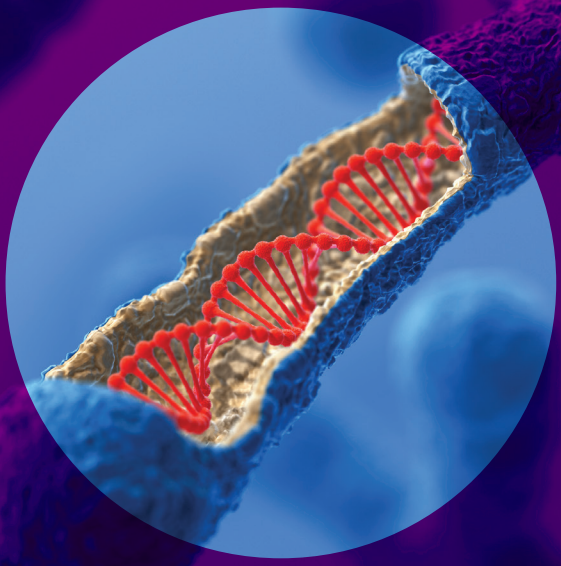
Histone Modifications and Chromatin Structure Symposium

OCTOBER
17–18
2024

Hosted by the Center of Excellence in Chromosome Biology

National Institutes of Health • Building 45 • 45 Center Drive • Bethesda, MD 20892 • Natcher Auditorium

This symposium honors the legacies of Dr. C. David Allis and Dr. Gary Felsenfeld, pioneers in the study of chromatin and gene expression.



Organizers:

Shiv Grewal, Ph.D.
Center for Cancer Research, NCI

Gordon Hager, Ph.D.
Center for Cancer Research, NCI

Alexander Kelly, Ph.D.
Center for Cancer Research, NCI

For more information about the symposium or to see the most recent list of speakers, please scan the QR code or visit: https://go.cancer.gov/hrxQPuE

Center of Excellence in Chromosome Biology

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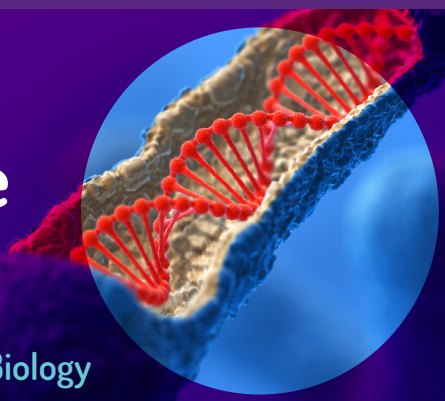
Histone Modifications and Chromatin Structure Symposium



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AGENDA

Thursday, October 17, 2024

8:30 a.m. **Welcome & Introductions**

Session 1: Histone Modifications and Chromatin Structure

Chair: David Clark, Ph.D., *National Institute of Child Health and Human Development, NIH*

8:45 a.m. **New Insights Link the Histone Methyltransferase SETD2 with Nuclear Lamina Integrity and Genome Stability**
Brian Strahl, Ph.D., *University of North Carolina at Chapel Hill*

9:15 a.m. **A New Layer of Gene Regulation Exploited in Human Disease**
Shelley Berger, Ph.D., *University of Pennsylvania*

9:45 a.m. **Molecular Insight into Epigenetic Regulatory Mechanisms Delivers Critical Targets for Cancer Therapeutics**
Ali Shilatifard, Ph.D., *Northwestern University*

10:15 a.m. **Chemical Approaches to Studying Chromatin**
Tom Muir, Ph.D., *Princeton University*

10:45 a.m. **BREAK**

Session 2: Chromatin Remodeling and Histone Dynamics

Chair: Alexander Kelly, Ph.D., *Center for Cancer Research, NCI*

11:00 a.m. **The Biogenesis and Regulatory Dynamics of Chromatin Accessibility for Gene Expression**
Carl Wu, Ph.D., *Johns Hopkins University*

11:30 a.m. **Structure and Function of Mammalian SWI/SNF Chromatin Remodeling Complexes in Health and Disease**
Cigall Kadoch, Ph.D., *Harvard Medical School*

12:00 p.m. **Shaping Chromatin and Cell Fate, a Choreography Involving Histones and Partners**
Genevieve Almouzni, Ph.D., *Curie Institute*

12:30 p.m. **Genome-Wide Mapping of Chromatin Dynamics in Situ**
Steve Henikoff, Ph.D., *Fred Hutch Cancer Center*

1:00 p.m. **LUNCH & POSTER SESSION**

Session 3: Chromatin Modifications and Gene expression

Chair: Eros Lazzerini Denchi, Ph.D., *Center for Cancer Research, NCI*

3:00 p.m. **Chromatin Dynamics in Cancer**
Emily Bernstein, Ph.D., *Icahn School of Medicine at Mount Sinai*

3:30 p.m. **Epigenetic Regulation by the MSLs: Old Complex, New Facts**
Asifa Akhtar, Ph.D., *Max Planck Institute*

4:00 p.m. **Role and Mechanism of Polycomb Silencing in Gastrulation**
Yi Zhang, Ph.D., *Harvard Medical School*

4:30 p.m. **Epigenetic Mechanisms of Tissue Aging**
Payel Sen, Ph.D., *National Institute of Aging, NIH*

5:00 p.m. **Adjourn for the Day**

Friday, October 18, 2024

Session 4: Chromatin Organization and Genome Stability

Chair: Elissa Lei, Ph.D., *National Institute of Diabetes and Digestive and Kidney Diseases, NIH*

9:00 a.m. **Proximity Copy Paste (PCP): A New Method to Map the 3D Organization of Chromatin**
Iestyn Whitehouse, Ph.D., *Memorial Sloan Kettering Cancer Center*

9:30 a.m. **Rogue Chromosome Duplication in Cancer**
Mirit Aladjem, Ph.D., *Center for Cancer Research, NCI*

10:00 a.m. **A Histone Methylation Writer/Reader System that Specifies the Location of Recombination**
Todd Macfarlan, Ph.D., *National Institute of Child Health and Human Development, NIH*

10:30 a.m. **Epigenetic Control of Enhancers in Cell Fate Transition and Cancer**
Bing Ren, Ph.D., *University of California, San Diego*

11:00 a.m. **BREAK**

Session 5: Histone Modifications and Development

Chair: Yamini Dalal, Ph.D. *Center for Cancer Research, NCI*

11:15 a.m. **Human Development and Evolution Through the Gene Regulatory Lens**
Joanna Wysocka, Ph.D., *Stanford University*

11:45 a.m. **Cryo-EM Insights into the Structure and Regulation of Chromatin Modifying Complexes**
Eva Nogales, Ph.D., *University of California, Berkeley*

12:15 p.m. **Overcoming Chromatin Barriers to Change Cell Fate**
Kenneth Zaret, Ph.D., *University of Pennsylvania*

12:45 p.m. **How Pioneer Factors Open Chromatin in Cell Reprogramming**
Yawen Bai, Ph.D., *Center for Cancer Research, NCI*

1:15 p.m. **LUNCH & POSTER SESSION**

Session 6: Histone Modifications and Gene Silencing

Chair: Alan Hinnebusch, Ph.D., *National Institute of Child Health and Human Development, NIH*

3:00 p.m. **Understanding Heterochromatin Dysregulation in Childhood Cancers**
Peter Lewis, Ph.D., *University of Wisconsin, Madison*

3:30 p.m. **New Insights into the Role of the lncRNA Xist and X-Chromosome Dosage Compensation**
Kathrin Plath, Ph.D., *University of California, Los Angeles*

4:00 p.m. **DNA-Based Instruction for Heterochromatin Formation in the Mouse Epigenome**
Thomas Jenuwein, Ph.D., *Max Planck Institute*

4:30 p.m. **The Molecular Basis of Heterochromatin Assembly and Epigenetic Inheritance**
Shiv Grewal, Ph.D., *Center for Cancer Research, NCI*

5:00 p.m. **Adjourn**

Histone Modifications and Chromatin Structure Symposium

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PP 01

COHESIN CONTROLS CCAN ASSEMBLY AND STABILIZATION IN MITOSIS.

JULIAN HASSE, MARY KATE BONNER, **KOLY AKTAR** & ALEXANDER E KELLY[1]

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The inner kinetochore, defined by the Constitutive Centromere-Associated Network (CCAN) complex, directly interacts with CENP-A-containing nucleosomes at centromeres. Upon entry into mitosis, the CCAN undergoes reorganization and becomes immobilized in preparation for chromosome segregation, yet the mechanisms facilitating the formation of the mitotic CCAN remain unclear. In this study, we demonstrate that cohesion is crucial for the stabilization and complete assembly of the CCAN In mitosis. Our findings reveal that a pool of cohesion localizes to centromeres in early mitosis and is safeguarded by a chromosomal passenger complex (CPC)-dependent pathway that counteracts the cohesin agonist WAPL. We show that the CPC and cohesin are essential for maintaining proper CCAN levels and stabilization. Interestingly, WAPL activity is still necessary for CCAN assembly; its depletion results in defects in CCAN levels at kinetochores and fails to rescue CPC depletion, indicating a non-cohesive role of cohesin in CCAN maturation and a need for cohesion turnover. Moreover, CPC-dependent WAPL regulation is vital for primary constriction formation, accurate kinetochore orientation, and the localization of the CENP-A binding region of CENP-C to centromeres. We propose that coheisn stabilizes the CCAN by facilitating the mitosis-specific organization of centromeric chromatin required for mitotic CCAN assembly.

PP 02

PROTEIN PHOSPHATASE 1 NUCLEAR TARGETING SUBUNIT PNUTS PREVENTS THE MISLOCALIZATION OF CENP-A AND CIN

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Chromosomal instability (CIN), a major hallmark of cancer, can be driven by defects in the integrity of centromeric chromatin or kinetochore structure. The histone 3 variant CENP-A, which normally localizes to the centromere, is overexpressed in many cancers. Mislocalization of overexpressed CENP-A to non-centromeric regions contributes to CIN. Despite advances in understanding the consequences of CENP-A mislocalization, pathways that regulate its mislocalization have not been comprehensively studied. In a genome-wide siRNA screen for gene depletions that lead to increased nuclear CENP-A levels, we identified Protein Phosphatase 1 (PP1) nuclear targeting subunit (PNUTS) that confers substrate specificity to PP1, among the top candidates. In the present study, we uncovered a role for PNUTS in preventing the mislocalization of CENP-A and CIN. We developed a degron-based PNUTS-FKBP12 fusion cell line to rapidly deplete PNUTS upon addition of dTAG-13. Cells depleted for PNUTS showed increased nuclear intensity of ectopically expressed YFP-CENP-A in HeLa cells at all cell cycle stages. Increased mislocalization of CENP-A and CENP-C were observed in PNUTS-depleted cells along with increased CIN phenotypes and reduced kinetochore integrity. Previous studies have reported a role for the H3.3 chaperone DAXX in promoting CENP-A mislocalization. The CIN phenotypes in PNUTS-depleted cells was rescued by suppressing CENP-A mislocalization through depletion of DAXX. Expression of a PNUTS mutant defective for PP1 binding did not suppress CENP-A mislocalization suggesting a PP1-mediated role of PNUTS in preventing CENP-A mislocalization. We hypothesize that defects in the regulation of phospho-proteome upon PNUTS depletion may favor CENP-A deposition to non-centromeric regions. We are currently characterizing changes in global proteome and phospho-proteome to gain mechanistic insights into the role of PNUTS in regulating CENP-A mislocalization. In summary, our studies define a novel role for PNUTS in preventing CENP-A mislocalization and CIN, and advance our understanding of pathways that regulate CENP-A overexpression and mislocalization.

PP 03

UNDERSTANDING HOW PIONEER FACTOR DIFFERENTIALLY TARGET H3K9ME3-HETEROCHROMATIN.

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During development, cells require establishment of new gene expression profiles, thus necessitating transitions from a closed and repressive chromatin structure to an open and active chromatin structure on *cis*-regulatory elements. The transition is often initiated by pioneer factors which bind closed and unmodified chromatin and enable it remodelling into an open chromatin structure. However how pioneer factors target and reorganize H3K9me3-heterochromatin which represses lineage-specific genes during development, is not clearly understood. Indeed various pioneer factors, such as pluripotent factor SOX2 which is able to bind and reorganize unmodified closed chromatin, are unable to bind H3K9me3-regions. Recently specific pioneer factors, such as PAX7 which drives muscle cell differentiation, has been identified as able to weakly bind H3K9me3-regions but not elicit their opening while few others, such as ESRRB which drives ectoderm differentiation, is able to bind and initiate their opening. Thus, I developed a technique allowing identification of pioneer factor and H3K9me3-heterochromatin interactions. Using sucrose gradient sedimentation, I purified native gene-associated H3K9me3-heterochromatin regions from human embryonic stem cells and human BJ fibroblast cells. I found that pluripotent pioneer factor OCT4 is associated with heterochromatin regions whereas SOX2 is only associated with non-heterochromatin regions in human stem cells. Pull-down of pioneer factors, such PAX7 and ESRRB, from heterochromatin-enriched sucrose gradient sedimentation fractions allows the identification of proteins involved in chromatin decompaction. In parallel, I also reconstituted *in-vitro* H3K9me3/HP1-compacted nucleosome arrays in order to validate the interactions pioneer factors and H3K9me3-domains and identify protein domains mediated those interactions and heterochromatin reorganization.

PP 04

INVESTIGATING DIRECT INTERACTIONS OF THE MYC ONCOPROTEIN WITH TRANSCRIPTIONAL ELONGATION FACTORS FOR DRUG TARGETING

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The MYC oncoprotein is a potent transcription factor that is dysregulated in a majority of human cancers, resulting in more aggressive disease and poor patient prognosis[1]. Despite the high therapeutic potential of MYC inhibitors, targeting MYC has been largely unsuccessful, partially due to the intrinsically disordered nature of the protein. However, several globular proteins have been identified as MYC binding partners and map to highly conserved but disordered regions of MYC[1]. It is at these sites of interaction where we aim to target MYC activity by disrupting MYC protein-protein interactions (PPI). We have previously identified protein phosphatase 1 nuclear targeting subunit (PNUTS) as a direct interactor of MYC and an important regulator of MYC stability[2,3]. PNUTS interacts with a disordered region of MYC known as MYC-box 0 (MB0) through PNUTS' alpha-helical bundle, annotated as a TFIIS N-terminal Domain (TND)[2]. Interestingly, the TND is a highly conserved structural domain shared among several proteins involved in transcriptional initiation and elongation[4]. Due to our interest in targeting the MYC-PNUTS interaction, we investigated whether MYC is capable of interacting with other TND-containing proteins. We show several TND-containing proteins interact directly with MYC through the same conserved MB0 regions as the PNUTS-MYC interaction using biolayer interferometry (BLI) and NMR. We also used a proximity ligation assay to confirm these interactions occur within the cellular environment. Future work will look to evaluate these TND-containing proteins in functional assays to determine the role of these interactions with MYC at the transcriptional level. These data will also help prioritize which MYC interactors to further investigate as potential therapeutic targets and enable data-driven design of selective small molecule inhibitors for future drug development.

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PP 05

T HELPER CELL FATE ORCHESTRATED BY H3K4ME1-CHROMATIN LOOP-GATA3 TANGOS

LIU S[1,5,8], CAO Y[1,8], CUI K[1,8], REN G[1,6], ZHAO T[1,7], WANG X[1], WEI D[2], CHEN Z[3], GURRAM RK[2], LIU C[4], WU C[3], ZHU J[2], ZHAO K[1,9,*]

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The development and function of the immune system are controlled by temporospatial gene expression programs, which are regulated by cis-regulatory elements, chromatin structure, and trans-acting factors. In this study, we catalogued the dynamic histone modifications and chromatin interactions at regulatory regions during T helper (Th) cell differentiation. Our data revealed that the H3K4me1 landscape established by MLL4 in naive $CD4^+$ T cells is critical for restructuring the regulatory interaction network and orchestrating gene expression during early phase of Th differentiation. GATA3 plays a crucial role in further configuring H3K4me1 modification and chromatin interaction network during Th2 differentiation. Furthermore, we demonstrated HSS3 anchored chromatin loops function to restrict the activity of Th2 locus control region (LCR), thus coordinating the expression of Th2 cytokines. Our results provide insights into the mechanisms of how the interplay between histone modifications, chromatin looping, and trans-acting factors contributes to the differentiation of Th cells.

PP 06

ENHANCERS ON THE LOOSE: UNVEILING THE DETERMINANTS OF SUSCEPTIBILITY TO DISRUPTION OF CHROMATIN STRUCTURE

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CTCF-mediated chromatin loops play a crucial role in facilitating interactions between distal genomic regions. These loops have also been proposed to insulate enhancers from contacting with promoters in neighboring domains to prevent ectopic gene activation. However, the *in vivo* significance of this model has not been thoroughly tested. To test whether chromosome domains with higher density of developmental regulators are more susceptible to disruption of chromatin structure, we deleted a 25kb region containing four CTCF motifs at the boundary of a domain harboring the *Fgf3*, *Fgf4*, and *Fgf15* loci. These genes with distinct spatiotemporal expression are critical for cell fate specification, patterning and organogenesis. Strikingly, heterozygous mutants showed perinatal lethality and encephalocele, a neural tube closure defect caused by over-proliferation of neural tissue, abnormal cranial morphology, and skull bone hypoplasia. To confirm that these defects arise from loss of CTCF mediated insulation, we replaced the 25kb boundary with a 672 bp transgene containing the four CTCF motifs, flanked by l*oxP* sites. Reintroduction of CTCF motifs rescued the phenotypes and confirmed the role of CTCF boundaries. The *Fgf3/4/15* genes were ectopically over-expressed in the midbrain and recapitulated the expression pattern of *Ano1* gene, located in an upstream domain. This suggested that loss of CTCF resulted in aberrant contact of the *Fgf* genes with *Ano1* enhancers. We performed region capture Micro-C in midbrain and visualized complete loss of loops, domain fusion and ectopic interaction of potential *Ano1* enhancer with *Fgf3* gene in mutant embryos. Interestingly, deletion of *Ano1* enhancers along with CTCF motifs completely prevented the deleterious phenotypes. Surprisingly, among the four motifs in the boundary, deletion of one CTCF motif oriented towards *Ano1* enhancer was enough to recapitulate encephalocele defect. Our work depicts how the effect of chromatin structure perturbation on gene regulation is highly dependent on developmental context, and that loss of a single CTCF motif in a gene-rich domain boundary can perturb higher-order chromatin organization and cause a detrimental effect in fetal development.

