Twisting chromatin in stem cells

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The EMBO Journal (2013) 32, 2304–2306. doi:10.1038/emboj.2013.179; Published online 6 August 2013

How transcriptional output is orchestrated in mammalian genomes is an area of intense research. The structural organization within the nucleus and the physical association between regulatory elements are considered critical components in this regulation. Recent work published in *Nature* (de Wit *et al.*, 2013) applied the Circular Chromosome Conformation Capture (4C) method at high resolution in pluripotent stem cells (PSCs), revealing that PSCs uniquely shape their genome around binding sites for pluripotency factors.

Many regulatory elements are located distal to regions of active transcription, but looping of DNA can juxtapose these

regulatory regions with gene promoters (Dekker *et al.*, 2013). The extent to which DNA elements display physical proximity during development and disease as well as the functional consequences of these three-dimensional (3D) chromosomal associations are poorly understood. Historically speaking, looping of DNA fibres was originally observed in bacteria and subsequently in mammalian cells, where it was proposed to serve a functional role (Splinter and de Laat, 2011). Microscopy-based methods were essential for characterizing the first looping interactions in mammalian cells, demonstrating that delineated territories are occupied by particular chromosomes in interphase nuclei (Dekker *et al.*, 2013).

Figure 1 Conformational properties of the PSC genome. Although overall genome topology is largely unchanged between PSCs and differentiated cells, the PSC genome does display unique 3D arrangements. Long-range interactions at inactive regions (red boxes) are mostly nonspecific (broken arrows). In contrast, clusters of pluripotency factor binding sites are capable of creating long-range interactions (green regions below) dependent on the *Nanog* and *Oct4* proteins, and such interactions are unique to the pluripotent state. These findings are depicted in reference to the 'dog-on-a-lead' model (de Wit *et al.*, 2013), whereby the region of interest (dog), as opposed to the rest of the chromosome (owner), can take on a weaker or a stronger role in regard to where it wants to walk (interact). Inactive regions assume a weaker role (small dog) in PSC, as their interactions are more restricted by local topology. Clusters of pluripotency factor binding sites are stronger (larger dog) in this cell type, as they efficiently form specific long-range contacts in PSC.

Microscopy, however, has limited resolution and thus new techniques were developed to characterize and quantify interactions between chromosomal regions. A seminal advance was the development of chromosome conformation capture, or 3C (Dekker *et al*, 2002), which allows the determination of whether particular DNA regions were in sufficient proximity in living cells so that they could be crosslinked by formaldehyde, and thus were likely to physically interact. Subsequent procedures were developed from this methodology that allow different levels of scale and resolution, including 4C (Simonis *et al*, 2006) to map multiple interactions starting from a particular genomic 'viewpoint'. A similar high-resolution application of 4C was recently employed by the developers of this method (de Wit *et al*, 2013), using many genomic regions in mouse embryonic stem cells and providing novel insights into the chromosome topology of PSCs. PSCs represent a highly suitable model system to study chromosome topology, as they are non-transformed (as compared to most other cell lines) primary cells that can be cultured in a pluripotent state with high purity. Furthermore, PSCs can be differentiated into specialized cell types and thus provide an *in vitro* model of developmental changes in gene expression and cellular function. Studies delineating transcriptional regulation in PSCs have the advantage that insight gathered here can have implications for understanding the nature of pluripotency and its application in regenerative medicine.

Current understanding of chromosome organization is that the majority of the genome is separated into topological-associated domains (TADs; Dekker *et al*, 2013) and that these TADs are mostly invariant between cell types. de Wit *et al* (2013) indeed observe this as well, but through artificial targeting of *Nanog* and removal of either *Nanog* or *Oct4* protein, demonstrate that clustered pluripotency factor binding sites are involved in higher-order PSC-specific topological organization. Additionally, they observe that long-range interactions are less specific at transcriptionally inactive regions in PSCs compared to astrocytes differentiated from these cultures. The authors then convincingly demonstrate that this structural order is reestablished when differentiated cells are reprogrammed to induced PSCs, stressing that this unique 3D conformation is a property of the pluripotent state. Because overall chromosome topology remains largely intact in these experiments, these data lend support to their proposed 'dog-on-a-lead model' (Krijger and de Laat, 2013; see Figure 1).

In light of these findings, the authors further argue that juxtaposition of regions rich in pluripotency factor binding sites may help to ensure the robust transcriptional output of engaged target genes, contributing to the maintenance of the pluripotent cell state. It should be noted that the necessity of pluripotency factors in PSC-specific 3D organization agrees

with previous studies (Apostolou *et al*, 2013; Wei *et al*, 2013; Zhang *et al*, 2013), yet others have reported that cohesin and mediator can engage in PSC-specific contacts independent of pluripotency factor binding (Phillips-Cremins *et al*, 2013). To what actual degree the structural proteins versus specific transcription factors mediate tissue-specific chromosomal looping remains to be seen. Simultaneous detection of transcription and looping in individual cells should allow testing of this model. Taken as a whole, these data argue that overall chromatin structure is highly dynamic between different cell types both at active and inactive loci.

de Wit *et al* (2013) then designed a computational approach called PE-SCAN to correlate ChIP-seq data with Hi-C interaction profiles. PE-SCAN determines whether particular transcription factors or histone marks identified by Chromatin-IP tend to colocalize in nuclear space over long distances as measured by 4C. This investigation resulted in the observation that promoter contacts, judged by enrichment for H3K4me3, were largely invariant across mammalian cell types as previously described (Dixon *et al*, 2012; Nora *et al*, 2012). In contrast, contacts by enhancer regions, as judged by H3K27Ac and H3K4me1, are much more tissue-specific and dependent on pluripotency factors *Nanog*, *Sox2* or *Oct4* in PSCs. In addition to providing novel insights in this study, the pipeline will undoubtedly be useful in integrating ChIP-seq data with long-range chromosome interactions to reveal factors that shape the 3D genome in different cell types.

The insights provided by de Wit *et al* (2013) raise important questions regarding chromosome topology. For example, how do these regions find each other in the nucleus? The dog-on-a-lead model would propose that this is a random walk of chromosomal regions that have high affinity when they come into contact. Yet to bridge large domains, additional factors might be necessary for mediating these interactions.

It will be interesting to determine whether the observed organizing function of pluripotency factors in stem cell is taken over by other tissue-specific transcription factors once cells differentiate. In other words is any of the observed regulation unique to stem cells or is this currently more a reflection of a cell type where the most information has been gathered? Future studies will undoubtedly reveal further insights into the connection between chromatin looping and gene regulation.

Acknowledgements

We apologize to colleagues, whose work we could not mention due to restricted number of citations. PG is supported by an EMBO long-term fellowship. DS acknowledges support from the Novartis Research Foundation.

Conflict of interest

The authors declare that they have no conflict of interest.

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