are unclear. Through genetic knockout and replacement of PRC2 subcomplex specific accessory proteins, we discover that PRC2.1 and PRC2.2 function through distinct mechanisms. During the transition from naïve to primed pluripotency, PRC2.1 accumulates in sharp defined peak-like profiles at CpG islands, dependent on the DNA and histone modification binding activities of MTF2. In contrast, PRC2.2 is recruited in broader profiles, mirroring H2AK119ub1 accumulation, and dependent on the ubiquitin binding abilities of JARID2 and AEBP2. Surprisingly, while PRC2.1 mediates the majority of H3K27me3 deposition at Polycomb target genes, it is insufficient to promote recruitment canonical PRC1 (cPRC1). In contrast, while PRC2.2 is poor at mediating H3K27me3 deposition, it is essential and sufficient for H3K27me3 independent binding of cPRC1 and consequent 3D chromatin interactions at Polycomb target genes. We therefore discover distinct contributions of PRC2.1 and PRC2.2 to Polycomb mediated repression and provide a surprising H3K27me3 independent logic for cPRC1 recruitment.

JAZF1-SUZ12 dysregulates PRC2 function and gene expression during cell differentiation

Manuel Tavares[1], *Garima Khandelwal*[1], *Joanne Muter*[2], *Keijo Viiri*[1], *Manuel Beltran*[1], *Jan J. Brosens*[2], *Richard G. Jenner*[1]*

1 UCL Cancer Institute and Cancer Research UK UCL Centre, University College London
2 Warwick Medical School, Division of Biomedical Sciences, University of Warwick,

Polycomb repressive complex 2 (PRC2) methylates histone H3 lysine 27 (H3K27me3) to maintain gene repression and is essential for cell differentiation. In low-grade endometrial stromal sarcoma (LG-ESS), the PRC2 subunit SUZ12 is often fused with the NuA4/TIP60 subunit JAZF1. We show that JAZF1-SUZ12 dysregulates PRC2 composition, genome occupancy, histone modification, gene expression and cell differentiation. Loss of the SUZ12 N-terminus in the fusion protein abrogated interaction with specific PRC2 accessory factors, reduced occupancy at PRC2 target genes, and diminished H3K27me3. Fusion to JAZF1 increased H4Kac at PRC2 target genes and triggered recruitment to JAZF1 binding sites during cell differentiation. In human endometrial stromal cells, JAZF1-SUZ12 upregulated PRC2 target genes normally activated during decidualization while repressing genes associated with immune clearance, and JAZF1-SUZ12-induced genes were also overexpressed in LG-ESS. These results reveal defects in chromatin regulation, gene expression and cell differentiation caused by JAZF1-SUZ12, which may underlie its role in oncogenesis.



Understanding and reconstructing small RNA-mediated heterochromatin formation

Yuka W. Iwasaki

Department of Molecular Biology, Keio University School of Medicine and Japan Science and Technology Agency (JST), Precursory Research for Embryonic Science and Technology (PRESTO)

Heterochromatin is vital to sustaining stable chromosome structure and gene expression patterns, and its dysregulation can cause various diseases. Some classes of small RNAs can regulate their target genes via heterochromatin formation. PIWI-interacting RNAs (piRNAs) are germline-specific small RNAs that form effector complexes with PIWI proteins to preserve genomic integrity by repressing transposable elements (TEs). Importantly, this regulation mechanism is conserved among a wide range of species. Among PIWI-clade proteins in *Drosophila*, Piwi transcriptionally silences its targets via heterochromatin formation characterized by H3K9me3 marks and the linker histone H1. We identified a silencing complex that plays a central role in transcriptional silencing mediated by Piwi-piRNA. This complex consists of four proteins, Piwi-Panx-Nxf2-p15. Additionally, we identified that this complex is capable of regulating not only the local chromatin state but also nuclear localization and chromatin conformation. Piwi and Nxf2 localize chromatin regions that encode piRNA target TEs to the nuclear periphery. Furthermore, depletion of Piwi or Nxf2 results in decreased intra-TAD interactions in those regions. Ectopic targeting of Nxf2 indicated that the regulation initiates by co-transcriptional repression of the target reporter coupling with the removal of active histone marks and nuclear periphery localization. Continuous silencing involves the increase of H3K9me3 marks and H1 and the decrease of intra-TAD interactions. These results suggest that Piwi-piRNA complexes promote heterochromatin formation by causing step-wise changes in nuclear architecture.

Investigating the effects of LINE1 promoters' activity in colon cancer

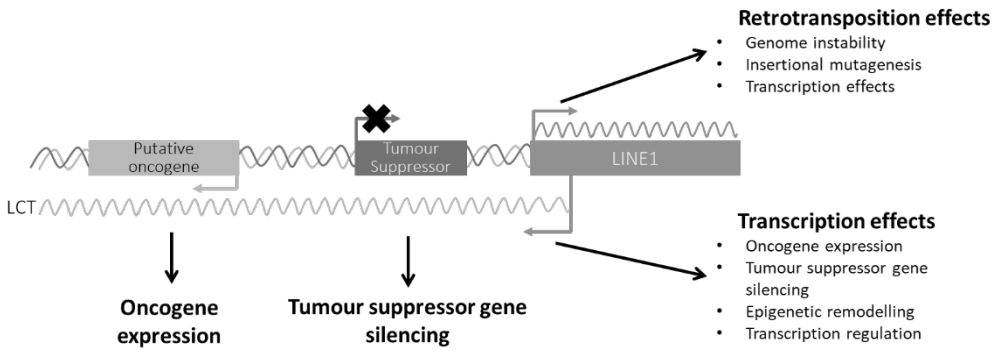
Chris Neophytou[1], Natasha Vafadar-Isfahani[1], Hang Xu[1], Inna Guterman[2], Raquel Palacios Gallego[2], Daniel Faulkner[2], Maria Mintseva[2], Emma Parrot[2], Karen Brown[2], Alessandro Rufini[2], Richard M Badge[3], Jonathan N Lund[1] and ***Cristina Tufarelli***[2]

1 School of Medicine, University of Nottingham, Royal Derby Hospital,
2 Leicester Cancer Research Centre, University of Leicester
3 Department of Genetics and Genome Biology, University of Leicester

Activity of transposable elements such as LINE1s is now recognised as a hallmark of epithelial cancers. These elements are best known for their ability to copy and paste themselves and other elements to new locations in the DNA therefore contributing to genome rearrangements and mutagenesis. A less studied aspect is the effects caused by the LINE1 promoters' transcriptional activity that can for example produce transcripts coding novel oncogenes or drive epigenetic silencing of tumour suppressor genes.

LCT13 is a LINE1 driven transcript we isolated whose expression in colon cancer that is linked to silencing of the metastasis suppressor gene *TFPI2* (a gene located within the first intron of LCT13) and causes ectopic expression of GNGT1, the gamma subunit of a retina-specific heterotrimeric G-protein with oncogenic potential. GNGT1 protein levels are higher in tumors than in matched normal tissues and in sera from CRC patients compared to healthy volunteers. In TCGA, colon cancer patients with high levels of GNGT1 transcripts have worse survival and we now show that all the GNGT1 transcripts in these samples arise from LINE1.

