Is There a Third Photoreceptor Involved in the Control of Chloroplast Movements in Mougeotia? ¹

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ABSTRACT

The photometric method was used to test a possibility proposed recently that a new photoreceptor with maximum activity at 620 nm is involved in mediating chloroplast rotation in Mougeotia (Z Lechowski, J Białczyk [1988] Plant Physiol 88: 189–193). The hypothesis was tested under conditions of continuous dichromatic unilateral or mutually perpendicular irradiation with red light of wavelengths 620 or 680 (660) nanometers and far-red. When the red light was polarized parallel to the long cell axis, chloroplast rotation could be monitored by changing the direction of far-red irradiation. The level of the response obtained with red and far-red applied from the same direction depended on far-red intensity: at higher fluence rates the maximum response was shifted to longer wavelengths of red light. A high fluence rate of far-red inhibited the response. The absorption coefficients of Mougeotia chloroplasts were measured for the studied wavelengths using the microphotometric method. Possible impact of absorption by the chloroplast on photoreception has been discussed. Current and previous results can be interpreted in terms of photochrome action and do not support the involvement of the hypothetical 620 nanometer photoreceptor.

Two main photoreceptor systems involved in the control of light-induced chloroplast movements in green plants have been well established: a blue-UV absorbing pigment and phytochrome (13). The most likely candidate for the blue-UV absorbing receptor is a flavoprotein (14) and, according to the evidence available to date, most plants exhibiting chloroplast rearrangements make use of this photoreceptor (1, 4, 14, 15, 20, 26, 34, 35). Both systems have been shown to control chloroplast orientation in the conjugate green algae Mougeotia and Mesotaenium (12, 17, 25) and in the fern Adiantum (33). Phytochrome appears to be the main photoreceptor in Mougeotia and Mesotaenium (12). The short-wavelength-absorbing pigment was shown to cooperate with phytochrome in mediating the strong-light, i.e. face-to-profile response in Mougeotia (7, 25). A possibility of its independent action was also raised (6).

The existence of several other photoreceptors has been assumed for various photomorphogenetic processes on the basis of action spectra and kinetic analyses. Some of them absorb in the blue region, while the others fill the spectral range between blue and red (2, 8, 9, 16, 19, 28, 29). In chloroplast movement, however, the blue-absorbing pigment and phytochrome are the only photoreceptors that have been considered over the years, notwithstanding occasional evidence suggesting that photosynthetic pigments can play an auxiliary role (3, 24, 27).

Recently, a third photoreceptor with an action maximum at 620 nm was reported in Mougeotia (21, 22). The postulate was based on experiments in which two different colors of light (730 nm and another varying from 500–680 nm) were used to elicit chloroplast movement. The concept deserves detailed consideration because it introduces a new, apparently overlooked factor into the process of light perception in Mougeotia chloroplast movement. The present work aims at testing the hypothesis of an additional photoreceptor for other irradiation programs, employing mutually perpendicular beams and higher fluence rates. The use of higher fluence rates was particularly important because the authors of the hypothesis assumed that the FR² used in their experiments canceled any Pb gradient in the cell. According to our previous experience the fluence rates used were insufficient to do so.

MATERIALS AND METHODS

Mougeotia sp. (strain from Botanical Institute, University of Erlangen, FRG) was cultured in Petri dishes as described previously (32). The algae were grown in a controlled environment chamber at 9 μmol m⁻² s⁻¹ PAR, 12 h photoperiod. The temperature in the culture chamber and during all the experiments was 17°C.

Experimental Procedure

All experiments were carried out according to the same general scheme. First, chloroplasts in all algal filaments were brought to a defined starting position which was either profile (Φ) or flat (Θ) (preorientation). Then, the response was elicited by constant illumination with two independent light beams.

² Abbreviations and symbols: FR, B, G, R, far-red, blue, green, and red light; ‡, polarization perpendicular to long cell axis; ||, polarization parallel to long cell axis; ‡, irradiation from the side; ‡, irradiation from above; Φ, profile position of chloroplasts; Θ, face position of chloroplasts; Pb, red light-absorbing form of phytochrome; Pb, far-red light-absorbing form of phytochrome.

