Direct correlation between the concentration of microtubules in the basal body and concentration of acetylated tubulin in the associated cilium of cells in sea urchin embryos

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Independent Research Project Report

Bio254 – Developmental Biology

May 3, 2012

Introduction

Cilia are microtubule based membrane-bounded projections out of the surface of a cell (P. Satir and S.T. Christensen, 2007). Cilia have been shown to play an important role in many signaling cascades in embryonic development. Problems in important developmental signaling pathways such as Hedgehog and Wnt, both important signaling pathways in gene expression, have been shown to be caused by defective cilia and ciliary disorders. This is due to the dependence of these pathways on cilia for normal signaling to occur (P. Satir and S.T. Christensen, 2007). Every cilium grows out of the cell from a basal body beneath the surface of the cell membrane. Each basal body is made of a modified centrosome from which the ciliary axoneme projects (J.T. Eggenschwiler and K.V. Anderson, 2007) The basal bodies and axoneme are comprised of microtubules made of α and β-tubulin. Post translational modifications (PTMs) can change the tubulin microtubules in a number of ways. Acetylation/deacetylation of tubulin has been shown to be a stabilizing factor in many different types of microtubules (G. Piperno, M. LeDizet, and X. Chang, 1987). Microtubules in ciliary axonemes are mostly acetylated and are more stable than those in the cytoplasm. Such PTMs as acetylation to tubulin are not specific to cilia, but are more concentrated in the microtubules that compose the axonemes of cilia. Hyperacetylation and irregular deacetylation patterns disrupt cell function and can ultimately lead to organismal disorders (J.W. Hammond, D. Cai, and K.J. Verhey, 2008).

As the importance of functional cilia in development becomes clear with ongoing research, it is important to investigate the factors effect ciliary strength and functionality. If acetylation adds to the strength of ciliary axonemes, then those acetylating enzymes themselves could potentially have an important role in embryonic development. As cilia are rooted in and arise from the basal body just below them, it is possible that factors affecting the basal body also affect the associated cilium. The following experiment explored the idea that cells with a greater concentration of microtubules near their basal body also have a greater concentration of acetylated tubulin in the associated cilium.

Materials and Methods

Sea urchin embryos were stained following the protocol in *Immunofluor staining of SU embryos – MeOH fixation* (Morris, 2012) with a few modifications in dilutions and incubation periods. The Hoechst staining solution was prepared with 4.5µL of Hoechst stock solution in 45mL block buffer in order to obtain a 1:10,000 dilution. The final concentration of the Hoechst solution was 1µg/mL. The anti-acetylated tubulin antibody solution was prepared with 12µL anti-acetylated tubulin antibody added to 6mL block buffer in a 1:500 dilution. The FITC-DM1A anti-alpha tubulin antibody solution was prepared by mixing 80µL FITC-DM1A with 4mL block buffer for a 1:50 dilution. Finally, the Alexafluor 546 Goat anti-mouse secondary antibody was made using 4µL Alexa546 GAM in 4mL block buffer for a 1:1000 dilution. The anti-acetylated tubulin Ab incubation was run for 8 hours, the FITC-DM1A anti-alpha tubulin Ab incubation was run for 3 hours, and the Hoechst solution incubation was run for an hour. Negative controls were only incubated with the primary antibody so the negative coverslip would only glow with the dim fluorescence all cells pick up during the Immunofluor staining. This allowed the dim fluorescence experienced by all cells unrelated to the antibody tagging to be ignored when observing the staining images.

The images generated from the staining were then analyzed and data was gathered about the concentrations of α-tubulin in the basal bodies and the concentration of acetylated tubulin in the related cilium. Alpha-tubulin concentration was assumed to be directly related to the mean brightness of the immunofluorescent staining done by anti-α-tubulin antibodies at the basal body of a cilium. Acetylated tubulin concentration was assumed to be directly related to the mean brightness of the immunofluorescent staining done by anti-acetylated tubulin antibodies in the cilium. Both the green α-tubulin immunofluorescence staining image and the red acetylated tubulin immunofluorescence staining image at one image location showing multiple sea urchin gastrulae were analyzed to measure the concentrations of the α-tubulin and acetylated tubulin respectively. Using Image J, the mean brightness of a single basal body was measured using a standard circular selection with an area of 44-pixels. Basal bodies which appeared to have their brightness reading affected by the background glow of the archenteron were not measured. After the mean brightness value of a basal body was recorded, the corresponding cilium was located using pixel coordinates. The mean brightness of the cilium was measured using a segmented line selector which reached from the base to the tip of the cilium. If two cilia intersected, the line selection was measured from the ciliary base to just before the point of intersection to avoid false brightness readings. The mean brightness measurements for the basal body and cilium were listed together to track the relationship between α-tubulin concentration and acetylated tubulin concentration. A sampling of basal body/cilium

