Comparing flagellar regeneration between wild-type and mutant *Chlamydomonas reinhardtii*

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Abstract

Our study of *Chlamydomonas reinhardtii*, a single-celled alga that has been used as a model organism for studying flagellar structure and function, compares flagellar regeneration between wild-type (CC-1690 *mt+ 21 gr*) and mutant (CC-3913 *pf9-3 mt-*) phenotypes. This comparative study measures the length of flagellar regeneration after deflagellation in order to characterize the mutant *pf9-3 mt-* phenotype. Four replicates of each of mutant and wild-type organisms were exposed to acetic acid shock for 30 seconds to detach flagella and potassium hydroxide was added to stop the reaction. The cells were then allowed to regenerate their flagella in fresh media and a compound microscope was used to observe the process at time points 0 mins, 15 mins, and 30 mins post deflagellation. With our data and statistical analysis, we found that with increased time the wild-type organisms showed increased flagellar length with *p*-value of 0.01; We also found that the mutation did not affect flagellar regeneration with *p*-value of 0.55; Finally, our data supports that time has a different effect on wild-type and mutant strains with a *p*-value of 0.03. Qualitatively, mutants showed flagellar defects, abnormal responses to the deflagellation treatment and inconsistent regrowth patterns.

Introduction

Chlamydomonas reinhardtii are unicellular, green algae that have been used as a model organism to study flagellar structure and function. This area of research has been vital, as flagella are the most common organelle used for movement by microscopic organisms in aqueous conditions (Silflow & Lefebvre 2001). *C. reinhardtii*, has a pair of flagella protruding from the cell body, which are used for movement towards light, a phenomenon called phototaxis, which helps the organism obtain nutrients (Stavis & Hirschberg 1973). They are also used for mating where the organisms use their flagella to move towards each other and intertwine to hold themselves in place (Silflow & Lefebvre

2001). Their flagella protrude from the basal body, which is a protein structure derived from a centriole. Each flagellum is made up of microtubules in a 9 + 2 conformation. In addition, it is possible to stimulate deflagellation by various methods, such as organic acids or detergents which induce the separation of the flagella from the basal body structure. Deflagellation itself causes the cell to upregulate transcription of genes encoding flagellar components and the organism can regenerate their flagella over a three-hour period (Harris 2001). Taking advantage of these qualities, our objective was to study the effect of time on flagellar regeneration of the wild-type *Chlamydomonas reinhardtii* and a mutant (CC-3913 – *pf9 – 3 mt*-), which exhibits defective motility due to atypical flagellar structure.

Our hypotheses to be tested are as follows:

Ho_1: Time has no effect on the length of the regenerated flagella of wild-type *Chlamydomonas reinhardtii.*

Ha_1: Time has an effect on the length of the regenerated flagella of wild-type *Chlamydomonas reinhardtii.*

We predict that given more time, the length of the regenerated flagellar will increase in the wild-type *C. reinhardtii*. As previously mentioned, literature supports that when the cell is deflagellated, it will upregulate the genes encoding structural components of their flagella and regrow their flagella in a short period (Harris 2001).

Ho_2: The mutation has no effect on the flagellar length of *Chlamydomonas reinhardtii* during flagellar regeneration.

Ha_2: The mutation has an effect on the flagellar length of *Chlamydomonas reinhardtii* during flagellar regeneration.

We predict that the mutation does affect the regenerated flagellar length. Specifically, we believe that the mutation will cause a decrease in regenerated flagellar length due to the nature of the mutation. Our mutant strain experiences impaired motility due to improper assembly of the inner arm dynein complex (Myster et al. 1997), which is a group of proteins that move along the microtubules of the flagella and drives its movement (King & Dutcher 1997). Since the mutant experiences these abnormalities regarding flagella assembly, we believe that they will be less successful in regenerating flagella as compared to the wild type.

Ho_3: The effect of time on the length of the regenerated flagella of *Chlamydomonas reinhardtii* is the same in wild type and mutant.

Ha_3: The effect of time on the length of the regenerated flagella of *Chlamydomonas reinhardtii* is not the same in wild type and mutant.

