

Contribution of 3D Chromatin Architecture to the Maintenance of Pluripotency

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Abstract Maintenance of pluripotency, lineage commitment and differentiation of mammalian embryonic stem cells into all somatic cell types involves differential regulation of different subsets of genes, as does reprogramming of somatic cells back into a pluripotent state. It is now understood that the three-dimensional organization of the human genome asserts a key role in these processes in two ways. First, by providing a largely invariable scaffold onto which dynamic changes in chromatin may manifest; second, by allowing the spatial clustering of genes contributing to the same functional pathways. In this review, we discuss the rapidly growing volume of literature on the structure-to-function relationship of mammalian genomes as regards key developmental transitions of stem cell populations.

Keywords Pluripotency · Chromosome conformation capture · Topological domains · Chromatin interactions · Enhancers · Transcription factories

Introduction

Lineage commitment and differentiation of embryonic stem (ES) cells into all somatic cell types involves upregulation and downregulation of different subsets of genes [1]. This cell

type-specific regulation of gene expression occurs predominantly at the level of transcription, and this was exemplified by a seminal experiment where mammalian fibroblasts were reprogrammed into an induced pluripotent cell state via the overexpression of only four transcription factors, Oct-4, Myc, Klf4, and Sox2 [2, 3]. Similarly, the key pluripotency factors, Oct-4, Sox2, and Nanog, have been shown to maintain ESCs in an undifferentiated state by driving the expression of genes associated with cell identity, including those encoding these TFs themselves [1]. The maintenance of pluripotency also requires the repression of lineage-specific genes that can stimulate various differentiation pathways; this is mediated by Polycomb group proteins that bind these genes and their cognate *cis*-regulatory elements, thus introducing an epigenetic environment preventing the loss of ESC identity. Given the diverse, sometimes overlapping [4] binding patterns of these factors, it has become evident that control of transcription programs is manifested via organizational features that extend beyond the mere linear order of primary genomic sequence.

These organizational features of the genome stem from the need to fit mammalian chromosomes within the confined dimensions of cell nuclei. As a result, DNA molecules are wrapped around histones and the resulting chromatin fibers are compacted into interphase chromosomes, which in turn assume a non-random arrangement and form spatially distinct territories [5]. Hence, even at this large-scale view of the nucleus, physical proximity of regions that are otherwise non-adjacent on the linear fiber (or even between regions lying on different chromosomes) becomes apparent. Most of the aforementioned knowledge comes from cell biology approaches, which—however powerful at the single-cell level—suffer from limitations in resolution and throughput [6]. But, as such, the need to study regulatory interactions between defined, sub-resolution, genomic regions called for the development of novel technologies able to capture these at the molecular

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level. In this review, we will discuss how new insight into the three-dimensional (3D) organization of mammalian genomes reshapes our understanding of pluripotency, cell differentiation, and organismal aging.

A New Tool to Study Chromatin Organization at Sub-Genome Resolution

Already in 1993, Cullen and colleagues described the elegant idea of “proximity ligation” by which one could detect short-range chromatin looping [7]. But, it was not until several years later that the introduction of the chromosome conformation capture (3C) technology revolutionized the study of chromosome folding at high resolution [8]. Within the last decade, a number of 3C variants have emerged, like circularized 3C (4C), carbon copy 3C (5C), or chromatin interaction analysis coupled to paired-end tagging (ChIA-PET) that exploit latest sequencing technologies to analyze events of chromatin looping at an increasingly higher genome coverage [9]. The key principles of 3C-based methods involve formaldehyde fixation to preserve chromatin interactions, digestion of DNA using a restriction enzyme, and cross-linked complexes undergo ligation under conditions that favor fusions also between fragments originally not in close proximity on the linear fiber; finally, identification of these ligation junctions, and of the frequency by which these occur, provides a snapshot of the *in vivo* genomic architecture.

