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Photoorientation of Chloroplasts in Protonemal Cells of the Fern *Adiantum* as Analyzed by Use of a Video-tracking System

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Photoorientation of chloroplasts mediated by phytochrome and blue light-absorbing pigment in protonemal cells of the fern *Adiantum* was studied by use of inhibitors of the cytoskeleton and was analyzed with a video-tracking system. The photoorientation responses were inhibited by cytochalasin B and by N-ethylmaleimide (NEM) but not by colchicine, suggesting that the photomovement depends on the actomyosin system. In the dark, chloroplasts moved randomly, being independent of one another. After induction of photoorientation by polarized red light, most chloroplasts that had been located at the margin of cells moved almost perpendicularly to the cell axis toward the site of photoorientation. This type of movement was hardly ever observed in the dark. Under polarized blue light, such specific movements were less evident but were still observed in the case of a few chloroplasts. After photoorientation was complete, chloroplasts still moved in random directions but their mobility was lower than that in the dark, indicating the presence of some anchoring mechanism.

When EGTA was applied, photoorientation was inhibited but this inhibition was overcome by the addition of $CaCl_2$. Video-tracking of chloroplasts in the dark revealed that the mobility of chloroplasts was higher in medium with EGTA than in medium with EGTA plus $CaCl_2$ and that many of the chloroplasts moved jerkily in the medium with EGTA. This change in the nature of movements was also seen under polarized light, resulting in the disturbance of photoorientation. These results indicate that the inhibition of photoorientation at low concentrations of Ca^{2+} ions may be due to changes in the nature of chloroplast movement.

Key words: Blue light-absorbing pigment (cryptochrome) — Chloroplast movement — Fern protonema — Photomovement (chloroplast) — Phytochrome

In plant cells, chloroplasts respond to unilateral light by intracellular orientation, perhaps in order to maximize photosynthetic activity in the case of weak irradiation and to avoid damage to the photosynthetic apparatus in the case of strong irradiation. The photoorientation responses have been investigated in some detail from the photobiological point of view (Haupt 1982, 1987, Haupt and Scheuerlein 1990, Schönbohm 1980, Zurzycki 1980). In most cases, the responses are induced by blue light,

Abbreviations: DMSO, dimethylsulfoxide; EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*, *N*, *N'*, *N'*-tetraacetic acid; MES, 2-(*N*-morpholino)-ethanesulfonic acid; NEM, *N*-ethylmaleimide

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and the blue light-absorbing pigment (cryptochrome) has been assigned as the photoreceptor. Occasionally, phytochrome also functions as the receptor pigment (Haupt 1959, Haupt and Thiele 1961, Izutani *et al.*, 1990, Yatsuhashi *et al.*, 1985). However, the mechanism of chloroplast translocation and its regulation during the orientation response are poorly understood even though the response has potential as a model system for the study of the movement of intracellular organelles in plant cells. By contrast, our understanding of cytoplasmic streaming is much more extensive (Kamiya 1986, Shimmen 1988). One of the difficulties encountered in attempts to analyze chloroplast movement stems from the fact that the speed of movement is much lower than that of cytoplasmic streaming. It is not easy to trace the path of a chloroplast or to measure the speed of movement under a microscope. To solve these problems, we have developed a video-tracking system that automatically tracks the chloroplasts in a cell and allows both reconstruction of the path of movement and measurement of the speed of movement. Chloroplast photoorientation mediated by phytochrome and blue light-absorbing pigment in protonemal cells of the fern *Adiantum* was analyzed by use of this tracking system.

Materials and Methods

Plant material and aseptic culture

Spores of *Adiantum capillus-veneris* L. were collected in the summer of 1982 in a greenhouse at the Botanical Gardens, University of Tokyo, Koishikawa, Tokyo, and were stored in the dark at about 5 C until use.

Spores were sterilized with 0.1-strength "Purelox" (4-6% solution of sodium hypochlorite ; Oyalox Co., Tokyo) and sown in a line on a membrane placed on a cover slip. The membrane was prepared from a mixture of 0.75% agar and 0.3% gelatin (Murata and Wada 1989). Spores were covered with another agar-gelatin membrane and were placed at the bottom of a Petri dish that contained ten-fold diluted modified Murashige and Skoog's mineral salt solution (Kadota and Furuya 1977). After imbibition for 1 day in the dark, spores were cultured for 9 days at 25 C under continuous red light at 0.5 Wm^{-2} which was applied horizontally. Protonemata cultured in this way were irradiated for 6 h with continuous white light at 3.6 Wm^{-2} and then kept in the dark for 2 days (Yatsuhashi *et al.*, 1987). This treatment resulted in two-celled protonemata of more than 1 mm in length. The photoorientation response of chloroplasts was monitored in the basal cell of these non-growing two-celled protonemata (Yatsuhashi *et al.*, 1987).

