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GENOME ORGANIZATION

Zooming in on nuclear organization

Chromatin conformation capture (3C) and its derivatives have provided insight into the three-dimensional (3D) organization of the nucleus. However, their ability to identify simultaneous chromatin contacts and to assess genome structure at the single-cell level was limited. Now, Beagrie *et al.* report a method for measuring three-way contacts between loci across the genome, and Stevens *et al.* couple imaging with enhanced 3C followed by high-throughput sequencing (Hi–C) to produce 3D structures of whole genomes in single cells.

In the genome architecture mapping (GAM) method developed by Beagrie *et al.*, the nuclear organization profile is extracted from thin cryosections of fixed cells by microdissection, amplified and sequenced; notably, unlike for 3C techniques, ligation is not required to capture interacting portions of DNA. Nuclear profiles contain loci that are in close proximity, even if they are distant on the linear genome; scoring the co-segregation of loci across many cryosections can infer preferred contacts. The authors collected 471 profiles from mouse embryonic stem (mES) cells using GAM and further assessed 408 of the highest quality profiles (the 'mES-400 dataset'); this dataset enabled the study of chromatin contacts at a resolution of 30 kb and could replicate data on chromatin architecture that was previously produced using 3C-based approaches. Beagrie *et al.* also developed a mathematical model called SLICE (statistical interference of co-segregation) that determines which chromatin contacts are likely to be specific. SLICE revealed that specifically interacting chromatin regions in their mES-400 dataset contained enhancers and active genes. It also identified the top 2% of triplet contacts (that is, contacts involving three genomic regions), which were detected at the same organizational level as topologically associated domains (TADs). These triplet contacts spanned genomic distances of up to 116 Mb and were often found to connect three TADs, all of which contained super-enhancers, or to connect highly transcribed TADs.

Stevens *et al.* imaged haploid mES cells expressing fluorescently labelled CENP-A and histone H2B proteins to select for cells in G1 phase. They then subjected these cells to Hi–C, which produced 37,000–122,000 contacts per cell, and computationally generated 3D genome folding structures at a resolution of 100 kb. Superimposing the computationally generated 3D structure on the microscopy image of the same cell demonstrated that the 3D structures are consistent with the cell imaging and Hi–C data. Stevens *et al.* used the structures to gain insight into the genomic details of individual cells, sometimes by mapping chromatin immunoprecipitation followed by sequencing (ChIP–seq) or RNA sequencing (RNA-seq) data sets from cell populations onto them. They determined that, although the structure of TADs and the organization of loops that are generated by the CCCTC-binding factor and the cohesion complex (these loops help to stabilize enhancer–promoter interactions) varies between cells, the organization of the A (active) and B (inactive) genome compartments, lamina-associated domains and active enhancers and promoters persists between individual cells. Furthermore, the position of pluripotency transcription factor binding on the 3D structures validated and extended previous data suggesting that Krueppel-like factor 4 (KLF4) organizes long-range chromosomal interactions. The biological relevance of this technique was further highlighted by the novel finding that genes regulated by the nucleosome remodelling and deacetylase (NuRD) complex cluster in 3D.

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ORIGINAL ARTICLES Beagrie, R. A. *et al.* Complex multi-enhancer contacts captured by genome architecture mapping. *Nature* http://dx.doi.org/10.1038/nature21411 (2017) | Stevens, T. J. *et al.* 3D structures of individual mammalian genomes studied by single-cell Hi-C. *Nature* http://dx.doi.org/10.1038/nature21429 (2017)

FURTHER READING Schmitt, A. D., Hu, M. & Ren, B. Genome-wide mapping and analysis of chromosome architecture. *Nat. Rev. Mol. Cell Biol.* **17**, 743–755 (2016)