Evidence That the Number of Pseudopodia Exhibited over Time by Amoeba Proteus is Reduced When Treated with Nocodazole

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Introduction

Interactions between cytoskeleton filaments, specifically actin and microtubule complexes, are the determining factors of fundamental cell process such as cell division and motility (Schober et al., 2007). Amoeba cell motility is achieved by pseudopodia extensions which require the structural support of both microtubule and actin filaments (Schober et al., 2007). The absence of these filaments would negatively affect pseudopodia activity and therefore amoeba movement. The importance and action of these filaments is well illustrated by the organism *Amoeba proteus* through its pseudopodial extensions. *Amoeba proteus* are single celled eukaryotic organisms that inhabit aquatic environments. These sizable complex cells can extend to be as large as 1mm in diameter (Cooper et al., 2007). Amoebas are highly motile and use cellular extensions called pseudopodia to walk (Cameron et al., 2007).

Pseudopodia are temporary cytoplasmic cellular extensions formed in response to environmental stimuli and supported by actin filaments; they are responsible for amoeboid activity such as motility and phagocytosis (Cooper et al., 2007). For this experiment, and in general, pseudopodia were characterized as long thin cellular extensions with a rounded terminal end protruding far enough out from the main cellular body to be easily distinguished. Pseudopodia are structurally supported by actin but require the forces of tension exhibited by microtubules in order to remain as temporary cellular structures (Cooper et al., 2007). The opposing forces of actin and microtubule filaments, along with regulation of their polymerization and deconstruction, control pseudopodial formation (Morris, 2008).

Microtubules, actin and intermediate filaments are the three cytoskeletal components of the inner cell structure. Microtubules are responsible for cellular structure and support. They are composed of tubulin which forms rigid hollow shafts extending outward from the centrosome to the cell periphery (Cooper et al. 2007; Schober et al., 2007). Microtubules exhibit treadmilling; the cytoskeleton filaments are in dynamic equilibrium and under normal environmental conditions individual microtubules continually cycle between growth and reduction (Cooper et al. 2007). Pseudopodia are formed and maintained by the regulatory formation of microtubule and actin filaments. Maintenance of pseudopodia shape and structure depends on intact microtubule structures (Rodionov et al., 1993).

Initial pseudopodia formation and extension is an osmotic process (Morris, 2008). First the actin filaments depolymerize at the site of decided pseudopodia formation. In response to this increase in cytoplasm subunits water defuses into this area in order to establish equilibrium and the pseudopodial extension begins to form as the phospholipid bilayer expands outward (Morris, 2008). The actin subunits then repolymerize providing the initial structure of newly formed pseudopod (Cooper et al., 2007). Microtubules then polymerize into the cellular extension, which is not yet filled with other organelles at this time, and provide lasting structural strength.

The presence of both the microtubules and actin filament in the pseudopodia balances the forces of compression and tension

necessary to sustain the pseudopodia structure and shape (Schober et al., 2007). Though the actin filaments are the primary filaments necessary for pseudopodia formation, without microtubules pseudopodia structure would not be stable and the pseudopodia would not maintain their shape or length (Rodionov et al., 1993). To illustrate the importance of microtubules in pseudopodia structure the drug Nocodazole is used.

Nocodazole is a drug that interferes with cytoskeletal filaments (Morris, 2008). Specifically, it is a synthetic microtubule polymerization inhibitor and depolymerizer, therefore it affects cellular processes that require microtubule assembly such as cell division or pseudopodial structure (Nocodazole, 2008). In light of this information it was hypothesized that *Amoeba proteus* immersed in a 2 μm/ml solution of the drug Nocodazole would exhibit fewer pseudopodia over time compared to amoeba subsisting in pond water. This hypothesis will be tested by comparing the number of pseudopodia exhibited by control amoebas submerged in pond water to amoeba immersed in a solution of Nocodazole.

