The effects of light intensity on oxygen production in CC 3913 *pf9-3* mutant and CC-1690 *mt+21 gr* wild-type *Chlamydomonas reinhardtii*

Melissa Choi, Sogand Goharpey, Brian Infanti, Emma Peachey

Abstract

The purpose of our study was to examine if wild type strain CC-1690 *mt+21 gr* and mutant strain CC 3913 *pf9-3 Chlamydomonas reinhardtii* exhibit different photosynthetic activities under different light intensities, as measured by oxygen production. Oxygen production of the wild-type and mutant *C. reinhardtii* was measured at average light intensities of 442 lux, 3055 lux, and 13300 lux. A two-way ANOVA indicated that there was a statistically significant relationship between light intensity and oxygen production in *C. reinhardtii* ($p = 0.00016$). Furthermore, it was determined that oxygen production of the mutant was different than the wild type ($p = 6.62 \times 10^{-7}$), and that the wild type and mutant responded differently to changes in light intensity ($p = 0.01354$). Oxygen production increased as the light intensity increased due the greater availability of light energy for photosynthesis. The mutant strain of *C. reinhardtii* has impaired flagellar movement and is observed to produce significantly less oxygen than the wild-type strain, thus suggesting that flagellar movement is important for detecting light and producing oxygen.

Introduction

Chlamydomonas reinhardtii is a biflagellate, eukaryotic, haploid and unicellular alga that is roughly 10 μm in diameter (Pröschold, Harris & Coleman 2005). *C. reinhardtii* contains a single chloroplast in each cell and its photosynthetic apparatus is similar to that of vascular plants (Erickson, Setsuko & Krishna 2015). *C. reinhardtii* has an eyespot apparatus that is composed of two layers of globuli for the detection of light direction and intensity (Wagner et al. 2008). This light-sensing organ contains rhodopsin photoreceptors that enable *C. reinhardtii* to respond to different light environments by triggering ion currents across the membrane (Dieckmann 2003).

Photosynthesis is a primary process used by *C. reinhardtii* to use sunlight to generate an energy source in the form of reduced carbon (Figure 1; Erickson, Setsuko & Krishna 2015).

During photosynthesis, the photoreceptors located in the chloroplast use light energy to convert carbon dioxide and water to produce oxygen and organic matter (Figure 2; Erickson, Setsuko & Krishna 2015). In our experiment, we measured oxygen produced per cell in response to the exposure of various light intensities in order to analyze the photosynthetic rate of *C. reinhardtii*. Our objective was to investigate if different light intensities have an effect on the photosynthetic rate of wild-type and mutant *C. reinhardtii*.

