

Effects of Nocodazole on Pseudopodium Growth in Amoeba

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Introduction:

Amoebae are single cell eukaryotes. They extend temporary projections called pseudopodia, which are extension of their cytoplasm, out from their cell body. These extensions assemble and disassemble actin subunits and microfilaments to extend and contract. The interaction of filaments near the wall end of the pseudopodia with myosin causes contraction (Wikipedia, 2008). Pseudopodia extend for motion and to ingest nutrients.

Nocodazole is a drug used in cell biology to inhibit the polymerization of microtubules. The way Nocodazole works is by blocking the disulfide linkages, and the alpha and beta tubulin subunits which make up microtubules. It binds to beta tubulin and prevents the interaction with alpha tubulin. Nocodazole also blocks metaphase of the cell cycle. Because of the absence of microtubules, the cell is unable to form the spindle needed to divide the chromosomes of the cell. After the cell checkpoint is activated, the cell undergoes apoptosis (Sigmaaldrick, 2008).

Because of the nature of Nocodazole, applying it to an amoeba should disband its microtubule network causing behavioral changes. The purpose of this study is to determine the effect of Nocodazole on the growth of pseudopodia in Amoebae. Pseudopodia are important structures of amoeba and allow for their mobility and consumption of nutrients.

Materials and Methods:

To conduct this experiment, we needed an array of different material. These materials included forceps, cover slip chips, 1 slide, VALAP, 1 cover slip, an upright microscope, Kim wipes, paper towels, 3 milliliters of 2µm/ml solution of Nocodazole and Dimethyl Sulfide (DMSO), and a transfer pipette, all obtained from Dr. Bob Morris in the

ICUC.

Two flow chambers were created to expose the Amoeba to the Nocodazole. One with amoeba A and the other with amoeba B. While making these chambers and using the drug Nocodazole, you must wear gloves at all time (Morris, 2008). We obtained each item from the lab instructor, Bob Morris. After receiving the materials, we placed a few cover slip chips in a circle, leaving space for the drop of pond water containing the amoeba, on the slide. We then placed the cover slip on the slide. After receiving the amoeba, we then searched for the amoeba under the microscope to make sure there was amoeba in the chamber. After that, we placed VALAP on the horizontal sides of the cover slip, making sure the sides are sealed. We did this twice to make two flow chamber, Only one flow chamber was used because the amoeba on the second chamber were pushed off as we added Nocodazole to it. After further observation of the amoeba, we captured images of the amoeba every 15 second for 2 minutes. Then we added the Nocodazole to the chamber and repeated the image capturing steps. After that was finished, we observed the 2 image sequences and compared them to see if our hypothesis was tested.

Results:

Amoeba A before it was treated with Nocodazole, exhibited very controlled patterns of streaming and pseudopodial growth. The amoeba grew wide oval shaped pseudopodia and moved quite quickly throughout the solution (Fig 2). Amoeba A usually had a leading pseudopodium. Cytoplasmic streaming was directed to that pseudopodium until the streaming direction changed where another pseudopodium would form. All cytoplasmic streaming would be directed toward that newly formed pseudopodium.

After being treated with the Nocodazole solution, pseudopodial growth changed. Many of the pseudopodia that were present before treatment, shot outward to form long thin pseudopodia (Fig 3). After more observation, cytoplasmic streaming became more random. The organelles moved about the cytoplasm forming new pseudopodia but at many different sites of the Amoeba. When streaming was directed to one place, it would diverge too quickly to form a well defined pseudopodium.

To test whether Nocodazole had an effect on the growth pseudopodia in amoeba, the control was observed for 2 minutes and after the Nocodazole was applied, we observed it for another 2 minutes. Over all, the mean pseudopodia of amoeba increased after treatment with Nocodazole (Fig 1).

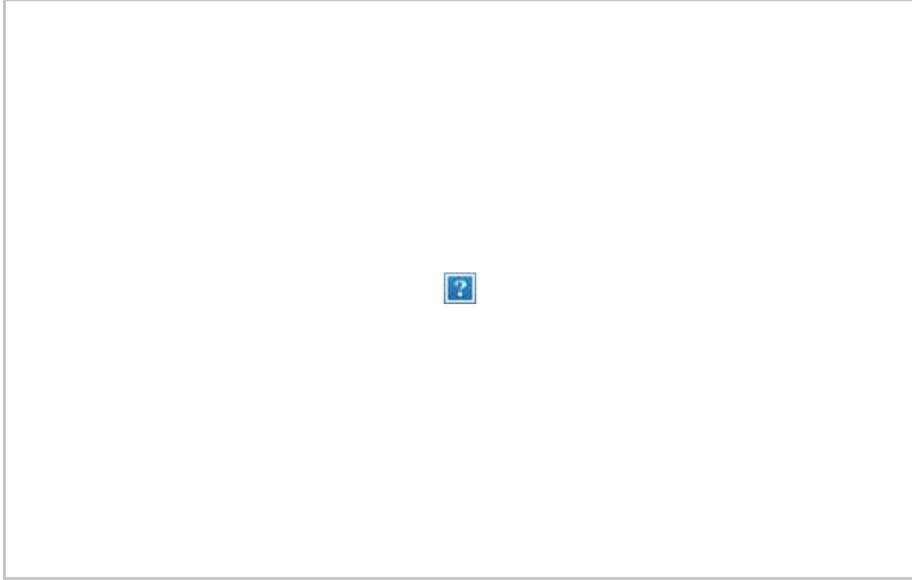


Figure 1. Mean pseudopodial growth of Amoeba A before it was treated with Nocodazole and after it was treated with Nocodazole. For the control, pond water was used. After, the amoeba was treated with 3 milliliters of 2 μ g/ml solution of Nocodazole.