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One beam was always FR of 725 nm; the second, R or G light.
Preparations were made at least 12 h before the experiment, kept overnight in darkness at 17°C, and then illuminated in the culture chamber for about 2 h from above with 9 μmol m⁻² s⁻¹ cool-white light from fluorescent tubes. Photometric preparations consisted of several hundreds of filaments mounted parallel on a glass plate in a drop of culture medium. The filament bands were fastened at both ends with parafilm strips and covered with a 30 μm thick transparent polyethylene membrane stretched over a metal ring. The membrane provided undisturbed and rapid gas exchange with the atmosphere. Microscope preparations were made in the same manner, but the number of filaments was reduced to 10 to 15 and they were placed at a distance of several cell diameters from one another. In experiments starting with chloroplasts in profile position, preparations were preirradiated laterally with R ⊥ of 3.3 μmol m⁻² s⁻¹.

The response was recorded using the photometric technique described elsewhere (31). The wavelength of the measuring beam was 656 nm and its fluence rate 0.11 μmol m⁻² s⁻¹. To eliminate the actinic effect of the measuring light it was switched on for 20 s each 10 to 20 min. In two experiments the level of the response was evaluated under a microscope after 30 min and/or 1 h of the irradiation in the photometer. The response was calculated as the percentage of cells with chloroplasts seen in face position plus 50% of partially responding cells, i.e., with chloroplasts in positions intermediate between face and profile. Chloroplast positions were counted each time in 100 cells.

Measurements of light transmission through single chloroplasts were carried out in a microscope-photometer, Amplival of Zeiss (Jena, GDR).

Light Sources and Filters

The actinic light was supplied from three halogen lamps (100 W, 12 V, PZO, Poland) with IR cut off by a 3 mm C805 heat-absorbing filter. The samples were irradiated with two beams either unidirectionally from above (with the use of a semitransparent mirror) or with the beams mutually perpendicular. In one set of experiments all three beams were used, two from above and one lateral. The lateral irradiation of the sample was provided by a glass plate cut obliquely at both ends, guiding the light to the preparation by total internal reflections (for details see Gabrys [5]). Monochromatic light was obtained using interference filters: IF 550 (6.0 nm half-band width), 620 (6.0), 660 (9.0), 680 (7.5), and 725 (11.0) nm. G and R light was polarized parallel (⊥) or perpendicular (∥) to the filament axis with a polarizing foil, FR was unpolarized in most experiments. For preirradiation, R⊥ light was used, obtained with an IF 656 nm. In test experiments we also used strong white light produced with a 2 mm thick BG14 broad-band filter and an additional C805 filter (3 mm). The filters served to change the spectral distribution of light from the halogen lamp and thus to imitate white light used in Lechowski and Bidaczky (21–22). Fluence rates were adjusted with a set of neutral density filters and measured with a silicon photovoltaic cell Hamamatsu S 122733 BQ. White light was measured with a quantum sensor LI-190SB (Li-Cor, USA). All filters were from Schott, Jena (GDR).

RESULTS

Dependence of the Response on Preirradiation

A number of preliminary experiments were carried out to determine whether strong white light should be used for preorientation of chloroplasts. Two preorientation modes were compared: one with strong white light of 250 μmol m⁻² s⁻¹ applied from above, second with R ⊥ of 3.3 μmol m⁻² s⁻¹ from the side. The former mode corresponded to conditions described (21–22), the latter to the preirradiation routinely used in our former investigations. The profile-to-face response was induced by a combination of R and FR typical of subsequent experiments.

Figure 1 shows a typical example of the recorded time-courses. After preirradiation with R ⊥ the response was significantly higher than after strong white light. The ratio of respective transmission changes after 30 min was in the range of 2.3 to 2.6. Moreover, transmission levels of the preparations preoriented with white light were always lower by about 20% when compared to those preirradiated with the weak R ⊥. Microscopic observation showed that chloroplasts attained full profile position following white light preorientation but they underwent a contraction characteristic of strong light (30). Therefore, to avoid the inhibitory effect of preirradiation on chloroplast responses, R ⊥ was used for preorientation in all experiments starting with chloroplasts in profile position.

