

The Affects of Salinity on Mitosis in *Lytechinus Variegatus* Sea Urchin Embryos

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I. Introduction

This lab will be examining the effect that salinity has on the development of sea urchin embryos. Rhodamine Phalloidin will be used as a means of highlighting actin filaments with florescent microscopy. By doing this actin will be able to be photographed and the role of actin during mitosis and how varying salinities affect its role in mitosis will be recorded. In the second portion of the experiment Hoescht, another fluorescent stain, will be used to observe the nuclei in the embryos and how the nuclei are affected by varying salinities. Salinity is an important variable to test in sea urchin embryos because sea urchins live in tide pools that have varying salinity concentrations depending on weather. For example, when tide pools are exposed to extreme sunlight evaporation occurs and the salinity concentration increases. In contrast when tide pools are exposed to excessive rains the salinity decreases. Sea urchin embryos must be able to adapt to these constantly changing conditions. It is for this reason that studying the carrying effects of salinity on the mitosis in sea urchin embryos is significant

Mitosis is a process of cellular division. After fertilization has occurred the sea urchin zygote begins a process of cell division to form the early embryo. The process of mitosis consists of six stages: interphase, prophase, metaphase, anaphase, telophase and cytokinesis. Interphase is the longest phase of mitosis during which DNA replicates, the centrioles divide, and proteins are actively being produced. During prophase, the first mitotic stage, nucleolus fades, chromatin condenses into chromosomes and the microtubules within the cytoskeleton disassemble. The microtubules are used to form the mitotic spindle. Following prophase is metaphase. During metaphase the spindle fibers align the chromosomes along the metaphase plate. Throughout anaphase the spindle fibers shorten and the daughter chromosomes are pulled apart. Finally during telophase the daughter chromosomes arrive at separate poles and the spindle fibers begin to disappear. To end the cell division cytokineses occurs when the actin filaments pinch the cell membranes resulting in two identical daughter cells. The microtubules then reorganize and prepare for interphase again (Cells Alive, 2004). It takes the sea urchin embryo approximatley one hour to undergo it's first mitosis.

Actin is a globular protein with an ATP binding site in the center of the molecule. Monomers of actin polymerize to form long thin fibers that are approximately 8nm in diameter and are known as microfilaments. The actin filaments have multiple functions. They form a band beneath the plasma membrane that provides mechanical strength to the cell, links transmembrane proteins to cytoplasmic proteins, anchors the centrosomes during mitosis and pinches the dividing cells apart during cytokinesis (Kreis, 1993 p. 13). The Rhodamine Phalloidin will allow the experimenter to see the role of actin in mitosis and other cell processes by highlighting the actin filaments (Wilt & Hake, 2004 p. 24).

The nucleus is the region in the cell that contain the DNA. During mitosis the DNA in the nucleus turns into chromosomes and is then duplicated to create another copy of DNA for the newly forming cell. Towards the end of mitosis two new nuclei form on the opposite sides of the cell and when cytokinesis occurs, the two nuclei form the

nucleus' in each daughter cell (Biology Project, 2004). The Hoescht stain will highlight the DNA within the nuclei a blue color, so the nuclei will be visible on each side of the cell on the fixed slides. This will allow the experimenter to determine the effect of salinity of the nuclei of the sea urchin embryo (Belmont Lab, 2004).

The average salinity for northeastern seawater is approximately 35 parts per thousand (ppt) (US Hydrology Investigation, 2004). I predict that the eggs will be more sensitive to a higher concentration of salt than to a lower salinity and that the effect of the high salinity on the embryos will prohibit the actin from initiating cytokinesis, likewise I do not think that the nuclei will develop properly, if at all, due to the stressful conditions. I believe the low salinity will not have an effect on the mitosis of the embryo and therefore the actin and nuclei will act accordingly during mitosis.

II. Materials and Methods

- female and male sea urchins
- KCl
- Filtered Natural Sea Water
- Distilled Water
- Rhodamine Phalloidin
- Poly Lysine
- PBS buffer
- 3% BSA PBS buffer
- 3% BSA PBS buffer with Rhodamine Phalloidin
- Slides and cover slips
- Hoescht stain
- Pipettes
- Frozen Methanol
- Varying concentrations of sea water (33ppt and 39ppt)
- Sea Salt
- Pedestals
- Culture Cluster dish
- Salinity Tester
- Microscope that can observe fluorescence

Procedure:

- Create the different concentrations (33ppt as average salinity and 39ppt as high salinity) of filtered natural seawater by diluting natural seawater with distilled water and by adding sea salt to the natural seawater. Test using salinity tester.
- Inject the male and female sea urchins with KCl to shed them. Shed the female urchin eggs into natural seawater and shed the male sperm onto parafilm.
- Rinse the sea urchin eggs in the different salinity concentrations (33ppt and 39ppt).
- Observe a drop of the eggs in the filtered natural seawater (33ppt) under the microscope to make sure that the eggs are viable.
- View the eggs in the different salinities under a microscope to see the effect that the salinities have on the unfertilized eggs. View the eggs in the different salinities three times and record observations.
- Observe sperm in the different salinities (33ppt and 39ppt) of filtered natural sea water to see if they are viable. Observe the sperm in each salinity three times and record data.
- Obtain PBS buffer. This will be used to wash the eggs.
- Create 3% BAS PBS buffer solution. Do this by adding 1.5g of BAS to 50 mL PBS. This will be used to but the embryos in block.
- Then create another 3% BAS PBS buffer solution with Rhodamine Phalloidin in it. Do this by adding 30 micro liters

- of Rhodamine Phalloidin to 600 micro liters of 3% BAS PBS buffer solution. This will be used to put the embryos in block and to stain them. Be sure to keep this mixture refrigerated and in the dark because the Rhodamine Phalloidin cannot be exposed to light.
- To create a 3% BAS PBS buffer solution with Hoescht by adding 50 micro liters of Hoescht to 900 micro liters of 3% BAS PBS buffer. Keep this solution refrigerated and in the dark because the Hoescht cannot be exposed to the light (Cell Biology Education, 2003).
 - Obtain Methanol and put it in the culture cluster dish and place in a subzero freezer.
 - Add polylysine to two cover slips. Allow the polylysine to sit on the slides for 30 minutes, then rinse with distilled water and let them dry.
 - Rinse eggs in 33ppt FNSW and activate sperm in 33ppt FNSW
 - Fertilize eggs.
 - As soon as the embryos begin cytokinesis, place the embryos on the cover slip that has been covered with poly lysine. Place the embryos on the side of the cover slip with the poly lysine on it.
 - Place the cover slip into frozen methanol in the culture cluster dish overnight to fix the slides.
 - Use a pipette to remove the methanol from the culture cluster dishes.
 - Wash the cover slip 3 times with PBS buffer by putting enough buffer in the culture cluster dishes to cover the slips. Let them sit in the PBS buffer for three minutes before rinsing them each time. Rinse them by removing the PBS buffer with a pipette and putting fresh PBS in the dishes.
 - Once the eggs are washed with the PBS buffer rinse them with the 3% BSA PBS buffer without Rhodamine Phalloidin, using the same method as the PBS buffer. Let them sit in this block solution for five minutes.
 - Following that wash, place the cover slips on pedestals, wash one of the cover slips with the 3% BSA PBS with Rhodamine Phalloidin solution. Let it incubate in this solution for one hour. Remember to do this in the dark because the Rhodamine Phalloidin cannot be exposed to light.
 - Leave the other slide on the pedestal and do not use and stains on it, it will be a control.
 - Then place the cover slip back in the culture cluster dishes and wash the slide with PBS buffer three times, letting it sit in the buffer for three minutes each time. And then rinse it in 3%BAS PBS buffer to block them. Use the same method of washing as used before (Cooper Lab, 1997).
 - Place the cover slip back on the pedestal and cover it with the Hoescht 3%BAS PBS solution. Let it sit for five minutes. Remember to do this in the dark as well because the Hoescht cannot be exposed to the light (Cell Biology Education, 2003).
 - Then place the cover slips on slides, put the side with the embryos face down on the cover slip. Remember to label slides with a sharpie.
 - Let excess liquid dry, then seal the slide with nail polish and place the slide in the refrigerator until ready to observe.
 - Repeat procedure for 39ppt FNSW except only use one slide, there is no need for a control with this salinity.
 - To observe the slides obtain a microscope used for observing micro-fluorescents.
 - Turn off all the computers and other electronic equipment surrounding the fluorescent scope.
 - Turn on the mercury bulb. After the mercury bulb is lit the other electronic equipment surrounding it can be turned back on.
 - Let the mercury bulb warm up for ten minutes.
 - Turn off the lights in the lab and remove the slides from the refrigerator. This will allow for the fluorescents to not be exposed to the light and also will make viewing the fluorescents easier.
 - Then view the slides under the regular setting on the microscope to find cells.
 - Once a cell is found center the microscope on the cell under the 40x setting.
 - Then cover the light lens and turn on the microscope onto the mercury bulb.
 - Look at the cells under the 330nm setting to see the Hoescht fluorescents. Take pictures and record data.
 - Look at the cells under the 400nm setting to see the Rhodamine Phalloidin fluorescents. Take pictures and record data. (Cell Biology: Microscopy Lab, 2004).

