# Increase of the Mitotic Spindle Length to the Mitotic Cell Diameter Ratio During Sea Urchin Embryonic Development

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## Introduction

During mitosis, tubulin comprises the mitotic spindles that attach to the chromosomes, physically segregating the chromosomes and pulling them to opposite poles (Desai & Mitchison, 1997). During this processes, the microtubules lengthen and shorten by means of GTP disassociation, enabling the cell to divide numerous times throughout its lifetime (Desai & Mitchison, 1997). Recently it has been considered that the mitotic spindle and the actin cortex “talk” during mitosis; a discovery that could help explain changes in cell size through mitosis and the ability of the cell to correctly cleave at the metaphase plate during division (Rodriguez et. al, 2003).

The purpose of this lab was to investigate the cellular and developmental changes that occur as sea urchin embryos progress through development, specifically *Lytechinus pictus*. This analysis was done using immunofourescent staining.

In this lab, it was hypothesized that the ratio of the size of mitotic spindles to the diameter of the mitotic cell

increases during development. Sea urchin embryos were fixed with methanol, and then cells were directly immunoflouresced with FITC-DM1A anti-alpha tubulin antibody. Once the fluorescence process was complete the cells were observed under a microscope and images were taken using a Nikon E400 epiflourescence microscope (See Figures 3,4).

## Materials

The materials used are those outlined in the procedure of Dr. Robert Morris made in consultation with Dr. J. Henson and Dr. B. Shuster, "Immunofluorescent staining of sea urchin embryos, MeOH fixation" (2008). The only addition material used was a ruler to measure the length of the mitotic spindles and the diameter of the cells.

## Methods

The procedure of Dr. Robert Morris made in consultation with Drs. J. Henson and B. Shuster, "Immunofluorescent staining of sea urchin embryos, MeOH fixation," was followed with the following modifications (2008). 1% protanenesulfate in water was used instead of polylysine in water to make the glass surface cationic. Also, the slides were left in an incubation chamber in a drawer for four days rather than in the refrigerator prior to imaging.

Images were acquired on a Nikon E400 epifluorescence microscope with standard Hoechst, FITC, and Rhodamine fluorescent filter set, using spot advantage software on a spot instant camera from Diagnostic instruments. 40x plan fluorescent object was used for all images.

Measurements of the mitotic spindles and cells were done using a centimeter ruler. Each image was enlarged to equal size on the computer screen. The ruler was used directly on the computer screen. The length of the spindle was measured from the center of one aster pole to the center of the opposing aster pole. The diameter of the cell was measured along the same axis of the aster poles. The mitotic spindle sizes and cell diameters for each stage were then averaged. The mitotic spindle size was then subtracted from the cell diameter to find the difference in size between the two measurements (See Figure 1). These measurements were then averaged and used as a ratio: mitotic spindle length (cm): mitotic cell diameter (cm).

# Results

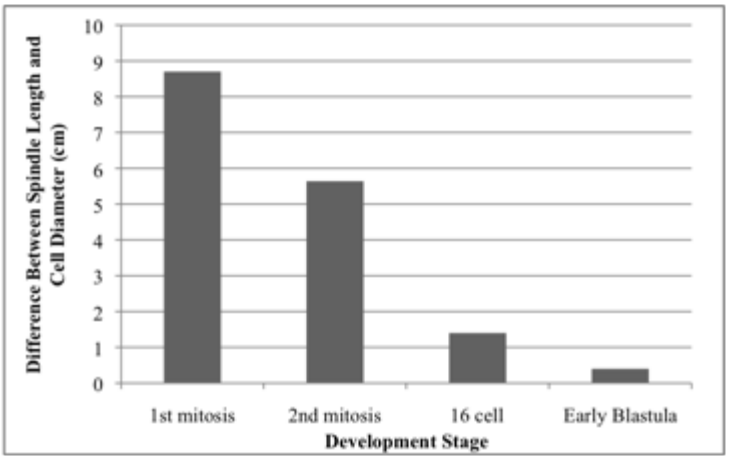


**Figure 1:** This graph shows the average difference between the mitotic spindle length (cm) and mitotic cell diameter (cm) at four stages of development: 1st mitosis, 2nd mitosis, 16 cell, and early blastula. The difference was taken by subtracting the length of the spindle from the length of the cell. The diameter of the cell was taken along the same axis as the poles of the mitotic spindles.

