Acetylation of Tubulin Oscillates Between High and Low Levels along the Length of the Sperm Tail

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Introduction

Microtubules are found in almost all eukaryotic cells and are associated with cellular functions such as cell division, morphogenesis, motility, intracellular transport and cytoskeletal organization (Kierszenbaum, 2002 ; Maruta, Greer, & Rosenbaum, 1986). This diversity of function is due to the differentiation of various intracellular microtubule populations (Kierszenbaum, 2002). Diverse populations of microtubules are generated by tubulin types, products of multigenic families, as well as post-translational modifications of tubulins (Mancini & Báo, 2005; Piperno, LeDizet, & Chang, 1987).

Microtubules are comprised of two types of heterodimeric proteins: alpha and beta tubulin (Mancini & Báo, 2005; Fuller, 1985). Alpha and beta tubulin associate to form one protofilament, and thirteen protofilaments make up one microtubule (Mancini & Báo, 2005; Fuller, 1985). Alpha and beta tubulin undergo a variety of post-translational modifications that include acetylation, detyrosynation, tyrosynation, phosphorylation, polyglutamylation and polyglycylation (Mancini & Báo, 2005; (Maruta, Greer, & Rosenbaum, 1986; Fuller, 1985). Acetylation involves the substitution of an acetyl group for an active hydrogen atom (Piperno, LeDizet, & Chang, 1987). Acetylated tubulin is present in various microtubule structures, and plays a role in stabilizing the structures of all microtubules (Piperno, LeDizet, & Chang, 1987).

In this study, I tested the hypothesis that the acetylation of tubulin in sperm tails will oscillate between high and low levels along the length of the tail. This is significant to investigate because the functional role of acetylated tubulin

in the axoneme is not yet understood.

Research has shown that microtubules with acetylated tubulin are more stable under depolymerizing conditions than the majority of cytoplasmic microtubules (Kierszenbaum, 2002;Maruta, Greer, & Rosenbaum, 1986). However, this relationship between post-translational acetylation and stability remains unclear and is currently being elucidated (Kierszenbaum, 2002;Piperno, LeDizet, & Chang, 1987).

Sea Urchin sperm were used in this study. The sperm were treated with three antibodies that were conjugated to a different colored fluorescent pigment (Morris, 2008). Hoechst stain bound to DNA and fluoresced blue, Fluorescein isothiocyanate (FITC) bound to total tubulin and fluoresced green, and Alexa 546 was a secondary antibody which bound to acetylated tubulin and fluoresced red. The sperm were then photographed using epifluorescence microscopy.

Materials and Methods

Immunofluorescent Staining

This experiment followed the protocol outlined in *Immunofluorescent staining of sea urchin embryos, MeOH fixation* (Morris, 2008).

Imaging of Data

A Nikon E400 epifluorescence microscope with standard Hoechst, FITC, and Rhodamine fluorescent filter sets was used with Spot Advanced software on a Spot Insight camera from Diagnostic Instruments. A 40X plan fluor objective was used for all images. The image of acetylated alpha tubulin was cropped to 1/8 of its linear dimension.

Results

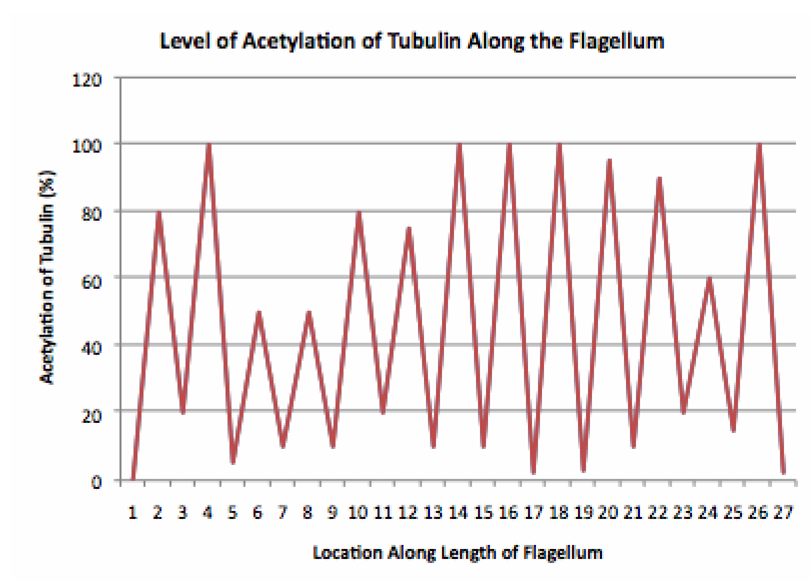

Figure 1

This graph shows the relationship between the acetylation of alpha tubulin and its location along the length of the flagellum. This semi-quantitative data was generated by presuming that the degree of fluorescence was proportional to

the degree of acetlyation of alpha tubulin.

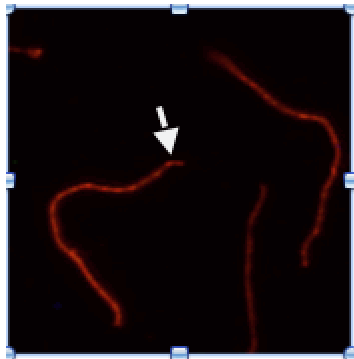

Figure 2
An image of acetylated alpha tubulin in sperm tails stained with Alexa 546 and cropped at 1/8 of its linear dimension. Notice the oscillation between bright and dark fluorescence along the length of the tail. The arrow indicates the flagellum from which the semi-quantitative data were collected.

The brightness of the red fluorescence was assessed on a percent scale and was proportional to the degree of acetylation of tubulin. The brightest portions were given a score of 100%. The black background was given a score of 0%. Along the length of the flagellum, the brightness changed dramatically. There was a high percent of acetylation near the sperm head as well as at the terminus of the sperm tail.

Discussion

The present experimental data demonstrate that the acetylation of tubulin oscillates between high and low levels along the length of the sperm tail. These semi-quantitative data regarding the acetylation of alpha tubulin support my hypothesis. It can be concluded that along the length of sea urchin flagella, the acetylation of tubulin does not remain constant, and oscillates between high and low levels.

One source of error in this study was the absence of a proper control population. We were not able to compare the experimental results to a control in order to assess any innate autofluorescence within the sperm tails.

If a very large data set was obtained, and I was convinced that the differences between the experimentals and controls were statistically significant, then some developmental process must be the driving factor. The oscillation in the levels of acetylated of tubulin along the sperm tail might aid in the motility of sperm because the flagellum needs to bend to carry out its functional role (Kierszenbaum, 2002). The combination of acetylated tubulin and total tubulin may help the dynein motor proteins move the sperm tail (Kierszenbaum, 2002). However, it is important to understand that the functional role of differentiated tubulins is not well understood (Fuller, 1985). One study suggests that the architecture and motility of the axoneme is dependent upon modified tubulins in *Drosophila melanogaster* (Hoyle & Turner, 2008).

To refine the protocol for this study I would make a control in order to look for any autofluorescence. Additionally, I would process the images in Photoshop to measure exact pixel values on the sperm axoneme, and quantify the levels of acetylation of tubulin more precisely.

In future experiments I would raise Alexa 546 against acetylated tubulin in sperm cells from different animals in order to see if this phenomenon occurs across species. I would be particularly interested to raise Alexa 546 against acetylated tubulin in human sperm cells.

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