Sea Urchin Coelomocytes Mitochondrial Activity Is Affected by the Addition of Yeast Cells

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I. Introduction

The behavior of mitochondria in sea urchin coelomocytes was observed with and without the addition of yeast cells. Fluorescence microscopy was used to capture images of coelomocytes with and without yeast cells to be analyzed. The sea urchin specimens used for this experiment were Lytechinus variegatus. They were taken from off the coast of North Carolina and stored in the Science Center. The coelomocytes of these animals were studied because

they are immune cells in the sea urchin specimen. Because these cells are quite similar to erythroid cells in other organisms such as humans they can undergo drastic morphological changes, and can be removed by exocytosis (PubMed). The mitochondria of these organisms were the focus of this experiment. Mitochondrai play a critical role in the metabolic energy in eukaryotic cells and are responsible for energy converted to ATP by oxidative phoshphorylation (Cooper and Hausman, 2004). They consist of an inner and outer membrane which are separated by an intermembrane space (Cooper and Hausman, 2004). This inner membrane is in folds called cristae that go into the interior matrix of the mitochondria itself (Cooper and Hausman, 2004). The hypothesis that more activity would be going on in the cell due to the addition of yeast cells to that cell, the more dispersal of the mitochondria was tested. This hypothesis will be investigated because it gives us information about whether or not activated mitochondria move toward the centrosome of the cell or if they move outward and scatter when at higher energy levels. The importance of this is to prove whether or not dispersal of the mitochondria depends on where the active part of the cell is. When mitochondria stray to the outer parts of the cell, there is activity there and when they stay towards the center, there is activity there. In other words, mitochondria move to where the activity is occurring within the cell. In this study, coelomocytes were extracted from the coelom of sea urchins, stained with Rhodamine 123, yeast was added to selected slides, and viewed under a microscope in order to obtain images that could be analyzed. Rhodamine 123 was used because it fluoresces the mitochondria of cells. These images were then used to measure inter-mitochondrial distance and brightness levels to determine whether the scattering reflected the addition of yeast and whether mitochondrial activity increased and therefore brightness increased with this addition.

II. Materials and Methods

In order to carry out this experiment, several materials were needed.

- Falcon 14mL – 17x100mm Sterile/Gamma Irradiated Polystyrene, round bottom tube with snap cap
- 3 c.c. syringe with 16.5 gauge needle
- VALAP (Vaseline, Lanolin, and Parafin)
- Nikon Eclipse 80I microscope at 63x with a .76x magnifying lens with Differential Interference Contrast and fluorescence microscopy
- Sea urchins
- Microscope connected to SPOT RT Color Camera by Diagnostic Instruments, Inc with a .76x magnifying lens
- Poly-lysine
- Rhodamine 123 at a concentration of 4μL
- PBS Buffer (Phosphate Buffer Solution)
- 4 coverslips and 4 slides
- 6-well plate
- Falcon Blue Max Jr. 15mL Polypropylene Conical Tube 17x120mm style Nonpyrogenic 50/rack with twist cap

- Aluminum foil
- Yeast cells
- Distilled water
- Adobe Photoshop 7.0

Sea urchin coelomocytes were extracted by injecting a 16.5 gauge needle into the peritoneal cavity and using a 3 c.c. syringe to gather them. The cells were then placed in a Falcon 14mL – 17x100mm Sterile/Gamma Irradiated Polystyrene, round bottom tube with snap cap. Rhodamine 123 was prepared to 4μL in PBS. A six well plate was made up with 4 coverslips in the first four wells as shown in Figure 1.

