The effect of light intensity on the growth rate of wild-type and *tla3* mutant *Chlamydomonas reinhardtii*

Shinhwo Bak, Julia Lee, Kangchi Lee, Seungwon Lee, and Hyeseon Sohn

Abstract

The *TLA3* gene in *Chlamydomonas reinhardtii* encodes the chloroplast signal recognition particle protein CpSRP43, which has a role in coordinating signal recognition events in the chloroplast during photosynthesis (Kirst *et al.* 2012, Gao *et al.* 2015). This study examined the effect of light intensity and the effect of a deletion in the *TLA3* gene, which resulted in the truncated light-harvesting antenna3, on the growth rate of the organism. We incubated both the wild type and the *tla3* mutant under three light intensities of 1200 lux, 3000 lux, and 10000 lux at a constant temperature of 17˚C for a total of 14 days. We counted the cells 8.92, 11.96, and 13.96 days of incubation, plotted the counts using exponential equations, and obtained the growth rates from the slopes. We then used the two-way ANOVA to test our results for statistical significance. We observed a significant increase in the average growth rate of both the wild type and the *tla3* mutant with increasing light intensity (p-value = 2.05×10^{-8}). We also found that the reduction (in the photosynthetic apparatus) of photosynthetic pigments in the light-harvesting complexes from the deletion in the *TLA3* gene caused the *tla3* mutant to have a significantly lower average growth rate than the wild type at all the light intensities tested (p-value = 4.99×10^{-7}). There was a statistically significant difference in the effect of light intensity on the growth rate between the wild-type and the *tla3* mutant *C. reinhardtii* (p-value = 1.84×10^{-4}). The average growth rate of the wild type increased sharply, whereas the *tla3* mutant had a constant steady increase as light intensity increased.

Introduction

Chlamydomonas reinhardtii are biflagellate unicellular eukaryotic green algae with well-understood haploid genetics (Pröschold *et al.* 2005). They are characterized by the large cup-shaped chloroplast containing light-harvesting antenna complexes with pigments that can extract photon energy in light to allow photosynthesis (Pröschold *et al.* 2005, Mayfield *et al.* 2007). *C. reinhardtii* are frequently used as model organisms due to their short life cycle, rapid reproduction, and easily manipulated genome (Funes *et al.* 2007). In the light-dependent reactions of photosynthesis of *C. reinhardtii*, the energy from light is absorbed by the chlorophyll pigments of the light harvesting complex, a functional unit consisting of proteins and photosynthetic molecules surrounding reaction centres (Caffarri *et al.* 2009). The energy

absorbed is then used to make nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), which are subsequently used in the Calvin cycle to produce chemical energy, stored in the form of sugars, which can be released to fuel the organism's activities and growth (Bryant and Frigaard 2006).

The study of the cellular processes of *C. reinhardtii*, such as cell cycle control, regulation of gene expression, and photosynthesis, will provide insight into the biological processes of higher eukaryotic organisms at a molecular level (Lefebvre and Silflow 1999). Moreover, the potential use of *C. reinhardtii* as a biofuel and for high-value biopharmaceuticals has garnered the efforts of scientists to study the maximization of the growth rate and photosynthetic efficiency of the organism (Melis 2012). For instance, Sorokin and Krauss (1958) suggest that the growth rates of *C. reinhardtii* vary depending on varying level of photosynthesis under different light intensity. Mussgnug *et al.* (2007) found that the growth rate of the organism depended on the assembly of the light-harvesting antenna complexes that are responsible for light capture.

Our objective is to study the effect of specific light intensities on the growth rates of wild type and the *tla3* mutant, which has a deletion in the *TLA3-CpSRP43* gene and consequently a reduced antenna size (Kirst *et al.* 2012). To do this, we generated three sets of hypotheses. The first null hypothesis is that light intensity has no effect on the growth rate of *C. reinhardtii* and the alternate hypothesis is that light intensity has an effect on the growth rate of *C. reinhardtii*. We predict that higher growth rates would be observed with higher light intensity based on the findings by Brown and Richardson (1968). Our second null hypothesis is that a deletion in the *TLA3-CpSRP43* gene has no effect on the growth rate of *C. reinhardtii* and the alternate hypothesis is that presence of the mutation has an effect on the growth rate of *C. reinhardtii.* We