Induction of chloroplast photoorientation

Intracellular photoorientation of chloroplasts was induced by continuous irradiation with linearly polarized red or blue light vibrating horizontally, which was given from the tip of the protonema (Fig. 1). Red and blue light were obtained by passing light from a fluorescent lamp (FL20SD ; Toshiba Corp., Tokyo) through a red plastic plate (Shinkolite A, #102 ; Mitsubishi Rayon Co., Ltd., Tokyo) and a blue plastic film

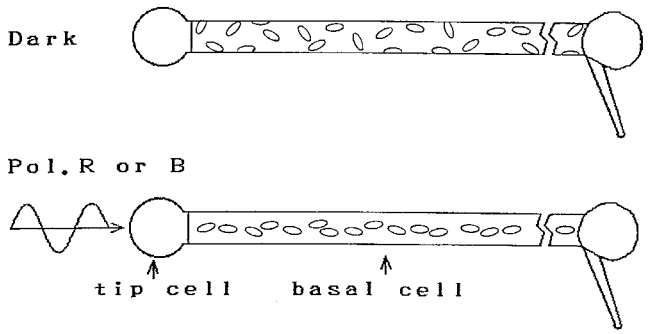

Fig. 1. Scheme showing the intracellular orientation of chloroplasts induced by irradiation with polarized light in a protonemal cell of *Adiantum*.

(Ryutate #63; Ryudensha, Tokyo), respectively. The colored lights were polarized through a linear polarizer (Polaroid HN22; Polaroid Corporation of Japan, Tokyo). The fluence rate at the sample level was measured with a radiometer (model 65A; Yellow Springs Instrument Co., Inc., OH, U.S.A.). Chloroplast photoorientation was monitored in the apical 150 μm region of the basal cell of the two-celled protonema and the responses were quantified in terms of the percentage of protonemata in which chloroplasts gathered at the upper or lower surface of the cylindrical cell while none remained along the margins (Fig. 1). Since the basal cells in the present system were highly vacuolated, chloroplasts always moved along the cell periphery in the cortical layer.

Analysis of the effects of various agents

Colchicine (Sigma Chemical Co., St. Louis, MO, U.S.A.) and *N*-ethylmaleimide (NEM; Sigma Chemical Co.) were dissolved in the above-mentioned liquid culture medium at concentrations of 5 mM and 10 μM, respectively. Cytochalasin B (Aldrich Chem. Co., Milwaukee, WI, U.S.A.) was dissolved in dimethylsulfoxide (DMSO) as a stock solution at a concentration of 10 mg/ml. For use, the stock solution was diluted with the liquid culture medium and the final concentrations of cytochalasin B and DMSO were 0.1 mM (50 μg/ml) and 0.5%, respectively. For the study of the effects of Ca^{2+} ions, 1.3 mM ethyleneglycol-bis-(β-aminoethylether)-*N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA) and/or 3 mM $CaCl_2$ was included in 10 mM 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffer, pH 6.0 (adjusted with KOH).

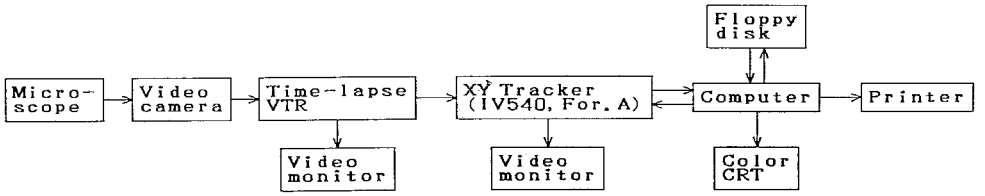

Fig. 2. Diagram of the video-tracking system.