LCT13 highlights functional potential of LCTs in cancer and we are investigating how this LINE1 promoter is regulated. However, LINE1s are a large and diverse group of elements with different 5'UTR likely to be differentially regulated. We have been developing bioinformatics approaches to identify and uniquely map LCTs from transcriptome datasets to enable the study of the different families and how their expression relates to epigenetic and expression profiles of neighboring chromatin.



Simultaneous analysis of cell lineage and the regulatory mechanisms by single-cell multi-ChIL-seq

Takeru Fujii[1], *Akihito Harada*[1], *Kaori Tanaka*[1], *Kazumitsu Maehara*[1], *Hiroshi Kimura*[2], *Yasuyuki Ohkawa*[1]

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Cell differentiation is the process of acquiring a terminal phenotype through a series of different stages. Due to the transient expression of various cell types during differentiation, it is possible to trace the differentiation lineage by tracking the expressed genes. At the same time, gene expression is regulated by dynamic changes in chromatin structure through the binding of transcription factors and post-translational modifications of histones. Therefore, simultaneous analysis of gene expression and chromatin structural changes at the single-cell level in the same cell is expected to elucidate the regulatory mechanisms as well as the differentiation lineage. Therefore, we attempted to develop a technology that enables simultaneous analysis of the regulation of gene expression and the expression information at the single-cell level.

Here, we have developed single-cell multi ChIL-seq (scmtChIL-seq) and realized simultaneous and high-throughput measurement of the genomic localization of two types of proteins. Using skeletal muscle differentiation as a model, we performed a simultaneous and comprehensive analysis of both differentiation state tracking and the changes in chromatin structure. In this presentation, we will discuss the implementation of these analysis methods and their applications.

Unlocking the differential activities of HDAC1/2 complexes: How many machines does it take to build a road..?

Ahmad Alshehri[1], India May Baker[1], David M English[1], Mark O Collins[2,3] Louise Fairall[4] John WR Schwabe[4] and ***Shaun M Cowley[1].***

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Histone deacetylase 1 (HDAC1) and HDAC2 are critical catalytic subunits of six distinct coregulator complexes (SIN3, NuRD, CoREST, MiDAC, MIER and RERE) that help regulate histone acetylation levels across the genome. One of the major unanswered questions in the HDAC field is why the cell needs such an array of HDAC containing complexes? To try to understand how these different molecular machines are assembled and what they all do, we have been using the structure of HDAC1 bound to MTA1 (part of the NuRD complex) to design mutations on the surface of HDAC1 which discriminate binding to the different complexes. We have identified two HDAC1 mutants, one which binds only to SIN3 and a second that interacts with SIN3 and CoREST complexes, but not to NuRD or MiDAC. Surprisingly, retention of SIN3 binding alone is sufficient for cells to retain their viability, demonstrating the essential nature of this complex. Both mutations cause differential gene expression and a gradual loss of pluripotency in embryonic stem cells. We have therefore defined new tools and first of their kind mutations, which will help to discriminate the function of these different chromatin modifying machineries.

Control of signal-responsive enhancer resetting by chromatin remodelling proteins

Brian Hendrich

Wellcome-MRC Cambridge Stem Cell Institute, University of Cambridge

During the course of development cells must respond to extracellular cues imparted by their environment. Appropriate cellular responses are vital to ensure development proceeds normally, whereas inappropriate responses, or a failure to respond, can result in developmental defects and/or oncogenesis. A cell responding to instructional cues must rapidly change its gene expression programme. This requires substantial remodelling of chromatin and of the topological organisation of key regulatory sequences: enhancers and promoters. Exactly how extracellular signals are transmitted to chromatin, and how the signal can then instruct initiation of a new transcriptional programme is not known. We sought to precisely determine how the ERK extracellular signalling pathway and chromatin remodelling activity work together to induce transcriptional change in mouse ES cells to instruct lineage decisions. We found that ERK activation results in a rapid and dramatic change in transcription factor binding kinetics at enhancers. I will describe our progress in characterising this enhancer resetting event, and the role played by the Nucleosome Remodelling and Deacetylation (NuRD) complex, and other chromatin remodelling proteins, in transmitting this extracellular signal to chromatin and instructing transcriptional changes which instruct lineage decisions.

Understanding how CpG islands regulate gene expression

Rob Klose

Department of Biochemistry, University of Oxford.

Precise control of gene expression is fundamental to cellular homeostasis and normal multicellular development. Central to the control of gene expression are gene promoters, where RNA Polymerase II engages to initiate transcription. In vertebrates, most gene promoters are embedded within epigenetically specified elements called CpG islands. Yet, despite their intimate relationship with gene promoters, how CpG islands contribute to gene regulation has remained enigmatic. Here I will describe our work examining how CpG island-binding proteins are leveraged to regulate gene expression. In particular, I will focus on an unexpected new role we have uncovered for a CpG island binding protein in regulating transcription.

Investigating the roles of BET bromodomain proteins in DNA replication and repair

Akhil Bowry, Ann Liza Piberger, Siobhan Murphy-Hollies, Patricia Rojas, Marco Saponaro, ***Eva Petermann***

Institute of Cancer and Genomic Sciences, University of Birmingham

Targeted therapeutics that inhibit epigenetic factors such as BET proteins or histone deacetylases (HDACs) aim to reverse aberrant transcriptional programmes in cancer cells. BET inhibitors (BETi) were rapidly progressed into clinical settings, but disappointing responses highlight the need for better mechanistic understanding. Our group investigates how BETi influence DNA replication and DNA repair in human cells.

We previously reported that BETi and BRD4 depletion cause DNA replication stress through conflicts between transcription and replication. Mechanistically, BRD4 inhibition promotes release of active P-TEFb from its inhibitor 7SK-snRP, leading to a net increase in RNA synthesis. This increased RNA synthesis correlates with slowed replication fork progression. We now focus on the role of homologous recombination (HR), an important DNA repair pathway at replication forks, in this process. Our data suggest that BETi and other agents that release P-TEFb activate several HR pathways. Firstly, the HR protein RAD51 actively slows down the progression of replication forks in BETi-treated cells. Secondly, RAD51 is recruited behind the forks at DNA gaps formed by the repriming enzyme PrimPol. RAD51 prevents BETi from activating the ATR and ATM-mediated DNA damage responses. We propose a model whereby P-TEFb release and increased RNA synthesis promote RAD51 recruitment to slow replication and prevent DNA damage at and behind forks. Unlike with other DNA damaging treatments, these BETi responses are cell line specific.

Understanding BETi-induced HR could aid the development of combination therapies and have implications for other epigenetic therapies that disrupt the P-TEFb/7SK-snRP complex, including HDAC inhibitors and azacitidine.

Dynamics of Histone and RNA Polymerase II Modifications in Living Cells

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Posttranslational modifications of histones and RNA polymerase II (RNAP2) play a critical role in gene regulation during development and differentiation. To understand the mechanism how genes are regulated in chromatin context in living cells, we have developed modification-specific antibody-based fluorescent probes, such as dye-labeled Fabs and fluorescent protein-tagged single-chain variable fragments. During the zygotic genome activation in zebrafish embryos, histone H3 Lys27 acetylation (H3K27ac) preceded RNAP2 transcription at miR430 gene loci. We have recently generated genetically encoded probes that detect Ser5- and Ser2-phosphorylated forms of RNAP2, which can be markers of transcription initiation and elongation, respectively. In living HeLa cells, RNAP2 Ser5ph and Ser2ph probes were concentrated in numerous foci, as observed in fixed cells by immunofluorescence. RNAP2 Ser5ph (initiation) foci were generally separated from RNAP2 Ser2ph (elongation) foci and more closely associated with p300-enriched foci. In addition, RNAP2 Ser5ph foci were less mobile than RNAP2 Ser2ph foci. These data suggest that the elongating RNAP2 becomes separated from the site of initiation. We will discuss how transcription initiation and elongation foci are organized in living cells.

