Planta (2000) 210: 932–937

Planta

Intracellular chloroplast photorelocation in the moss *Physcomitrella patens* is mediated by phytochrome as well as by a blue-light receptor

Akeo Kadota[1], Yoshikatsu Sato[1], Masamitsu Wada[1,2]

[1]Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Minami-Ohsawa 1-1, Hachioji, Tokyo 192-0397, Japan
[2]National Institute for Basic Biology, Myodaiji, Okazaki 444-8585, Japan

Received: 7 September 1999 / Accepted: 15 October 1999

Abstract. The light-induced intracellular relocation of chloroplasts was examined in red-light-grown protonemal cells of the moss *Physcomitrella patens*. When irradiated with polarized red or blue light, chloroplast distribution in the cell depended upon the direction of the electrical vector (E-vector) in both light qualities. When the E-vector was parallel to the cross-wall (i.e. perpendicular to the protonemal axis), chloroplasts accumulated along the cross-wall; however, no accumulation along the cross-wall was observed when the E-vector was perpendicular to it (i.e. parallel to the protonemal axis). When a part of the cell was irradiated with a microbeam of red or blue light, chloroplasts accumulated at or avoided the illumination point depending on the fluence rate used. Red light of 0.1–18 W m^{-2} and blue light of 0.01–85.5 W m^{-2} induced an accumulation response (low-fluence-rate response; LFR), while an avoidance response (high-fluence-rate response; HFR) was induced by red light of 60 W m^{-2} or higher and by blue light of 285 W m^{-2}. The red-light-induced LFR and HFR were nullified by a simultaneous background irradiation of far-red light, whereas the blue-light-induced LFR and HFR were not affected at all by this treatment. These results show, for the first time, that dichroic phytochrome, as well as the dichroic blue-light receptor, is involved in the chloroplast relocation movement in these bryophyte cells. Further, the phytochrome-mediated responses but not the blue-light responses were revealed to be lost when red-light-grown cells were cultured under white light for 2 d.

Key words: Blue light – Chloroplast – Photomovement – *Physcomitrella* (moss) – Phytochrome

Introduction

As photoautotrophic organisms, plants have developed two fundamental survival photoresponses to sense and adapt to the environmental light conditions: intracellular chloroplast photorelocation and phototropism. Photorelocation movements of chloroplasts allow them to move to a suitable position in the cell for efficient photosynthesis, and also to escape from potentially hazardous strong illumination. As a result of their ability to detect the direction of sunlight, plant shoots can grow by phototropic bending into a favourable position for photosynthesis. In higher plants, these two photoresponses are exclusively controlled by blue light (Haupt and Scheuerlein 1990; Wada et al. 1993; Briggs and Liscum 1997). In lower green plants, on the other hand, it has long been known that, in addition to the blue-light receptor, phytochrome also plays a part in these phenomena (Wada and Kadota 1989). In many fern species, such as *Adiantum capillus-veneris*, *Dryopteris filix-mas*, *Pteridium aquilinum* and *Onoclea sensibilis*, tip-growing protonemal cells show phototropism towards red light (Bünning and Etzold 1958; Miller and Greany 1974; Davis 1975; Kadota et al. 1989). As evidenced by the phenomenon of polarotropism under polarized light, the tropic responses are governed by dichroic phytochrome molecules which are assumed to be aligned at the cell periphery, possibly on the plasma membrane (Etzold 1965; Kadota et al. 1982). Fern protonemal cells also show red-light-induced chloroplast relocation, another response regulated by dichroic phytochrome (Yatsuhashi et al. 1985). Protonemata of the fern *Pteris vittata*, which lack red-light phototropism, do not show red-light-induced chloroplast relocation (Kadota et al. 1989). We recently isolated several strains of red-light-aphototropic (rap) mutants in *Adiantum* and found that all the rap strains also lack a red-light response with respect to chloroplast photorelocation while the blue-light responses are retained (Kadota and Wada 1999). Thus, parallelism exists in the occurrence of red-light-induced phototropism and chloroplast relocation movements in ferns, indicating

Abbreviations: E-vector = electrical vector; LFR = low-fluence-rate response; HFR = high-fluence-rate response

Correspondence to: A. Kadota;
E-mail: kadota-akeo@c.metro-u.ac.jp; Fax: +81-426-77-2559

that the same phytochrome species regulates both responses.

