Phytochrome Action on the Timing of Cell Division in 
Adiantum Gametophytes¹

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ABSTRACT

When filamentous protonemata of Adiantum capillus-veneris L. precultured under continuous red light were transferred to the dark, the apical cell divided about 24 to 36 hours thereafter. The time of the cell division was delayed for several hours by a brief exposure to far red light given before the dark incubation. The effect of far red light was reversed by a small dose of red light given immediately after the preceding far red light. The effects of red and far red light were repeatedly reversible, indicating that the timing of cell division was regulated by a phytochrome system. When a brief irradiation with blue light was given before the dark incubation, the cell division occurred after 17 to 26 hours in darkness. A similar red far red reversible effect was also observed in the timing of the blue light-induced cell division. Thus, the timing of cell division appeared to be controlled by phytochrome and a blue light-absorbing pigment.

An atypical red far red reversible system was reported to control the elongation of protonemata in Onoclea sensibilis (7), and red light promoted the elongation of young filaments although it inhibited the elongation of older ones (9). An interaction of blue light with the photo-reversible reaction was shown in this filament elongation (8). Still, little is known with respect to possible roles of phytochrome in cell division of fern gametophytes.

Synchronous cell division was induced by blue light irradiation in single celled protonemata of Pteris vittata (5). Moreover, the orientation of the mitotic cell plate in protonemata of Adiantum capillus-veneris was controlled by the sequence of treatment with red light, white light, and darkness given at appropriate intervals (16) and by the direction of irradiation (17).

In the present work, a red far red reversible effect on the timing of cell division was investigated.

MATERIALS AND METHODS

Plant Material. The experiments were done using spores of Adiantum capillus-veneris L. collected in the summer of 1970 in a greenhouse of the Koishikawa Botanical Garden of the University of Tokyo and stored in a plastic tube at room temperature.

Aseptic Culture. The culture medium consisted of one-tenth strength of modified Murashige and Skoog’s (11) mineral salts solution with 0.5% agar (Wako Pure Chemical Industries, Ltd., Osaka), adjusted to pH 5.7 to 6.0, sterilized by autoclaving at 120°C for 15 min, and poured into Petri dishes (3 cm diameter) which had been sterilized at 160°C for 2 hr. All preparations were done in a sterile room. Spores were sown on the surface of the solidified medium using Ito’s (4) aseptic technique, imbibed for 1 day in the dark, and cultured under continuous red light of about 500 ergs cm⁻²sec⁻¹ for 6 days at 25 ± 1°C.

Light Sources. Red light was provided by fluorescent tubes (Toshiba, FL 40 S-D/NL for preculture, and FL 20 S-W/NL for red light treatment) with a 3 mm thick red plastic filter (Torayglas 130, Toray Co., Ltd., Tokyo). Blue light was supplied by 20 w fluorescent lamp (Toshiba FL 20 S-W/NL) with a filter of 3 mm thick blue Plexiglas (Rohm and Haas, 2045). The far red light was provided by 300 W incandescent lamp (Iwasaki, Eye lamp PSR 300) filtered through a 10 cm depth of water and a 3 mm thick black Plexiglas (Rohm and Haas, V-58015). White light was provided by fluorescent tubes (Toshiba, FL 40 S-D/NL). The intensities of blue, red, far red, and white light for light treatment at the plant level, determined with a radiometer (YSI Kettering, Model 65), were about 850, 850, 3 × 10⁴ and 4000 ergs cm⁻²sec⁻¹, respectively.

Fig. 1. Timing of cell division induced by dark incubation without (O) or with pretreatment of far red light for 10 min (+), in single celled protonemata grown under continuous red light.

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RESULTS

Red Far Red Reversible Effect on the Timing of Cell Division Induced in the Dark. Continuous red light resulted in single celled protonemata after germination. The average proportion of two celled protonemata was less than 4% at the 6th day. When the single celled protonemata so cultured were transferred to the darkness, cell division occurred in the apical portion of the protonemata about 24 to 36 hr after the transfer to the dark (Fig. 1). However, the cell division was markedly delayed if far red light was given for 10 min before the dark incubation (Fig. 1).