PP 07

HIERARCHICAL CHEMICAL INHIBITION OF MYST ACETYLTRANSFERASE ENZYMES

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Lysine acetyltransferases (KATs) catalyze protein acetylation which is a post-translational modification playing a critical role in the regulation of transcription, metabolism, and other central biological functions. MYST family is one of the subfamilies of KATs. It is composed of 5 KATs: KAT5, KAT6A, KAT6B, KAT7, and KAT8. Dysregulation of MYSTs has been implicated in the activation of oncogenic gene expression programs in human cancers such as acute myeloid leukemia, breast cancer and so on.
Pfizer reported a very potent KAT6 (KAT6A and KAT6B) inhibitor named PF-9363 recently. It inhibits the activity of KAT6 leading to growth suppression in ER+ breast cancer. Due to PF-9363 binds to the active site of KAT6 and enzymes in the same subfamily have similar active sites, we supposed that a specific inhibitor of KAT6 may hit other subfamily members at higher concentrations, rendering them 'pan-MYST' inhibitors. This could be harmful or beneficial depending on the context.
To understand the anticancer properties of PF-9363, we characterized the target engagement of inhibitor PF-9363 in cancer cells. First, we incubated nuclear extracts from Hela with different concentrations of PF-9363, then used KATs-capturing beads to enrich KATs. If PF-9363 occupies active site of KATs in advance, these KATs will be less enriched. Using this competitive method conjugated with proteomic profiling, we found that PF-9363 engages MYST KAT complexes in a dose-dependent hierarchical manner, from most potent to least potent is: KAT7>KAT8>KAT5. We also measured the enzyme activity of different MYSTs by measuring their acetylation level. The inhibition of enzyme activity is consistent with the potency of engagement between MYSTs and PF-9363. Using NCI60 screening to test the cytotoxicity of PF-9363, we found that the cytotoxicity of PF-9363 is strongly dose-dependent and greatest at high concentrations in multiple cell lines especially breast cancer cell lines. Analysis of CRISPR knockout in the DepMap dataset also shows that breast cancer cells such as BT-549 is more dependent on other MYST proteins than KAT6. All these results suggest that the cytotoxicity of PF-9363 at high concentration is caused by its pan-inhibition of MYST family members.
Overall, our study defines hierarchical target engagement of MYST acetyltransferases by PF-9363 at enzyme and cellular level and provide the first evidence that engagement of other MYST proteins can be exploited for anticancer therapy.

PP 08

GRANULE ASSOCIATION DRIVES SMALL RNA BINDING SPECIFITY FOR NUCLEAR ARGONAUTE PROTEINS

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RNA interference (RNAi) is an evolutionarily conserved gene silencing process that protects genome integrity and regulates gene expression. The core components of RNAi are Argonaute proteins and the associated small RNAs. In *C. elegans*, there are 19 functional Argonaute proteins and diverse small RNAs. Correct small RNA binding of Argonaute proteins is essential for accurate gene regulation. However, it is unknown how the small RNA binding specificity is achieved. Most of the RNAi components localize in phase-separated germ granules at *C. elegans* germline nuclei periphery. SIMR foci is a newly discovered germ granule compartment with unknown protein components and functions.
Here we discovered several new SIMR foci components including HRDE-2 and ENRI-2. HRDE-2 and ENRI-2 interact with unloaded nuclear Argonaute protein HRDE-1 and NRDE-3 respectively in SIMR foci, drive spatial separation from non-target small RNAs, and contribute to their nuclear Argonaute binding specificity. In addition, we found that the somatic nuclear Argonaute NRDE-3 switches small RNA targets during embryogenesis, suggesting a dual role in of Argonaute protein in *C. elegans* development. Therefore, our study contributes to a deeper understanding of the function of phase-separated germ granule compartments in *C. elegans* small RNA pathway and elucidates a new Argonaute binding specificity mechanism.

PP 09

TOPOISOMERASE-MODULATED GENOME-WIDE DNA SUPERCOILING DOMAINS COLOCALIZE WITH NUCLEAR COMPARTMENTS AND REGULATE HUMAN GENE EXPRESSION

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DNA supercoiling is a biophysical feature of the double helix with a pivotal role in biological processes. However, understanding of DNA supercoiling in the chromatin remains limited. Here, we developed azide-trimethylpsoralen

sequencing (ATMP-seq), a DNA supercoiling assay offering quantitative accuracy while minimizing genomic bias and background noise. Using ATMP-seq, we directly visualized transcription-dependent negative and positive twin-supercoiled domains around genes and mapped kilobase-resolution DNA supercoiling throughout the human genome. Remarkably, we discovered megabase-scale supercoiling domains (SDs) across all chromosomes that are modulated mainly by topoisomerases I and IIβ. Transcription activities, but not the consequent supercoiling

accumulation in the local region, contribute to SD formation, indicating the long-range propagation of transcription-generated supercoiling. Genome-wide SDs colocalize with A/B compartments in both human and Drosophila cells but are distinct from topologically associating domains (TADs), with negative supercoiling accumulation at TAD boundaries. Furthermore, genome-wide DNA supercoiling varies between cell states and types and regulates human gene expression, underscoring the importance of supercoiling dynamics in chromatin regulation and function.

PP 10

HIGH RESOLUTION MAPS OF CHROMATIN REORGANIZATION REVEAL A MEIOTIC-SPECIFIC ARCHITECTURAL ROLE FOR CTCF IN MOUSE MEIOSIS

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When germ cells transition from the mitotic cycle into meiotic prophase I, chromosomes condense into an array of chromatin loops that are required to promote homolog pairing and genetic recombination. To identify the changes in chromosomal conformation, we used a unique nuclei sorting strategy to isolate germ cells at stages ranging from spermatogonia, through the mitotic-to-meiotic transition stages, and up to the end of meiotic prophase I. By performing in-situ Hi-C and Micro-C, we explored the trajectory of chromatin reorganization at the highest resolution to date.

We find that the stereotypical large-scale A and B compartmentalization is lost during meiotic prophase I alongside the loss of topological associating domains (TADs). Still, local sub-compartments and the interactions between regulatory elements were detected and maintained throughout the stages analyzed. This establishes a potential mechanism for how the meiotic chromatin maintains active transcription within a highly structured genome. The isolation of a previously unrecognized cell population that has exited the spermatogonia stage yet has not entered meiosis enabled us to uncover a concurrent decline in mitotic cohesion and a rise in meiotic cohesin complexes before meiotic entry, initiating meiotic chromatin reorganization. Enhanced Micro-C resolution revealed that, despite the loss of TADs, higher frequency contact sites between two loci were detectable during meiotic prophase I coinciding with CTCF bound sites. The pattern of interactions around these CTCF sites with their neighboring loci showed that CTCF sites were anchoring meiotic loops. Additionally, the localization of CTCF to the meiotic axes and its interaction with meiotic-specific cohesins indicated that these anchors were at the base of loops. Strikingly, we find that double-strand break (DSB) hotspot sequences interact with CTCF bound loci at a high frequency. In summary, our high-resolution data reveals previously unappreciated aspects of mammalian meiotic chromatin organization. Our data indicate that CTCF plays a role in stabilizing cohesin complexes and anchoring meiotic loops. Having a flexible but reproducible looping point might be an important feature that allows both homologs to have an array of loops in register that in turn would facilitate homology search, homologous pairing and subsequent double-strand break repair.

PP 11

A CHEMICAL GENETIC APPROACH UNCOVERS CHROMATIN-ASSOCIATED PROTEINS AS NOVEL SUBSTRATES OF CYCLIN D-CDK4/6

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The G1-phase cyclin-dependent kinases, CDK4 and CDK6, partner with D-type cyclins to drive cell proliferation. The best-known targets for cyclin D-CDK4,6 complexes are the pocket protein family members retinoblastoma protein (RB), p107, and p130, which they inactivate through phosphorylation. Besides deactivating RB, cyclin D-CDK4,6 complexes also initiate other processes in G1-phase. However, the exact targets, through which they do so are not known.
Here, to determine novel targets of cyclin D-CDK4,6 complexes in a high-throughput manner, we have developed modified analog-sensitive versions of CDK4 and CDK6. These versions of CDK4 and CDK6 can use bulky ATPgS analogs as substrates and can be inhibited by bulky ATP-competitive inhibitors in *in vitro* biochemical assays. Furthermore, we have generated cell lines with conditionally expressing these analog-sensitive CDK4 or CDK6. This approach has allowed us to label potential new CDK4,6 targets in cells and to determine the labeled proteins using mass-spectrometry. As a result, we have determined numerous chromatin-associated proteins as targets for CDK4 and CDK6, suggesting an important role for these CDKs in modulating chromatin structure during cell cycle. Our results identifying novel targets of cyclin-CDK4,6 complexes extend our understanding of how cyclin D-CDK4,6 drive cell-cycle progression and provide novel means for cancer therapeutics.

PP 12

DNA-PAINT TECHNOLOGY FOR MINFLUX IMAGING OF NUCLEAR PROTEINS

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Super-resolution microscopy techniques have been crucial for examining molecular distributions and assemblies in biological systems [1-5]. Notably, MINFLUX [4] and DNA-PAINT [5] methods have recently achieved nanometer resolution, allowing detailed exploration of sub-cellular processes and structures at the molecular scale [6,7]. MINFLUX, for example, has been instrumental in studying motor protein dynamics [8], the actin cytoskeleton [9], and various other sub-cellular components. Likewise, DNA-PAINT has been used to reveal nanoscale details of nuclear pore complexes [7], as well as the spatial distribution of CD20 receptors [7] and kindlin-2-GFP [6], among others.

Typically, DNA-PAINT images are acquired on camera-based imaging systems. However, DNA-PAINT is also compatible with MINFLUX acquisition [10]. MINFLUX provides higher level of nano-resolution than camera-based systems due to the imaging technology itself. On the other hand, camera-based systems can also achieve nano-scale resolution through data post-processing [6,7]. Here, we address an important question: does MINFLUX-based acquisition produce molecular distributions and patterns that are different from those acquired by camera-based technologies due to inherent differences between these two imaging methods? To address this, we image samples stained by DNA-PAINT both on MINFLUX and a conventional camera-based system (Carl Zeiss Elyra). We estimate the localization precision and accuracy on samples with well-defined structures, such as DNA-origami constructs. We then compare the resulting molecular distributions and nanoscale structures in nuclear pore complexes (NPCs) and the glucocorticoid receptor (GR) [11] associated with an array of binding sites in the nucleus by evaluating common metrics: 1) localization precision, 2) resolution of the final reconstructed images.

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PP 13

STRUCTURAL MECHANISM OF TAF-Iβ CHAPERONE FUNCTION ON LINKER HISTONE H1.10

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Linker histone H1 plays an essential role in the regulation of gene activity through the maintenance of higher-order structure and epigenetic state of chromatin, which is facilitated by its chaperones. However, little is known about the structural mechanism of how the chaperones recognize linker histones and conduct their function. Here, we investigated the recognition of human linker histone isoform H1.10 by the TAF-Iβ chaperone using solution NMR in combination of spin labeling, modeling, mutagenesis, and isothermal titration calorimetry. Both H1.10 and TAF-Iβ proteins consist of folded cores and disordered tails. We found that H1.10 formed a complex with TAF-Iβ in a 2:2 stoichiometry. Using distance restraints obtained from methyl-NMR and spin labels, we built a structural model for the core region of the complex. In the model, TAF-Iβ core interacts with the globular domain of H1.10 mainly through electrostatic interactions. We confirmed the interactions by measuring the effects of mutations on the binding affinity. Comparison of our structural model with the chromatosome structure shows that TAF-Iβ blocks the DNA binding sites of H1.10. Thus, our study provides insights into the structural mechanism whereby TAF-Iβ functions as a chaperone by preventing H1.10 from interacting with DNA directly. Our study also demonstrates that methyl-TROSY NMR combined with spin-labeling can be useful for obtaining structural information of the protein complexes with similar sizes, providing insights into their biological functions.

PP 14

EVERY AMINO ACID MATTERS, BUT SOME MATTER MORE: INSIGHTS FROM POPULATION-WIDE HISTONE MISSENSE MUTATION LANDSCAPE

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Core histone proteins are encoded by dozens of paralogous genes. This complicates the interpretation of the biological significance of histone missense mutations frequently observed in malignancies and developmental disorders, as a single allele contributes only a fraction of total protein. Several dominant "oncohistone" alleles represent a notable exception, where a single copy is sufficient to inactivate a "writer" enzyme *in trans* (Lewis et al., 2013, Lu et al, 2016) or *in cis* (Nacev et al., 2024), or disrupt the chromatin fiber structure (Bennett et al., 2019) upon incorporation. However, the vast majority of histone mutations in disease remain difficult to interpret.

We predicted that critical variants will be absent in the missense variant landscape in healthy population – thus allowing us to refine the true "driver" mutations in disease and uncover biologically significant residues. To this end, we analyzed germline histone alleles reported in Genome Aggregation Database (gnomAD4.1) encompassing over 730,000 exomes and 76,000 whole genome assemblies, revealing both entirely invariant residues, and patterns in missense variants suggesting yet-uncharacterized dominant mechanisms.

We find that, while many residues demonstrate distinct constraint patterns, only three residues across four core histones are entirely invariant: H4 lysine 91 (K91), H4 tyrosine 98 (Y98), and H2B lysine 116 (K116). Alterations at any of these three thus must have a strong dominant effect, critically impairing development and/or survival. As proof of principle, H4 K91 mutations were reported previously in a severe developmental disorder (Tessadori et al., 2017), and our own studies suggest H4 K91 mutations alter H3/H4 deposition (Feng et al., 2023). Little is known about the other two residues, with the dominant mechanisms likely involving distinct chromatin systems. Further, we reasoned that any lysines that do not tolerate M/I substitutions represent likely targets of SET methyltransferases with critical developmental function. Out of 58 lysine residues in core histones where codon structure permits a K>M/I substitution from a single nucleotide change, 31 do not tolerate either of these variants. Importantly, both H3 K27 and K36 are found within this group, providing convenient positive controls. Absence of M/I variants in this subset of lysine residues suggests that (a) these represent likely targets of SET domain methyltransferases *in vivo*, and (b) these methyltransferases in turn have a critical role in development. Remarkably, no "writers" or "readers" of methylation are reported for many of these residues, offering a starting point for new investigations into the biologically significant histone modifications.

PP 15

THE CYSTEINE-RICH DOMAIN IN CENP-A CHAPERONE SCM3^{HJURP} ENSURES CENTROMERE TARGETING AND KINETOCHORE INTEGRITY

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Centromeric chromatin plays a crucial role in kinetochore assembly and chromosome segregation. Centromeres are specified through the loading of the histone H3 variant CENP-A by the conserved chaperone Scm3/HJURP. The N-terminus of Scm3/HJURP interacts with CENP-A, while the C-terminus facilitates centromere localization by interacting with the Mis18 holocomplex via a small domain, called the Mis16-binding domain (Mis16-BD) in fission yeast. Fungal Scm3 proteins contain an additional conserved cysteine-rich domain (CYS) of unknown function. Here we find that CYS binds zinc and centromeric DNA in vitro, and it is essential for the localization and function of fission yeast Scm3. Disrupting CYS by deletion or introduction of point mutations within its zinc-binding motif prevents Scm3 centromere localization and compromises kinetochore integrity. CYS alone can localize to the centromere, albeit weakly, but its targeting is greatly enhanced when combined with the Mis16-BD. A truncated protein containing Mis16-BD and CYS, but lacking the CENP-A binding domain, causes toxicity and is accompanied by considerable chromosome mis-segregation and kinetochore loss. These effects can be mitigated by mutating the CYS zinc-binding motif. Collectively, our findings establish the essential role of the cysteine-rich domain in fungal Scm3 proteins and provide valuable insights into the mechanism of Scm3 centromere targeting.

PP 16

LIVE-CELL IMAGING UNCOVERS DYNAMIC COUPLING BETWEEN ENHANCER TRANSCRIPTION AND GENE ACTIVATION

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Despite 40 years of investigation, the mechanisms by which enhancers locate and activate their target genes remain a central unanswered question in the field. Recent studies have identified enhancer transcription as a robust signature of enhancer activity, yet the regulation of this process and its potential role in gene activation are still poorly understood. To address these fundamental questions, we characterized the transcriptional dynamics of the endogenous MYC gene and its upstream enhancer in a non-transformed human cell line, focusing on the coordination between enhancer and promoter activities. Using dual-color live-cell imaging, we discovered that transcription from both the MYC enhancer and promoter occurs in bursts, but with markedly different dynamics. Intriguingly, we found that the MYC gene and its enhancer are transcribed in alternating phases, suggesting a potential relay-like mechanism where these processes are sequentially coordinated. Importantly, perturbation of the MYC enhancer or its RNA results in a significant reduction in the MYC gene transcription. Taken together, these results suggest that enhancer transcription may prime the gene for activation while also limiting the duration of its activity. In summary, our work uncovers dynamic coupling between enhancer and gene transcription, previously obscured by the lack of temporal information, offering new insights into the mechanisms of enhancer function.

