5th International Conference on Epigenetics and Bioengineering (EpiBio)

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Abstract

The 5th International Conference on Epigenetics and Bioengineering (EpiBio) brought together pioneers in the field of Epigenetics and Biotechnology, working in both academia as well as industry to discuss the gaps as well as advancements in the field. The workshop included various session topics, including the Biophysics of Chromatin: Measurements and Models, Emerging Technologies, Epigenetics in Health and Development, Sensing Epigenetic Modifications, Perturbing Chromatin and Epigenetic Editing, and Nuclear Organization. The conference included various other events, including a Phase Condensate Panel, a Black in Chromatin networking session dedicated to increasing diversity in the field, and various Q&A discussions. This paper will highlight the topics of discussion from select sessions and select talks from the Conference.

Session 1: Sensing Epigenetic Modifications: Author: Lexi Bounds

Dr. Gang Greg Wang, from the University of North Carolina, Chapel Hill, discussed his lab's work towards understanding how chemical chromatin modifications regulate gene expression and contribute to cell fate specification. Specifically, he presented results showing a conserved domain of BAHCC1 recognizes the repressive mark, H3K27me3, and enforces silencing of H3K27me3-demarcated genes in mammalian cells. Disruption of this domain resulted in loss of suppression of oncogenesis demonstrating its role in human disease.

Alex Chialastri, a trainee from the University of California, Santa Barbara, introduced new methods to identify CpG dyad sites with single-cell resolution (single-cell CpG-Dyad-seq) and showed how the technique can be applied to quantify changes in DNA methylation across mouse embryonic development. The method uncovered a subset of cells that escape reprogramming by retaining high levels of 5mC.

Dr. Kavitha Sarma, from the Wistar Institute Cancer Center, shared her work developing targeted nuclease technologies for mapping R-loop chromatin structures (MapR). MapR enables sequencing-based detection of R loops at both promoters and active enhancers and provides a platform to study aberrant R-loop formation, which has been implicated in neurological disorders and cancers.

Dr. Karolin Luger, from the University of Colorado in Boulder, delivered the keynote presentation, entitled "Histones, histones everywhere: chromatin structure in non-eukaryotic organisms". She discussed her lab's work combining structural biochemistry with quantitative assays to study nucleosome structure and dynamics. Shehighlighted how the combination of these techniques has improved understanding of histone function across multiple organisms and can be leveraged to provide mechanistic insight into chromatin remodeling.

Session 2: Emerging Technologies: Author: Lexi Bounds

Dr. Lacra Bintu, from Stanford University, presented her lab's work with her talk, titled "High-throughput development of nanobodies for transcriptional and epigenetic control". To address the size limitations of chromatin regulators, her group showed that a stably-integrated, doxycycline-inducible reporter system with the nanobodies for HP1 and DNMT1 effectively recruited the chromatin regulators to the genomic target site. They next tested whether combining the nanobodies could augment reporter gene silencing and found the fusion demonstrated a synergistic reduction in reporter expression, with the total silencing from the fusion being greater than either nanobody alone. The work demonstrates the utility of chromatin regulator-targeted nanobodies to control gene expression and epigenetic memory, with potential applications in therapeutics and synthetic biology.

Dr. Aaron Streets, from the University of California, Berkeley, gave a talk "Measuring protein DNA interactions with long read sequencing", which showcased his lab's work towards leveraging long-read sequencing to map the interaction sites of various proteins and histone modifications with single molecule resolution. Their approach overcomes limitations of current

methods that do not retain methylation information and cannot be used in genomic regions with poor mapping quality. He demonstrated how the method can be applied to simultaneously analyze the DNA interactions of a specific histone variant and the endogenous methylation levels in a repetitive genomic region.

Janina Licyte, a trainee from Vilnius University, presented methods for a bisulfite-free approach to analyze genomic 5-carboxylcytosine with base-pair resolution. Specifically, these methods can be used to capture demethylation dynamics and effects on gene expression and were applied to uncover distinct characteristics of subpopulations within mouse ESC cultures.

Lexi Bounds, a trainee from Duke University, introduced her work towards developing single-cell noncoding CRISPR/dCas9 regulatory element screening methods and applying the platform to understand regulatory mechanisms within complex genetic loci. Moreover, she presented results demonstrating cell-type specific and shared regulatory mechanisms governing gene expression across three diverse cell types and showcased the scalability of her approach.