pairs from two different embryos in the same image plane used to create the data pool. Each pair's brightness measurements were plotted as cilium brightness over basal body brightness in order to observe trends in the data.

Results

Figure 1 shows the FITC immunofluorescent staining of α-tubulin in the basal bodies of the sea urchin embryos' cells. The archenteron that caused diffuse background fluorescence in an area that was avoided in data collection is clearly visible in the left-most embryo. Figure 2 shows the AF546 immunofluorescent staining of acetylated α-tubulin in the cilia of the sea urchin embryos' cells. When the mean brightness of each cilium was plotted on a graph over the mean brightness of the basal body, a trend appeared and is represented by a linear trend line in Figure 3 below. The slope of this line shows a direct correlation between the brightness of the basal body and the brightness of the associated cilium. The slope of the trend line is steep as the variance in mean basal body brightness was only 36 bits while the variance in mean cilium brightness was 92 bits. These preliminary data suggested that if the α-tubulin stain in the basal body of a particular cell was bright, the associated cilia had a brighter acetylated tubulin stain than another cell with a dimmer α-tubulin staining in the basal body. Following the assumption that tubulin Immunofluor staining brightness is representative of the tubulin concentration in that area, the data show that the concentration of acetylated tubulin in cilia is directly related to the concentration of α-tubulin in the basal body out of which that cilium grew.