PP 17

TOPOLINK™: A HIGH-RESOLUTION, HIGH-THROUGHPUT, PROXIMITY LIGATION METHOD THAT ENABLES DETECTION OF STRUCTURAL VARIANTS AND CHROMATIN TOPOLOGY FEATURES IN CANCER.

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Despite numerous technological advances, including the widespread adoption of massively parallel genome sequencing, many clinically relevant cancer driver mutations go undetected. Additionally, even with the most comprehensive cancer profiling using a combination of whole genome and whole transcriptome sequencing, a driver mutation goes undetected in approximately 20% of cancers, making targeted treatment of these patients challenging. The reason for this gap in understanding is two-fold.: 1) current technologies do not have the required sensitivity for detection of the causative variants; or 2) causative variants are epigenetic or regulatory in nature and are not captured by current technologies. An explanation for 1) is that structural variants (SVs) are particularly challenging to detect using shotgun or long read-based approaches, particularly in heterogenous samples. This is due to the absolute requirement that a read spans the breakpoint of a chimeric molecules. For 2) it is increasingly described that epigenetic dysregulation may be a driver of disease state through mechanisms such as enhancer hijacking or changes to 3D genome organization.

To address the gap of current technologies outlined above, we developed TopoLinkÔ – a dual-purpose proximity ligation library protocol that yields high-quality, high-resolution, unbiased Hi-C libraries capable of highly sensitive detection of structural variants and chromatin topological features from a single data set. Importantly, TopoLink sample-to-library preparation can be performed in under 8 hours – less than half of the required time using conventional Hi-C protocols. With this technology, we demonstrate enhanced sensitivity for detection of variants found within the primary sequence (such as SVs, SNVs/Indels, and CNVs) in addition to high resolution of 3D chromatin conformation.

In this study we performed benchmarking analyses for both genetic variant detection and chromatin topology in two well characterized lymphoblast cell lines, GM12878 and K562. We highlight the capability of TopoLink data to detect interchromosomal translocations with 10-fold more sensitivity over shotgun. Furthermore, using hybridization capture to detect the BCR-ABL1 fusion of K562 cells, we show that a fraction of 1M read pairs is sufficient to detect this fusion with high confidence in TopoLink libraries. In addition, we highlight that shotgun-like, uniform coverage of TopoLink data additionally enables detection of SNV/Indel, with improved sensitivity and precision over conventional HiC methods. Importantly, we demonstrate that TopoLinkÔ libraries maintain topological features consistent with biologically relevant topologically-associated domains (TADs) and chromatin loops, and enable insight into novel epigenetic cancer drivers. Remarkably, TopoLink data enables higher resolution assessments of chromatin topology as demonstrated by 50% more TADs and loops called at 5kb resolution over conventional, multi-RE-based Hi-C technologies sampled to the same sequencing depth. Taken together, we demonstrate TopoLinkÔ proximity ligation libraries as a complementary technique that offers enhanced sensitivity of clinically relevant structural variants with the added benefit of improving discovery of novel epigenetic mechanisms.

PP 18

TARGETING LNCRNA WITH SMALL MOLECULES TO INVESTIGATE CHROMOSOME INSTABILITY IN CANCER

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Centromeric protein A (CENP-A), a histone H3 variant, is essential for recruiting microtubule attachment to the centromere, ensuring proper chromosome segregation during cell division. Accurate localization of CENP-A is mediated by the centromeric-specific chaperone HJURP and centromeric non-coding RNA. In cancer cells, CENP-A is overexpressed and mislocalized to fragile chromosomal regions facilitated by non-centromeric chaperones, ultimately leading to mitotic defects. Recent findings indicate that oncogenic long non-coding RNAs (lncRNAs) from these ectopic sites also play a role in CENP-A recruitment. Oncogenic lncRNA has emerged as a promising therapeutic target because of its specificity and often structure-based function that can be targeted with small molecules. Small molecule microarrays enable screening of thousands of drug-like small molecules to identify selective lncRNA binders that could disrupt or stabilize functional interactions. Using this technology, we set out to identify small molecule lncRNA binders that target ectopic CENP-A deposition as a novel therapeutic route to treat cancer progression. We focused our work on an oncogenic lncRNA, prostate cancer associated transcript 2 (PCAT2), that has been shown to recruit CENP-A to the c-MYC locus. Using in vitro transcription, we prepared PCAT2 RNA which we subsequently fluorescently labeled allowing for small molecule screening. With the fluorescently labeled PCAT2 RNA, we screened through 21,000 drug-like small molecules using small molecule microarrays. In total, we identified 30 selective hits. We are now using chromatin immunoprecipitation and immunofluorescence combined with DNA Fluorescence in Situ Hybridization (FISH) to test if CENP-A loading at the c-MYC locus is altered in the presence of these binders. We anticipate these small molecule lncRNA binders will provide novel drugs that regulate ectopic CENP-A for the first time in cancer. Furthermore, this approach is broadly applicable and expands the chromatin toolbox for deciphering epigenetic roles of lncRNA.

PP 19

IMAGING-BASED CRISPR KNOCKOUT SCREEN IDENTIFIES REGULATORS OF CENTROMERE CLUSTERING

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Centromeres are specialized genomic loci that assemble the multi-protein kinetochore complex, which interacts with the spindle apparatus to ensure faithful chromosome segregation during cell division. Centromere function is highly conserved across species although the structure of kinetochores and the underlying DNA sequences can be diverse. For example, the primary function of both single nucleosome-length centromeres of baker's yeast and the megabase long centromeres in humans is to ensure proper chromosome segregation. Faithful centromere function enables sister kinetochores to correctly attach to the spindle fibers from opposite poles, congregate into the metaphase plate and to help align spindle apparatus along the axis of cell division - all within a given time frame for a smooth completion of cell division. Therefore, physical location of centromeres in the 3D space of an interphase cell nucleus is a crucial determinant of genomic stability. While in most yeasts all centromeres form a single cluster at the nuclear periphery, multiple heterochromatin embedded clusters of centromeres are observed in *Drosophila* and mice. In human cells clustering is less pronounced, but some centromeres tend to cluster near the nucleolus. The molecular mechanisms that determine the location and extent of clustering of centromeres in the 3D space of the human cell nucleus are largely unknown. By quantitatively measuring the degree of centromere clustering across several cell lines, we find cell type specific differences. For example, colon cancer epithelial cells have a central arrangement of centromeres, while centromeres are well distributed in retinal epithelial cells. To address the molecular mechanisms of cell type specific centromere clustering patterns, we performed an arrayed high-throughput CRISPR knockout screen for 1068 chromatin-associated proteins in these two cell types. We identified several genes encoding components of nucleoli, kinetochore, the cohesin-condensin complex, nuclear pores, and the replication machinery that regulate centromere clustering in both cell types. We also found cell type specific effects. For example, CRISPR knockout of a substantially larger number of nucleolar components perturb centromeres clustering in one cell type compared to the other. These experiments are providing novel insights into mechanisms of centromere clustering and of higher order genome organization in general.

PP 20

THE CENP-A ASSEMBLY MACHINERY REGULATES CENTROMERE ARCHITECTURE

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The equal segregation of chromosomes during proliferation is essential for proper cell function. The kinetochore, a large multiprotein complex, facilitates this process by connecting the spindle microtubules to segregating chromosomes during cell division by assembling at a specific locus of the chromosome called the centromere. Centromeres are defined by nucleosomes containing the centromere-specific histone H3 variant CENP-A, which is epigenetically maintained by the chaperone HJURP and its cofactors. CENP-A nucleosomes assemble the inner kinetochore that associates with the centromeric chromatin throughout the cell cycle. Studies in different organisms, including humans, have shown that the centromere contains only a small fraction of CENP-A nucleosomes compared to histone H3 nucleosomes, and they exist as a cluster at the kinetochore formation site. However, the molecular mechanisms by which centromeres and kinetochores are spatially organized are unknown. In this study, we used super-resolution microscopy and *Xenopus* embryo extracts to examine the *de novo* assembly of kinetochore architecture. Strikingly, we observed that the spatial arrangement of the inner kinetochore is dependent on the cell cycle. The architecture of inner kinetochore proteins CENP-C and CENP-T transform from irregular puncta in early interphase to ring-like structures with a ~220-280 nanometer diameter in late interphase, which then reverts back to punctate structures in mitosis. Importantly, CENP-A also forms a ring-like structure in late interphase, and disruption of CENP-A loading destabilized the ring architecture of the inner kinetochore components without affecting their centromeric levels. The CENP-A loading factor HJURP and its cofactors localize inside of the ring-like structures and are required for centromere and kinetochore architecture. Altogether, our data reveal a new role for the CENP-A assembly machinery in the spatial organization of the kinetochore. We propose that these cell cycle-dependent changes to centromere architecture are important to prepare kinetochores for segregation.

PP 21

PATTY: A BIAS ESTIMATION AND CORRECTION MODEL FOR BULK AND SINGLE-CELL CUT&TAG DATA

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The accurate detection of transcription factor (TF) binding sites and histone modifications (HM) on the genome-wide scale is essential for studying functional epigenetics and gene regulation. Cleavage Under Targets & Tagmentation (CUT&Tag) is a low-cost and easy-to-implement epigenomic profiling method that can be performed on a low number of cells and on the single-cell level. CUT&Tag experiments use the hyperactive transposase Tn5 for tagmentation. We find that Tn5 is subject to intrinsic sequence insertion bias (intrinsic bias). Additionally, preference of Tn5 insertion toward accessible chromatin also affects the distribution of CUT&Tag reads (open chromatin bias). Both types of biases can significantly confound bulk and single-cell CUT&Tag data analysis, which requires careful assessment and new analytical methods. To address this challenge, we present PATTY (Propensity Analyzer for Tn5 Transposase Yielded bias), a computational method for systematic characterization and correction of biases in CUT&Tag data. We demonstrate that histone modification signals (H3K27me3 and H3K27ac) detected from CUT&Tag data after bias correction using PATTY are better associated with orthogonal biological features such as gene expression. PATTY-corrected single-cell CUT&Tag signals for histone modification can better cluster cells into their true cell types. This new computational method can improve both bulk and single-cell CUT&Tag data analysis.

PP 22

MOLECULAR SIGNATURES OF CB-6644 INHIBITION OF THE RUVBL1/2 COMPLEX IN MULTIPLE MYELOMA

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Multiple Myeloma is the second most hematological cancer. RUVBL1 and RUVBL2 forms a subcomplex of many chromatin remodeling complexes implicated in cancer progression. As an inhibitor specific to the RUVBL1/2 complex, CB-6644 exhibits remarkable anti-tumor activity in xenograft models of Burkitt's lymphoma and multiple myeloma (MM). In this work, we defined transcriptional signatures corresponding to CB-6644 treatment in MM cells and determined underlying epigenetic changes in terms of chromatin accessibility. CB-6644 upregulated biological processes related to interferon response and downregulated those linked to cell proliferation in MM cells. Transcriptional regulator inference identified ATF4/CEBP and E2Fs as regulators for downregulated genes and MED1 and MYC as regulators for upregulated genes. CB-6644-induced changes in chromatin accessibility occurred mostly in non-promoter regions. Footprinting analysis identified transcription factors implied in modulating chromatin accessibility in response to CB-6644 treatment, including ATF4/CEBP and IRF4. Lastly, integrative analysis of transcription responses to various chemical compounds of the molecular signature genes from public gene expression data identified CB-5083, a p97 inhibitor, as a synergistic candidate with CB-6644 in MM cells, but experimental validation refuted this hypothesis.

PP 23

DIFFERENTIAL HETEROCHROMATIN TARGETING BY PIONEER TRANSCRIPTION FACTORS

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At the onset of cell fate transitions, pioneer transcription factors target silent chromatin and enable the activation of regulatory elements required for the future cell state. Yet pioneer factors do not bind all their cognate DNA motifs across the genome, often binding with cell-type specificity. How specific heterochromatin states govern pioneer factor binding is unknown. Here, I systematically assess the ability for seven lineage-specifying pioneer factors and a non-pioneer factor, ectopically expressed at equivalent levels in a reprogramming cell model to target the dominant forms of mammalian heterochromatin, H3K27me3 and H3K9me3. I find that the transcription factors TCF-1, ESRRB, and EOMES bind targets enriched for H3K9me3 and H3K27me3. In contrast, PAX3, PAX7, SOX2, and FOXA bind within the cracks of broad heterochromatin domains, targeting unmodified nucleosomes flanked by heterochromatin enriched for the repressive modifications. In contrast to all seven pioneers, the non-pioneer HNF4A is confined to targeting nucleosome-free chromatin. Through global depletion of H3K9me3, I observe that SOX2 gains access to key developmental genes, demonstrating that H3K9me3 is a direct barrier to its binding. By engrafting non-DNA binding protein domains from TCF-1, ESRRB, and EOMES onto SOX2, I engineered hybrid SOX2 proteins with the goal of overcoming the H3K9me3 and H3K27me3 barrier. I found that a protein domain of ESRRB enables SOX2 to bind H3K9me3 and H3K27me3 targets. My results demonstrate that pioneer factors differentially target heterochromatin, enabled by protein segments outside of the DNA binding domain and may provide novel tools to enhance gene activation of heterochromatin targets during cellular reprogramming.

PP 24

NUCLEOSOME REMODELER EXCLUSION BY HISTONE DEACETYLATION ENFORCES HETEROCHROMATIC SILENCING AND EPIGENETIC INHERITANCE

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Eukaryotic genomes harbor heterochromatin domains, which assemble at repetitive elements and gene-containing regions, playing a critical role in maintaining genome stability and preventing aberrant gene expression. Heterochromatin enforces transcriptional gene silencing and can be epigenetically inherited, but the underlying mechanism has remained elusive. We demonstrate that histone deacetylation—a conserved feature of heterochromatin from yeast to mammals—restricts the accessibility of SWI/SNF subfamily nucleosome remodelers responsible for chromatin unraveling. This restriction stabilizes modified nucleosomes and promotes gene silencing. Our findings reveal that histone hyperacetylation, induced either by the loss of a histone deacetylase (HDAC) or by the direct targeting of a histone acetyltransferase to heterochromatin, allows remodeler access, leading to silencing defects. Interestingly, the requirement for HDAC in heterochromatin silencing can be bypassed by impeding the activities of these nucleosome remodeling enzymes. Highlighting the crucial role of SWI/SNF, merely targeting this complex to heterochromatin, even in cells with functional HDAC, increases nucleosome turnover, causing defective gene silencing and compromised epigenetic inheritance. Our study elucidates a fundamental mechanism whereby histone hypoacetylation, maintained by high HDAC levels in heterochromatic regions, ensures stable gene silencing and epigenetic inheritance, by thwarting nucleosome remodeling activities.

PP 25

DIRECT OBSERVATION OF TRANSCRIPTION FACTOR BINDING AT TARGET GENES IN LIVING CELLS

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Transcription factors (TFs) bind to upstream regulatory elements, initiating downstream cascades involving transcriptional regulators to activate or repress gene transcription. While each gene is typically regulated by a limited number of “specific” TFs, these TFs are vastly outnumbered by “non-specific” TFs in the nucleus. A key question is how genes discriminate specific TF binding from the overwhelming presence of non-specific TFs. Moreover, although TF dynamics in live cells have been extensively studied, their binding kinetics in the context of transcription at endogenous gene loci remain largely unknown. Here, we use single-molecule imaging in living cells of endogenous transcription factors binding at native genes, coupled with a model of kinetic proofreading to address the question of TF specificity. As a member of the conserved nuclear receptor superfamily, the glucocorticoid receptor (GR) mediates transcription of glucocorticoid-responsive genes in a ligand-dependent manner, making it an ideal candidate for studying inducible gene transcription. Using single-molecule tracking (SMT), we first examined the global dynamics of endogenously Halo-tagged GR. Notably, GR residence times on chromatin significantly increased in the presence of ligand, with minimal stable-binding in the inactive state, suggesting that long-lived GR residence times lasting tens to hundreds of seconds are relevant for transcriptional activity. To spatially resolve specific from non-specific TF binding, we endogenously tagged *ERRFI1* (GR-responsive gene) with MS2 stem loops to fluorescently mark the gene in the transcriptionally active state. Using dual-color, single-molecule imaging of GR and *ERRFI1*, we observed that GR exhibits substantially longer residence times at regions near the *ERRFI1* compared to the *MYH9* locus (non-target gene). In combination with the conceptual framework of kinetic proofreading, these data suggest promoters are ‘dwell time detectors’ rather than simply ‘occupancy detectors.’ Overall, our findings support the hypothesis that dissociation rates could serve as a kinetic signature for distinguishing specific from non-specific TF binding events.