predict that the *tla3* mutant would grow slower due to its reduced content of key pigments in the light-harvesting complexes (Kirst *et al*. 2012). The third hypothesis is that the effect of light intensity on the growth rate of *C. reinhardtii* is the same in the wild type and the *tla3* mutant and the alternate hypothesis is that the effect of light intensity on the growth rate of *C. reinhardtii* is not the same in wild type and mutant. We predict that the change in growth rate will be greater in the wild type than in the *tla3* mutant with increasing light intensity since the *tla3* mutant has a fixed and reduced number of photosynthetic complexes (Kirst *et al.* 2012).

Methods

Experimental setup

The *C. reinhardtii* cell culture was originally grown in Tris-Acetate-Phosphate (TAP) medium. Cells of both the wild type and the *tla3* mutant were then transferred and allowed to grow for two weeks in standard medium which contained sodium citrate but lacked tris and acetate. Both the wild type and the *tla3* mutant had an inoculating concentration of $5x10^5$ cells/uL, as counted using a haemocytometer viewed under the Axio compound microscope. The starting cell concentration was specifically chosen to ensure optimal growth exhibiting exponential growth, as suggested by previous studies (Bölling and Fiehn 2005). As shown in Figure 1, the experiment had three light intensity treatments of 1200 lux, 3000 lux, and 10000 lux, each approximately corresponding to low light conditions, optimal conditions, and high light conditions (Bonente *et al.* 2012),. We placed four replicates of each of the wild type and *tla3* mutant into a 17˚C incubator for a period of two weeks, during which time we removed samples and counted the number of cells.

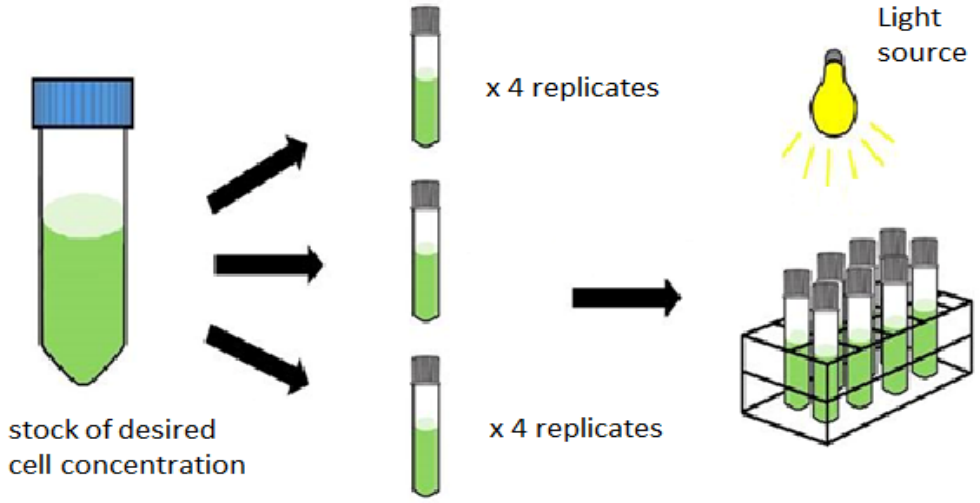

Figure 1. The steps taken to prepare the wild-type and *tla3* mutant replicates for the three light-intensity treatments.

Data collection

We counted the cells after 8.92, 11.96, and 13.96 days of incubation (after the cultures started to show an increase in cell numbers) and graphed the counts. Since growth of *C. reinhardtii* is exponential (Cooper 2006), we used the exponential equation to determine the growth rate of the organism from 8.92 to 13.96 days. We calculated the 95% confidence intervals for the average growth rate of each treatment for both the wild type and the *tla3* mutant to examine the variation of growth rate from the mean. We used the growth rates from all the replicates of the wild type and *tla3* mutant in a two-way ANOVA (analysis of variance) to calculate the *p*-values to determine the statistical significance of the effect of light intensity and the *TLA3* gene deletion, as well as the of the interaction of the two factors on the growth rate of *C. reinhardtii* at a significance level of 0.05.

