Trends in Genetics

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Spotlight

Cell type-specific chromatin topology and gene regulation

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Chromatin structure is critically involved in gene regulation and cell fate determination. How this structure is established and maintained in distinct, terminally differentiated cells remains elusive. Winick-Ng *et al*. address this puzzle by applying immunoGAM in different brain cell types and reveal cell type-specific chromatin topologies, long gene decompaction, and the involvement of transcription factors (TFs).

Reorganization of 3D chromatin structure occurs during cell lineage specification and is believed to regulate gene transcription and determine cellular states. Chromatin topology formation is driven by multiple dynamic, coexisting, and sometimes opposing forces, including cohesin complex-mediated loop extrusion and compartmentalization of self-associating genomic regions [1]. Loop extrusion is executed by the multi-subunit ring-like cohesin complex, which extrudes DNA in an ATP-dependent manner until it encounters CTCF proteins bound in a convergent orientation, thereby stabilizing point-to-point interactions termed chromatin loops. In contrast, compartmentalization results in the segregation of the genomes into active and inactive nuclear areas. The combined forces of loop extrusion and compartmentalization give rise to contiguous regions of self-associating DNA, termed topologically associating domains (TADs), which often demarcate regulatory and/or transcriptional units [2]. TFs and chromatin-modulatory proteins also represent an important driving force in shaping 3D chromatin structure; however, their exact mechanisms of action are not fully understood. Increasing evidence suggests that some TFs and chromatin-modulatory factors influence 3D chromatin landscape via liquid–liquid phase separation (LLPS), a type of weak multivalent interactions, to form membraneless condensate of biomolecules [1,3,4]. Deregulation of chromatin structure, exemplified by disruption of TADs, loops, and other 3D chromatin structures, is recurrently detected in human diseases and, often, is causal for pathogenesis [5]. Faithful, efficient mapping of chromatin topology is important for an improved understanding of normal and pathological development; however, characterization of these structures in complex tissues remains challenging with traditional techniques.

In a recent issue of *Nature*, Winick-Ng *et al*. developed and applied immunoGAM [an extension of genome architecture mapping (GAM)] (Figure 1) [6], a chromatin topology profiling method using ultra-thin nuclear slices (~220 nm), generated by cryosectioning fixed tissues in random directions, and laser-capture microdissection of immunofluorescence-labeled nuclear slices. Physically distant genomic sites co-segregate less frequently in the same thin slice than physically proximal sites. Sequencing of amplified DNA from each nuclear slice and plotting of pair-wise co-segregation matrices produce the frequencies of chromatin contacts between any number of genomic loci in a specific cell type. GAM-based chromatin contact maps have been found to be highly correlated with those from chromosome conformation capture (3C)-based approaches, particularly Hi-C [7,8]. Compared with Hi-C, immunoGAM offers several noted advantages [6], which include: (i) no need of tissue dissociation in order to profile complex tissues and multiple cell types based on cryo-sectioning and immunolabeling; (ii) a relatively small number of cells (400–1000) required to produce contact heatmap with a resolution of about 30–50 kb; and (iii) no involvement of restriction enzyme-mediated DNA digestion and subsequent ligation, thus avoiding the related bias [9]. ImmunoGAM, however, requires specialized equipment for cryo-sectioning and laser-capture microdissection of nuclear slices. The team has previously applied GAM to profile mouse embryonic stem cells (mESCs) [8] and, in the work of Winick-Ng *et al*. [6], applied immunoGAM to mouse brain tissue and examined cell type-specific genome organization among three functionally distinct cell types: oligodendroglia [oligodendrocytes and their precursors (OLGs)], pyramidal glutamatergic neurons (PGNs), and dopaminergic neurons (DNs). There exist cell type-specific 3D chromatin structures at both short- and long-range genomic scales, and these chromatin topology changes are correlated to gene expression patterns in brain cells. For example, protocadherin cluster genes display the increased long-range contacts in neurons and OLGs, relative to mESCs, which is associated with higher expression [6]. Previous Hi-C profiling of other systems also uncovered cell type-specific rearrangements in 3D chromatin architecture, such as those related to macrophage differentiation [10].