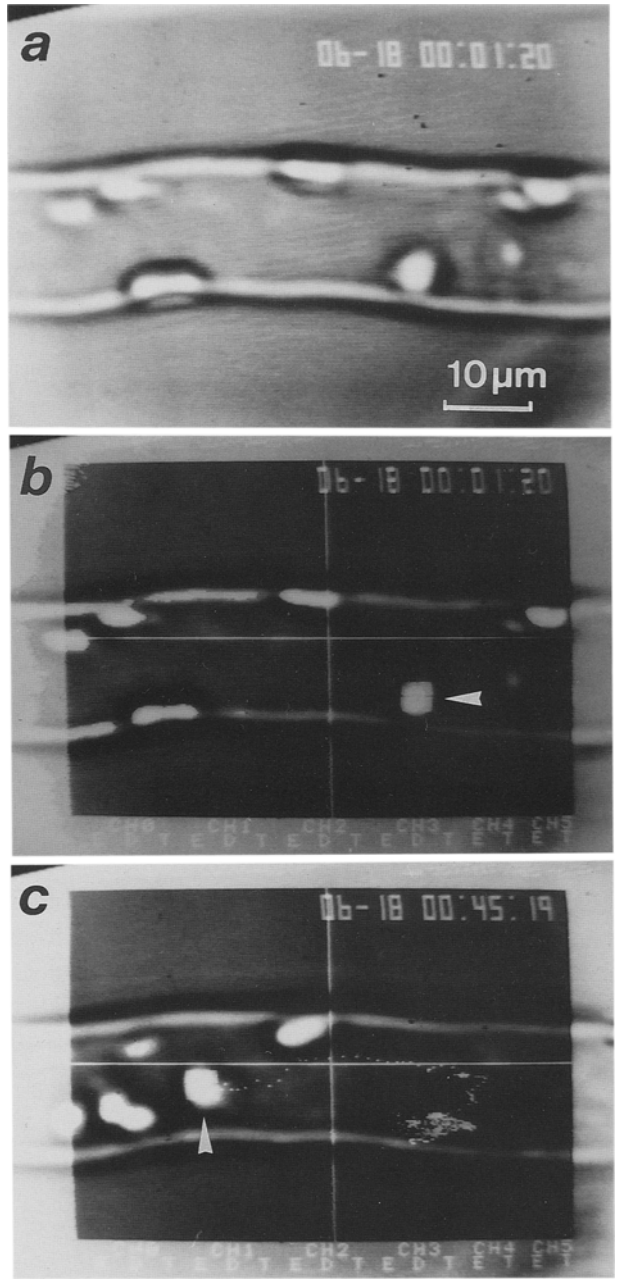

Fig. 3. Photographs showing the video-tracking of chloroplast movement. a, Raw video image of a protonema; b, video image after binarization of a. The rectangular tracking area divided into quadrants by vertical and horizontal lines is shown. An arrowhead shows the chloroplast on which the "window" was placed to track its movement; c, video image showing the path of movement of the chloroplast indicated in b over the course of 44 min. An arrowhead shows the chloroplast that was tracked. White dots show the positions of the chloroplast on every frame of the video record (positions at intervals of 2.4 s in this particular case).

Two hours before the photoinduction of chloroplast movement, sample protonemata were transferred to medium that contained the agent of interest under a dim, green safe light (Kadota *et al.*, 1984) and were kept in the dark until the light treatment.

Video-tracking of the path of chloroplast movement

A diagram of the video-tracking system is shown in Fig. 2. Movement of chloroplasts during photoorientation was monitored under infrared light through an inverted microscope (TMD; Nikon, Tokyo) equipped with a video camera with an infrared light-sensitive tube (Newvicon S4140; Matsushita Electric Corp., Tokyo). Movements were recorded at intervals of 1.2 sec with a time-lapse videotape recorder (VTR; NV-8030; Matsushita Electric Industrial Co., Ltd., Kawasaki, Japan). The videotape was replayed and the movement of each individual chloroplast was automatically tracked by an XY tracker (IV540, For. A Corporation, Tokyo). After binarization of the recorded image to white and black, the "window" was placed over the white (or black) target object (=chloroplast) in the tracking area (Fig. 3). The XY tracker calculated the center of gravity of the white (or black) area inside the "window" in every frame of the video record and automatically tracked the movement of the target. Data on the coordinates of the chloroplast in the tracking area were sent every 72 sec (in real time) to a personal computer (PC9801E; NEC Corp., Tokyo) and stored on a floppy disk. The path of chloroplast movement was reconstructed on a CRT and the distance traveled every 30 min was calculated from the stored data. The tracking system allows the analysis of the two-dimensional movement of a chloroplast projected onto the XY plane, but it does not follow movements in the vertical direction (Z-axis). Such movement occurs particularly when the chloroplasts are located at the margins of the cell. Therefore, for the control cells, the mobilities of chloroplasts located at the center (upper or lower surface) and at the margins were calculated and are presented separately. To determine the noise level from the tracking system itself, protonemata were fixed with 3% glutaraldehyde and used as samples. The noise level was 1.8 ± 0.3 μm/0.5 h.

Results

Photoorientation of chloroplasts during irradiation with polarized red or blue light

Photoorientation of chloroplasts was induced by polarized red light at 0.27 Wm^{-2} or by polarized blue light at 0.37 Wm^{-2}. As shown in Fig. 4, in most of the protonemata chloroplast photoorientation occurred within 1 h after the onset of irradiation with polarized light, and the response remained at a plateau level thereafter. In a certain percentage of cells, one or two chloroplasts remained at the margins while others responded by photoorientation and accumulated at the upper or lower surface of the cell. As a result, the maximum response level did not reach 100%. When the effects of inhibitors of cytoskeleton were examined (Table 1), we found that photoorientation responses were inhibited by 0.1 mM cytochalasin B and by 10 μM NEM but

