The Presence of Histone Variant H2A.Z in *Drosophila melanogaster* Egg Chambers

Sarah Hileman and John Condie

Department of Biology, Fort Lewis College, Durango, CO

ABSTRACT

This study explores the relationship between *Drosophila melanogaster* egg development and the appearance of histone variant H2A.Z in the developing egg chambers during stages 1-13. Female wild type fruit flies were dissected and egg chambers in various stages were extracted from the ovaries. The egg chambers were stained by immunofluorescence using antibodies for H2A.Z. Although fluorescence was present in the final egg chamber slides, there was no indication of obvious locations within individual areas of the chambers to decisively show H2A.Z formation. There was a difference in fluorescence between egg chambers incubated with both 1° and 2° antibodies and egg chambers stained with only 2° antibodies, showing that egg chambers incubated with both antibodies fluoresced more. These results are still inconclusive as to when H2A.Z first begins to develop in egg chambers, but literature suggests its appearance is after stage 10.

INTRODUCTION

- Histones are a type of chromosomal protein that associate with DNA and can act to regulate transcriptional activity. Histone H2A is a type of histone that plays a role in chromatin remodeling, cellular homeostasis, and interaction with histone H1. H2A.Z is a variant of this histone.

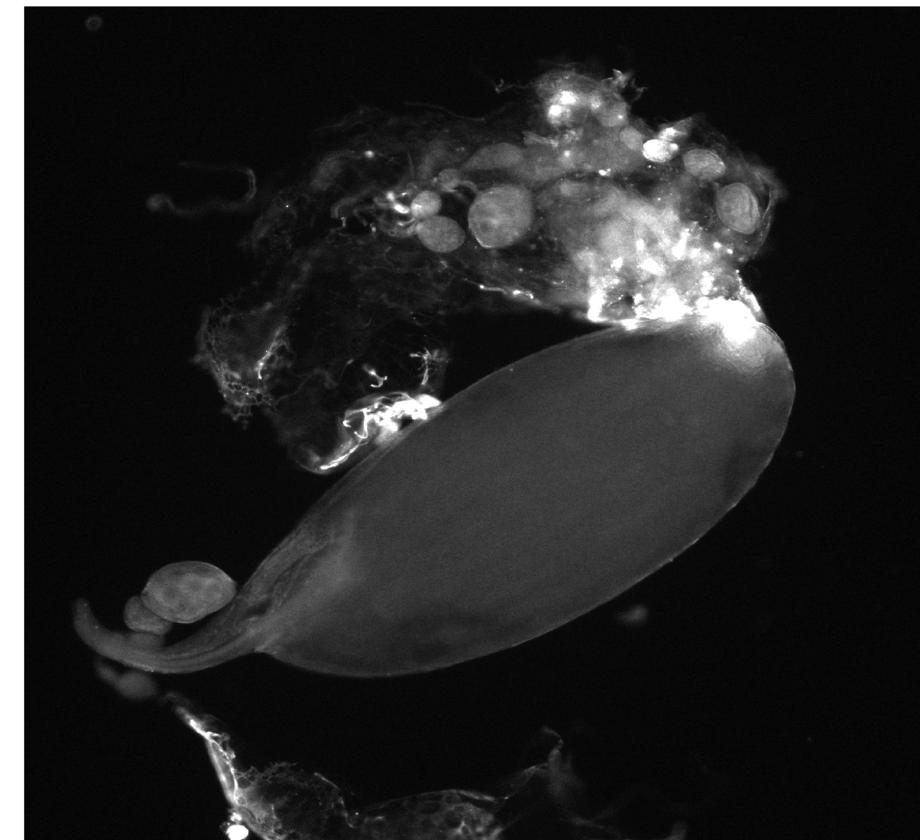
- Although histones have been studied for years, there are still gaps of understanding concerning their roles in gene expression. *Drosophila* are a good model to study histones because they reproduce frequently and their developmental stages are easy to observe.

