

The Hyper-acetylation of Pre-fertilization sperm Axonemes is Diminished Following Fertilization

Alison Mehlhorn
Independent Research Project Report
Developmental Biology
December 1, 2009

Introduction

Successful fertilization and zygote development in Sea Urchins requires the successful achievement of a variety of specifications. One such requirement may involve the structural properties of the sperm tail, specifically the axoneme, as fertilization and the subsequent stages of development proceed (Fechter et. al., 1996). The structure and stability of the sperm tail axoneme is thought to be partially dependent on the presence and patterning of acetylation along the microtubules that compose the axoneme (Fechter et. al., 1996). Though the exact role that acetylation may play is not specifically understood it is thought to affect the overall properties of the sperm tail and therefore may impact the process of fertilization (Fechter et. al., 2000).

The sea urchin sperm tail axoneme is composed of multiple microtubules. Microtubules are constructed from tubulin which is a heterodimer consisting of α -tubulin and β -tubulin (Sigma, 2009). Alpha-tubulin can be post translationally modified by the reversible addition of an acetyl group to Lys40 (Sigma, 2009). The presence of acetylation along the microtubule has been correlated with microtubule stability, though this relationship is not well understood (Schatten et. al., 1988).

Previous experiments, performed by Fetcher et. al. and published in 1996, studied the fate of sperm tail microtubules of Sea Urchins pre-fertilization and then at multiple time points following fertilization. These experiments identified the presence of sperm tail microtubules through the technique of immunofluorescence with the use of a monoclonal antibody raised against acetylated α -tubulin. The dynamic nature of microtubules as related to the presence of acetylation reported by this study compels further examination. Like the study by Fetcher et. al. the following experiment utilizes a similar technique to examine the acetylation patterns along the axoneme of sea urchin sperm.

By the technique of immunofluorochemistry and the use of a secondary antibody, tagged with FITC, against a primary antibody against acetylated α -tubulin, the distribution and concentration of acetylated tubulin along the sperm axoneme will be compared between pre-fertilization sperm and post-fertilization sperm of the species *Lytechinus pictus*. It is hypothesized that there will be a higher level of acetylation along the axoneme of pre-fertilization sperm compared to post-fertilization sperm due to the structurally stabilizing properties of acetylation. The pre-fertilization sperm will require a more structurally stable axoneme in order to propel the sperm cell, while the post-fertilization sperm axoneme is in the process of being disassembled and will therefore not have an as abundant level of acetylation.

Material and Methods

The procedure of Morris 2009, in consultation with Drs. J Henson and B. Shuster, was utilized with the following procedural modifications. The primary antibody against acetylated tubulin was incubated for thirty minutes. The secondary antibody, against primary antibody against acetylated tubulin, was incubated overnight for about twenty hours. The FITC-conjugated DM1A primary antibody against alpha-tubulin was incubated for about three hours. All cover slips were washed once with the block buffer to remove this final antibody before being stained with Hoechst, which was not required by the given procedure. All washes were performed by removing cover slips from wells, dumping out block buffer, replacing with fresh block buffer and repositioning cover slips within designated well for required four minutes before repeating as necessary.

Images were taken of the resulting stained cells with the help of Professor Morris. The images were obtained with the use of a Nikon E400 epifluorescence microscope with standard FITC filter set using Spot Advanced Software on a Spot Insight Camera and diagnostic instruments (Morris, 2009). A 40x plan fluor objective was used for all images.

Results

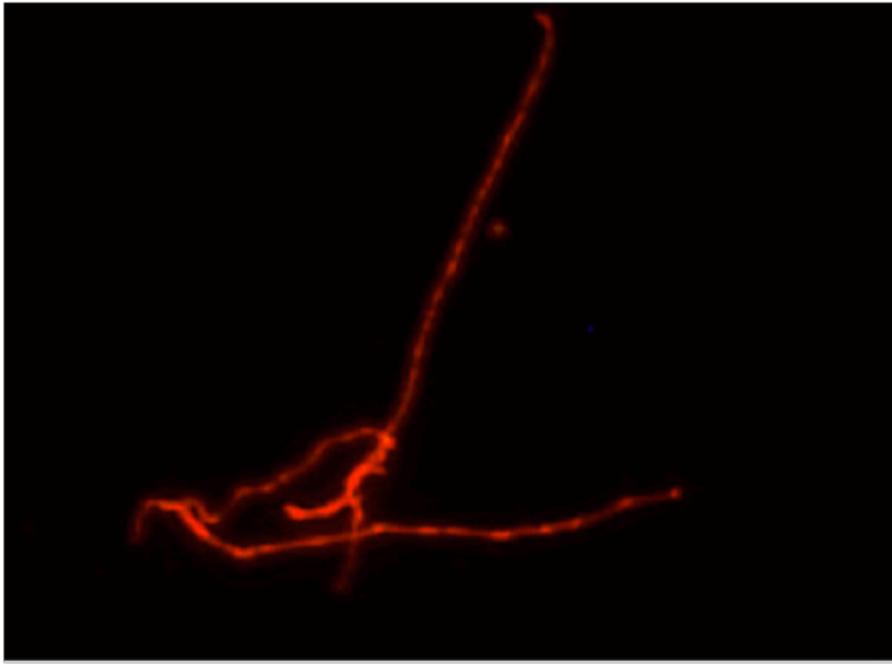


Image 1: Distribution of acetylated alpha-tubulin along the length of pre-fertilization sperm axoneme as illustrated by brightness of immunofluorescence with FITC tag.

40x magnification

25% of linear dimension of image (2.5x enlargement), 71.75 mm total horizontal length

Nikon E400 epifluorescence microscope

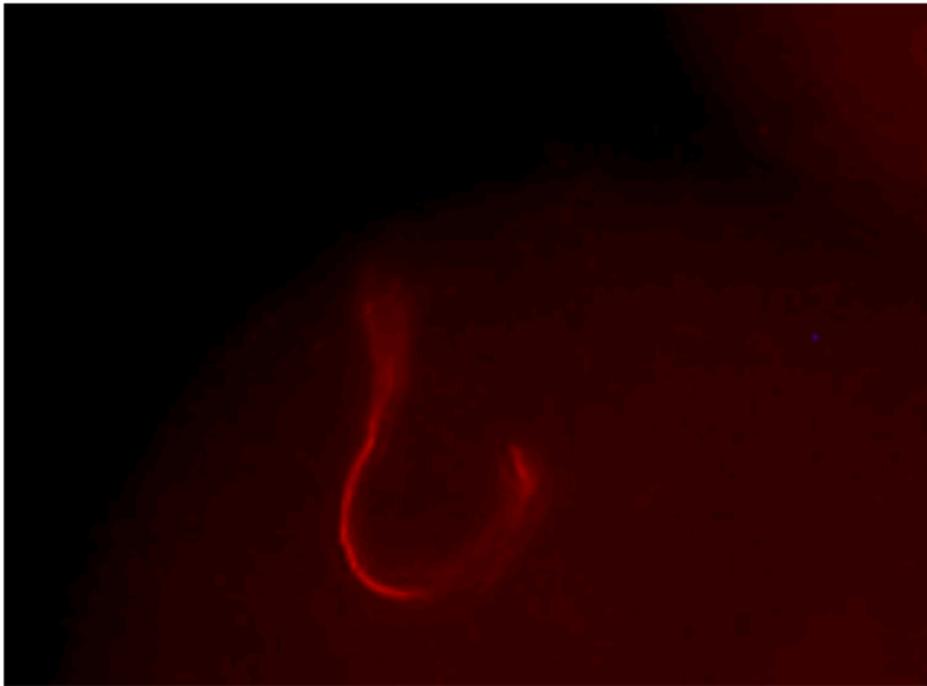


Image 2: Distribution of acetylated alpha-tubulin along post-fertilization sperm axoneme as illustrated by brightness of immunofluorescence with FITC tag.

40x magnification

25% of linear dimension of image (2.5x enlargement), 71.75 mm total horizontal length