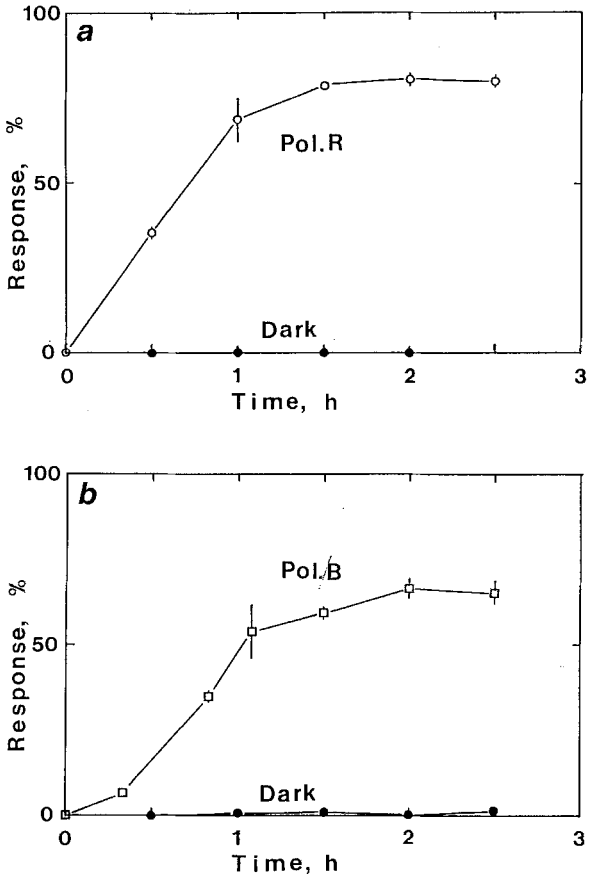

Fig. 4. Time course of the intracellular orientation of chloroplasts induced by irradiation with polarized light in a protonemal cell of *Adiantum*. Protonemata were irradiated with polarized red (Pol. R ; 0.27 Wm^{-2}) or blue light (Pol. B ; 0.37 Wm^{-2}). Means and standard errors were derived from triplicate samples in which 50 protonemata were examined.

Table 1. Effects of colchicine, cytochalasin B and *N*-ethylmaleimide (NEM) on the photoorientation of chloroplasts induced by polarized red or blue light

Treatment	Response (%)	
	Red light	Blue light
None	80.7±1.8	66.7±2.9
Colchicine	82.4±7.2	66.0±7.2
Cytochalasin B	13.7±8.5	6.7±2.4
NEM	0.0±0.0	0.0±0.0
DMSO	80.2±0.7	63.3±2.9

5 mM colchicine, 0.1 mM cytochalasin B, and 10 μM NEM were applied 2 h before the light treatment. Cytochalasin B was dissolved in 0.5% DMSO and the control results with DMSO alone are also presented. Photoorientation responses were assessed 2 h after the onset of irradiation with polarized light. Means and standard errors were derived from triplicate samples in which 50 protonemata were examined.

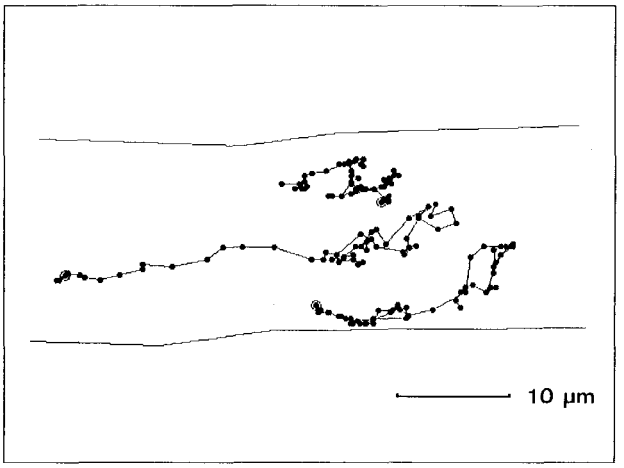

Fig. 5. Movements of 3 individual chloroplasts in a single cell in the dark over the same time period. Chloroplast movement was tracked for 1 h with the video-tracking system and positions are shown at intervals of 72 sec. The protonemal tip was on the left. Initial positions of chloroplasts are indicated by ◉. Lines above and below the paths are the edges of the cell.

not by colchicine at 5 mM, a concentration known to be sufficient to destroy the microtubule architecture within the cell (Murata and Wada 1989). Thus, it was evident that the movement of chloroplasts was dependent on the actin-myosin interactions and not on microtubule system.

Chloroplast movement in the dark

Movement of chloroplasts in the dark was monitored with the video-tracking system. Figure 5 shows the paths of movement of three individual chloroplasts in a single cell over the course of 1 h in the dark. Apparently, the chloroplasts moved independently of one another. The direction and speed of movement differed from one chloroplast to another even in the same cell and even during the same period of time. Moreover, the speed of movement of a single chloroplast was not uniform but varied over time.

