The Ca²⁺ ion on has a positive affect on the regeneration of flagella in the Chlamydomonas cell

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**ABSTRACT:**

Much is understood about the affect of the Ca2+ ion and its affect on flagellar excision and flagellar motion in Chlamydomonas, however little data is provided on the affect of this ion on flagellar regeneration. This study seeks to determine the affect the Ca2+ ion has on flagellar regeneration in the Chlamydomonas cell. To accomplish this, tests were run observing the regeneration of shocked cells treated with different solutions. One containing Ca2+, one without additional Ca2+, and one with Ca2+ and a known Ca2+ inhibitor (EDTA). In the current experiment it was found that in solutions with increased Ca2+ concentration there was an increased rate of flagellar regeneration. In the solution with the inhibitor there was a severe retardation of flagellar regeneration and a complete lack of motility. These observed results support the hypothesis that the Ca2+ ion has a positive affect on, and is indeed necessary for the regeneration of flagella in the Chlamydomonas cell.

**INTRODUCTION:**

Temporal and spatial changes in Ca2+ concentration are thought to transduce environmental signals into distinct and appropriate cellular responses (Berridge and Dupont, 1994). Previous studies have done a great deal of research on the affects of the Ca2+ ion on the Chlamydomonas cell and have determined that a sophisticated interplay between the regulated release of internal stores of Ca2+ and the influx of extracellular Ca2+ across the plasma membrane contribute to the dynamic nature of Ca2+ signaling (Quarmby, 1996)
In fact, it appears that the presence Ca2+ ion (or lack thereof) is a central player in many of Chlamydomonas’s most important functions. Among these processes are flagellar excision, flagellar resorption, flagellar retraction, and flagellar movement. As aforementioned, it is believed that these processes are in response to a flow of Ca2+ ions across the plasma membrane in the Chlamydomonas cell. For the purpose of this study, it will be important to remember that Calcium plays a roll in all of the following processes, however, we will be focusing specifically on a new hypothesis. The goal of this present study is to determine if the Ca2+ ion also plays a roll in the regeneration of flagella after exposing Chlamydomonas to a pH shock which deflagellates them.

It is important also to understand the mechanism by which a low pH excises the flagella of Chlamydomonas. “pH shock” creates an acidification of the cytosol. This however is not sufficient in and of itself to remove the flagella of Chlamydomonas. In other words, the acid does not simply “burn” off the flagella of Chlamydomonas. Acidification causes deflagellation only if it is accompanied by an influx of Ca2+. (Quarmby and Hartzel, 1994). This influx of Ca2+ is not caused because of an increase in the total amount of Ca2+ in the system, but rather because the calcium is actively transported through the plasma membrane of Chlamydomonas. This transport occurs through channels used specifically during acid shock. When used in this way, the calcium triggers the shearing of the flagella. The result of this reaction is then a solution somewhat depleted of Calcium and full of non-flagellated Chlamydomonas.

