Investigation of the Double-Anteriorized Egg Phenotype as a Consequence of eRpL22-like Knock Out in *Drosophila melanogaster*

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Abstract & Hypothesis

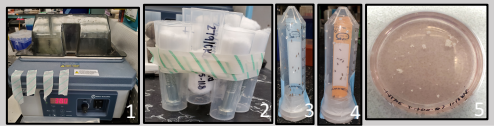
eRpL22-like and eRpL22 are essential for development. eRpL22 is ubiquitously expressed but eRpL22-like has a tissue-specific pattern of expression. Although each paralogue is an essential component of the ribosome itself in fruit flies, it is also possible that these proteins function in pathways apart from translation. Genetic depletion of eRpL22-like generates a host of phenotypes in the developing egg within the ovaries. One particularly striking phenotype we named "neopolitan" is defined as a double-anteriorized egg. What pathways are disrupted by eRpL22-like knock-out to create this phenotype are unknown. To further investigate this phenotype, we used heat shock to induce a flippase to knock out the *eRpL22-like* gene at different times in fly development to score fertility changes and egg phenotypes in eRpL22-like mutants compared to heat-shocked wild-type controls. Several phenotypes were observed including one named "turkducken", dorsal appendage mutant phenotypes, and eggs with dual micropyles. These phenotypes all suggest a range of egg polarity defects. Immunohistochemistry of pre-egg stage ovarioles revealed a spectrum of egg-chamber and morphology defects prior to the stage at which a "neopolitan" egg phenotype would form.

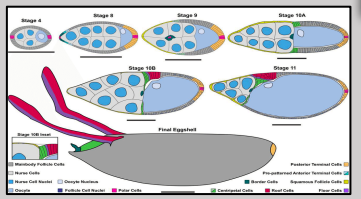
eRpL22-like has an essential role during early stages of oogenesis and in specifying egg polarity.

- Preliminary data shows knock out of eRpL22-like in early development results in severe fertility defects, including double-anteriorized eggs
- We used a heat shock to induce flippase to knock out the *eRpL22-like* gene at different times in fly development to score fertility changes and egg phenotypes in eRpL22-like mutants compared to heat-shocked wild-type controls

Experimental Methods

The conditional knockout of eRpL22-like occurs due to heat-shock (HTS) flippase activity. The chosen conditions for this experiment were 38 C for thirty minutes. These conditions were carefully chosen for this experiment, as the goal is to stunt development of the egg in the ovary, but not to kill the adult flies. Thirty minutes of heat shock allows for the activation of the flippase, which excises the gene eRpL22-like. Eight days after heat shock, the adults were then sorted and put into collection cages, as seen in images 3 and 4. Grape juice agar plates were made, and then labelled and sealed with parafilm after being covered with a confocal tube lined with holes to allow air flow. After an average of twenty-four hours, these cages were disassembled and the grape juice plates (image 9) were analyzed and the eggs laid were scored for both number and phenotype, as seen in images 1, 2, 3, and 4. Lastly, heat shocked female flies were dissected and an immunohistochemistry experiment was performed on ovary tissue from both wild-type (WT) and 279/CK) genotypes.