Chloroplast movement after induction of photoorientation

Movement of chloroplasts was tracked after the induction of photoorientation by polarized light. The time course study in Fig. 4 revealed that the orientation responses reached a plateau within 1 h after the onset of light. Therefore, chloroplast movement was analyzed in two phases: from 0 to 1 h after the start of light irradiation, during which time chloroplasts gather at the upper or lower surface of the cell and from 2 to 4 h after the onset of light, during which time the photooriented chloroplasts remain in position. During the first phase, the chloroplasts located at the margins initially were tracked, and during the second phase the chloroplasts that had accumulated at the upper or lower surface of the cell were examined. Under red light, light-directed movement in the first phase could be detected as early as 15 min after the

onset of light and most chloroplasts completed their orientational movement within 0.5 h (Fig. 6b). The paths of their movements were almost perpendicular to the cell axis (Fig. 6b). This type of movement was barely ever observed in the dark (Fig. 6a). Under blue light, by contrast, such a specific movement was less evident but was still observed in the case of a few chloroplasts (Fig. 6c). One hour was needed before most of the chloroplasts had gathered at the upper or lower surface of the cell (Fig. 6c). No significant difference in the mobility of chloroplasts from 0 to 0.5 h or from 0.5 to 1 h after the onset of light was detected under the two sets of light conditions (Table 2; compare the values with those in the dark for chloroplasts located at the margins and at the upper or lower surface, respectively).

After the photoorientation of chloroplasts was complete with either type of light

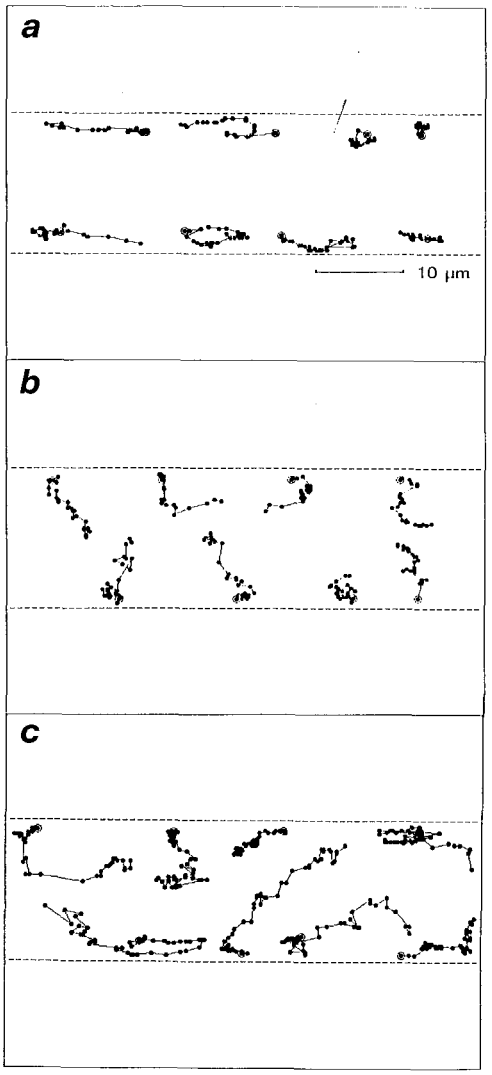

Fig. 6. Movements of chloroplasts in the dark (a) and after induction of photoorientation by polarized red (b) or blue light (c). Chloroplasts located initially at the margins were tracked. Chloroplast positions for 0.5 h (a, b) and for 1 h (c) are indicated at intervals of 72 sec. In b and c, movements immediately after the onset of polarized light are presented. Movements of chloroplasts in more than 3 cells are shown. Dashed lines indicate the edges of a hypothetical cell with a diameter equal to the mean of the cells examined. The protonemal tip was on the left. Initial positions of chloroplasts are indicated by ◉.

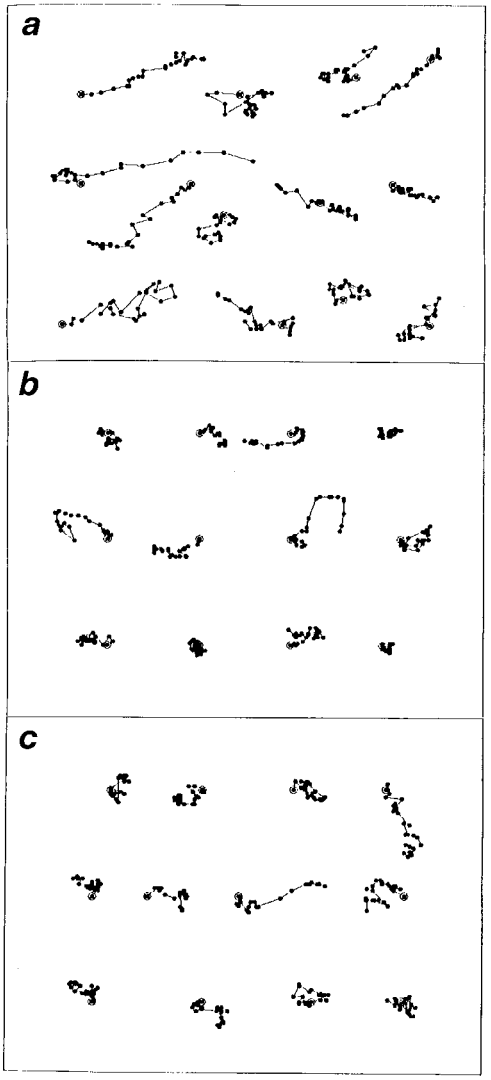

Fig. 7. Movements of chloroplasts in the dark (a) and after completion of photoorientation induced by polarized red (b) or blue light (c). Movements of chloroplasts located at the upper or lower surface of the cell at the beginning of video-tracking are presented. Movements of chloroplasts for 0.5 h are shown at intervals of 72 sec. In b and c, positions over the course of 0.5 h during the period from 2 to 4 h after the onset of polarized light are indicated. Movements of chloroplasts in more than 3 cells are shown. The protonemal tip was on the left. Initial positions of chloroplasts are indicated by ◉. Same magnification as in Fig. 6.

