Direct proportionality of acetylation of microtubules in sperm tails to their adhesivity

Ariel Eaton

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INTRODUCTION

The hypothesis for this experiment is that the degree of acetylation of microtubules in sperm tails is directly proportional to their adhesivity for each other. The immunofluorescent staining done in this experiment affected the DNA, tubulin, and acetylated tubulin in sea urchin sperm and egg, gastrulas, blastulas, and a zygote in first mitosis. The experiment's hypothesis is concerned with the staining results from the tails of the sea urchin sperm.

The axoneme of a sperm's tail is constructed of microtubules, which polymerize from α-tubulin and β-tubulin heterodimers (Satir & Christensen 2007). Acetylated tubulin has acetyl groups attached to its α-tubulin subunits and this reduces the likeliness of depolymerization of the microtubule, therefore making the sperm's microtubules more stable (Professor Morris, personal communication).

The immunofluorescent staining isolates the acetylated tubulin and tublin with the corresponding stain, AF 546 and FITC, respectively. The hypothesis was based off the acetylated tubulin and tubulin images. The staining shows the acetylated tubulin organized in large, tangled clusters, which could be an indicator of adhesiveness between the individual acetylated tubulin.

MATERIALS & METHODS

The materials and methods for this experiment were provided by Immunofluorescent staining of SU embryos - MeOH fixation (Morris, 2012, in consultation with Hensen and Shuster), with exceptions. The samples were prepared, fixed, and permeabilized before beginning the experiment, so the first step was to rehydrate the samples with saline solution; the samples I analyzed were fixed sea urchin sperm and eggs. When the experiment was completed and the coverslips were prepared, Professor Morris aided in the digital imaging analysis.

From the data, the qualitative images were turned into quantitative data. The methods for creating a three-cover overlay of the three fluorescent images were provided by How to create three-color overlays of your fluorescent pictures using Adobe Photoshop, (Morris, 2012). After creating the three-color overlay, I used that image in addition to the tubulin and acetylated tubulin images and made observations that then led me to forming my hypothesis. To quantify my data for the experiment, I isolate a section of the images and counted the numbers of pairs in contact with one another. After, I created a bar graph to present my findings.

