Spatiotemporal Regulation of Neurogenic Networks by Opa and Oc

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BACKGROUND

- Early embryonic development begins with the conserved MZT where control shifts from maternal to zygotic by initiating germ layer formation and neurogenesis.
- In *Drosophila*, the second wave of gene expression regulation is driven by **Odd-paired (Opa)/Zic** and **Ocelliless (Oc)/Otx2**, which regulate key neural genes.
- *Opa* and *Oc* co-regulate transcription of early brain markers such as *empty* spiracles *(ems)* and twin of eyeless (*toy*), a conserved homolog of vertebrate Pax6, essential for neural precursor specification.
- In mice, a similar transcriptional pathway regulates early brain patterning paralleling the roles of Opa and Oc in *Drosophila*.

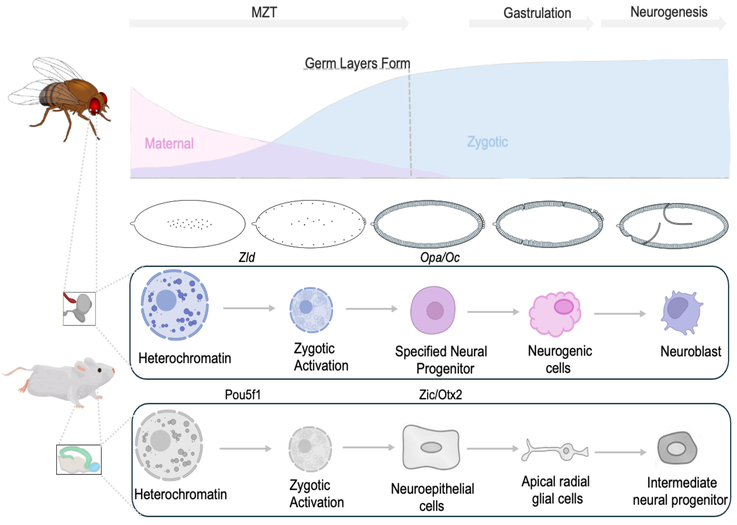

Fig.1 *Epigenetic Dynamics of Gene Regulation in Neurogenesis*

METHODS

- To visualize spatial gene expression in *Drosophila* embryos, we made fly crosses, collected embryos on apple juice agar plates and fixed them at relevant developmental stages.
- We then conducted FISH using gene-specific fluorescent probes to label mRNA transcripts of interest.
- Labeled embryos were mounted and imaged using super-resolution confocal microscope, allowing us to detect gene expression patterns at single-cell resolution during early embryonic development.

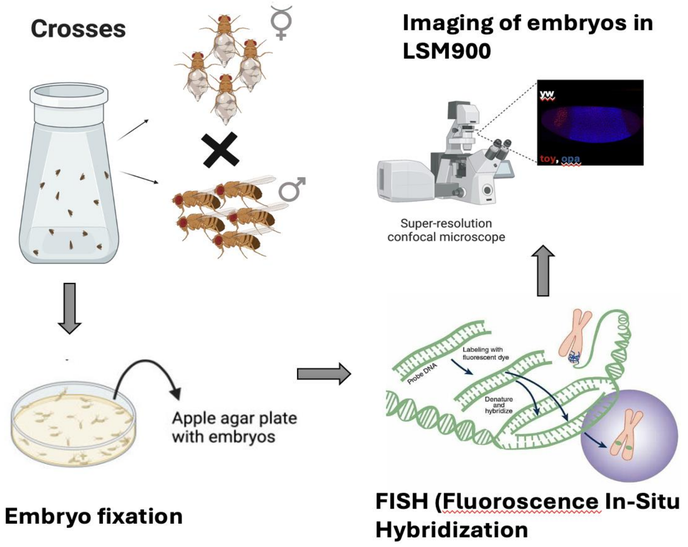

Fig.2 *Embryonic gene expression visualized by FISH and super-resolution confocal microscopy*