At the whole-genome level, development and application of Hi-C [10] has verified older findings and at the same time uncovered novel organizational principles of mammalian genomes. For example, the preferential co-associations between euchromatic and heterochromatic regions—termed compartment “A” and “B,” respectively—were not unforeseen, but were extensively charted by Hi-C [10, 11, 12•]. At the sub-compartment scale, a striking new feature was that of topologically associating domains (TADs); these represent spatial neighborhoods that harbor high-frequency interactions and are insulated from adjacent TADs by sharp boundaries of low interaction capacity [12•, 13, 14••]. Finally, at the sub-TAD level, the dynamics of promoter-promoter and enhancer-promoter crosstalk are the highlights of 3D architecture and change considerably upon establishment of lineage-specific transcriptional programs [15, 16•]. Under the light of a multi-level hierarchical genome organization, the control of cell fate can be studied in all four dimensions, in 3D space and over time [17–19], with the capacity to investigate variations down to a ~1-kbp resolution [20] or even at the single-cell level [21]. Notably, the recent emergence of “targeted” 3C variants will allow lower cost, single-restriction-fragment resolution analysis of selected loci involved in cellular pathways of particular interest [16•, 22, 23].

Large-Scale Reorganization of Higher-Order Chromatin Structure During Differentiation

A more careful look into the pioneering Hi-C studies reveals that genomic regions correlating with both genetic features (e.g., gene density, GC content) and epigenetic indicators of transcriptional status (e.g., chromatin accessibility, transcriptional co-regulation, “activating” or “repressive” histone marks, early or late replication timing) preferentially interact with regions exhibiting similar characteristics [10, 24, 25]. This is consistent with numerous studies linking transcriptional co-regulation to spatial clustering of the relevant genes and their *cis*-regulatory elements [26–32] around the nucleoplasmic supra-molecular entities that harbor most nuclear transcription—around transcription factories [19].

Advances in sequencing capacity, accuracy, and read length allowed Hi-C studies to achieve resolution down to ~10 [33] and then ~1 kbp [20]. In a recent analysis, comparison of high-resolution Hi-C data generated using human ES cells and four ES-derived cell types revealed extensive A/B compartment rearrangements. Transition from the ES cell state to lung fibroblasts or mesenchymal stem cells coincides with a marked expansion of the B compartment [12•]; this is in agreement with the previously documented spread of heterochromatic modifications upon differentiation [34]. As a result, changes in expression correlate with genes switching from compartment A to B (or the converse); however, these are in average small, indicative of a contributory rather than deterministic role of compartments during lineage commitment [12•].

The Role of TADs in the Regulatory Landscape

At a 0.1–1-Mbp resolution, TADs represent a prominent module of 3D genome organization. Dixon and colleagues identified a partitioning of the genome in ~2000 such TADs, covering more than 90 % of its length [11]. More recently, Rao and colleagues, pushing resolution to the 1-kbp limit, claim a reduced number of TADs with a median loop size of 185 kbp [20]. Either way, cell types of endodermal, mesodermal, and ectodermal origin share between 55 and 75 % of such loops, while ~45 % of TADs called in mouse are also seen in man [20]. In addition, TADs are restricted to interphase chromosomes, when transcription is widespread, suggestive of their influence in regulation of gene expression [35]. As a result, transitions between the A/B compartments during cell differentiation, which rearrange TAD boundaries, are accompanied by the gain or loss of interactions within these TADs. This occurs in parallel with concomitant changes in replication timing, histone mark profiles, and association to the nuclear lamina [12•, 14••, 36•]. Especially, the latter lead to the repositioning of TADs within the typically repressive context of

the lamina and often harbor genes silenced along the course of differentiation [37].

Nevertheless, as the majority of TADs and their boundaries remain largely invariable, an overarching architecture of the genome can be imagined that acts as an evolutionarily selected scaffold onto which finer-scale changes allow for lineage commitment. It is worth noting here that both inflammatory and hormonal signaling only marginally remodel (<10 %) TAD boundaries, as well as that pro-inflammatory, pluripotency-specific, and key developmental enhancers have been seen pre-looped onto the gene promoters they control before these become activated [33, 38, 39••, 40, 41, 42••]. Collectively, these beg the question of how this overall “stable” overarching architecture allows for the gene expression changes seen along the different differentiation paths [1].