RESULTS

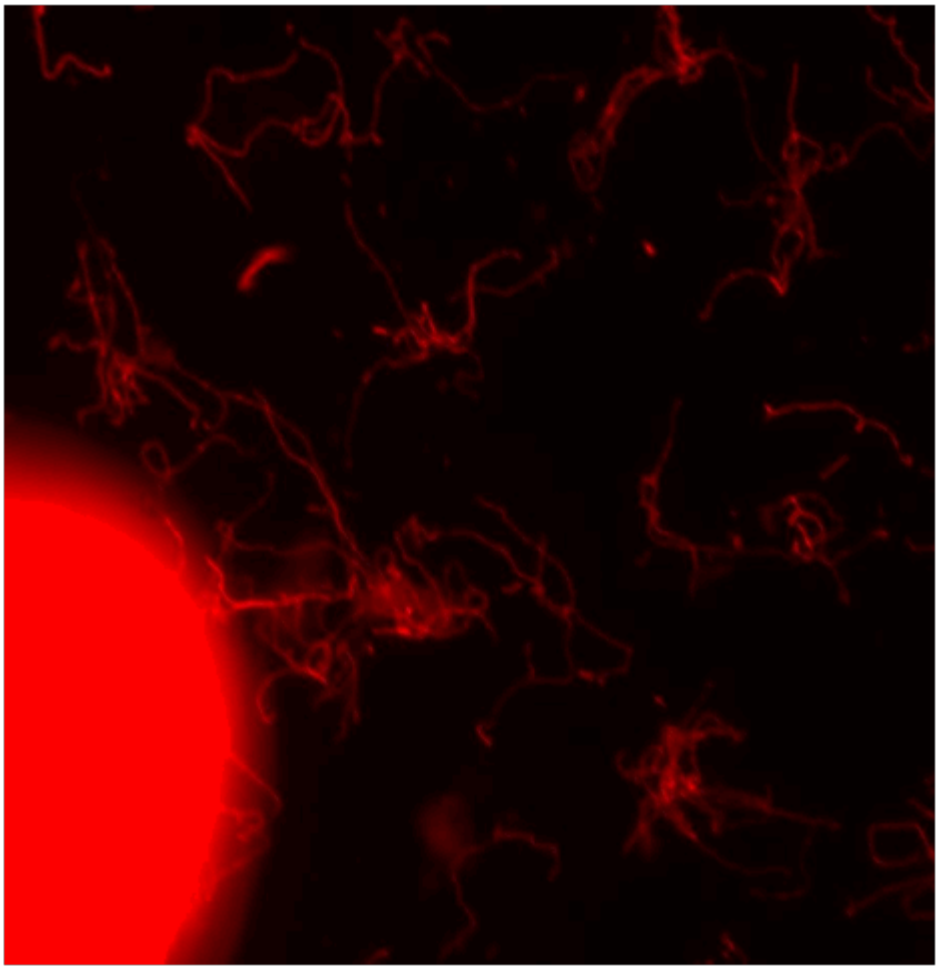

Figure 1: Distribution of acetylated tubulin (stained with AF 546 to emit red) in sea urchin sperm tails and in the bottom, left corner, a portion of a sea urchin egg.