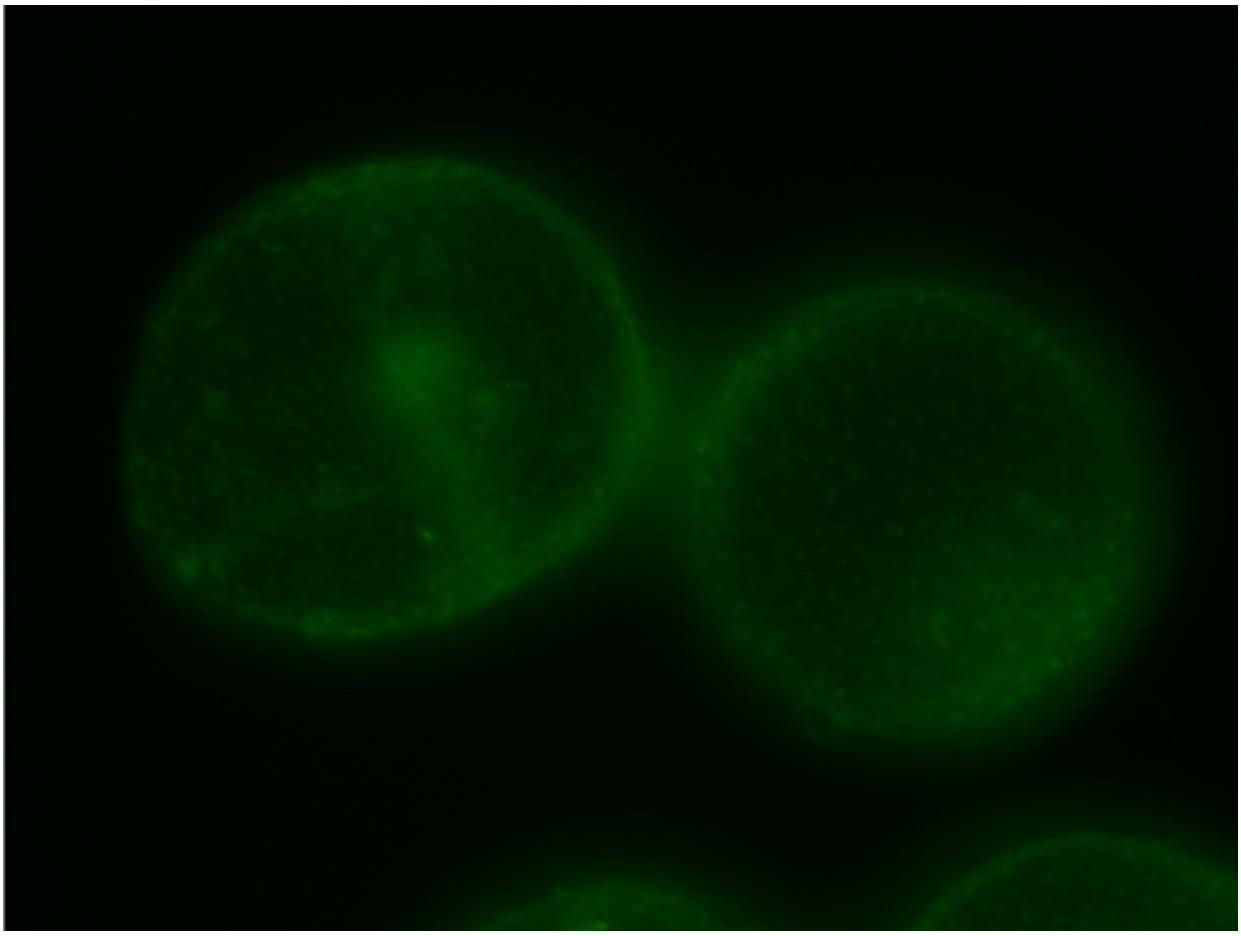

Figure 1: FITC Immunofluorescent Staining of α-tubulin in Sea Urchin Gastrulae

