Acetylated Tubulin in Sea Urchin (*Lytechinus pictus)* Sperm Cells

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Introduction:

Immunofluorescent staining of cells can be very useful for helping scientists break down the sub cellular make-up, or visualizing the inner workings of cells. The technique works by labeling antibodies with fluorescent dyes which bind specifically to targeted antigens. In order to target such specific regions of the cell often primary and secondary antibodies are used, where the primary antibody targets a specific antigen of interest and the secondary, or "indirect" antibody recognizes the primary antibody (RLMorris, 2009). In this experiment, antibodies were introduced to the sperm cells of sea urchins (*Lytechinus pictus).* Those antibodies were used to indicate the presence of total tubulin (tubulin that was polymerized but not stabilized) and acetylated tubulin, or mature tubulin, which has polymerized and stabilized. Using fluorescent microscopes, we were able to obtain images showing tubulin distribution in the sperm tails of the sea urchins.

Tubulin is a globular protein that makes up the microtubules found in eukaryotic cells and some prokaryotic cells (Wilt and Hake, 2005). Microtubules are extremely important in many cellular processes such as mitosis, cytokinesis, and vesicular transport. Microtubules in sperm tails are important for the sperm's movement and structural makeup as they are part of the structure called the axoneme, which allows the swimming movement by which the sperm finds the egg (Fechter et. al., 1998). Scientists have used antibody targets on the microtubules of sperm tails in order to determine the fate of the sperm tail as the sperm fuses with the egg during fertilization (Fechter, 1998). Analyzing the presence of total and acetylated tubulin in *Lytechinus pictus* has not been done, but could help scientists understand how the sperm tail might contribute to the zygote and what structures it fuses with the egg.

Because all of the tubulin is not stable in the sperm tail, there will be inconsistencies with the pictures provided by the immunofluorescent imaging. Total tubulin will be seen throughout the tail but the tubulin that is stable and

polymerized will hypothetically be in concentrated areas in the tail of the sperm. Florescent microscopes can be used to get images of the stained sperm cells and can then be analyzed to find trends in light concentration with special software. In the example of tubulin in sea urchin sperm tails if acetylated tubulin is unevenly distributed throughout the sperm tail then the images will show patchy areas of red fluorescence while the total tubulin (which will be green) will be evenly throughout the tail.

Method:

This experiment was preformed using the method instructions developed in consultation with Drs J. Henson and B. Shuster by RLMorris in the summer of 2008. All materials presented in the instructions were used. The following minor adjustments were observed as the experiment was preformed:

Students performing the experiment started at roman numeral II, the immunofluorescent labeling of methanol-fixed embryos. They were not required to personally complete methanol-fixation of the sea urchin embryos. Also, in step 13 of this section, 1% protamine sulfate was used instead of polylysine in water. For most steps, the minimum required amount of incubation time was used but at step 36 where the “Secondary Antibody against Primary Antibody against acetylated tubulin” were introduced, the embryos were kept in their wells and incubated in a refrigerator overnight. At step 50, though there was no indication that a rinse with PBS-T needed to be used, it was preformed but should not have effected the results in any way.

Images were acquired with Professor Robert Morris on a Nikon E400 epifluorescence microscope with standard Hoescht, FITC, and Rhodamine fluorescent filter sets, using Spot Advanced Software on a spot insight camera from Diagnostic Instruments with a 40X plan fluorescent objective. Images absorbing at a blue scale and emitting at a green scale indicated total tubulin and were acquired using a ND16 filter on the microscope. Images were then overlaid by the professor to show one coherent image which indicated the presence of specific molecular structures.

Results:

Figure 1:

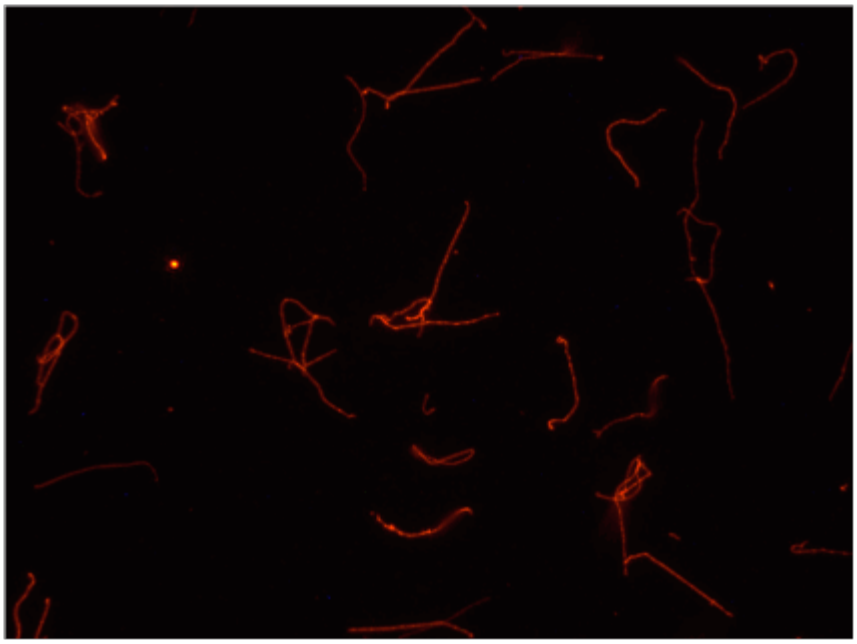

Figure 1 depicts an image of the alpha tubulin fluorescence exhibited on the tail of the sperm cells. It shows where the anti-acetylated tubulin antibody binded and shows that it binded in a patchy, concentrated, pattern along the sperm tail. This image measures 287 mm across the horizontal length.

Figure 2:

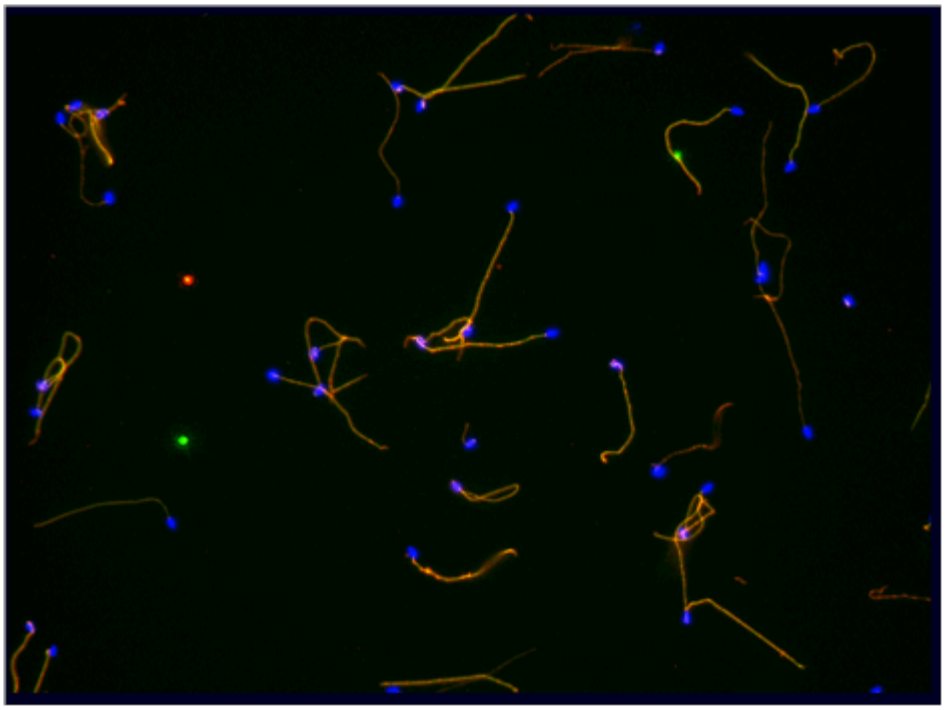

Figure 2 depicts an overlapped image of all three antibodies that were used in the experiment. Yellow areas indicate where both total tubulin and acetylated tubulin were present. The image show that areas of yellow uniform throughout the tail, yet there are clearly concentrated areas that exhibit a more orange-red tint. This image measures 287 mm across the horizontal length.

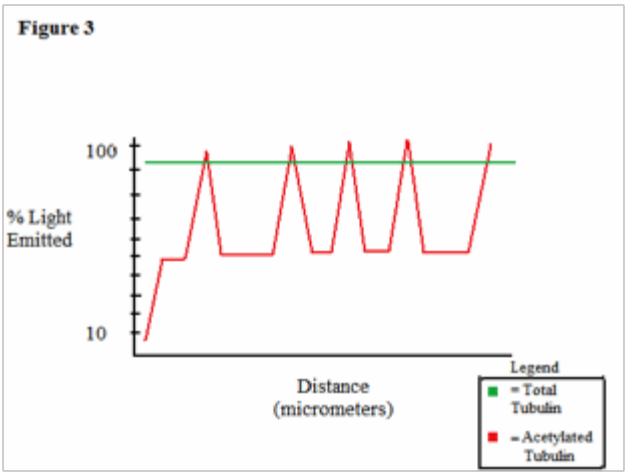

Figure 3 depicts a quantitative measurement of the percent light concentration along the sperm tail of the sea urchin sperm. It depicts an example of what one sperm tail would be expected to exhibit for light emitted. The graph shows that total tubulin has a continuous percent of light and the alpha tubulin shows a much less uniform percent as one moves down the tail.

