Evidence that Pseudopodia Formation Decreases with Cytochalasin B

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Introduction

Amoeboid movement is the result of a combination of chemical reactions, one of which involves the actin cortex. An Amoeba has projections off the main body called pseudopodia, or false feet. The actin cortex is a thick structure made up of small repeating subunits just under the plasma membrane; it forms lamellapodia in the pseudopodia. This cortex undergoes a gel to sol transition, where the filamentous actin depolymerizes. The depolymerization causes water to flood into the cell, down its concentration gradient. The globular actin reassembles into a gel form with filamin and fimbrin holding the actin strands in gel form. Water then moves out of the amoeba. This transition enables the amoeba to crawl (The Cell, 2007).

This lab focused on the number of pseudopodia present when an amoeba was exposed to cytochalasin B. Cytochalsin B has a large impact on this function as the fungal toxin binds to the barbed end of the filamentous actin molecule. It is often used for studying contractile microfilaments (Sigma Aldrich). This particular cytochalasin has many side effects and so is excellent for short tissue experiments, but not ideal for studies for over an hour or two (Morris 2008). Studying the role of actin in Amoeba Proteus amoeboid movement is critical because without filamentous actin cortexes, the amoeba cannot move. The specific actin dynamics of this motion were studied previously, and it was found that actin polymerisation is involved in formation of the contractile and adhesive layers of the cytoskeleton (Pomorski 2007). Destroying or inhibiting the formation of the actin cortex leaves the organism without the ability to carry out critical functions, such as moving away from harmful areas of its environment.

The actin cortex of the Amoeba Proteus is well studied. Research has been done exploring how injected muscle actin impacts the normal behavior of an amoeba. The foreign actin is incorporated into the actin component of the amoeba, and the behavior of the new actin can be observed (Hoffmann 1983). Research is also done where Amoeba proteus kill other organisms, such as salmonella. In order for the amoeba to kill another organism, it needs to be motile. Since motility is based in the actin cortex and microtubule complex, it is crucial to understand these structures

thoroughly (Savant 1964).

There has been research that specifically looks at the effects of fungal toxins, such as cytochalasin B, on cytoplasmic streaming. Studies found that cytochalasin B causes irreversible changes to the cell that inhibits cytoplasmic streaming (Florescence 1981). In this lab, we looked at a slightly different behavior, the formation and size of pseudopodia. Polymerization and depolymerization behavior is important to study because it can give quantifiable information about the effects of fungal toxins on the actin cortex, which can have implications in other research projects. To test the effects of cytochalasin B on the actin cortex, an amoeba was exposed to a forty micrograms/ milliliter solution and observed, along with a control group, over the course of thirty minutes. We tested the hypothesis that the cytochalasin B would cause the amoeba to have fewer pseudopodia.

Materials and Methods:

Two chip chambers were made using cover slip glass chips. Amoebae were injected onto the slides using a transfer pipette. A cover slip was then placed on top of the liquid and any excess water removed with a transfer pipette. Two sides of the cover slip were sealed using valap and a small brush to form a flow chamber. Each of the slides was labeled with sharpie and then observed. Pictures were taken of each amoeba, one picture every five seconds over the course of a minute. These pictures and all others in the lab were taken with the 10x lens at 100 magnification with a Sony DFW-X700 camera mounted on a Nikon Eclipse E200 microscope and viewed with Cancer Imac using BTV. The first and last pictures were then compared and the number of pseudopodia counted. A pseudopod was defined as a finger-like projection off the main body of the amoeba that was at least 8 microns wide and 9.5 microns tall (Jahnke, Glass 2008). The base was defined as where the two sides of the pseudopodia diverged from being parallel (Morris 2008). After the preliminary observations were made, those involved in the experiment donned gloves.

The test slide was placed on a paper towel and cytochalasin B, purchased from Sigma Chemical Company and prepared at a concentration of forty micrograms per milliliter was applied to one side of the flow cell with a transfer pipette and wicked through with a Kim wipe three times. The volume of fluid under the cover slip was replaced three times. This process was completed with the experimental slide while the control was left alone. The treated slide was then observed for a minute twenty minutes after the drug application. Pictures were taken every five seconds for one minute under the same conditions as outlined above. Two more pictures were taken thirty and thirty-one minutes after the drug application. The number of pseudopodia was counted in one picture from the twenty-minute series and also in the picture taken thirty-one minutes after application. Over the course of the lab, the cells were hydrated with the same pond water they came from. Water was applied with a transfer pipette at the side of the cover slip and any overflow was dried with a Kim wipe.

