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Blue Light Regulation of Cell Division in *Chlamydomonas reinhardtii*[1]

Petra Münzner and Jürgen Voigt*[2]

Institut für Allgemeine Botanik und Botanischer Garten, Universität Hamburg, Ohnhorststraße 18, D-2000 Hamburg 52, Germany, and Botanisches Institut der Technischen Universität Braunschweig, Mendelssohnstrasse 4, D-3300 Braunschweig, Germany

ABSTRACT

A delay in cell division was observed when synchronized cultures of the unicellular green alga *Chlamydomonas reinhardtii* growing under heterotrophic conditions were exposed to white light during the second half of the growth period. This effect was also observed when photosynthesis was blocked by addition of the photosystem II inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Light pulses of 10 minutes were sufficient to induce a delay in cell division in the presence or absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. A delay in cell division was induced by blue light but not by illumination with red or far-red light. The equal intensity action spectrum revealed two peaks at 400 and 500 nm.

Photoautotrophically growing cultures of unicellular green algae can be synchronized by alternating periods of light and darkness (18, 20, 23). In the case of *Chlamydomonas reinhardtii*, synchronization by light/dark cycling was also achieved in the presence of acetate (8, 26), which is metabolized by this phytoflagellate. *C. reinhardtii* cultures synchronized in the presence of acetate continue to divide synchronously for one cell cycle period when transferred to heterotrophic growth conditions (26). This finding enabled us to investigate the differential effects of light on cell growth and cell division. Illumination at the beginning of the growth period caused an increased growth rate. As a consequence, the cells entered the division phase earlier than dark-grown cells (26). However, when the cultures were exposed to light after the cells had doubled their mass, a considerable delay in cell division was observed, which was accompanied by an extended growth period (26). The light-induced delay in cell division was also observed in the presence of DCMU, an inhibitor of PSII.

These observations indicate that the transition from cell growth to cell division is regulated by a light/dark-responsive cell cycle switch in *C. reinhardtii* (26) and that photosynthesis is apparently not involved in this process. Therefore, we have investigated which wavelengths and intensities of light are effective in inducing a delay in cell division. The aim of these studies was to identify the light receptor(s) involved in the light/dark control of cell division because both phytochrome (19) and at least one blue light receptor (1–7, 9, 11, 14, 24) have been implicated in *C. reinhardtii*.

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[2] Present address: Botanisches Institut, Technische Universität Braunschweig, Mendelssohnstraße 4, D-3300 Braunschweig, Germany.

MATERIALS AND METHODS

Chlamydomonas reinhardtii 137C mt+ (a wild-type strain from the collection of W.T. Ebersold) and the mutant strain ls mt− (15, 25) were obtained from Professor Dr. D. Mergenhagen (University of Hamburg, Germany). A modified high-salt medium (22) supplemented with sodium acetate (26) was used for all the experiments. Cultures were grown without aeration and synchronized by alternating periods of 14 h light and 10 h darkness as previously described (26). For subsequent growth in the dark, cultures were wrapped in a double layer of aluminium foil and bubbled with filtered air. Aeration was started 24 h after the beginning of the preceding light period. Cell concentrations were determined by hemocytometer counting. DCMU was obtained from Sigma (München, Germany) and added from a 2 mmol L^{-1} stock solution in 95% (v/v) ethanol.

Light Sources

Unless otherwise stated, the cultures were irradiated laterally using a combination of white incandescent lamps (type 36W/25, Osram, München, Germany) and daylight fluorescent lamps (Osram type 36W/11) at a photon fluence rate of 20 μmol m^{-2} s^{-1}. As a standard red light source, we used a combination of red fluorescent lamps (TL15/40W; Philips, Eindhoven, The Netherlands) and red Plexiglas (No. 501; Röhm and Haas, Darmstadt, Germany) as described by Mohr *et al.* (16) with a standard photon fluence rate of 20 μmol m^{-2} s^{-1}. As a far-red source, we used fluoresent lamps (Sylvania type F36T12/HO special phosphor No. 232 combined with Roscolux filters Nos. 83 and 27). The broad-band standard blue light source used throughout the experiments consisted of blue fluorescent lamps (TL18/36W; Philips) and an additional filter (Blaues Signalglas, 2 mm; Schott, Mainz, Germany). The standard photon fluence rate was 20 μmol m^{-2} s^{-1}. Most experiments with monochromatic light were performed using a Hitachi F-3000 fluorescence spectrophotometer as light source. At wavelengths below 400 nm and above 600 nm, additional interference filters were required to obtain monochromatic light.