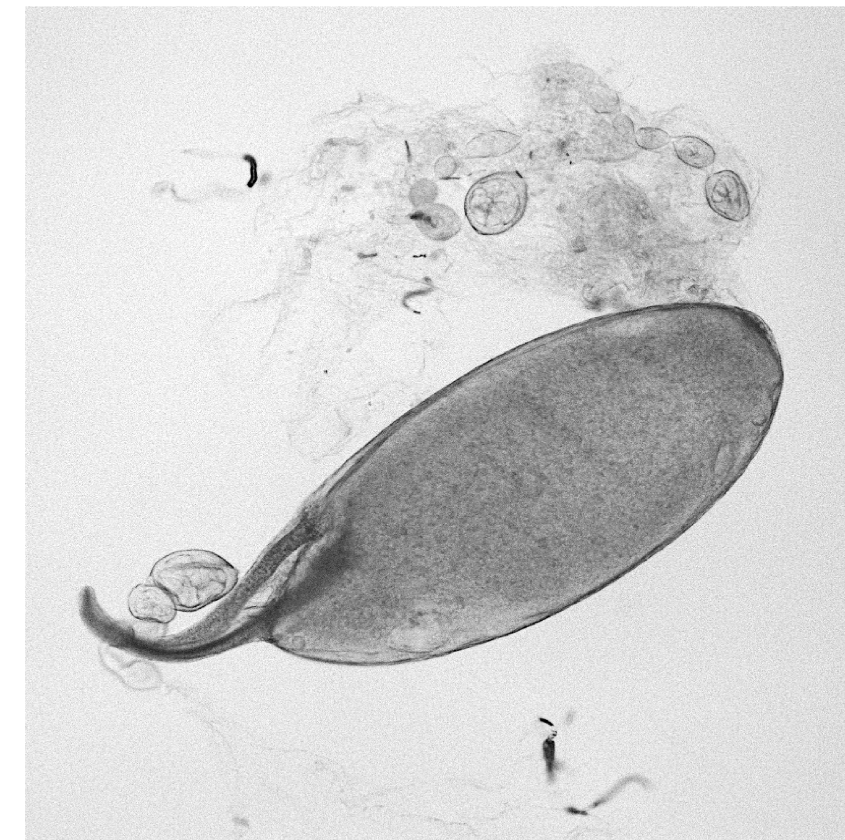
- This study examined the presence of histone variant H2A.Z as it is introduced in the developing egg of *Drosophila*. Our objective was to track the onset of accumulation of H2A.Z in egg chambers during development.

METHODS

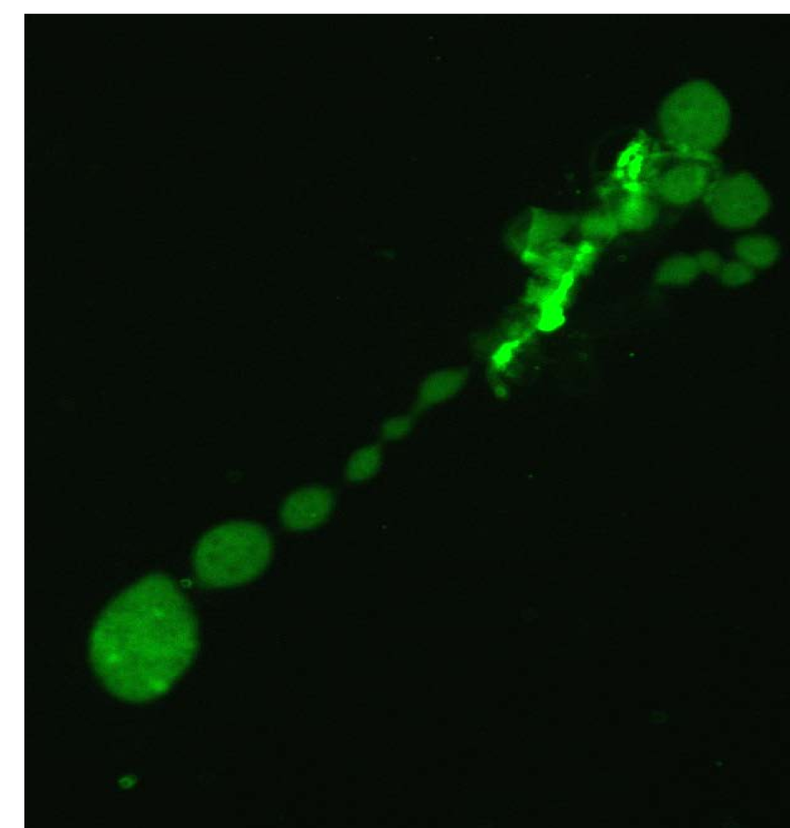
- Female wild-type *D. melanogaster* flies were dissected in 1X PBS, and ovaries were dissected to expose egg chambers.
- Sets of egg chambers from 6-10 ovaries were washed 3x for 10 min. at $20^{o}C$ in a 500µL portion of 100mL sucrose-fixing solution titrated to a pH of 7.2.
- Egg chambers were then washed for 2.5 hrs. in a 1% Triton solution, again for 10 min. in a 0.5% Triton solution, and then blocked 3x in a 0.3% BSA solution for 10 min.
- The egg chambers were washed at room temperature for 2.5 hours or incubated in a cold room over night in 500 µL with 0.5 µL 1° sheep H2A.Z antibody and washed again 3x for 10 min. in a 0.3% BSA solution*.
- Egg chambers were washed at room temperature for 2.5 hours in 400 µL 0.3% BSA solution and 10 µL 2° anti-sheep IgG conjugated to FTIC and then 3x for 10 min. with 0.3% BSA solution.
- The chambers were finally mounted on slides with an 80% Glycerol solution and observed at 100x magnification for evidence of florescence, which would indicate the presence of H2A.Z.