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$$

Figure 1. Photosynthesis Equation

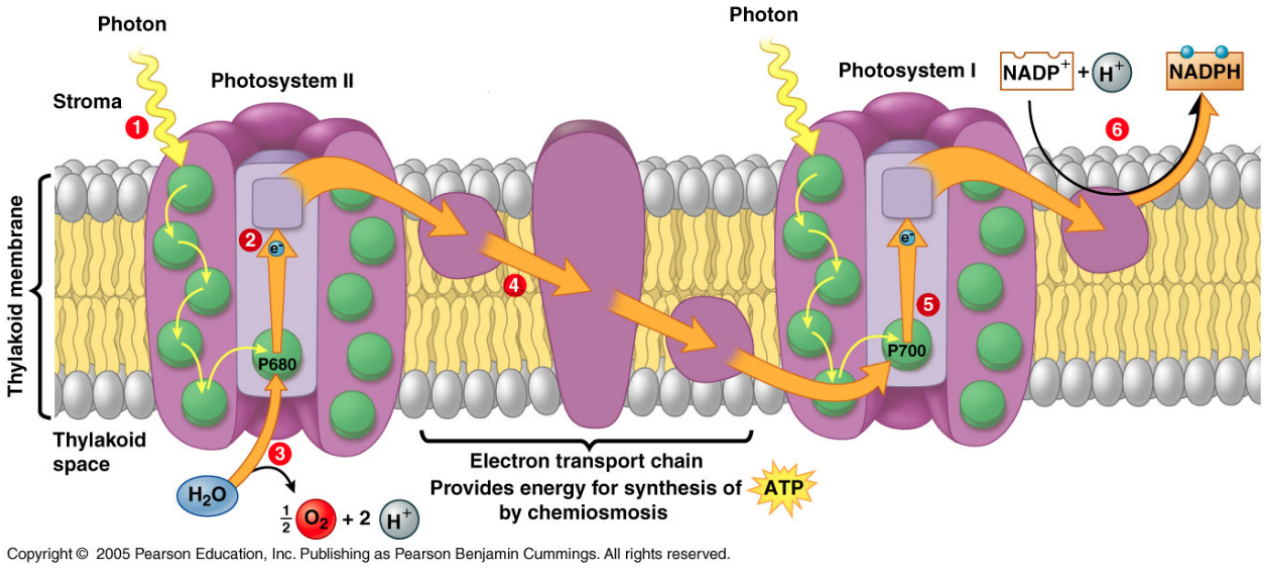

Figure 2. Diagram illustrates the process of photosynthesis occurring in the thylakoid membrane in the chloroplast. (Campbell et al. 2005)

In this experiment, we focused on the photosynthetic function of *C. reinhardtii* wild-type strain CC-1690 *mt+21 gr* and mutant strain, CC 3913 *pf9-3.* The wild type and mutant show a strong phenotypic difference in their flagella, as the mutant strain lacks flagella that enable proper swimming behaviour (Perrone et al. 1998). Previous research has demonstrated that the mutant type exhibited slower forward swimming velocities and an inability to exhibit phototaxis,

a process that enables cells to swim towards optimal light conditions for photosynthesis (Perrone et al. 1998). Since their function in the formation of flagellar waveforms is impaired, the mutant *C. reinhardtii* cannot quickly recover when illuminated by high light intensity (Perrone et al. 1998).

Therefore, we predict that both wild-type and mutant *C. reinhardtii* will have greater photosynthetic rates at greater light intensities as observed by the increase in oxygen production. However, we also predict that wild type will show greater photosynthetic rates due to their ability to control their motility easily in a liquid media for better photosynthetic function. Accordingly, we propose three sets of hypotheses, which are:

Ho1: Light intensity has no effect on oxygen production of *Chlamydomonas reinhardtii.*

Ha1: Light intensity has an effect on oxygen production of *Chlamydomonas reinhardtii.*

Ho2: Presence of the mutation has no effect on oxygen production of *Chlamydomonas reinhardtii.*

Ha2: Presence of the mutation has an effect on oxygen production of *Chlamydomonas reinhardtii.*

Ho3: The effect of light intensity on the oxygen production of *Chlamydomonas reinhardtii* is the same in wild type and mutant.

Ha3: The effect of light intensity on the oxygen production of *Chlamydomonas reinhardtii* is not the same in wild type and mutant.

This investigation is important as it provides implications for the relationship between light harvesting functions and the photosynthetic apparatus of *C. reinhardtii* as well as vascular plants. In a broader context, this can provide an insight to the evolution of photoreception, which can be utilized to study eyes of eukaryotes (Gehring 2004).

Methods

We had three treatments each with four replicates of mutant and wild-type *C. reinhardtii.* We set our first treatment as our control under a light intensity of 442 lux. We performed our second treatment at a light intensity of 3055 lux and our third treatment at 13300 lux.

. We made four 27mL replicates of wild-type and mutant *C. reinhardtii* for each treatment. We determined cell concentrations with a haemocytometer after the cells were fixed with $10\mu L$ of IKI (Iodine- potassium iodide).

We used the lamp and light meter to achieve light intensities of 442 lux, 3055 lux and 13300 lux. To measure the initial and final oxygen concentrations in each vial, we used an oxygen meter. We kept the temperature constant between treatments by using a water bath and a thermometer to maintain an average temperature of 21℃. To ensure that the light was distributed evenly throughout each sample, we turned each vial upside down in the water bath. We distributed the five vials in the water bath, one being the procedural control (as shown in Figure 3), to ensure that the change in oxygen production was only due to exposure of *C. reinhardtii* to different light intensities. We exposed them to the light for 60 minutes. After 60 minutes, we measured the O_2 concentration in each vial. We used this method for all treatments and replicates. The wild type were tested in one session and we used the same setup the following week to test the mutant.

