P5

3D genome structure dynamics during *T. castaneum* axis formation

GönomiX

State of the art

The function of the genome is closely related to its 3D structure in the nucleus [1]. Genome structure emerges during early embryonic development, in parallel to the maternal to zygotic transition (Fig. 1) [2]. However, the precise (causal) relationship and interplay between genome structure and function remain unclear. Furthermore, the general principles underlying genome organization are not completely understood. Mammalian chromosomes are organized into compartments and Topologically Associating Domains (TADs). Compartments reflect separation of euchromatin and heterochromatin, whereas TADs are local domains formed by an active process of loop extrusion, in which Cohesin molecules translocate along the chromatin fiber in opposite directions and create a progressively larger “loop” until they encounter CTCF-binding boundary elements [1]. Although compartmentalization appears to be conserved across eukaryotes, TADs may reflect specific features of large mammalian or vertebrate genomes, since it has been shown that the structure of the *D. melanogaster* genome is not dependent on CTCF and other boundary proteins. However, it is unclear whether *D. melanogaster* is representative for insect species and/or invertebrates, considering its adaptation to extremely rapid development. In addition, it not known whether additional mechanisms contribute to shaping the 3D genome across species. With the exception of the *D. melanogaster* genome, relatively little is known about the organization of the genomes of invertebrate metazoan species. Characterization of genome architecture, its establishment during development, and the factors that drive its 3D organization in other model organisms has strong potential to generate insight into the fundamental processes driving genome organization and its functions across species. Because of the availability of a high-quality reference genome [3] and a wide range of functional genetics techniques, *T. castaneum* provides an excellent model system to investigate c[illegible]pects of 3D genome structure and th[illegible]unction within insects.

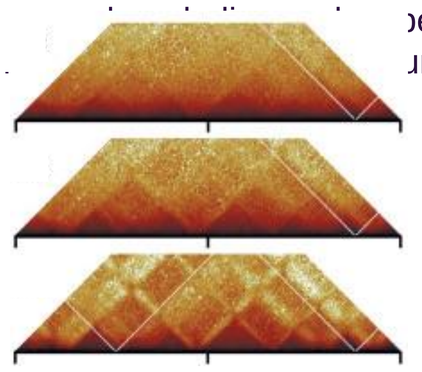

Fig. 1 Emergence of 3D genome architecture in D. Melanogaster *embryos (top to bottom: nuclear cycle 12, 13, and 14. Adapted from Hug et al. Cell 2017 [2].*

Primary Questions

- How is the *T. castaneum* genome organized in the 3D nuclear space?
- What drives 3D genome organization in *T. castaneum*?
- How do principles of 3D genome organization in *T. castaneum* compare to other species?
- How does the 3D genome modulate the regulation of genes involved in Wnt signalling?

Objectives

- Identify principles of 3D genome organization in *T. castaneum* and compare these across species.
- Integrate 3D genome analyses with other genomics datasets to characterize the regulation of genes involved in Wnt-mediated axis formation.

Workplan

1. **Developing Chromosome Conformation Capture (3C) protocols for *T. castaneum* embryos.** We will leverage our expertise in developing 3C methods to establish 3C in *T. castaneum* embryos, including the genome-wide Hi-C approach (moderate resolution) and the targeted Capture-C approach (high resolution).
2. **Performing Hi-C analysis across development stages.** We will generate Hi-C data in *T. castaneum* across developmental stages [stage 0: before initiation (before zygotic genome activation (ZGA)); stage 3: maturing GRN (full ZGA); stage 5: tissues specified].
3. **Investigating the drivers of genome organization with RNAi.** We will test the contribution of architectural proteins conserved in *T. castaneum* by performing Hi-C experiments upon RNAi mediated knockdown [CTCF, Cohesin, Zw5, GAGA Factor, and Su(Hw)].
4. **Integrating 3C data with RNA-seq and ATAC-seq data.** We will generate ATAC-seq data across developmental stages and integrate these with 3C and RNA-seq data (generated in P1) to characterize *cis*-regulatory landscapes in *T. castaneum* and the relationship between chromatin accessibility, 3D genome, and gene expression.
5. **Investigating the regulation of genes involved in Wnt-mediated axis formation.** We will generate high-resolution Capture-C data for selected genes of interest involved in Wnt-mediated axis formation and study their tissue-specific 3D regulation in embryos with a double abdomen phenotype and double head phenotype.

Synergy and collaborations

- **Collaborative Project 2: Reconstructing evolving GRNs**
  Contribute 3C and ATAC-seq data across *T. castaneum* developmental stages (**GB**); integrated analysis of *cis*-regulatory landscapes in anterior and posterior regions of *T. castaneum* embryos (**GB**).
- **Collaborative Project 3: Novel bioinformatics and genetic tools**
  Establishment of 3C in emerging model species (**GB, JR**); 3C data analysis pipelines; integration of RNA-seq, ATAC-seq, and 3C datasets (**GB, NP, TB**).

Technical innovations

- Establish Chromosome Conformation Capture in *T. castaneum*
- Integration of RNA-seq, ATAC-seq, and 3C data for understanding GRN dynamics and function

Specific qualifications

- Chromosome Conformation Capture (experiments and computational analysis)
- RNAi-mediated knock-down gene function
- Bioinformatic integration of RNA-seq, ATAC-seq, and 3C datasets

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References

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