PP 26

UNRAVELLING CENTROMERIC EVOLUTION BY NUCLEOSOME ELASTICITY

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Faithful chromosome segregation, which is indispensable for survival of all organisms, is evolutionarily conserved across most eukaryotes. Despite the function being preserved, both centromeric DNA and kinetochore proteins are rapidly evolving. These contrasting aspects of centromere biology are known as "centromere paradox". This motivates us to understand mechanistic basis of centromeric evolution. Unlike canonical histone proteins which are nearly invariant through evolution, centromeres are epigenetically defined by the fast-evolving histone H3 variant CENP-A. Recently we reported that CENP-A nucleosomes are two-fold more elastic than canonical H3 nucleosomes, and get stiffer when bound to the essential kinetochore protein CENP-C. In this work, we investigate whether CENP-A elasticity is a conserved feature across eukaryotes and co-evolves with kinetochore proteins. To study nucleosome elasticity in different eukaryotic models, we selected CENP-A from fungi kingdom (*Saccharomyces cerevisiae*), plant kingdom (*Arabidopsis thaliana)*, and animal kingdom (*Homo sapiens*). Preliminary results indicate that human CENPA nucleosomes are the most elastic followed by canonical H3, budding yeast Cse4 and *Arabidopsis* CenH3 nucleosomes. Although the size of human centromere tandem repeat (171 bp) is in the same range as that of *Arabidopsis* with 178 bp in length, there is no resemblance in elasticity between human CENP-A and *Arabidopsis* CenH3. Whereas budding yeast point centromere is 125 bp in size with AT rich CDEII DNA elements. Therefore, considering the genetic diversity of centromeres, CENP-A elasticity may be centromere specific and not a conserved feature among eukaryotes. To expand this study further H3.3 histone variants are under investigation. We have specifically chosen H3.3 glioblastoma mutants to understand the relationship between cancer mutations and mechanical characteristics at single nucleosome level and how this modulates chromatin fiber dynamics.

PP 27

AGO2'S CATALYTIC FUNCTION REGULATES PLACENTAL ENDOTHELIAL CELL AND VASCULAR DEVELOPMENT

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Mammalian Argonaute (AGO) proteins are prototypically involved in gene regulation via the microRNA (miRNA) pathway, which uses a multiprotein complex to promote translational repression or decay. However, AGO proteins can also repress transcripts through direct AGO2-mediated cleavage, a conserved mechanism that utilizes catalytic tetrad (DEDH) in its PIWI domain. This mode of gene regulation is well established in the siRNA pathways, only few mammalian miRNAs are known that has full complementarity to the bound RNA that can direct AGO2 for cleavage with unknown physiological significance. Surprisingly, although poorly understood, AGO2 cleavage activity is essential for mammalian viability since mice carrying a single point mutation in AGO2's catalytic tetrad (*Ago2*$^{ADH/ADH}$)-a mutation that abolishes the ability of the AGO2 protein to cleave RNA targets-die soon after birth due to unknown reasons. Yet, the broader roles of AGO2-mediated RNA cleavage in mammals and its implications to human health remain unknown. My unpublished work shows that *Ago2*$^{ADH/ADH}$ mice display placental defects characterized by placentomegaly. By RNA sequencing I found that genes deregulated in *Ago2*$^{ADH/ADH}$ placentas are largely linked to angiogenesis and frequently implicated in embryonic lethality. Upregulated genes in our mutants are also largely related to vascular endothelium functions. Amongst these genes, I found paternally imprinted domesticated retrotransposon gene *Rtl1* is a direct cleavage target of AGO2 and that its upregulation in *Ago2*$^{ADH/ADH}$ mice causes endothelial cell defects that lead to animal lethality within a few hours of birth. Specifically, my data shows that *Rtl1* cleavage is driven by miRNAs expressed from a maternally imprinted miRNAs that is antisense to *Rtl1*. The *in-vitro* established endothelial cell (ECs) line from *Ago2*$^{ADH/ADH}$ placentas shows upregulation of *Rtl1* including higher levels of proliferation, which recapitulates findings *in-vivo* as well as increase cell permeability compared to *Ago2*$^{ADH/+}$ ECs as a control. The rescue of *Rtl1* overexpression using dCas9:CREB system in *Ago2*$^{ADH/ADH}$ ECs successfully rescued the levels of *Rtl1* to levels close to those of control *Ago2*$^{ADH/+}$ ECs. Importantly, this was sufficient to rescue the increased permeability of the mutant lines indicating the phenotype is indeed caused by deregulated *Rtl1* expression. Currently I am doing *in-vivo* rescue experiment to test if *Ago2*$^{ADH/ADH}$ animals are viable postnatal. In summary, my data suggest that cleavage of a domesticated transposon by AGO2 is a crucial event in endothelial cell development and animal viability, indicating important physiological requirement of the conserved but poorly understood catalytic activity of AGO2 in mammals.

PP 28

INVESTIGATING WHY DNMT3A METHYLATES CA IN NEURONS

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DNA cytosine methylation is an epigenetic modification that plays a role in the regulation of gene expression. While CG methylation is most common, neurons exhibit unusually high rates of CA methylation (mCA), solely deposited by DNA methyltransferase three alpha or DNMT3A. DNMT3A mutations cause the neurodevelopmental disorder Tatton-Brown-Rahman-Syndrome and various cancers such as Lung Adenocarcinoma. Here, we explore why its methylation pattern is unique among neurons, albeit DNMT3A is expressed broadly across cell types. We hypothesize that DNMT3A methylates CA DNA in neurons because: 1) Neuron-specific protein interactors “guide” DNMT3A to CA sequences, or 2) The high levels of DNMT3A expression in neurons, in addition to the non-dividing nature of neurons, lead to increased mCA.

To test our hypothesis, we first quantified the levels of DNMT3A and mCA across mouse tissue types at postnatal day (P)15, when DNMT3A levels peak in neurons. DNMT3A levels varied across tissues, with the lung being the highest non-brain region. mCA levels were consistently low across all non-brain tissues. Based on these results, we immunoprecipitated DNMT3A in P15 mouse brain and lung and used Mass spectrometry to compare its interacting proteins in the two tissue types. The results identified several brain-enriched DNMT3A-interacting proteins involved in different biological processes such as chromosome organization and regulation of gene expression. Next, we validated the Mass Spectrometry results by western blot targeting several identified proteins and confirmed their interaction with DNMT3A.We next plan to knock-down the brain-enriched DNMT3A-interacting proteins in neurons to see if they are required for mCA.

To analyze whether the high levels of DNMT3A expression in neurons lead to mCA, we cloned DNMT3A into an overexpression construct and transfected it into HEK293T cells. Genomic DNA and protein were extracted 36 hours post-transfection. DNMT3A overexpression in HEK293T was shown successfully by western blot, and dot blot showed an increase in mCA levels upon DNMT3A overexpression. Based on these results, overexpression of DNMT3A is sufficient to induce mCA in HEK293T cells. We are currently preforming these experiments in Mouse Embryonic Fibroblasts to reproduce our results in a biologically relevant cell type.

Taken together, our results so far suggest that both neuron-specific DNMT3A-interacting proteins and high DNMT3A expression are involved in CA methylation in neurons.

PP 29

ISWI ENSURES PRECISE GENOMIC TARGETING OF MASTER TRANSCRIPTION FACTORS

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ISWI chromatin remodeling complexes use one of the two ATPase subunits Snf2h (Smarca5) and Snf2l (Smarca1) to regulate nucleosome spacing. How ISWI regulates stem cell identity and cell fate transition remains largely unclear. Using gene knockout and acute protein degradation approaches, here we show Snf2h and Snf2l are partially redundant and are essential for embryonic stem cell (ESC) self-renewal, muscle satellite cell identity maintenance, myogenesis and adipogenesis. Mechanistically, depletion of Snf2h and Snf2l results in marked genomic redistribution of ESC master transcription factors (TFs) Oct4 and Nanog, myogenic TF MyoD, adipogenic TFs C/EBPα/β and PPARγ, and other lineage-determining TFs, leading to generally impaired cell identity. In comparison, inactivating SWI/SNF chromatin remodeling complexes generally reduces TF genomic binding. Together, our findings highlight an essential role of ISWI in precise genomic targeting of master TFs critical for stem cell identity maintenance, cell differentiation and tissue development.

PP 30

EPIGENETIC CONTROL OF TOPOISOMERASE 1 ACTIVITY PRESENTS A CANCER VULNERABILITY

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DNA transactions introduce torsional constraints that pose an inherent risk to genome integrity. While topoisomerase 1 (TOP1) activity is essential for removing DNA supercoiling, aberrant stabilization of TOP1:DNA cleavage complexes (TOP1ccs) can result in cytotoxic DNA lesions. What protects genomic hot spots of topological stress from aberrant TOP1 activity remains unknown. Here, we identify chromatin context as an essential means to coordinate TOP1cc resolution. Through its ability to bind poly(ADP-ribose) (PAR), a protein modification required for TOP1cc repair, the histone variant macroH2A1.1 establishes a TOP1-permissive chromatin environment, while the alternatively spliced macroH2A1.2 isoform is unable to bind PAR or protect from TOP1ccs. Mechanistically, we find that macroH2A1.1 facilitates the recruitment of the TOP1cc repair factor XRCC1 in response to both endogenous and drug-induced topological stress. Impaired macroH2A1.1 splicing, a frequent cancer feature, was predictive of increased sensitivity to TOP1 poisons in a pharmaco-genomic screen in breast cancer cells, and macroH2A1.1 inactivation mirrored this effect. Consistent with this, low macroH2A1.1 expression correlated with improved survival in cancer patients treated with TOP1 inhibitors (TOP1i). We propose that macroH2A1 alternative splicing serves as an epigenetic modulator of TOP1-associated genome maintenance and a potential cancer vulnerability.

PP 31

NON-CODING GENETIC VARIANTS DOMINANT IN AFRICAN AMERICAN REVEAL PROSTATE CANCER RISK

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Prostate cancer (PrCa) manifests substantial variation in incidence rates among distinct populations. African American (AA) men are more likely to be diagnosed with and die from PrCa than European American (EA) men. Despite ongoing advancements in identifying polygenic risk variants from large genome-wide association study (GWAS) cohorts, the genetic mechanisms underlying the higher prevalence of PrCa in AA men remain unclear. A systematic approach that does not rely on extensive cohorts to identify causal regulatory variants contributing to PrCa development is still lacking. Here, by employing a sequence-based deep learning model of prostate regulatory enhancers, we identified ~2,000 essential SNPs (eSNPs) with increased alternative allele frequency in AA and which potentially affect the enhancer function leading to greater PrCa susceptibility. The identified eSNPs potentially mediate PrCa development through two complementary mechanisms: alternative alleles with increased enhancer activity are associated with immune system suppression, while those with decreased enhancer activity are linked to differentiation processes. Interestingly, the eSNPs disrupt the binding of key prostate transcription factors including FOX, AR and HOX families, collectively contributing to PrCa predisposition. Together these eSNPs can be used to assess polygenic risk score that is more effective than previous GWAS-based risk scores in distinguishing individuals with PrCa from the control.

PP 32

THE TRANSCRIPTOMIC AND CHROMATIN LANDSCAPE OF MGE AND HYPOTHALAMUS IN WT AND H3K4M HOM MICE

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Methylation of lysine 4 on histone H3.3 (H3K4) is required for promoter activation and undergoes dynamic regulation during neurodevelopment. Perturbation in H3K4 methylation is associated with several neurodevelopmental and psychiatric disorders, but the specific role of H3K4 methylation in distinct brain regions has not been characterized. Here, we explore the role of H3K4 methylation in medial ganglionic eminence (MGE)-derived interneurons and hypothalamus by mating H3K4M mice (in which the H3K4 lysine is mutated to methionine, preventing methylation) with Nkx2.1-Cre mice. As expected, H3K4 methylation in the MGE and hypothalamus is decreased at E13.5. H3K4M homozygous (hom) mice show significant growth retardation and increased death during the 2nd-3rd postnatal weeks, and they have smaller brains and enlarged lateral ventricles. Notably, ~80% of H3K4 hom females showed spontaneous seizures, and both male and female hom mice were more susceptible to developing seizures upon pentylenetetrazol injection. Relatedly, H3K4M hom mice have significantly fewer MGE-derived cortical and hippocampal interneurons at P21. H3K4M homo mice showed less ambulation and more fine movements at 5-6 weeks old mice, but they became more hyperactive and display impulsive behaviors in adults. The mutation of H3K4M also caused cognitive and motor dysfunctions and increased anxiety. To determine how loss of H3K4M methylation affects gene expression and chromatin organization, we performed single cell Multiome analysis on the MGE and hypothalamus from embryonic WT and H3K4M hom mice. We found expression of specific disease-associated genes were downregulated in the MGE and/or hypothalamus. In summary, our study demonstrates that downregulation of H3K4 methylation in the MGE and hypothalamus decreased interneuron density, leading to increased seizures, and causes behavior deficits that are consistent with neurodevelopmental and psychotic disorders.

PP 33

PDAIP-SEQ CAPTURES GENOME-WIDE DNA TOPOISOMERASE CLEAVAGE COMPLEXES WITHOUT PRIOR DRUG ENRICHMENT

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DNA topoisomerases modulate chromatin structure and coordinate spatiotemporal regulation of gene expression in living cells by regulating DNA topology. During the catalytic cycle, topoisomerases break DNA before religation, transiently forming topoisomerase cleavage complexes (Topcc) as covalent protein-DNA adducts. Therefore, profiling Topcc reveals topoisomerase in action more accurately than profiling topoisomerase binding sites via ChIP-seq. However, current Topcc profiling methods apply drugs of topoisomerase poison to enrich Topcc, leading to biased Topcc mapping, as these drugs impact topoisomerase binding and the distribution of Topcc sites.

Here, we developed pDAIP-seq (**P**rotein-**D**NA **A**dduct **I**mmuno-**P**recipitation followed by sequencing), that enables the genome-wide mapping of human Top1cc, Top2acc, and Top2bcc without prior drug enrichment. We found that at transcription start sites (TSS), Top1cc level was low, despite an enrichment of Top1 binding. In contrast, downstream of the TSS along the gene body, the abundance of Top1cc, but not of Top1 binding, was sharply elevated. On the other hand, both Top2acc and Top2bcc showed an enrichment upstream of the TSS and a depletion at transcription termination sites (TES), that was different from Top1cc, indicating distinct roles of different types of human topoisomerases during transcription regulation.

Furthermore, genome-wide analysis revealed that Top1cc enrich at L1 elements while Top1 binding enrich at Alu elements. Co-analysis of topoisomerase binding sites and cleavage complexes with our previous genome-wide DNA supercoiling data showed that Top1 binding, but not Top1cc, was well-correlated with the distribution of DNA supercoiling across the human genome, indicating potential regulation of topoisomerase by DNA supercoiling in human cells. The treatment of camptothecin, an inhibitor of Top1, increases Top1cc levels but also shift Top1cc and Top1 binding patterns, impacting more on actively transcribed genes with a similar distribution pattern of Top1cc and Top1 binding at TSS. Finally, using pDAIP-seq, we mapped native PARP1-DNA crosslinks associated with DNA damage and revealed its colocalization with Top1cc and R-loops across the human genome, indicating potential consequences of Top1cc in genome instability via R-loops and regulation through PARP1.

PP 34

DROSOPHILA HNRNP M HOMOLOG RUMPELSTILTSKIN PROMOTES HOMIE INSULATOR BARRIER ACTIVITY AND REGULATES POLYCOMB-DEPENDENT 3D INTERACTIONS

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Chromatin insulators are DNA-protein complexes that help maintain genome organization by creating boundaries that separate active and inactive chromatin and control promotor-enhancer interactions. In *Drosophila,* Homing insulator at eve (Homie) is an insulator sequence that can regulate interactions between *even-skipped* (*eve*), a Polycomb Group (PcG) regulated gene, and its cis-regulatory elements. Homie also acts as a barrier to prevent PcG repressive chromatin spreading into the neighboring ubiquitously expressed gene *TER94*. The mechanism behind Homie insulator activity and the requirement of trans-acting factors is unknown.
To this end, we determined that Homie barrier activity in all tissues tested relies on Rumpelstiltskin (Rump), as well as two chromatin insulator proteins, Centrosomal Protein 190kD (Cp190) and CCCTC-binding factor (CTCF). To address whether chromatin compaction at the *eve-TER94* locus may be altered, we performed 3C analysis in Kc167 cells after depletion of Rump, Cp190, or CTCF. We found an increase in *cis*-looping within the PcG domain after Rump but not Cp190 or CTCF depletion. Further analyzing the PcG domain, we detected H3K27me3 spreading onto *TER94* in Rump-depleted cells and increased H3K27me3 levels at PcG domains genome-wide by ChIP-seq. Interestingly, this increase in H3K27me3 is accompanied by a global reduction in chromatin bound Polycomb (Pc), a protein in the PRC1 complex, after Rump depletion. To visualize potential changes in 3D genome organization after Rump depletion, we utilized Oligopaint DNA FISH to analyze pairwise PcG domain interactions in Kc167 cells. This analysis revealed that distances between distal PcG domains increase after Rump depletion. However, depletion of Cp190 or CTCF showed no effect, suggesting Rump regulates PcG through a mechanism unrelated to insulator regulation. Taken together, in Rump-depleted cells we observe increased compaction of the PcG domains *in cis* yet decreased compaction *in trans*. To elucidate how Rump affects the localization of Pc to chromatin and influences opposite *cis* and *trans* PcG interactions, future experiments to test how Rump impacts other aspects of both PcG domain formation and maintenance will be performed. Ultimately, through understanding these interactions, we seek to gain additional insight into how Rump promotes Homie barrier activity.

