Cohesin's role as an active chromatin domain anchorage revealed

Christine Feig and Duncan T Odom*

University of Cambridge, Cancer Research UK Cambridge Institute, Cambridge, UK.

*Correspondence to: Duncan.Odom@cruk.cam.ac.uk

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Cohesin is a conserved protein complex indispensable for proper cell division, because it secures sister-chromatid cohesion following DNA replication until segregation is required at the onset of anaphase. Recent studies have revealed functions beyond this, showing that cohesin binds to interphase chromatin regulating gene expression at select loci via long-range chromosomal interactions. In this issue of *The EMBO Journal*, Sofueva *et al* (2013) use a combination of chromatin conformation capture methods, classical FISH imaging, and loss-of-function studies to elegantly demonstrate how cohesin controls the 3D architectural organization of the genome.

Packing the 3 billion bases of a typical mammalian genome, which if extended would be 2 m long, into the cell's 10- μ m diameter nucleus is an evolutionary and architectural marvel. To achieve that level of compaction, DNA is wrapped around nucleosomes, and condensed further into ever tighter chromatin structures. Because genes and their corresponding *cis*-regulatory elements are often separated over long distances precise DNA folding is necessary to bring them into close contact and achieve proper gene regulation. Recently, methodologies have been developed that can map long-range chromatin contacts (e.g. 3C, 4C, Hi-C etc.). Several groups using these methods have shown chromosomes to be partitioned into topological domains that presumably provide a structural framework, in which genes and their corresponding regulators can interact selectively (Dixon *et al*, 2012; Nora *et al*, 2012; Andrey *et al*, 2013).

Cohesin, a protein complex comprised of three subunits (Smc1, Smc3, and Rad21/Sccl), is a likely candidate to help establish these domains, and to mediate inter- and intra-chromosomal interactions. Cohesin binds interphase chromatin and often colocalizes with the CCCTC binding factor (CTCF) across mammalian genomes. At a handful of genomic locations, cohesion has been shown to form long-range chromatin loops, and through those to regulate gene expression (Nativio *et al*, 2009; Kagey *et al*, 2010; Schmidt *et al*, 2010; Seitan *et al*, 2011).

On a genome-wide basis however, it has been unclear how functionally relevant cohesin is to both genome architecture and gene expression. In this issue of *The EMBO Journal*, Sofueva *et al* (2013) use an elegant and comprehensive approach that combines carefully selected primary and derived terminally differentiated cells, high-resolution

chromatin conformation mapping approaches, and classical fluorescent *in situ* hybridization (FISH) experiments to explore cohesin's roles *in vivo*.

First, Sofueva *et al* (2013) generate chromatin contact maps using Hi-C in proliferating neural stem cells (NSCs) and NSC-derived post-mitotic astrocytes (AST). These confirm the topological domain structures described in other cell types in earlier studies (Dixon *et al*, 2012; Nora *et al*, 2012; Sexton *et al*, 2012), which cluster into transcriptionally active and inactive regions. Sofueva *et al* (2013) note novel features:

Figure 1 Genetic depletion of the cohesin subunit Rad21 to 11% of control (depicted as blue dots) in terminally differentiated astrocytes leads to decompaction of topological domains (top panel) and consequently to an overall relaxed genome architecture with enlarged nuclei (middle panel). A select number of altered cohesin-mediated contacts were confirmed by DNA FISH (bottom panel).

passive domains are larger and characterized by homogenous internal structures, whereas active domains are smaller yet contain more internal contact complexity. Overlaying Hi-C maps with chromatin immunoprecipitation profiles for Rad21 and CTCF uncovered a striking correlation between domain boundaries and cohesin/CTCF binding. More specifically, the study revealed that active domains contain more cohesin/CTCF co-bound sites, thus suggesting an explanation for their enhanced complexity. Interestingly, an inverse correlation appeared between (i) the number of cohesin/CTCF binding sites separating two chromosomal locations and (ii) the likelihood that they contact each other, supporting a model where cohesin/CTCF binding acts as a contact insulator. Importantly, contact insulation was a unique feature of cohesin/CTCF co-bound sites, and not observed at sites solely bound by Rad21 or CTCF.

To avoid confounding influences from cohesin's well-known roles during cell cycle, Sofueva *et al* (2013) perform loss-of-function studies in post-mitotic ASTs derived from conditional Rad21-knockout mice. These Rad21-depleted ASTs had reduced intra-domain contacts, and while domain boundary locations were unchanged, they became more permeable (Figure 1). Sofueva *et al* (2013) go on to validate individual contact points perturbed by Rad21 knockout (increased or reduced contact) using 3D DNA FISH (Figure 1). Perhaps unsurprisingly, the decompaction of the genome from loss of Rad21 also resulted in an increased nuclear volume (Figure 1).

Ultimately, what are the downstream consequences of a perturbed genome architecture? Sofueva *et al* (2013) continue with an mRNA-seq analysis of wild-type versus Rad21-knockout ASTs and find hundreds of genes with altered transcription. While the majority of those perturbed genes did not have a

cohesin/CTCF binding site near their transcription start site, those that did showed reduced chromosomal looping upon Rad21 loss between cohesin/CTCF sites located in their promoter region. Thus, cohesin indeed establishes chromatin contacts essential for proper genome architecture and maintains domain insulation. Sofueva *et al* (2013) provide direct evidence that these genomic features are necessary to safeguard the stability of transcriptional programmes.

A recent paper used a similar approach to explore cohesin's role in genome structure maintenance (Seitan *et al*, 2013). Seitan *et al* (2013) also used Hi-C to map the effect of cohesin ablation in terminally differentiated, and thus post-mitotic, T-cells—but present somewhat different conclusions. Both groups observed similar gene expression perturbations caused by similar reductions in Rad21 protein levels. Interestingly, however, in the system employed by Seitan *et al* (2013), Rad21 deficiency resulted in very few observable changes in the large-scale genome structure. They identified fewer and exclusively intrachromosomal changes, most of which were confined to individual compartments. A number of reasons could account for the discrepancies between these studies including different cell types used in the assays, different computational approaches, and different types of supporting and functional data.

Future work will surely resolve these disparate results, as the architectural mechanisms that determine the spatial shape and function of the mammalian genome are an increasingly exciting and dynamic area of investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

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