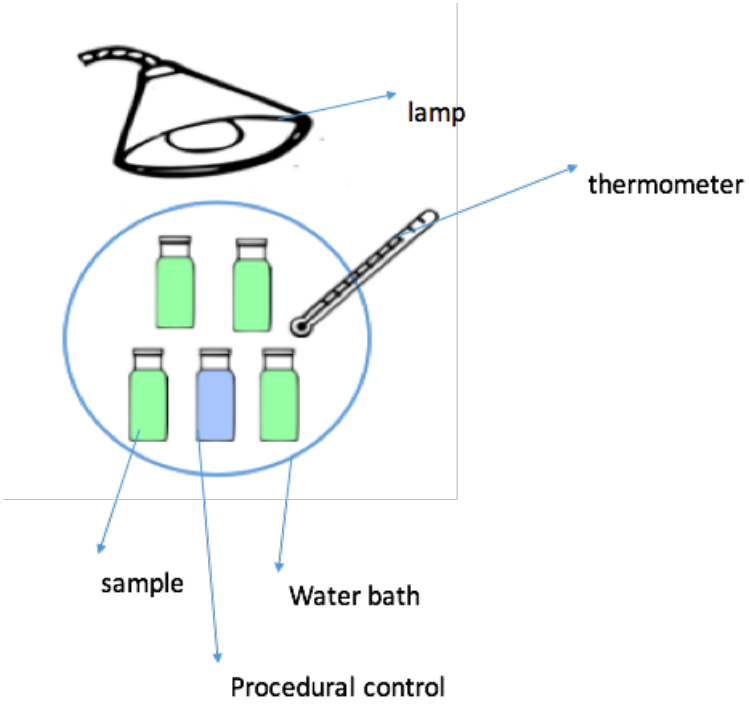

Figure 3. Setup of our samples in the water bath

From this, we then calculated the oxygen produced per cell in each treatment. We used the average light intensities and change in oxygen production per cell to calculate 95% confidence intervals. Finally, we performed a two-way ANOVA test to analyze our data.

Results

It is apparent from Figure 4 that the mean oxygen production per cell for both the wild-type and mutant *C. reinhardtii* increases as light intensity increases. At 442 lux, the mean oxygen production per cell was the lowest for wild type ($1.62 \times 10^{-7} \pm 4.97 \times 10^{-8}$ mg O_2 L^{-1} $cell^{-1}$) and the lowest for the mutant ($2.405 \times 10^{-10} \pm 2.11731 \times 10^{-8}$ mg O_2 L^{-1} $cell^{-1}$). At this light intensity, we observed a negative change in oxygen production per cell in some mutant samples. Oxygen production increased at the 3055 lux treatment, and oxygen production per cell was measured as $2.16 \times 10^{-7} \pm 1.07 \times 10^{-7}$ mg O_2 L^{-1} $cell^{-1}$ for the wild type and $5.80437 \times 10^{-8} \pm 4.03178 \times 10^{-8}$ mg

O_2 L^{-1} $cell^{-1}$ for the mutant. The greatest oxygen production produced per cell was at 13300 lux, with wild type at $4.73 \times 10^{-7} \pm 1.35 \times 10^{-7}$ mg O_2 L^{-1} $cell^{-1}$ and mutant at $9.31643 \times 10^{-8} \pm 3.23088 \times 10^{-8}$ mg O_2 L^{-1} $cell^{-1}$. We also observed during our experiment that some vials had air bubbles present. It is evident from Figure 4 that the 95% confidence intervals at 442 lux and 13300 lux do not overlap for both wild type and mutant. Furthermore, 95% confidence intervals do not overlap at each treatment between wild-type and mutant *C. reinhardtii.*