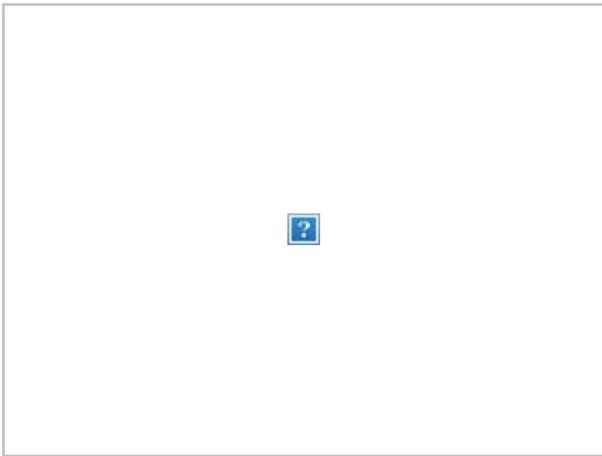


Figure 2. Amoeba A is shown before treatment with Nocodazole. Amoeba A is shown in pond water solution (scale bar = 200 μ m).

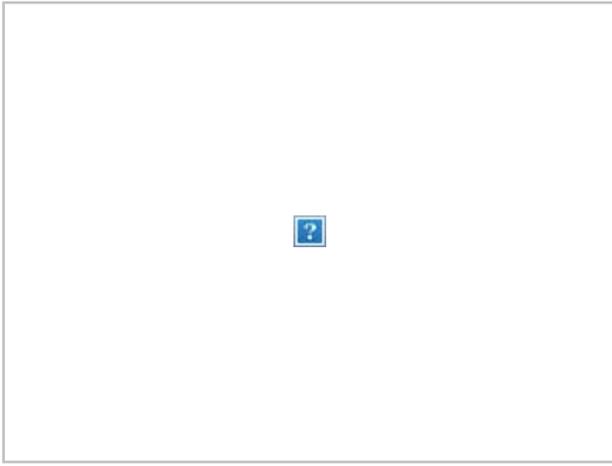


Figure 3. Amoeba A is shown after it has been treated with Nocodazole. Amoeba A has been treated with three milliliters of a $2\mu\text{g}/\text{ml}$ solution of Nocodazole (scale bar = $200\mu\text{m}$).

Discussion:

After conducting this experiment, I concluded that my hypothesis was refuted by the gathered results. After treatment of the amoeba, I observed more pseudopodia growth. From graphing the mean pseudopodia growth, the data obtained from the control and experimental amoeba shows that the amoeba treated with Nocodazole has a higher mean growth (Fig 1). According to Rosnia and Swanson, amoeba placed in Nocodazole or any other microtubule depolymerizer, its asymmetrical shape should change to symmetrical. The depolymerization of the microtubules within the amoeba should have caused the amoeba to relax and in the end, turn into a spheroid figure.

After many minutes of observation, the amoeba observed, continued its pseudopodial extensions and continued to migrate across the slide. The migration of the amoeba across the cover slip continued but with no set direction. The pseudopodial extensions shifted from one side to another. The amoeboid extensions erratically formed with what seemed like no direction or purpose. The directional movement of the amoeba could not be determined. Because of the lack of the microtubule network, the amoeba lost its shape and was unable to move directionally (Rodionov, et al.1993). Microtubules serve as a means of transportation for organelles to migrate through the cell.

Without the microtubule network, streaming in amoeba becomes less directed. While observing the amoeba when it was treated with Nocodazole, I noticed the amoeba continued to stream but, streaming was more widespread

over almost the entire amoeba. Also, the streaming caused the erratic growth of pseudopodia over the entire cell. It was not longer directed to only the leading edge. Vasiliev says that efficient transfer of cortex components from the central parts to the active edge may happen through the presence of microtubules and intermediate filaments. Without the cytoskeletal structures present throughout the amoeba, the transfer of the structures needed to form elongated pseudopodia can not be properly conducted.

After much observation, the higher mean of pseudopodial growth in the treated amoeba refuted our hypothesis. The lack of pseudopodial growth at the leading edge along with the erratic spurs of pseudopodia suggests that the microtubule network indeed broke down. Streaming was not longer directional and concentrated. Many different streams, all leading from the central body began to form. Studies show that microtubules are still intact near the nucleus, allowing for directional streaming.

Only one experiment was conducted. More experiments conducted on this topic along with its experimental data will yield more concise results about the effects of Nocodazole on amoeba. Because of a short time lapse that happened before we observed the amoeba, data may have been lost and the resulting effect of the drug may have been missed.

Future Experiments:

An experiment of a more precise hypothesis can be conducted. Instead of looking at only pseudopodial growth, as there are other factors that suggest microtubule depolymerization, we can also hypothesize on the effects of streaming on pseudopodial growth. Also, more flow chamber with amoeba could be studied to increase the amount of data.

References:

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I collaborated with Kathleen Knapp in this work.

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