Results

There was an initial lag phase in the growth rate of both the wild type and *tla3* mutant as we observed a slight decrease in the number of cells. The wild type had a mean growth rate of

23.51 ± 2.21 (95% confidence interval), 25.23 ± 5.15, and 54.31 ± 5.17 cells/day under 1200 lux, 3000 lux, and 10000 lux of light respectively. The *tla3* mutant had a mean growth rate of 15.12 ± 3.04, 19.84 ± 6.00, and 27.17 ± 2.87 cells/hour under 1200 lux, 3000 lux, and 10000 lux respectively. In the 1200 lux treatment, the wild type had a maximum growth rate of 26.23 cells/day and a minimum growth rate of 21.38 cells/day, whereas the mutant grew at a maximum rate of 17.86 cells/day and a minimum rate of 11.21 cells/day. In the 3000 lux treatment, the wild type grew at a maximum rate of 32.94 cells/day and a minimum rate of 21.71 cells/day, while the mutant had a growth rate of 28.27 cells/day maximum and a minimum rate of 13.61 cells/day. In the 10000 lux treatment, the wild type had a maximum growth rate of 59.27 cells/day and a minimum growth rate of 49.20 cells/day, as compared to the mutant which grew at a maximum rate of 30.93 cell/day and minimum rate of 24.46 cells/day.

The variances of the growth rates for both the wild type and the *tla3* mutant were represented by the 95% confidence intervals of the means of the average growth rates. For the wild type, we observed the greatest variation in growth rate in the 10000 lux treatment and the least in the 1200 lux treatment. The 10000 lux treatment had the largest 95% confidence interval of 49.14 to 59.48 cells/day and the 1200 lux treatment had the narrowest 95% confidence interval of 21.39 to 25.63 cells/day. For the *tla3* mutant on the other hand, we found the greatest variation in growth rate in the 3000 lux treatment and the least in the 10000 lux treatment. The 3000 lux treatment had the largest 95% confidence interval of 13.84 to 25.84 cells/day, while the 10000 lux treatment had the narrowest 95% confidence interval of 24.31 to 30.04 cells/day as shown by the length of the error bars in Figure 2.