Replication dynamics identifies the folding principles of the inactive X chromosome

Ichiro Hiratani

RIKEN Center for Biosystems Dynamics Research

The chromosome-wide late replication is an enigmatic hallmark of the inactive X chromosome (Xi). How it is established and what it represents has remained obscure. By single-cell DNA replication sequencing, here we show that the entire Xi replicates rapidly and uniformly in late S-phase upon X chromosome inactivation (XCI), which reflects its uniformly compacted structure revealed by 4C-seq. Despite this uniformity, in Smchd1-mutant cells, only a subset of the Xi became earlier replicating, which protruded out of the Xi core, made long-range contact with each other, and became transcriptionally reactivated. These Smchd1-dependent late-replicating domains constituted the outermost layer of the X chromosome territory, suggesting that their default position predisposed them to become reactivated upon the absence of the global Xi-binding protein Smchd1. Coincidentally, the Xi's outermost layer was rich in genes that escape XCI. These observations demonstrate how the default 3D genome folding impacts heterochromatin stability and gene regulation.

Chromatin dynamics during transcription activation in real time

Silvia Kocanova[1], Flavien Raynal[1,2], Nawel Fellouah[1], Lea Costes [1], Vera Pancaldi [2], Job Dekker[3], Marc Marti-Renom[4], Tom Sexton[5], ***Kerstin Bystricky[1]***

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The three-dimensional organization of the genome is tightly linked to gene expression. We investigate how dynamic changes in this organization respond to and participate in regulation of transcription activation. Using live cell imaging of single loci, whole chromatin nanoscale tracking as well as modeling of 5C, promoter-capture HiC (PCHiC) and ChiP-seq data, we found that transcription activation rapidly confines motion and describe how pre-existing structures reorganize.

We use estrogen inducible loci in human mammary tumour cells as a model system in which chromatin remodelling via looping allows priming of the gene environment for hormone-induced transcription. Live cell imaging and image analysis at nanoscale resolution identifies areas of constraint motion and abrupt boundaries between domains of transcription dependent correlated movement but independent of chromatin density. High-resolution 5C, PCHiC combined with FISH analysis reveals that pre-established local folding of estrogen inducible genes reorganizes. Confined motion predominates dynamic regimes of both chromatin and transcription factors. The link between transcriptional activity, domain folding, frequencies of long and short range contacts and real time dynamics, will be discussed.

Cohesin-independent STAG proteins interact with RNA and localise to R-loops to promote complex loading

***Yang Li**, Hayley Porter, Suzana Hadjur*

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Most studies of cohesin function consider the Stromalin Antigen (STAG/SA) proteins as core complex members given their ubiquitous interaction with the cohesin ring. Here, we provide functional data to support the notion that the SA subunit is not a mere passenger in this structure, but instead plays a key role in the localization of cohesin to diverse biological processes and promotes loading of the complex at these sites. We show that in cells acutely depleted for RAD21, SA proteins remain bound to chromatin, cluster in 3D and interact with CTCF, as well as with a wide range of RNA binding proteins involved in multiple RNA processing mechanisms. Accordingly, SA proteins interact with RNA and are localised to R-loops where they contribute to R-loop regulation. Our results place SA1 within R-loop domains upstream of the cohesin complex and reveal a role for SA1 in cohesin loading which is independent of NIPBL, the canonical cohesin loader. We propose that SA1 takes advantage of structural R-loop platforms to link cohesin loading and chromatin structure with diverse functions. Since SA proteins are pan-cancer targets, and R-loops play an increasingly prevalent role in cancer biology, our results have important implications for the mechanistic understanding of SA proteins in cancer and disease.

Epigenetic Regulation, Heterochromatin and Antifungal Resistance

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Wellcome Centre for Cell Biology, School of Biological Sciences, The University of Edinburgh

Heterochromatin that depends on histone H3 lysine 9 methylation (H3K9me) renders embedded genes transcriptionally silent. In the fission yeast, *Schizosaccharomyces pombe*, heterochromatin is normally concentrated at telomeres and around centromeres where it contributes to centromere identity and is required for normal chromosome segregation.

Under certain conditions heterochromatin islands can arise over genes. We demonstrated that H3K9me heterochromatin can be transmitted through cell division provided the counteracting demethylase Epe1 is absent. Heterochromatin heritability might allow wild-type cells under certain conditions to acquire epimutations, which could influence phenotype through unstable gene silencing rather than DNA change. We have found that heterochromatin-dependent epimutants that are resistant to caffeine arise in fission yeast grown with threshold levels of caffeine. Isolates with unstable resistance have distinct heterochromatin islands with reduced expression of embedded genes, including some whose mutation confers caffeine resistance. Our analyses reveal that epigenetic processes promote phenotypic plasticity, letting wild-type cells adapt to unfavourable environments without genetic alteration. Caffeine affects two anti-silencing factors: Epe1 is downregulated, reducing its chromatin association, and a shortened isoform of Mst2 histone acetyltransferase is expressed. Thus, heterochromatin-dependent epimutation provides a bet-hedging strategy allowing cells to transiently adapt to insults while remaining genetically wild type. Isolates with unstable caffeine resistance show cross-resistance to antifungal agents, suggesting that related heterochromatin-dependent processes may contribute to resistance of plant and human fungal pathogens to such agents.

Mechanisms regulating Clr4/SUV39H histone methyltransferase activity

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In eukaryotic cells, the assembly of higher-order chromatin structure, known as heterochromatin, plays an important role in diverse chromosomal processes. Heterochromatin assembly is intimately associated with changes in post-translational histone-tail modifications. In fission yeast, the methylation of histone H3 at lysine 9 (H3K9me), a hallmark of heterochromatin structure, is catalyzed by the methyltransferase Clr4/SUV39H, and functions as a binding site for recruiting the HP1 family proteins. Clr4/SUV39H has two functional domains, the N-terminal chromodomain (CD), which recognizes H3K9me, and the C-terminal SET domain responsible for its enzymatic activity. Since Clr4/SUV39H's promiscuous activity leads to inappropriate H3K9me and aberrant gene silencing, its enzymatic activity needs to be strictly controlled. However, the detailed mechanisms regulating Clr4/SUV39H's activity are incompletely understood. Here we show that Clr4/SUV39H's CD binds nucleic acids, and that this binding is important for its function in heterochromatin assembly. We further demonstrate that Clr4/SUV39H's activity is regulated by a novel crosstalk between histone modifications and also by intramolecular interactions.

Histone ubiquitination marks antagonize to compartmentalise the genome

Thomas Schalch [1,2]

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The timely and accurate compartmentalization of eukaryotic genomes into euchromatin and heterochromatin is critical for their regulation, organization, and protection. Understanding the mechanisms that control chromatin status is highly relevant for understanding processes such as differentiation or aging as well as diseases like cancer. Fission yeast has highly conserved chromatin and RNAi machinery that play a critical role in defining the sites of heterochromatin formation. Intriguingly, the Cullin4-RING E3 ligase Rik1 is a key component of this system and has recently been found to target specifically histone H3 lysine 14 for ubiquitination (H3K14ub). Using structure-function analysis, we have shown that this very poorly studied mark strongly activates Clr4, the methyltransferase responsible for depositing the hallmark of heterochromatin, histone H3 lysine 9 di- and trimethylation (H3K9me2/3). These findings suggest that H3K14ub has the power to control where and when heterochromatin can form.