For determination of the wavelength dependence curve, equal photon fluence rates (2, 5, or 10 μmol $m^{-2}s^{-1}$) were provided to the cell suspensions by use of neutral density filters. In some experiments, the bandpass of light was adjusted to 10 nm. In other experiments, a half-band width of 20 nm was chosen. At illumination times of 10 min, no differences were observed between these two sets of experiments. Some experiments were also performed with light produced by an XBO 450-W xenon lamp. In these experiments, monochromatic light was obtained by either the combined use of interference filters with half-band widths of 10 to 20 nm and appropriate color filters or splitting up the light by a Zeiss (Oberkochen, Germany) M4Q III monochromator. Photon fluence rates were measured with a quantum photometer (Li 185-A; Lambda Instruments Corp., Lincoln, NE) or—below 400 nm—by a thermophile attached to a Microva AL4 microvoltmeter (Kipp & Zonen, Delft, The Netherlands).

Determination of Protein

Cell growth was measured by determination of protein accumulation in the cells as recently described (26).

RESULTS

The vegetative cell cycle of the unicellular green alga *C. reinhardtii* is not only influenced by light via photosynthesis and cell growth but is also regulated by a light/dark-responsive cell cycle switch (26). This regulatory effect of light has been studied by investigating the effects of light on synchronized cultures of *C. reinhardtii* growing under heterotrophic conditions (Fig. 1; ref. 26). When these cultures were transferred to light during the second half of the growth period, a considerable delay in cell division was observed even in the presence of DCMU, an inhibitor of PSII (Fig. 2, A and B). A light-induced delay of sporangia formation and zoospore release was observed at photon fluence rates <5 μmol m^{-2} s^{-1} (Fig. 3), whereas photon fluence rates >10 μmol m^{-2} s^{-1} were required for photosynthesis and increased growth under comparable conditions (25). These findings indicate that the

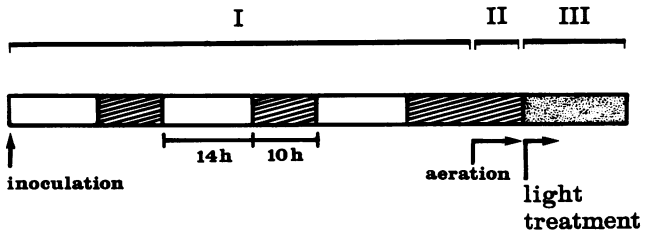

Figure 1. Experimental protocol for studying the regulatory effects of light on cell division in *C. reinhardtii*. I, Synchronization. Cultures were synchronized by growing mixotrophically (without aeration) for 3 d under a 14-h light/10-h dark regimen. II, Heterotrophic growth. Cultures were bubbled with filtered air (starting 10 h after the end of the preceding light period as indicated by arrow) and incubated in the dark for 8 h. III, Experiment (differential illumination in the absence or presence of DCMU). The bars show the light conditions: open bar, light; hatched bar, dark; dotted bar, varying light conditions.