III. Results

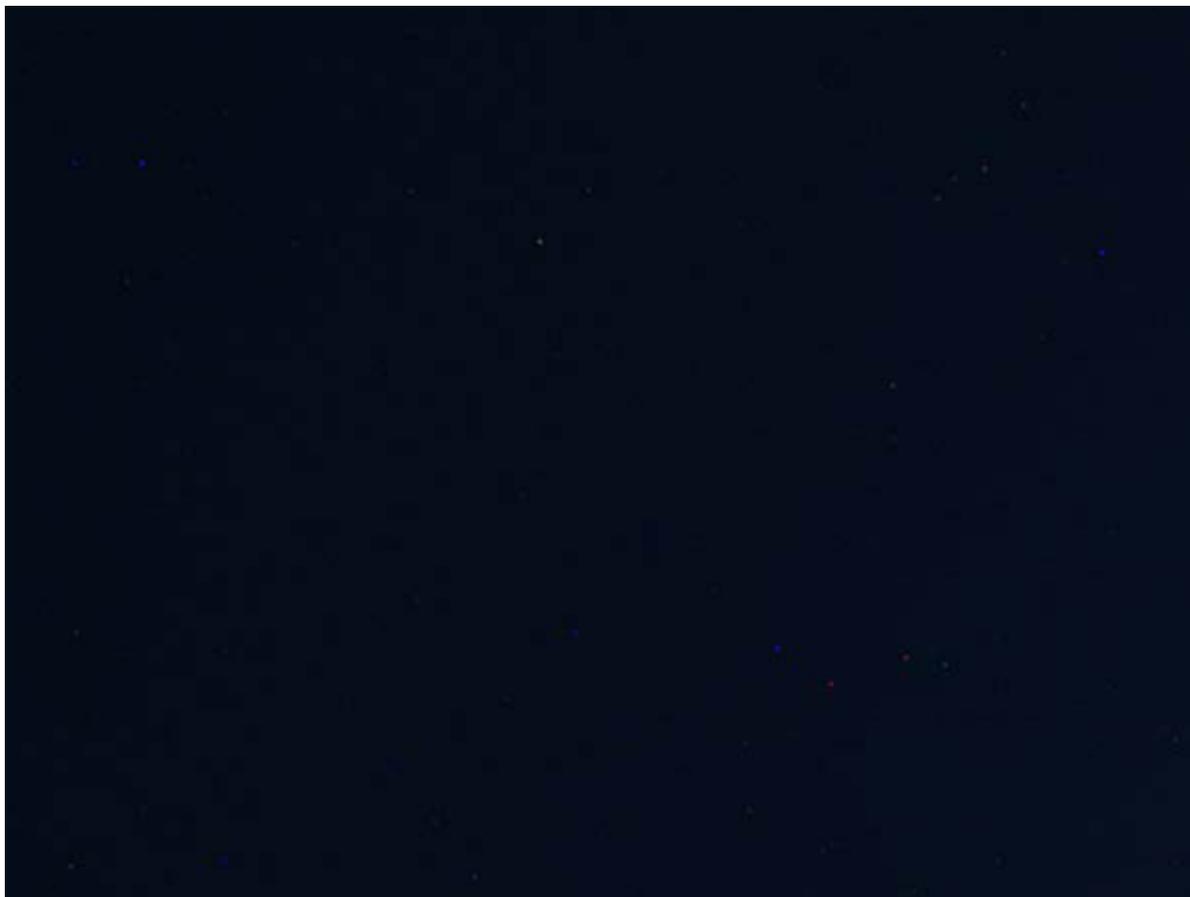


Figure 1: 33ppt Negative Control



Figure 2: 33ppt with Rhodamine Phalloidin and Hoescht Staining



Figure: 39ppt with Rhodamine Phalloidin and Hoescht Staining

The data that was gathered in this lab was unexpected. When the eggs and sperm were observed in the 33ppt filtered natural sea water they were viable. When the eggs were fertilized with the sperm in the 33ppt sea water the first time, fertilization envelope lift off occurred; however, the eggs never underwent mitosis. This also occurred in the eggs that were in 39ppt filtered natural sea water; the eggs and sperm were viable and were fertilized successfully, but they never underwent mitosis. Because the eggs did not undergo mitosis the experiment was started a second time. The second time, the eggs and sperm in the 33ppt filtered natural sea water were viable again. This time the eggs were successfully fertilized and mitosis did occur. The same happened in the 39ppt filtered natural sea water. As soon as the cell membrane began to pinch the eggs were placed on the cover slips and dropped in the cold methanol. The eggs sat in the cold methanol over night and then they were washed with buffers and stained with the fluorescents. Then the eggs were placed on the slides and sealed with nail polish. They were kept in the refrigerator and then observed using the microscope. However, there were no embryos on the slides and there were also no remaining sperm on the slides. The embryos had either been washed off in the process of preparing the fixed slides or no embryos made it on the cover slip from the slide. Therefore, there were no results for how the salinity of the water affects actin in the embryos during mitosis or how salinity affects the nuclei during mitosis. For this reason I cannot support or refute my hypothesis about the effect of salinity on sea urchin embryos.

IV. Discussion and Conclusions

The slides that were created ended up having no cells on them. This could have happened for two reasons. One of the reasons could have been that no embryos were obtained from the slides that had the fertilized embryos on them when I was trying to transfer them to the cover slips. The other reason is that there could have been embryos that were washed off by the buffer when they were washed. To avoid having this happen the next time this experiment is conducted there are several changes that can be made. One of the changes would be to make the concentration of fertilized eggs on the slide, which contain the fertilized embryos that will be transferred to the cover slip, greater than it was on the original slide. This would make it easier to ensure that there would be embryos on the cover slip. To prevent the embryos from washing off the slides when they are washed with the buffer, the slides may need to be fixed