Examining the Role of Enhancers in Regulating Nuclear-Content-to-Cytoplasm Ratio-Dependent Genes in *Drosophila* Embryos

Anthony Senouillet (SEAS Class of 2026)
Advised by Shufan Lin & Dr. Bomyi Lim | Funded by the CURF Grants for Faculty Mentoring Undergraduate Research
Department of Chemical and Biomolecular Engineering

Background

Cell cycle timings and zygotic gene activation (ZGA) are controlled by the ratio of nuclear content to cytoplasmic volume (N/C ratio).[1] Some genes, called N/C ratio-dependent genes, are directly regulated by the N/C ratio via one of three overlapping modes: changing the timing of nascent RNA output (changes in cell cycle duration), the rate at which transcription occurs (kinetics of expression), or the probability of transcription initiation.[2]

In eukaryotes, genes are regulated by noncoding regions of DNA, such as enhancers and promoters. These sequences bind to proteins called transcription factors (TFs) that in turn regulate whether a gene is expressed and how. While ZGA is controlled by the N/C ratio, whether this regulation acts through enhancers remains unexplored.[3]

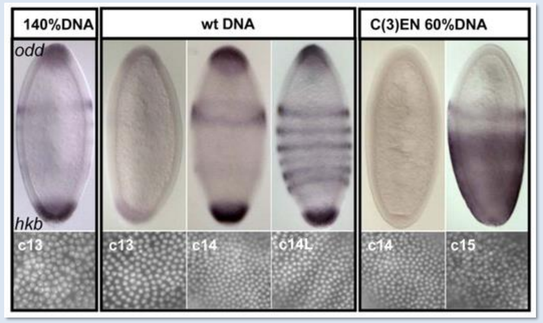

Figure 1: N/C ratio regulates gene activation

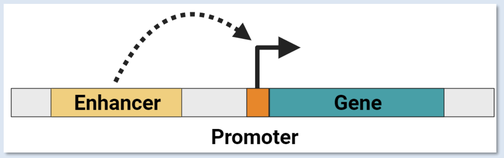

Figure 2: Enhancer-promoter interactions are facilitated by TFs binding to both sites

Goals & Experimental Questions

1. Identify more N/C ratio-dependent genes in *Drosophila* embryos and their respective modes of regulation
2. How does expression vary temporally and spatially for different N/C ratio-dependent genes in wild type (WT), haploid (*ssm*), and rapidly-dividing diploid (*chk*)?
3. Do enhancers take charge of sensing changes in the N/C ratio?
4. What mechanism allows enhancers to sense changes in the N/C ratio?