WELL 1	**WELL 2**	**WELL 3**
Rhodamine 123 and Yeast	Rhodamine 123 only	
WELL 4	**WELL 5**	**WELL 6**
Rhodamine 123 and Yeast	Rhodamine 123 only	

Figure 1. The six well plate with the four coverslips and the contents of the coverslips

Poly-lysine was added to all 4 coverslips by covering the coverslips with it and waiting 5 minutes before rinsing and washing with PBS. Two drops of coelomocytes were added to each coverslip and then each well was filled half way with the PBS buffer. After 5 minutes, the PBS was drained off and the cells were stained with the Rhodamine 123. The Rhodamine 123 needed to be covered with aluminum foil directly after making because of the loss of fluorescence in light. The stain was added to all 4 coverslips and the 6-well plate was then covered with aluminum foil and left on the counter-top at room temperature for 30 minutes. After the 30 minutes was up, Rhodamine 123 solution was drained off the cells and they were re-suspended in PBS buffer for 5 minutes while still being covered with the foil. After 5 minutes, the PBS was drained off. Yeast was then added to coverslips 1 and 4 in a PBS solution. The coverslips were mounted onto slides using the slip-chip method. The coverslips were sealed onto the slides using VALAP (Vaseline, Lanolin, and Parafin) and rinsed with distilled water. Then, the slides were examined under a Nikon Eclipse 80I microscope at 63x with a .76x magnifying lens with Differential Interference Contrast and fluorescence microscopy and a SPOT RT Color Camera by Diagnostic Instruments, Inc was used to capture images, either DIC or fluorescent.

In order to measure the dispersal of mitochondria the pictures of choice were opened and analyzed using Adobe Photoshop 7.0 on the PISCES computer. In order to analyze these images, we went to the VIEW tab and clicked on the RULERS option so that the page would now be lined with rulers. Finally, the spots on the image that were furthest

away within each cell were measured using the centimeters on the rulers given in the photoshop. On each slide there were either 2 or 3 cells measured and 12 cells were measured in total. This was only for one trial of slides made with the described procedure.

III. Results

After performing this experiment as described in the methods section before, I got results that were easy to interpret and therefore easy to understand. The table below shows the outcome of the measurements taken during the procedure. The cells were numbered from left to right in each image taken as in Figure 3 below.

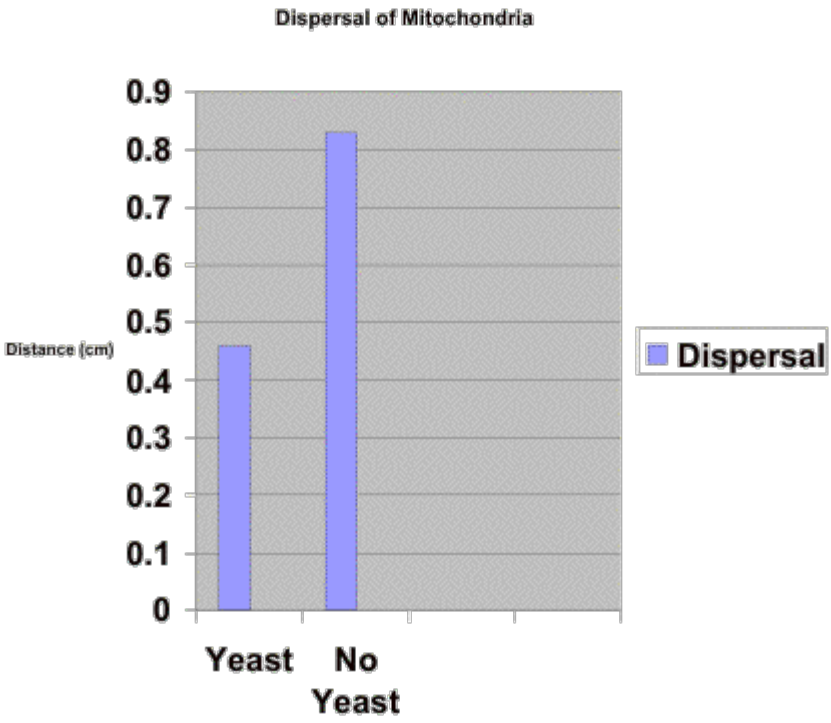

Figure 2. This is a graphical representation of the dispersal of the mitochondria in coelomocytes with and without the presence of yeast.