Materials and Methods

Amoeba protei were isolated and arranged on slides using the flow cell technique. Two microscope slides were prepared; amoeba where positioned on the slides, one cell was focused on per slide, and sealed with VALAP to create a flow cell. The specific amoeba to be observed per slide was identified with a Sharpie ® black dot in order to clearly identify the correct cell. The slides were labeled “1” for control and “2” for experimental.

The control amoeba (labeled 1) was observed with the aid of a light microscope, Nikon Elipse E200 set for Koehler illumination, and the BTV imaging program on the Virgo computer in the ICUC located at Wheaton College. Images were taken, by the Sony Digital Interface camera, of the control organism submerged in pond water at an ocular magnification power of 4x so that the entire cell could be easily viewed. One photo was taken every minute for a total of twenty-one minutes and the number of pseudopodia exhibited by the cell was recorded. This data were used to establish the average number of pseudopodia exhibited by an amoeba proteus under normal conditions.

The experimental amoeba was treated with the drug Nocodazole. The flow cell was flushed three times with the drug solution producing an approximately complete fluid transfer. The 150 μL spring water surrounding the amoeba was replaced with a 2μg/ml solution of Nocodazole. This was accomplished with the aid of a Kimwipe ® which, when positioned on the opposite side of the flow cell as the drug being applied, pulled the pond water out from under the cove slip and replaced it with the drug solution being applied with a transfer pipette. The amoeba was immediately observed in the same manner as the control cell. Images were again taken every minute and the number of pseudopodia was recorded over time. The treated amoeba was observed for fifty-eight minutes in which results were continually recorded.

The data were analyzed and graphs were produced displaying the change in the number of pseudopodia formed over time in control amoeba proteus and those treated with the drug Nocodazole. An additional graph was also produced, based on the results, which averaged the number of pseudopodia present over a ten minute period in both the control organism and the treated cell at the beginning and end of the experiment. Images, of both the control and experimental amoeba at the beginning and end of the procedure, were also isolated using the programs Adobe Photoshop and ImageJ. This was done to display the cellular changes over time.

Results

Images: Ameoba proteus exhibiting pseudopodia at beginning and end of experiment.

Using Sony Digital Interface camera on Virgo computer in the ICUC

4x power objective

Using BTV, ImageJ and Adobe Photoshop programs

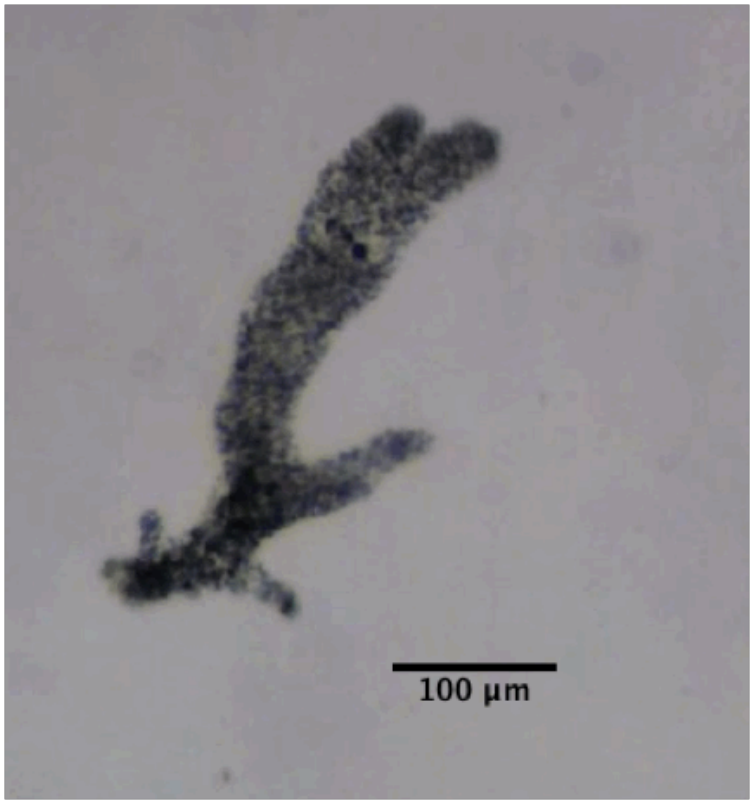

Image 1: Control at time 0 minutes
(Six pseudopodia)