The session ended with a panel discussion of phase condensates and phase separation, led by **Dr. Clifford Brangwynne**, **Dr. Mitchell Guttman**, and **Dr. Geeta Narlikar**, and moderated by **Dr. Albert Keung**.

Session 3: Nuclear Organization: Author: Anibal Tornes Blanco

The Nuclear Organization session began with a thought-provoking talk from **Dr. Edith Heard**, a pioneer in the field of X-chromosome inactivation and director general of the EMBL Heidelberg.

Dr. Ana Pombo from the Max Delbrück Center for Molecular Medicine shared her group's work and collaborations working with "Specialization of 3D genome topologies in specific cell types and states". This presentation was then followed by **Dr. Mitchell Gutman**, from the California Institute of Technology, discussing "How lncRNAs shape nuclear structure to regulate gene expression".

David Podorefsky, from University of California Santa Barbara, described a single-cell sequencing framework that provides "Simultaneous Measurement of DNA Methylation and Genome-Nuclear Lamina Interactions in Single Cells" using chronic myelogenous leukemia cells (KBM7). The tool dubbed sc5mC+DamID-seq barcodes 5mC as well as genome -Nuclear Lamina (NL) interactions for individual KBM7 cells. Such an approach allowed the group to observe hypomethylation states at regions contacting the NL, known as Lamina Associated Domains (LAD) as well as tracking correlations between LADs and 5mC. Interestingly, by demethylating the epigenome they observed relocated variable genome-NL contact regions towards the nuclear interior. Dysregulation of epigenetic features such as DNA methylation (5-methylcytosine or 5mC) as well as the 3-dimensional genome organization interactions are commonly associated with cancers. This tool provides a deeper understanding of how the DNA methylation landscape of the epigenome relates to the genome organization of individual cells.

Dr. Dmitri Kireev, from the University of North Carolina, presented his work titled "Submolecular-Resolution 3D Dynamics of Heterochromatin Formation". Our current knowledge about heterochromatin and its relation to a repressed chromatin landscape during cell development is limited, as there exists a notable gap in detailing the structural mechanism of heterochromatin formation. Using molecular simulations and setting parameters for key molecular events, the group was able to obtain structural interpretation of micro- and macroscopic heterochromatin properties. More importantly, this work elucidated structural mechanisms of chromatin bridging and condensed chromatin formation. Once complemented by experimental chromatin in vivo assays they were able to obtain unbiased estimates of time scale responses to heterochromatin triggering events.

Session 4: Perturbing Chromatin: Author: Anibal Tornes Blanco

Dr. Henriette O'Geen, from the University of California Davis, gave a presentation titled "Determinants of Heritability in Epigenetic Editing". This talk was followed by **Dr. Stanley Qi**, from Stanford University, sharing his perspectives on "Engineering the Spatial Genome to Study Epigenetics".

Dr. Elizabeth A.Heller, from the University of Pennsylvania, shared their work titled "Chromatin-Mediated Alternative Splicing Regulates Reward Behavior" looking into neuronal alternative splicing to understand the implications of chromatin perturbations. This splicing event is a key gene regulatory mechanism whose activity can be altered by histone modifications such as H3K36me3 suggested to be a putative splicing regulator. This murine cocaine addiction study looked into self-administration impact in widespread differential alternative splicing, showing enrichment of H3K36me3 at differentially spliced junctions. When the group looked further into localized splice factor expression by targeting Srsf11 they found both alternative splicing as well as H3K36me3 enrichment in the brain following cocaine self-administration. Therefore, they were able to trace alternative splicing drivers which provides valuable insight into H3K36me3 relevance in alternative splicing of targeted epigenetically edited Srsf11 to impact reward behavior after cocaine self- administration.

Dr. Kaiyuan Wang gave a talk which described a "Systematic Comparison of CRISPR-Based Transcriptional Activators Uncovers Gene-Regulatory Features of Enhancer-Promoter". The goal of the project was to further characterize Nuclease-inactivated CRISPR/Cas-based (dCas-based) systems at human enhancer-promoter pairs. They compared the transcriptional effects between more characterized dCas platforms and novel activators such as dCas9 fused to the catalytic core of the human CBP protein, at human enhancer-promoter pairs. Their findings showed transactivation of targeted human loci at variable expression levels in a cell dependent manner. Importantly, they showed that the effector domain used would impact the recruitment architecture, target locus and cell type. In conclusion, this work was able to 1) provide insights into enhancer-mediated control of gene expression and 2) use engineerable dCas9-based transcriptional activators at human regulatory elements to demonstrate that contact frequencies between an enhancer-promoter highly correlate to transcriptional outputs.