We predict that time will affect the length of the regenerated flagella in both wild type and mutant in that both will exhibit increased flagellar length as time increases. This is because while our mutant strain exhibits anomalies in their flagella, they are still able to produce the structure. We believe that since they could form flagella, then they will be able to regenerate them given enough time and nutrients. Hence, increased time should allow both wild-type and mutant strains to increase their flagellar length.

Methods

Replicates and controls

We conducted our experiment with four replicates each from wild type and mutant. We pipetted 10 mL four times from a wild-type *C. reinhardtii* culture, placed them in separate 50 mL beakers and repeated for a mutant *C. reinhardtii* culture (Figure 1). We made samples of untreated culture cells that did not undergo deflagellation, added fixative, and observed them under the microscope. We took pictures with DinoXcope equipment for our records.

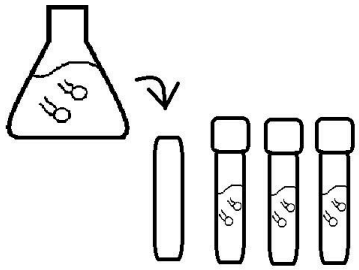

Figure 1. Diagram showing four replicates taken from a culture of wild type or mutant into separate containers.

Treatment Set-up

We placed the beakers in an ice bath on top of a magnetic stirrer and added 0.5 N acetic acid dropwise until the pH of the culture reached 4.5 (Figure 2). We let the deflagellation proceed for 30 seconds then we added 0.5N KOH dropwise to each beaker until the pH returned to 7. We kept track of the pH of each beaker using pH paper. We then transferred the culture to a 15 mL centrifuge tube and centrifuged at maximum speed for three minutes to pellet the cells. We then decanted the supernatant and resuspended the

cells in fresh culture medium. At this point (time 0 minutes), we pipetted 200 µL samples from each replicate into microcentrifuge tubes and added 20 µL iodine potassium iodide (IKI) fixative. We repeated this step twice, at 15 minutes and 30 minutes post-deflagellation to obtain samples at these times. We placed each sample on a microscope slide and focused on a single cell at 400 X magnification using an Axio compound microscope. We then switched to 1000 X magnification (oil immersion) and took a picture of the cell with a DinoXcope ocular camera, calibrated the photo and measured the flagellar length.

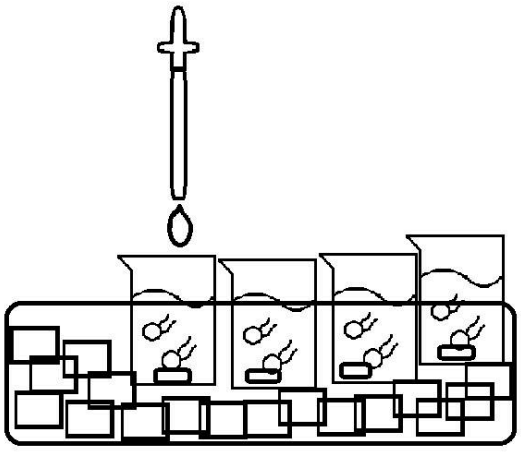

Figure 2. Deflagellation protocol set up where our cultures are in four beakers in an ice bath with a magnetic stirrer while acid is added dropwise with a pipette.

Data Collection and Analysis

Photos of wild type and mutant before deflagellation were observed. Photos post deflagellation confirmed that the deflagellation process succeeded. Regenerated flagellar

length at time 15 and 30 best showed the differences in regeneration rates between mutant and wild type.

We analyzed our data using Microsoft Excel software. First, we obtained the mean of our measurements for wild-type cells at each time, and repeated the same steps for the mutant samples. We then calculated standard deviation and 95% confidence intervals and graphed our results. We then statistically compared the two sets of data using two-way ANOVA to obtain our *p*-values.

Results

Figures 3 and 4 show the mutant and wild-type and samples without undergoing deflagellation. The wild type has two flagella protruding from the same spot on the cell body, which is the normal phenotype. In contrast, the mutant shows two flagella at opposites ends of the cell body, which is an abnormal expression of flagella and accounts for the impaired motility. In Figure 5, the cell has no observable flagella showing our deflagellation procedure was successful. This was the case for all wild-type replicates, but there was one replicate of the mutant where there were short flagella remaining after the deflagellation (Figure 6).