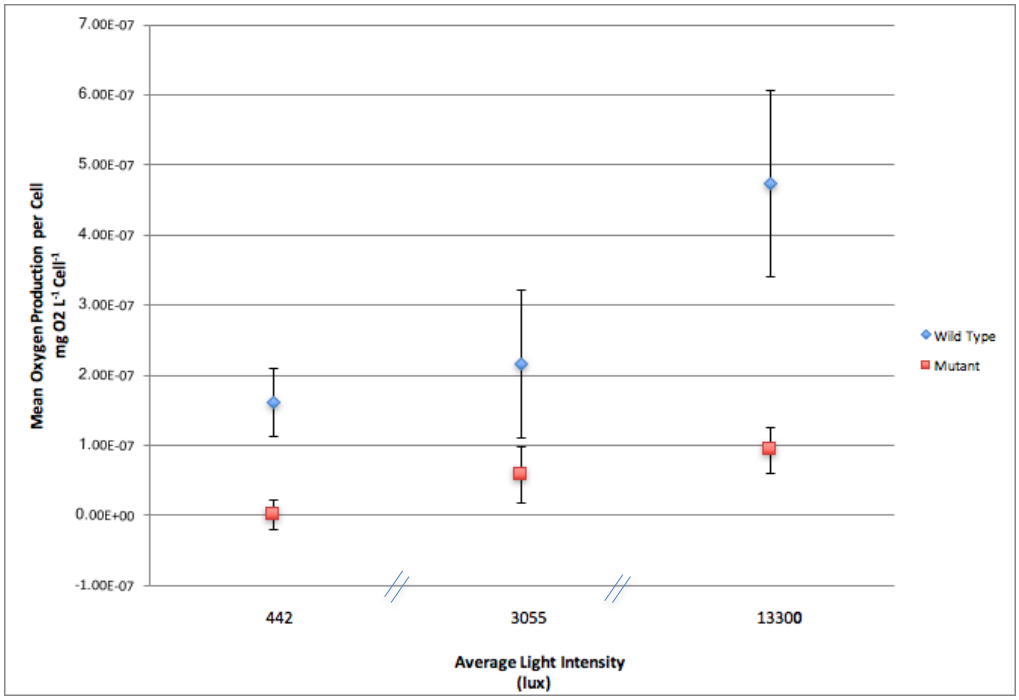

Figure 4: Mean oxygen production per cell of wild-type and mutant *Chlamydomonas reinhardtii* measured at light intensities of 442, 3055, and 13300 lux. Error bars represent 95% confidence intervals, n = 4 per treatment, p-values = 0.00016, 6.62×10^{-7}, 0.01354 for hypothesis 1, hypothesis 2, and hypothesis 3 respectively.

After performing a two-way ANOVA test between the mean oxygen production per cell at different light intensities, we obtained three different p-values for each of our hypotheses. The p-value calculated was 0.00016 for our first hypothesis, whether or not light intensity has an effect on oxygen production in *C. reinhardtii*. For our second hypothesis determining whether the presence of a mutation has an effect on oxygen production in *C. reinhardtii*, we obtained a p-

value of 6.62 X 10^{-7}. Lastly, the p-value calculated was 0.01354 for our third hypothesis on the effect of light intensity on oxygen production between wild-type and mutant *C. reinhardtii.*

Discussion

Based on the results from the two-way ANOVA, we reject all three of our null hypotheses and provide support for the alternate hypotheses. Our first null hypothesis that light intensity has no effect on oxygen production in *C. reinhardtii* had a p-value =0.00016. The null hypothesis is rejected since the p-value is smaller than 0.05, i.e., light intensity has a significant effect on oxygen production in *C. reinhardtii*. Similarly, our second null hypothesis is rejected ($p=6.62 \text{ X } 10^{-7}$); the presence of the mutation has a significant effect on oxygen production. Lastly, our third null hypothesis is rejected (p=0.01354), indicating there is a significant difference in oxygen production between wild-type and mutant *C. reinhardtii.*

The results obtained from the experiment provide support for our prediction that light intensity does have an effect on the oxygen production of *C. reinhardtii*. *C. reinhardtii* produces oxygen when consuming CO_2 through a process necessary for cell growth known as photosynthesis (Klein, Chen, & Gibbs 1983). As seen in Figure 2, photosynthesis is carried out by two multiprotein complexes, known as photosystems, located in the organism's chloroplasts, which split water into oxygen and hydrogen (Rochaix 2002). As a larger quantity of photons comes into contact with *C. reinhardtii's* photosystems, the organism is able to distribute the light excitation energy between its two photosystems and is therefore able to oxidize more water and produce a greater amount of oxygen (Rochaix 2002). This could be why we observe an increasing amount of oxygen produced per cell as light intensity increases and the number of photons *C. reinhardtii* are exposed to increases, not only in the wild type but also in the mutant.

