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Seeing Is Believing: ORCA Allows Visualization of Three-Dimensional Genome Organization at Single-Cell Resolution

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Recent studies using chromosome conformation capture methods have begun to decode how eukaryotic genomes are organized in the three-dimensional (3D) space of the nucleus and the principles underlying this organization.[1] However, methods such as Hi-C require the use of millions of cells and therefore provide a population view of the 3D organization that is an ensemble of that of the individual cells present in the population. In a recent issue of *Nature*, Mateo et al. developed a microscopy-based approach named optical reconstruction of chromatin architecture (ORCA) to visualize the 3D chromatin architecture inside the nucleus of individual cells[2] (Figure 1A). Utilizing high-resolution microscopy in combination with Oligopaint and RNA-FISH, ORCA can simultaneously infer cell type and 3D chromatin organization for individual loci. Compared to Hi-C, ORCA and other microscopy-based approaches offer the advantage of being able to examine the heterogeneity of interactions present in individual cells.

Contrary to the case in mammals, where the CTCF protein plays a dominant role in 3D chromatin organization by forming loops, results from Hi-C experiments suggest that CTCF and other architectural proteins appear to play a minor role in *Drosophila* nuclear architecture. Instead, *Drosophila* cells appear to organize their genome by forming self-interaction domains that correspond to the transcriptional state of the chromatin.[3] To explore the principles of the 3D organization in individual cells, Mateo et al. applied ORCA to study the 3D organization of the bithorax complex (BX-C), at 3 kb resolution in cryosectioned *Drosophila* embryos. From anterior to posterior, *Drosophila* embryos contain a head, three thoracic segments (T1−T3), and eight abdominal segments (A1−A8). The identity of these body segments is controlled by the precise regulation of gene expression of homeotic genes located in the ANT and BX-C loci.[4] *Drosophila* BX-C (>300 kb) encodes three protein-coding homeotic genes, *Ubx*, *abd-A*, and *abd-B*, that are sequentially activated in an anterior-to-posterior manner, plus several lncRNAs (Figure 1B). The segment specific expression of these homeotic genes is regulated by multiple cell-type specific *cis*-regulatory elements identified by classical genetics experiments. Using ORCA, Mateo et al. revealed for the first time that cells present in different body segments exhibit distinct patterns of chromatin organization at BX-C and identified segment specific self-interaction domains similar to topologically associating domains (TADs) found in population Hi-C experiments. For example, the 3D chromatin organization of the locus in cells present in the head is different from that found in cells of the genitalia.

What contributes to the formation of segment specific TADs at BX-C, and is the transcriptional state a main driver of this organization? It has been shown that Polycomb (Pc) and H3K27me3 coat the BX-C in a body segment specific manner, corresponding to the silenced or active state of genes in each segment, with sharp borders that coincide with the BX-C regulatory domains and binding sites for architectural proteins. However, it is not known how these chromatin states correlate with the 3D chromatin organization of the BX-C region in cells of different segments. Mateo et al. found correlations between chromatin states and body segment specific 3D chromatin organization. For example, in segment T2, where the three homeotic genes are silenced and H3K27me3 coats the entire region of BX-C, one single TAD with no obvious partitioning was identified. However, starting at segment T3, where *Ubx* first turns on, TAD boundaries can be found at the borders of H3K27me3 that also represent the borders between transcriptionally active and inactive regions (Figure 1C). Thus, these observations support the idea that the sequential establishment of chromatin domains at BX-C correlates with segment specific transcription activation and function in the anterior−posterior axis,[4] although the causal relationship between these two events is not clear.