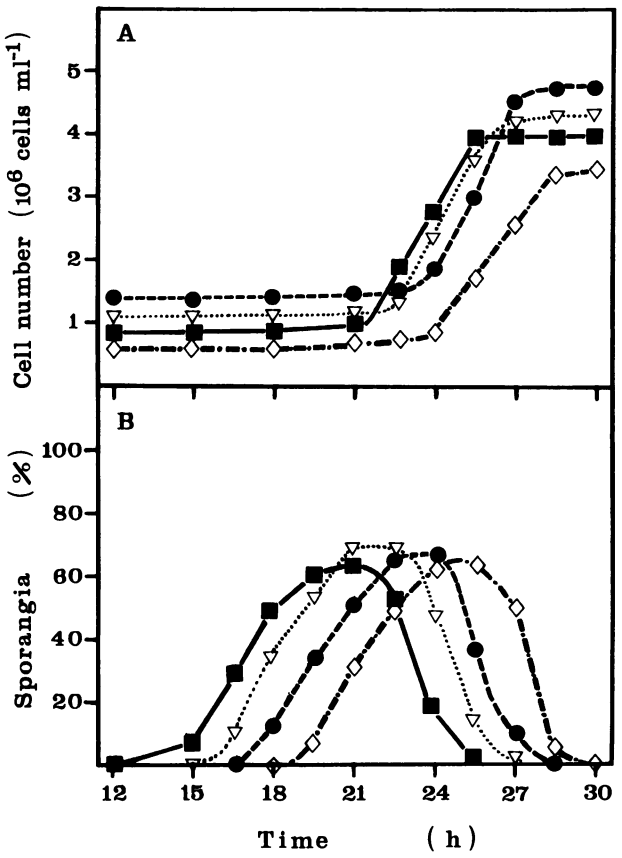

Figure 2. Effects of light on the vegetative cell cycle of *C. reinhardtii* strain Is grown synchronously under heterotrophic conditions. A, Cell number; B, proportion of sporangia. Ten hours after the beginning of aeration (start of heterotrophic growth = zero time), DCMU was added to a final concentration of 8 μmol L^{-1}, and the cultures were illuminated at photon fluence rates of 2.5 μmol m^{-2} s^{-1} (▽), 5 μmol m^{-2} s^{-1} (●), or 10 μmol m^{-2} s^{-1} (◇). The control culture was incubated in the dark (■).

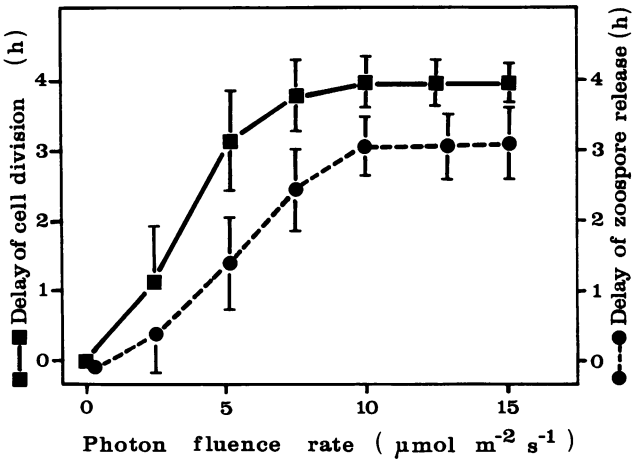

Figure 3. Irradiance effect curves for the delay of accumulation of sporangia (■) and the delay of the release of zoospores (●). The results are the mean ± SE from six independent experiments. Similar results were obtained with the wild-type strain 137C.

light-induced delay of cell division is not mediated by effects on photosynthesis.

Studying the effects of light on the formation of gametes by *C. eugametos* and *C. moewusii*, Förster (5) obtained wavelength dependency curves with two peaks at 460 and >600 nm, indicating that there are two light receptors in *Chlamydomonas*. Several blue light effects have been described for *C. reinhardtii* by different authors, such as induction of carbonic anhydrase (3, 4), nitrogen metabolism (1, 14), survival of dark-lethal mutants (24), and phototaxis (6, 7, 9, 11). On the other hand, Ruyters *et al.* (19) reported evidence for the presence of phytochrome in *C. reinhardtii*. Therefore, we have investigated whether phytochrome or a blue light receptor is involved in the light/dark control of cell division. In the presence of DCMU, an inhibitor of PSII, neither red light nor far-red light affected the transition from cell growth to cell division (Fig. 4, A and B). A considerable delay in cell division and sporangia accumulation was observed, however, when the dark-grown cells were exposed to blue light in the second half of the growth period (Fig. 4, C and D). To extend these results, experiments were performed at different intensities of blue, red, far-red, and white light. An effect of red or far-red light could not be observed at any light intensity tested (Fig. 5). Blue light, however, caused a delay in cell division at a considerably lower photon fluence rate than white light (Fig. 5).