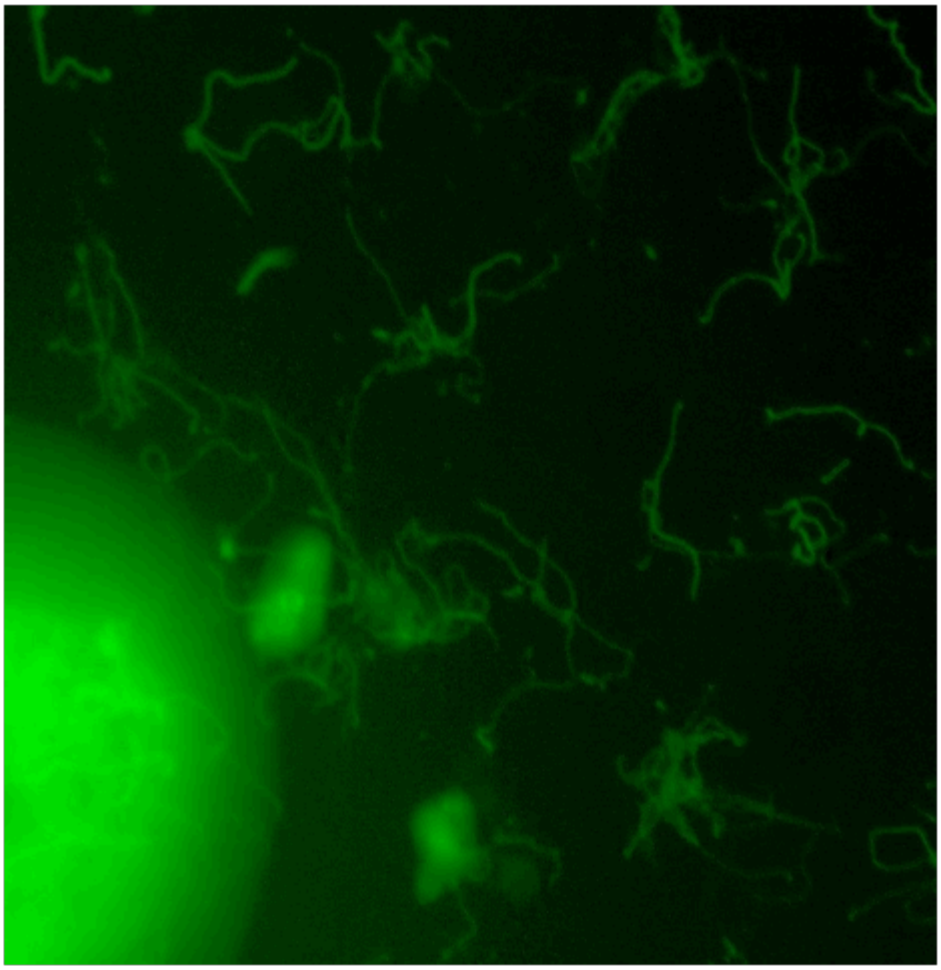

Figure 2: Distribution of tubulin (stained with FITC to emit green) in sea urchin sperm tails and in the bottom, left corner, a portion of a sea urchin egg.

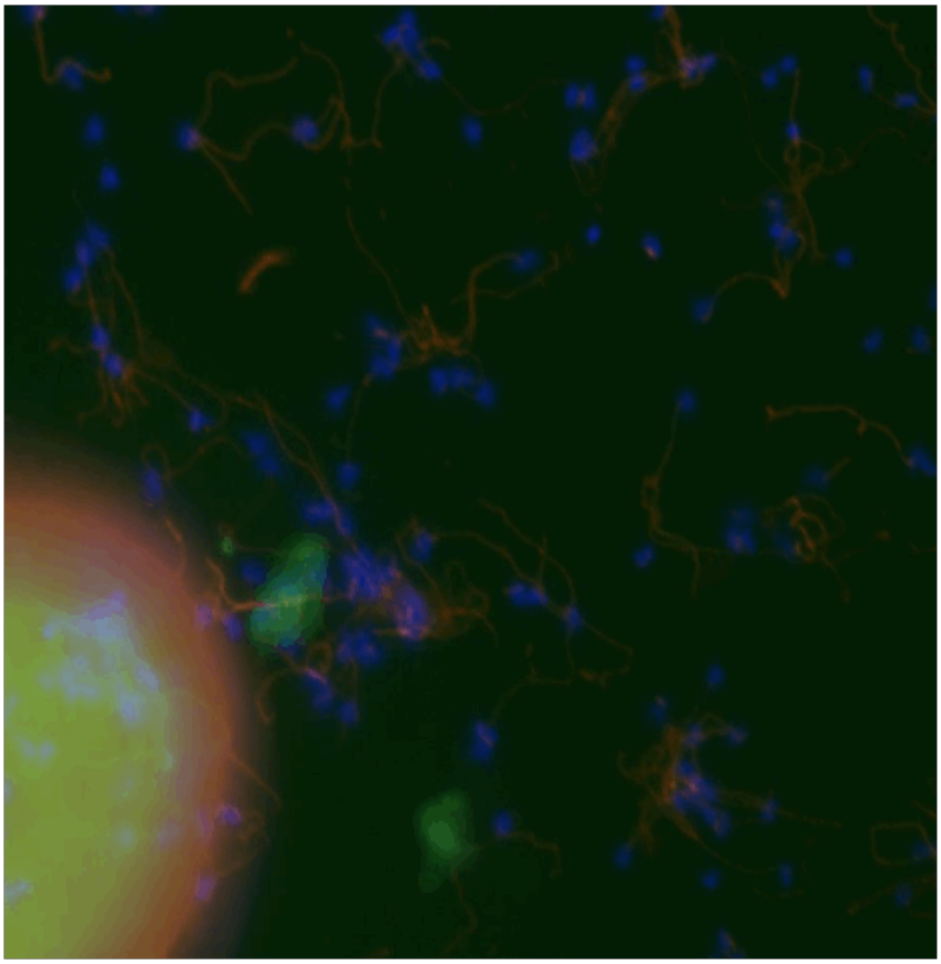

Figure 3: Distribution of DNA, located in the sea urchin sperm heads, acetylated tubulin, and tubulin, both located in sea urchin sperm tails.