As shown by Figure 1, the average difference between the length of the mitotic spindle and the diameter of the cell decreased as development progressed. At the early blastula stage, the average length of the spindle and the average diamter of the cell are very similar, only differing by 0.4 cm. The greatest difference is in the 1st mitosis, during which the average difference of the two measurements was 8.70 cm. There was a decrease of 95.5% in the average difference of lengths from 1st mitosis to early blastula stage. The average difference in 2nd mitosis was 35.2% less than in the 1st mitosis; this was the smallest percent change of difference. The difference in lengths in the 16 cell stage was 75.2% smaller than in 2nd mitotis, and 71.4% smaller in the early blastula stage than the 16 cell stage.

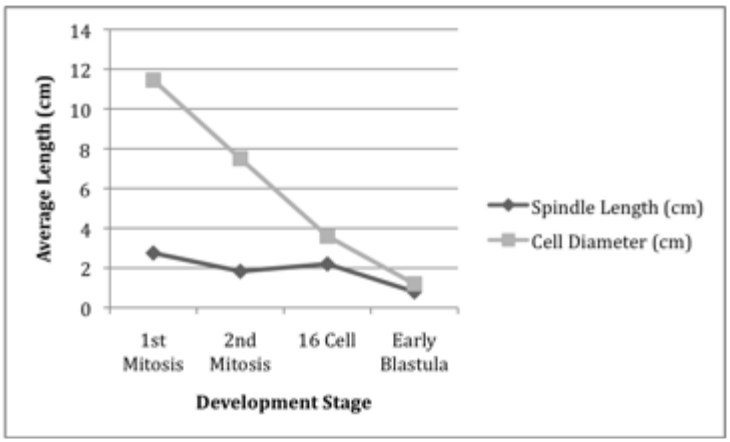

**Figure 2**: This graph shows the decrease in both average spindle length and average cell diameter as development progressed from 1st mitosis to early blastula stage. These lengths were measured using a ruler on images of cells which were magnified on a computer screen, thus all measurements should be considered relative rather than actual.

The diameter of the mitotic cell decreases at a much faster rate than the spindle length (Figure 2). The average spindle length decreases from 2.75 cm to .80 cm from the 1st mitosis to the early blastula. The average cell diamter decreases from 11.45cm to 1.2cm, a much larger change than the spindle length. The spindle length does decrease in size as development progresses, an average decrease of 1.95 cm, however it does not decrease as greatly as the diamter of the cell, which becomes smaller in size by 10.25 cm.

# Discussion

The hypothesis was supported in that the ratio of the lengths of mitotic spindles to the diameter of the mitotic cell increased during development. This was shown graphically in the results by calculating the difference between the length of the spindles and the diamter of the mitotic cells; as the ratio increased the difference decreased (Figure 1).

One possible explanation of this that is not yet completely understood is the possibility of communication between the mitotic spindle and the actin cortex. As development continues cell divisions occur more rapidly, which could be a result of better, faster, stronger communication between the tubulins in the mitotic spindle and the actin cortex. As the ratio between spindle lentgh and cell diamter increases, the distance a signal would need to travel between the spindle and the actin cortex decreases. This decrease in distance enables the actin cortex to properly cleave the cell at the metaphase plate at a faster rate. There is evidence that astral microtubules connect the spindle to the cortex, helping direct the spindle to the correct spot in the cell prior to mitotic division (O'Connell & Wang, 2000). It is probable that as the cell divides and develops these astral microtubules can direct the spindle more easisly, allowing for more rapid divisions.

Although the difference between the spindle length and diamter of the mitotic cell decreases significantly, it is mostly a result of the change in cell diameter rather than spindle length (Figure 1 & 2). The fact that the average diameter of the cell decreases in length much more than the spindle length could help explain why the difference

between the two lengths decreases as development progresses (Figure 2).