In contrast to H3K14ub, histone H2B ubiquitination (H2Bub) is tightly associated with transcription elongation. Here we will present evidence that the H2B ubiquitin ligase complex (HULC) protects active transcription from heterochromatin silencing when challenged by small RNAs. Like H3K14ub, H2Bub engages in crosstalk with a histone methyltransferase to promote lysine methylation, in this case H3K4me2/3. We postulate that H2Bub drives a euchromatic feedback loop, which keeps heterochromatin in check by acetylation of specific histone residues, first and foremost H3K14 and H3K9.

Proteomic profiling reveals distinct phases to the restoration of chromatin following DNA replication.

Vanesa Alvarez[1], Jens Hukelmann[2], Diana Rios-Szwed[1,4], Elisa Garcia-Wilson[1], Hao Jiang[2], Nicola Wiechens[3], Eva Greisser[2], Tom Owen-Hughes[3], Angus Lamond[2], ***Constance Alabert***[1,*]

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2 Laboratory of Quantitative Proteomics, Centre for Gene Regulation and Expression, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK.
3 Centre for Gene Regulation and Expression, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.
4 Present address: MRC Human Genetics Unit, EH4 2XU Edinburgh Scotland

Chromatin organization must be maintained during cell proliferation to preserve cellular identity and genome integrity. However, DNA replication results in transient displacement of DNA bound proteins, and it is unclear how they regain access to newly replicated DNA. Using quantitative MS-based proteomics coupled to Nascent Chromatin Capture, we provide time resolved binding kinetics for thousands of proteins behind replisomes within euchromatin and heterochromatin in human cells. This shows that most proteins regain access within the first 15 minutes after the passage of the fork. In contrast, 30% of the identified proteins do not, and this delay cannot be inferred from their known function, physicochemical properties, or nuclear abundance. Instead, differential chromatin organization affect their reassociation. We provide evidence that DNA replication not only disrupts but also promotes recruitment of transcription factors and chromatin remodellers, providing a significant advance in understanding how DNA replication could contribute to programmed changes of cell memory

Epigenetic changes arising from acute depletion of the tumour suppressor ARID1A

Tom Owen-Hughes

School of Life Sciences , University of Dundee

The ARID1A subunit of SWI/SNF chromatin remodelling complexes is a potent tumour suppressor. It is a major driver for ovarian clear cell carcinoma and endometrial cancer. Like many genes involved in signalling, the relationship between mutations to the gene and the phenotype is complexes. We have attempted to address this using an auxin degron to trigger acute depletion of ARID1A. Loss of chromatin accessibility is detected at thousands of loci within a couple of hours. At these sites ARID1A containing complexes act to generate accessible minidomains of nucleosomes. ARID1A also interacts with the co-activator EP300. When ARID1A is degraded EP300 dissociates from many locations. The locations where both ARID1A dissociates and chromatin accessibility is lost are strongly associated with downregulated transcription. In contrast, sites of gained EP300 occupancy are linked to genes that are transcriptionally upregulated. These chromatin changes are associated with a small number of genes that are differentially expressed in the first hours following loss of ARID1A. Indirect or adaptive changes dominate the transcriptome following growth for days after loss of ARID1A and result in strong engagement with cancer pathways. The identification of this hierarchy suggests that upstream steps may represent new sites for intervention in ARID1A-driven diseases. This may be generally applicable for cancers driven by alterations to epigenetic regulators.

Structure and mechanism of the SWR1 histone exchange complex

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Eukaryotic DNA is packaged into nucleosomes in which the DNA is wrapped around a histone protein core. While this provides greater stability and protection for the DNA, this causes problems for processes that require access to the genetic material. Access is required for processes such as transcription but also for DNA repair. Access is regulated by chromatin remodelling complexes that shuffle nucleosomes along the DNA. Signalling at DNA damage also involves covalent modification of histones and exchange of histones within the nucleosome core *in situ*.

The SWR1 complex has the specific role of replacement of histone H2A with H2AZ within nucleosomes as a signal for DNA damage repair. The multiprotein enzyme complex comprises 14 proteins with a molecular weight in excess of 1 MDa that includes 11 ATP-binding subunits. We have initiated studies on the SWR1 complex to try and understand more about the structure and mechanism of this system using cryoEM, biochemistry and single-molecule approaches. Progress to date will be presented which is already providing insight into the workings of this complex machine

Poster Abstracts

Poster 1

Non-repetitive nucleosome arrays form disrupted, fragile chromatin fibres.

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MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh

Chromatin is the substrate for all DNA-associated processes and revealing its structure is key to understanding the regulation of nuclear functions. DNA sequence influences the primary positioning and binding affinity of histone octamers, but how this affects higher-order chromatin folding is not well understood. Many in-vitro chromatin structure studies utilize the Widom-601 DNA template, which strongly positions nucleosomes, and when reconstituted in the presence of linker histones folds into regular fibres. However, as this template uses a synthetic tandem repeat with strong nucleosome positioning properties that are not commonly found *in-vivo* it does not sample the sequence complexity within cells.

To explore the properties of more physiological DNA sequences, analogous to what may be observed in cells, we synthesized novel DNA templates that contain 25 unique nucleosome positioning sequences derived from the ovine β-lactoglobulin (BLG) gene. Using MNase-seq, BLG sequences position nucleosomes weakly and form irregularly spaced nucleosome arrays. Structural analysis using sucrose gradient sedimentation and small angle X-ray scattering showed that non-repetitive fibres form disrupted and heterogeneous structures when folded in the presence of the H5 linker histone. Then, using single-molecule force spectroscopy with magnetic tweezers to analyse the mechanical properties of fibres we showed that non-repetitive fibres have less intra-nucleosome interactions and are more fragile under tension compared to 601 fibres. Together, these findings indicate that DNA sequence heterogeneity contributes to chromatin structure variability observed *in vivo* and can reconcile some of the divergent data observed between in-vitro and in-vivo studies.

Poster 2

Investigating the role of Raf2 in heterochromatin formation in *S. pombe*

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Institute of Cell Biology, University of Edinburgh

In fission yeast, heterochromatin formation requires RNAi and the histone H3K9 methyltransferase complex CLRC which contains Raf2, a protein with unknown function. Raf2 has an RFTS domain which is also found in DNMT1 in higher eukaryotes, and this domain of DNMT1 binds to histone H3. Using protein modelling of Raf2 to published DNMT1 structures, we identified residues of Raf2 RFTS domain that could be involved in H3 binding. Reporter gene silencing assays along with RT-qPCR and ChIP-qPCR analyses showed that mutating some of these residues in Raf2 leads to defects in heterochromatin formation, possibly because Raf2 is not binding to H3.

One of these mutants showed different effects at different heterochromatic loci, similar to what was previously reported for mutants of the FACT complex. Preliminary mass spectrometry data showed that wild-type Raf2 interacts with both FACT components Spt16 and Pob3, and that this interaction is reduced in the mutant, suggesting that the Raf2 RFTS domain may mediate interaction with FACT and possibly help recruit it to heterochromatin. As this mutant also has a severe silencing defect at the subtelomere, and Raf2 has recently been implicated in mediating recruitment of CLRC to telomeres, it is also possible that the RFTS domain is involved in this locus-specific recruitment.