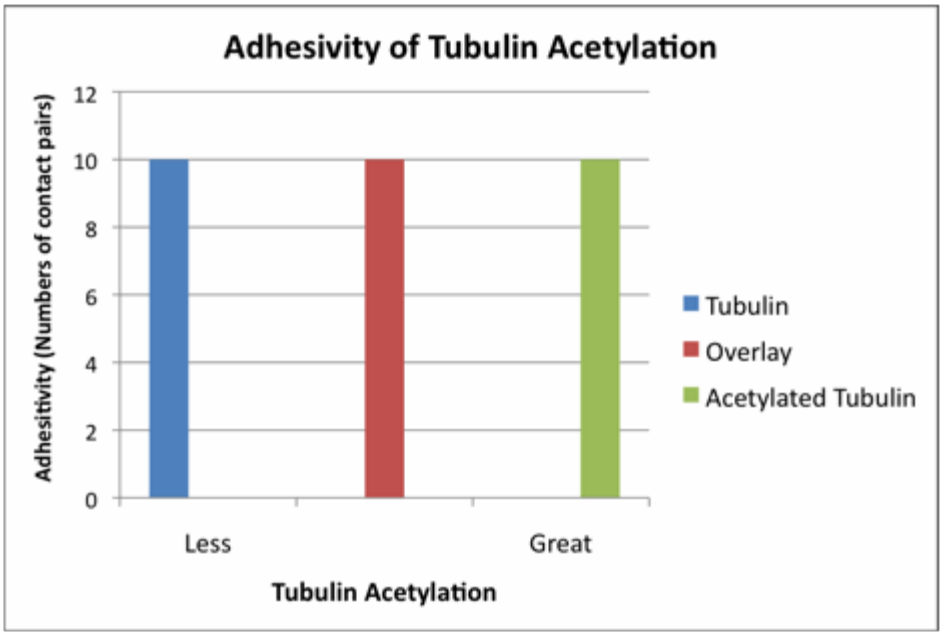

Figure 4: A bar graph showing adhesivity in tubulin acetylation with the numbers of pairs between single tubulin and single acetylated tubulin.

The results of the experiment very clearly show the sea urchin sperm and eggs. The number of pairs in contact between tubulin and acetylated tubulin are all equal, ten pairs. Figure 1 shows the acetylated tubulin in the sperm tails and to explain the clustering of the acetylated tubulin is higher adhesivity between each other. Figure 2 shows the tubulin, which is also entangled. Figure 3 shows the overlay between tubulin (green) and acetylated tubulin (red); where the color is tinted yellow is where the tubulin and acetylated tubulin overlay one another. Figure 4 is the quantification of the data and is a bar graph showing the adhesivity for Figures 1, 2, and 3.

DISCUSSION

The results from the experiment do not support my hypothesis. The bar graph created to quantify my data very obviously shows my hypothesis is not supported, as there are ten pairs of tubulin and acetylated tubulin in contact, corresponding to each image of my results. The adhesivity of the acetylated tubulin and tubulin could be determined; however it would not be possible with the time constraint in lab. While doing this experiment with my hypothesis, I was also considering the idea that maybe if the acetylated tubulin is more adhesive than tubulin, it would allow the sperm to better travel during fertilization. Unfortunately, the data from the experiment cannot determine this idea.

In the three-image series included in the results section, the tubulin and acetylated tubulin is very tangled and led me to develop my hypothesis on considering adhesivity, but the most likely cause for the tubulin entanglement is due to the experiment itself. Because the sea urchin sperm are so small, the many washes from the experiment would have moved the sperm along the cover slip, therefore causing the clustering which remained when the coverslip was mounted on the slide. If the sperm could be spread out, I believe the clustering would not occur. In conclusion however, the

experiment was a success. Although my hypothesis was no supported by the data, I was still able to learn a great deal through this lab, the technique of immunofluorescent staining and turning qualitative data into quantitative data.

References

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Satir, P., Christensen, T. (2007). Overview of Structure and Function of Mammalian Cilia.

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