The rate at which cells in the Animal Hemisphere undergo mitosis compared to those in the vegetal hemisphere in Sea Urchin Blastula

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I. Introduction:

In this study, we tested the hypothesis that cells in the Animal hemisphere of a late sea urchin blastula (*Lytechinus pictus*) undergo more rapid mitosis than cells in the vegetal hemisphere. It was noticed in this study that there seemed to be more cells undergoing mitosis in the animal hemisphere (above the blastopore) than in the vegetal hemisphere. Immunoflourescence was used to measure the brightness of DNA which allowed for the different steps in mitosis to be clearly identified. DNA stained with Hoerchst appears brighter when it is more compacted and concentrated, which are both things that occur while a cell undergoes mitosis. Therefore, we could see evidence to support our hypothesis by measuring the brightness of the nuclei of the cells in the blastula. The Sea Urchin (*Lytechinus pictus*) (Uniprot, 2009) was used because it is an organism that is easily acquired by the Wheaton College lab and undergoes development fairly rapidly compared to other organisms which is advantageous when studying beginning development.

This hypothesis is supported by previously performed experiments and discoveries such as fate maps. The fate map, first primitively discovered by Vogt through experimentation in 1928, allows us to see the patterns that different tissues follow throughout development (Morris, 2009). The main tissue present in the animal hemisphere of the sea urchin blastula is ectoderm. This tissue undergoes a process called convergent extension where the cells must move inwards to cover the blastopore. It is logical that the cells must divide more rapidly to cover a larger area based on what we know about cell fates and how different tissues behave during differentiation and early development.

There have also been other experiments that produced results supporting my hypothesis. One experiment studied the patterns of cell division in embryonic tissues of amphioxus. In their results they found that in an early blastula the cell divisions in the vegetal hemisphere lag behind those in the animal hemisphere (Holland,

2006). They also found that once the cells reach early blastula phase they begin to divide much more asynchronously. This type of division is typical of more rapid cell division with a larger group of cells (Holland, 2006). Another experiment that was testing the evolutionary acceleration of regional differentiation in early development of the Ilyanassa embryo came up with similar results regarding a difference in cell proliferation between different fated tissues in gastropod embryos (Goulding, 2009). Yet another study reported results that support the hypothesis that the rate of mitosis in cells present in the animal hemisphere is greater than that of the vegetal hemisphere (Flickinger, 2001).

II. Materials and Methods

This experiment involved sea urchin blastulae, transfer pipettes, kimwipes, humidity chamber(petri dish, damp kimwipe, parafilm, three epitube caps), three coverslips, coverslip chips, three slides, VALAP, 6 well plate, sterilized forceps, latex gloves, Spot Insight camera, Spot software, Nikon E400 epiflourescence microscope, aluminum foil, hand centrifuge, waste beaker, polylysine, distilled water, 1.5 ml Eppendorf centrifuge tube, and pre-made solutions of PBS-T, MeOH, Hoechst stain, block buffer, anti-acetylated tubulin primary Ab, FITC-DM1A anti-alpha tubulin Ab, and Alexaflour 546 GAM.

The method for this experiment was to follow the directions provided by Henson and Shuster's <u>Immunoflourescent staining of sea urchin embryos, MeOH fixation</u> (Shuster, 2008). The incubation times in this procedure were changed due to the essence of time in our lab, so it did not follow this procedure exactly. The sea urchin embryos went through the process of methanol fixation previous to this experiment and were supplied already fixed. The embryos were then rehydrated with PBS-T solution while being rocked to prevent the embryos from clumping together. The embryos were mounted on three different coverslips that are sterile and cationic. The coverslips were placed in the prepared humidity chamber and the embryos were covered with block buffer. The block buffer was refreshed twice and then the coverslips were labeled. There was one negative control and two experimental coverslips. The negative control only ever had block buffer added to it until the Hoechst was added at the end.

The experimental embryos were stained with the primary antibody solution against acetylated tubulin and incubated first. The embryos were washed off with PBS-T three times and then stained with the secondary antibody against acetylated tubulin and incubated again. The secondary antibody is light sensitive, so the samples were shaded with aluminum foil from then on as much as possible. The embryos were washed off three times with PBS-T again and then the labeled with FITC-conjugated DM1A primary antibody against alpha

tubulin and incubated a third time. This was removed and the embryos were again rinsed with PBS-T three times. Lastly, the Hoechst was added three of the embryos and incubated. Then the embryos were washed with PBS-T buffer three times.