We exposed *C. reinhardtii* to light intensities ranging from 442 lux to 13300 lux and observed a continuous increase in oxygen production, even at 13300 lux, which had been reported by Matsuda, Kikuchi and Ishida (1971) to inhibit photosynthesis. *C. reinhardtii* possess two photoreceptors, known as rhodopsins, which sense incoming light and function at low and high light intensities to allow the cell to respond to a wide range of light intensities (Sleneshchekiv, Jung, & Spudich 2002). This may explain why our populations of *C. reinhardtii* were able to produce oxygen at a range of light intensities. Our cells may not have exhibited an inhibitory response to light at 13300 lux as *C. reinhardtii* has been shown to stop photosynthesizing after two and a half hours of light exposure, and we measured oxygen production after only one hour (Matsuda, Kikuchi, & Ishida 1971).

Our results also provide support for our prediction that light intensity will have an effect on oxygen production in the mutant strain of *C. reinhardtii*. The CC-3913 *pf9-3* mutant strain that we used in our experiment has a mutation in the *pf9-3* gene, resulting in a non-functioning protein that fails to assemble the inner arm of the flagellum and thus *C. reinhardtii* is unable to initiate a phototactic response to light (Myster et al. 1997; Li et al. 2009). The phototactic response causes *C. reinhardtii* to stop flagellar motion and reorient the cell, which is a process used to respond to increases in light intensities in their environment (Li et al. 2009). While the *pf9-3* mutant strain is unable operate their flagella, they still have fully functioning chloroplasts and are therefore able to photosynthesize when exposed to light and produce oxygen, which may be why an increase in oxygen production is observed over increasing light intensities in our results. However due to their inability to move their flagella, they cannot use the phototactic response to respond to increases in light intensities, and therefore cannot orient themselves in the water column to maximize the use of incoming light to produce oxygen. This may be why we

don't see as much oxygen produced in the mutant strain of *C. reinhardtii* as in the wild-type strain.

Furthermore, the results from our experiment support our prediction that the effect of light intensity on oxygen production is not the same in the mutant and wild-type strains of *C. reinhardtii*. As observed in our results, the wild-type strain produces significantly more oxygen than the mutant strain when compared at the same light intensity for three different treatments. This suggests that flagellar movement is very important in order for *C. reinhardtii* to maximize photosynthesis. At the light intensity of 442 lux, almost no oxygen was produced by the mutant strain. This could be due to the fact that *C. reinhardtii's* photoreceptors are located on the eyespot of the cell, and these photoreceptors are what respond to light and initiate the phototactic response (Sleneshchekiv, Jung, & Spudich 2002; Li et al. 2009). Without the ability to move to initiate the phototactic response, the *pf9-3* mutant cannot orient the cell's eyespot to face the incoming light source, which may be why the mutant does not produce as much oxygen as the wild type.

There are many sources of uncertainty and variation that we encountered when performing our experiment and collecting our data. As mentioned previously, we recorded the concentration of cells for each replicate of each treatment. However due to spillage of the stock solution of cells from the vials when using the oxygen meter, we had to add more solution to each vial. As a result, our cell concentrations may not reflect the true cell concentration in each vial; therefore, our results for oxygen production per cell may not be accurate. Another source of error was that some of our vials contained small air bubbles when we put the caps on the vials. Since the solubility of oxygen is greater in air than in water, oxygen produced through photosynthesis by *C. reinhardtii* may have escaped into these small air bubbles and then into the

atmosphere when the caps were removed (Manahan 2005). As a result, the total oxygen produced per cell by *C. reinhardtii* may be more than our recorded values, which could be why we see negative oxygen produced per cell in some samples of mutant *C. reinhardtii* at 442 lux. Furthermore, there were some errors in our data collection due to use of the haemocytometer and calculating cell density. Some replicates had clusters of cells located in the haemocytometer whereas other replicates had cells dispersed throughout. Due to this variation in cell dispersal viewed in the haemocytometer, our recorded cell densities for each replicate may be overestimated or underestimated, thus affecting our calculation for oxygen produced per cell. Another source of error that we encountered in our experiment was that we obtained a low concentrated stock solution of cells. In another study, a starting concentration of 2×10^6 cells per mL was used when examining the growth rate of *C. reinhardtii* at different light intensities (Bonente et al. 2012). If we were to repeat this experiment, starting with a greater concentration of cells and with the same concentration in wild-type and mutant *C. reinhardtii* in order to reduce variation in cell concentration between replicates could contribute to more accurate results.

