Evidence that Cytochalasin B depolymerizes F-actin filaments involved in Pseudopod formation in Amoeba proteus

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Introduction

Amoeba proteus is a unicellular organism of the genus protozoa the moves by the means of pseudopods (McGrath and Blachford 2001). Actin is a globular protein found in all eukaryotic cells. It is a monomeric subunit of microfilaments and thin filaments which are the part of the contractile apparatus in muscle cells (Vandekerckhove and Webber 1978). Actin monomers (globular [G] actin) polymerize to form filaments (filamentous [F] actin) (Cooper and Hausman 2007). Pseudopodia are temporary cytoplasm filled projections of the cell membrane that the cell uses for motion and obtaining nutrients (Neff 1955). Pseudopodia extend and contract by the polymerization and depolymerization of F-actin. The pseudopodium extends itself until the actin reassembles itself into a network. This explains amoeboid movement (Pseudopodial locomotion, Encyclopedia Britannica 2008). Cytochalasin B is a cell permeable mycotoxin that blocks the formation of contractile microfilaments such as F-actin and inhibits cell movements as well as cytoplasmic division. Cytochalasin B shortens actin filaments by blocking monomer addition at the fast-growing end of polymers (Tanaka, Y., et al. 1994). In this study we tested the hypothesis that stated: if we expose Amoeba to the drug Cytochalasin B then the area between the streaming organelles and the cell membrane in the forming pseudopod will quantitatively decrease. We measured the area in the cellular margins of the forming pseudopod as well as gathered data about pseudopod formation. This is because amoeba is an organism that exaggerates pseudopod formation and we chose to study pseudopodia in Amoeba because an amoeba is an excellent organism that exhibits pseudopodial formation. We exposed our experimental amoeba to Cytochalasin B in order to measure and compare the cellular marginal area of a pseudopod between the control and experimental amoeba. During cytoplasmic streaming we observed the unaffected control amoeboid and noticed that clear marginal cytoplasm existed between the cell membrane and the streaming organelles during pseudopod formation (depicted in figure 2.1). This clear marginal cytoplasm was occupied with polymerized F-actin filaments and when depolymerized with Cytochalasin B that clear marginal cytoplasm filled with streaming organelles (depicted in figure 2.2). In addition to that we also observed that

the exposed experimental amoeboid extended numerous pseudopods in several directions as opposed to the unaffected control amoeboid.

Materials and Methods

Materials

The control and experimental amoeba were kept in two separate flow cell chambers and we employed a Nikon Eclipse E400 with Spot Insight Firewire 2 for imaging and microscopy. The concentration of Cytochalasin B was 40 mg/ml. Adobe Photoshop CS3 was used for image manipulation and ImageJ was used for image analysis. The images of the pseudopods (depicted in figure 2.1 and 2.2) were taken on the 40X objective and the images of the entire amoeba (depicted in figure 3.1 and 3.2) were taken on the 10X objective.

Methods

In this experiment: we constructed two separate flow cell chambers for at least two amoebae. One was designated control and the other experimental. The experimental chamber was exposed to Cytochalasin B and in comparison the control chamber was left unaltered. We observed the effects of the Cytochalasin B on the experimental amoeba as well as observed the natural behavior of the control amoeba. While observing we took comparable images of the entire amoebae (depicted in figure 3.1 and 3.2 at 10X) and we also took comparable images of the forming pseudopods (depicted in figure 2.1 and 2.2 at 40X). We observed the amoebae for an hour and used Photoshop to assign scale bars to the resulting images. The quantitative values for cellular margin distance explained in the Results section for figure 1 were analyzed using the line selections tool on ImageJ and then converted to micrometers. The quantitative assessment that resulted in figure 1 was performed on ImageJ.

Quantitative Calculations for Control

Area in pixels^2 was calculated on image J using the freehand selections and measure tools: for example: 4806 pixels^2.

Length was converted from pixels to area in pixels by squaring the scale bar length: for example: 134^2= 17956 pixels^2.

Length was converted from microns to area in microns by squaring the scale bar length: for example: 25^2 = 625 microns^{2}Area of cellular margin in microns^2 was solved for by comparing ratios of the scale bar area and the cellular margin area. For Example:

(4806 pixels^2)/(X microns^2) = (17956 pixels^2)/(625 microns^2)

X= 167.3 microns^2

Repeat for Experimental.

