Genome Biology

MEETING REPORT

Epigenomics and chromatin dynamics

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Abstract

A report of the 'Joint Keystone Symposium on Epigenomics and Chromatin Dynamics', Keystone, Colorado, 17-22 January 2012.

This year's Joint Keystone Symposium on Epigenomics and Chromatin Dynamics was one of the largest Keystone meetings to date, reflecting the excitement and many developments in this area. Richard Young opened the meeting by giving a historic overview before sharing more detailed insights from his recent work in describing the role of the lysine demethylase Lsd1 in mouse embryonic stem (ES) cell differentiation. He also set the broader stage and highlighted the excitement concerning recent advances in epigenetic drugs such as the new bromodomain inhibitors.

DNA methylation

Plants and algae

Co-organizer Steven Jacobsen reported his team's discovery of two novel ATPase-encoding genes in *Arabidopsis thaliana* that are involved in maintaining the repression of endogenous transposable elements (TEs). Using a double mutant strain, they discovered that the activation of these genes was concomitant with increased histone acetylation, but that it did not require loss of DNA methylation. Robert Fischer discussed the role of DEMETER (DME) in transposon silencing. Loss of DME does not affect methylation in endosperm cells but increased it within maternal TEs. Activation of TEs through demethylation by DME in vegetative cells produces small RNAs, which Robert Martienssen described as epigenetically activated siRNAs (easiRNAs). These easiRNAs translocate to sperm and silence the same TEs that they were generated from. Joseph Ecker reported that epivariations occur five orders of magnitude more frequently than spontaneous genetic mutation in genetically matched *Arabidopsis thaliana* cohorts. Using DNA methylation patterns in different families of distantly related algae, Daniel Zilberman identified a distinct DNA methylation pattern that supports the evolutionary origins of the DNMT5 protein, a DNA methyltransferase with ATP-dependent nucleosome remodeling capabilities.

Mammals and methylomes

By examining DNA methylation in early developmental stages of heterozygous *Dnmt3l* mice, Deborah Bourc'his identified several novel imprinting control regions (ICRs), including a new class of transiently methylated ICRs. Alexander Meissner presented a comprehensive characterization of DNA methylation dynamics in the early mouse embryo and in reconstructed embryos after nuclear transfer. Andrew Feinberg provided evidence for the existence of large genomic regions that are characterized by high variability in DNA methylation between individual tumors, and proposed an evolutionary model that suggests a positive selection for molecular mechanisms that allow epigenetic drift. Timothy Bestor showed results from a restriction enzyme-based genome-wide DNA methylation assay. This assay suggests that DNA methylation levels have been overestimated by bisulfite-based approaches, which he believes erroneously led to the notion that DNA hypermethylation at genes encoding tumor suppressors contributes to cancer.

Peter Jones described the use of nucleosome occupancy methylome sequencing (NOMe-Seq) to confirm that active enhancers exhibit nucleosome depletion in conjunction with low levels of DNA methylation. He also presented evidence that nucleosomes assemble first, before acquiring DNA methylation during the silencing of enhancers. Jones concluded his talk by presenting data on the promoter of *MLH1*, an example of a tumor suppressor gene that is aberrantly silenced by DNA methylation in primary colon tumors. Asaf Hellman reported studies on the methylation state of 93 CpG sites in regions that have previously been associated with type 2 diabetes. This work led to the identification of a subset

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of hypomethylated regions that are unique to the afflicted cohort. Additionally, he provided evidence suggesting that the establishment of these hypomethylated regions precedes disease onset.

Bing Ren described the use of divergent mouse strains to track parent-of-origin methylation, unveiling several novel allele-specific methylated regions. Gregory Hannon showed that hypomethylated regions can impact gene expression and genome organization. Keji Zhao characterized the spatial interactions of gene regulatory elements within the genome using chromatin interaction analysis by paired-end tag sequencing (CHIA-PET). He also presented evidence that Mecp2 (methyl CpG binding protein 2) and gene body DNA methylation are involved in facilitating faithful exon inclusion, providing a direct link between DNA methylation and splicing.