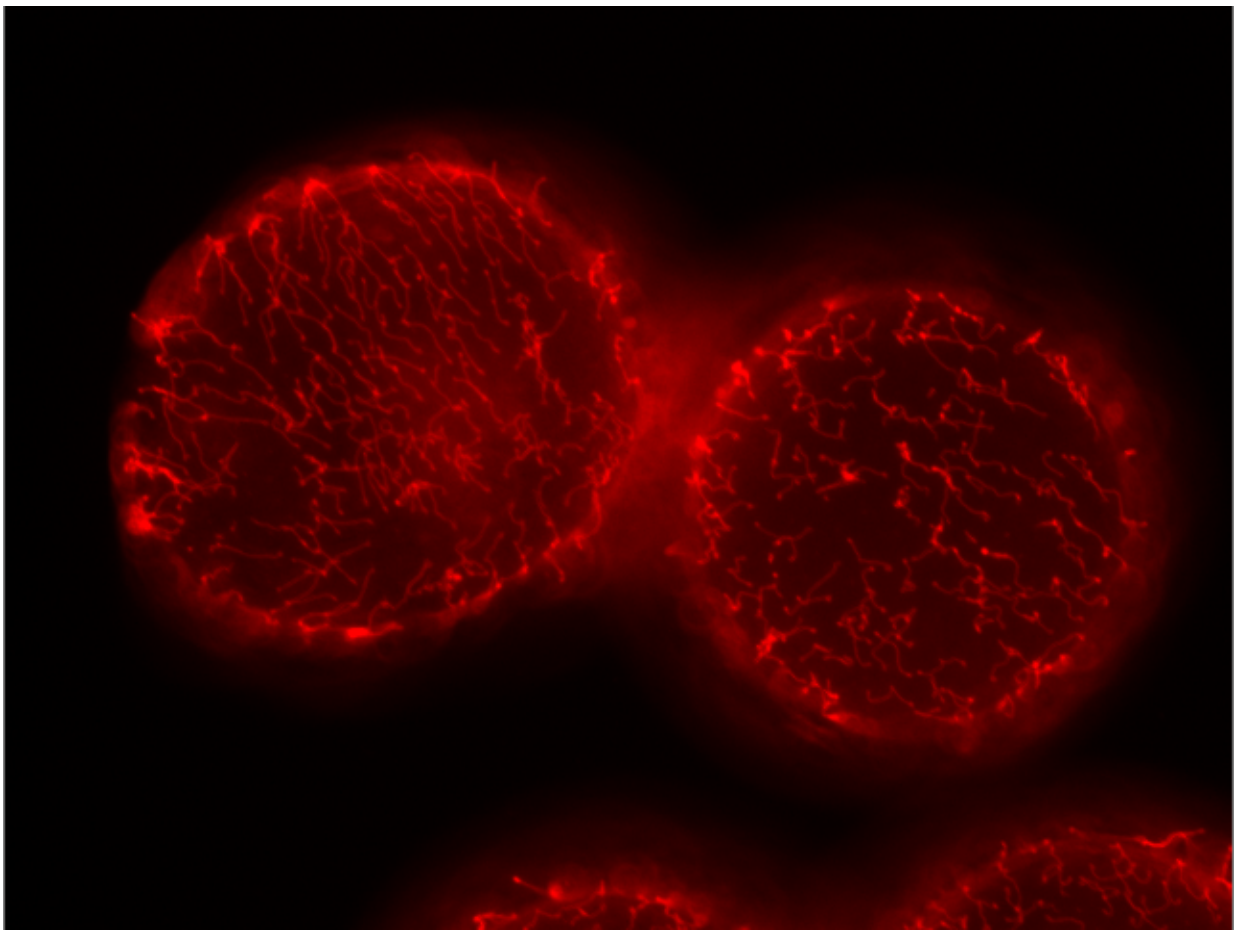

Figure 2: AF546 Immunofluorescent Staining of acetylated α-tubulin in Sea Urchin Gastrulae