for longer in the methanol than they were, or fixed in a colder methanol solution so that they are more fixed to the cover slip. Another solution would be to not wash the cover slips as much as the protocol called for in this experiment. This would give the embryos less of a chance to wash off of the slide and therefore providing a better chance of acquiring data. Another problem may have been that the poly lysine coat, on the cover slips, did not work, which would allow the embryos to wash off the slides and not stick to them. One more thing that should be changed is that there should have been more trials done in this experiment. Instead of doing three trials there should have been nine trials, three trials for each salinity and three trials for each control. By doing this the chances of getting data would be greater than it was by having so few trials. By making these changes to the protocol, the odds that the fixed fluorescent slides may have embryos on them, that would provide results as to what the effect of high and low salinities are on the actin and nuclei of sea urchin embryos during the mitosis, I believe would greatly increase.

V. Bibliography

Belmont Lab. Large Scale Chromatin Structure and Chromonema Fibers.

Retrieved November 14, 2004 from

<http://www.life.uiuc.edu/belmont/WhatWe'veDone/lscs.html>

Trial User Page 9 12/2/2004 Trial User Page 9 12/2/2004

Cells Alive. (2004). Animal Cell Mitosis. Retrieved October 24, 2004 from <http://www.cellsalive.com/mitosis.htm>

Cell Biology Education (2003). Apoptosis: A Four-Week Laboratory Investigation for Advanced Molecular and Cellular Biology Students. Retrieved November 14, 2004 from

<http://www.cellbioed.org/articles/vol2no4/article.cfm?articleID=85>

Cell Biology: Microscopy Lab. Introduction to Fluorescence Microscopy.

Retrieved November 14, 2004 from

<http://dept.kent.edu/projects/cell/FLUORO.HTM>

Cooper Lab. (1997). Rhodamine Phalloidin Staining. Retrieved November 14, 2004 from

<http://www.cooperlab.wustl.edu/Methods/PhalloidinStain.html>

Kreis T & Vale R. Guidebook to the Cytoskeletal and Motor Proteins. New York. Oxford University Press. 1993.

Hydrology Investigation. Salinity: Hydrology Investigation. Retrieved October 24, 2004 from

<http://www.globe.gov/hq/trr/hydro/hydsal.ppt>

The Biology Project, the Department of Biochemistry and Molecular Biophysics University of Arizona. (October 2004). The Cell Cycle and Mitosis Tutorial. Retrieved November 14, 2004 from

www.biology.arizona.edu/cell_bio/tutorials/cell_cycle/cells3.html

Wilt F. & Hake S. Principles of Developmental Biology. W.W. Norton & Company, Inc. 2004.

I also used multiple handouts from Professor Morris' Developmental Biology 254 Wheaton College Fall 2004 and class notes and information from Professor Morris during office hours. This information was used as background knowledge.