irradiation (that is, during the second phase, 2–4 h after the beginning of light treatment), chloroplasts continued to move randomly (Fig. 7) but the mobility of chloroplast was lower than that in the dark control (Table 2 ; compare the values with that in the dark for chloroplasts located at the upper or lower surface of the cell).

Effects of EGTA on the photoorientation of chloroplasts

The photoorientation response was inhibited in 10 mM MES buffer that contained 1.3 mM EGTA and the inhibition was overcome by the addition of 3 mM $CaCl_2$ to the medium (Table 3).

The video-tracking analysis of the paths of chloroplast movement in the dark revealed that the mobility of chloroplasts was higher in the medium with EGTA than

Table 2. Movement of chloroplasts during the course of photoorientation from margins to upper or lower surfaces of cells

Treatment	Distance traveled in 0.5 h (μm)	(n)[a]
Dark (upper or lower surface)[b]	23.9±1.7	25
Dark (margin)[b]	18.6±1.0	25
Red light (0–0.5 h)[c]	17.8±1.4	9
Red light (0.5–1 h)[c]	22.4±3.7	7
Red light (2–4 h)[c]	13.2±0.8	32
Blue light (0–0.5 h)[c]	14.9±1.6	11
Blue light (0.5–1 h)[c]	24.2±3.6	11
Blue light (2–4 h)[c]	16.9±0.7	34

[a] Number of chloroplasts examined.
[b] Upper or lower surface and margin denote the location of chloroplasts in the cell when the tracking was performed.
[c] Period during which the mobility of chloroplast was analyzed. The period is indicated in terms of the time after the onset of irradiation with polarized light.
Mobility is expressed as the distance traveled in 0.5 h. The noise level from the video-tracking system was 1.8±0.3 μm.

Table 3. Effects of EGTA and of EGTA plus $CaCl_2$ in the medium on the photoorientation of chloroplasts induced by polarized red or blue light

Treatment	Response (%)	
	Red light	Blue light
None	76.9±7.7	75.3±3.3
EGTA	36.0±5.0	48.0±4.0
EGTA→EGTA+$CaCl_2$	72.7±4.1	76.7±5.8
EGTA+$CaCl_2$	77.3±3.5	70.7±1.8
$CaCl_2$	80.0±3.1	74.0±3.5

10 mM MES buffer (pH 6.0) containing 1.3 mM EGTA, 3 mM $CaCl_2$ or 1.3 mM EGTA plus 3 mM $CaCl_2$ was applied 2 h before the light treatment. One group of protonemata was treated for 2 h with medium containing 1.3 mM EGTA and then with medium containing 1.3 mM EGTA plus 3 mM $CaCl_2$ medium for 2 h before light treatment. Photoorientation response was assessed 2 h after the onset of irradiation with polarized light. Means and standard errors were derived from triplicate samples in which 50 protonemata were examined.

in the medium with EGTA plus $CaCl_2$ (Table 4), and that many of the chloroplasts moved jerkily in the medium with EGTA (arrows in Figs. 8a and 9a). The jerky movements were seen only in the medium with EGTA and chloroplasts moved in an uncoordinated fashion, showing frequent and sudden changes in the direction of movement. The change in the nature of their movements was also seen after the onset of polarized light (arrows in Figs. 9b, c), with resultant disturbance of the directional