Session 5: Epigenetics in Health and Development: Author: Anibal Tornes Blanco

Dr. Mary Goll from the University of Georgia walked us through her work on the "Developmental regulation of global heterochromatin establishment during early embryogenesis". Following this talk, **Dr. Greg Davis** from Sangamo discussed "Epigenetic Applications of Zinc Finger Proteins in Genomic Medicine".

Saahj Gosrani from Emory University gave a talk titled "Observing neuro degeneration in Real Time Using 2-Photon Microscopy in the LSD1 Inducible Mouse Model". The work sought to better characterize Alzheimer's Disease (AD). Previous work by the lab members unveiled that H3K4 histone demethylase LSD1/KDM1A mislocalized to cytoplasmic neurofibrillary tau tangles (NFTs) in AD cases. By using two-photon live imaging in the LSD1 inducible knockout mouse, the group was able to visualize neurons dying in the cerebral cortex using a Thy1-YFP neuronal reporter. With this live imaging approach, the group will complement previous findings which suggested that loss of LSD1 induced inflammatory responses and thereforelinking microglia to neurodegeneration. In conclusion, this method provides valuable insight to how inhibition of the chromatin repressor LSD1 contributes to neurodegeneration, with the ultimate goal of generating novel therapeutic strategies targeting LSD1.

Yu Bai from Emory University gave a talk titled "The Inhibition of LSD1 VIA Sequestration Contributes to Tau-Mediated Neurodegeneration". With an unknown molecular mechanism underlying neuronal cell death, the lab group members were able to find that LSD1 is mis-localized with cytoplasmic pathological tau in human AD cases. This finding suggests that LSD1 may be inhibited by tau and to study the function of LSD1, the lab members induced deletions on LSD1 in adult mice to induce cortical and hippocampal neurodegeneration, learning and memory deficits and transcription alterations to match human AD. The lab members hypothesized that pathological tau contributes to neuronal cell death by sequestering LSD1 into the cytoplasm. To achieve this, they used a PS19 tauopathy mouse model to examine functional consequences of changing LSD1 expression. The group was able to support their hypothesis of neurofibrillary tangles of hyperphosphorylated tau (NFTs) involved in Alzheimer's Disease sequestering LSD1 through its N-terminus. These findings provide essential information for understanding as to how sequestered LSD1 can prevent blocking inappropriate gene expression.

Dr. Toshio Tsukiyama, from the Fred Hutchinson Cancer Research Center, gave a talk titled "Regulation of quiescence through 3D chromatin structure", which discussed his lab's work on nucleosome folding arrays and nucleosome interactions within the quiescent phase of the cell cycle in yeast. During quiescence, both mammalian and yeast cells experience transcriptional repression and increased chromatin condensation; however, the role of 3D chromatin structure and the respective functional implications in cells during quiescence remains to be understood. The data presented demonstrated that, as opposed to actively dividing yeast cells, nucleosome folding arrays in quiescent cells experience distinct H4-dependent local fiber folding that may play a role in condensin-regulated gene transcription.

Dr. Jessica Williams, from the King Lab at Yale University, presented her work in a presentation titled “Phase-Separated Heterochromatin Domains Impart Mechanical Stiffness to the Nucleus”. She discussed whether phase-separated heterochromatin resulting from liquid-liquid phase separation has distinct mechanical roles and whether the perturbation of functions can negatively impact the cell by compromising nuclear stiffness. The lab members found that, in fission yeast, disrupting the methylation of histone 3 lysine 9 (H3K9) and compromising its interactions with HP1-binding homologue, Swi6, causes downstream defects in nuclear mechanics.

Dr. Kaushik Ragunathan, from the University of Michigan, gave a talk titled “HP1 oligomerization Compensates for Low-Affinity H3K9me Recognition and Provides a Tunable Mechanism for Heterochromatin-Specific Localization”, in which he discussed the complexity of Swi6 single-molecule dynamics in fission yeast heterochromatin. He addresses the conundrum of HP1’s ability to navigate the chromatin landscape with low affinity but manages to bind to H3K9 with high specificity by understanding the protein dynamics and biochemical properties of individual Swi6 molecules and their biochemical intermediates. In his talk, he proposes that the higher order complexes formed via HP1 oligomerization perturb the inhibitory effects of nucleic acid binding and permit specific H3K9 recognition.

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