Building (Cellular) Barriers: Identifying cellularization dynamics involving somatic X chromosome eliminations in Bradysia coprophila development

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Embryonic cellularization is an early developmental process common in insects where early nuclear divisions are characterized by occurring syncytially, in the absence of plasma membranes and cytoskeletal structures, until a specific time where those cell structures form around the syncytial nuclei and create discrete cells. The exact timing of cellularization in the dark-winged fungus gnat, *Bradysia coprophila*, was unclear with estimates ranging from nuclear division cycles ten through twelve. This timing also suggested that the somatic X chromosome eliminations that *B. coprophila* embryos undergo during cycles seven through nine occur syncytially.

I demonstrated using antibody markers for f-actin and the integral membrane protein neurotactin that structures associated with cellularization are present as early as cycle six. I found that condensed DNA buds consistent with previous descriptions of the eliminated chromosomes lack a nuclear envelope-derived vesicle structure unlike the micronuclei formed by chromosome eliminations in other systems. Additionally, based on the visualized actin structures through mitosis, I identified a lack of actin enrichment that characterizes the contractile ring formation during cytokinesis and visualized actin structure morphology after elimination cycles that suggest that a breakdown in these structures enable the elimination of the X chromosome during elimination cycles.

I also analyzed RNA-seq data of male and female embryos 0-4 hours after deposition and 4-8 hours after deposition and used differential expression analysis to identify putative gene candidates that may be involved in cellularization or chromosome eliminations as well as used reciprocal BLAST searches between the *Drosophila* and *Bradysia* genomes, where I found a lack of *Bradysia* orthologs for multiple genes implicated in Drosophila cellularization. Finally, I successfully developed an adult injection protocol and trialed implementing direct parental injection based CRISPR-Cas9 gene editing in the system.

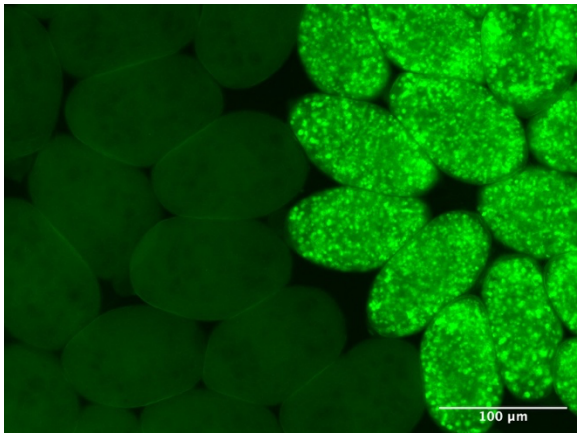

Figure 1. *Drosophila* yolk peptide fused green-fluorescent protein present in developing *Bradysia* oocytes after adult injections, suggesting successful uptake of yolk-tagged proteins into developing

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