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GENOME ORGANIZATION

3D genome architecture — of loops and globules

> higher-order chromatin organization is essential for the proper regulation of gene expression

Substantial efforts have been made to understand the fundamental aspects of three-dimensional (3D) genome organization within the nucleus. Using chromosome conformation capture (3C)-based technologies, two studies provide new insights into the factors shaping higher-order chromatin structures from yeast to mammals and the pivotal role of genome organization in regulating gene expression.

In cancer and other diseases, specific chromosome structures that are important for the control of normal cell identity are altered. The first study, led by Keji Zhao and Richard A. Young, thus focused on the relationship between local chromosome structure and the transcriptional control of cell identity genes in embryonic stem cells. Using ChIA-PET (chromatin interaction analysis by paired-end tag sequencing), which combines chromatin immunoprecipitation-based methods with 3C, the researchers searched for a link between looped chromosome structures and cell identity-defining genes that are regulated by 'super-enhancers'.

"High-resolution identification of cohesin-mediated long-range chromatin interactions was critical for us to find the loops between two CTCF (CCCTC-binding factor) sites bracketing a domain that harbours a super-enhancer-driven pluripotency gene or Polycomb-repressed differentiation gene," reflects Zhao. Not only did the team find that genes regulated by super-enhancers occur within large looped chromosome structures that are connected through interacting CTCF sites co-occupied by cohesin but, more importantly, they also showed that higher-order chromatin organization is essential for the proper regulation of gene expression. "CTCF and cohesin organize the loops in such a way that protects key cell identity genes from dysregulation by other regulatory elements outside the domain," explains Zhao. In other words, the 'super-enhancer domains' restrict super-enhancer activity to genes within the domain, as evidenced by the fact that loss of a boundary delineated by CTCF resulted in the inappropriate activation of genes located outside that boundary. "Many of the loops are retained throughout differentiation," comments Young, "so in this study we define the chromosome structures that are the foundation for differentiation into the broad range of cell types found in mammals."

The second study, led by Shiv I. S. Grewal, used Hi-C methodology — which combines 3C with deep sequencing to investigate long-range chromatin interactions genome-wide and at high resolution — to determine the role of heterochromatin and its associated factors on 3D genome organization in fission yeast. *Schizosaccharomyces pombe* has a small genome of 13.8 Mb and only 3 chromosomes but is a highly malleable genetic system with conserved heterochromatin assembly pathways. Although heterochromatin domains had previously been suggested to play a part in higher-order genome organization, its exact role was unknown.

Grewal and co-workers found that chromosome arms are organized into locally compacted globules of 50–100 kb in size that require cohesin enrichment at their boundaries. Impairment of cohesin resulted in disruption of these structures and led to loss of chromosome territory restriction and genome-wide transcriptional readthrough. "These results reveal that cohesin-dependent globules are basic architectural components of arms and are, in fact, the smallest structural unit yet discovered," says Grewal. As for the function of globules on arms, Grewal posits that globules are likely to "promote functional annotation of the genome, perhaps by ensuring confinement of RNA polymerase II transcriptional activity". Surprisingly, heterochromatin provided additional structural constraints at centromeres and telomeres, which in effect shape 3D genome architecture by constraining interactions between chromosome arms. "Unconstrained interactions could allow deleterious events such as recombination to occur, which could ultimately lead to genome instability," explains Grewal.

Jointly, these studies highlight the potential of 3C-based technologies to explore aspects of genome structure and organization at high resolution, and bring us a step closer to elucidating the factors controlling genome organization and regulation.

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ORIGINAL RESEARCH PAPERS Dowen, J. M. *et al.* Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. *Cell* **159**, 374–387 (2014) | Mizuguchi, T. *et al.* Cohesin-dependent globules and heterochromatin shape 3D genome architecture in *S. pombe*. *Nature* http://dx.doi.org/10.1038/nature13833 (2014)