Gene Regulation and Chromatin Architecture in Developmental Dynamics

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Abstract

Genome organization is emerging as a major aspect of gene regulation. Precise regulation of gene expression is essential to establish different cellular identity and changes in gene activity will result in different outcomes[1]. Transcriptional enhancers often reside over large genomic distances from their target promoters. Three-dimensional chromosome architecture facilitate the establishment of these long-range regulatory interactions, and thus, the precise regulation of gene activity across developmental stages and tissues. However, it remains unclear how spatial genome organization undergirds complex developmental regulation by vast multi-enhancer regulatory landscapes. One intriguing gene that form the basis of this genomic examination is *Sex combs reduced* (Scr). While it regulation has been thoroughly studied[2] the aspect of genome organization had remained partly unexplored, leaving way for a detailed investigation. My supervisor, Dr. Philippe Batut, obtained some preliminary results showing that a particular region of interest located 40 kb away from the locus regulate its expression. Here, (1) integrating genome editing methods & quantitative single cell live imaging we investigate how chromatin architecture regulate Scr expression. The Mediator kinase module (MKM) has been proven to be a master regulator of transcription[3] but does it also plays a role in establishing loops and transcription bursting? We (2) also intended to determine if there is a co-dependency transcriptional dynamics between MED12 (a subunit of the MKM) and different developmental genes.

Biological system

Very early *Drosophila melanogaster* embryos (~ 90 min after fertilization)

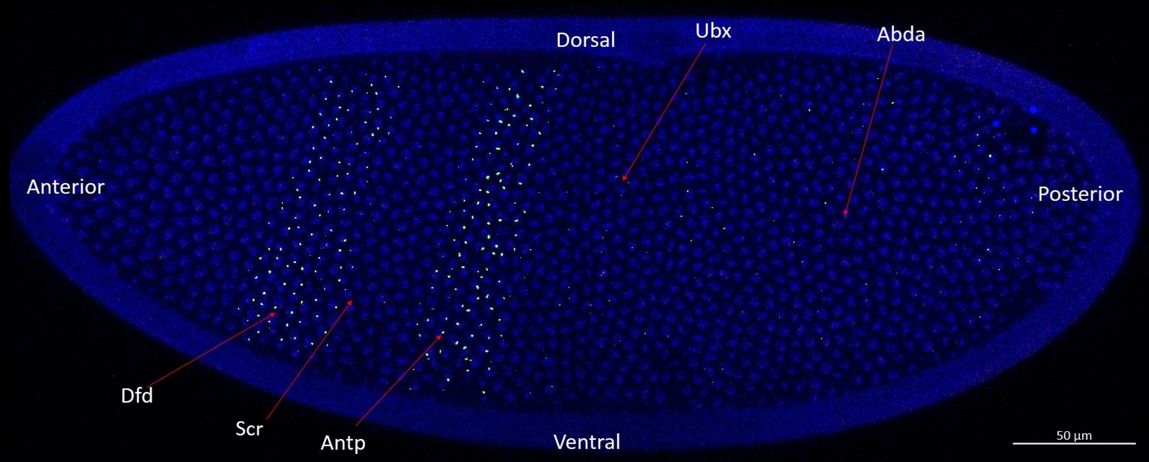
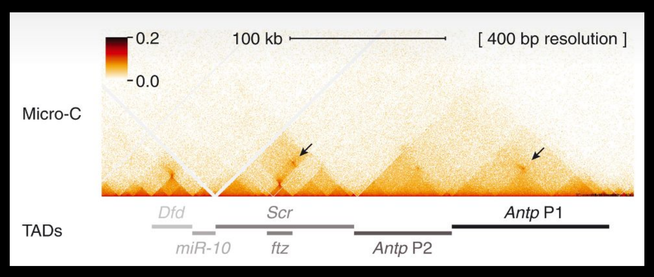


Image of a live embryo (mid cycle 14) showing transcription of Dfd, Antp and Abda in green, with Scr and Ubx in red, nuclei in blue. Screenshot from a movie.



Micro-C contact map showing the organization of the *Antennapedia* gene complex (one of two Hox genes clusters)

Discussion

Our first results points towards a co-transcriptional dynamics of MED12 & even-skipped but quantitative analyses are needed. We obtained the different mutant lines at the very end of my stay. There is a lot of imaging coming up to gather quantitative data. We are also planning to conduct Micro-C experiment for the distal regulatory region deletion mutant. We expect that the matrices will demonstrate a loss of focal contacts at relevant developmental stages. The finely tuned dynamic of Scr expression should be disrupted. The impact of the deletion of the Polycomb response element (PREs) is more unpredictable. PREs are indeed involved in the epigenetic silencing of genes by recruiting Polycomb Group (PcG) protein complexes, which lead to the repression of nearby genes[4]. Deletion of a PRE could result in the loss of PcG-mediated repression, leading to an increase in gene expression.

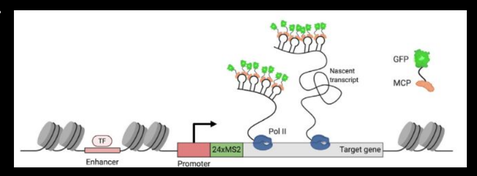
The present and future outcomes from this work may provide valuable hints at how spatial genome organization offers the physical infrastructure for the long-range regulation of genes activated by multiple enhancers throughout development. Enhancers are central to transcriptional regulation, but it is unclear whether they play any role in establishing or upholding chromatin structure. Still, there's a chance that they could contribute to reshaping the genome organization at the Scr locus.