Next, experiments were performed to see whether the process was under the control of a red far red reversible system. The percentage of divided cells was determined 32 hr after the cyclic treatment with red and far red light. Table I shows that cell division was induced in 17.7% of the protonemata transferred directly to the dark, but the rate decreased to 1.3% if an irradiation of far red light for 10 min was given. The decrease in percentage of cell division was fully reversed by subsequent

Table I. Effects of Red and Far Red Light on the Timing of Cell Division in Adiantum Protonemata

Protonemata cultured under continuous red light for 6 days were irradiated with about 850 ergs cm$^{-2}$ sec$^{-1}$ red light (R) and about 3 x 10$^4$ ergs cm$^{-2}$ sec$^{-1}$ far red light (FR) for 10 min for each treatment, transferred to the dark, and observed 32 hr after the light treatment. Values represent mean values ± one standard error obtained from triplicate samples of 100 gametophytes.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Division</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>17.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>R/FR</td>
<td>1.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>R/FR/R</td>
<td>17.0 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>R/FR/R/FR</td>
<td>2.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>R/FR/R/FR/R</td>
<td>24.0 ± 2.5</td>
<td></td>
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</tbody>
</table>

Fig. 2. Effects of red or far red light irradiation on the blue light-induced cell division. Single celled protonemata were grown under continuous red light and then exposed to blue light for 10 min (●), to red light for 10 min immediately after the blue light (○), and to blue light followed by far red light for 10 min (+). They were transferred to the dark after the light treatments.

Fig. 3. Effect of blue light intensity on cell division rate in single celled protonemata grown under continuous red light. Blue light was given for 10 min, and the percentage of dividing cells was measured at 24 hr after the irradiation.

Fig. 4. Effect of irradiation time of blue light on the cell division rate in single celled protonemata grown under continuous red light. The percentage was counted 24 hr after the irradiation.

red light irradiation for 10 min given immediately after the far red light exposure. The effect of red and far red light on the rate of cell division was repeatedly reversible when cyclic treatments with red and far red light were applied to the protonemata precultured under continuous red light.

Effect of Blue Light on the Timing of Cell Division. When protonemata precultured under continuous red light were treated with 10-min irradiation of blue light and then kept in
the dark, the protonemata underwent the first transverse division after 17 to 26 hr (Fig. 2). This timing of the division was significantly earlier than those treated with red and far red light.

In preliminary experiments, the dose-response curve for three different intensities of 10-min blue light was determined after 24 hr of dark incubation, and the results are plotted in Figure 3. The frequency of cell division at the 24th hr also depended on the stimulus duration of blue light (Fig. 4).

**Effect of Far Red Light on the Timing of Blue Light-induced Division.** The relation between the blue light effect and the red far red reversible effect in terms of the timing of cell division was examined. Six-day-old protonemata exposed to far red light for 30 sec to 16 min immediately after 10-min irradiation with blue light were transferred to the dark, and the percentage of divided cells was counted 24 hr after the blue light exposure (Fig. 5). The result clearly showed that 2-min or longer irradiation was enough to lower the frequency of cell division. Further, a time-course study was carried out after exposures to blue light for 10 min followed by far red irradiation for 10 min, and the cell division was observed to occur in a range of 20 to 34 hr in the dark (Fig. 2). Thus, the timing of blue light-induced division was remarkably delayed by a small dose of far red light, but division under these conditions was still earlier than in the absence of blue light.

**Red and Far Red Reversible Effect on the Timing of Blue Light-induced Division.** The effect of red light given immediately after irradiation with far red light following blue light.