Results from this experiment very clearly show visually and quantitatively that the alpha tubulin antibody stain binded to specific, concentrated areas of the sperm tail while the total tubulin antibody stain binded uniformly along the sea urchin sperm tail. Graphs made my analyzing the percent light concentration along the length of the sperm tail show that the percent light concentration of total tubulin was at the same concentration along the entire length of the tail. The graph line is completely straight while the line for alpha tubulin is much less uniform and shows peaks in high light concentration where more acetylated tubulin was present.

Conclusion:

The hypothesis that images would yield patchy results for the acetylated tubulin while it would yield continuous results for the total tubulin was supported with this experiment. With this evidence we are able to conclude that there is some form of tubulin throughout the entire length of the tail of sea urchin sperm cells, but there is not mature tubulin found along the entirety of the tail. Mature tubulin, which is both polymerized and stable, appears in tight clusters along the length of the tail. This can be seen in the graphs and by the naked eye in auto-fluorescent images. It is unclear why this clustering occurs but one can not deny that it does. Scientists have been working for many years to discover all of the different types of tubulin that are present in the sperm cell structure (Kierszenbaum, 2002). The discoveries have yielded results suggesting there is a whole wealth of different types of microtubule structures that play important roles in the structure and development of sperm (Kierszenbaum, 2002). In the context of these previously researched experiments, our experiment holds interesting information on the placement of such tubulin structures.

The data collected shows us that there is a concentrated presence of alpha tubulin in certain areas of the sperm tail, but it also tells us that there must be some sort of *other* tubulin in the regions *between* the highly acetylated areas. Because a stain for the presence of total tubulin showed even distribution, we can successfully conclude that there is tubulin present between areas of alpha tubulin and thus along the whole sperm tail. To determine what form of tubulin these structures are, further experiments would have to be preformed. The images do, however, confirm that the sperm tail contains a large amount of microtubule material and they support the original hypothesis that acetylated tubulin would not be uniform throughout. Why this happens can only be hypothesized with the information available, but it may have to do with the production of the structure and its polymerization. Because alpha tubulin is stable it may have different properties than other forms of tubulin and may separate itself from those other forms (Fechter et. al, 1998).

This experiment was quite a success based on the number of clear images that were obtained and based on the time constraints that we had to face. Taking the time to repeat the methods for the experiment would yield more successful data in the sense that any human error that occurred whilst preparing the slides could be avoided with more practice. The images, however, were very clear and conclusive so the most prominent problem with this experiment was the lack of time that we had to analyze our findings. More advanced equipment could also have been used in order to get more detailed pictures, and if there were more time it would have been better to be more well-versed in the knowledge of sperm tail tubulin. Repeating the experiment more than a couple of times would not yield any better or different results. Since results in this are based on fixed characteristics of the sperm cells, it may lead us to more confident results because of ability to repeat the results but would not yield any particularly different findings.

There is an overwhelming abundance of options for future experiments in which more time could be allotted to actually measuring the % light concentration for the areas of alpha tubulin. Further experiments could also be conducted where different antibodies could be used to target different types of tubulin. Results may show that, when narrowing down the spectrum of “total tubulin” to more specific types, other kinds of the structures may also have patchy results as the alpha tubulin did. In order to know what results the tests would provide, the experiment would need to be done. Another characteristic that could be tested would be if there is a specific number of alpha tubulin “clusters” on each sperm tail. The number of concentrated areas could be counted for many independent sperm tails and could then be compared to one another or even compared to other species. There may be correlations in how many “clusters”, or concentrated areas, each tail has and these could then be analyzed for their importance.

In all, the experiment was a success and supported our hypothesis very confidently. Unfortunately, the scope of this experiment does not cover such further investigation to determine *why* the alpha tubulin exhibits patchy positioning within the tail. In the future this question may be answered, but for now there is limited literature on the subject.

References

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