If all we have mentioned about the Calcium ion then is true, it stands to reason that calcium may be a signaling medium through which Chlamydomonas can react to its surrounding environment. If this is true, it stands to reason that Chlamydomonas needs
calcium in order to signal that the environment (post shock) is suitable for regenerating flagella. To test this hypothesis three tests will be run. Each will subject chlamydomonas cells of the same culture, to acid shock and then to neutralization through the base KOH. This shocked culture will then be treated with 3 different solutions. These three different mixtures will define the three different tests. One will be treated with regular TAP, another with a TAP solution lacking the Ca2+ ion, and another with TAP solution containing EDTA, a known Ca2+ inhibitor. All of these solutions will be observed over time and measured for flagellar regeneration. Flagellar regeneration will be judged on two aspects; motility, and flagellar length. The combination of these two observations will give us not only data on the extent of flagellar regeneration but a measure of how useable the regrown flagella are. Ideally, we would expect to see a regeneration of the flagella in the TAP treatment test reaching completion around 45 minutes after the initial treatment. This would correspond to our previous work in lab. Comparatively, we would expect to see a slightly retarded regeneration in the test that added no additional Ca2+. This is due to the fact that calcium concentration would have been depleted during the acid shock procedure (aforementioned research). It is, as of now, unclear to what extent the acidification will deplete the calcium supply. If there is calcium left in the solution, regeneration would be expected to continue, but at a somewhat slower rate than the calcium enriched TAP solution. If the solution is completely void of Ca2+ no regeneration should occur. In effect we predict that if there are fewer signaling ions in a solution (calcium) the regeneration process will proceed at a slower rate. This however in itself will not prove that Calcium is necessary for signaling flagellar regeneration. To prove this, we have treated one solution with EDTA, a known
Ca2+ inhibitor. This will prevent Ca2+ from entering the cell via calcium ion transport channels. If flagellar regeneration is signaled by Ca2+ crossing the plasma membrane of Chlamydomonas, this test should result in severely stunted growth of flagella in this solution.

**MATERIAL & METHODS:**

For this experiment we used a modified version of the procedure for Flagellar Excision and Regrowth of Chlamydomonas as detailed on pages 74-76 of the laboratory manual provided for students in MSU’s BS 111L course (Urbance, 2007). The primary difference between our procedure and the lab manual’s is that we are testing for the effect of the Ca 2+ ion and its inhibitor EDTA on flagellar regrowth as opposed to the caffeine treatment given in the lab manual. Thus, the following changes were made to the Lab Procedure:

*Flagellar Excision:*

This follows the same procedure as detailed in the manual; however we used only 100mL of Chlamydomonas cell culture. Therefore, our acid and base volumes used were decreased in equal proportion; 4 mL of acetic acid was used to shock the culture and 4mL of base was used to neutralize the culture after the acid shock. It is also important to note that we took a sample of the cell culture immediately before the shock and immediately preceding the neutralization. These samples served as our control groups for our data.
*Treating Shocked Cultures:*

10 mL of the shocked culture were then placed into 3 appropriately labeled conical tubes (10mL into each tube). The three tubes were then treated in the following manner:

- **Tube 1:** Treated with 1 mL of TAP Solution
- **Tube 2:** Treated with 1mL of TAP Solution w/o Calcium
- **Tube 3:** Treated with 1mL TAP Solution + EDTA (.5mL)

These solutions were inverted to mix the sample and then placed under a lamp for incubation.

*Collecting and Examining Samples:*

Samples were collected much like those in the Lab Manual’s procedure as outlined on page 74. However, our samples were collected at times 5, 10, 15, 30, and 45 minutes. Samples fixed in Lugol’s solution were examined for flagellar presence and length. Flagellar length was then rated on a scale of 0-5 according to their length in relation to the length of the chlamy cell (see table 1).

Additionally, we collected live samples and examined them for percent motility prior to fixing them in Lugol’s solution.

*Data Collection:*

All fixed samples (17 in all) were viewed under a compound microscope at 100x magnification to view the flagella and to determine their length. The percent motility was determined by viewing the live chlamy on a hemiacytometer. However, due to the nature of the movement, percent motility was an estimation.
RESULTS:

Two basic sets of data were collected from our experiment. We first determined the percent motility of each solution at intervals of 5, 10, 15, 30, and 45 minutes after having been shocked with acid and put into their respective treatments. However, it is not easy to accurately and precisely determine the percent motility of such a large number of cells simply by viewing them, nor is it easy to ascertain to what extent all of the cells are motile. To attain a more accurate view of flagellar regeneration, we fixed each of our live samples in Lugol’s solution as mentioned in the Method’s section. In each sample, 10 cells were selected at random, and viewed under a compound microscope so as to measure the length of their flagella. Flagellar length was rated on a scale of 0-5 as seen in table 1. By measuring both the percent motility and the flagellar length we were able to ascertain not only how well the flagella had regrown, but to what extent the Chlamydomonas were able to utilize their regenerated flagella.