PP 35

DORY: A COMPUTATIONAL METHOD FOR DIFFERENTIAL CHROMATIN TRACING ANALYSIS

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Three-dimensional (3D) genome structure in mammalian cell nuclei is dynamic and complex. The 3D chromatin architectures can be measured by sequencing-based techniques such as Hi-C on the ensemble level or by imaging-based techniques such as chromatin tracing on the single-molecule level. Chromatin tracing is a multiplexed DNA fluorescence in situ hybridization (FISH) technique that can directly trace the 3D positions of genomic loci along individual chromosomes in single cells. A major challenge in analyzing chromatin tracing data is the missing values in the 3D coordinates of some loci, caused by technical limitations in capturing certain genomic regions. Furthermore, there have not been computational methods developed for differential analysis of chromatin traces. Here, we present Dory, a statistical method for differential chromatin tracing analysis that does not require imputation of missing data. Dory measures spatial distance between each pair of regions in each chromatin trace and employs multi-level statistical tests to quantify the difference between different groups of chromatin traces. The output of Dory includes a differential score for each region pair comparing chromatin traces between two biological conditions or cell types. We applied Dory to chromatin tracing data from six cell types in mouse fetal liver samples and from a lung cancer progression model. We observed that the changes in chromatin structure are associated with alterations in A-B compartment interactions. Additionally, we found that differential chromatin region pairs likely indicate promoter-enhancer interaction changes for differentially expressed genes. Dory enables quantification of 3D chromatin structure dynamics across cell states.

PP 36

DELINEATING MYC PROTEIN-PROTEIN INTERACTIONS REGULATED BY PHOSPHORYLATION REVEALS A NOVEL DIRECT BINDING PROTEIN

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Targeting MYC directly has proven difficult and novel strategies to inhibit MYC-driven cancers are urgently needed. MYC is known to harbour many post-translational modifications (PTMs) and have a large network of protein-protein interactions (PPIs) that are responsible for regulating MYC function. Identifying those MYC-protein interactions that are important for oncogenesis could provide novel opportunities to target MYC. We previously identified a MYC alanine phosphorylation mutant as gain-of-function (GOF) for transformation, indicating that the two phosphorylation sites altered in this mutant are important for regulating MYC activity. We leveraged this knowledge to conduct an in-situ labeling technique (BioID) to identify MYC PPIs that specifically interact with the GOF mutant and are thus putative drivers of the transformed phenotype and attractive therapeutic targets. From a list of 38 significant GOF mutant interactors, we prioritized ASF1A as it is overexpressed in many cancers, is involved in H3.3 deposition at transcriptionally active sites and is a structured protein that is targetable. Interestingly, we also detected HIRA as a significant GOF mutant interactor, which belongs to the HIRA complex that directly interacts with ASF1A. To validate the interaction between MYC and ASF1A, we conducted proximity ligation assay (PLA). We demonstrated using phosphorylation mutants that the interaction is phosphorylation-dependent, and showed that phosphorylation via JNK can negatively regulate the interaction under stress conditions. As our goal is to inhibit direct MYC PPIs, we then evaluated whether the interaction was direct using biophysical methods. By both nuclear magnetic resonance (NMR) and bio-layer interferometry (BLI), we determined that the interaction with MYC is direct with low micromolar affinity, and that phosphomimetic mutants disrupt the direct interaction by BLI. Overall, disrupting the MYC:ASF1A interaction in MYC-driven cancers is attractive, and has the potential to further advance cancer therapy and patient outcomes.

PP 37

MECHANISM OF CHROMATIN REMODELING BY SNF2-TYPE ATPASE

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Chromatin remodeling enzymes use Snf2-type motor ATPases to alter histone–DNA interactions through a conserved DNA translocation mechanism. They play a crucial role in the organization of chromatin, enabling both stability and plasticity of genome regulation. Chromatin remodelers slide nucleosomes, but how they move DNA around the histone octamer during this process is unclear. We used cryo-EM to visualize the continuous nucleosomal DNA motion induced by human chromatin remodeler SNF2H, an ISWI family member. Our data show SNF2H actively remodeling nucleosomes, revealing how the chromatin remodeler moves DNA on the nucleosome. We observe conformational changes in SNF2H, DNA and histones during the process of DNA translocation. As the catalytic motors of chromatin remodelers are highly conserved, the mechanisms we describe likely apply to other families.

PP 38

DIFFERENTIAL ACTIVITY OF THE TRANSCRIPTION FACTOR NKX2.1 IN EMBRYONIC MOUSE BRAIN, LUNG, AND THYROID

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Individual transcription factors are often expressed in multiple cell and tissue types during development, but with varying roles and genomic targets. This in part explains why transcription factor mutations in humans can lead to complex, multi-organ phenotypes that are challenging to identify and treat. The factors that guide differential transcription factor activity in distinct tissues are not well understood. Possible mechanisms that guide transcription factor specificity are (1) tissue-specific chromatin accessibility, (2) tissue-specific co-factors and adaptor proteins, and/or (3) tissue-specific genomic binding motifs that recruit a specific set of transcription factors. The transcription factor Nkx2.1 is expressed in the embryonic thyroid, lung, hypothalamus, and medial ganglionic eminence (MGE), where it plays critical roles in development of these tissues. In the MGE, Nkx2.1 serves as a 'master regulator' for all MGE-derived GABAergic inhibitory neurons, while in the lung, it regulates differentiation of alveolar cell subtypes. Humans with NKX2.1 mutations suffer from the aptly named Brain-lung-thyroid syndrome and display neurodevelopmental symptoms, impaired respiration and hypothyroidism. Thus, Nkx2.1 represents an ideal candidate to explore differential transcription factor binding in distinct tissues and its role in disease. To probe differential Nkx2.1 binding in these tissues, we collected the MGE, hypothalamus, lung, and thyroid at embryonic day 13.5 (E13.5) and performed CUT&RUN to identify Nkx2.1 binding sites in each tissue. We also performed single cell Multiome assays to analyze gene expression and chromatin accessibility in Nkx2.1-expressing cells. By integrating gene expression, chromatin accessibility, and Nkx2.1 binding sites, we have gained mechanistic insight into differential Nkx2.1 function in distinct tissue. At some loci, differential accessibility in tissues drives differential Nkx2.1. binding. At other loci, accessibility is similar between tissue, implying that other regulatory mechanisms drive this differential binding. These insights improve our understanding of not only Nkx2.1 regulation during development, but also how transcription factors regulate distinct genetic programs in different tissue during development.

PP 39

COMPACTION OF ACTIVE CHROMATIN DOMAINS BY H3S10-PHOSPHORYLATION IN MITOSIS PRESERVES THEIR INTERPHASE-SPECIFIC 3D CHROMATIN STRUCTURE AND TRANSCRIPTION

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Mitotic chromosomes lose interphase-specific genome organization and transcription but gain histone phosphorylation, specifically H3S10p. This phosphorylation event compacts chromosomes in early mitosis by reducing inter-nucleosomal distance before the loading of condensins. However, it is unclear if H3S10p in mitosis preserves the identity of lost chromatin domains and promoters, both physically and functionally. Here, using the premitotic expression of histone H3S10 and its mutants H3S10A and H3S10D, we show that H3S10p hyper-phosphorylates active promoters and spreads into super-domains A in mitosis, causing compaction of these regions. By spreading into active domains in the absence of genome organization, H3S10p retains their identity physically. Functionally, H3S10p ensures optimal closing of promoters by stabilizing the nucleosomes, thereby protecting them from excess loading of transcription machinery post-mitosis. In the H3S10p phospho-mutants, these chromatin regions fail to condense properly during mitosis. As a result, they exhibit enhanced accessibility and transcription of active genes in the next interphase. We propose that the spreading of mitotic H3S10p into active domains preserves their identity during mitosis and, in subsequent interphase, acts as a rheostat to fine-tune transcription and chromatin domain re-formation.

PP 40

CELL CYCLE DEPENDENT METHYLATION OF DAM1 REGULATES KINETOCHORE INTEGRITY AND FAITHFUL CHROMOSOME SEGREGATION

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The kinetochore, a megadalton structure composed of centromeric (CEN) DNA and several evolutionarily conserved protein complexes, is required for the faithful chromosome segregation in eukaryotes. The evolutionarily conserved Dam1/DASH complex (Ska1 in metazoans) is one of the essential protein sub-complexes of the budding yeast kinetochore. Moreover, Set1-mediated methylation of Dam1 lysine residue 233 is essential for viability as mutation of K233 to alanine in Dam1 causes lethality. In this study, we defined a molecular role for Dam1 lysine methylation in faithful chromosome segregation. Our results show that Dam1 methylation is cell cycle regulated with the highest levels of methylation in metaphase cells. Consistent with these results, co-immunoprecipitation experiments showed an interaction of Dam1 with methyltransferase Set1 in metaphase cells. Previous studies have shown that Set1 colocalizes with Jhd2 a histone lysine demethylase and Jhd2 demethylates Set1-methylated histones. Our genome-wide mass spectrometric analysis of proteins that associate with Jhd2 identified 7 of the 10 subunits of the Dam1 complex; an association of Jhd2 with non-histone proteins such as Dam1 has not been previously reported. We confirmed the interaction of Jhd2 with Dam1 and showed that cells overexpressing *JHD2* exhibit reduced levels of methylated lysine in Dam1, defects in kinetochore assembly, synthetic growth defects in kinetochore mutants, reduced levels of kinetochore proteins at centromeric chromatin and chromosome missegregation. In summary, we have defined a novel role for methylation of Dam1 in kinetochore assembly and demonstrated that a dynamic methylation of Dam1 is essential for faithful chromosome segregation.

PP 41

MINIATURIZING BRCA2 USING CRISPR/CAS9 IN MOUSE MODELS

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BRCA2 is an essential gene for survival and biallelic loss of function is embryonic lethal. It is a huge protein (3418aa) that has multiple domains interacting with different proteins and complexes. We plan to generate a smaller BRCA2 protein by deleting the dispensable regions of BRCA2; this can be used for restoration of protein functions in cells mutated/absence for BRCA2. BRCA2 helps in error free repair of DNA double strand breaks via homologous recombination (HR). The 8 BRC repeats of BRCA2 help in loading RAD51 at the damaged DNA site to facilitate HR. These repeats are highly conserved across species and have differential affinity towards RAD51 speculating functional redundancy. There have been many missense mutations reported in the BRC repeats of BRCA2 but none of them have been found to be associated with cancer progression, further hinting towards their functional redundancy. We wanted to find the minimum number of BRC repeats needed to suffice BRCA2 functions. mES cells-based studies revealed BRC1-4 are enough for fully functional BRCA2 and BRC5-8 are dispensable. Further we made BRCA2 with single BRC repeats (1,2,3 and 4 individual) and found high rate of functional redundancy in them. BRCA2 having just BRC2 and BRC4 were enough for cell survival and RAD51 loading at DNA breaks and performed as good as full length BRCA2. This excited us to check if a single BRC repeat is enough for mouse viability. To our surprise, we were able to generate two mouse models with *Brca2* having just BRC2 and BRC4 using CRISPR/CAS9 technology. These animals breed well and produced homozygous and hemizygous animals in regular Mendelian ratios. To get BRCA2 with single BRC repeat around 1100aa were deleted and it is surprising that removal of about one-third of the protein is functional and supports mouse survival. Reports suggests that the DNA binding domain of BRCA2 (residue 2474–3190) is dispensable for mouse survival. Also, we in our lab have previously generated mouse model lacking *Brca2* exon4-7. These animals are also viable and fertile. We further plan to remove the whole DNA binding domain (716aa) along with exon4-7 (104aa) in BRCA2 having just BRC4 intact which will reduce the protein size less than half of its original.

PP 42

UNRAVELING MOT1'S ROLE IN ORCHESTRATING TBP DYNAMICS IN STRESS-INDUCED GENE REGULATION BY GCN4

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In budding yeast, Mot1, is a member of a Snf2 family, a DNA-dependent ATPase and is essential for the cell viability. Mot1 dissociates TATA binding protein (TBP) from DNA in an ATP-dependent manner to control the unregulated TBP binding and thus maintain a reservoir of unbound TBP for gene expression. Previous studies have shown that Mot1 acts to dissociate TBP preferentially from strong TATA (consensus) elements with attendant increased TBP occupancy at weak TATA-like (non-consensus TATA sequence) elements. In yeast, promoters of the stress-induced genes predominantly rely on the coactivator SAGA complex for TBP recruitment that generally consist of strong TATA element. Conversely, constitutive genes, mostly containing weak TATA promoters are largely TFIID-dependent. So, we tested the regulation of TBP binding at the genes activated during amino acid starved (stress) conditions by Gcn4 in absence of Mot1. Contrary to early findings, we observed that Mot1 is needed for high-level TBP binding at Gcn4-responsive promoters harboring strong TATAs. Expanding our analysis to the ~3600 constitutively expressed genes not regulated by Gcn4 in starved cells, we found that Mot1 depletion generally reduces TBP binding at highly expressed genes while reducing TBP occupancies at highly expressed genes in starved cells. By examining the effects of Mot1 depletion on different sets of genes shown previously to depend on SAGA versus TFIID for wild-type transcription in non-starved cells, stratified according to their expression levels in starved cells, we found evidence that Mot1 acts to redistribute TBP from weakly expressed to strongly expressed genes in starved cells regardless of their reliance on SAGA vs TFIID in non-starved cells. We predict that the genes showing increased TBP binding in Mot1 absence will behave as poorly expressed constitutive genes under non-starved conditions. As a result, will exhibit decreased TBP binding suggesting a role of Mot1 in transcriptional reprogramming during stress-induced conditions. This shift in Mot1-associated TBP dynamics appears critical for cell survival, as lower levels of BTAF1, the human homolog of Mot1, have been linked to lethal skin cancer.

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WITHDRAWN

PP 44

INVESTIGATING THE ROLE OF SMURF2 IN THE REGULATION OF COHESIN PROTEIN COMPLEX

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Abstract:
Cohesin, a multiprotein complex composed of SMC1, SMC3, and Rad21 is indispensable for chromosomal architecture maintenance, particularly in sister chromatid cohesion, DNA repair, and gene expression regulation. Cohesin is known to be ubiquitinated, although the mechanism, regulation, and effects of cohesin ubiquitination remain poorly defined. Recent investigations have shed light on the intricate regulatory mechanisms governing cohesin dynamics, with a spotlight on Smad ubiquitin regulatory factor 2 (Smurf2), a member of the HECT domain E3 ubiquitin ligase family, as a pivotal regulator. Smurf2 was initially recognized as a negative regulator of transforming growth factor-b (TGF-b) and bone morphogenetic protein (BMP) signaling, but recent studies identified its function to regulate genomic stability and carcinogenesis. Therefore, our study provides a focused examination of Smurf2's role in modulating SMC1, SMC3, and Rad21 within the cohesin complex. We report here that Smurf2 is a novel cohesin-interacting protein identified by mass spectrometry analysis of dual-affinity purifications. Subsequent immunoprecipitation/Western blots and immunofluorescence staining confirmed the endogenous interaction and colocalization of Smurf2 and cohesin complex in MCF10A cells. Our study also revealed that the WW and hect domains of Smurf2 are crucial for cohesin binding and Smurf2 negatively regulates cohesin protein levels in MCF10A cells. Furthermore, we also found that Smurf2 and cohesin protein complex colocalized at the mitotic spindle. According to the above results, we summarize that Smurf2 may modulate the assembly or stability of the cohesin complex through direct interactions with its components. By influencing the formation or stability of the cohesin complex, Smurf2 could impact chromosomal processes such as genomic stability and gene regulation.

PP 45

SPECIALIZED REPLICATION OF HETEROCHROMATIN ENSURES SELF-TEMPLATED CHROMATIN ASSEMBLY AND EPIGENETIC INHERITANCE

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Heterochromatin, defined by histone H3 lysine 9 methylation (H3K9me), spreads across large domains and can be epigenetically inherited in a self-propagating manner. Heterochromatin propagation depends upon a read–write mechanism, where the Clr4/Suv39h methyltransferase binds to preexisting trimethylated H3K9 (H3K9me3) and further deposits H3K9me. During DNA replication nucleosomes are disassembled posing challenges for the stability of epigenetic marks. Recent studies suggest an active role for the replication machinery in the recycling of modified histones contributing to the transmission of chromatin states during cell division. However, how exactly the parental methylated histone template is preserved during DNA replication and consequently how this process affects the read-write mediated maturation of chromatin is not well understood. Here, we demonstrate using *Schizosaccharomyces pombe* that heterochromatic regions are specialized replication domains demarcated by their surrounding boundary elements. DNA replication throughout these domains is distinguished by an abundance of replisome components and is coordinated by the heterochromatin protein Swi6/HP1. Although mutations in the replicative helicase subunit Mcm2 that affect histone binding impede the maintenance of a heterochromatin domain at an artificially targeted ectopic site, they have only a modest impact on heterochromatin propagation via the read–write mechanism at an endogenous site. Instead, our findings suggest a crucial role for the replication factor Mcl1 in retaining parental histones and promoting heterochromatin propagation via a mechanism involving the histone chaperone FACT. Engagement of FACT with heterochromatin requires boundary elements, which position the heterochromatic domain at the nuclear peripheral subdomain enriched for heterochromatin factors. Our findings highlight the importance of replisome components and boundary elements increasing a specialized environment for the retention of parental methylated histones, which facilitates epigenetic inheritance of heterochromatin.