Preliminary Results

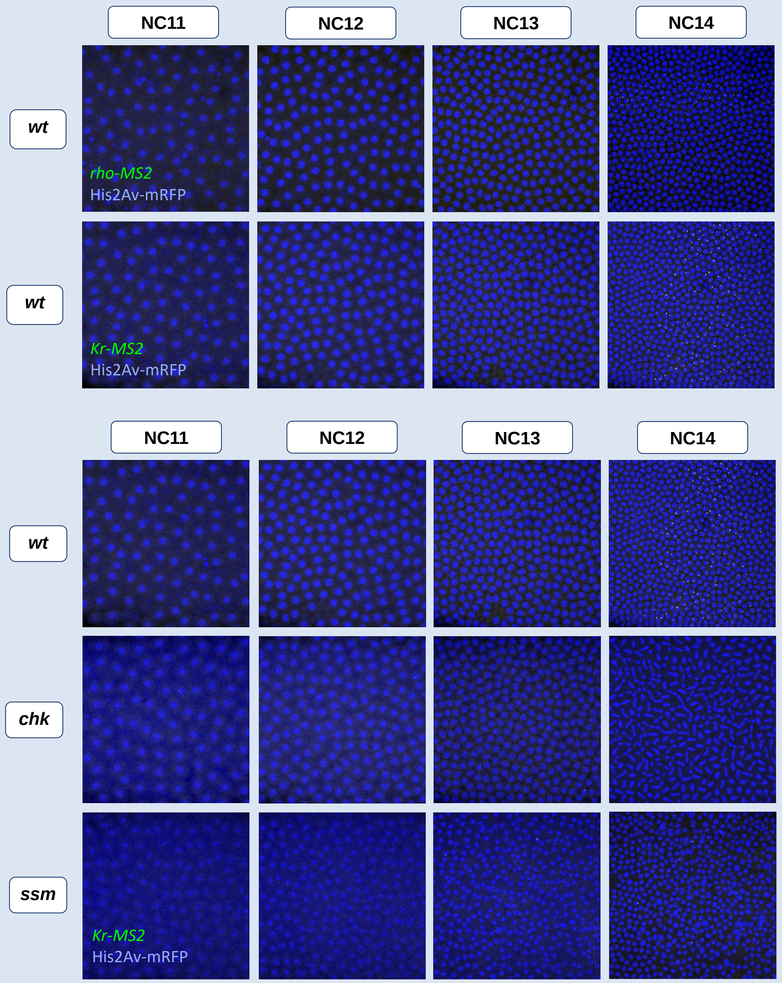

Figure 3: Demonstration of N/C ratio increase between nuclear cycles and the expression domains of *rho* and *Kr*

Figure 4: *Kr*-MS2 expression showing N/C ratio-dependent properties

Discussion & Proceeding Forward

As progress on this project continues, the main goal is to continue collecting images of embryos with target genes tagged using the MS2/MCP reporter system. As imaging and analysis are performed, another goal is to study expression at the endogenous locus rather than utilizing a reporter system to produce more accurate results. This entails inserting the MS2 repeats adjacent to the target gene rather than in the regulatory region, which will provide a direct quantification of expression rather than indirectly assaying the gene via a reporter. Upon analyzing collected data, we hope to uncover the mechanism behind the enhancer's ability to sense and react to changes in the N/C ratio in N/C ratio-dependent genes.

Methods

- Spatial and temporal expression of target genes visualized by MS2/MCP visualization technique[4] (Figure 5A)
- Generate haploid embryos through genetic crosses (Figure 5B)
- Procure wild-type diploid embryos, haploid embryos with the *sesame* (Hira185b, or *ssm*) mutation, and rapidly-dividing diploid embryos with the *checkpoint* (*chk*) mutation[5,6,7]
- Mount the *Drosophila* embryos in an optimal orientation for imaging using a standard mounting protocol (Figure 5C)
- Images are taken using a confocal laser-scanning microscope, and we use MATLAB to trace the expression inside each nucleus (Figure 5D)

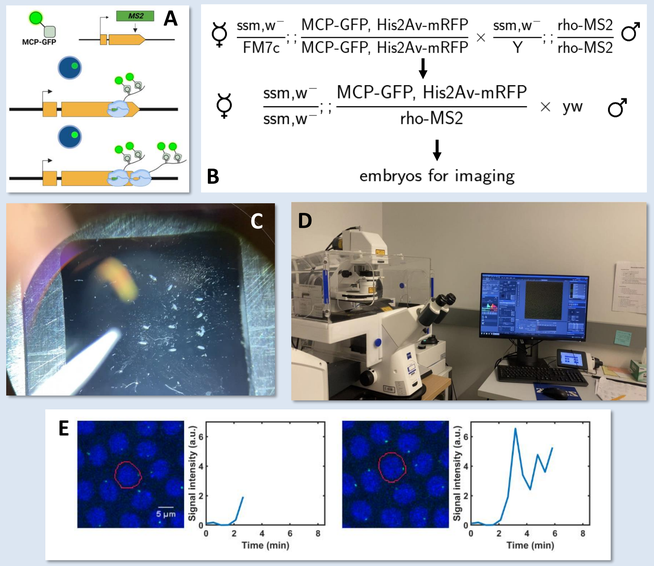

Figure 5: Experimental setup

References & Acknowledgments

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[2] See Footnote 1
[3] See Footnote 1
[4] Hoppe, C., & Ashe, H. L. (2021). Live imaging and quantitation of nascent transcription using the MS2/MCP system in the Drosophila embryo. STAR Protocols, 2(1), 100379. https://doi.org/10.1016/j.xpro.2021.100379
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