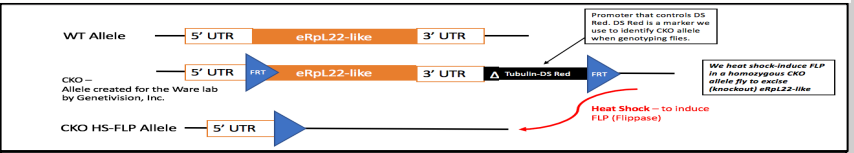


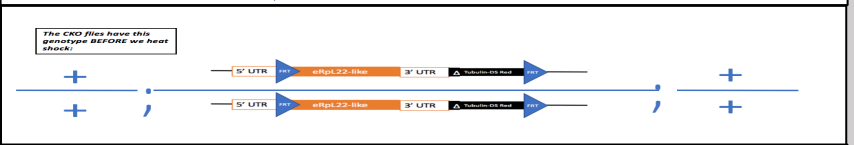


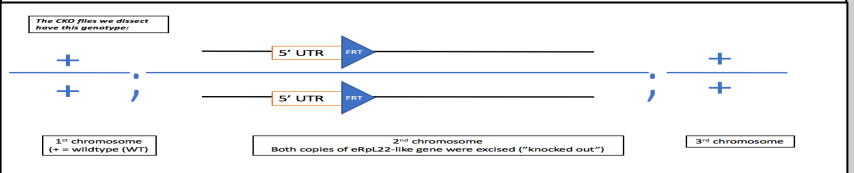
Adapted from: Duhart, J. C., Parsons, T. T., & Raftery, L. A. (2017). The repertoire of epithelial morphogenesis on display: Progressive elaboration of Drosophila egg structure. *Mechanisms of Development*, *148*, 18-38.

Figure 1. Heat shock apparatus
Figure 2. Fly set-up for heat shock experiment
Figure 3. WT flies in egg lay apparatus
Figure 4. CKO flies in egg lay apparatus
Figure 5. CKO egg lay apparatus ready to be scored

CKO Mutant Origin







Egg Lay Phenotype Analysis

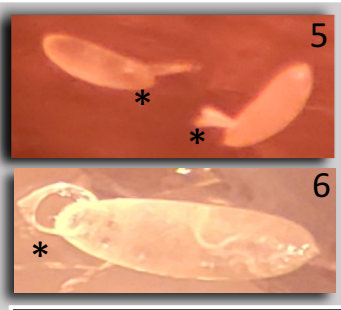
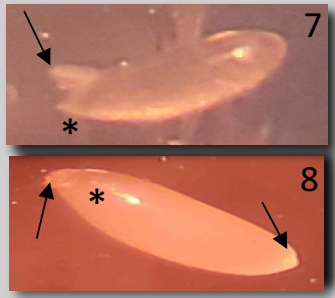


Figure 5. WT HTS (Heat shock) - right
Translucent WT HTS– left
Figure 6. Translucent CKO HTS with fused dorsal appendages (breathing antennae-like structures)
Figure 7. Translucent CKO HTS with short dorsal appendages
Figure 8. CKO HTS– Double anteriorized with two micropyles (pointed tip between the two dorsal appendages)

* Indicates the anterior end of the egg



Results

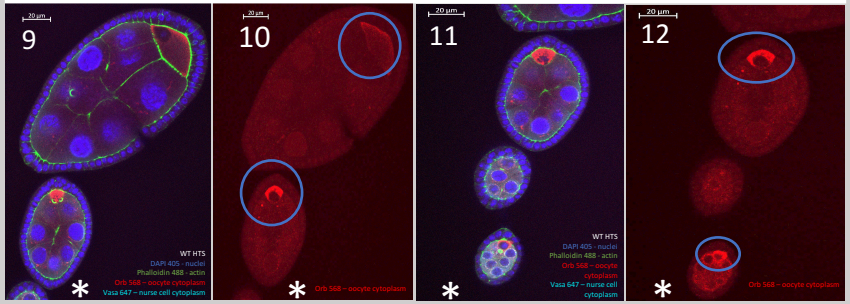


Figure 9. Concentration of Orb 568 at posterior ends of ovarioles
Figure 10. Red-channel orb only is the same slice as image 9, showing the red channel alone to emphasize expression pattern
Figure 11. Concentration of Orb 568 at posterior ends of ovarioles
Figure 12. Red-channel orb only is the same slice as image 11, showing the red channel alone to emphasize expression pattern
Orb marks position of the oocyte