A most apparent chromatin reorganization event in brain cells, when compared with mESCs, occurs at extremely long genes (with a size of >300 kb) [6]. These long neuronal genes are involved in neuronal lineage-specialized cell functions. Compared with what was seen in mESCs, long neuronal genes (such as Grik2 and Dscam) lose local contact density in PGNs and/or DNs, a phenomenon termed gene decondensation or 'melting' [6]. Long genes with 'melting' generally exhibit higher levels of transcription and chromatin accessibility in neurons [6]. The mechanism underlying gene 'melting' remains unknown and the relationship to gene

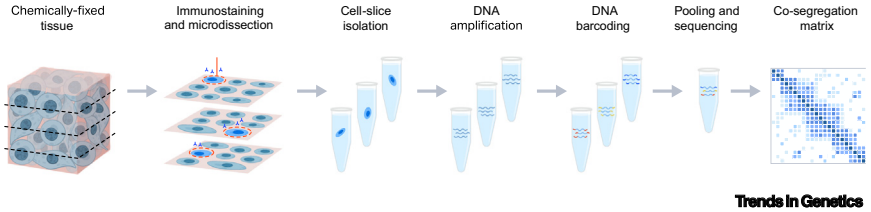

Figure 1. Overview of the immunoGAM protocol. Chemically fixed tissue is isolated and processed into ultra-thin slices. Cells of interest are identified by immunostaining and/or morphology and excised using laser microdissection. One or more slices are combined. DNA is amplified, barcoded, pooled, and sequenced. The resulting data are aligned to the genome, and co-segregation frequencies are calculated and used to infer 3D chromatin structure.

transcription is unclear. Melting was suggested to stem from specialized changes of long genes, such as those related to topoisomerase-induced topology resolution [11], to accommodate the transcriptional and spliceosome machineries for gene activation and/or RNA processing. This phenomenon is reminiscent of 'puffing' of *Drosophila* polytene chromosome, in which active transcription is induced in response to hormone or heat shock. It is also possible that the causal arrow points in the other direction and that transcriptional machinery antagonizes loop extrusion at highly expressed genes, leading to a more 'open' chromatin conformation. Indeed, several previous studies have documented the accumulation of cohesin at transcription end sites, suggesting that polymerase complexes can push and/or displace cohesin [12,13]. Further work is required to understand the causal relationships between melting and transcription at long genes in neuronal lineages.

Winick-Ng *et al*. provide exciting evidence that chromatin contacts regulate transcription over short and long distances and are associated with both activation and repression [6]. At shorter scales (<5 Mb), neuron-specific contacts were enriched for neuronal-specific TFs such as Neurod1/2 dimers, Egr1, and Foxa1 and contained genes differentially expressed in brain cells, including synaptic plasticity-related genes in PGNs and drug addiction-related genes in DNs [6]. These findings corroborate previous studies that demonstrate a correlation between gene expression and DNA looping across cell types [10,14]. In contrast, at longer distances Winick-Ng *et al*. found neuron-specific interactions between repressed genes, including olfactory receptor genes (Olfr). This agrees with previous studies demonstrating how long-range (even interchromosomal) interactions between Olfr genes function to repress all but one Olfr gene in olfactory sensory neurons [15]. The molecular mechanisms underlying the aforementioned gene-regulatory effects await further investigation.

Cell-specific chromatin topologies are correlated to gene regulation and cell type-specific functions. Genomic tool kits (such as immunoGAM, Hi-C, RNA-seq, ATAC-seq, ChIP-seq, CUT&RUN, and CUT&Tag), which can be performed in bulk cells or at single-cell levels, have revolutionized current understanding of spatial and temporal organization of the genomes. During cellular differentiation, complex interplays exist among chromatin structure and modifications, master TFs and chromatin-modulating factors, and transcription itself. Efforts shall also be directed towards dissecting the causal relationship among these molecular events. Moreover, interplays between the forces underlying 3D chromatin (re) organization can also be complex. Conceivably, they can coexist, cooperate, or oppose one another, profoundly shaping the chromatin topologies. An example of their opposing effect is that chromatin looping driven by LLPS of NUP8-HOXA9, a leukemia-causing onco-TF, can result in decreased CTCF looping at nearby genomic sites [4]. How exactly the forces interact to establish and rearrange the chromatin conformation during organismal development and how these changes relate to transcription merits detailed investigation. Dissecting the molecular mechanisms underscoring chromatin topology organization shall greatly improve our understanding of development and pathogenesis.

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Declaration of interests

No interests are declared.

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