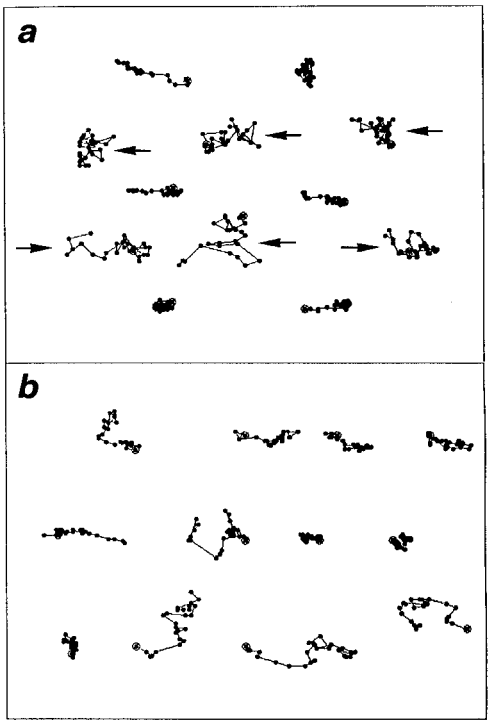

Fig. 8. Movements of chloroplasts in medium containing EGTA (a) and in medium containing EGTA plus $CaCl_2$ (b) in the dark. 10 mM MES buffer (pH 6.0) containing 1.3 mM EGTA or 1.3 mM EGTA plus 3 mM $CaCl_2$ was applied 2 h before the analysis. Movements of chloroplasts located at the upper or lower surface of the cell at the beginning of video-tracking are indicated. Positions of chloroplasts over the course of 0.5 h are shown at intervals of 72 sec. Arrows in a indicate the paths of jerky movement. Same magnification as in Fig. 6. Other details are the same as in Fig. 7.

Table 4. Effects of EGTA and of EGTA plus $CaCl_2$ in the medium on the mobility of chloroplasts during the course of photoorientation

Treatment	Distance traveled in 0.5 h (μm) (n)[a]	
	EGTA	EGTA+$CaCl_2$
Dark (upper or lower surface)[b]	22.1±2.1 (24)	15.5±1.6 (18)
Dark (margin)[b]	20.2±1.4 (30)	16.4±0.8 (41)
Red light (0–0.5 h)[c]	29.9±2.5 (10)	20.0±0.9 (11)
Blue light (0–0.5 h)[c]	33.4±3.2 (7)	22.0±1.8 (13)
Blue light (0.5–1 h)[c]	33.0±2.6 (7)	24.7±1.8 (12)

[a] Number of chloroplasts examined.

[b] Upper or lower surface and margin denote the location of chloroplasts in the cell when the tracking was performed.

[c] Period during which the mobility of chloroplasts was analyzed. The period is indicated in terms of the time after the onset of irradiation with polarized light.

10 mM MES buffer (pH 6.0) containing 1.3 mM EGTA or 1.3 mM EGTA plus 3 mM $CaCl_2$ was applied 2 h before the light treatment. Mobility is expressed as the distance traveled in 0.5 h. The noise level from the video-tracking system was 1.8±0.3 μm.

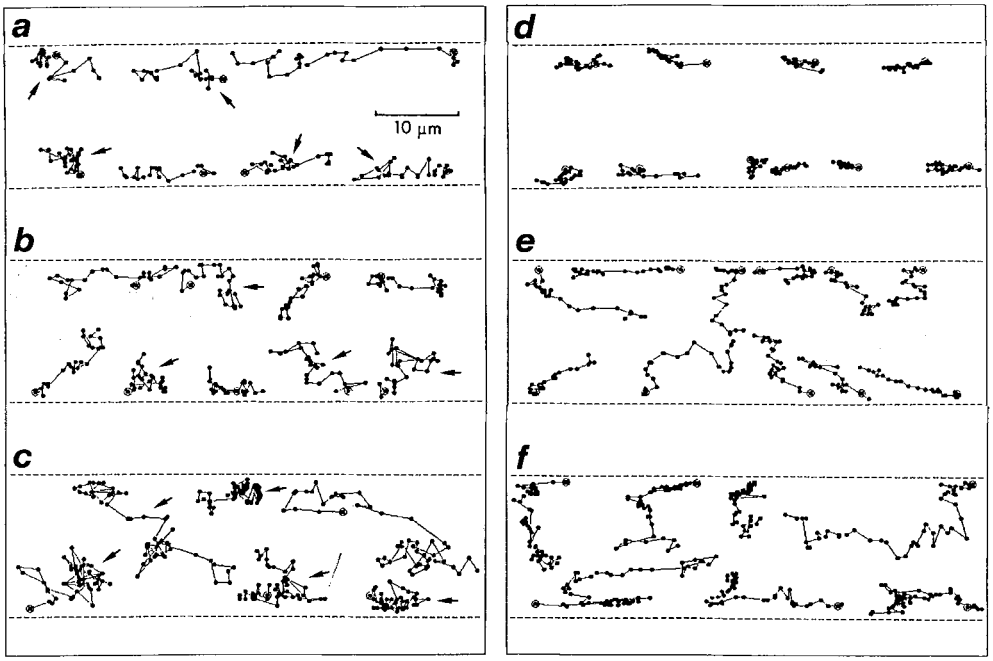

Fig. 9. Movements of chloroplasts in the dark (a, d) and immediately after the onset of polarized red (b, e) or blue light (c, f) in medium containing EGTA (a-c) and in medium containing EGTA plus $CaCl_2$ (d-f). 10 mM MES buffer (pH 6.0) containing 1.3 mM EGTA or 1.3 mM EGTA plus 3 mM $CaCl_2$ was applied 2 h before the light treatment. Chloroplasts located initially at the margins were tracked. Positions of chloroplasts over the course of 0.5 h (a, b, d, e) and 1 h (c, f) are indicated at intervals of 72 sec. Arrows in a-c indicate the paths of jerky movement. Other details are the same as in Fig. 6.

movement of chloroplasts. Directional movement under polarized light was evident in the medium with EGTA plus $CaCl_2$ (Fig. 9e, f). In media with EGTA and with EGTA plus $CaCl_2$, the mobility of chloroplasts was somewhat higher in the light than in the dark (Table 4).