Our work suggests several potential functions of Raf2 that have not previously been described, including interaction with H3 to tether CLRC to chromatin, and association with the FACT complex. Future work will aim to describe these functions in more detail.

Poster 3

PROTAC Mediated Degradation of Class-I HDACs Leads to Cell Death and Profound Transcriptional Defects in Colon Cancer Cells

India-May Baker[1], *Joshua Smalley*[2], *James Hodgkinson*[2], *Shaun Cowley*[1]

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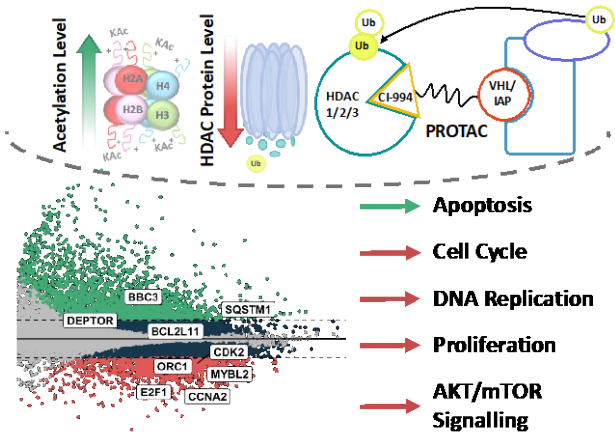
Class-I histone deacetylases (HDACs) play a pivotal role in the regulation of gene expression, acting as the catalytic core of seven multiprotein co-repressor complexes, responsible for the removal of lysine residues from histone tails. The aberrant activity of these enzymes has many repercussions in diseases including colon cancer, making them an auspicious therapeutic targets. However, current HDAC inhibitors lack enzyme and complex specificity, often linked with unfavourable patient side-effects and drug resistance. Proteolysis targeting chimeras (PROTACs) offer the potential to overcome many of these caveats through the targeted degradation of a protein of interest. Smalley et al. (2022) have recently reported an updated library of novel PROTACs, capable of degrading HDAC1, 2 and 3. These PROTACs utilise the benzamide-based inhibitor CI-994, coupled to either a Von Hippel–Lindau (VHL) or inhibitor of apoptosis (IAP) E3-ligase ligand, via an alkyl linker. Although, the underlying mechanisms and transcriptomic effects of HDAC inhibition and PROTAC-mediated degradation in HCT116 cells are poorly defined. A comprehensive transcriptomic analysis of seven novel PROTACs utilising RNA-sequencing revealed many underlying patterns in differential gene expression. It was found that VHL-based JPS016 and IAP-based JPS026, both potent inducers of cell death, led to an enrichment in genes pertaining to key processes including apoptosis, cell cycle and the AKT1/mTOR signalling pathway. In summary, a novel combination of CI-994 and IAP or VHL ubiquitin ligase ligands, coupled via an optimized alkyl linker generates a PROTAC with a potent ability to stimulate apoptosis and differential gene expression in human colon cancer cells.



Reference:

Joshua P. Smalley, India M. Baker, Wiktoria A. Pytel, Li-Ying Lin, Karen J. Bowman, John W. R. Schwabe, Shaun M. Cowley, and James T. Hodgkinson. Optimization of Class I Histone Deacetylase PROTACs Reveals that HDAC1/2 Degradation is Critical to Induce Apoptosis and Cell Arrest in Cancer Cells. Journal of Medicinal Chemistry 2022 65 (7), 5642-5659

Poster 4

Understanding the RERE Deacetylase Complex role in transcription

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The modifications on the histone tails of nucleosomes regulate transcription by recruiting protein complexes to control chromatin structure. Histone Deacetylases (HDACs) are important transcriptional regulators, as they remove acetyl groups from the histone tails. HDACs 1-3 form the catalytic subunit of several large co-regulator complexes. The RERE complex has been shown to both activate and repress transcription and is associated with developmental defects and neurological disorders. The mechanism by which RERE regulates gene expression is unknown. We sought to identify new components of the RERE complex and to investigate the mechanism in which RERE recognises and binds to chromatin.

We have shown that the BAH domain of RERE recognises methylated H3R26 histone peptides, an active transcription mark. Chromatin immunoprecipitation experiments show RERE is located upstream and downstream of the transcriptional start site and located on genes which are known to be regulated by methylated H3R26. We have identified potential novel components of the complex which are involved in splicing and transcriptional activation suggesting RERE maybe involved in multiple stages of transcription. Finally using structural techniques and prediction tools, we propose a structural model for the RERE complex.

Poster 5

Investigating the Role of LSD1 in Early Development

***Megan Broderick**, Shaun Cowley*

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LSD1 (Lysine specific demethylase 1) specifically demethylates H3K4me2 and H3K4me1, thereby repressing transcription of previously permissive genes. LSD1 constitutes part of the CoREST complex and is required for its stability. Knockout of LSD1 in developing embryos is lethal at e6.5, which aligns with the beginning of gastrulation, showing that LSD1 is essential in early development. To investigate the role of LSD1 at this stage, we employed the use of gastruloids as a model system. Produced originally by Beccari et al. (2018), gastruloids closely mimic the multiple germ layer organisation and morphology of gastrulating embryos. Gastruloids were produced using LSD1 KO and control mESCs. RNAseq was then performed on Day 0, Day 3, and Day 5 gastruloids. Analysis has shown differential expression in several genes associated with gastrulation, as well as pluripotency associated genes. These results suggest an impaired ability to switch from a pluripotent state to progression of gastrulation.

Poster 6

The context-dependent of MGA during primordial germ cell development

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The MAX giant associated protein (MGA) is a member of the non-canonical polycomb repressive complex 1.6 and is expressed in the pre-and post-implantation epiblast during mouse embryonic development. Loss of MGA causes early embryonic lethality. Recent studies show that MGA is required for maintaining pluripotency and preventing premature induction of meiotic gene expression in mouse embryonic stem cells (ESCs). However, the role of MGA in the specification and differentiation of primordial germ cells, the embryonic precursors of sperm and egg, is not known. To address this, we used an *in vitro* model, in which mESCs are induced into epiblast-like cells (EpiLCs), which in turn can give rise to functional PGC-like cells (PGCLCs). Using an inducible degron system, we show that depletion of MGA results in loss of PGCLCs concomitant with the upregulation of meiotic genes. CUT&RUN and mass-spectrometry data give an insight into cell-type-specific genomic binding sites as well as interaction partners of MGA. Together, these results point out a context-dependent role of MGA during PGCLC differentiation.