Table II. Reversible Effect of Red and Far Red Light on the Timing of Blue Light-induced Cell Division in Adiantum Protonemata

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Division (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>56.0 ± 5.0</td>
</tr>
<tr>
<td>B/R</td>
<td>51.0 ± 1.0</td>
</tr>
<tr>
<td>B/R/FR</td>
<td>13.3 ± 1.7</td>
</tr>
<tr>
<td>B/R/FR/R</td>
<td>45.7 ± 4.2</td>
</tr>
<tr>
<td>B/FR</td>
<td>11.0 ± 1.5</td>
</tr>
<tr>
<td>B/FR/R</td>
<td>43.3 ± 1.4</td>
</tr>
<tr>
<td>B/FR/R/FR</td>
<td>11.0 ± 1.7</td>
</tr>
<tr>
<td>FR</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Dark</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>FR/R</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>FR/B</td>
<td>21.0 ± 2.5</td>
</tr>
</tbody>
</table>

Fig. 6. Effects of red light irradiation dosage on cell division rate in single celled protonemata with 10 min blue light followed by 10 min far red light. The percentage of cell division was counted at 24th hr of dark incubation after the light treatment.

Fig. 7. Timing of cell division induced by incubation under continuous blue light (●) and continuous white light (○) in single celled protonemata grown under continuous red light.
was examined. Six-day-old red light-precultured protonemata were exposed to 10 min of blue light followed by 10 min of far red light, and then immediately thereafter were irradiated with red light for 30 sec to 16 min. The percentage of divided cells was determined after 24 hr of dark incubation (Fig. 6). Two minute or longer irradiation with red light was enough to reverse the far red light-induced suppression of cell division. The effects of red and far red light on the percentage of divided cells at 24 hr after blue light exposure were repeatedly reversible when cyclic treatments with red and far red light were applied (Table II).

Figure 2 shows the time course of the percentage of divided cells in protonemata treated with red light for 10 min immediately after 10 min of blue light. Division occurred after 19 to 29 hr, and a slight delay of the time of division as a result of such treatment, as compared with blue light alone, was observed.

When protonemata precultured under continuous red light were transferred to continuous white light rather than darkness, cell division occurred slightly later than under continuous blue light (Fig. 7). The delay caused by white light may be due to the effect of red light from the white fluorescent lamp, as indicated by the results in Figure 2.

**DISCUSSION**

Since the early work of Borodin (1), it has become evident that developmental processes in the fern gametophyte are markedly influenced by wavelength: blue light induces cell division and red light induces cell elongation (6, 10, 15). The present work shows that red, far red, and blue light greatly influence the timing of cell division in *Adiantum* protonemata. The time required for 50% cell division was about 22 hr after an exposure to blue light, 31 hr for red light, and 37 hr for far red light (Figs. 1, 2).

The action spectra for photochemical transformation of oat phytochrome in vitro (2) showed that light in the blue and near ultraviolet regions will transform phytochrome, although the longer wavelengths are much more effective. However, phytochrome is not likely to be the photoreceptor for the blue light-induced cell division in *Adiantum*. If phytochrome were the only pigment controlling the timing of cell division, the results should be dependent on the final state of phytochrome irrespective of the sequence of light treatments. For example, the treatment with far red light and that with blue followed by far red light should induce the same result; however, the average time to 50% cell division was about 37 hr for the former (Fig. 1) and only 27 hr for the latter (Fig. 2). Thus, a phytochrome-dependent timing mechanism appears to overcome the blue light-absorbing system.

Similar interaction between phytochrome and a blue light-absorbing pigment in ferns has been reported for cell elongation of *Onoclea sensibilis* (8) and spore germination of *Pteris vititata* (13). The elongation of *Onoclea* protonemata was reversibly controlled by red and far red light (7), and far red irradiation alone promoted filament elongation to a greater extent than blue light; but a blue light irradiation, either following or preceding far red treatment, strongly inhibited the far red promotion (8). The spore germination of *Pteris* was typically controlled by phytochrome, but, when the imbibed spores were briefly exposed to low energy blue light either immediately before or after red irradiation, the germination was completely inhibited (13). The blue light-induced inhibition was reversed by intermittent red light, this effect in turn being reversibly annulled when each exposure was followed by a brief far red irradiation (14). Action spectra for the blue light effect had peaks at 440 nm and 380 nm with a shoulder between 440 and 480 nm (12).

In an earlier study, a red and far red reversible effect on the timing of cell division was also observed in *Pteris vititata*, when protonemata precultured under continuous red light were transferred to darkness (3). Therefore, the phenomenon reported in the present paper seems to be general in fern development.

**LITERATURE CITED**