Effect of FR Direction on the Response

As shown in Figure 2A, a simultaneous irradiation of Mougeotia with R∥ 620 nm, 3.3 μmol m⁻² s⁻¹ and FR 725

![Figure 1](https://example.com/image1.png)

**Figure 1.** Effect of preirradiation on chloroplast movement induced by continuous R∥ light of 680 nm ⊥ + 725 nm ⊥. Fluence rates were 3.3 and 12.2 μmol m⁻² s⁻¹, respectively. Solid curve, preirradiation with R ⊥ = 3.3 μmol m⁻² s⁻¹; dashed curve, preirradiation with white light ⊥, 250 μmol m⁻² s⁻¹. Ordinates: left, absolute transmission; right, percent of the maximum transmission change corresponding to full ⊥ → ⊥ rotation. Note that the preparations preirradiated with white light had a lower transmission level at the starting profile position of chloroplasts. This was most probably due to chloroplast contraction (see text).
Figure 2. Dependence of chloroplast response on the direction of FR combined with R parallel (A) or G (B). Photon fluence rates [\( \mu \text{mol m}^{-2} \text{s}^{-1} \)]: A, R parallel 9.1 or 98 (lower curve); B, G 13.8, FR 12.2.

nm, 9.1 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) brought about the rotation of chloroplasts from \( \Theta \rightarrow \Theta \) or from \( \Theta \rightarrow \Theta \), depending on the direction of FR; the chloroplast always turned with its face toward FR. G of 550 nm combined with FR led to identical responses in terms of the direction, with a fourfold higher fluence rate needed for attaining the same response level (Fig. 2B).

The chloroplast rotation could be inhibited only by using substantially stronger FR irradiation. The increase in the fluence rate by one order of magnitude reduced the response after 1.5 h to 15%, a level only slightly higher than that obtained when the 620 nm light only was used (Fig. 2A). No increase in the FR intensity was necessary to stop the rotation of chloroplasts completely when FR was applied from both directions (FR and R), as shown by the G-FR combination in Figure 2B.

Only one response, i.e., \( \Theta \rightarrow \Theta \) was possible for R of 620 nm polarized perpendicular to the long cell axis, combined with FR. It occurred when both wavelengths were applied from the same direction (Fig. 3).

**Effect of the Direction of FR Polarization**

The response \( \Theta \rightarrow \Theta \) was induced by 620 nm light from above in the presence of the polarized, lateral FR. Figure 4 shows that the parallel polarization of FR hindered the chloroplast rotation which attained only 12% of the full response, i.e. the level of the control at 620 nm light alone (cf. with Fig. 2A). On the contrary, chloroplasts rotated to the complete profile when FR polarized perpendicular to the long cell axis was used.

**Dependence of the Response Level on the Fluence Rate of FR for Two Red Light Wavelengths**

We investigated the response \( \Theta \rightarrow \Theta \) induced by R and FR of increasing intensities, both applied from one direction.

The levels of the response were compared for two R parallel wavelengths: 620 and 660 nm, both of 3.3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). As shown in Figure 5, the maximum response was shifted to a higher fluence rate of FR with increasing wavelength of R. At the same time, the rotation was slower at higher FR fluence rates as one can see from the comparison of the curves obtained after 30 min and 1 h (Fig. 5A).

The density of algae in photometric preparations was greater than in those used in the optical microscopy and the filaments were not ideally parallel. Therefore, we repeated the experiments described above, on preparations consisting of several filaments and microscopically evaluated the number of rotating chloroplasts. The results for 660 nm and for a longer wavelength of 680 nm are presented in Figure 5B. The levels of the response calculated for the respective FR fluence rates were somewhat higher than those measured photometrically but the maximal response was attained at the same FR fluence rate.

**Red Light Absorption by Chloroplasts of Mougeotia**

Evaluation of light transmission through the turning chloroplast, that is the irradiation of the distal part of the cell was carried out in order to discuss the results in more detail. The data obtained for four different wavelengths are given in Table 1. Marked differences in transmission percent can be noticed for different red light wavelengths. They become still larger if an approximately four time increase in chloroplast thickness is taken into account. The latter corresponds roughly to the situation when light passes through the chloroplast in the profile position.

**DISCUSSION**

The results presented in Figures 2 and 3 clearly demonstrate that the chloroplast movement to the profile position proceeds solely following the direction of FR, directions of R parallel or G being insignificant in this respect. A \( \Theta \rightarrow \Theta \) response was obtained when FR was applied from above and an inverse response when FR was given laterally. Only the former re-
polarized parallel to the long cell axis: the direction of the tetrapolar \( P_r \) gradient depends on the direction of FR irradiation (Fig. 6, d, e, f). Thus, by changing the direction of FR from top to lateral, both types of chloroplast responses may be induced.