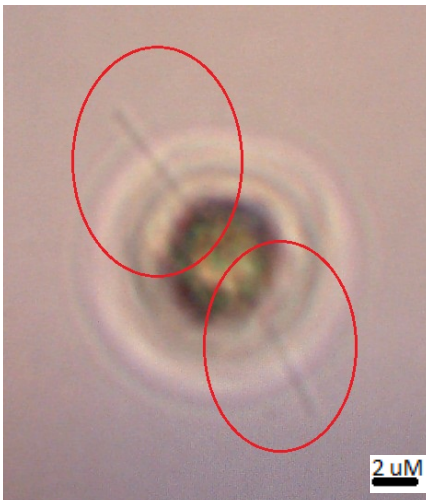

Figure 3. Mutant *C. reinhardtii* as viewed under the compound microscope at 1000X magnification prior to deflagellation.

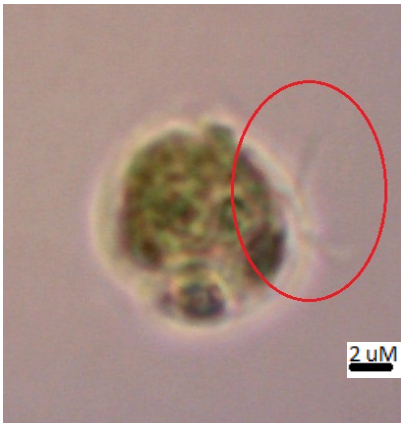

Figure 4. Wild-type *C. reinhardtii* as viewed under the compound microscope at 1000X magnification prior to deflagellation.

Figure 5. Wild-type *C. reinhardtii* as viewed under the compound microscope at 1000X magnification at t = 0 mins after deflagellation.

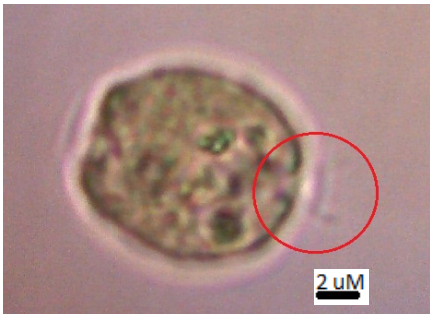

Figure 6. Mutant *C. reinhardtii* as viewed under the compound microscope at 1000X magnification at t = 0 mins after deflagellation.

Figure 7 shows that the mutant has a slightly higher mean flagellar length at time = 0 mins. Figure 7 also shows that error bars overlap between the wild type and mutant and that the mutant means have larger error bars, which indicates there was more variation in the data. The *p*-values for H_1, H_2 and H_3 are 0.01, 0.55, and 0.03 respectively as calculated by a two-way ANOVA.

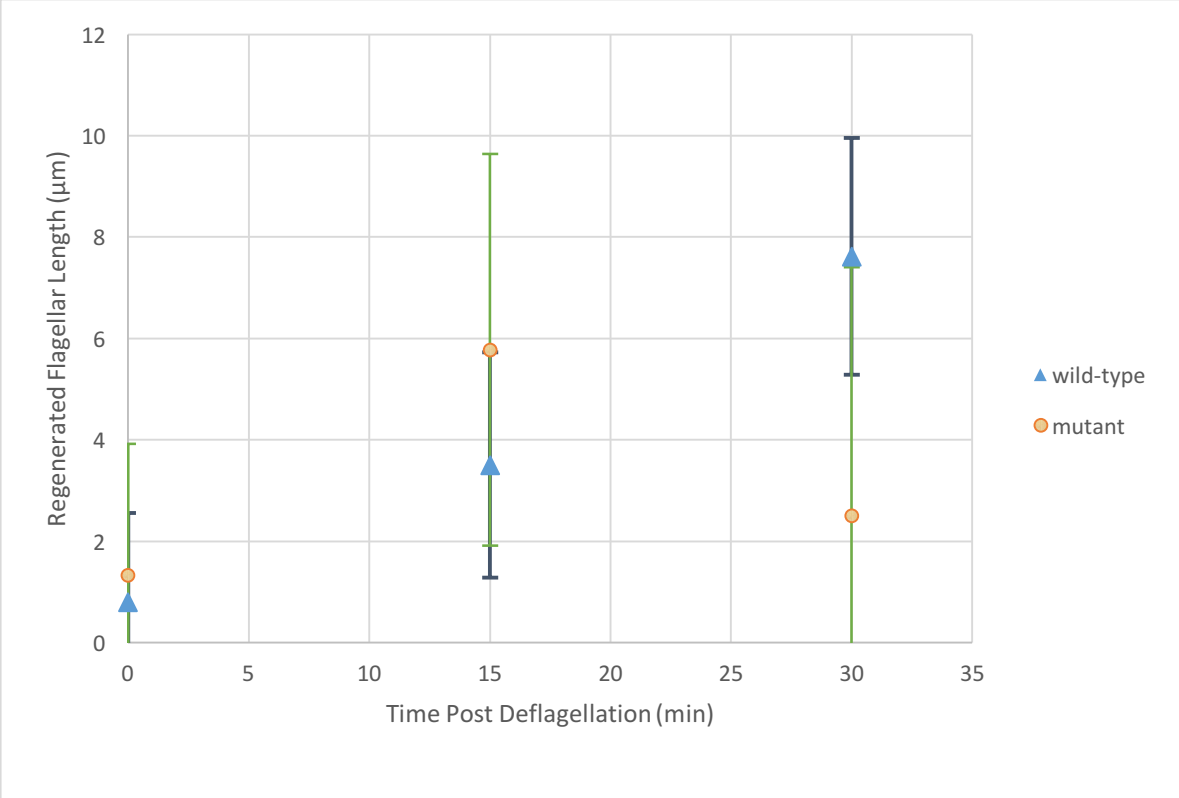

Figure 7. Mean regenerated flagellar lengths of wild-type and mutant, *Chlamydomonas reinhardtii* measured at specific times, t= 0, 15, 30 mins, after deflagellation with 95% confidence intervals as error bars with p_1 = 0.01, p_2 = 0.55, and p_3 = 0.03; n = 4.

We observed consistent increase of flagellar length during regeneration of the wild-type samples, but the mutant flagella did not get longer with time. Figure 7 shows that the data points for mutant did not exhibit linearly increasing behavior as you would expect in this situation. In fact, three of the four mutant replicates did not regrow their flagella by t = 30 mins, thus the average was far lower compared to the wild-type samples at the same time (Figure 7). In addition, we observed that the regenerated flagella in the mutant had a more crooked, deformed appearance compared to the wild-type regenerated flagella (Figure 8), which were straight and long.

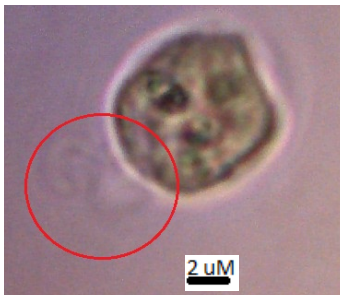

Figure 8. Mutant *C. reinhardtii* as viewed under compound microscope at 1000X magnification at t = 15 mins after deflagellation, replicate #2.

Lastly, we observed that regenerating wild-type cells aggregated into a concentrated green film near the top of the culture media by 10 – 15 mins post deflagellation. However, the mutant cells were concentrated at the bottom of the flask throughout the 30 mins of regeneration.

Discussion

We reject our Ho_1 and provide support for Ha_1 because $p_1 = 0.01$ which is less than 0.05. Therefore, time does have an effect on the regenerated length of flagella in wild-type *C. reinhardtii.* This is consistent with our prediction as well as the literature that *C. reinhardtii* will regrow their flagella after deflagellation given enough time (Harris 2001).

We fail to reject our Ho_2 and fail to provide support for Ha_2 because $p_1 = 0.55$ which is greater than 0.05. This means the presence of the mutation does not have an effect on the length of regenerated flagella. This is not consistent with our prediction; there is no significant difference in the lengths of regenerated flagella.

We also reject our Ho_3 and provide support for Ha_3 because $p_3 = 0.03$ which is less than 0.05. Hence, our data support that time does not have the same effect on both wild-

type and mutant *C. reinhardtii.* This is not consistent with our prediction that both strains will increase their flagellar length as time increases.

These results could be due to a variety of possible biological and experimental factors. Firstly, we consider that although our mutant strain has a defect in flagellar assembly and inhibited motility, it has normal, functional regenerative abilities. While our mutant strain lacks a functional inner arm dynein complex, which causes motility dysfunction (Myster et al. 1999), it does not necessarily mean that their ability to regenerate flagella will be impaired. We initially predicted that they will be slower to regenerate flagella due to irregular structural assembly; however, flagellar generation depends mostly on ample cytoplasmic microtubule proteins and kinases that regulate the microtubule dynamics (Wang et al. 2013). Assuming those components were normally expressed in our mutant strain, it is reasonable that there was no significant difference in flagellar length between wild-type and mutant cells.

Secondly, uncertainty was introduced at various times throughout the experiment. For example, our data showed that there was one mutant sample (Figure 6) where flagella were observed at t = 0 mins after deflagellation. We expected all cells to be deflagellated at this point, when in fact a small percentage of cells may not have lost their flagella. More rigorous studies using advanced techniques could achieve 98% deflagellation of the *C. reinhardtii* cells, but our simple procedure may not have been enough to achieve a high success rate (Rosenbaum et al. 1969). In addition, there is biological variability in the rate of regeneration of each organism; in wild-type *C. reinhardtii* the time it takes to fully regenerate flagella could vary between 70 – 90 minutes (Rosenbaum et al. 1969). Due to

time constraints of this experiment, we observed regeneration up to 30 minutes post-deflagellation, we also measured only a single cell per replicate. This may not accurately represent the status of the whole population of cells in each replicate sample. It was also not possible for us to look at the same cell over time due to constraints in our microscope technique; therefore, we looked at a different cell at each time. This may explain our inconsistent results for the mutant phenotype, for example, three cells from mutant replicates had flagella at t = 15 mins, yet another three cells taken from the same replicates had no flagella at all by t = 30 mins (Figure 7: mean at t = 15 mins is 5.8 μm, and t = 30 mins is lower at 2.5 μm). Due to this variation and experimental difficulties, our data could show that the presence of the mutation does affect the regeneration of flagella.

As for our qualitative results, wild-type *C. reinhardtii* usually have long flagella around 10-15 μm, but most flagellar mutants have uneven, stumpy flagella that grow to a range of different lengths (Tam et al. 2003). This matches the flagella we observed in mutant samples, which showed strange physical appearance of being short and/or crooked (Figure 8). These can be compared to the long straight flagella observed in our wild-type samples to characterize the mutant. Since we observed mutant cells could regenerate some flagella at t = 15 mins (Figure 7: mean length of mutant is 5.8 μm at t = 15 mins), yet we observed macroscopically that they were unable to swim to the surface, it shows that their motility was still impaired. This is consistent with our microscopic observation of the unusual physical qualities of our mutant samples. Since a pair of flagella allows movement by beating in asymmetrical patterns at one end of the organism (Brokaw 1982), short, crooked, or uneven flagella can explain lack of motility seen in the mutants (Figures 3 and

6). In contrast, the wild-type cells were first concentrated at the bottom after deflagellation, but as regeneration proceeded, they were able to use their new flagella and regain motility to swim to the surface.

Conclusion

We were able to reject our Ho_1 and provide support that time does have an effect on regenerated flagellar length in wild-type, as we predicted. We failed to reject our Ho_2: the mutation does not have an effect on the regeneration of the flagella, which is contrary to our predictions. Finally, our data cause us to reject Ho_3 which states that time has the same effect on both wild-type and mutant flagellar regeneration, and thus support that time does not have the same effect on the two strains. This contradicts our predictions as well. We also documented abnormal physical appearance of mutant cells and its ability to regenerate flagella comparatively to the wild-type despite lacking certain assembly components.

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