The coverslips were mounted on the slides and prepared with coverslip chips, block buffer, and VALAP. They were also labeled with a sharpie. They were then analyzed with a Nikon E400 epiflourescence microscope with standard Hoechst, FITC, and Rhodamine fluorescent filter sets. Pictures were taken using Spot Advanced software on a Spot Insight camera from Diagnostic Instruments with a 40x plan fluorescent objective. To acquire these photos Dr. Bob Morris's protocol was followed. This experiment took a total of around 6 hours collectively. The data gathered was qualitative which I then translated into semi-quantitative data based on a qualitative brightness scale that I created. Each area of the picture is assigned a level of brightness and compared to the brightness of other areas.

III. Results

We know that the brightness of the DNA and the total tubulin will be greater in cells that are undergoing mitosis because the DNA and the tubulin are more concentrated and more compacted during this process. Therefore, measuring the brightness of the cells in general will gives us evidence of which cells are going through mitosis and then we can analyze whether or not they are in the animal cap based on their location in relation to the blastopore. The results of the control slide showed no immunoflourescence for any tubulin because it was not dyed with any of the stains. These were the results expected for the negative control and proved that the reason the tubulin was glowing is solely because of the solutions the blastulae were placed in.

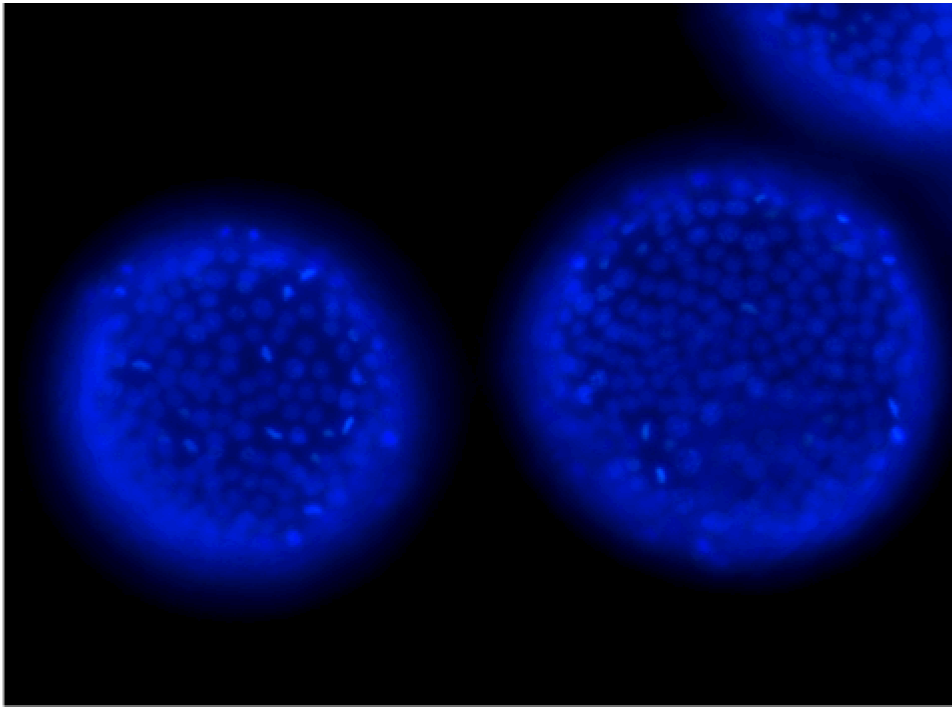


Image 25 – This image is 287 microns across the horizontal axis

This image shows the fluorescence from the Hoechst stain which displays the DNA. Here, the brightness of the nuclei of cells in mitosis is much brighter than the cells not in mitosis. The compact DNA gives off a more concentrated light and appears brighter. Notice the frequency of the cells in mitosis compared to the location of the blastopore (circled) in the right hand blastula. The number of cells in mitosis seems to be greater in the same area than the rest of the cell. This may indicate the location of the animal cap.