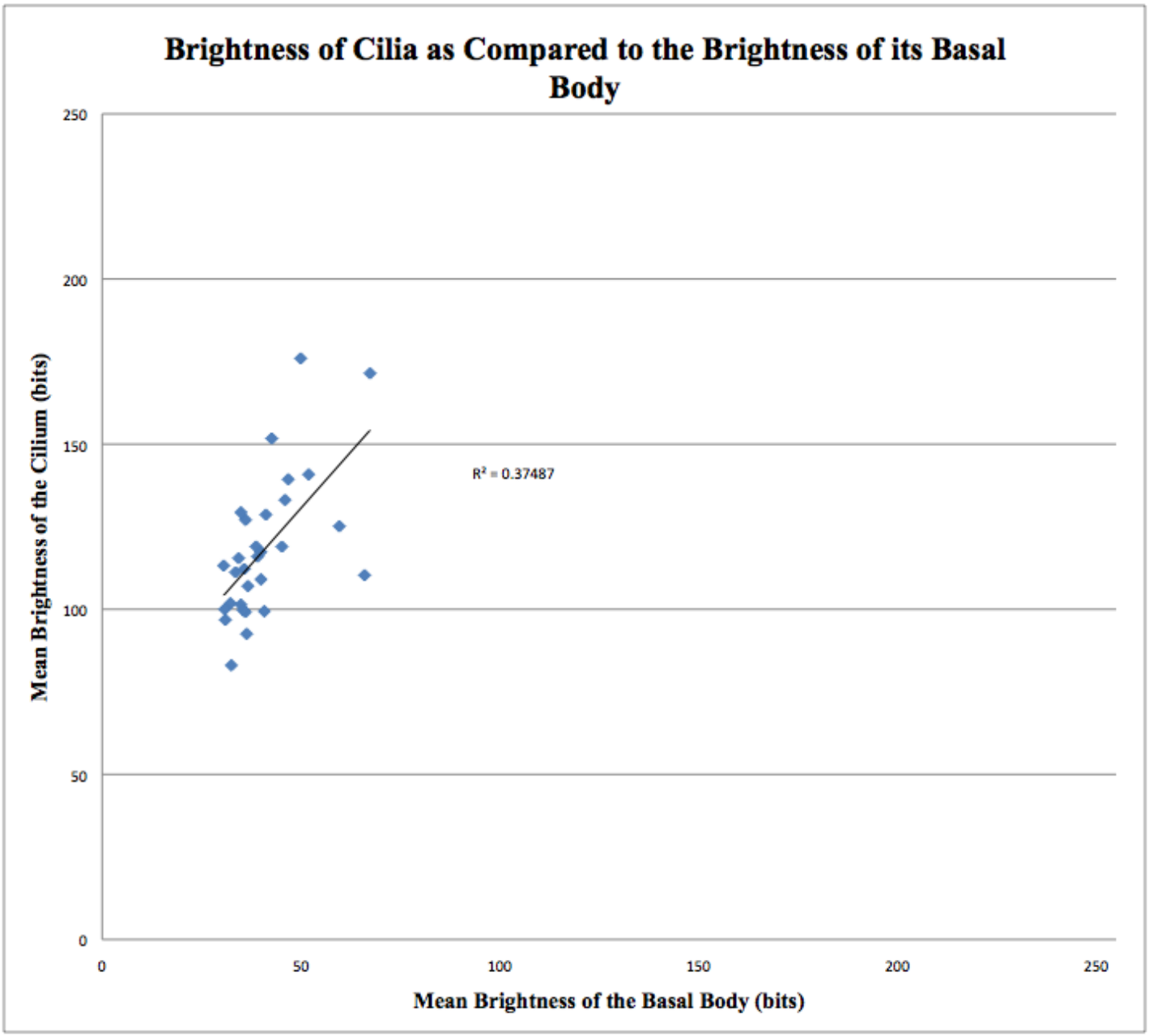

Figure 3

Discussion

Due to the small sample size of the data pool, the trend seen in the graph of mean cilia brightness over mean basal body brightness is suggestive of a correlation between basal body tubulin concentration and acetylated tubulin concentrations in the associated cilium, but no conclusions can be drawn. The R^2 value for the linear fit line added to Figure 3 was a mere 0.3749 and statistically, this trend is not a significant. Since a suggestion of a trend is seen, more

data should be collected to investigate this relationship further.

There are many factors contributing to the insignificance of these data. One issue with the assumption that basal body brightness and cilia brightness are directly related to the α-tubulin and acetylated tubulin concentrations, respectively, is that the images collected werewhole, three-dimensional gastrulae. This means that the immunofluorescence seen by the camera could be a combination of the layers of cells that experienced staining. This layering effect could cause false brightness readings for the stained basal bodies and cilia. The archenteron seen faintly glowing in the background of Figure 1 is an obvious example of how background fluorescence can affect the brightness of a certain point in the foreground. Therefore, although it may appear that there is a direct correlation between the concentration of α-tubulin in the basal bodies and the concentration of acetylated tubulin in the cilia, the brightness data used to generate this trend may have been skewed by the nature of a three-dimensional immunofluorescent image and the relationship assumed between fluorescent brightness and tubulin concentration. It is also possible that the method of measurement in Image J was not an accurate method. The mean brightness value was taken of the same sized circular area for basal bodies and varied lengths of cilia. This could lead to inconsistencies within the data set.

If a correlation was seen with a more controlled system of data collection and a larger sample size, then a connection between the concentration of α-tubulin in the basal body and the amount of acetylated tubulin in the cilium could be drawn which could lead to further connections between basal body α-tubulin concentration and ciliary strength. This would be useful information due to the importance of cilia in embryonic gene expression signaling pathways during development (P. Satir and S.T. Christensen, 2007).

References

R. Morris. Developmental Biology Laboratory Manual. 2012.

Wheaton College. Norton, Massachusetts

P. Satir and S.T. Christensen. 2007. Overview of Structure and Function of Mammalian Cilia.

Annual Review of Physiology, 69, 377-400. DOI: 10.1146/annurev.physiol.69.040705.141236

J.T. Eggenschwiler and K.V. Anderson. 2007. Cilia and developmental signaling.

Annual Review of Cell and Developmental Biology, 23, 345-373.

G. Piperno, M. LeDizet, and X. Chang. 1987. Microtubules containing acetylated alpha-tubulin in mammalian cells in culture.

Journal of Cell Biology, 104(2), 289-302.

J.W. Hammond, D. Cai, and K.J. Verhey. 2008. Tubulin modifications and their cellular functions.

Current Opinion in Cell Biology, 20, 71-76. DOI: 10.1016/j.ceb.2007.11.010

I have abided by the Wheaton College Honor Code in this work. Kathryn V. Svec