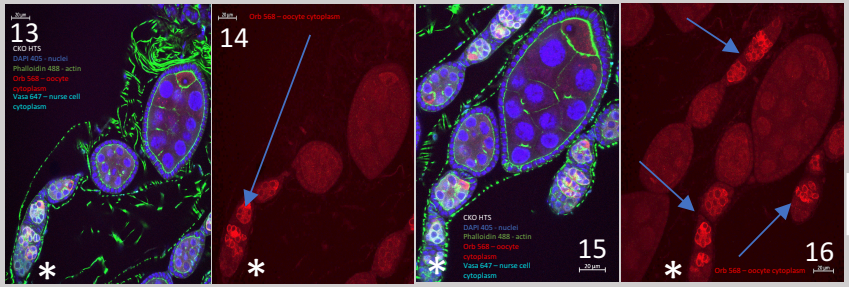
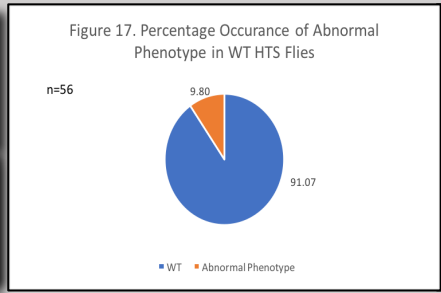
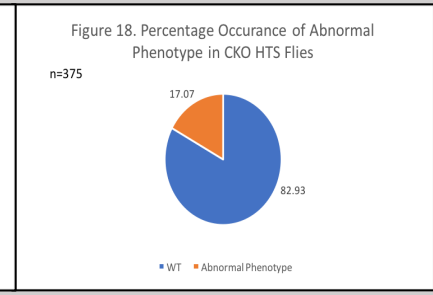


Figure 13. No concentration of orb at posterior ends seen, rather spread over misshaped cells
Figure 14. Red-channel orb only image is the same slice as image 13, showing the red channel alone to emphasize expression pattern
Figure 15. No concentration at posterior ends seen, rather spread over misshaped cells
Figure 16. Red-channel orb only image is the same slice as image 15, showing the red channel alone to emphasize expression pattern
* Indicates the anterior-most egg chamber for WT and CKO HTS sets of images

Conclusions





Abnormal Phenotypes: translucent, turducken (translucent casing with white egg inside), short dorsal appendages, uneven dorsal appendages, and absent dorsal appendages

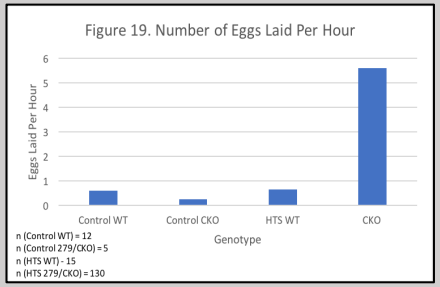


Figure 19 demonstrates that CKO flies have increased egg laying, however, because they have laid more eggs, indicating increased fertility, does not mean the viability is the same as WT flies. Many of these eggs were defective, as having a missing or malformed breathing apparatus , which is not compatible with life. It may appear as if more eggs were laid, but this does not mean that CKO flies produce many, if any, viable eggs, or eggs that survive development into adulthood.

- Knock-out of eRpL22-like in the adult ovary causes less severe polarity phenotypes than knockout in eggs alone
- The adult ovary phenotypes reveal subtle changes in expression of oocyte markers and changes in later-stage egg chamber shape
- Reduction of oocyte marker orb expression suggests loss of oocyte fate in the posterior of the egg – a potential precursor to the neopolitan double anteriorized egg phenotype

Future directions include:
1) Immunohistochemistry using the oocyte marker orb to study polariziation defects
2) Repitition of egg laying experiments with heat shock conditions to collect double anteriorized eggs
3) Manipulating heat shock conditions in first and third instar larvae to attempt greater success in inducing double anteriorized egg phenotype
4) Investigation of egg lethality and egg development into larvae, pupae, and adults under heat shock conditions

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