IMS Seminar: Mini workshop on Chromatin Regulation
May 27, 2021 — Zoom online seminar

Abstracts

Introduction

Chromatin conformation and its role in enhancer-promoter interactions and regulation in the cell nucleus is increasingly an important topic at our Center, with multiple groups engaged in data generation (Hi-C and other methods), bioinformatics analysis, and technology development.

At this workshop, we are planning to bring these groups together to foster collaborations between complementary groups and to encourage future joint grant applications.

Chromatin conformation analysis for functional genomics

Michiel do Hoon
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DNA is packaged in the cell nucleus by histone proteins, forming a chromatin macromolecular complex that is folded in an intricate 3D structure. The cell type and cell state-dependent architecture of chromatin provides the structural basis for gene regulation, in particular through physical interactions between distant genomic regulatory elements such as promoters and enhancers by chromatin looping. Such interactions can be found on a genome-wide scale using Hi-C, a chromatin conformation capture technology that takes advantage of next-generation sequencing to identify spatially proximal chromatin regions. Hi-C is particular powerful when used in conjunction with Cap Analysis Gene Expression (CAGE) data to identify the genomic locations of the expressed promoters of coding and non-coding genes, as well as bi-directionally transcribed enhancers.

We analyzed Hi-C data to determine the chromatin structure in 18 human cell types and tissues, and overlapped with CAGE data in the same cell types and tissues to create a high-resolution interaction map of promoters and enhancers. Using this map, we could associate long non-coding RNAs with protein-coding genes and use them to generate a cell type-specific functional annotation of 7,688 (54.15%) out of 14,198 long non-coding RNAs (lncRNAs) expressed in the 18 cell types and tissues. For comparison, currently only about 5% of lncRNAs are functionally described in the scientific literature.

To make the Hi-C derived functional annotations available to the scientific community, we used ZENBU, a genome viewer and data visualization system under active development in our team, to create a web portal allowing users to browse and compare the functional annotations for each lncRNA in individual cell types. ZENBU was further extended with modules specifically designed to show chromatin-chromatin and RNA-chromatin interactions, making it a generally usable platform to visualize Hi-C and similar data.

Complementary to these genome-wide analyses, our team is currently implementing chromatin electron microscope tomography as an imaging method to visualize the conformation of chromatin at single nucleosome resolution in an individual cell. By landmarking enhancers, promoters, and RNA transcripts of specific genes to identify their position in the electron tomography images, we aim to validate regulatory interactions deduced from Hi-C data and to directly visualize local chromatin structures in their nuclear environment.

Controlling sample/library preparation of Hi-C for the acquisition of high-quality data for genome scaffolding and epigenomic analysis.

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Genome-wide chromatin conformation capture (Hi-C) is a method based on proximity DNA ligation to analyze chromatin organization in the nuclei. Traditional Hi-C utilizes a single restriction enzyme (e.g., HindIII, MboI, DpnII) to generate chromatin fragments. More recently, multiple restriction enzymes or non-sequence specific nuclease (e.g., DNaseI, Micrococcal nuclease) are being used to overcome the limitations of Hi-C with single restriction digestion, i.e., low-resolution and non-uniform coverage of the sequencing reads. In addition to the nuclease enzyme, choices extend to kits from several biotech companies (e.g., Arima Genomics, Dovetail Genomics, Phase Genomics). I will summarize the differences in the available approaches for Hi-C, including our Hi-C sample/library preparation procedure for diverse plant and animal samples. I will also introduce key sample/library preparation steps for successful Hi-C data acquisition

HiCap: a novel tool for functional association

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Hi-C has been instrumental at resolving mid- to large-scale structural features of mammalian genomes, however it provides limited information regarding chromatin loops that mediate regulatory interactions between promoters and enhancers. To address this limitation, we developed HiCap (Hi-C coupled with sequence capture) to obtain chromatin interaction profile of a large number of selected regions (many-to-all) in a single experiment. We applied HiCap in cardiovascular and skin inflammatory disease, macrophage stimulation and aortic valve pathology settings using capture probe sets targeting circa 22,000 promoters as well as few thousand relevant disease associated variants. Our enhancer resolution is around 600 bases and more importantly HiCap can provide allele-specific interaction information. In this talk, I will briefly describe HiCap and go over some of our recent results showcasing the benefits of using high-resolution interaction maps in understanding the role of noncoding genome in disease and health.

Modulating enhancer regulation: mechanisms and applications

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Enhancer regulation is a key for organisms to diversify gene expression patterns, as enhancers can confer new expression domains to genes when placed in the vicinity along the genome. Intriguingly, gene expression patterns are not determined just by the enhancer activity; the interaction between genes and enhancers is under some regulation and greatly modulates gene expression patterns. However, the complexity of enhancer regulation at the level of the interaction is still elusive. In this seminar, I would like to discuss several layers of regulation for such enhancer modulation, introducing some of our previous studies on regulation of duplicated photoreceptor genes as well as on CTCF regulation for gene-enhancer interaction at other loci. One of our studies exemplifies that modulation of gene-enhancer interaction can even be a target of artificial manipulation for controlling gene activity. Our studies emphasize the importance of gene regulation at the level of gene-enhancer interaction to understand genome function and evolution.

Chromatin regulation by transcription factors in myeloid cell development

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We have been studying the mechanism of myeloid cell development from the viewpoint of chromatin regulation by transcription factors. The transcription factor IRF8 is essential for the development of monocytes and dendritic cells (DCs), whereas it inhibits neutrophilic differentiation. We have demonstrated that IRF8 establishes enhancer landscapes in myeloid progenitors to epigenetically prime these cells to differentiate into monocytes or DCs. Recently, we began investigating the regulation of *Irf8* itself and have identified a RUNX–CBFβ-driven enhancer 56 kb downstream of the *Irf8* transcription start site. Deletion of this enhancer *in vivo* significantly decreased *Irf8* expression throughout the myeloid lineage from progenitor stages, resulting in loss of DCs, but unexpectedly monocytes were overproduced, unlike *Irf8*$^{-/-}$ mice that lack monocytes. We demonstrated that high, low or null expression of IRF8 in hematopoietic progenitor cells promotes differentiation toward DCs, monocytes or neutrophils, respectively, via epigenetic regulation of distinct sets of enhancers in cooperation with other transcription factors. Thus, IRF8 controls the lineage choice via chromatin regulation in a dose-dependent manner within the myeloid cell system.

More recently, we focus on higher-order chromatin structure during DC development. Our Hi-C data revealed that DC-specific active compartments are gradually established throughout the course of differentiation, while most of the intra-topologically associating domain (TAD) interactions specific for the DC lineage are formed with slower kinetics. We also found that the activation of DC-specific enhancers precedes the compartment switch and subsequent gene expression. In addition, our data suggest that IRF8 is required at least partially for the formation of DC-specific active compartments.

We are now investigating higher-order chromatin structure around the *Irf8* gene, which has at least 4 enhancers including the one we identified above. To achieve enough resolution, we will employ the Tiled-C method by designing 7,303 RNA probes for the surrounding 3 MB region.

Hopefully, we would be eventually able to understand the meaning of the changes in higher-order chromatin structure during cell differentiation. We speculate that at least at some key genes, the high-order chromatin structure has an instructive role, rather than a passive phenomenon.

References: Kurotaki et al, *Cell Rep* 22, 2628–2641, 2018; Kurotaki et al, *Blood* 133, 1803-1813, 2019; Murakami et al, *Nat Immunol* 22, 301-311, 2021