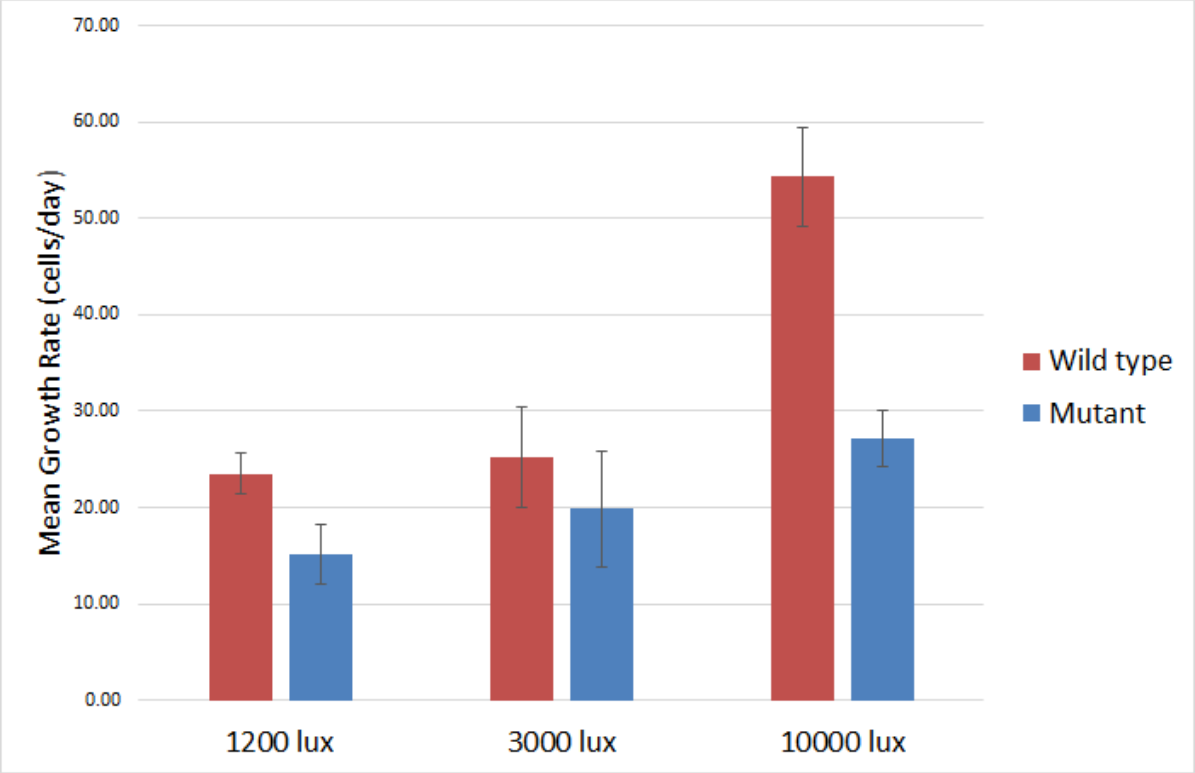

Figure 2. Mean growth rates of the wild-type (red) and *tla3* mutant (blue) *Chlamydomonas reinhardtii.* Bars represent 95% confidence interval. n = 4 for each cell type in each treatment of 1200 lux, 3000 lux, and 10000 lux light intensity.

The growth rates of both the wild type and the *tla3* mutant increased with increasing light intensity. We used a two-ANOVA test and found there was a significant difference between the growth rates of the wild type and the *tla3* mutant (*p*-value = 4.99×10^{-7}). It is likely that the significant difference can be accounted for by the lack of overlap of the 95% confidence intervals in the 1200 lux and 10000 lux treatments (Figure 2). Furthermore, Figure 2 shows that the wild type has a steep increase in the growth rate when the light intensity increased from 3000 lux to 10000 lux, whereas the *tla3* mutant had a constant increase in the growth rate across all light intensities. The other two *p*-values generated by the two-way ANOVA for hypotheses 1 and 3 were 2.05×10^{-8} and 1.84×10^{-4} respectively.

Discussion

With a *p*-value of 2.05×10^{-8}, we rejected our first hypothesis and provided support for the alternate hypothesis that light intensity had an effect on the growth rate of both the wild type and *tla3* mutant. Prior to the experiment, we had predicted that higher growth rate of *C. reinhardtii* would be associated with higher light intensity as long as light intensity was below a level that would cause photoinhibition to produce a stationary growth phase (Bonente *et al.* 2012, Falk *et al.* 2006). Our prediction was consistent with the experimental results in that the average growth rate of both the wild type and *tla3* mutant significantly increased with increasing light intensity. Both the wild type and *tla3* mutant became more photosynthetically active and exhibited faster growth rate when light intensity was increased from 1200 lux to 10000 lux. This result could be explained by the photoautotrophic or light dependent, nature of our organism (Brown and Richardson 1968). Kirst *et al* (2012) found that a linear increase in photosynthetic efficiency was associated with increasing light intensity until light saturation of photosynthesis was reached at 500 µmol photons m^{-2} s^{-1} (approximately 14000 lux). Higher light allows the chlorophyll pigments of *C. reinhardtii* to trap more energy for photosynthesis, the primary process that the organism uses to produce the carbohydrates needed for energy, growth and reproduction (Mayfield *et al*. 2007).

The *p*-value of 4.99×10^{-7} led us to reject our second hypothesis and provide support for the alternate hypothesis that the mutation in the light-harvesting antenna complexes had an effect on the growth rate of *C. reinhardtii*. Based on the findings by Kirst *et al.* (2012), we predicted that the mutant would have a lower growth rate due to its functional reduction in the structures that facilitate photosynthesis. Our prediction was confirmed by the results that at the same light intensity, the *tla3* mutant had a significantly slower growth rate than the wild type. The wild type

was able to absorb more light due to its higher content of chlorophyll and other accessory pigments for more photosynthesis (Bonente *et al*. 2012, Duanmu *et al.* 2013). The reduction in the antenna size and in the amount of light-harvesting complexes caused the *tla3* mutant to require a relatively higher light intensity to achieve the same maximum photosynthesis rate as the wild type (Kirst *et al.* 2012, Polle *et al.* 2003). Consequently, this resulted in the slower growth rate observed in our study. Because chlorophyll a, b, and carotenoids were the primary pigments that extracted photons for photosynthesis, the significant reduction of 30%, 5%, and 35% respectively of these pigments in the *tla3* mutant suggested a compromised capability of photosynthesis for the *tla3* mutant. The reduction in the development of thylakoid membrane also indicated a reduced ability to assemble functional photosystems (Kirst *et al*. 2012), further rationalizing the lower photosynthetic activity and the significantly lower growth rate of the *tla3* mutant, when compared to the wild type.