Poster 7

Identifying the acute effects of HDAC1 removal in mouse embryonic stem cells

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Histone Deacetylase 1 (HDAC1) plays a crucial role in embryonic development as evidenced by mouse knockout embryos dying by embryonic day 10.5 (1). The Cowley lab has previously characterised the effects of the double knockout (DKO) of *Hdac1/2* on mouse embryonic stem cells (2). While these efforts have been very informative, they have also been hamstrung by the length of time it takes for protein levels to be completely reduced following the induction of genetic knockout (at least 4 days). This prevents the delineation between primary and secondary effects of the removal of HDAC1/2. To overcome this, we have used the degradation tag (dTag) system to study the acute effects of HDAC1 removal on embryonic stem cells (3). Using the dTag system we were able to remove HDAC1 protein in less than 1 hour thus allowing for the study of effects of HDAC1 removal on a time scale that has never previously been possible. Mass spectrometry revealed increases in acetylation at residues on core histones H2B, H3 and H4 including at key residues such as H3K27. Additionally, RNA-seq revealed that the primary effect on gene expression is largely an upregulation of expression while the secondary effects are more biased towards downregulation. Overall, our results have revealed that the removal of HDAC1 leads to rapid increases in acetylation at key sites on the core histones which are reflected in widespread changes in gene expression.

References

1. Lagger G, O'Carroll D, Rembold M, Khier H, Tischler J, Weitzer G, et al. Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. EMBO J. 2002 Jun 03,;21(11):2672-81.

2. Jamaladdin S, Kelly RDW, O'Regan L, Dovey OM, Hodson GE, Millard CJ, et al. Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells. Proc Natl Acad Sci U S A. 2014 Jul 08,;111(27):9840-5.

3. Nabet B, Roberts JM, Buckley DL, Paulk J, Dastjerdi S, Yang A, et al. The dTAG system for immediate and target-specific protein degradation. Nature Chemical Biology. 2018 - 05;14(5):431-41

Poster 8

Interaction of the MiDAC histone deacetylase complex with chromatin

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Class I histone deacetylase complexes are involved in development, differentiation, metabolism, DNA damage and X-chromosome inactivation. The specificity of these complexes is determined by the accessory proteins that recruit the deacetylase and results in each complex serving an individual role within the cell. Understanding the assembly of these different complexes will enable exploration of their individual functions.

We have solved the structure of the tetrameric MiDAC complex using cryo-EM, which reveals a unique and distinctive mode of assembly. Four HDAC1 proteins are positioned at the periphery with outward-facing active sites suggesting that the complex may target multiple nucleosomes implying a processive deacetylase function.

We are now investigating the interaction of the MiDAC complex with chromatin using cryo-EM and engineered nucleosomes.

References:

Millard CJ, Watson PJ, Celardo I, Gordiyenko Y, Cowley SM, Robinson CV, Fairall L**, Schwabe JW** (2013) Class I HDACs share a common mechanism of regulation by inositol phosphates. Mol Cell 51: 57-67

Turnbull RE*, Fairall L*, Saleh A*, Kelsall E*, Morris KL, Ragan TJ, Savva CG, Chandru A, Millard CJ, Makarova OV, Smith CJ, Roseman AM, Fry AM, Cowley SM**, Schwabe JWR** (2020) The MiDAC histone deacetylase complex is essential for embryonic development and has a unique multivalent structure. Nat Commun 11: 3252

Wang ZA, Whedon SD, Wu M, Wang S, Brown EA, Anmangandla A, Regan L, Lee K, Du J, Hong JY, Fairall L, Kay T, Lin H, Zhao Y, Schwabe JWR**, Cole PA** (2022) Histone H2B Deacylation Selectivity: Exploring Chromatin's Dark Matter with an Engineered Sortase. J Am Chem Soc 144: 3360-3364

Poster 9

Structural insights into the 3D genome folding and expression

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Chromatin is the complex structure formed by the association of nuclear proteins and DNA. It brings about condensation and organisation of chromosomes during the cell cycle, enabling fundamental processes such as gene expression, recombination, DNA repair, and mitosis. A large number of protein complexes form a network within chromatin, carrying out different tasks and distinguishing the high versatility and dynamicity of this structure. My PhD project is centred around the structural and functional characterisation of three protein complexes that carry out fundamental chromosomal transactions. In the first part of my PhD project, I studied the assembly of the IFN-β enhanceosome and the chromatin modifier p300 as a prototypical system to acquire insights into the molecular crosstalk that regulate transcription through enhancers. Moreover, I investigated different assemblies of the cohesin complex with its partners and the way these interactions determine specific roles in different cellular contexts. In particular, I studied the association of cohesin with two factors that play key roles during mitosis and meiosis, respectively. By deepening the understanding of how these molecular machineries play their role in the three-dimensional organisation of the genome it will be possible to broaden the knowledge of the complexity that lays behind essential cell activities, opening new doors to the way these subjects are currently studied.

Poster 10

The Role of Cohesin in 3D Genome Architecture

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Leicester Institute of Structural and Chemical Biology

DNA folding via ‘loop extrusion’ has been proposed to be a key process in the organisation and regulation of the genome. SMC complexes such as condensin and cohesin catalyse this folding of the DNA, bringing distant loci into proximity, regulating many DNA-based processes. It was recently discovered that cohesin can bind to CTCF through a conserved essential surface (CES). An interaction that was shown to be essential for DNA loop formation and stabilisation at TAD boundaries. The fundamental binding motif of CTCF that binds to the CES is also present in other nuclear proteins that regulate transcription, replication, and other important genome functions. These proteins have been referred to as ‘CES’ ligands and are hypothesised to bind and compete for this conserved site on cohesin in order to control various genomic processes. By utilising biochemical assays and structural biology techniques such as X-ray crystallography and cryo-EM I intend to study these CES ligands in greater detail to understand how the control of 3D genome folding contributes to various fundamental genome processes.

Poster 11

Investigating the role of Ying-Yang 1 (YY1) and its recruitment to chromatin in hormone-sensitive breast cancer

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Ying-Yang 1 (YY-1) is a ubiquitously expressed transcription factor, with an eponymous, dual, context-dependent role, as both a transcriptional activator and repressor, involved in both chromatin looping and remodelling, and often dysregulated across cancers. However, the puzzling roles of YY1 are merely disentangled. We recently identified YY1 as a major player in hormone-sensitive breast cancer, essential in cell lines resistant to endocrine therapy. Intriguingly, we found evidence of YY1 binding to chromatin - independent of its consensus DNA binding motif - in MCF7 cells stimulated with oestradiol. Furthermore, rapid immunoprecipitation mass spectrometry (RIME) suggested that YY1 might be able to bind a certain combination of H3 and H4 histone modifications. Nevertheless, YY1 chromatin recruitment did not change in male-specific lethal (MSL) complex knock-out skin fibroblasts, revealing that deficiency in H4K16ac is not sufficient to abrogate YY1 binding in this *in vitro* system. Similarly, YY1 binding is independent of lipopolysaccharide (LPS) stimulation in murine macrophages, suggesting that this inducible DNA sequence - independent binding of YY1 might also be highly context-dependent, in line with the current paradigms. We now further continue exploring the specific dynamics of oestradiol - induced YY1 binding to chromatin of MCF7. SILAC-mass spectrometry revealed that oestradiol also induces changes in some of the relevant histone modifications. Recently performed ChIP-Seq and RIME time course experiments will reveal the YY1 interactome dynamics underlying its recruitment to chromatin in MCF7. We heretofore propose that YY1 contributes to hormone-sensitive breast cancer evolution through a novel, complex mechanism of inducible chromatin remodelling.