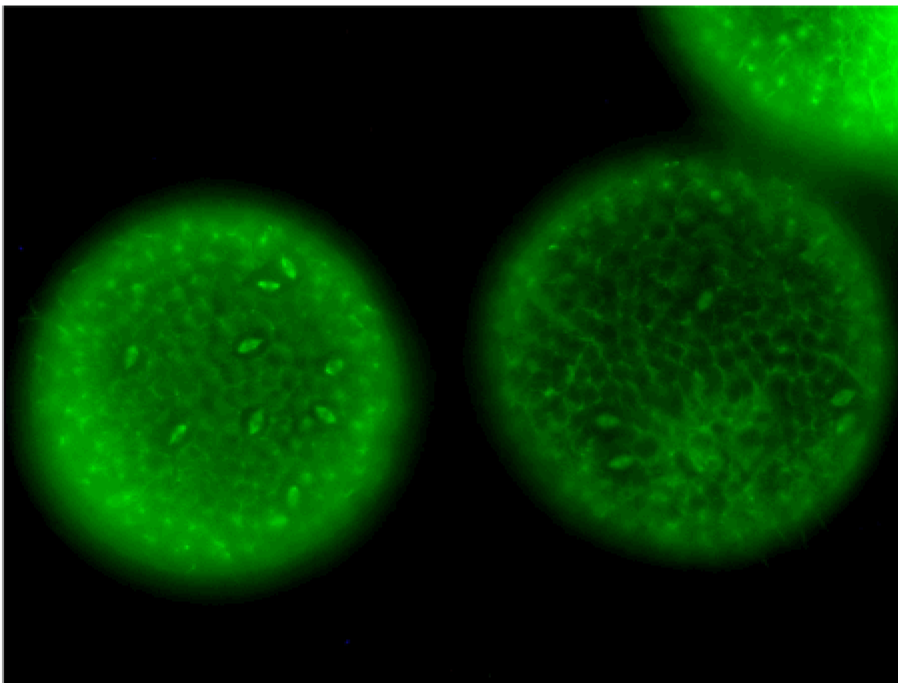


Image 26 – This image is 287 microns across the horizontal axis

This image shows the alpha tubulin present in the blastulae. Notice the cells in mitosis glow a brighter green because they have more concentrated tubulin in the form of the spindle fibers whereas the other cells in interphase are dimmer. The cells in mitosis appear in pairs in certain areas indicating that these areas are experiencing more rapid mitosis than other areas of the blastula which appear to be in temporary mitotic arrest or interphase. The area where the much of the mitosis is occurring may be the animal cap.

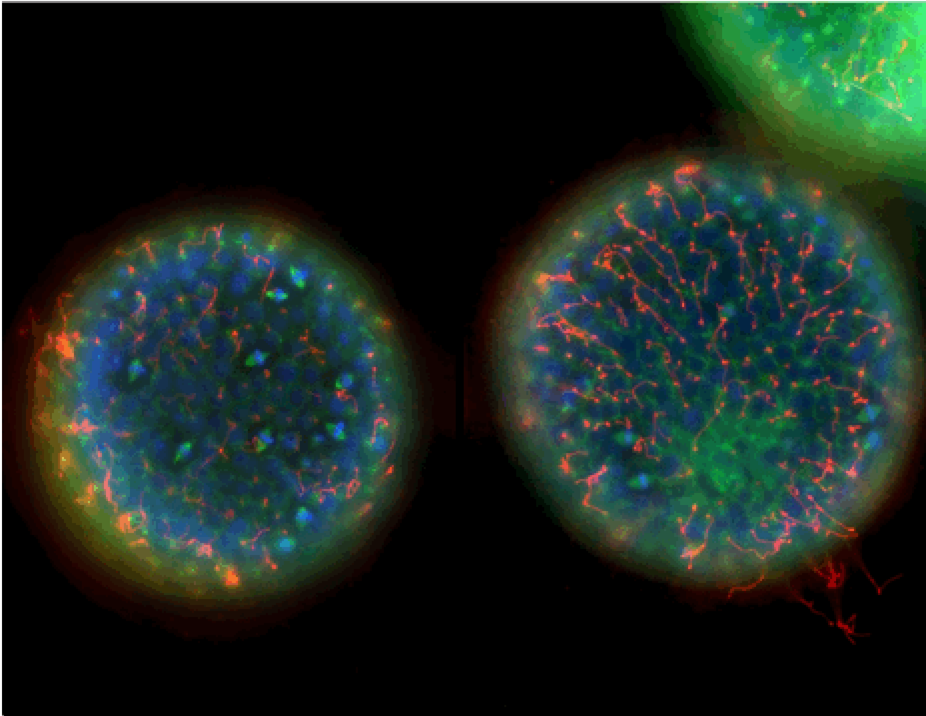
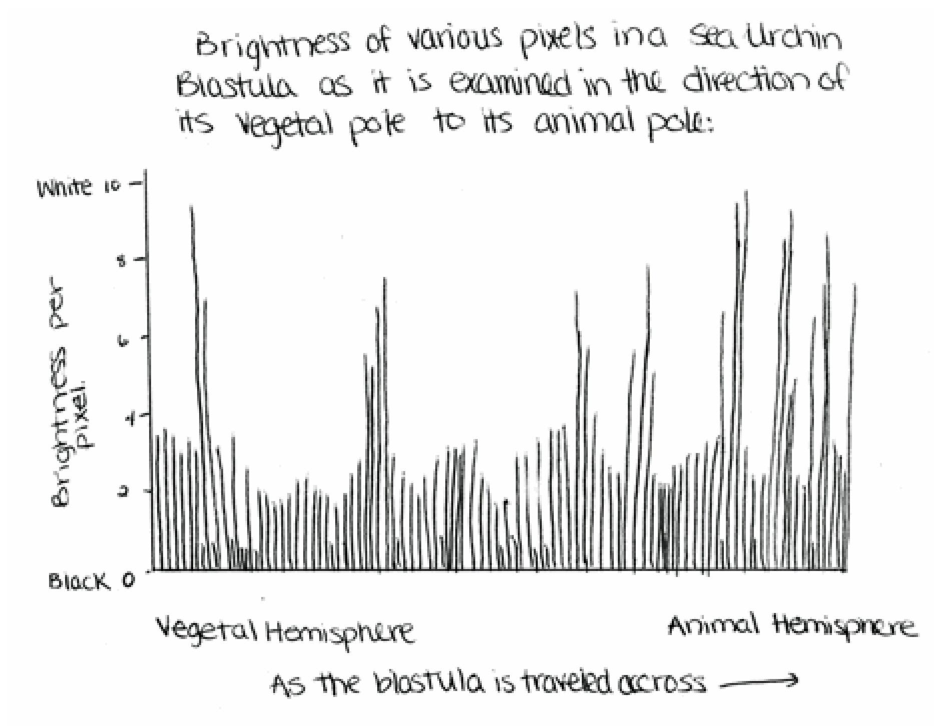


