GENOME ORGANIZATION

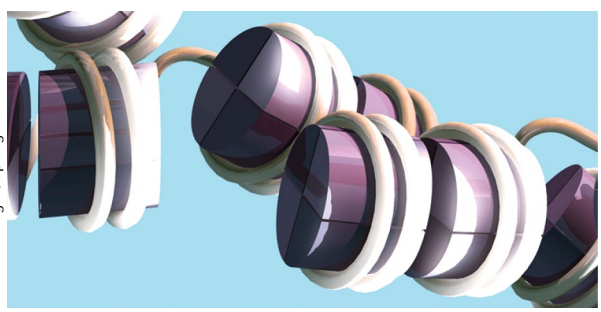
Chromosome structure at micro-scale

> functional interplay between transcription and fine-scale chromatin structure

Various sequencing-based methods for characterizing 3D genome structure are revealing hierarchical organization of chromosomes across different scales. A major goal is to characterize fine-scale structure and to decipher its functional links to gene expression. Two new studies report fine-scale structural profiles of mammalian genomes using Micro-C, indicating that fine structure is regulated by diverse transcription-related features.

Chromosome conformation capture methods such as Hi-C are based on the principle that when fixed and sheared genomic DNA is re-ligated, the pairs of re-ligated loci are those that were in close 3D proximity in the nucleus. Micro-C is a derivative of Hi-C that includes a micrococcal nuclease (MNase) digestion step to specifically probe proximity between pairs of nucleosomes. Until now, Micro-C studies have focused on yeast rather than on complex genomes.

In their study, Krietenstein et al. applied Micro-C to human embryonic stem cells and fibroblasts, generating 2.6–4.5 billion uniquely mapped reads per sample (~150× coverage per nucleosome). By comparing with existing Hi-C data sets, they showed that Micro-C recapitulates the known hierarchical structural features of chromosomes but has substantially superior signal-to-noise ratios for detecting close-range contacts at the 1–100 nucleosome scale. Additionally, analysing the same data in a single-ended manner (rather than explicitly considering ligation pairs) provides a genome-wide MNase-based map of nucleosome occupancy, so that structural features can be linked to chromatin accessibility.



Credit: P. Morgan/Springer Nature Limited

In their study, Hsieh et al. applied Micro-C to mouse embryonic stem cells, analysing 2.64 billion reads. Similarly to Krietenstein et al., the Micro-C structural information was consistent with that from Hi-C but with increased power for detecting short-range interactions.

One debated feature of chromosome structure is how nucleosomes pack together locally. For both teams, the Micro-C data indicated that small 'clutches' of nucleosomes form a 'zig-zag' arrangement, whereby for a series of nucleosomes the even and odd numbered nucleosomes form separate stacks.

Both studies identified boundaries that demarcate chromosomes into domains with high intra-domain contact frequency but lower inter-domain contact frequency. The best characterized chromosomal domains are topologically associating domains (TADs) of 100 kb–1 Mb, which are generally demarcated by inward-facing CTCF-binding sites from which cohesin-based loop extrusion is thought to occur during TAD formation. However, disruption of CTCF or cohesin function has negligible or modest effects on gene expression, leaving a conceptual gap for how 3D chromosome structure is linked to transcription.

From the Micro-C data, both teams characterized the boundaries of fine-scale domains within TADs by analysing the sequence features at these sites, as well as layering on existing epigenomic profiles. They found that boundaries of fine-scale domains are highly diverse in nature and can comprise various transcription-related features, such as enhancers, promoters, nucleosome depletion and RNA polymerase II occupancy. Moreover, Hsieh et al. showed that chromatin accessibility and transcriptional activity are stronger predictors of these domain boundaries than are CTCF–cohesin sites.

These boundary analyses indicate that whereas CTCF–cohesin might define the overall structure and boundaries of TADs, gene-regulatory interactions within these TADs are likely to be more diverse and involve sequences and chromatin features more directly linked to transcription.

In further characterization of the interplay between chromosome structure and transcription, Hsieh et al. showed that gene structural compaction correlated positively with gene transcription, although this is the opposite relationship to what was previously demonstrated in Micro-C studies in yeast (where transcription was associated with less-compact chromatin). Furthermore, although inhibitors of transcription had negligible effects on large-scale chromosome structure, there were finer-scale alterations to the local interactions of enhancers and promoters, providing further support for the functional interplay between transcription and fine-scale chromatin structure.

Owing to the power of Micro-C data to reveal focal interactions, both teams identified many additional local interactions between regulatory elements, which will serve as a valuable resource for further characterization of gene regulatory mechanisms.

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ORIGINAL ARTICLES Krietenstein, N. et al. Ultrastructural details of mammalian chromosome architecture. *Mol. Cell* https://doi.org/10.1016/j.molcel.2020.03.003 (2020) | Hsieh, T.-H. S. et al. Resolving the 3D landscape of transcription-linked mammalian chromatin folding. *Mol. Cell* https://doi.org/10.1016/j.molcel.2020.03.002 (2020)
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