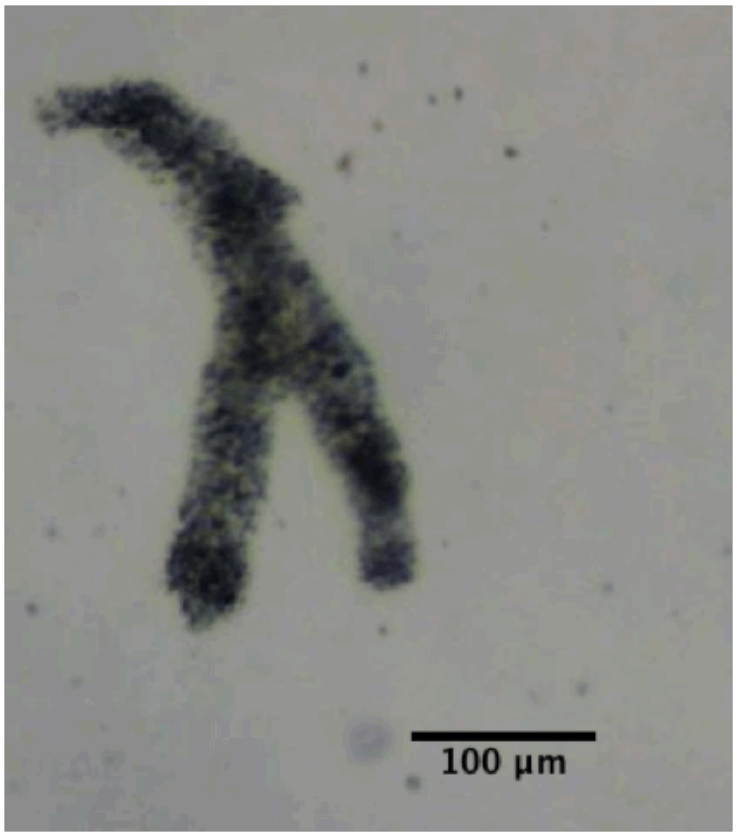

Image 2: Control at time 21 minutes
(Four pseudopodia)