Poster 12

Investigating the role of Sin3a

***Samuel Lee**, Shaun Cowley, John Schwabe, Katy Kettleborough*

University of Leicester

Gene regulation through histone acetylation is dynamically regulated through the opposing functions of two families of enzymes, the histone acetyltransferases (HATs) and the histone de-acetylases (HDACs). HDACs are recruited into several different complexes, each with their own plethora of auxiliary proteins, to ensure site specific deacetylation. HDACs are often dysregulated in many types of cancer, and HDAC inhibitors (HDACi) have shown promising effectiveness in the treatment of certain cancers. HDACi unfortunately present with serious side effects due to the large number of complexes effected. One of these complexes, the Sin3 complex, seems best suited for further targeting, as it regulates cell cycle and cell proliferation. Specific functions of the Sin3 complex, such as which genes it regulates and how it regulates cell cycle, are poorly defined. To study Sin3 we created a Sin3a-FKBPF12V fusion protein, which can be selectively degraded within two hours upon the addition of the ligand DTAG-13. In mouse embryonic stem cells, 6 hours after the addition of DTAG-13 we identified using RNA-seq 800 genes that were differentially expressed (fold change > 2, p-value <= 0.01), and approximately 1500 genes after 24 hours. Mimicking early development through the creation of gastruloids showed Sin3a was critical for the effective elongation and enlargement of gastruloids.

Poster 13

Import and chaperoning of monomeric histones

Alonso Javier Pardal *, Andrew James Bowman*

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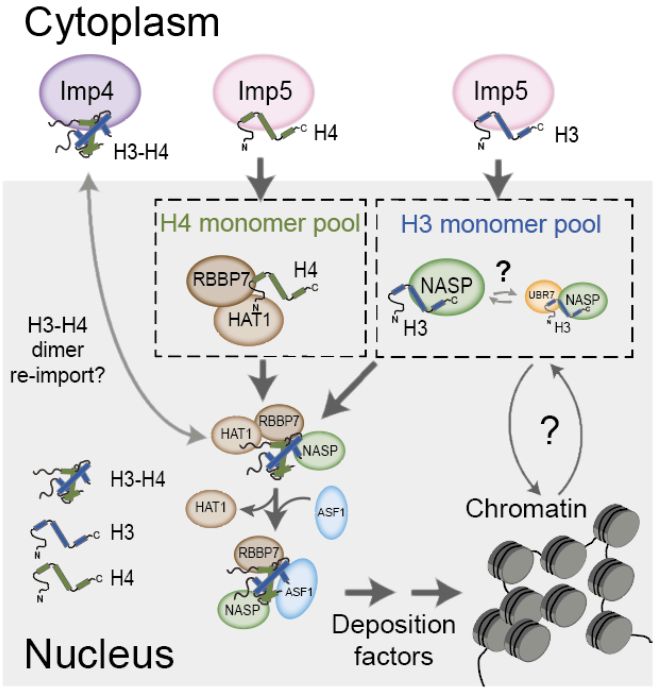
Core histones package chromosomal DNA and regulate genomic transactions, with their import and deposition involving a dedicated repertoire of molecular chaperones. Recent findings allude to a previously unsuspected pool of monomeric histones in the nucleus. Here we show that monomeric H3 and H4 use preferentially an Importin 5 (Imp5) dependent pathway for their nuclear import, distinct from known Importin 4 (Imp4), characteristic of the dimer H3-H4 translocation pathway. Newly synthesized histones H3 and H4 as monomers associate with Imp5 to translocate into the nucleus. Imp5 and NASP are mutually exclusive in their binding, suggesting a facilitated hand-off mechanism facilitated by RanGTP upon nuclear ingress. Furthermore, new H3 accumulates rapidly in a NASP-bound complex after nuclear translocation, with H4 associating with the HAT1-RBBP7 complex. These complexes are known to exist together as the major soluble complex. Thus, we propose that NASP for H3 and the HAT1 complex for H4 buffer any uneven histone synthesis/degradation acting as a docking platform for H3-H4 dimerization and subsequent association with ASF1, the central hub for the histone chaperoning network.



Poster 14

Dissecting the regulatory mechanisms governing histone lysine 9 methyltransferase Clr4

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The formation of heterochromatin plays a key role in the regulation of transcription, genome repair, and replication. The SUV39 family of histone methyltransferases catalyses the methylation of histone lysine 9, a hallmark of heterochromatin. Their crucial role in this process has prompted an increased research interest in the mechanisms of their regulation. The effects of histone post-translational modifications (PMTs) have been extensively studied in literature and ubiquitination has emerged as a key regulatory mechanism associated with the deposition of H3K9me2/3 marks on the genome. Clr4, the sole SUV39 H3K9 methyltransferase in fission yeast Schizosaccharomyces pombe, is highly regulated by PMTs and recent work in our laboratory revealed that ubiquitination of H3K14 stimulates the activity of Clr4 on H3K9. In this project, high resolution x-ray crystallography, cryo-EM, NMR, and biochemical approaches will be used to elucidate on the regulation of Clr4 by PMTs, such as ubiquitination and phosphorylation. The techniques that will be developed in this project will be used as a structural toolbox for probing the regulation of Clr4 and other enzymes of the SUV39 family, such as the human SUV39H1/SUV39H2 homologues, to broaden our understanding and to gain insights into the mechanisms underlying heterochromatin formation.

Poster 15

Histone demethylase KDM4A can modulate the efficiency of post-stress recovery and the efficacy of Pol I inhibitors.

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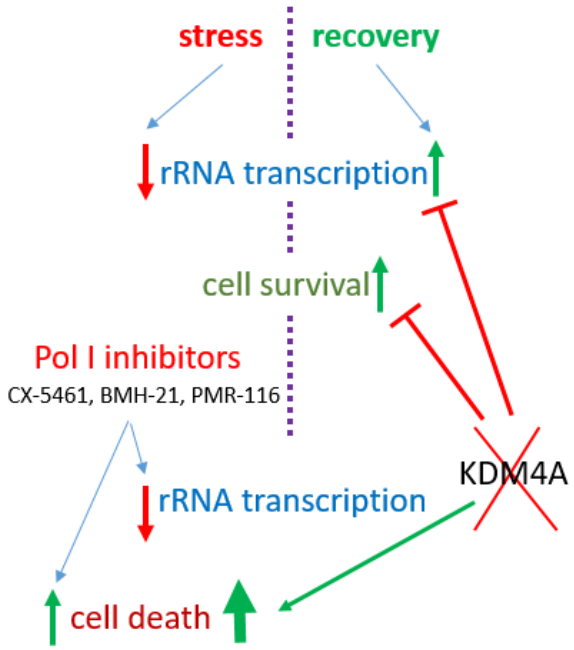

Histone demethylase KDM4A is a member of KDM4 family of 5 histone demethylases and plays an important role in diverse biological processes by regulating differentiation, double-strand break repair, proliferation, metabolic response, genomic stability. Recent work from our lab and others has identified an important role for KDM4A in regulating rRNA transcription on stress dependent manner.[1,2]

Cancer cells are constantly subjected to diverse stresses imposed by the primary or metastatic tumour microenvironment (e.g. nutrients deprivation, lack of oxygen) or by anticancer treatments (e.g. radiotherapy, chemotherapy). In order to survive, cancer cells not only must adapt to these conditions through activating stress-response mechanisms (e.g. energy saving, autophagy, DNA repair *et cetera*), but must be able to efficiently recover once conditions return to normal.