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MAPPING THE EARLY AND LATE EPIGENETIC AND TRANSCRIPTIONAL LANDSCAPE OF DOXORUBICIN-INDUCED SENESCENCE

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After sustaining DNA damage, cells choose between several cell fates, including: (1) continued proliferation, (2) programmed cell death, and (3) senescence. Senescence is characterized by long-term cell cycle arrest, high expression of CDK inhibitors, flattened morphology, and secretion of pro-inflammatory cytokines known as the senescenceassociated secretion phenotype (SASP). After treatment with DNA damaging agents, the secretion of SASP cytokines by senescent tumor cells is considered deleterious to patient outcomes by promoting drug resistance in neighboring tumor cells and creating a pro-tumorigenic tumor microenvironment. Thus, understanding the molecular mechanisms by which cancer cells commit to senescence is critical for designing therapeutic interventions to push cells to an alternative cell fate. We have previously shown that blocking the activity of the AP-1 transcription factor c-Jun within the first 12 hours after low-dose doxorubicin-induced DNA damage partially blocks senescence in U2OS osteosarcoma cells, demonstrating that early c-Jun binding to chromatin promotes senescence after DNA damage. In addition, previous studies have shown that changes in chromatin accessibility are required to drive senescenceassociated transcriptional programs. Therefore, we hypothesize that AP-1 binding events lead to changes in chromatin accessibility and gene expression that enforce the commitment to senescence following DNA damage. To link the early epigenetic changes to late transcriptional changes, we performed ATAC-seq, RNA-seq, and CUT&RUN experiments at 4 and 24 hours and 5 days after treating U2OS cells with a senescence-inducing dose of doxorubicin. We observed significant changes in chromatin accessibility binding of the AP-1 transcription factors c-Jun and JunB as early as 4 hours after treatment. These early doxorubicin-specific c-Jun binding sites are in specific enhancer regions that promote activity at later times. Using an activity-by-contact model to predict target genes of enhancers, we link specific differentially-expressed genes upregulated 5 days after doxorubicin treatment to these early c-Jun bound enhancers. Together, these results identify specific changes in both chromatin accessibility and the binding of specific AP-1 transcription factors to the enhancers of late expressed genes as early as 4 hours after doxorubicin treatment.

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KINETICS OF THE PAF1C–SPT5 INTERACTION BY SINGLE-MOLECULE TRACKING IN LIVE CELLS

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Transcription of genes by RNA polymerase II (RNAPII) demands its association with factors that enhance its processivity and help overcome the barrier imposed by chromatin during transcription elongation. A key factor in the elongation machinery is the polymerase-associated factor 1 (Paf1) complex, a five-subunit transcription complex that is universally conserved throughout all kingdoms of life. Paf1C has significant direct and indirect roles in nuclear gene regulation, being involved in nucleosome remodeling, histone ubiquitylation, genome stability, RNA export, and transcriptional elongation. Paf1C's regulatory processes require its association with the transcription elongation complex (EC), an interaction mediated by Paf1C's binding to the intrinsically disordered C-terminal repeats (CTR) of transcription factor Spt5 (subunit of DSIF). While Paf1C's binding specificity to Spt5 and *in vitro* activities have been well-characterized, its spatial and temporal coordination to RNAPII-EC *in vivo* remain elusive.

Live-cell single-molecule tracking (SMT) enables real-time observation of elongation factor interaction dynamics as they search for their targets, allowing for precise quantification of both free- and chromatin-bound protein populations and their residence times. We explore the kinetics of Paf1C and other early elongation factors on chromatin as they associate with RNAPII using SMT in live yeast. Our study reveals that the majority of Paf1C and its recruiter, Spt5, is chromatin-bound, with stable-binding events occurring at residence times closely aligning with RNAPII's (~20s). Additionally, assistive elongation factors such, Bur1 and TFIIS, are highly diffusive and display shorter interactions with chromatin (~5s). Following up on Paf1C dynamics, we demonstrate that the chromatin association kinetics of the complex is highly sensitive to loss of the C-terminal repeats of Spt5, but does not require the full complement of repeats for its function. Overall, our study quantitatively characterizes the key regulatory interaction between Paf1C and Spt5 and provides unique insights to the downstream transcriptional kinetics. With dysfunctional Paf1 complex implicated in human cancers and developmental disorders, these findings are crucial for understanding the role of Paf1C in nuclear processes.

PP 48

ONCOHISTONE H3 E97K MUTATION FACILITATES CENP-A MISLOCALIZATION IN BUDDING YEAST

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Centromeric localization of histone H3 variant, CENP-A (Cse4 in *Saccharomyces cerevisiae*, Cnp1 in *Schizosaccharomyces pombe*, CID in *Drosophila melanogaster*, and CENP-A in humans), is essential for chromosomal stability. Overexpressed CENP-A leads to its mislocalization to non-centromeric chromatin and contributes to chromosomal instability (CIN) in yeasts, flies, and humans. Overexpression and mislocalization of CENP-A is observed in many cancers and this correlates with poor prognosis. Understanding pathways that contribute to mislocalization of CENP-A will help us understand CIN an important hallmark of many cancers. We have defined a role for histone stoichiometry in preventing mislocalization of Cse4 and shown that gene dosage of histone H4 and the interaction of Cse4 with histone H4 facilitates a conformational state of Cse4 *in vivo* from a “closed” to an “open” state facilitating Cse4 mislocalization (Au *et al.,* 2008, Eisenstatt *et al.,*2021, Ohkuni *et al.,* 2022, 2024). Furthermore, studies with human cells depleted for H3-H4 chaperones such as DNAJC9 and CHAF1B exhibit defects in CENP-A mislocalization and CIN (Shrestha *et al.,* 2023, Balachandra *et al.,* 2024). Since histone H3 is an obligate partner of histone H4, we hypothesized that reduced gene dosage or mutants of histone H3 will facilitate Cse4-H4 interaction and promote mislocalization of Cse4. These studies are clinically relevant because mutations in histone H3 (oncohistone) in the histone hold domain, are frequently seen (hotspots) in many cancers.

In this study, we used histone H3 deletion (*hht1*Δ and *hht2*Δ), structurally defective (*hht1 Y99A*), and oncohistone (*hht1 E97A/K*) H3 mutants to examine Cse4-H4 interaction, Cse4 structure, and chromosomal localization of Cse4. Our results showed that reduced gene dosage of histone H3 (*hht1*Δ and *hht2*Δ) and oncohistone H3 mutants *hht1 E97A/K* enhance Cse4-H4 interaction facilitating an “open” state of Cse4 *in vivo* and Cse4 mislocalization. In addition, oncohistone H3 mutants *hht1 E97A/K* exhibit defects in H3-H4 interaction and H3 stabilization. We propose that defects in the interaction of H3 E97A/K with histone H4 result in increased levels of free histone H4 thereby enhancing the interaction of Cse4 with histone H4 resulting in Cse4 mislocalization. These studies advance our understanding for how histone gene dosage and oncohistone mutations contribute to CIN in CENP-A overexpressing cancers.

PP 49

HETEROCHROMATIN IS GENERALLY ACCESSIBLE IN LIVING HUMAN CELLS

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The eukaryotic genome is packaged into chromatin, which resembles beads on a string. Each 'bead' consists of a nucleosome containing ~147 bp of DNA wrapped around a histone octamer. The nucleosomal filament undergoes additional folding to create a complex genomic organization which is thought to restrict the access of gene-regulatory transcription factors to the genome. Consequently, it is widely accepted that DNA accessibility, modulated by chromatin architecture, is pivotal in gene regulation. Here, we measure genome accessibility at all GATC sites in living human MCF7 and MCF10A cells using an adenovirus vector to express the sequence-specific *dam* DNA adenine methyltransferase. We find that the human genome is globally accessible in living cells, unlike in isolated nuclei. Active promoters are methylated somewhat faster than gene bodies and inactive promoters. Remarkably, heterochromatic sites are methylated at a rate that is only marginally slower than that of euchromatic sites. In contrast, subsets of centromeric sites are methylated more slowly and are partly inaccessible. We conclude that both euchromatin and heterochromatin are in a state of flux in living human cells. This dynamic chromatin architecture implies that simple occlusion of transcription factor binding sites is unlikely to be critical in gene regulation.

PP 50

ACETYL-HISTONE READERS BDF1 AND YAF9 GUIDE TARGETING OF SWR1 REMODELER TO +1 NUCLEOSOME

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The histone variant H2A.Z is known to mark permissive chromatin and plays a crucial in gene regulation. H2A.Z is predominantly deposited at promoter-proximal +1 nucleosomes by the ATP-dependent chromatin remodeler SWR1. Although SWR1 targeting to nucleosome-free regions (NFRs) is directed by its free DNA length sensing module, how the remodeler is guided preferentially to flanking +1 nucleosomes has been enigmatic. SWR1 contains acetyl-histone readers, the YEATS domain in Yaf9 subunit and bromo domain in Bdf1 subunit. Despite *in vitro* characterization of these domains, their *in vivo* role in targeting SWR1 to chromatin remains unclear.

Using single-molecule tracking (SMT) in live yeast cells, we investigated the dynamics of H2A.Z and SWR1. Our study indicates that acetyl histone reader domains in Bdf1 and Yaf9 constrains the diffusion of free SWR1 molecules. These reader domains are required for effective chromatin binding via distinct kinetic mechanisms: Bdf1 enhances SWR1 association, Yaf9-YEATS reduces dissociation (thereby stabilizing the interaction). Notably, genome-wide ChIP-exo shows that once recruited to the NFR at gene promoters, the directionality of SWR1 to the +1 nucleosome is regulated by the Bdf1 and Yaf9-YEATS domain. In mutant cells, the loss of this directionality impacts site-specific H2A.Z incorporation.

These findings suggest a two-stage mechanism: acetyl-histone interactions initially constrain three-dimensional diffusion of SWR1, increasing its local concentration, followed by stochastic one-dimensional diffusion at NFRs for directional capture by acetylated +1 nucleosomes. This dual mechanism offers new insights into the precise targeting of chromatin remodelers, enhancing our understanding of chromatin dynamics and gene regulation.

PP 51

EZH2 METHYLTRANSFERASE ACTIVITY ORCHESTRATES CELL FATE CHOICE BETWEEN SKELETAL MYOCYTES AND BROWN ADIPOCYTES

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Ezh2, a histone H3K27 methyltransferase in the Polycomb repressive complex 2 (PRC2), is a critical regulator of transcriptional repression and a recognized key player in cancer biology. The recent FDA approval of an Ezh2 inhibitor Tazemetostat (EPZ-6438) for various cancers underlines its clinical significance. We have shown previously that Ezh2 promotes adipogenesis but suppresses myogenesis in cell culture. However, whether Ezh2 functions as a master regulator in determining the fate of skeletal myoblasts and brown adipocytes, originating from Myf5-expressing precursors, remains unclear. Our current study revealed that mice lacking Ezh2 enzymatic activity in $Myf5^{+}$ cells exhibit reduced interscapular brown adipose tissue mass and enlarged neck muscles, suggesting a critical role for Ezh2 enzymatic activity in orchestrating a cell fate choice between skeletal myocytes and brown adipocytes *in vivo*.

To reinforce our observations, various cell lines were treated with the Ezh2 inhibitor EPZ-6438 to assess functions of Ezh2 enzymatic activity. Inhibition of Ezh2 activity in brown preadipocytes blocked the induction of adipogenesis and lipogenesis gene expression, emphasizing its involvement in promoting adipogenesis. Conversely, inhibiting Ezh2 activity in C2C12 myoblasts increased the expression of myogenic markers, including Muscle creatine kinase and Myosin heavy chain, highlighting Ezh2's role in repressing myogenesis. Further characterization of Ezh2 enzymatic activity in adipogenesis and myogenesis was pursued using C3H10T1/2 mesenchymal stem cells as a model. Our findings showed an elevation in myogenic markers and a reduction in adipogenic markers upon treatment with EPZ-6438, providing insights into the intricate regulatory role of Ezh2 enzymatic activity in lineage determination. We are currently performing single-cell RNA-seq on mouse embryos from E12.5 to E15.5 to provide a more comprehensive understanding of how Ezh2 enzymatic activity regulates cell fate choice during critical stages of embryogenesis.

PP 52

BRCA2 LOSS INDUCES TRANSLESION SYNTHESIS VIA COHESIN STABILIZATION

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Breast cancer type2 susceptibility gene (BRCA2) is a tumor suppressor gene involved in double strand break repair by homologus recombination (HR) and replication fork protection. Hence loss of BRCA2 results in genomic instability and cell death in normal cells. However, few cells in the breast epithelium overcome BRCA2 null crisis and develop into BRCA2 deficient tumors. Molecular mechanism underlining the survival of such cells are still not understood. Here, we hypothesize that over expression of few genes in breast epithelium could help to overcome the BRCA2 null crisis by compensating some of the functions of BRCA2, leading to the development of BRCA2 deficient breast tumors. To understand that we carried out CRISPR activation screening using CRISPR/Cas9 Synergistic Activation Mediator (SAM) library wherein we over expressed ~23000 genes individually and identified 189 genes whose over expression rescues the BRCA2 null crisis. One such factor is Ubiquitin like protein 5 (UBL5). We found that UBL5 supports the very early BRCA2 deficient cells by promoting genomic stability. Mechanistically, we observed that UBL5 stabilizes the cohesin ring (constitutes SMC1, SMC3, RAD21, STAG1/2) efficiently in BRCA2 deficient cells, which is crucial for sister chromatid segregation and DNA replication. Interestingly, wild type BRCA2 itself destabilizes the cohesion ring. Hence, dual effect of BRCA2 loss and UBL5 over expression retains stable cohesin on chromatin in BRCA2 deficient cells. Further, stable retention of cohesin on chromatin upon UBL5 over expression promotes PCNA retention in BRCA2 deficient cells. Since PCNA is the downstream effector of this pathway, cohesion defect and associated cell death by UBL5 loss could be restored by retaining PCNA stable on chromatin by silencing PCNA unloader, ATAD5 in BRCA2 deficient cells. PCNA retention by UBL5 induces stable replication fork progression and relieves replicative stress to promote the survival of BRCA2 deficient cells. Mechanistically, PCNA recruits Translesion synthesis(TLS) polymerases to promote stable replication fork progression in BRCA2 deficient cells and these cells can be effectively targeted by TLS inhibitor (JH-RE-06). Potentially, this study provides the mechanistic understanding of how cohesion retention on chromatin relieves replicative stress in BRCA2 deficient cells to overcome the detrimental effect of BRCA2 crisis to establish BRCA2 deficient tumors.

PP 53

GENOME-WIDE SCREENING IDENTIFIES NUA4 HAT COMPLEX MEMBERS EP400 AND KAT5 THAT PREVENT CENP-A MISLOCALIZATION AND CIN

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Aneuploidy, a hallmark of many cancers, arises due to chromosomal instability **(**CIN). Centromeric localization of evolutionarily conserved histone H3 variant, CENP-A is essential for chromosomal stability. We have shown that mislocalization of overexpressed CENP-A to non-centromeric regions contributes to CIN in human cells and xenograft mouse model. Overexpression of CENP-A is observed in several cancers, and this correlates with poor prognosis and therapeutic resistance. However, the molecular mechanisms that prevent CENP-A mislocalization remain poorly defined. Here, we used an image-based genome-wide high-throughput siRNA screen to identify gene depletions that show high nuclear levels of CENP-A. Among the top ten candidates were EP400 and KAT5, which are components of the multi-subunit NuA4 histone acetyl transferase (HAT) complex. In-depth studies have uncovered roles for EP400 and KAT5 in preventing mislocalization of overexpressed CENP-A and CIN in near stable diploid hTERT-RPE1 cells. Mislocalization of CENP-A to non-centromeric regions on mitotic chromosomes and and an enrichment of CENP-A was observed in the chromatin fraction of EP400 and KAT5 knockdown (KD) cells. Cells expressing an EP400 mutant that is defective for ATP-dependent chromatin remodeling activity showed increased stability and mislocalization of CENP-A with CIN phenotypes such as lagging chromosomes and micronuclei. Depletion of the histone H3.3 chaperone DAXX suppressed CENP-A mislocalization in EP400 KD cells. We conclude that ATPase activity of EP400 prevents mislocalization of CENP-A and CIN and defects in EP400 lead to mislocalization of CENP-A by factors such as DAXX. In summary, our studies define novel regulators of CENP-A localization and highlight the multifaceted roles of chromatin remodelers such as EP400 and KAT5 in preventing CENP-A mislocalization and CIN. We propose that expression of EP400 and KAT5 may serve as important prognostic biomarkers for CENP-A overexpressing cancers.

PP 54

COACTIVATOR SAGA PLAYS A CRUCIAL ROLE IN TBP RECRUITMENT FOR THE GCN4 TRANSCRIPTOME VIA SUBUNITS SPT3/SPT8

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Transcription initiation by RNA polymerase II is facilitated by coactivators that recruit general initiation factors to promoters, including TATA-binding protein (TBP). Coactivator complexes TFIID and SAGA recruit TBP, while SAGA also enhances transcription by histone acetylation via the HAT Gcn5. It was proposed that most yeast genes depend exclusively on TFIID, with only ~10% requiring cumulative contributions by SAGA and TFIID for efficient transcription. It wasurther suggested that genes induced by Gcn4, transcriptional activator of amino acid biosynthetic genes induced by amino acid starvation, depend on the HAT, but not TBP-recruitment function of SAGA. At odds with this model, ChIP-sequencing of TBP and Pol II subunit Rpb1 revealed that deleting *SPT3* or *SPT8*, but not *GCN5*, reduced TBP binding at many Gcn4 target genes, whereas deleting *GCN5* selectively impaired promoter histone eviction; and all three SAGA mutations reduced transcription comparably. Nuclear depletion of TFIID subunit Taf1 generally reduced TBP recruitment only in cells lacking Spt3 or Spt8. Thus, SAGA is crucial for TBP recruitment via Spt3/Spt8, beyond its role in histone acetylation, whereas TFIID plays an ancillary role in the Gcn4 transcriptome. Deleting *SPT3/SPT8* also reduced Taf1 occupancies, suggesting that SAGA stimulates recruitment of TFIID in addition to TBP.