However, unexpectedly from the original prediction suggesting that TAD boundaries would form at the edges of H3K27m3 domains, Mateo et al. also found TADs whose boundaries do not coincide with the distribution of this histone modification. For example, there are multiple small TADs found in a large region devoid of H3K27me3 located 5′ to an H3K27me3-containing domain in cells of segments A2−A4 (Figure 1C). Boettiger and colleagues suggest that these smaller TADs represent domains defined genetically that harbor segment specific promoters and enhancers. This region also contains several lncRNA genes, and it is therefore possible that these small TADs are formed by interactions within and between these genes. For example, three separate TADs were found in cells of segment A2 in a region devoid of H3K27me3. One TAD is present in the region containing the *Ubx* gene; one encompasses an enhancer and a lncRNA gene, and a third TAD contains the gene, enhancer, and promoter of *abd-A*. The authors suggest that these H3K27me3-independent TADs reflect the promoters and their specific enhancers in this actively transcribed region in cells located in segment A2. However, it is also possible that the repressed regions marked with H3K27me3 form distinct compartmental domains, while in the absence of H3K27me3, the smaller compartmental domains are formed on the basis of the transcriptional activation of homeotic genes and lncRNAs at BX-C.

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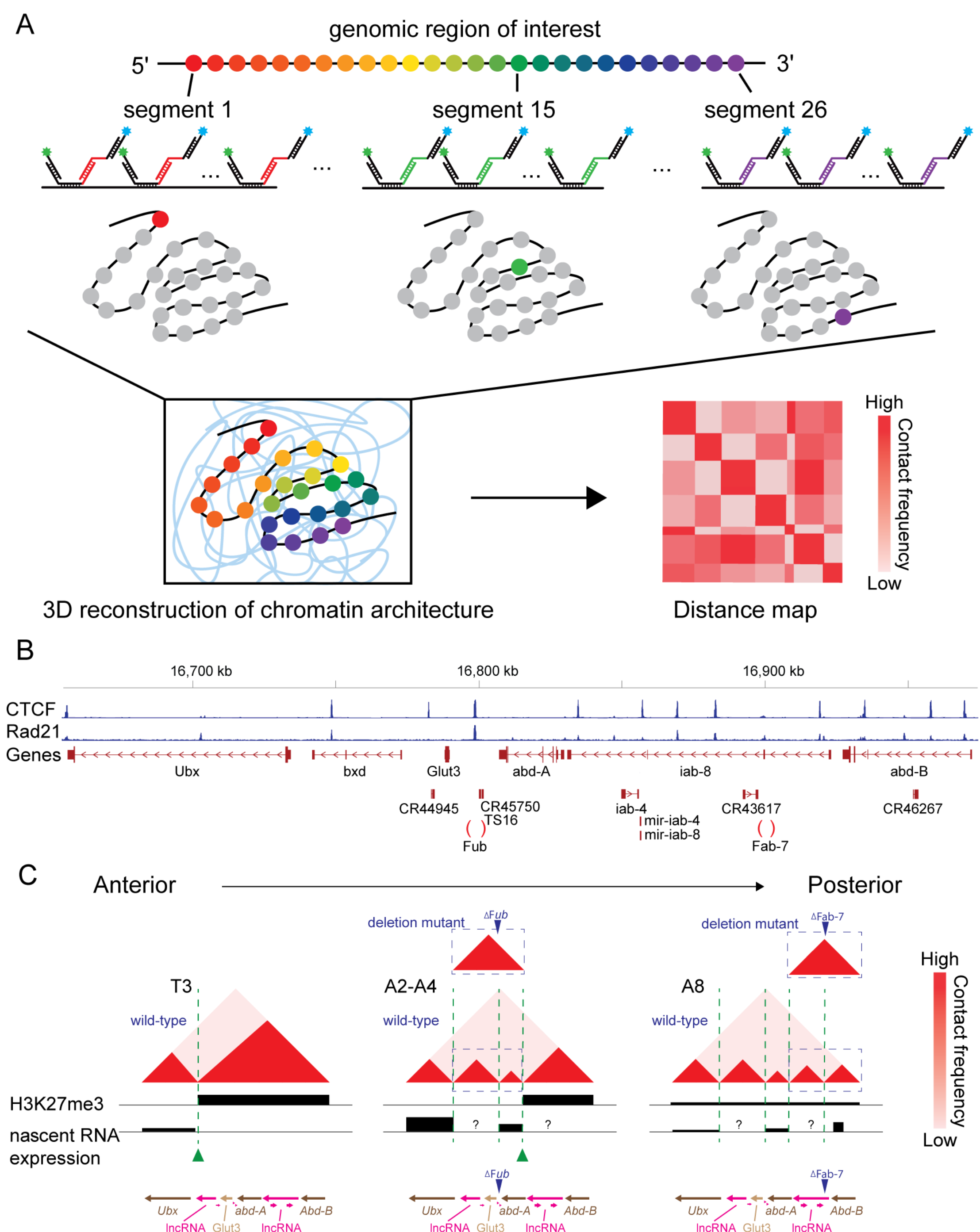