Tubulin assembles and dissasembles by means of tyrosination and detyrosination of the c-terminus (Gunderson & Bulinksi, 1986). The data from this lab supports the idea that the products necessary for tyrosination and detyrosination remain in the same quantity throughout development, based on the relatively minimal decrease in mitotic spindle length as development progresses. Rather, it is the contents of the cell, such as the cytoplasm, that decrease as divisions continue thus increasing the ratio of spindle length to cell diameter.

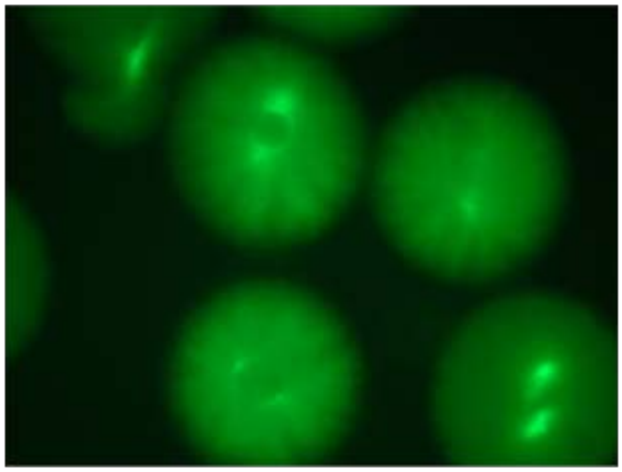

**Figure 3:** This image shows FITC stained sea urchin cells during the first mitosis. The mitotic spindle can be clearly viewed on the top middle cell. This image shows 287 mm of sample across the horizontal axis. (Carson & Seiburth, 2009)

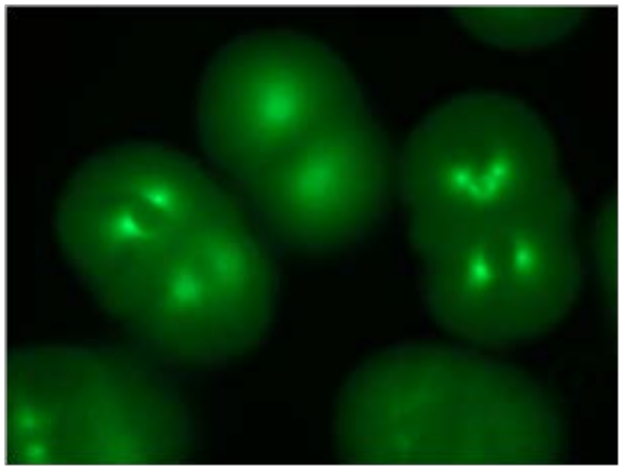

**Figure 4:** This image shows FITC stained sea urchin cells during the second mitosis. The mitotic spindles can be clearly seen in the second cell from the left. This image shows 287 mm of sample across the horizontal axis. (Greenstein & Shintaku, 2009)

It is easier to see the similarity in spindle size when comparing Figure 3 and Figure 4. The diameters of the cells are noticably different while the spindle sizes appear similar.

This possibility of crosstalk between the actin cortex and the mitotic spindle would explain the benefits of a decreasing cell size. As development continues and the growth of the organism progresses, more cells are needed to enable this growth. Thus, the ability for the spindle to communicate with the actin cortex due to a decrease in cell diameter is highly beneficial and enables more rapid divisions and in turn more cells for growth (Rodriguex et. al, 2003).

To improve on the results in this lab it would be beneficial to increase the amount of data by increasing the number of images taken for each stage. It was also improve the results to print the images out in color prior to taking the measurements so as to reduce glare on the computer screen that could effect distinct staining edges. It would also be better to keep the slides in the refridgerator prior to taking the images. Putting the slides in the refridgerator maintains the staining pattern more effectively than leaving the slides at room temperature.

Although there is still much to be learned regarding how the spindle interacts with the actin cortex during mitosis, understanding the tubulin to cell relatoinship is a step in the right direction. The hypothesis of this lab was supported in that the ratio between the length of the mitotic spindle to the diameter of the cell did in fact increase as development progressed. Futher studies should be done to better understand this size relationship and the effects it has on the mitotic process.

Works Cited

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