PP 55

PHPC^{NF-Y} TRANSCRIPTION FACTOR INFILTRATES REPRESSIVE HETEROCHROMATIN TO GENERATE TRANSCRIPTS CRUCIAL FOR SPLICEOSOME-DEPENDENT SMALL RNA PRODUCTION

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The assembly of repressive heterochromatin in eukaryotic genomes is crucial for silencing lineage-inappropriate genes and repetitive DNA elements. Paradoxically, transcription of repetitive elements within constitutive heterochromatin domains is required for RNA-based mechanisms, such as the RNAi pathway, to target heterochromatin assembly proteins. However, the mechanism by which heterochromatic repeats are transcribed has been unclear. Using fission yeast, we show that the conserved trimeric transcription factor (TF) PhpC^{NF-Y} complex, which includes histone fold domain proteins, can infiltrate constitutive heterochromatin to transcribe repeat elements. PhpC^{NF-Y} collaborates with a Zn-finger containing TF to bind repeat promoter regions with *CCAAT* boxes. Mutating either the TFs or the *CCAAT* binding site disrupts the transcription of heterochromatic repeats. Although repeat elements are transcribed from both strands, PhpC^{NF-Y}-dependent transcripts originate from only one strand. These TF-driven transcripts contain cryptic introns that are processed via a mechanism requiring the spliceosome to generate small interfering RNAs (siRNAs) by the RNAi machinery. Our analyses show that siRNA production by this TF-mediated transcription pathway is critical for heterochromatin nucleation at target repeat loci. This study reveals a mechanism by which heterochromatic repeats are transcribed, initiating their own silencing by triggering a primary cascade that produces siRNAs necessary for heterochromatin nucleation.

PP 56

REGULATION OF EPIGENETICS AND CHROMOSOME STRUCTURE BY HUMAN ORC2

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The six subunit Origin Recognition Complex (ORC) is a DNA replication initiator that also promotes heterochromatinization in some species. A multi-omics study in a human cell line with mutations in three subunits of ORC, reveals that the subunits bind to DNA independent of each other rather than as part of a common six-subunit ORC. While DNA-bound ORC2 was seen to compact chromatin and attract repressive histone marks, the activation of chromatin and protection from repressive marks was seen at a large number of sites. The epigenetic changes regulate hundreds of genes, including some epigenetic regulators, adding an indirect mechanism by which ORC2 regulates epigenetics without local binding. DNA-bound ORC2 also prevents the acquisition of CTCF at focal sites in the genome to regulate chromatin loops. Thus, individual ORC subunits are major regulators, in both directions, of epigenetics, gene expression and chromosome structure, independent of the role of ORC in replication.

PP 57

DECIPHERING THE GENOMIC LANDSCAPE OF BUDDING YEAST CELL CYCLE CYCLINS

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The conventional understanding has strictly separated the cell cycle cyclin-dependent kinases (CDKs) from transcriptional CDKs. Recent studies in budding yeast have identified a novel mechanism in the transcriptional regulation by cell cycle CDKs. According to this novel model, the G1 phase cyclin-CDK (Cln3/Cdk1) complexes are recruited to promoters regulated by G1-specific transcription factor SBF and directly phosphorylate the RNAPII CTD, leading to transcriptional changes required for proper G1 to S phase transition. In addition to Cln3, other cell cycle-dependent cyclin-Cdk1 complexes could also phosphorylate RNAPII in vitro, although at lower efficiency. However, it is unknown whether this also happens in cells, and what role this phosphorylation could play in the regulation of transcription and cell cycle progression.

Here, we hypothesized that other cell cycle cyclin-Cdk1 complexes in budding yeast in addition to Cln3-Cdk1 regulate transcription by being recruited to specific promoters via transcription factors, and subsequently phosphorylate RNAPII. To investigate this, we have: (1) determined the genome-wide occupancy of cell cycle-associated cyclins using ChIP-sequencing; (2) screened for novel docking interactions between the cell cycle cyclins and nuclear proteins, including transcription factors and transcriptional co-regulators using Alphafold Multimer structure prediction; (3) studied the functional consequences of the identified novel docking interactions between cyclins and transcription factors on cell cycle progression.

Our study reveals novel mechanistic insights into how cell cycle cyclins/cyclin-dependent kinase complexes bring about transcriptional changes throughout the cell cycle in budding yeast, and pave way to understanding the universal principles underlying the cell cycle and transcriptional regulation across eukaryotes.

PP 58

ACUTE INTERVENTION REVEALS DYNAMIC INTERPLAY OF ENHANCER REGULATORS BAF, KMT2D, AND P300

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Lineage-determining transcription factors (LDTFs) bind to specific DNA sequences to mediate cell type-specific enhancer activation and gene expression. Activated enhancers are typically open regions marked with histone H3K4 mono-methylation (H3K4me1) and histone H3K27 acetylation (H3K27ac). Chromatin factors such as the canonical SWI/SNF chromatin remodeling complex BAF, H3K4 mono-methyltransferase KMT2D, and H3K27 acetyltransferase p300 are reported to play important roles in LDTF-mediated enhancer activation. Recent findings further indicate that BAF and KMT2D reciprocally regulate each other on enhancers.

Functions of BAF, KMT2D, and p300 are usually investigated using stable gene knockout/knockdown strategies and continuous LDTF expression models. Although they provide significant insights, these traditional experimental designs are prone to accumulated secondary effects that might conceal the direct functions of BAF, KMT2D, and p300 on chromatin. These designs also lack the time resolution for a dynamic study. Hence, early dynamics and sequential events of enhancer activation following LDTF binding remain largely unknown.

In this study, we investigate early dynamics of enhancer activation mediated by the myogenic LDTF MyoD, utilizing a 4-OHT inducible translocation of MyoD-ER system. After one hour of 4-OHT treatment, we observed that MyoD binding is induced at over 70,000 *de novo* sites. 50% of *de novo* MyoD binding sites are co-enriched for the chromatin factors BAF, KMT2D, and p300. By acute inhibition of BAF enzymatic subunit BRG1, we showed that BAF chromatin remodeling activity is essential for the *de novo* binding of MyoD, BAF, KMT2D and p300 at these enhancer sites. Meanwhile, acute loss of KMT2D proteins has significant but less pronounced effects on the binding of MyoD, BAF, and p300. Interestingly, acute inhibition of acetyltransferase activities of p300 – a factor reported to be downstream of KMT2D – strongly decreases *de novo* binding of MyoD, BAF, KMT2D, and p300 at 1-hour MyoD binding sites. This suggests that p300 may play a more upstream role than previously thought, and p300 exhibits inter-dependent relationships with BAF and KMT2D. Collectively, our data highlight the dynamic interplay of chromatin regulators BAF, KMT2D and p300 in the early phase of transcription factor-mediated enhancer activation.

PP 59

GENOME-WIDE BIVARIATE ANALYSIS OF GENOMIC FOOTPRINT (BaGFoot) IDENTIFIES A NOVEL THERAPEUTIC TARGET IN SMALL CELL LUNG CANCER

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Small Cell Lung Cancer (SCLC) is an aggressive form of lung cancer, accounting for 13% of all lung cancer cases. Despite advancements in cancer treatments, survival rates for SCLC have remained stagnant. Fourteen cell lines from SCLC, 10 non-small cell lung cancer (NSCLC) lines, and human induced pluripotent stem cell (iPSC) lines derived from human small airway epithelial cells were used for genome-wide analysis of identifying novel therapeutic targets in SCLC. RNA-seq and DNA methylome analyses revealed significant similarities between iPSCs and SCLC but did not yield insights into potential therapeutic targets. We compared 4 cell lines representative of distinct molecular SCLC subtypes to iPSCs using DNase-hypersensitivity sequencing (DHS-seq) for deeper analysis. Over 95% of changes in SCLC were not near promoters and thus represented massive remodeling at enhancers. In addition, peak-to-gene links showed that each open chromatin site was linked to at least 9 genes and less than 25% of peaks were linked to its predicted nearest neighbor gene. Using DHS-seq data from SCLC and iPSC, we performed Bivariate analysis of Genomic Footprint (BaGFoot) which correlates chromatin accessibility with digital footprint of all known TFs. The results were consistent with some TFs identified through HOMER suite analysis, such as FOXA1, which showed high occupancy at open chromatin sites in SCLC. However, BaGFoot analysis revealed no significant change in footprint depth, suggesting low level of functional relevance. Additionally, several stem cell TFs, including OCT2 and SOX2, exhibited negative associations, as indicated by the closing of DNase hypersensitive sites (DHS) and reductions in footprint depths, which corresponded to their lower expression in SCLC as compared to iPSCs.

Unexpectedly, we identified Nuclear Factor I C (NFIC) as being uniquely positioned with high chromatin occupancy at differentially open chromatin sites and showing an increased footprint in SCLC. NFIC knockdown resulted in delayed growth of human SCLC cells *in vitro*, reduced colony formation, and inhibited tumor formation *in vivo*. Furthermore, we discovered that the NFIC promoter region contains several Bromodomain 4 (BRD4) binding sites, and treatment with three different BET inhibitors reduced NFIC expression and inhibited SCLC growth *in vitro* and tumor formation of cell lines and PDX models *in vivo*.

Our results underscore the critical role of unbiased chromatin accessibility analysis to study malignant transformation and to identify novel therapeutic targets. We present preliminary evidence suggesting the involvement of NFIC in the pathobiology of SCLC and propose NFIC inhibition as potential therapeutic strategy.

PP 60

PROBING TOPOISOMERASE ACTIVITY DURING MITOTIC CHROMOSOME CONDENSATION

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DNA topoisomerases catalyze changes in the topological states of DNA. Previous experiments demonstrate topoisomerase IIa (Top2a) scaffolds DNA loops during prometaphase, maintaining chromosome condensation. A characteristic of topoisomerase activity is the covalent binding to DNA (Topo-CC) before catalytic cycle completion. We enrich and sequence DNA fragments with Topo-CC to reveal genome-wide activity site distributions in HCT116 cells. A combination of visual and statistical analyses suggests that these distributions differ between interphase and prometaphase cells. Top2a activity sites during interphase may be more transcriptionally driven than those during prometaphase. Many Top1 and Top2b activity sites specific to prometaphase appear to colocalize with Top2a activity sites also specific to prometaphase. These data support the presence of Top1 and Top2b in proximity to Top2a at the anchor points of loop domains in mitotic chromosomes and have important implications for the structural mechanisms that regulate changes in genome organization across the cell cycle.

PP 61

THE HOMEOBOX TRANSCRIPTION FACTOR DUXBL CONTROLS EXIT FROM TOTIPOTENCY

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Mammalian development initiates with the formation of a totipotent zygote which can develop into a whole organism. In mice, zygotic genome activation (ZGA), the process resulting in the transcriptional awakening of the embryonic genome, occurs at the onset of the two-cell stage in embryonic development and coincides with the exit from totipotency. Furthermore, ZGA is characterized by the activation of a two-cell stage-specific gene expression program that needs to be decommissioned during the transition from the two-cell to the four-cell stage to allow further embryonic development. However, the molecular mechanisms involved in this process remain poorly understood. Our work identifies DUXBL as an essential regulator of totipotency exit enabling the first divergence of cell fates. We demonstrate that the two-cell specific transcription factor DUX, which induces the two-cell stage-specific gene expression program, mediates an essential negative feedback loop by inducing the expression of DUXBL to promote this silencing. We show that DUXBL gains accessibility to DUX-bound regions specifically upon DUX expression. Moreover, we determine that DUXBL interacts with TRIM24 and TRIM33, members of the tripartite motif superfamily involved in gene silencing and co-localizes with them in nuclear foci upon DUX expression. Importantly, DUXBL overexpression impairs two-cell associated transcription, whereas Duxbl inactivation in embryonic stem cells (ESC) increases DUX-dependent induction of the two-cell transcriptional program. Consequently, DUXBL deficiency in embryos results in sustained expression of two-cell associated transcripts leading to early developmental arrest. In summary, we identified a mechanism whereby a transcription factor (DUX) and its paralog (DUXBL) act as an activator-repressor pair that mediates an efficient negative feedback loop during ZGA in mice.

PP 62

H3.3K18R DEPLETES A SUBSET OF ACTIVE ENHANCER MARK H3K18AC AND CAUSES EMBRYONIC LETHALITY

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CBP and p300 are the histone acetyltransferases responsible for H3K18ac and H3K27ac in mammalian cells. While H3K27ac marks active enhancers, the genomic distribution of H3K18ac remains unclear. Here we have generated K-to-R mutation on K18 of histone H3.3 (H3.3K18R) in embryonic stem cells (ESCs) and mice to investigate the role of H3.3K18 and H3K18ac in gene activation, ESC differentiation and embryonic development.

By ChIP-Seq analyses, we observed that H3K18ac marks active enhancers and shows similar genome-wide binding profiles as H3K27ac. By generating H3.3a$^{-/-}$; H3.3b$^{K18R/K18R}$ ES cell lines, we show that H3.3K18R mutation led to a decrease in H3K18ac levels on ~37% of active enhancers but had little effects on associated gene expression in undifferentiated ESCs. Further, H3.3K18R mutation did not affect *de novo* enhancer activation and gene activation in epiblast-like cell (EpiLC) differentiation. H3.3K18R also had no effect on ESC differentiation including embryoid body differentiation, neural differentiation, and extraembryonic endoderm (ExEn) differentiation.

To rule out the potential functional redundancy between H3.3K18ac and H3.3K27ac, we generated H3.3K18/27R double knockin ESCs. H3.3K18/27R mutations have little effects on maintaining associated gene expression in undifferentiated ESCs or gene activation during ESC differentiation towards neuron progenitor cells and EpiLC. These findings suggest that while H3K18ac and H3K27ac both mark active enhancers, they are dispensable for gene activation and cell fate transition during ESC differentiation.

To study the role of H3.3K18 and H3K18 in mice, we introduced a K-to-R mutation on K18 of H3.3b. The resulting H3.3b$^{K18R/K18R}$ mice showed perinatal lethality. Further, by intercrossing H3.3a$^{-/+}$; H3.3b$^{K18R/+}$ mice, we found that H3.3a$^{-/-}$; H3.3b$^{K18R/K18R}$ embryos show a severe developmental delay at embryonic day 9.5, suggesting that H3.3K18 is required for embryonic development.

PP 63

BENCHMARKING COMPUTATIONAL TOOLS FOR PREDICTING FUNCTIONAL TRANSCRIPTION FACTORS FROM SINGLE-CELL OMICS DATA

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Transcription factors (TF) are essential for the regulation of the synthesis of RNA from a DNA template. TFs bind to specific DNA sequences in the promoter or enhancer regions of genes. TF-driven transcriptional regulation is known to play a critical role in a wide range of biological processes including oncogenesis. Therefore, identifying functional TFs in various cell systems will enhance our understanding of gene regulation and aids in the discovery of potential therapeutic targets of cancer.

With the rapid development of high-throughput sequencing technologies, especially single-cell sequencing technology, inferring transcription factor activity from single-cell omics data has become an important and feasible task. Computational tools based on different principles have been developed, including SCENIC, SCENIC+, MAESTRO and BITFAM. However, each tool has its limitations and does not have a perfect performance in all application scenarios. Therefore, we developed BARTsc (Binding Analysis for Regulation of Transcription for Single Cell), a computational method for TF prediction for single-cell data. BARTsc uses a model-based approach to predict active TFs for each cell type from an input single-cell RNA-seq or single-cell ATAC-seq dataset by leveraging a collection of over 13,000 high-quality TF binding profiles.

To systematically assess the performance of these computational tools, we benchmarked their power of predicting cell-type marker TFs. Using single-cell datasets from human peripheral blood mononuclear cells (PBMC) and mouse cerebral cortex, we evaluated the accuracy, specificity, and sensitivity of predicting known marker TFs for each cell type. Moreover, we examined the correlation between the marker TFs predicted by each method and the expression levels of their downstream target genes. In every benchmarking metric, BARTsc outperformed other methods by successfully identifying more known marker TFs in most cell types. The marker TFs predicted by BARTsc also demonstrated a stronger correlation with the expression level of their putative targeted genes in specific cell types. This benchmarking study provides evidence-based useful information for choosing a powerful computational tool for transcriptional regulation and cancer genomics research.

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EXPLORING VIRAL-HOST TRANS-INTERACTIONS IN HPV31 POSITIVE CERVICAL KERATINOCYTES BY CHROMOSOME CONFORMATION CAPTURE TECHNIQUES

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During persistent infection, Human Papillomavirus (HPV) genomes are maintained as low-copy extrachromosomal elements in the basal epithelium by tethering to host chromosomes. Persistence of oncogenic alpha-HPVs can result in carcinogenesis, which is often associated with integration of viral DNA into host chromatin. We have shown previously that recurrent integration “hotspots” are regulatory hubs for cell-identity genes and propose that extrachromosomal HPV genomes associate with these transcriptional epicenters to ensure persistent infection. To investigate global viral-host interactions during viral maintenance, we performed chromosome conformation capture techniques in the 9E clone of CIN612 cervical keratinocytes that maintain extrachromosomal HPV31 genomes. Another clone containing integrated HPV31 DNA (CIN612-6E) was used as a control to monitor local interactions at the viral integration locus. Here we compare HPV attachment sites identified through HiC (detects all pairwise chromatin interactions within and between the viral and host genomes) and 4C (detects viral-host interactions using sequences within the HPV genome as bait). HiC is an unbiased approach for studying viral-host interactions but requires high sequencing depth to achieve good resolution of HPV genome association with host chromatin. In our study, HiC identified <100 trans-interactions between HPV31 and host chromatin in CIN612-9E cells, compared to >400 hits from 4C, with ~60 sites common in both datasets. As expected, viral-host trans-interactions were limited to the integration locus on chromosome 4 in CIN612-6E cells. Active (A) and repressive (B) chromatin compartments were defined from our HiC dataset and showed enrichment of HPV genomes at euchromatic regions of the host genome. Furthermore, HPV31 genomes showed a strong tendency to associate with regions of host chromosomes that contain super-enhancers and regions that are frequently observed as integration sites for HPV in various cancers. This supports that extrachromosomal HPV genomes localize at key transcriptional regulatory hubs within the host genome to ensure an active infection during viral persistence.