Nikon E400 epifluorescence microscope

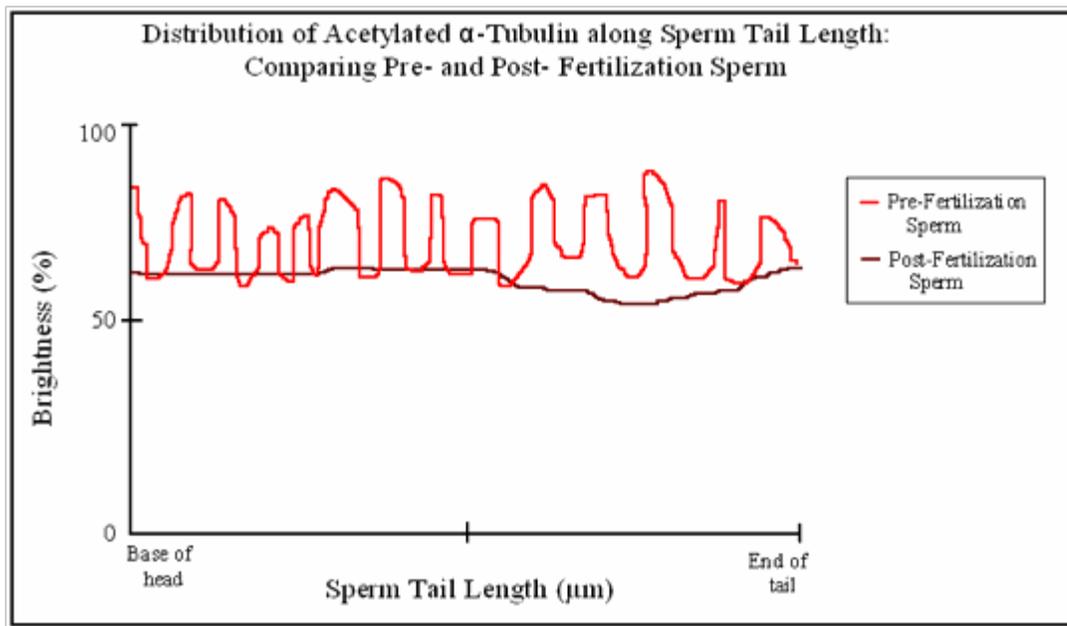


Figure 1: Concentration and distribution of acetylated α -tubulin along the length of sperm axoneme, comparing sperm tails before and after fertilization, based on the brightness of the immunofluorescence.

Both pre- and post-fertilization sperm axonemes displayed high levels of brightness, through fluorescent imaging, along their lengths. This fluorescence directly corresponds to the level of acetylation of α -tubulin. Pre-fertilization sperm axonemes displayed varying levels of acetylation along their lengths with areas of hyper-acetylation and regions with lower levels of acetylation. Post-fertilization sperm axonemes exhibited a more even distribution of acetylation along their lengths, but at a lower concentration. This lower concentration of acetylation along the length of post-fertilization sperm axonemes corresponds to the intermittent lower levels of acetylation observed in the pre-fertilization sperm axonemes. Therefore the level of acetylation along the post-fertilization sperm axoneme is more uniform but not as abundant as the acetylation along the axoneme of pre-fertilization sperm.

Discussion

The results of this experiment suggest that the hypothesis may be supported. The images and resulting graph illustrate the higher levels of acetylation along pre-fertilization sperm axonemes compared to the level of acetylation along post-fertilization sperm axonemes. These findings may correspond to the role of acetylation in microtubule stability and when this stability is required.

Microtubules that need to be highly stable and persist for an extended period of time, such as those that make up a

sperm axoneme prior to fertilization and therefore must be highly stable in order to propel the sperm cell forward, would have high levels of stabilizing acetylation. In comparison, microtubules of a post-fertilization sperm axoneme would no longer function to propel the sperm cell and would be depolymerized; therefore they would not require high levels of stabilizing acetylation. The reversible process of acetylation would most likely occur making these microtubules less stable and more easily broken down by the fertilized cell. (Morris*, 2009)

The results of this experiment relate to those of Fechter et. al. (1996) in which the presence and level of acetylated alpha-tubulin along sea urchin sperm axonemes was study over a period of time including pre- and post-fertilization points. In this experiment high levels of acetylation were observed in pre-fertilization sperm axonemes. Following fertilization the level of acetylation slowly diminished and the axoneme began to fragment. At one hundred minutes post-fertilization the axoneme was no longer detectable. These findings correspond with the provided possible explanation for why acetylation levels vary between pre- and post-fertilization sperm axonemes.

Overall, this experiment was a success with a minimum of errors. The only major source of error pertains to the control cells which were dyed with Hoechst stain instead of being incubated with the block buffer solution. By simply repeating the experiment now that the technique has been practiced and better understood through experience, the amount of overall minor experimental errors would most likely be avoided.

In order to learn more about acetylation patterns of sperm axonemes it is suggested that multiple time points both pre- and post-fertilization be examined. Also many more sperm should be studied in order to get a more general understanding of axoneme acetylation patterns. Though this experiment only begun to examine the acetylation properties of sea urchin sperm axonemes it provokes further study towards better understanding of the properties of fertilization and development.

Literature Cited

- Fechter, J., A. Schoneberg, and G. Schatten. "Excision and Disassembly of Sperm Tail Microtubules During Sea Urchin Fertilization: Requirements for Microtubule Dynamics." *Cell Motility and the Cytoskeleton* 35.4 (1996): 281-88. Print.
- Moreno, Ricardo D., and Gerald Schatten. "Microtubule Configuration and Post-Translational α -Tubulin Modifications During Mammalian Spermatogenesis." *Cell Motility and the Cytoskeleton* 46 (2000): 235-46. Print.
- Morris, Robert and Drs. J. Henson and B. Shuster. *Immunofluorescent staining of sea urchin embryos, MeOH fixation (for classroom use, adherent embryo technique)*. Nov. 2009.
- Morris*, Robert. Personal communication. 30 Nov. 2009.
- Schatten, Gerald, Calvin Simerly, David J. Asai, Ester Szoke, Perter Cooke, and Heide Schatten. "Acetylated α -Tubulin in Microtubules during Mouse Fertilization and Early Development." *Developmental Biology* 130.1 (1988): 74-86. Print.
- SIGMA. Advertisement. *Monoclonal anti-acetylated tubulin*. Sigma-Aldrich, Inc. Web. 29 Nov. 2009. <<http://www.alzforum.org/res/com/ant/tubulinredo/sigmaT6793.pdf>>.