A View of Gene Expression Regulation at the Sub-TAD Scale

A key functional feature of TADs is the insulation of intra-domain interactions from “leaking” into neighboring domains, and the disruption of TAD boundaries was shown to cause gene expression misregulation [42••]. For example, in the well-studied *HoxD* gene cluster, early and late developmental genes are differentially induced via a switch between the two TADs that span the locus and act to direct promoter-enhancer crosstalk [43].

This crosstalk has been globally investigated using ChIA-PET and a targeted Hi-C approach. ChIA-PET connectivity maps have charted ~40,000 interactions amongst mouse enhancers and promoters; the majority of which involve enhancers contacting promoters that are located beyond their nearest active gene but are contained within the same TAD. Again, genes pivotal for stem cell identity are found in physical proximity with one another, indicative of co-regulation at the surface of “specialized” transcription factories [15]; this non-random 3D clustering was verified in a comparison of the interactomes of >22,000 mouse promoters in ES versus fetal liver cells using Capture Hi-C, its most emphatic manifestation seen in genes controlling key developmental transitions [16•]. It is worth noting however that a large number of associations persist throughout differentiation, pointing to a robust spatial network underlying cellular homeostasis, which — nonetheless — is characterized by the preferential clustering of genes involved in the same biological processes or pathways [15, 16•]. Similarly, the application of 5C at six developmentally regulated genes in ES and neural progenitor cells (NPCs) revealed additional sub-domains within a single TAD, indicating reorganization at the sub-Mbp scale during differentiation [44]. Owing to the single-fragment resolution achieved here, these “sub-TAD” chromatin loops can be classified into three groups. First, constitutive looping interactions

seen in both cell types; second, enhancer-promoter loops specific to the pluripotent state in ESCs; third, lineage-specific looping occurring only upon differentiation. Taken together, changes in chromatin architecture interplay with gene expression during the process of cell-type commitment, but the question whether 3D genome topology is a functional cause or a result of transcription is still unresolved.

Finally, using the more focused 4C approach, where all interactions of a single genomic “viewpoint” are recorded [45], the promoters of a number of key pluripotency genes have been studied in both mouse and human ES cells. Again, long-range looping appears mostly confined within TADs, and co-associations, completely absent from differentiated cells, preferentially involve sites bound by the Oct-4, Sox2, and Nanog (OSN) transcription factors [38, 46]. Critically, Oct-4 or Nanog knock-down diminishes contact frequencies between OSN-bound regions [38, 39••], and insertion of a Nanog-binding array in the ESC genome led to the nucleation of endogenous OSN-bound loci around this ectopic array [46]. Not surprisingly, reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) by OSN overexpression recapitulates the pluripotency-specific genome configuration [38, 47]. Taken together, this data convincingly demonstrate that the three key pluripotency factors drive spatial organization via a specialized network of interactions as proposed [19] (Fig. 1a).

Genome organization does not solely owe to the “active” compartment of chromatin. The “inactive” ES cell compartment contains, amongst others, genes that are to be activated later in development. As it has been demonstrated that some of these associate with a subset of developmentally important “poised” promoters also marked by H3K27me3 [4] and that this Polycomb-instated histone mark is a key structural feature of the *Drosophila* genome [48], Denholtz and colleagues investigated Polycomb contribution to stem cell chromatin folding. A segregation between OSN-occupied and Polycomb-bound and H3K27me3-marked loci was revealed, and this feature is largely specific to the ES cell state. Moreover, loss of a central component of the Polycomb complex partially disassembles clusters formed around such H3K27me3-marked loci [49•]. Hence, changes in both the transcriptionally permissive and non-permissive parts of the ES cell genome contribute to the spatial arrangement and facilitate the state of pluripotency (Fig. 1b).

Protein-Mediated 3D Organization of the Pluripotent Genome

All the aforementioned novel insight into genome 3D organization has prompted researchers to look for proteins other than transcription factors that may contribute to looping, both on its “static” and on its dynamic aspects. These proteins, usually

folding obtained using independent methods [63] highlight the need for the development of novel tools for interrogating the *in vivo* architecture of the genome in the absence of crosslinking and from increasingly lower cell counts.

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Compliance with Ethics Guidelines

Conflict of Interest Lilija Brant and Argyris Papanonis declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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