While in many ways enhancers are the star players in transcriptional activation, polycomb response element could be considered the guiding hands that steer them precisely to their intended destinations.

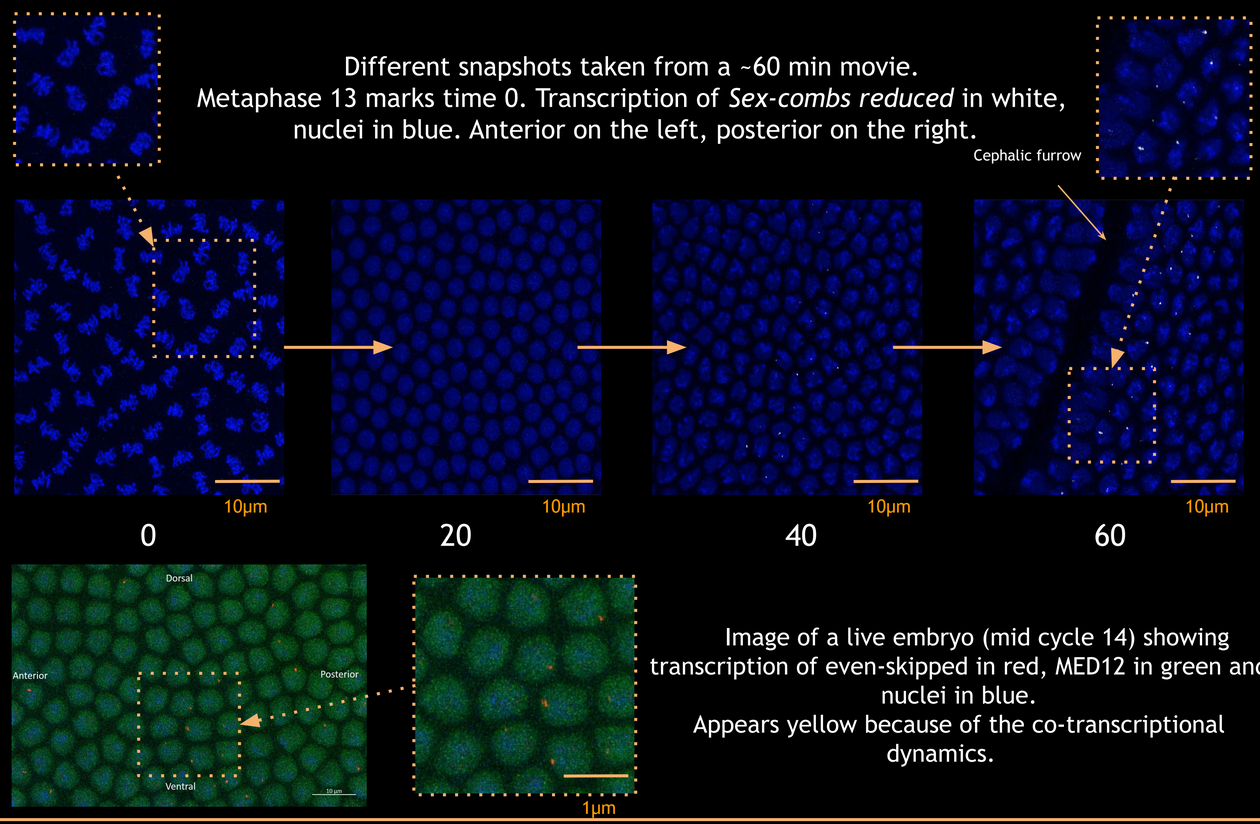
Experimental approach

Based on genome-wide datasets we determined candidate developmental enhancers and polycomb-response elements located within or near the distal region. We then used the CRISPR/Cas9 system to independently remove from the Drosophila genome candidate sequences as well as the entire 23 kb distal region. We cloned candidate regulatory regions into synthetic reporter genes utilizing the MS2 detection system to allow live transcriptional measurements in embryos. We established two crosses to visualize simultaneously MED12 recruitment with two gene's transcription: even-skipped (Eve), a pair-rule gene and Antennapedia (Antp), a hox gene. To better characterize hox-genes expression patterning we also set up a cross that enables us to visualize the expression of 5 hox genes at the same time.

Visualization of transcription with the MS2/MCP imaging system.



Single Cell Live transcriptional measurements



Different snapshots taken from a ~60 min movie.
Metaphase 13 marks time 0. Transcription of *Sex-combs reduced* in white, nuclei in blue. Anterior on the left, posterior on the right.

Image of a live embryo (mid cycle 14) showing transcription of even-skipped in red, MED12 in green and nuclei in blue.
Appears yellow because of the co-transcriptional dynamics.

References

1. Chen, L.-F. et al. Structural elements promote architectural stripe formation and facilitate ultra-long-range gene regulation at a human disease locus. Mol Cell, (2023).
2. Calhoun, V. C. & Levine, M. Long-range enhancer-promoter interactions in the Scr-Antp interval of the Drosophila Antennapedia complex. Proc Natl Acad Sci USA 100, (2003).
3. Richter, W.F. et al. The Mediator complex as a master regulator of transcription by RNA polymerase II. Nat Rev Mol Cell Biol 23, 732–749 (2022).
4. Ogiyama, Y. & Cavalli, G. Polycomb-Dependent Chromatin Looping Contributes to Gene Silencing during Drosophila Development. Mol Cell 71, (2018).