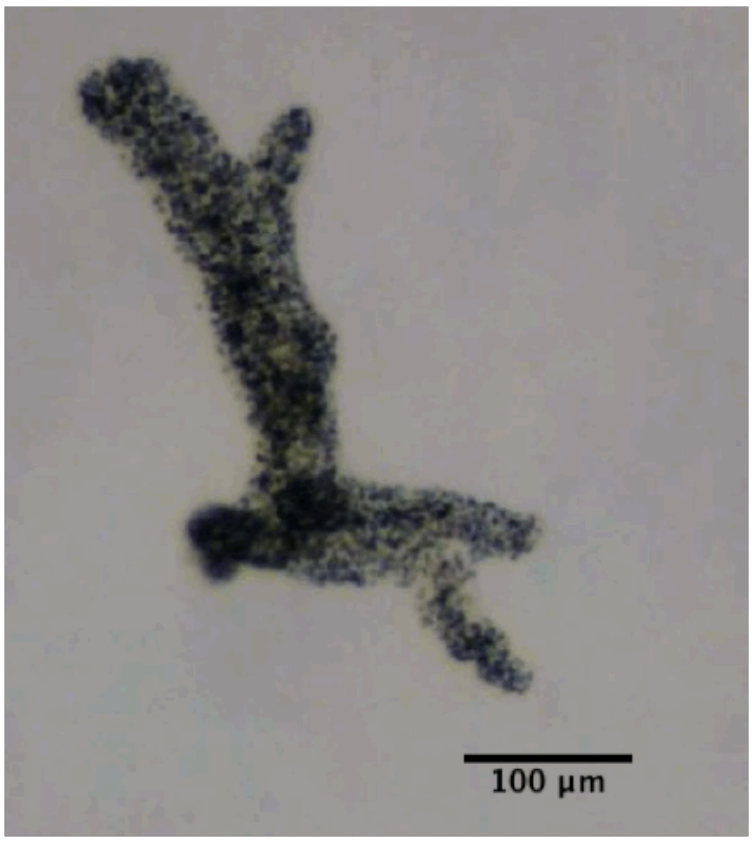

Image 3: Experimental at time 0 minutes
(Five pseudopodia)

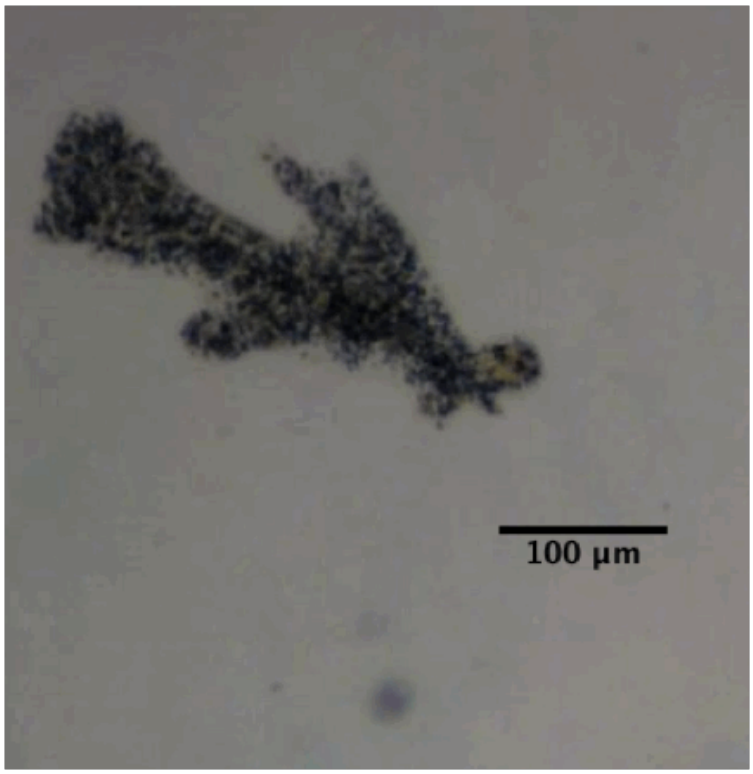

Image 4: Experimental at time 58 minutes. (Four pseudopodia)

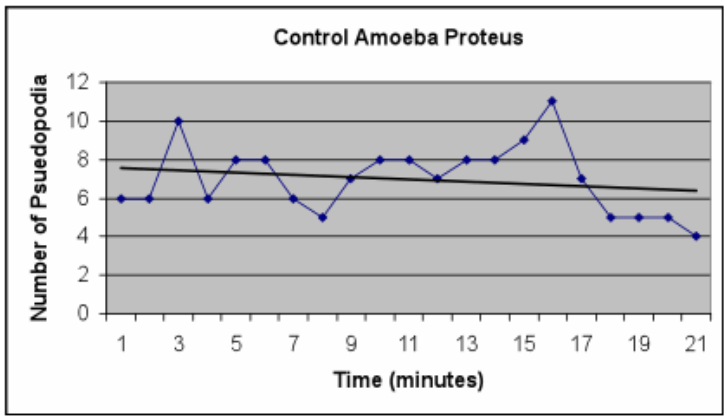

Figure 1: Change in number of pseudopodia over twenty minute time interval of control amoeba proteus subsisting in pond water, including best fit line. (n=1)