*In one trial the egg chambers were not washed with this 1° antibody.





Figures 1. Stage 14 egg with early-stage egg chambers under fluorescence (L) and bright field (R) microscopy with a 10x ocular lens. Divisions within the earlier-stage egg chambers are visible in both pictures with and without fluorescence. The exposure on the left shows where the 2° antibody had bound with the 1° antibody. These areas in which the exposure is white depict tubules filled with the antibodies. This indicates that the one or both of the antibodies became trapped in air pockets in these tubules.



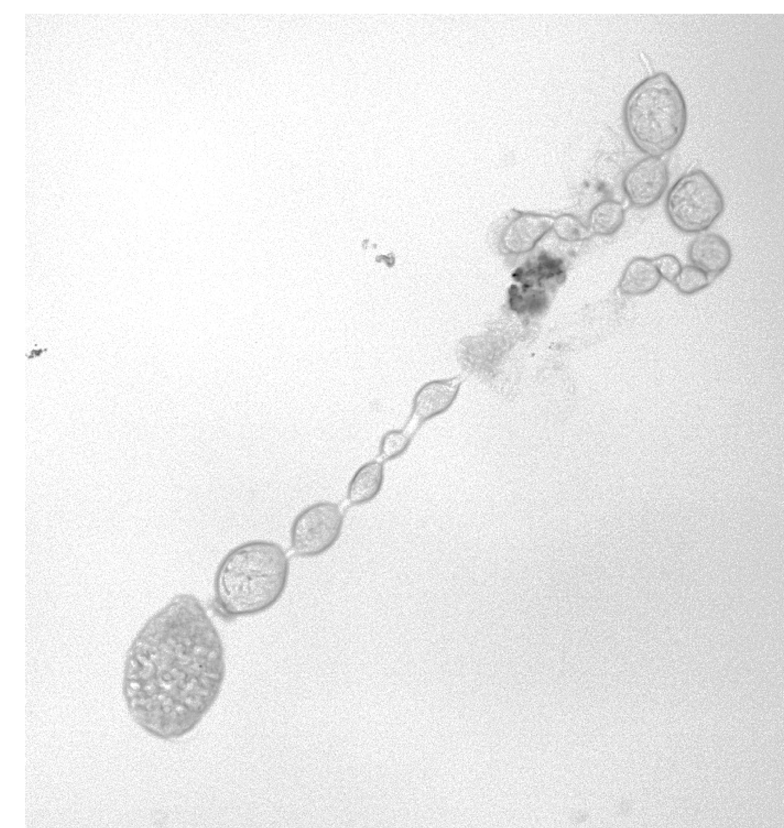
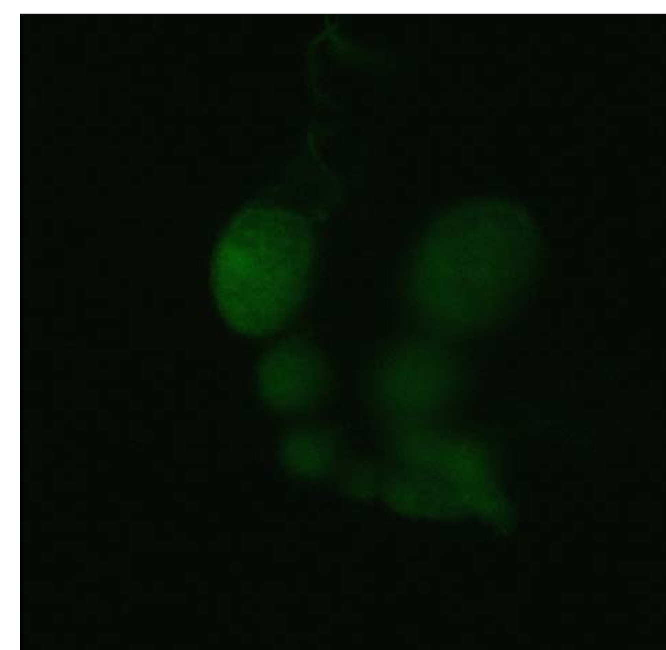


