3D GENOMICS

TADs without borders

Genomes are highly organized in space and time. Compartments, topologically associating domains (TADs) and loops are three dimensional (3D) genome features that have been extensively studied. Among these three levels of organization, TADs have sparked the most debate. New microscopy data shed light on how TADs and their leaky borders contribute to gene regulation.

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Genomes fold into higher-order chromatin domains ranging in size from a few kilobases to hundreds of kilobases. These domains, referred to as TADs, are self-interacting regions of the genome that appear as 'triangles at diagonal'[1] in population-based Hi-C chromatin-contact maps. TADs exist in many organisms and have been associated with a plethora of genome-regulatory roles. However, the biological relevance of TADs has led to vivid discussions, because they were originally defined from a population-based experiment (that is, a bulk analysis of thousands to millions of cells). In this issue, Luppino et al.[2], using oligopaint-based fluorescence in situ hybridization imaging, describe, at the single-cell level, the existence of TADs with permeable boundaries that play a role in the regulation of nearby genes.

TADs, which were originally described almost 10 years ago (refs. [3,4]), encompass all DNA between the so-called boundaries or borders, which are often demarcated by architectural proteins such as CTCF[5]. On the basis of cell-population experiments, TADs remarkably correlate with coordinated gene expression, replication timing and histone modifications[6], thus suggesting that these structures may have a regulatory role. Currently, the most accepted mechanism for TAD formation is based on a dynamic process described by the loop-extrusion model[7–9], in which cohesin complexes, loaded onto chromatin fibers, extrude progressively larger loops until they dissociate from chromatin, bump into each other or are halted by insulator complexes such as a pair of convergent CTCF molecules[5]. This dynamic model explains the presence at the population level of various types of domains—some characterized by strong boundaries, which are indicative of a separation between highly discrete TADs, and others delineated by weaker insulation between adjacent domains, which are seen as more transitional or dynamic boundaries. Notably, the difference in absolute contacts between pairs of intradomain or interdomain loci is typically only twofold to threefold (refs. [2,6]), thus suggesting that multiple low-frequency, low-affinity interactions are sufficient to create these structures[10]. Overall, this dynamic loop-extrusion model for TAD formation is consistent with the heterogeneity of TAD structures[11] and is supported by experimental evidence in which depletion of cohesin[12] or CTCF[13] results in the disappearance of TADs at the population level. However, recent reports have challenged the structural and regulatory roles of TADs. At the single-cell level, cohesin depletion is not sufficient to remove TAD-like structures that have high cell-to-cell heterogeneity at their boundary positions[11]. At the population level, local deletions and insertions of TAD boundaries have subtle effects on transcription[14].

Patrolling borders

Luppino et al.[2] used a combination of oligopaint-based[15] diffraction-limited[16] microscopy and super-resolution sequential single-molecule localization microscopy[11,17] to study chromatin interactions between TADs. These experiments have yielded several informative and important observations regarding the role of cohesin in boundary permeability, defined as the extent to which loci separated by a TAD border can interact. As expected, the authors observed high cell-to-cell heterogeneity in inter-TAD interaction levels, in which variability appeared to be locus specific. Interestingly, TAD border permeability was independent of the underlying chromatin state of the adjacent domains. The question then became what patrols such borders and makes them permissible to crossings.

In a series of very well controlled experiments, Luppino and colleagues next investigated whether cohesin, a clear candidate for border regulation, might be responsible for border leaking. To do so, they depleted HTC-116 cells of RAD21, a core component of the cohesin ring. The experiment broadly affected cohesin complexes, regardless of whether they contained SA1 or SA2 subunits, which have been reported to have distinct roles in 3D genome structure[18]. After cohesin depletion, the interactions within and across domain boundaries decreased overall, and weaker boundaries were more sensitive to cohesin loss. Interestingly, deletion of the cohesin loader NIPBL resulted in a greater decrease in inter-domain interaction, whereas deletion of WAPL, which promotes the release of cohesin from chromatin fibers, increased interdomain interactions. Finally, depletion of the CTCF insulator protein significantly increased contacts and spatial overlap between domains, and stronger TAD borders were more affected by CTCF depletion. These findings suggest that, at the population level, the disappearance of the typical triangle-at-diagonal shape of TADs after depletion of CTCF or cohesin is driven by opposite effects, thus complementing the understanding of factors involved in TAD border permeability.

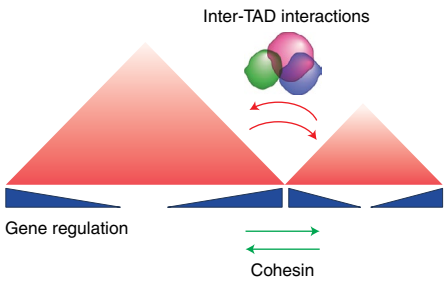

Fig. 1 | Using light microscopy to characterize border permeability in 3D genome structures. Whether TAD borders strongly insulate genes from neighboring genomic regulators has been a source of debate in the field of 3D genomics. The results from Luppino et al. indicate that such borders are leaky and regulate the expression of nearby genes.

Leaky borders for optimal function

Cohesin, while patrolling borders, thus appears to promote interactions between loci from two adjacent TADs. However, do leaky borders contribute to the regulation of gene expression? Luppino and colleagues then analyzed differentially expressed genes in HCT-116 cells after cohesin depletion and found that not all genes were equally affected. First, cell-type-specific genes, compared with housekeeping genes, were more affected by cohesin depletion. Second, and more interestingly, the expression of genes near TAD borders was most affected by cohesin depletion. The results suggest that expression of genes close to TAD borders is finely regulated by the balance between intradomain and interdomain interactions. If that conclusion holds true genome wide and for different cell types, highly regulated genes (for example, developmental genes, tissue-specific genes or genes that rapidly respond to external stimuli) might be expected to be found more often near less stable TAD boundaries. In fact, if TADs are constantly shifting, forming and reforming as cohesin extrudes loops and pauses at dynamically bound CTCF sites, why are some loci more affected by cohesin depletion than others? The dynamic nature and variability of chromatin conformations, rather than being an impediment to controlled gene expression, may thus facilitate optimal regulation of genes.

Altogether, the findings of Luppino et al. (Fig. 1) provide intriguing fodder for future research on the roles of the genome's variable organization in gene regulation. The observations that TADs are permeable and that this permeability affects gene expression in a locus-specific manner provide a reminder that one rule does not fit all. Many diverse ways of ensuring proper gene regulation are likely to exist, including cohesin simultaneously patrolling borders and promoting leaks. ❐

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Competing interests

The authors declare no competing interests.