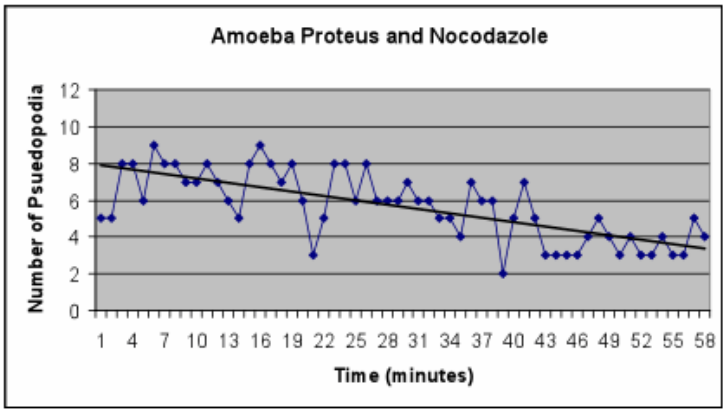

Figure 2: Change in number of pseudopodia over fifty-eight minute timer interval of amoeba proteus immersed in 2 µm/ml solution of Nocodazole, including best fit line. (n=1)

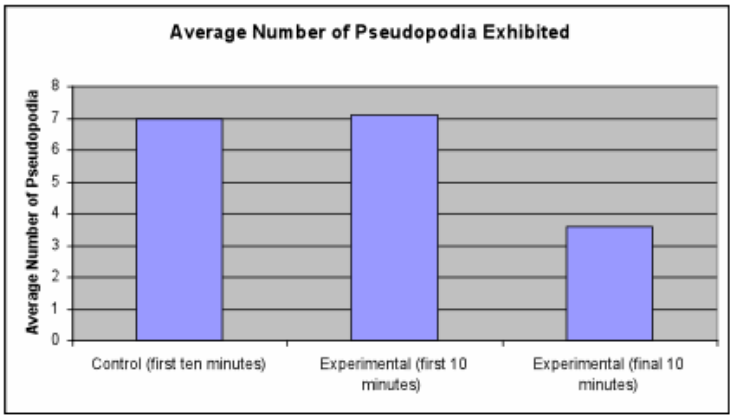

Figure 3: Comparison of average numbers of pseudopodia between control amoeba and amoeba immersed in Nocodazole at first ten minutes and last ten minutes of application of drug.

The control *Amoeba proteus*, submerged in pond water, exhibited a range in the number of pseudopodia; between four and eleven pseudopodia were present at any one time. This range was quite consistent throughout the twenty minute time interval. The average number of pseudopodia that this cell exhibited was seven, with a median also of seven pseudopodia. There was no noticeable trend, in terms of increase or decrease, in the number of pseudopodia over time in the control amoeba as is illustrated by the relatively horizontal trend line remaining at or near seven (See *Figure 1*). From a qualitative perspective the pseudopodia of the control amoeba were thin and long (See *Image 1* and *Image 2*).

The *Amoeba proteus* immersed in a solution of the drug Nocodazole also had a varied number of pseudopodia protruding off the main body at any one time. The number of pseudopodia exhibited by the experimental amoeba changed overtime in a directed fashion as is illustrated by the trend line which fits the data (See *Figure 2*). The average number of pseudopodia exhibited by the experimental amoeba in the first ten minutes was 7.1. This is not significantly higher than the overall average of the number of pseudopodia protruding from the control organism. In the final ten minutes of recorded data, beginning forty-nine minutes after the drug was applied, the experimental amoeba exhibited an average of 3.6 pseudopodia, a much lower quantity. This reduction in the number of pseudopodia exhibited by the treated cell over time compared to the control amoeba is illustrated by *Figure 3*.