Figure 2. Various stages of egg chamber growth before stage 14 under fluorescence (L) and bright field (R) microscopy with a 10x ocular lens. Progressive stages of egg chamber formation are visible. As in Figure 1, the bright fluorescence indicates probable trapping of 1° antibody in tubules, and not excessive presence of H2A.Z. There is no clear indication of H2A.Z spotting within egg chambers.



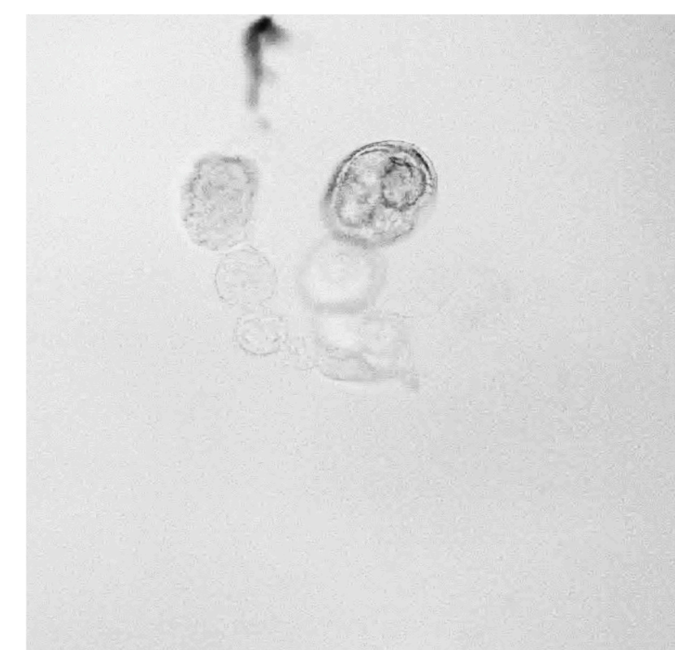


Figure 3. Egg chambers stained with 2^{0} antibody only under fluorescence (L) and bright field (R) microscopy with a 10x ocular lens. These egg chambers did not fluoresce as brightly as egg chambers washed with both antibodies. Although much of the green coloration seen under magnification is background color, this discovery indicates that there was some possible staining resulting from the presence of H2A.Z in most egg chamber stages when the 1° antibody was also employed, although specific points of fluorescence were not observed.

DISCUSSION

Our methods did not produce definitive results, indicating some problem with our protocols. We speculate that the assay may have been compromised for the following reasons:

1. Although the 1° antibody was reported to cross-react with *Drosophila* H2A.Z, it may not have done so in this experiment.
2. The absence of bright fluorescence within egg chambers may indicate that the outside of the egg chambers were not penetrated by the washes. Therefore any H2A.Z within the egg chambers was not marked by fluorescence due to lack of binding of the 1° antibody.
3. The possibility exists that the egg chambers do not have H2A.Z in large amounts at any stage during development. This situation would indicate that this histone variant is not implicated in the development of the egg, and that perhaps it is more crucial to the maturing fly's development.

Results of this study suggest that H2A.Z assays should focus on penetration of the outer cells of the egg chamber. This could be done by the following:

1. The concentrations of antibodies may not have been ideal for the washes that were used to locate any H2A.Z. Different antibody concentrations (especially with elevated levels of 1°) should be examined.
2. Alternative fixing and staining methods could be further researched and attempted.
3. Flies no more than three days old could be used in order to extract younger and more plentiful egg chambers.

According to literature, there are some levels of transfer to the egg of histone mRNA up to stage 10. Because of the major accumulation of histone mRNA later in oogenesis (after stage 10), the presence of histone variant H2A.Z should appear in larger quantities during stages 11-14. This suggests H2A.Z may not be available to the egg in major quantities until after stage 10, further implicating that H2A.Z does not perhaps play an important role in gene expression of early egg development.

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