Conclusion

Our results suggest that light intensity has a significant effect on oxygen production of *C. reinhardtii*. Furthermore, there was a significant difference in the effect of light intensity on oxygen production between wild-type and mutant *C. reinhardtii.* We observed that the mutant *C. reinhardtii* produced less oxygen; therefore, our results suggest that the presence of fully functioning flagella has a positive effect on oxygen production for this organism.

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Literature Cited

Bonente, G, Pippa, S, Castellano, S, Bassi, R & Ballottari, M 2012, Acclimation of *Chlamydomonas reinhardtii* to different growth irradiances, *The Journal of Biological Chemistry*, vol. 289, no. 8, pp. 5833-5847.

Campbell, NA, Reece, JB, Taylor, MR & Simon, EJ 2005, *Biology concepts and connections,* Pearson, Arizona.

Dieckmann, Cl 2003, Eyespot placement and assembly in the green alga *Chlamydomonas*, *Bioessays*, vol. 25, no. 4, pp. 410-416.

Erickson, E, Setsuko, W & Krishna, KN 2015, Light stress and photoprotection in *Chlamydomonas reinhardtii*, *The Plant Journal*, vol. 82, no. 3, pp. 449-465.

Gehring, W 2004, Historical perspective on the development and evolution of eyes and photoreceptors, *International Journal of Developmental Biology,* vol. 48, no. 8-9, pp. 707-717.

Klein, U, Chen, C & Gibbs, M 1983, Photosynthetic properties of chloroplasts from *Chlamydomonas reinhardtii*, *Plant Physiology*, vol. 72, pp. 488-491.

Li Z, Wakao S, Fischer BB & Niyogi KK 2009, Sensing and responding to light, *The Annual Review of Plant Biology*, vol. 60, pp. 239-260.

Manahan, SE 2005, *Environmental Chemistry*, CRC Press, Florida.

Matsuda, Y, Kikuchi, T & Ishida, MR 1971, Studies on chloroplast development in *Chlamydomonas reinhardtii* I. Effect of brief illumination on chlorophyll synthesis, *Plant and Cell Physiology,* vol. 12, pp. 127-135.

Myster, SH, Knott, JA, O'Toole, E & Porter, ME 1997, The *Chlamydomonas* Dhc1 gene encodes a dynein heavy chain subunit required for assembly of the I1 inner arm complex, *Molecular Biology of the Cell,* vol. 8, no. 4, pp. 607-620.

Perrone, CA, Yang, P, O'Toole, E, Sale, WS & Porter, ME 1998, The *Chlamydomonas* IDA7 locus encodes a 140-kDa dynein intermediate chain required to assemble the I1 inner arm complex, *Molecular Biology of the Cell,* vol. 9, no. 12, pp. 3351-3365.

Pröschold, T, Harris, EH & Coleman, AW 2005, Portrait of a species, *Genetics*, vol. 170, no. 3, pp. 1601-1610.

Rochaix, JD 2002, *Chlamydomonas*, a model system for studying the assembly and dynamics of photosynthetic complexes, *The Federation of European Biochemical Societies*, vol. 52, pp. 34-38.

Sleneshchekiv, OA, Jung, KH & Spudich, JL 2002, Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*, *Proceedings of the National Academy of Science*, vol. 99, no. 13 pp. 8689-8694.

Wagner, V, Ullmann, K, Mollwo, A, Kaminski, M, Mattag, M & Kreimer, G 2008, The phosphoproteome of a *Chlamydomonas reinhardtii* eyespot fraction includes key proteins of the light signalling pathway, *Plant Physiology,* vol. 146, no. 2, pp. 772-778.