Image 28 – This image is 287 microns across the horizontal axis

Here, the overlay of all of the fluorescence that we tested for was generated into one image. The combination of the brightness of the DNA and the alpha tubulin is very obvious in this image and stand out very well. The animal cap of the blastula on the left side may be facing outward towards the reader. This area of the blastula is growing more rapidly than the leftmost area of this blastula which seems to be lacking in many cells that are undergoing mitosis.



This graph shows the brightness of the cells in the blastulae as one looks at the blastulae from the vegetal hemisphere to the animal hemisphere. Notice how the highest brightness lines, while present in some areas on the vegetal hemisphere, are much more prevalent in the animal hemisphere. Since greater brightness of the DNA and the total tubulin is an indicator of the number of cells in mitosis this graph shows that there are a greater number of cells undergoing mitosis in the animal hemisphere of the blastulae than there is in the vegetal hemisphere.

IV. Discussion and Conclusions

From these data it can be concluded that the animal hemisphere may undergo more rapid mitosis in a late sea urchin blastula. It can also be concluded that mitosis tends to occur in clusters or pairs, indicating more rapid growth in specific areas of the blastula than in other areas. Based on the location of the blastopore in images 25, 26, and 28 it is possible that the areas that were experiencing rapid mitosis were in the animal hemisphere (Holland, 2006). In a sea urchin blastula when gastrulation begins and the archenteron is formed, the ectoderm (main tissue in the animal cap) must undergo a process called convergent extension (Gilbert, 2006). The ectoderm must extend and grow around the blastopore so that the entire blastula is covered in ectoderm (Gilbert, 2006). This process would require a faster generation of these cells to cover a greater area while the other cells in the vegetal hemisphere are dividing more slowly

or at their regular pace, which would seem slower in comparison to the rate of mitosis in the animal hemisphere.

If this experiment had been repeated a thousand times the sample size would be much larger. A larger sample size yields less percent error. If the sample size is increased by a factor of four, the percent error is decreased by a factor of two. Therefore, the conclusions would be supported by more data with less percent error creating greater confidence in the results if the experiment was performed one thousand times.

The sources of error for this experiment were present, but their effects were minimal due to the fact that the data gathered supported the hypothesis. Possible sources of error could have been a faulty humidity chamber. If the humidity chamber was defective then the embryos on the coverslips may have dried out which would produce faulty images. It is also possible that the deviation from the recommended incubation times in Shuster's directions affected the results. Altering the amount of time that each stain solution was applied may have altered the strength of the fluorescence in the results. If the antibody had been left on for longer it may have created a stronger fluorescence in areas that seem weak in the current results (Shuster, 2008).

To refine this experiment I would allow for more time in the future. I feel as though this experiment was somewhat rushed which created more room for error. It is always better in an experiment to have more time than you need rather than less. I would also use a more precise microscope if I was able to have access to the equipment. Lastly, I would include many more trials of different blastulae to determine if the same brightness and clustering of mitotic cells phenomena was happening in all of the different blastulae. The more data there is the more confident one can be in his/her results.

In future experiments it would be interesting to separate the animal cap from the rest of the blastula and perform the same immunofluorescence. This process may be able to allow a direct comparison of the proliferation of the ectoderm cells versus the proliferation of the cells in the vegetal hemisphere. By initially identifying these two hemispheres it would be much easier to compare the results and draw conclusions from them.

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