The behavior of chloroplasts subjected to dichromatic irradiation with the use of polarized FR (Fig. 4) also agrees with Haupt's model. As expected, FR \( \parallel \) from the side was ineffective in inducing the movement toward the profile position because in the whole cell the absorption vector of \( P_r \) was perpendicular to the \( E \) vector of FR. A small response in Figure 4 was most probably observed because the orientation of filaments was not perfectly parallel in the preparation. On the other hand, after changing the polarization of FR to \( \perp \), the \( P_r \) gradient established in the cell corresponded to the situation depicted in Figure 6f.

It would be noteworthy to discuss at this point the assumptions and arguments behind the new concept of a photoreceptor with an absorption maximum at 620 nm. First, the authors of the concept assumed that the FR background applied in their experiments canceled any gradient of \( P_r \) in Mougeotia (21, 22). In our opinion this is not true; on the contrary, FR of several \( \mu \text{mol m}^{-2} \text{s}^{-1} \) must have led to the formation of a larger gradient than one created by the R \( \parallel \) itself (cf. 18). An efficient abolishment of the \( P_r \) gradient in the cell irradiated continuously with R \( \parallel \) or G \( \parallel \) and FR is possible when two FR beams perpendicular to each other are applied (see Fig. 2B). FR from one direction may cancel the \( P_r \) gradient only when its fluence rate is an order of magnitude higher than that of R \( \parallel \) (Fig. 2B). Probably, in that case, the FR effect results from light scattering by intracellular structures.

An essential argument in favor of the new photoreceptor was the finding that the maximum of R \( \parallel \) activity against a constant background of 12 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) FR lay at 620 nm and that the response fell abruptly to 0 for red light of 675 nm (22). At that wavelength, the strongest induction of the phytchrome-controlled chloroplast movement in Mougeotia should be anticipated (10). The results shown in Figure 5 demonstrate that the maximum sensitivity to R \( \parallel \) shifts toward longer wavelengths with the increase in the intensity

![Figure 4](image-url)  
**Figure 4.** Dependence of chloroplast response on the polarization of FR applied simultaneously with R \( \parallel \) 620 nm. Fluence rates as in Figure 3.

response was possible for R \( \perp \) and required irradiation with both wavelengths from the same direction. These results are entirely compatible with the model of phytchrome orientation in the cell membrane proposed by Haupt (11; cf. also 12 and 13), which is schematically illustrated in Figure 6. The strongest \( P_r \) gradient is produced by red light polarized perpendicular to the cell axis (a). A simultaneous irradiation with FR from the same direction (b) causes only a narrowing of the region of high \( P_r \) concentration between Oi axis since, for the axis OY, the absorption vector of \( P_r \) is perpendicular to the direction of the electric vector in FR. In the case of FR perpendicular to R \( \perp \) (c) the \( P_r \) absorption vector and \( E \) vector of FR are parallel. This leads to the \( P_i = P_r \) cycling on the walls situated in the directions OY and O(–Y). The concentration of the \( P_r \) form on these walls depends on the ratio of quantum flux densities. In that case the rotation \( \Theta \rightarrow \circ \) is impossible (Fig. 3). The situation changes when R is polarized parallel to the long cell axis: the direction of the tetrapolar \( P_r \) gradient depends on the direction of FR irradiation (Fig. 6, d, e, f). Thus, by changing the direction of FR from top to lateral, both types of chloroplast responses may be induced.

![Figure 5](image-url)  
**Figure 5.** Effect of the FR fluence rate on the chloroplast response for two R wavelengths, measured photometrically (A) and calculated under a microscope (B). R \( \parallel \) and FR were applied from above. Shorter and longer wavelength red lights had fluence rates of 3.3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \); in the experiment shown in B, right curve, higher fluence rates of 5.7 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) were used. Standard deviations are indicated only when exceeding 4%.
of the background FR. At the FR fluence rate higher than 42 μmol m⁻² s⁻¹ 660 nm is more effective than 620 nm. Consequently, if the action spectrum were measured at the FR fluence rate higher than 42 μmol m⁻² s⁻¹ it would have a maximum at λ ≥ 620 nm.