The timing of cell division was not only affected when heterotrophically growing cultures were transferred to continuous light (Figs. 2 and 6, A and B) but also by a short pulse of white light (Fig. 6, D and E). Illumination for 10 min at a photon fluence rate of 5 μmol m^{-2} s^{-1} was sufficient to induce a measurable delay in sporangia formation (Fig. 6E) and in the increase in cell density (Fig. 6D). No effect of DCMU on sporangia formation (Fig. 6E), cell number (Fig. 6D), or cell growth, as measured by determination of the accumulation of protein (Fig. 6F), was observed under these conditions. When the cultures were transferred to continuous light, differential effects on sporangia formation (Fig. 6B), increase in cell number (Fig. 6A), and accumulation of protein (Fig. 6C) were observed in the presence and absence of DCMU. The delay in the increase in cell number (Fig. 6A) and the formation of sporangia (Fig. 6B) was considerably more pronounced in the presence than in the absence of DCMU. The increased accumulation of protein observed in the absence of DCMU (Fig. 6C) indicates a light-induced increase of cell growth, which might be related to the less pronounced effect on the timing of cell division (Fig. 6B), because it has been shown that light/dark control of cell division no longer occurs when the cells attain a strain-specific maximal cell size that causes the transition to the cell division phase (26). In the presence of DCMU, when light is not affecting cell growth, continuous light caused a considerably more pronounced delay in sporangia formation (Fig. 6B) than a light pulse of 10 min (Fig. 6E).

Illumination with a short pulse of blue light caused a more pronounced delay in cell division than a pulse of white light at the same photon fluence rate (Table I). The blue light effect on cell division was not modified by additional pulses of red or far-red light (Table I).

The wavelength dependency of the light-induced delay in cell division is shown in Figure 7. The equal intensity action spectrum revealed two peaks at 400 and 500 nm (Fig. 7B). Similar equal intensity action spectra were obtained when

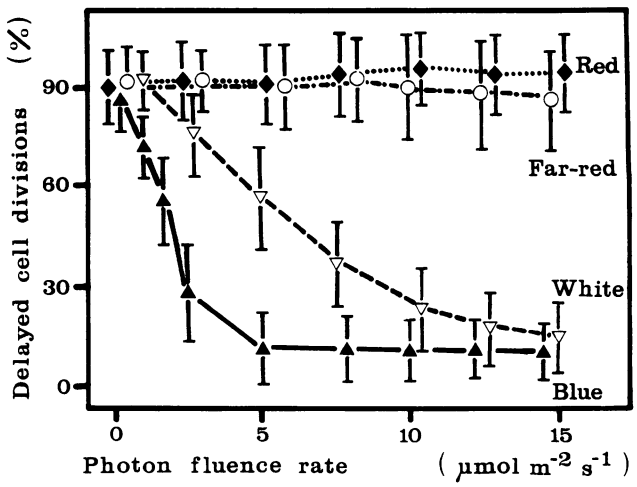

Figure 5. Effects of light quality and photon fluence rate on the delay in cell division in dark-grown cultures of *C. reinhardtii*. Dark-grown cultures of *C. reinhardtii* were adjusted to 8 μmol L^{-1} DCMU and transferred to the light as indicated 9 h after beginning of aeration. All the cultures were continuously bubbled with filtered air.