RESULTS

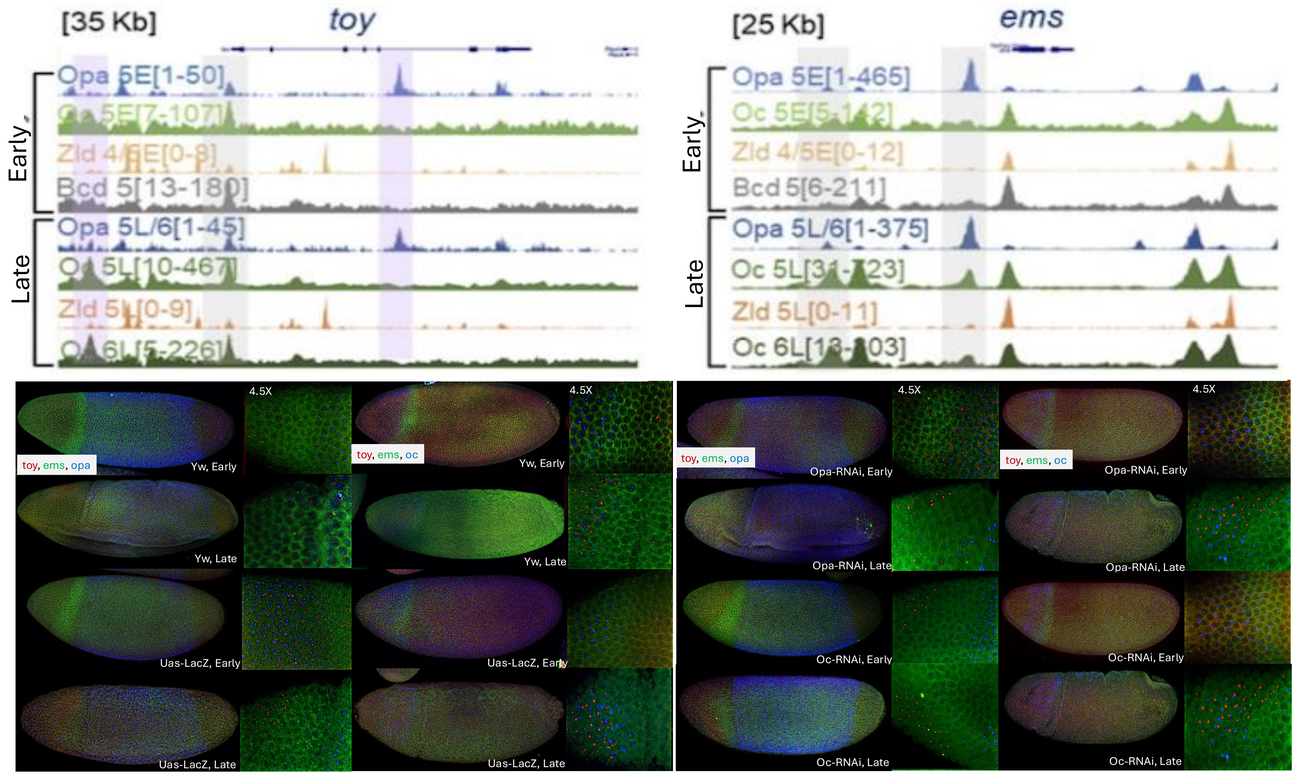

Fig.3 *Combined ChIP-seq and RNAi knockdown reveal Opa/Oc-dependent control of toy and ems.*

DISCUSSION/FUTURE WORK

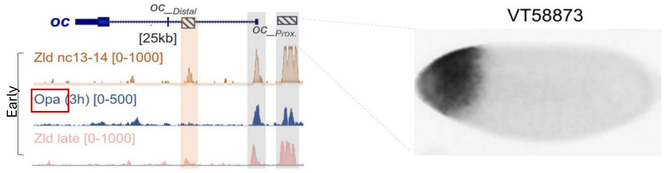

Fig.3 *Enhancer activity reflects regulation of Oc by Opa*

- CHIP-Seq data of *toy* and *ems* shows, Opa and Oc bind upstream regulatory regions of both, indicating direct transcriptional control.
- By Knockdown of Opa and Oc leads to change in *toy* and *ems* expression in embryos, demonstrating their essential role in anterior gene activation.
- We are also generating CRISPR/Cas9 lines (*ems.CRISPR* and *toy.CRISPR*) to study the function of brain-specific enhancers regulated by Opa and Oc.
- We use super-resolution live imaging to track nascent transcripts and uncover single-cell expression dynamics of brain genes during neurogenesis.
- This integrated approach will help define a transcriptional network model for early brain specification and explore how its disruption may contribute to neurodevelopmental disorders like ADHD.

REFERENCES

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