Hydroxymethylcytosine and the Tet family

Nathaniel Heintz reported on how his group had profiled hydroxymethylcytosine (hmC) conversion in three types of neural cells. High levels of methylcytosine (mC) clustered in nuclei and correlated with heterochromatin, but hmCs are dispersed and appear to be located predominantly in gene bodies. Anjana Rao showed that, within the Tet family of nucleic acid-binding proteins, Tet1 mainly binds to transcription start sites, whereas Tet2 mainly localizes within gene bodies and exons, and that knockdown of Tet2 results in greater reduction in hmC levels than does depletion of Tet1 in ES cells. She also proposed that IDAX, the transposed CXXC domain of Tet2, regulates Tet2 post-transcriptionally.

Thomas Fazzio showed that the chromatin regulators Mbd3 and Brg1 regulate genes antagonistically by regulating promoter-proximal nucleosome occupancy and RNA polymerase II (PolII) recruitment. Notably, Mbd3 co-localizes with Tet1 and seems to bind hmC, which appears to be supported by the distinct tyrosine to phenylalanine switch in the binding pocket. Finally, Wolf Reik showed that in reprogramming to pluripotency, Tet1 preserved the unmethylated status of many pluripotency-related genes. Co-expression of Tet1 and the pluripotency maintenance factor Nanog dramatically enhanced the reprogramming of epiblast stem cells (EpiSCs) to induced pluripotent stem cells (iPSCs). The expression of Tet1 in EpiSCs leads to the demethylation of its own differentially methylated region (DMR), whereas deletion of Tet1 in ES cells leads to the methylation of its DMR.

Chromatin biology

Pluripotency

Kathrin Plath continued her detailed analysis of 'X reactivation' dynamics during reprogramming to pluripotency and compared it to X inactivation during embryonic development. She noted that reverse dynamics appear to be occurring, with the exception of DNA methylation, which remains a terminal barrier to reactivation. Amanda Fisher described the effect of different cell-cycle phases on reprogramming potential and found that S/G_2-phase mouse ES cells reprogram human lymphocytes more efficiently than do G_1-phase cells. She then showed that fusion with embryonic germ (EG) cells, but not ES cells, could erase genomic imprinting, and that this process was both replication dependent and independent. Bradley Bernstein presented chromatin state data for many cell types, and identified potentially novel transcriptional regulators on the basis of the chromatin signature at enhancers. He then presented genome-wide binding data for various chromatin modifiers that, in combination with transcriptional status, had allowed his group to define distinct chromatin state modules in two human cell types. Ana Pombo showed data describing a novel PolII variant that is specifically enriched at Polycomb repressive complex (PRC)-bound genes.

Regulators and remodeling

Genevieve Almouzni described how the histone chaperone NASP (Nuclear autoantigenic sperm protein) protects soluble H3/H4 dimers from chaperone-mediated autophagy, thus fine-tuning the amount of histones available for nucleosome assembly. Bradley Cairns provided evidence to support an autoinhibitory model governing sliding of the ISWI (imitation switch family) of chromatin remodeling ATPases. Interestingly, one domain present on the amino terminus serves as an H4 mimic, which conformationally inhibits ATP catalysis in the absence of histone binding. Timothy Richmond provided structural work supporting a dinucleosomal substrate for ISW1a binding: one nucleosome acts as a stable spool while the other is brought towards it. Blaine Bartholomew showed mutant data relating to the slide domain within the HSS (Hand Sant Slide) of ISW2. These data suggest that four basic residues are essential for DNA contact and for the entry of linker DNA into the remodeled nucleosome.