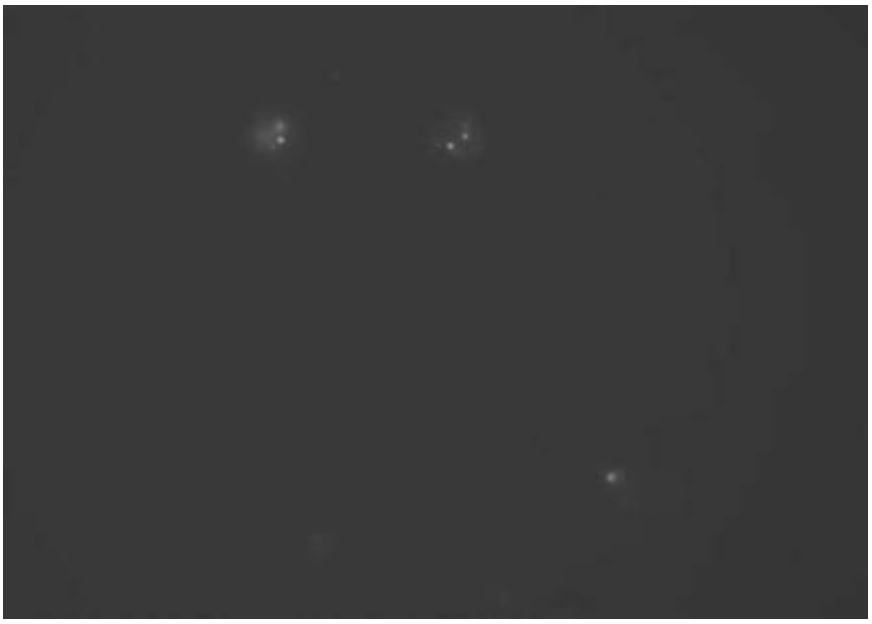

Figure 3. This image is fluoresced coelomocytes from the sea urchin. In this image there is the presence of yeast and the coelomocytes are the glowing spots.

Important trends exist in Figure 2 above showing that dispersal decreased when yeast cells were added.

IV. Discussion

The hypothesis is not supported by the results of this experiment. From Table 1 above, you can see that when there was yeast present in the sample, the inter-mitochondrial distance was shorter than when no yeast was present. This contradicts my hypothesis because I believed that the addition of yeast would increase the activity of the mitochondria, therefore they would scatter. This theory was introduced by the fact that neuronal cells display these properties where the mitochondria move toward the area with the most activity. Therefore, the activity in these cells was towards the center and not in the outer parts. However, it is known that when yeast is added the activity is increased. The activity increases when yeast is added because the coelomocytes cells are programmed to digest the yeast cells and other foreign cells upon them entering into the coelom of the sea urchin. Therefore, the mitochondria must have been in the center of the cell to begin with because that is where they were when the images were taken. There is no evidence suggesting that the mitochondria moved toward the center upon the addition of yeast cells because in the images with no yeast, the mitochondrial glow exists in the center with other mitochondrian that are dispersed farther away from the center.

There were some sources of error in my experiment. When adding yeast cells to the samples on the coverslips, 2 drops were added to each one. However, within these drops there were more yeast cells than coelomocytes present on our coverslips, inundating our coelomocytes cells. This over-abundance of yeast showed up in the images taken and it appeared as though the yeast actually made it onto the cell layer of the slide. This could have been caused because there is the slide layer and the coverslip layer with a space between provided by the coverslip chips. Therefore, the yeast cells could have been on the slide layer while the coelomocyte cells were on the coverslip. But, after careful searching with the microscope, yeast cells and coelomocytes were found on the same layer and interacting with each other. As another source of error, less light would be allowed into the slides after being stained with Rhodamine 123.

In comparison to my collaborator, Suzanne Frasca's, results, we achieved the same conclusion. We both found that with increased activity due to the addition of yeast cells, the mitochondria responded oppositely to our hypotheses. Her hypothesis was that with increased activity due to the addition of yeast cells, the brightness of the mitochondria under fluorescent microscopy would increase. However, the brightness was greater in those cells not exposed to the yeast. In order to refine this experiment and do it differently next time, a less concentrated amount of yeast would have been used.

This procedure produced results that were interpretable, even though they did not prove mine or my collaborators hypothesis. The Rhodamine 123 staining procedure worked because the images we gathered were fluoresced. Upon performing this experiment again, the same procedure can be followed but different food sources can be added or a bacteria instead.

Bibliography

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