Discussion

Systems in which photoorientation of chloroplasts has been examined can be classified roughly into two categories: one involves the rotation of a large plate-like chloroplast in a cell, as, for example, in the cells of the green algae *Mougeotia* and *Mesotaenium*; the other involves the "tactic" movement of many small chloroplasts along the interface between the plasma membrane and the highly developed central vacuole, as, for example, in higher plant cells (Haupt 1982, Haupt and Scheuerlein 1990). In the former system, filaments connecting the chloroplast edge to the cortical ectoplasm have been observed (Wagner and Klein 1978, Schönbohm 1980) and it has been suggested that the rotation of chloroplasts occurs via changes in their sites of

anchorage within the cortical ectoplasm (Grolig and Wagner 1987) and via the regulation of the actin-myosin system (Haupt 1987, Haupt and Scheuerlein 1990). In the latter case, exemplified by the cells in the present study, passive translocation of chloroplasts to the site of orientation has been suggested. In studies of the chloroplasts in *Vaucheria* cells, it appeared that the chloroplasts moved passively with the flowing endoplasm and the cytoplasmic streaming slowed down and stopped at the illuminated area, with resultant accumulation of chloroplasts at the lighted site (Fischer-Arnold 1963, Blatt and Briggs 1980).

In the present study of *Adiantum* cells, chloroplasts, after the induction of photoorientation, moved along paths that were almost perpendicular to the cell axis. This type of movement was barely ever seen in the cells in darkness. Cytoplasmic streaming always occurs along the long axis of the filamentous cells of *Adiantum* (Wada *et al.*, 1982) and the speed of chloroplast movement (Table 2) is much lower than that of the cytoplasmic streaming (Wada *et al.*, 1982). Furthermore, no change in the direction of streaming and no interruption of streaming was detected during the process of chloroplast orientation (data not shown). Thus, it is unlikely that the orientation response in *Adiantum* cells is due to the trapping of chloroplasts at sites at which the streaming has ceased. The photomovement of chloroplasts seems to be not dependent on the cytoplasmic streaming. Similar directional movement of chloroplasts was also reported in *Lemna* cells during the response to strong blue light (Zurzycki 1980). However, its relationship to the cytoplasmic streaming that involves smaller organelles remains obscure.

Even after completion of intracellular orientation, the chloroplasts still moved in random directions but their mobility was much reduced as compared to that in the dark (Table 2). It seems likely that the chloroplasts are somehow anchored at the sites to which they have moved. Recently, we investigated the organization of cortical actin filaments in the present material and found evidence for photoinduction of a structure that may serve to anchor chloroplasts in place. After the photoorientation of chloroplasts in the cell, a circular bundle of actin microfilaments was formed on the chloroplast, on the side facing the plasma membrane and along the edge of chloroplast (Kadota and Wada 1989). It was evident from a time course study that such structures appeared after the chloroplasts had moved to the sites of accumulation. Moreover, the structures disappeared before the chloroplasts left these sites when the stimulus light was switched off (Kadota and Wada 1992). As discussed by Kadota and Wada (1989, 1992), it is unlikely that chloroplasts with these circular structures move freely in the cell even when the putative myosin on the chloroplasts interacts with the circular actin bundles. Changes in the organization of actin microfilaments have also been postulated in *Vaucheria* cells since reticulation of the cortical layer of the illuminated part of the cell was observed under Nomarski differential interference contrast optics (Blatt and Briggs 1980, Blatt *et al.*, 1980). However, the organizations of the cortical cytoskeletons induced differ significantly from each other in the two systems.

In the phytochrome-mediated rotation of chloroplast in *Mougeotia*, several lines of

evidence suggest that regulation of the intracellular concentration of Ca^{2+} ions may be involved in the early steps of signal transduction from the phytochrome (see review by Haupt and Scheuerlein 1990). In the present study, Ca^{2+} ions were found to be essential for the photoorientation of chloroplasts in *Adiantum* cells in the case of both the response mediated by phytochrome and that mediated by blue light-absorbing pigment (Table 3). The video-tracking analysis revealed, however, that low concentrations of Ca^{2+} ions in the external medium changed the pattern of movement of chloroplasts in the dark as well as in the light. Treatment with EGTA induced jerky movements by many of the chloroplasts (Figs. 8 and 9) and their mobility was higher at low concentrations of Ca^{2+} ions (Table 4), as is the case for cytoplasmic streaming in plant cells (Shimmen 1988). The inhibitory effect of EGTA on the photoorientation responses can be ascribed to changes in the nature of the movements. It is likely that Ca^{2+} ions in the present system may affect the actomyosin system and/or the mechanical properties of the cytoplasmic matrix but not the early steps of the signal transduction from phytochrome or blue light-absorbing pigment.

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