To model compaction mediated by polycomb group components in a simplified system, Robert Kingston had examined the structural interaction between yeast Sir3 and the nucleosome, which is also responsible for heterochromatin compaction. He reported a large positively charged surface within the BAH domain that provides the largest contact with histones and few contacts with DNA. This interaction might serve as the primary docking mechanism through which larger heterochromatin structures are assembled. Gerald Crabtree expanded on the nuanced regulatory role of BAF complexes in neuronal differentiation and reprogramming. Switching of only a few components within this large complex appears to influence genomic targets strongly and can dramatically affect cell fate. Ali Shilatifard presented

evidence that leukemia-associated fusions between MLL and PolII elongation factors can recruit the SEC (super elongation complex) to target genes, licensing aberrant elongation by PolII. Joanna Wysocka presented recent findings on enhancer dynamics in development using a human ES-cell-based model of cranial neural crest formation. ChIP-seq profiling of the neural crest cell enhancer repertoire, along with sequence analyses of predicted transcription factor binding sites, enabled the identification of the COUP (chicken ovalbumin upstream promoter) family of orphan nuclear receptors as novel regulators of craniofacial development. This strategy also identified the transcription factor AP2 as a major specifier of the neural crest lineage.

Rena Levin-Klein described experiments elucidating the non-random nature of allelic exclusion in developing B cells, which has an epigenetic basis at immunoglobulin genes involving monoallelic H3K4me3 deposition at DJ exons slated for removal. Utilizing a large mutant library for histone residues and chromatin modifiers in yeast, Oliver Rando addressed the lingering discrepancy between the widespread association of chromatin modifications to transcriptional regulation and the limited phenotypic effects upon depletion. Inkyung Jung presented his most recent results from the gene expression profiling of a large histone mutant library in yeast, and was able to confirm an association of H3K56 methylation with nucleosome positioning. David Katz reported on the functional role of the histone demethylase LSD1/KDM1 during epigenetic reprogramming using *Caenorhabditis elegans* and mouse models. Paul Soloway presented a novel technique for the isolation of single nucleosomes using a microfluidics approach similar to that employed for flow cytometry, which could open the door to single molecule and single cell epigenomics.

Architecture

Job Dekker used 5C technology to reveal that the majority of genomic looping interactions are cell type specific and can skip CTCF binding sites, and that enhancers do not always interact with the nearest gene in genomic co-ordinates. Wendy Bickmore investigated the regulation of the HOX locus using 5C and fluorescence *in situ* hybridization (FISH) technology. Although the loss of PRC1 and PRC2 leads to chromatin decompaction, an increase in histone acetylation is also sufficient to induce chromatin decompression and basal expression, even in the presence of active PRC complexes at this locus. Shalini Oberdoerffer received the Herb Tabor young investigator award preceding her presentation on PolII pausing during alternative pre-mRNA splicing. She had found that in the absence of exon splicing enhancers, CTCF binding in the proximity of an exon leads to PolII pausing and exon inclusion. Consistently, global analysis of CTCF depletion revealed exon exclusion when the CTCF site was located downstream of the exon, highlighting another CTCF function. Bas van Steensel discussed lamina-associated domains (LADs), which predominantly include inactive genes and show low PolII occupancy. Using a GFP-fusion protein to observe the single-cell dynamics of LADs, he showed that although LADs are dynamic, their interaction with the nuclear periphery is spatially constrained. Matthias Merkenschlager's group performed gene expression analysis and chromatin conformation capture techniques to investigate the role of cohesin in gene expression and long-range chromatin interactions. Steven Henikoff presented results obtained using a micrococcal nuclease (MNase)-based approach to identify transcription factor footprints and the nearby chromatin-remodeling complexes. This novel approach provides striking resolution in revealing the interaction of gene regulatory elements and their associated protein complexes.

Conclusions and future outlook

Exciting technical advancements in recent years have influenced the scope of many projects and continue to drive the field forward at a rapid pace. Despite the increasing number of whole-genome datasets now available, many interesting questions remain unanswered. This brief report only begins to acknowledge many of the exciting projects and ideas presented at this meeting, but should serve as an indicator of the powerful information that will undoubtedly come out of the epigenomics and chromatin biology field in the coming years.

Abbreviations

DME, DEMETER; DNMT5, DNA METHYLTRANSFERASE 5; easiRNA, epigenetically activated siRNA; ES, embryonic stem; hmC, hydroxymethylcytosine; ICR, imprinting control region; LAD, lamina associated domain; PRC, Polycomb repressive complex; PolII, RNA polymerase II; TE, transposable element.

Competing interests

The authors declare that they have no competing interests.

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