For the last set of hypotheses, we rejected our null hypothesis (p-value = 1.84×10^{-4}.) and supported the alternate hypothesis that the effect of light intensity on growth rate was different between the wild type and the *tla3* mutant. The impact of light intensity on growth rate was more apparent in the wild type than the *tla3* mutant; the growth rate of the wild type increased sharply with increasing light intensity, whereas the growth rate of the *tla3* mutant increased steadily as shown in Figure 2. The reduction in light-harvesting complexes in the *tla3* mutant limited the amount of light that could be absorbed at the given light intensities to reach the optimum photosynthetic activity (Kirst *et al*. 2012). Photosynthesis in the *tla3* mutant increased more steadily because only a fixed, reduced number of pigments were available at all the light intensities examined (Kirst *et al.* 2012, Mitra and Melis 2010).

Cell cultures of wild type and mutants at all light intensities experienced an initial decrease in cell numbers, leading to a long lag phase. We suspect that this was due to the change to a new less-rich culture medium and a decrease from optimum cultivation temperature. Cells were initially grown in TAP medium containing acetate, but were transferred to standard growth medium that lacked acetate during the experiment. *C. reinhardtii* can grow photoautotrophically with light and CO_2, chemotrophically on acetate, or photomixotrophically in a combination of the two growth modes (Stern *et al.* 1989, Zabawinski *et al.* 2001). Since acetate is a co-substrate for optimal production and accumulation of organic compounds, such as carbon sugars and lipids (Therien *et al.* 2014), its absence could lead to a long lag phase as observed. Moreover, protein complexes of light-dependent reactions such as photosystem II are extremely thermosensitive (Berry and Bjorkman 1980). Thus, a decrease from the optimal growth temperature of 23-25˚C to 17˚C may have contributed to the extended log phase. After the initial decrease in cell numbers, *C. reinhardtii* showed an exponential growth which is consistent with the proposed universal pattern of exponential cell growth of unicellular organisms during cell cycles (Cooper 2006).

Fluctuations in the light intensities examined were a consistent problem during our experiment. This problem largely contributed to the variances of the growth rates shown between replicates. We had difficulty acquiring desired light intensity and were limited by the ability to assure consistent light intensity for all the cultures. Although the fluctuation was within 10%, it was possible that this contributed to variation observed. Data collections were done by five different people could also contribute to the large variations in recorded cell counts.

It would be interesting to investigate whether a significant increase or decrease in the growth rate would be observed in the *tla3* mutant under light intensities that would have been

excessive, or damaging, to the photosynthetic apparatus of the wild type. Since the photosynthetic activity of the wild type saturates at 14000 lux and the photosynthetic activity of the *tla3* mutant could continue to increase beyond 55000 lux, future studies examining these light intensities could provide further insights into determining the active photosynthetic range and exploring ways to maximize the photosynthetic potential of *tla3* mutant.

Conclusion

We rejected our first null hypothesis since our results indicated that light intensity significantly affected the growth rate of *C. reinhardtii*. We found that higher light intensity allowed a higher growth rate of organisms regardless of the cell type in that the growth rate of both the wild type and the *tla3* mutant increased significantly when the light intensity increased from 1200 lux to 10000 lux. We also rejected our second null hypothesis as we found that the deletion *TLA3-CpSRP43* gene had an impact on the growth rate of our organism in that the wild type had a significantly higher growth rate than the *tla3* mutant under all the light intensities tested. As well, we rejected our third hypothesis as light intensity had a significantly greater impact on the growth rate of the wild type than the *tla3* mutant. We found that the growth rate increased sharply for the wild type but not for the tla3 mutant when light intensity increased substantially.

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