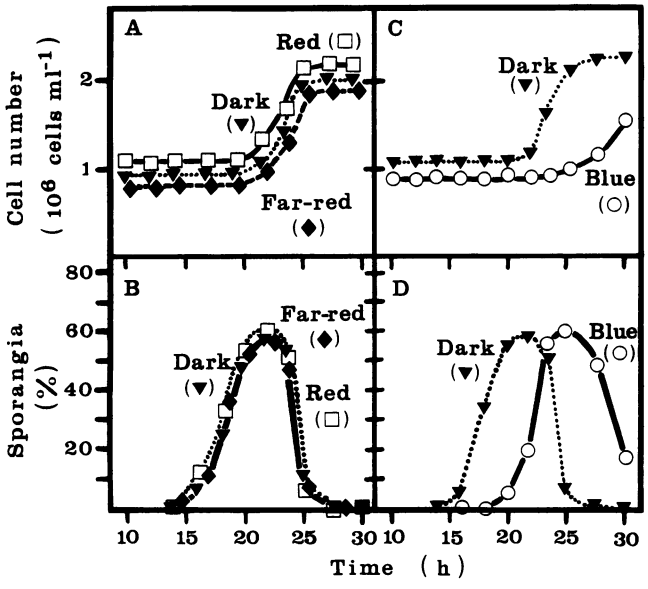

Figure 4. Effects of red, far-red, and blue light on cell divisions in heterotrophically growing cultures of *C. reinhardtii* strain Is. DCMU was added to the culture 9 h after beginning of heterotrophic growth to a final concentration of 8 μmol L^{-1}. Subsequently, the cultures were illuminated either with continuous red, far-red (A and B), or blue light (C and D) at photon fluence rates of 20 μmol m^{-2} s^{-1}. The increase in cell numbers (A and C) and the accumulation of sporangia (B and D) were compared with control cultures incubated in the dark. Similar results were obtained with the wild-type strain 137C.