Figure 1. Optical reconstruction of chromatin architecture (ORCA) was used to study the 3D organization in cells of the *Drosophila* embryo. (A) ORCA methodology. A set of primary probes is designed to tile the region of interest and partitions the region into short segments (rainbow circles). Each probe carries a unique barcode that is sequentially imaged to provide the 3D position of each probe/barcode in the nucleus. The 3D positions of barcodes are measured and presented as pseudocolors, leading to the 3D reconstruction of chromatin architecture in the regions of interest. The 3D positions between each pair of barcodes are used to construct the distance map that resembles the conventional Hi-C maps. (B) Distribution of CTCF and Rad21 in BX-C, including Glut3, lncRNAs, and miRNA genes. The organization of BX-C corresponds to the Dm6 *Drosophila* genome assembly. (C) Diagram showing distinct chromatin structures observed in cells of different body segments of the *Drosophila* embryo in both the wild type and the mutants in which sequences containing CTCF sites were deleted. The green triangles indicate the boundary of H3K27me3 domains, and the green lines indicate the boundaries of transcriptionally active regions. The homeotic genes (*Ubx*, *abd-A*, and *Abd-B*), the nonhomeotic gene (Glut3), lncRNAs, and deletions (*Fub* and *Fab-7*) at BX-C are shown.

To provide additional insights into the nature of the H3K27me3-independent boundaries, Mateo et al. utilized *Drosophila* embryos homozygous for deletions that span the border between TADs in regions that lack H3K27me3. Two separate mutants, *Fub* and *Fab-7*, spanning different TAD borders were examined in segments A2−A4 and A7−A9, respectively. Interestingly, in both cases, two distinct TADs present in wild-type embryos became one large domain (Figure 1C). Mateo et al. propose that the fusion of the TADs is likely due to the deletion of binding sites for

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architectural protein CTCF and cohesin subunit Rad21, which in turn suggests that architectural proteins are responsible for the formation of a boundary between TADs in regions that exhibit the same epigenetic state. However, the ~4 and ~3 kb deletions in *Fub* and *Fab-7* mutants span not only the binding sites for architectural proteins but also several lncRNA genes. In the case of *Fub* mutants, the deletion may also affect a nonhomeotic gene at BX-C, Glut3, which is located between *Ubx* and *abd-A*. Therefore, the deletion may not simply remove binding sites for architectural proteins but may also perturb the transcriptional activity of these genes. These changes in transcriptional activity may represent an alternative explanation for the fusion of TADs in the deletion mutants.

The novel method developed by Mateo et al. provides an optical approach to examine chromatin architecture in a region of interest with the advantage of single-cell resolution compared to data produced by Hi-C. Therefore, ORCA is a great resource for deciphering cell-type specific compartmental domain organization in individual cells, which remains largely unexplored. Future experiments should examine the contribution of various chromatin components to the establishment of 3D organization, including components of the basal transcription machinery, transcription factors, and complexes involved in histone modification and remodeling.

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Notes

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