Results:

Figure 1:

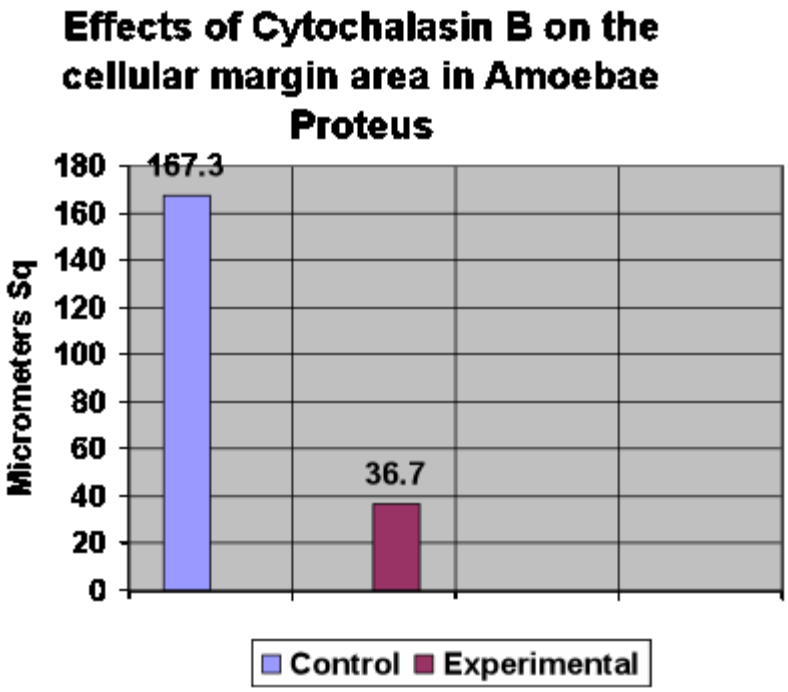
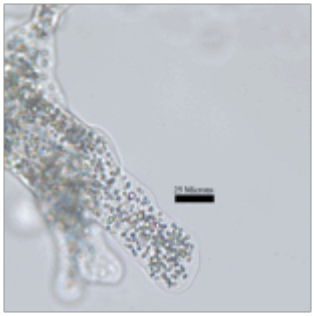
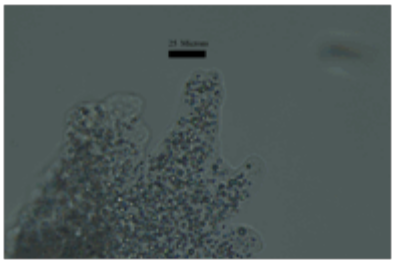


Figure 2.1:



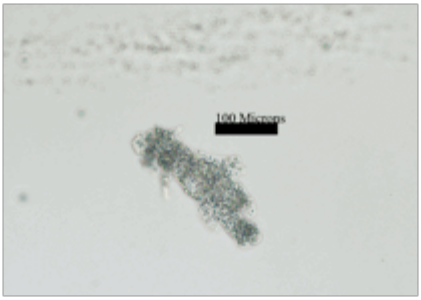
Control Image on 40X Nikon Eclipse E400 with Spot Insight Firewire 2
Note: Clear Margins during cytoplasmic streaming.

Figure 2.2:



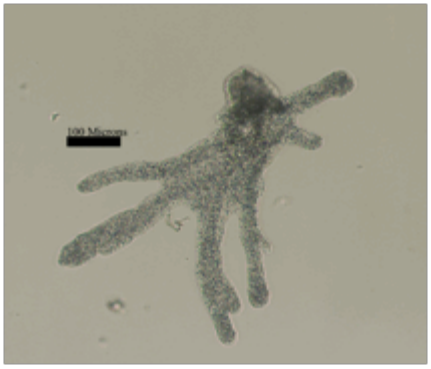
Experimental Image (Cell exposed to Cytochalasin B) on 40X Nikon Eclipse E400 with Spot Insight Firewire 2
Note: Organelles are closer to cell membrane during cytoplasmic streaming.

Figure 3.1:



Control Image on 10X Nikon Eclipse E400 with Spot Insight Firewire 2

Figure 3.2:



Experimental Image (Cell Exposed to

Note: Not all pseudopodia are extended.	*Cytochalasin B) on 10X Nikon Eclipse E400 with Spot Insight Firewire 2* *Note: Pseudopodia are extended in several directions at the same time.*

In Figure 1:

This graph depicts the quantitative difference in the cellular margins between the forming pseudopods of the affected experimental amoeboid and the unaffected control amoeboid. The cellular margin distance for the experimental amoeboid in the forming pseudopod was 2.61μm where as the cellular margin distance for the control amoeba was 5.19μm.

In Figure 2.1 and 3.1:

The control amoeboid exhibited cytoplasmic streaming with clear cellular margins. The clear cellular margins will filled with polymerized F-actin filaments. The amoeboid extended and contracted its pseudopodia. There were channels of streaming organelles between regions of stationary organelles.

In Figure 2.2 and 3.2:

In the experimental amoeboid the area between the organelles and the cell membrane visibly decreased after the experimental amoeba was exposed to the drug Cytochalasin B. During cytoplasmic streaming the organelles moved closer to cellular margins and there was a reduction in clear marginal cytoplasm. Cytoplasmic streaming was reduced in volume and speed. The amoeboid also extended numerous pseudopodia in several directions at the same time as shown in figure 3.2.

Discussion

Effects of Cytochalasin B on Cellular Margin Area

The results of this experiment support my hypothesis which states that when the amoeboid is affected by Cytochalasin B (CB) the area between the streaming organelles and the cell membrane in the forming pseudopod will quantitatively decrease.

CB is known to cause salivary gland epithelium in mice to lose its characteristic shape. This is because the drug causes disorganization of 50 Å microfilaments in epithelial cells that are thought to control cell shape because of their contractile properties (Spooner and Wessels 1970). Actin filaments along with myosin II are also known to form contractile rings in nonmuscle cells. This is best exhibited by cytokinesis (Cooper and Hausman 2007). In vitro evidence suggests that CB binds to the growing ends of F-actin filaments thereby blocking further G-actin monomer

addition and leading to a net reduction in the rate of filament growth. If this process is extended to a dynamic in vivo situation would result in a net depolymerization of cellular F-actin. (McIntosh, Painter and Whisenand 1981). In conclusion, CB inhibited F-actin growth in our experimental amoeboid and this lead to the depolymerization reaction of F-actin filaments. In the unaffected control amoeboid the clear cellular margins indicate polymerized F-actin filaments (figure 2.1) and in comparison in the affected experimental amoeboid the organelles were able to move closer to the cell membrane which indicates depolymerized F-actin filaments (figure 2.2). In figure 1, the cellular margin area of a pseudopod for the unaffected control amoeboid was 167.3 μm^2 compared to the cellular margin area of a pseudopod for the affected experimental amoeboid which was 36.37 μm^2. This reduction of clear cellular margin area can be explained through the depolymerizaion of F-actin filaments caused by microfilament growth inhibiting CB. This quantitative analysis was performed on ImageJ. However, manually outlining the cellular margin area using the freehand selections tool on ImageJ provides a source of error.

Effects of Cytochalasin B on Pseudopod formation

CB is known to inhibit pseudopodial activity and other cellular movement (Bliokh, Domnina and Ivanova 1980). According to the work of Bliokh and colleagues, cells spreading in a CB medium do not form well organized branched structures but extend and attach numerous unstable pseudopods. This evidence supports the data gathered in figure 3.2 as compared to figure 3.1. Therefore as noted in figure 3.2, the affected experimental amoeboid extended numerous pseudopodia in several directions at the same time. In comparison as noted in figure 3.1, the unaffected control amoeboid did not extend numerous pseudopodia in several directions. In conclusion the data shown in figure 3.2 shows evidence that CB inhibits pseudopodial formation and cells affected by CB extend numerous pseudopodia in several directions.

Future Experiments

For future experiments, since this experimental was only performed once, more trials need to be run in order to reduce that source of error because the lack of trials provides a source of error. This experiment should be further analyzed over time. This can be done by taking sequential images of the amoeba as it is affected by Cytochalasin B. This would provide us with the length of time it takes for the F-actin to depolymerize in the cellular margins of the forming pseudopods and we could draw further conclusions from these observations. Other drugs such as Nocodazole should also be tested on Amoeba Proteus in order to observe their effects on cytoskeletal filaments. Also rather than using two separate amoeba (one for control and one for experimental), we should use one amoeba for each trial, the initial unaffected observations and results would count as control and later when that same organism is exposed to the drug, the following observations and results would count as experimental. This would also reduce variation in results caused

by comparing two separate organisms to each other because we would use the same organism for control and experimental.

References

I collaborated with Joshua Cohen in gathering data and conducting this experiment.

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"I have abided by the Wheaton College Honor Code in this work."