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A MODIFIED SPRITE ANALYSIS PIPELINE REVEALS TELOMERE GENOMIC INTERACTIONS

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Proliferating cells must adopt a telomere maintenance mechanism to counteract the shortening of telomeres normally observed with successive cell divisions. A subset of tumors utilizes a telomerase-independent mechanism called Alternative Lengthening of Telomeres (ALT) which is associated with mutations in the ATRX/DAXX histone H3.3 chaperone complex. ALT tumors are characterized by highly rearranged genomes, with interstitial telomere sequence insertions being particularly common. To better understand the genetic evolution of these tumors, we seek to determine whether specific genomic loci tend to spatially contact telomeres and thus may be especially liable to experience recombination with telomeres. While the spatial organization of nonrepetitive regions of the genome has been extensively studied with Hi-C and SPRITE, little is known about the spatial distribution of telomeres and other repetitive genomic elements in the nucleus. Analysis pipelines for these next-generation sequencing-based methods generally discard reads aligning to repetitive elements since they cannot be uniquely mapped to a reference genome. Here, we present a novel approach to extracting a contact profile for telomere reads from SPRITE data. Reads containing large numbers of telomere repeats are identified, sorted into a single "bin," and contacts between the telomere bin and other genomic loci are quantified. Profiling of telomere contacts across multiple cell lines reveals some robust trends. As anticipated, contact probabilities decrease sharply with increasing chromosomal distance from the telomere. ALT cell lines commonly show spikes in telomere contact probability internal to the chromosome. Using customized analysis of Oxford Nanopore long read whole genome sequencing data, we show that the locations of these spikes strongly correlate with additions of telomere sequence at interstitial sites. Telomeres exhibit strong interactions with centromeric sequences and centromere-proximal loci, consistent with spatial clustering of heterochromatic regions. FISH experiments also reveal a consistent telomere-centromere association across cell lines, regardless of telomere maintenance mechanism. Collectively, our results demonstrate that extensive additions of interstitial telomere sequence are a hallmark of ALT cell lines and reveal which genomic loci spatially associate with telomeres. Future work will seek to determine whether telomere-proximal loci are more likely to experience telomere additions in ALT and investigate the relevance of telomere additions in driving tumor evolution.

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THE INTERACTION BETWEEN CTCF AND SMAD3 IS REQUIRED FOR CONTROLLING THE TGF-β INDUCED EPITHELIAL-MESENCHYMAL TRANSITION

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Epithelial-mesenchymal transition (EMT) is a critical biological process involved in tissue differentiation, wound healing, and cancer metastasis. MCF10A cells, a non-tumorigenic epithelial cell line derived from human mammary glands, are widely used in EMT research due to their well-characterized response to transforming growth factor-beta (TGF-β). Previous studies from our group demonstrated that reducing Smad3 protein levels led to significant alterations in the expression of several key EMT marker genes in MCF10A cells, suggesting that Smad3 plays a pivotal role in the regulation of EMT. However, the specific molecular mechanisms by which Smad3 influences EMT remained elusive.

In the present study, we uncover a novel interaction between Smad3 and the CCCTC-binding factor (CTCF) induced by TGF-β. CTCF is a versatile zinc finger protein known for its role in insulator function, transcriptional regulation, and the organization of topologically associated domains (TADs) in the genome. Our data reveal that knocking down either CTCF or Smad3 in MCF10A cells disrupts the normal expression patterns of major EMT marker genes, including Vimentin and Snai1, highlighting the importance of both proteins in maintaining EMT-associated gene expression.

Using chromatin immunoprecipitation sequencing (ChIP-seq) and high-throughput chromosome conformation capture (Hi-C) techniques, we discovered that the epithelial marker gene CDH1 is part of a gene cluster with other cadherin genes, such as CDH5 and CDH11. This cluster is situated within TADs defined by CTCF in the chromosomal region. Our findings indicate that CTCF not only demarcates the boundaries of these TADs but also plays an essential role in recruiting Smad3 to specific chromatin regions.

We demonstrate that the interaction between CTCF and Smad3 affects the promoter-proximal pausing of RNA polymerase II (Pol II) and is necessary for TGF-β-dependent repression of cadherin genes, including CDH1 and CDH3. This repression is crucial in the EMT process, as the downregulation of epithelial markers like E-cadherin is a hallmark of EMT. The requirement of CTCF for Smad3 recruitment to chromatin and the subsequent transcriptional repression of cadherin genes elucidates a previously unrecognized layer of regulation in the EMT process.

Our study provides a detailed mechanistic insight into how CTCF and Smad3 cooperate to regulate EMT. Understanding the interplay between these two factors offers potential new avenues for therapeutic interventions aimed at controlling EMT in cancer metastasis. By targeting the CTCF-Smad3 interaction pathway, it may be possible to develop novel strategies to inhibit EMT and, consequently, metastasis in cancer patients.

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CHARACTERIZING METHYLTRANSFERASE ACTIVITIES OF MLL FAMILY IN EMBRYONIC DEVELOPMENT AND ESC DIFFERENTIATION

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The MLL/KMT2 family of histone H3 lysine 4 (H3K4) methyltransferases play critical roles in gene regulation, cell differentiation, animal development, and human diseases. However, the exact roles of MLL family enzymatic activities and MLL-catalyzed H3K4me in these processes remain poorly understood. Here, we focus on two subgroups in the MLL family, MLL3/MLL4 (KMT2C/KMT2D) and MLL1/MLL2 (KMT2A/KMT2B), and characterize their methyltransferase activities during mouse development and cell differentiation.

MLL3 and MLL4 are the major H3K4me1 methyltransferases and key enhancer regulators in mammals. We reported that enzymatic activities of MLL3 and MLL4 are redundant during early embryonic development. Simultaneous elimination of both prevents gastrulation and leads to early embryonic lethality around E6.5. However, selective elimination of MLL3/4 enzymatic activities in embryonic, but not extraembryonic, lineages, leaves gastrulation largely intact. Consistently, embryonic stem cells (ESCs) lacking MLL3/4 enzymatic activities can differentiate towards the three embryonic germ layers but show aberrant differentiation to extraembryonic endoderm and trophectoderm. Our findings suggest a lineage-selective, but enhancer activation-independent, role of MLL3/4 methyltransferase activities in early embryonic development and ESC differentiation (Xie et al, *Nat Genet* 2023).

MLL1 and MLL2 mediate H3K4me3 on bivalent promoters, with MLL2 playing a more prominent role. MLL1 enzymatic activity is largely dispensable for mouse development. By generating MLL2 enzyme-dead knockin mice using CRISPR/Cas9, we show that the lack of MLL2 enzymatic activity leads to developmental retardation as early as E8.5 and embryonic lethality after E10.5. At E8.5 and E10.5, only a subset of MLL2 enzyme-dead mice display gross defects. Interestingly, further removing MLL1 enzymatic activity results in a complete penetrance of this severe phenotype. In culture, ESCs lacking MLL2 or MLL1/2 enzymatic activities prematurely differentiate into endoderm and mesoderm, and show defective neurogenesis. Our findings indicate that MLL2 enzymatic activity is required for embryonic development and coordinated ESC differentiation. Out data also suggest that MLL1 activity can partially compensate for the loss of MLL2 enzymatic activity in development and differentiation.

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PARALOGUE-SELECTIVE DEGRADERS OF THE LYSINE ACETYLTRANSFERASE EP300

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The transcriptional coactivators EP300 and CREBBP are essential regulators of gene expression, sharing a high degree of sequence similarity yet performing distinct functions in both normal and disease contexts. In this study, we introduce MC-1, a bifunctional small molecule designed to selectively degrade EP300 while sparing CREBBP. By combining a potent aminopyridine-based inhibitor (CPI-1612) targeting the EP300/CREBBP catalytic domain with a VHL ligand, we show that MC-1 preferentially induces the proteasome-dependent degradation of EP300. Our mechanistic investigations reveal that selective degradation is not solely determined by target engagement or ternary complex formation, implying that additional factors contribute to the paralogue-specific degradation. MC-1 also inhibits cell proliferation in certain cancer cell lines, offering a novel chemical biology tool to explore the noncatalytic roles of EP300 and CREBBP. This work broadens the scope of chemical probes targeting EP300/CREBBP and sheds light on the underlying mechanisms of selective degradation of structurally similar proteins.

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BARTSC: A TRANSCRIPTION FACTOR ANALYSIS SUITE FOR SINGLE-CELL OMICS DATA

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In Eukaryotic cells, the expression of a small set of transcription factors (TFs) is usually sufficient to set up a cell-type-specific gene expression program. The underlying complicated epigenetic mechanisms that explain how cells change their states in response to intra- and extra-cellular signals in different scenarios remain to be fully understood. Importantly, epigenetic regulation of gene expression has been considered a central regulatory mechanism of cell fate determination and cellular plasticity. Insights into it will also profoundly impact our understanding of misregulation of gene expression in diseases such as cancer. However, the functional TFs of many cell types remain unclear, which greatly hinders the study of epigenetic regulatory mechanisms.

The rapid development of single-cell technology is a promising opportunity that enables us to infer functional TFs for different cell types. Some computational methods have been published in this area. However, existing methods have various pitfalls. For instance, some methods use transcription factor binding motifs (TFBMs) as the reference of TF binding, overlooking widespread non-specific binding. Some other methods hold strict criteria for training data and thus can only predict a small number of TFs. To overcome these issues, we propose to leverage publicly available high-quality ChIP-seq data to predict TFs functional in single-cell omics data. Here, we present BARTsc, a comprehensive suite for a series of TF analysis functionalities, including inference of signature TFs of given cell types/clusters, cross-cell-type/cluster TF activity comparative analysis, and TF clustering based on their cross cell-type distributions. The input of BARTsc can be either scRNA-seq, scATAC-seq, or scMultiome data. We demonstrate that BARTsc outperforms several existing tools in both sensitivity and specificity in identifying key cell type regulators in single-cell datasets of well-studied systems. Applying BARTsc to a scRNA-seq dataset for cancer-associated fibroblasts (CAFs), we identified distinct regulatory activities of some TFs in different subtypes of CAFs, but the TF genes are universally expressed in these subtypes. In addition, by time course BARTsc analysis, we identified a list of TFs that may either promote or suppress CAF differentiation, offering a new approach to find potential candidate therapeutic targets.

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STRUCTURAL INSIGHTS INTO THE COOPERATIVE NUCLEOSOME RECOGNITION AND CHROMATIN OPENING BY FOXA1 AND GATA4

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Pioneer transcription factors can access DNA in closed chromatin and play crucial roles in cell differentiation and reprogramming. FOXA1 and GATA4 are the prototypes of pioneer factors, which initiate liver cell development by binding the N1 nucleosome in the enhancer of the ALB1 gene. However, the structural basis of the N1 nucleosome recognition by FOXA1 and GATA4 remains unknown. Here, using cryo-EM, we determined the structures of the free N1 nucleosome and its complexes with FOXA1 and GATA4 individually, as well as in combination. We found that the DNA binding domains of FOXA1 and GATA4 mainly recognize the linker DNA and an internal site in the nucleosome, respectively, while their intrinsically disordered regions interact with the acidic patch on histone H2A-H2B. FOXA1 repositions the N1 nucleosome to facilitate GATA4 binding. In vivo DNA editing and bioinformatics analyses suggest that the co-binding mode of FOXA1 and GATA4 play important roles in regulating the genes involving liver cell functions. Our results reveal the mechanism whereby FOXA1 and GATA4 cooperatively bind the nucleosome through nucleosome repositioning, opening chromatin through bending linker DNA, weakening H1 binding and obstructing nucleosome packing.

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THE ORIGINS OF DNA SUPERCOILING IN THE HUMAN GENOME

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DNA supercoiling is a fundamental biophysical property of chromatin that influences many critical biological processes and functions involving chromatin and DNA. Despite its importance, our understanding of DNA supercoiling in living cells, including its dynamics, regulatory mechanisms, and functions, remains limited. Using the azide-trimethylpsoralen sequencing (ATMP-seq) assay recently developed in our lab, we characterized DNA supercoiling across the human genome and uncovered distinct supercoiling patterns across various genomic scales. Notably, for the first time, we observed twin-supercoiled domains around genes at transcription start sites (TSS) and transcription end sites (TES), reported the accumulation of negative supercoiling around topologically associating domain (TAD) boundaries, and discovered megabase-scale supercoiling domains (SDs) that colocalize with nuclear compartments throughout the human genome.

Here, we investigated the origins of DNA supercoiling in the human genome.

Our findings suggest that RNA polymerase (RNAP) movement during transcription elongation, rather than transcription initiation, is the primary driver of the observed twin-supercoiled domains. Interestingly, contrary to predictions from biophysical models, the distribution of DNA supercoiling between the twin domains is asymmetric, with an excess of negative supercoiling at the TSS. This excessive negative supercoiling is not attributable to transcription initiation, nucleosome dynamics, or antisense transcription activity, but rather to processes associated with RNAP elongation. Specifically, we found that the asymmetry arises from the differential relaxation of supercoiling by topoisomerases during RNAP elongation. Both human topoisomerase I and topoisomerase II relax positive supercoiling ahead of the elongating RNAP more efficiently than negative supercoiling behind it, resulting in the accumulation of excessive negative supercoiling at the TSS.

We also explored the origins of negative supercoiling accumulation at TAD boundaries. After ruling out the contributions of cohesin loop extrusion and CTCF activity, we found that it originates from transcription, particularly RNAP elongation. Further investigation revealed that the negative supercoiling at TAD boundaries is primarily caused by the excessive negative supercoiling generated during RNA elongation, with local nucleosome dynamics at TAD boundaries also contributing to this accumulation.

This work offers new insights into the origins of DNA supercoiling in the human genome, deepening our understanding of its regulatory mechanisms, its interaction with chromatin features and 3D genome organization, and its role in human chromatin function and gene regulation.

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HISTONE N-TAILS MODULATE SEQUENCE-SPECIFIC POSTITIONING OF NUCLEOSOMES

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The precise mechanisms governing sequence-dependent positioning of nucleosomes on DNA remain unknown in detail. Existing algorithms, taking into account the sequence-dependent deformability of DNA and its interactions with the histone globular domains, predict rotational setting of only 65% of human nucleosomes mapped *in vivo*. To uncover additional factors responsible for the nucleosome positioning, we analyzed potential involvement of the histone N-tails in this process. To this aim, we reconstituted the H2A/H4 N-tailless nucleosomes on human BRCA1 DNA (~100 kb) and compared their positions and sequences with those of the wild-type nucleosomes. We found that removal of the histone N-tails promoted displacement of the predominant positions of nucleosomes, accompanied by redistribution of the AT-rich and GC-rich motifs in nucleosome sequences. Importantly, most of these sequence changes occurred at superhelical locations (SHLs) ±4, ±1 and ±2, where the H2A and H4 N-tails interact with the DNA minor grooves. Furthermore, a substantial number of H4-tailless nucleosomes exhibit rotational setting opposite to that of the wild-type nucleosomes, the effect known to change the topological properties of chromatin fiber. Thus, the histone N-tails are operative in selection of nucleosome positions, which may have wide-ranging implications for epigenetic modulation of chromatin states.

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HISTONE DEACETYLASES PRESERVE MODIFIED HISTONES THAT ARE ESSENTIAL FOR PROPAGATION OF SELF-TEMPLATED HETEROCHROMATIN AND EPIGENETIC MEMORY

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Extensive regions of eukaryotic genomes are organized into heritable heterochromatin structures that silence lineage-discordant genes and maintain cellular identity. The assembly of heterochromatin domains is initiated at specific loci through sequence-targeted mechanisms. However, the spreading of heterochromatin across extensive domains and their heritability necessitates the self-templated propagation of repressive histone modifications. Specifically, histone H3 lysine-9 trimethylation (H3K9me3) sustains heterochromatin propagation, even in the absence of initiating activity, by facilitating the targeted recruitment of the dual read-write activity of the H3K9 histone methyltransferase Clr4/SUV39H, which can both bind to and deposit histone methylation marks. It is noteworthy that the efficiency of self-templated propagation varies across different chromosomal contexts. Nevertheless, the factors that predispose chromatin to efficiently transmit epigenetic memory remain unknown. Our recent findings demonstrated that robust chromatin localization of the H3 lysine-14 histone deacetylase Clr3 is sufficient to propagate histone-templated heterochromatin in distinct contexts of mitotic chromosomes. We report that the suppression of histone acetylation hinders the turnover of epigenetically modified histones thereby maintaining a critical density of H3K9me3 that is essential for self-templated propagation. Furthermore, we will present our recent findings that elucidate the role of chromatin features in transgenerational heterochromatin inheritance. Our results suggest that the presence of specific DNA elements may predispose heterochromatin propagation in the absence of initiating activity.

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