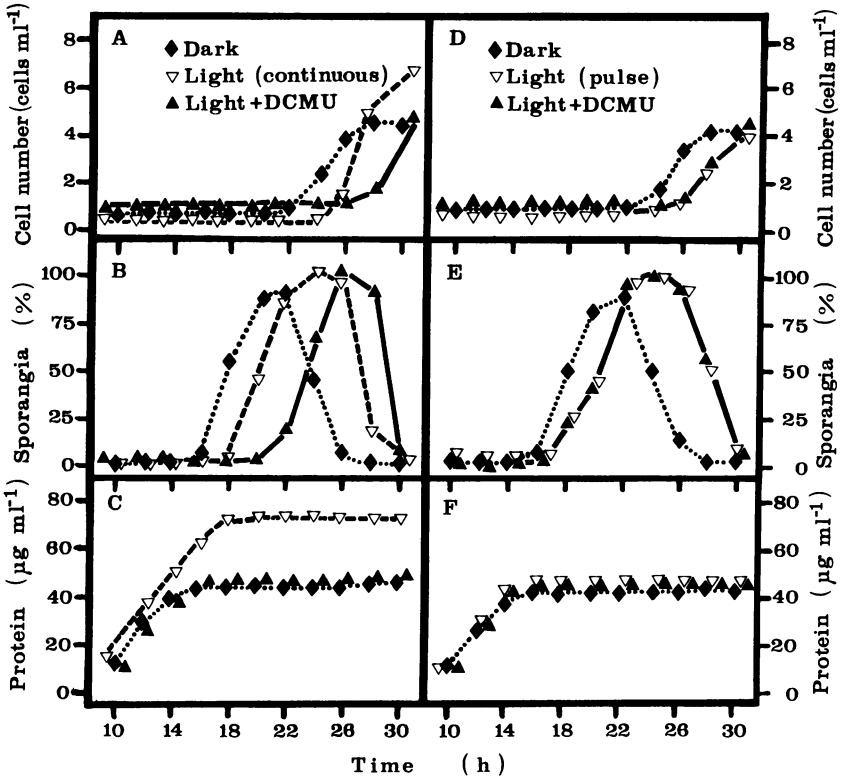

Figure 6. Effects of light pulses and continuous light in the presence or absence of DCMU on cell division in heterotrophically growing cultures of *C. reinhardtii*. Synchronized cultures of *C. reinhardtii* growing in the presence of sodium acetate were divided into six cultures at the end of the third light period. All of the cultures were subjected to aeration with filtered air 24 h after the beginning of the preceding light period (zero time) and incubated in the dark. After 8 h, two cultures were transferred to continuous white light (A–C) with or without addition of DCMU (final concentration, 8 µmol L^{-1}). Two cultures (one with and one without DCMU) were illuminated with white light at a photon fluence rate of 5 µmol m^{-2} s^{-1} for 10 min and then further incubated in the dark (D–F). The other two cultures were kept in the dark as controls. To determine the accumulation of protein (C and F), 1.5-mL aliquots of each culture were taken at the indicated times, and the cells were harvested by centrifugation, washed, and lysed by addition of 100 µL 1 mol L^{-1} NaOH. Aliquots were analyzed for protein as recently described (26).

Table I. *Effects of Light Pulses of Different Wavelengths on the Timing of Cell Division in Heterotrophically Growing Cultures of C. reinhardtii*

A synchronized culture of *C. reinhardtii* growing under heterotrophic conditions was divided into several daughter cultures 8 h after the beginning of the growth period. The cultures received light pulses (photon fluence rate, 5 µmol m^{-2} s^{-1}) as indicated and were transferred to the dark. Cell number and proportion of sporangia were determined in intervals of 30 min.

Treatment	Time of Cell Divisions[a]
	h
Continuous dark	16.5 ± 0.5
10 min white	18.0 ± 0.5
10 min blue	18.5 ± 1.0
10 min red	16.5 ± 0.5
10 min far-red	16.5 ± 0.5
10 min red, 10 min far-red	17.0 ± 1.0
10 min far-red, 10 min red	16.5 ± 0.5
10 min blue, 10 min red	19.0 ± 1.0
10 min blue, 10 min far-red	18.0 ± 0.5
10 min red, 10 min blue	19.0 ± 1.0
10 min far-red, 10 min blue	18.5 ± 0.5

[a] Time period after beginning of the heterotrophic growth where the proportion of sporangia reached its half-maximal level.

the cells were illuminated at photon fluence rates of 2 and 10 µmol m^{-2} s^{-1}, respectively (data not shown).

DISCUSSION

Both blue light and red light effects have been observed in the case of the unicellular green alga *C. reinhardtii* (1, 4–7, 9, 11, 14, 24). There is evidence for the presence of phytochrome (19) and at least one blue light receptor (1, 2, 4, 6, 7, 9, 11, 14, 24) in *Chlamydomonas*. Our data clearly demonstrate that the light/dark control of cell division is not mediated by phytochrome but that the light-induced delay of cell division is a blue light effect.

In the case of *Chlorella*, a light-induced stimulation of cell division has been described by Senger and Schoser (21). The action spectrum revealed two peaks, at 485 and 674 nm. Light induction of cell division was found to be sensitive to DCMU. However, DCMU inhibition of the induction of cell division was high in red and weak in blue light, indicating that the blue light effect is not exclusively due to photosynthesis. Apparently, blue light differentially affects cell division in *Chlamydomonas* and *Chlorella*.

Because nitrogen metabolism is similarly affected by blue light in *Chlamydomonas* and *Chlorella* (1, 10), the observed blue light effects on cell division are obviously not mediated by effects on nitrogen metabolism.