The first and last image from each series of pictures received a scale bar and was analyzed in Photoshop. Using the marquis tool to select a width of pixels equivalent to one hundred microns did this. The ratio of pixels to microns used was .588. After the selection was made, the selection was saved and then opened in a new document. The document was filled with black using the paint bucket tool, and then dragged with the move tool onto the image. The text tool was used to identify the length of the scale bar.

The number of pseudopodia in each was analyzed graphically using Microsoft Excel. The number of pseudopodia was entered under the cell corresponding to the slide. The information was then highlighted, and using the mapmaker tool, displayed as a bar graph.

It should be noted that the control group used for this experiment was not the correct control. The control used should have been exposed to DMSO, which was the solvent for cytochalasin B. The control slide should have been exposed to the DMSO three times so a complete exchange of fluid could occur (11/19/08 26 JAE N) (Morris 11/08). The graphs created in Microsoft Excel should contain error bars if the experiment included averaged data (Morris 11/08).

Results:

In the control group, some of the pseudopodia branched off the main body of the amoeba, while others branched off existing pseudopodia.

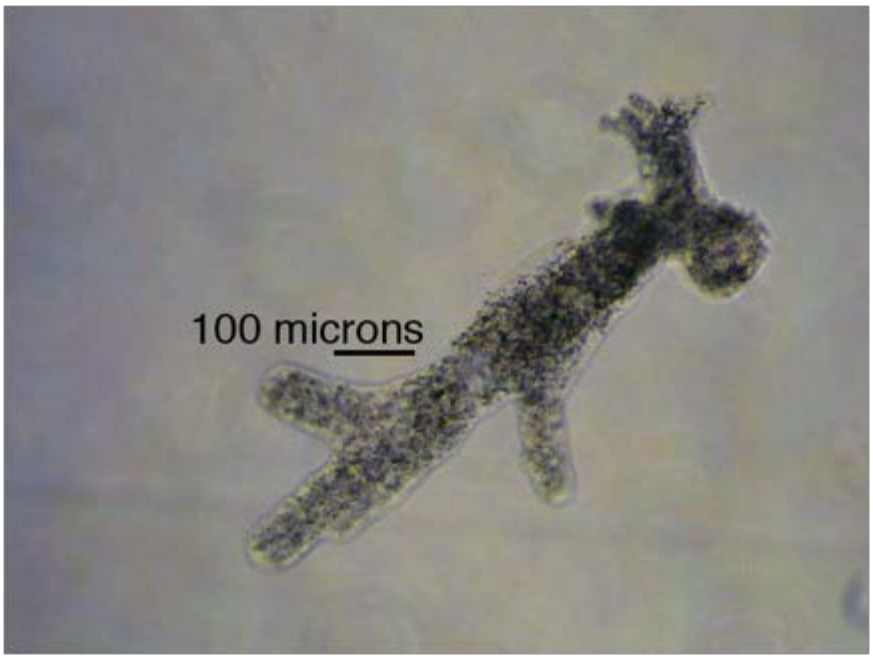

Figure 1. Scale bar 100 microns. In this image, the amoeba has six pseudopodia.

After the drug was added to the system and the amoeba was exposed for about twenty minutes, the amoeba formed one long pseudopod. The cytoplasm appeared to be flowing more slowly from one end to the other, and swirled more than before the drug was added.

Figure 2. Scale bar is 100 microns. This image was taken 20 minutes after the amoeba was exposed to Cytocholasin B. In this picture, the pseudopod is much more extended and is only forming in one direction.

After thirty minutes however, the amoeba was a round blob with no pseudopodia visible. There was no visible cytoplasmic streaming, and the amoeba appeared dark and unmoving. There were small clear regions around the outside perimeter of the amoeba, but there was no pseudopodia formation or cytoplasmic streaming.

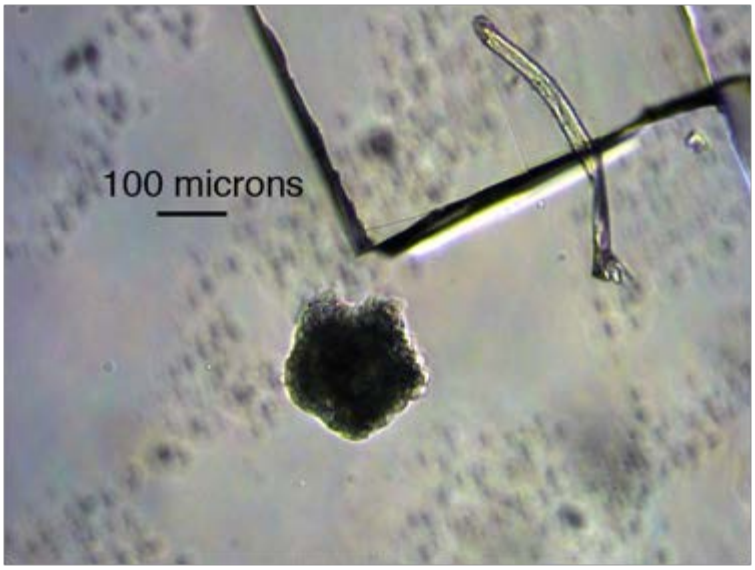

Figure 3. Scale bar is 100 microns. This image was taken after the amoeba had been exposed to the drug for about thirty

minutes. There are no evident pseudopodia.

The results of the experiment can be seen in the graph below.

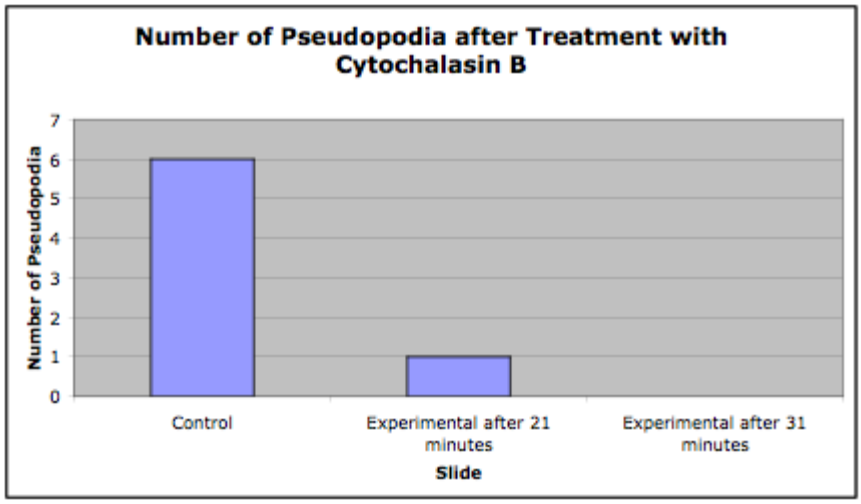

Figure 4. In this graph, it is clear that the number of pseudopodia visible decreased the longer the amoeba was exposed to cytochalasin B, until there were no visible pseudopodia.

After the amoeba had been exposed to the drug for twenty minutes, there was only one long pseudopod. After thirty minutes, it appeared rounded without any visible pseudopodia. These results are the work of Kyle Glass, Maris Jahnke, and myself.

Discussion:

The decrease in the number of pseudopodia visible after twenty and thirty minutes of exposure to the drug imply that the cytochalasin B rendered the amoeba incapable of forming pseudopodia by blocking the polymerization of actin. From the results collected in this lab, the hypothesis is supported.

There were sources of error in this experiment. The test and control slides were not viewed at regular intervals both before the drug application and after. The control was not viewed at all after the drug application. When the drug was applied, it was not applied in three stages as to completely replace the fluid under the cover slip. This means that the amoeba was not exposed to the full concentration of the drug, which could have an impact on the results, mainly the potency of the fungal toxin. After the drug application, we could not find our amoeba. It was probably wicked out of the flow cell when the drug was added too quickly. The drug application step was repeated with the control group, and a new control group was created.

To refine this experiment, the number of test groups should be expanded. Looking at more samples and how

they react to the addition of the drug would yield more results and give more conclusive evidence. Also, looking at different concentrations of the cytochalasin B would show how potent it is at different concentrations, and also how much cytochalasin B an amoeba can sustain without becoming incapacitated by a depolymerized actin cortex. Looking at more than one slide treated with the fungal toxin would give greater insight into the reactions and results of the experiment.

For future experiments, it would be very interesting to run the above-suggested experiments with amoeba that had been injected with human muscle actin. This kind of experiment would show the differences between human and amoeboid actin and the effects of cytochalasin B on both. An experiment like this one would give insight into the connections between amoeboid actin and human actin and how both interact with natural toxins.

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