We recently found that KDM4A is required for efficient recovery from external stresses such as heat shock and starvation and, interestingly, affects cell survival after treatment with Pol I inhibitors. We found that transient depletion or inhibition of KDM4A reduces the survival of stressed cells, and increases Pol I inhibitor-induced cell death without increasing the efficiency of Pol I transcription inhibition. We also found that depletion of KDM4A in stressed cells results in decreased rDNA chromatin accessibility even after stress relief.

Here we will discuss potential mechanisms of KDM4A dependent regulation of post-stress survival and modulation of Pol I inhibitors efficacy.

Poster 16

Investigations into the structure and chromatin interactions of the NuRD complex.

***Liam Regan**, Louise Fairall, John Schwabe.*

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The overall aim of this project is to gain more insight into the structure and function of the NuRD complex and its mode of recruitment to chromatin. Recently, the Schwabe lab has solved three sub-complexes of NuRD using single particle cryo-electron microscopy to high resolution. The complexes contain density for the flexible BAH domain of MTA, which was of too low resolution for de-novo building. The BAH domain of MTA1 is thought to enable chromatin binding. One solution to enhance resolution, could be to express the complex without MBD, thereby increasing particle symmetry for creating finer 2D class averages. This information may give greater insight into the functional role of the BAH domain. In addition, I would like to understand the mechanism and regulation of NuRD recruitment to its chromatin substrate. Therefore, electromobility shift assays (EMSA) with reconstituted chromatin have been utilised to investigate this interaction. Modified chromatin and additional macromolecular components will be incorporated in the future to further determine the nature of these interactions.

Poster 17

Investigating the Role of the Sin3A/HDAC1 Complex in DNA Replication and Mitosis

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The Sin3A co-repressor complex utilises HDAC1/2 to catalyse the removal of acetyl moieties from post-translationally modified histones. Unlike other prototypical co-repressor complexes, Sin3A can function as a co-activator, co-repressor and general transcription factor for many genes. Previous mouse knockout models have shown that Sin3A is essential for early embryonic development, cell survival and the maintenance of pluripotency. This project utilises mouse embryonic stem cells (mESCs) and a PROTAC system, where a mutant FKBP12 domain is conjugated to Sin3A and targeted for proteasomal degradation by a degradation tag, dTAG-13 (Nabet et al, 2018). Unlike conventional knockout methodologies, this system enables direct investigation into the acute and rapid effects experienced by cells post-Sin3A loss. Previous Sin3A knockout/null mESCs arrest in the G2/M phase of the cell cycle, triggering a DNA damage response, a phenomenon not observed with deletion of the remaining HDAC1/2 complexes. This suggests that the Sin3A complex plays a vital role in DNA replication and maintenance of genomic integrity (McDonel et al, 2012; Kelly and Cowley, 2013). This project will utilise the DNA fibre assay technique to establish the requirement of Sin3A during DNA replication and mitosis by evaluating the consequential loss of Sin3A on replication speed, fork progression And DNA damage markers such as yH2AX, RPA and RAD51 foci.

References:

Kelly, R.D.W. and Cowley, S.M. (2013) 'The physiological roles of histone deacetylase (HDAC) 1 and 2: complex co-stars with multiple leading parts', Biochemical Society transactions, 41(3), pp. 741-749.

McDonel, P. et al. (2012) 'Sin3a is essential for the genome integrity and viability of pluripotent cells', Developmental Biology, 363(1), pp. 62-73.

Nabet, B. et al. (2018) 'The dTAG system for immediate and target-specific protein degradation', Nature chemical biology, 14(5), pp. 431-441.

Poster 18

Investigating the specialized role of MiDAC- member of the Class 1 histone deacetylase complex family

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Class I histone deacetylases (HDACs) play a pivot role in the regulation of gene transcription acting as the catalytic center of seven co-repressor complexes including SIN3, NuRD and CoREST complexes (Hayakawa, Tomohiro & Nakayama, Jun-Ichi, 2011). The role of the MiDAC complex, a lesser-known complex to its widely studied counterparts, is yet to be fully elucidated. At the current time, there is an insufficient understanding of the complex functional specificity, resulting from the presence of authentic subunits that are unshared between the multi-protein structures.

To characterise the individual complex activity and identify the specific chromatin targets, a CRISPR/Cas9 knock-in degradation (dTAG) strategy has been employed. This system, first described by Nabet et al. (2018), involves the addition of the FKBP12F36V proteolytic tag, facilitating the rapid degradation of the target protein within 1 hour. The approach allows for the acute transcriptional and acetylome changes arising from the rapid loss of MiDAC to be observed.

The current computational analyses indicate gene expression changes of different magnitudes when compared between the co-repressors/complexes. Data has also shown significantly affected genes varying between complexes, which hints at potential complex-specific gene targets.

Building on these findings, the next steps will be conducting further experiments, such as mass spectrometry. The work will continue the characterization of the complexes, elucidating their activity and the specific role played in the cellular environment.

Poster 19

Defining the functional components of constitutive heterochromatin through genetic interaction screening

***Anna F. Townley**, Roopali Pradhan and Julie Ahringer*

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Constitutive heterochromatin makes up 20-50% of animal genomes and is an important modulator of developmental transitions. It is enriched for repressive histone modifications H3K9me2/3 and is associated with the silencing of gene expression, including that of repetitive elements. The mechanisms through which heterochromatin is established and maintained are not well understood.

We seek to identify and functionally dissect components of constitutive heterochromatin in *C. elegans.* We previously identified a network of five heterochromatin factors that genetically interact and co-localise with H3K9me2 at repetitive elements and other genomic regions (MET-2, SET-25, LET-418, LIN-13 and HPL-2*;* McMurchy et al, 2017*)*. These factors regulate transposable element repression, gene expression, DNA repair, and fertility and growth in *C. elegans,* in close collaboration with small RNA pathways. To expand this network, we carried out genetic interaction screens using RNAi. The strains screened were mutants of H3K9 methyltransferases (*met-2, set-25),* HP1 orthologues (*hpl-1, hpl-2*), and HP1-interacting genes (*lin-61, lin-13, tdp-1*). Using a panel of 2309 RNAi clones that target genes known or predicted to encode nuclear localised proteins, we identified 289 enhancers and 86 suppressors of the phenotypes of one or more of these heterochromatin mutants. Enhancers included components of ribosome biogenesis, ubiquitylation, SUMOylation, RNA splicing and chromatin remodelling pathways, and suppressors included proteins associated with active transcription; most hits have human orthologues. Our results expand knowledge of the network of functional players in heterochromatin formation and function in a metazoan organism and identify targets for in depth studies in *C. elegans* and human cells.

Poster 20

An unexpected histone chaperone function for the MIER1 histone deacetylase complex

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Histone deacetylases 1 and 2 (HDAC1/2) serve as the catalytic subunit of six distinct families of nuclear complexes. These complexes repress gene transcription through removing acetyl groups from lysine residues in histone tails. In addition to the deacetylase subunit, these complexes typically contain transcription factor and/or chromatin binding activities. The MIER:HDAC complex has hitherto been poorly characterized. Here we show that MIER1 unexpectedly co-purifies with an H2A:H2B histone dimer. We show that MIER1 is also able to bind a complete histone octamer. Intriguingly, we found that a larger MIER1:HDAC1:BAHD1:C1QBP complex additionally co-purifies with an intact nucleosome on which H3K27 is either di- or tri-methylated. Together this suggests that the MIER1 complex acts downstream of PRC2 to expand regions of repressed chromatin and to deposit histone octamer onto nucleosome-free regions of DNA.

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