Further, the authors excluded the involvement of the phytochrome system in the observed effect since they noticed a complete absence of 675 nm activity against 12 μmol m⁻² s⁻¹ FR (see refs. 21, Fig. 1 and 22, Fig. 1). Our experiments did not confirm this result: 50% of the chloroplast response was reached after 30 min as shown in Figure 1 for 680 nm || + 12 μmol m⁻² s⁻¹ FR. A similar result was obtained using microscope estimation (Fig. 5). Results in Figure 1 point to one of the possible reasons of the discrepancy: after the preirradiation of algae with strong white light the observed response was much lower than after weak R ⊥ preirradiation. The apparent inhibition may be interpreted in terms of the coaction between phytochrome and the blue-light receptor system, discussed in our earlier paper (8). After-effects of the irradiation with strong B + R which are the components of white light may occur in the cell up to several tens of minutes (cf. 8, Fig. 3) and may disturb the responses induced subsequently by relatively weak red light.

Another potentially interfering factor is the chloroplast contraction after white light. Return of the chloroplast to its normal state is a very slow process: the first, fastest phase was reported to last 1 h (30).

A possibility of occurrence of strong pH and oxygen concentration gradients after preirradiation of preparations closed tightly in a small volume of solution should also be taken into account. The employment of a CO₂- and O₂-permeable membrane in our experiments eliminates those physiologically harmful effects; as a consequence, algae survive up to 2 weeks in such preparations.

There remains an open problem why at lower FR fluence rates, i.e. I_R < 42 μmol m⁻² s⁻¹, the light of 620 nm was more active than 660 and 680 nm which are more efficient in phytochrome transformation. To answer that question we pose another: What is the difference between the action of 620 nm and that of a longer wavelength light in the studied system? One basic difference lies in the optical properties of the algal cell: Of three red wavelengths studied, 620 nm is the most weakly absorbed by the chloroplast and most strongly scattered. This may result in a partial depolarization. The studied response took place under specific conditions of constant cancellation of the P₉ form established continuously by R || on the side walls, i.e. under conditions of constant phytochrome cycling (Fig. 6e). The stationary state P₉ = P₉ depends on the ratio of quantum fluxes of both radiation types, their absolute values, and their polarization planes. The different light absorption and scattering by cell structures may therefore be essential for the establishment of a tetrapolar P₉ gradient: the combination of 620 nm || with an appropriate FR may result in the formation of a more effective gradient than a quantum-equivalent 680 nm ||.

### Table 1. Transmission of R and FR Through Single Chlooplasts

Mean values of 12 measurements. In the third column %T is given after recalculation for a four times greater chloroplast thickness (D).

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Mean Transmission</th>
<th>%T for 4 × D</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>720</td>
<td>92.1 ± 0.9</td>
<td>72.0</td>
<td>0.04</td>
</tr>
<tr>
<td>680</td>
<td>45.4 ± 7.6</td>
<td>4.2</td>
<td>0.34</td>
</tr>
<tr>
<td>654</td>
<td>69.5 ± 9.5</td>
<td>23.3</td>
<td>0.16</td>
</tr>
<tr>
<td>620</td>
<td>81.1 ± 6.6</td>
<td>43.3</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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Another aspect that needs investigation is a possible contribution of the intermediates of phytochrome phototransformation. Studies using laser flashes demonstrated that 620 nm could be more effective in creating a Pₐ gradient in Mougeotia than R of longer wavelengths (660, 690 nm) when the response was induced with multiflashes (23). The results were interpreted in terms of a photochromic system establishment between Pₐ and the very early intermediates.

In order to find out whether the observed spectral shift is really related to the level of cycling, experiments with the use of R // and FR light pulses have been planned. Their aim will be to compare the effectiveness of 620 nm and longer wavelength light for simultaneous and time-resolved pulses.

CONCLUDING REMARKS

Three basic facts argue against the existence of a novel photoreceptor; under conditions of dichromatic irradiation with parallel polarized visible light and FR: (a) chloroplasts turn with their face toward FR; (b) FR from both sides for to compare the critical reading of the manuscript.

ACKNOWLEDGMENTS

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LITERATURE CITED

27. Seitz K (1979) Light induced changes in the centrifugability of chloroplasts: different action spectra and different influence of inhibitors in the low and high intensity range. Z Pflanzenphysiol 95: 1–12