Regular TAP Treatment:

In this assay flagellar regeneration began to occur almost immediately. At the 5 minute mark there was already a marked improvement in the average length of flagella, however motility did not increase significantly until 15 minutes after the treatment had been administered. By the 45 minute mark, flagellar motility had reached near pre-shock levels and flagellar length was approaching normal with very few acceptations.

TAP w/o Calcium 2+ ion:

In this assay, flagellar regeneration occurred in much the same way as the regular TAP solution for the first 10-15 minutes of the experiment. However,
flagellar motility did not increase at the rate of the TAP solution with the Calcium ion. By the 45 minute mark, only 70% of these cells showed motility, whereas the Regular TAP treatment showed 90% motility.

**TAP with Calcium Inhibitor (EDTA)**

This solution showed a marked retardation in both cell motility and flagellar regeneration. Motility, in fact, never reached over 3% which was our baseline as determined by our negative control group. In essence, these cells never gained motility. Interestingly, there were however, signs of flagellar regeneration in these cells. The flagellar regeneration was not comparable to either of the previous assays, but it was existent. After 5 minutes, only 10 percent of these cells had a flagella rating of 2, and none were higher than 2. After 45 minutes, 10 percent of the cells had received a flagella rating of 3 while 60 percent received a rating of 2.

**DISCUSSION:**

*Data Analysis and conclusions:*

Our results support but do not prove our original hypothesis that Calcium is necessary in the process of flagellar regrowth following acid shock upon the Chlamydomonas cell. Results for the tests involving the regular TAP solution and the TAP solution without the Ca2+ ion went as expected. They underwent normal flagellar regeneration in accordance with our predictions. The solution with the regular TAP solution regenerated at a slightly higher rate than did the solution without the Ca2+ treatment. This is presumably because there was more Ca2+ to signal the protein synthesis required for flagellar regeneration. More specifically, there was a higher
concentration of Ca2+ outside the cell thus, transport of Ca2+ through the semi-permeable plasma membrane of the cell occurred at a faster rate. This in turn resulted in a faster regeneration period.

In contrast, the results from the test with the TAP and Ca2+ inhibitor were somewhat unexpected. The prediction was that the inhibitor would severely retard the growth of flagella, or stunt it altogether. Our results showed us that the EDTA did in fact severely retard the growth of flagella, as the flagella were significantly smaller than those in our other tests, and there was no increased motility of these cells observed. This would seem to support our hypothesis that EDTA blocks the channels which allow Ca2+ to enter the cell and signal for flagellar growth to begin. However, it is odd that any flagellar growth would occur at all. As most growth occurred in the later stages of the experiment, it is possible that the EDTA becomes less effective after time. It is also possible that more EDTA was initially needed to completely block all channels which the Ca2+ ion might use. This is our only known source of possible experimental error. Further tests should be done to see if we did not use a sufficient amount of EDTA to completely block the Calcium Ion channels.

Without better imaging technology it is impossible to establish that Ca2+ behavior we observed is the signaling mechanism used to initiate flagellar excision and regrowth in Chlamydomonas. However, we have established that Ca2+ is indeed an integral part of flagellar regeneration, as regeneration occurs slower when it is in short supply, and fails to occur at all when it is blocked from use.
Additional Suggestions:

Using the knowledge obtained in this experiment and the information already provided in the introduction to this paper, I would recommend research on a new hypothesis which states that flagella generation and excision is a process created by evolution allowing chamydomonas to maximize its energy use. According to our research and the research of others, only in correct Ca2+ concentrations can chlamy move correctly, therefore, only in correct Ca2+ conditions is it beneficial for Flagella to exist. In this hypothesis, flagella are removed in imperfect concentrations in order to benefit the cell.

RESOURCES:


