Deflagellation and Flagellar Regeneration in *Chlamydomonas*

I. Introduction

A. *Chlamydomonas*

In this experiment, you will be working with *Chlamydomonas reinhardtii*, a unicellular. *Chlamydomonas* is a photosynthetic organism which in the light, will grow in a defined medium containing inorganic salts and trace elements. *Chlamydomonas* has a cell wall and a single, large chloroplast, which occupies about 40% of the cell volume. Within the chloroplast there is a prominent pyrenoid, composed largely of protein and surrounded by starch deposits. On one side of the cell is a darkly pigmented “eye spot”. The contractile vacuole and nucleus are not always visible in live specimens, but you will be able to see all of these structures in the electron micrographs available in the lab.

B. Flagellar Structure

At one end of the organism there are two prominent flagella, which function in locomotion. The flagellar axoneme is comprised of a 9+2 arrangement of microtubules. The microtubules are always observed in a characteristic array of 9 outer doublet microtubules surrounding a central pair of two single microtubules. This 9+2 arrangement is highly conserved. You will be able to identify the axonemal microtubules as well as their associated accessory structures, such as the dynein arms and radial spokes, in the electron micrographs in lab. The axoneme is enclosed by an extension of the plasma membrane so that each flagellum is really a protruding region of the cytoplasm. At the base of each flagellum there is a basal body from which the doublet microtubules are assembled.

C. Assembly of Flagella

Flagellar length is tightly regulated in *Chlamydomonas*. Each time a cell undergoes mitosis, the flagella are resorbed, the cell divides, and each daughter cell produces two new flagella of the same length, about 10-12 µm. In addition, if one flagellum of the cell is amputated, the intact flagellum will disassemble as the new flagellum assembles, until the two flagella are the same length. Then both flagella will grow to their original length at approximately the same rate. How does the cell know what length its flagella should be? How does the cell know when the correct length has been achieved during the assembly process? How does the cell know when one flagellum is longer than the other?

Mutant *Chlamydomonas* strains that are defective in flagellar length control have been isolated. For example, some mutant strains have two flagella of unequal length (*ulf* strains); some mutant strains have long flagella up to 40 µm in length (*lf*).

D. Deflagellation and Flagellar Regeneration in *Chlamydomonas*

For today's exercise, you will observe and measure flagellar regeneration in a wild type strain of *Chlamydomonas*. First, you will induce flagellar excision by subjecting the organisms to acid pH shock. When *Chlamydomonas* cells experience solutions of acidic as part of a stress response. You will then neutralize the samples by adding base; the flagella should begin to regenerate. It is important to realize that the flagellar axoneme is comprised of approximately 300 polypeptides, yet the flagella completely regenerate within one hour of the treatment! You will also compare regeneration in the presence and absence of a specific inhibitor, cycloheximide. Cycloheximide inhibits protein synthesis by preventing translocation of the ribosome.
II. Procedures
You will be working together in pairs to observe flagellar regeneration in the presence and absence of cycloheximide. Each student will be involved in performing the experiment and observing the results. There is a handout chart on the last page for recording your measurements.

1. You will start with a 500µl aliquot of *Chlamydomonas* culture.

2. Mix the *Chlamydomonas* culture and then prepare a wet mount (as instructed by your TA) using 15µl of the culture. View the prep under 400X magnification using phase contrast. You should observe *Chlamydomonas* swimming.

3. Next, fix and prepare a wet mount of *Chlamydomonas* culture in the presence of Lugol’s. To do this, remove a 20µl aliquot from the culture into an eppendorf tube and add 1µl of Lugol’s. Using 20µl (culture and Lugol’s), prepare a wet mount as before. Observe the flagellated cell culture and measure the length of 10 flagella in this untreated culture. Calculate the average length of these flagella and record this value on the accompanying data sheet.

4. Transfer 400µl of culture to a fresh tube.

5. Add 24µl of 0.5N Acetic acid (this pH shock causes the deflagellation). Mix and wait 45 seconds. This timing is critical so as not to kill the cells. Then quickly add 24µl of 0.5N KOH (neutralization step) and mix. Note the time.

6. Transfer 200µl of the deflagellated cell culture into a pre aliquoted tube containing 2µl of cycloheximide and mix. Note the time. This tube will be termed “sample with cycloheximide”.

7. The remaining 200µl of deflagellated cell culture will be termed “sample with no cycloheximide”.

8. Remove a 20µl aliquot from the sample with cycloheximide and a 20µl aliquot from the sample with no cycloheximide. Add 1µl of Lugol’s to each 20µl aliquot to fix and stain cells. This will be the 0 time point.

9. Prepare a 20µl wet mount of each culture and view under the microscope. Repeat step 8 every 15 minutes (should continue for up to 1 hour). For each time point, you will measure the length of 5 individual flagella (see instructions below for measuring flagella using the microscope, camera, and accompanying computer software). Enter these measurements in the accompanying Table.

10. Report your measurements to the graduate and undergraduate teaching assistants. The teaching assistants will generate graphs of the combined class data which you will use to complete your assignment.

III. Measuring Flagellar Length:

1. Capture an image.
2. Under the Edit menu, select “Add Measurement.”

3. The measurement window will open. Within this window, select the objective you are using and select either “straight line” or “curved line”. This window must be kept open when making measurements. Note: Measuring using the “curved line” option take a clean mouse and very steady hand.

4. Identify the flagellum to be measured. Place the cursor at one end of the flagellum and left click. Move the cursor along the full length of the flagellum to its other end. Click the left mouse button when you have the cursor at the end. A line and its length (in microns) will appear on the image.

5. To take additional measurements, move the cursor to the next flagellum to be measured and repeat the steps used for the first measurement.

6. To remove a measurement, place the cursor on the line/measurement to be removed and double-click the right button.

7. To make a measurement a permanent part of the image, click on the “stamp” button in the measurement window. Note: Measurements can be made on captured images only.

IV. References


**Chlamydomonas Deflagellation and Flagellar Regeneration**

Average flagellar length \( n = 10 \) before deflagellation ____________

<table>
<thead>
<tr>
<th>Sample with no cycloheximide</th>
<th>Sample with cycloheximide</th>
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<tbody>
<tr>
<td>Time (min)</td>
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