Qualitatively the overall appearance of the pseudopodia exhibited by the experimental amoeba changed overtime compared to the static characteristics of the control cell (See *Images 3* and *Image 4*). The pseudopodia in the Nocodazole solution tended to be much

shorter and had a greater width; they did not exhibit the extended rounded shape characterized by the control pseudopodia. In addition there were a greater number of short, very pointed pseudopodia in the beginning stages of the introduction of the drug. The shape of the pseudopodia became altered overtime in the Nocodazole treated cell. These changes can be seen in the amoeba images provided.

Discussion

The hypothesis was supported in this experiment; the *Amoeba proteus* immersed in a 2 μg/ml solution of Nocodazole exhibited fewer pseudopodia over time compared to the number of pseudopodia displayed by the control amoeba submerged in pond water. The active presence of microtubules is therefore necessary for the proper stability of multiple pseudopodia. In the absence of microtubule polymerization, due to inhibition of this activity caused by an agent such as Nocodazole, pseudopodia exhibition is reduced in number. Though initial formation of pseudopodia is not inhibited by a lack of microtubules because this process is primarily preformed by actin filaments, the overall structure and stability of the pseudopodia is compromised without the microtubule components. In the absence of microtubules the pseudopodia are short-lived and altered in shape.

This decrease in number of pseudopodia may cause motility issues for the cell because the pseudopodial extensions are necessary for the walking motility exhibited by *Amoeba proteus*. Research preformed by Cameron et al. (2006) demonstrated that amoeba pseudopodia are necessary for *Amoeba proteus* motility and that this cell migrates by walking. This research demonstrates the importance of microtubules and cellular regulation of their polymerization and depolymerization.

Similar experiments preformed studying the affects of Nocodazole on pseudopodia yielded comparable results. Both Rodionov et al. (1993) and Baudoin et al. (2008) describe the affects of Nocodazole on pseudopodia exhibition. Rodionov et al. (1993) describes the importance of microtubules in localized pseudopodial activity at the leading edge of the cell for motility purposes. When treated with Nocodazole pseudopodia are no longer polarized and cannot work to move the cell. Baudoin et al. (2008) experimented with Medial Ganglionic Eminence cells and the affects of Nocodazole induced changes associated with changes in microtubule dynamics. As in the results of this experiment Baudoin et al. discovered that the cells treated with Nocodazole exhibited shorter, less stable pseudopodial extensions. Though these results were not quantified in terms of number of pseudopodia exhibited over time when treated with Nocodazole, the qualitative results are comparable.

There were complications with this experiment and possible improvements that could be made when repeating this procedure to improve this experiment. Only two amoebas were examined for this experiment and this small sample size affects the legitimacy of these results. Multiple organisms would need to be studied in order to clearly correlate the absence of microtubule polymerization with decreased number of pseudopodia.

Another difficulty of this experiment was the identification of pseudopodia. A definition of pseudopodia was established prior to the onset of this study, but it was strongly challenged throughout the experiment as the size and shape of cellular extensions changed. The identification of pseudopodia must be objective in order to obtain clear results. At times the ability to clearly and accurately identify the number of true pseudopodia being exhibited by the organism was complicated. In order to improve this experiment, and obtain more consistent interpretable results, it is suggested that the data be collected differently.

Though the overall number of pseudopodia did decrease the most remarkable difference observed, between the control and experimental cells, was the size and shape of the pseudopodia. In future experiments of this nature it is suggested that instead of observing

the change in the number of pseudopodia exhibited over time, the size of pseudopodia should be measured. This can be accomplished by finding the change in the area of one pseudopod over time using the ImageJ program. The longevity of the pseudopodia may also be measured in order to compare for how long the pseudopodia are extended in the absences of microtubules.

Overall the hypothesis was supported and fewer pseudopodia were exhibited over time when Amoeba proteus was treated with the drug Nocodazole. Similar experiments preformed corroborate these findings and explain why this occurs. Though overall this experiment was successful there are improvements that could be made to the procedure to improve the clarity and significance of the results. This experiment involves important cellular processes and suggests possible future studies to be performed to further knowledge in this biological area.

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