It has been reported that blue light stimulates carbohydrate degradation (13) and respiration (12). These effects might be involved in the blue light induction of cell divisions in *Chlo-*

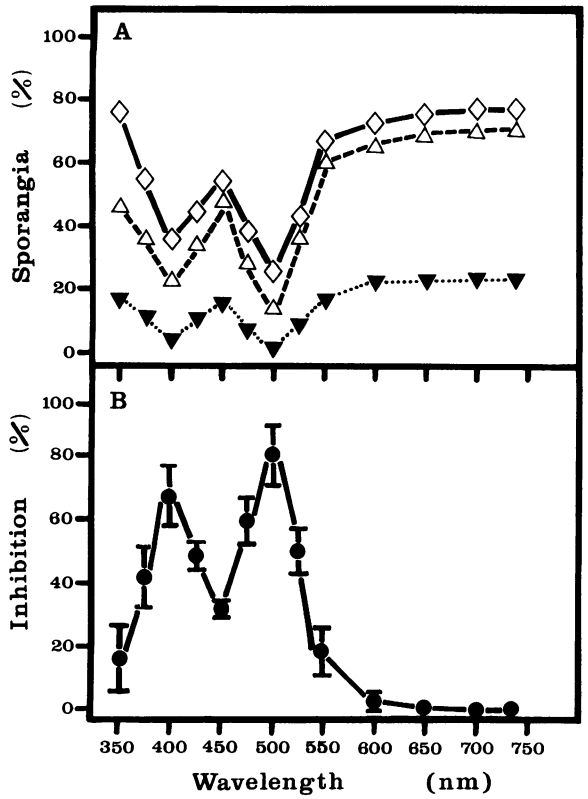

Figure 7. Wavelength dependency of the light-induced delay in cell division in heterotrophically growing cultures of *C. reinhardtii*. Synchronized cultures of *C. reinhardtii* growing under heterotrophic conditions were divided into 14 daughter cultures 8 h after the beginning of heterotrophic growth. One culture remained in the dark; the others were illuminated at a photon fluence rate of 5 μmol m^{-2} s^{-1} for 10 min as indicated and then further incubated in the dark. A, Cell number (not shown) and proportion of sporangia were determined in the different cultures 15 h (▼), 18 h (△), and 21 h (◇) after the beginning of heterotrophic growth. Results of a typical experiment of a series of five independent experiments are shown in A. B, Action spectrum of the light-induced delay in cell division. Mean values ± SD of five independent experiments are shown. The values are expressed as percentage reduction of the proportion of sporangia as compared with the culture remaining in the dark (not subjected to a light pulse).

rella observed in the presence of DCMU (21), because Senger and Schoser (21) also observed a blue light-induced stimulation of RNA synthesis. In the case of *C. reinhardtii*, however, a light-induced delay in cell division was also observed under conditions in which effects on cell growth can be excluded (Figs. 6 and 7, Table I). Therefore, the only remaining blue light effect observed in *C. reinhardtii* that might be somehow related to the blue light-induced delay in cell divisions is the blue light-induced survival of dark-lethal *C. reinhardtii* mutants (24). However, with the present state of knowledge, it would be too speculative to postulate an interrelationship between these two blue light effects.

More reliable conclusions can be drawn with respect to the photoreceptor(s) involved in the light-induced delay in cell divisions: the equal intensity action spectrum revealed two peaks at 400 and 500 nm (Fig. 7B). The peak at 500 nm might correspond to rhodopsin, which has been reported to be involved in the blue light effects on phototaxis (2, 6, 7, 9). The second peak at 400 nm might be an indication that there is a second blue light receptor that is also involved in light/dark control of cell division in *Chlamydomonas* (Fig. 7). Nossag and Kasprik (17) reported a strong light response of *Micrasterias thomasiana* Archer to blue light. The action spectrum revealed a peak at 390 nm. However, it is uncertain whether or not this effect is mediated by the same blue light receptor that, in addition to the putative rhodopsin, also affects cell division in *C. reinhardtii*. Because essentially the same equal intensity spectra were obtained at 2, 5, and 10 μmol m^{-2} s^{-1}, it seems also to be possible that there is a single blue light receptor involved in light/dark control of cell division in *C. reinhardtii*, which has absorption peaks at 400 and 500 nm.

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