Moss protonemata, including those of *Physcomitrella patens*, *Ceratodon purpureus* and *Funaria hygrometrica*, also exhibit red-light phototropism and polarotropism (Cove et al. 1978; Hartmann et al. 1983; Jenkins and Cove 1983; Bittisnich and Williamson 1985). However, no red-light-induced chloroplast relocation movement has been reported so far in mosses. Rather, Zurzycki (1967) and Kagawa et al. (1997) reported the absence of a red-light response in the chloroplast photorelocation of *Funaria* and of *Ceratodon*, respectively. Thus, the reported evidence in mosses is quite contrary to that for ferns.

In the present study, we analyzed light-induced chloroplast relocation movements in the moss *Physcomitrella patens* with special emphasis on the red-light response, and found that protonemal cells of this species do exhibit phytochrome-mediated, red-light-induced chloroplast relocation movements but that the response is hidden as a result of the usual culture conditions under white light.

Materials and methods

Plant material and aseptic culture. As a stock culture, *Physcomitrella patens* was grown in standard conditions as described by Ashton and Cove 1977. For experiments, an aliquot of protonemal cells from the stock culture was inoculated onto an agar medium in a 35-mm petri dish and covered with small coverslip. The composition of the medium was 10 mM KNO_3, 0.1 mM $CaCl_2$, 50 μM $MgSO_4$, 1 mM KH_2PO_4, 8 μM Fe-citrate, 10 mM glucose, 0.5% agar, pH 5.2 (Kagawa et al. 1997). Protonemata were cultured for 5–9 d under unilateral red light of ca. 0.5 W m^{-2}. The resulting protonemal cells growing towards the red light were used throughout the study. All procedures were performed at 25 °C.

Light sources and light treatments. For polarized light treatment, broad-band red and blue light were obtained from a fluorescent lamp (FL20SD; Toshiba Lighting & Technology Corp., Tokyo) covered by a 3-mm-thick, red, acrylic plastic plate (Shinkolite A, #102; Mitsubishi Rayon Co., Tokyo) or a blue plastic film (Ryutate #63; RDS Corp., Tokyo), respectively (Nakazato et al. 1999). The colored light was polarized through a linear polarizer (HN22; Polaroid Corp. of Japan, Tokyo).

Microbeam irradiation was performed on a custom-made microbeam irradiator, which was a modified version of an inverted microscope (TMD, Nikon, Tokyo) equipped with an epi-fluorescence unit. Red, blue and far-red lights were obtained from a 12-V 100-W halogen lamp coupled to interference filters (Vacuum Optics Co. of Japan, Tokyo). The interference filters had their peaks at 663.2, 452.5 and 743.8 nm with half-band widths of 32, 7.5 and 36 nm, respectively.

When necessary, neutral density filters (either Inconel-coated quartz glass from Fujitoku Corp., Tokyo, or an ND filter from Hoya Corp., Akishima, Japan) were used to attenuate the fluence rate. The fluence rate of the light was measured either with a radiometer (model 65A; Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) or with a silicon photodiode, the output of which had been calibrated against the radiometer.

All the experiments reported here were repeated at least twice on different occasions and the same results were obtained.

Observation of chloroplast movement. Protonemata in a dish were placed on the stage of the microbeam irradiator. During the partial irradiation of a cell with a red or blue microbeam, chloroplast movement in the irradiated cell was monitored continuously under infrared-light with the aid of an infrared-light-sensitive video camera (C-2400-07ER; Hamamatsu Photonics K.K., Hamamatsu, Japan). Infrared light was obtained by passing light from the light source (12-V 100-W halogen lamp) of the irradiator through an infrared-light-transmitting filter (IR85; Hoya Corp.). Images were acquired every 1 min by a personal computer (PowerMac 8100/80AV; Apple Computer) using the public domain software, NIH image ver. 1.61.

Results

Red-light-grown protonemata of Physcomitrella patens. When the inoculated protonemata were cultured under unilateral red light of ca. 0.5 W m^{-2}, many protonemata emerged and grew phototropically towards red light. Branching of filaments was rare (Fig. 1a). Many chloroplasts were found to have accumulated at the cross-walls that separated adjacent cells, and which faced the unilateral red light (Fig. 1b). When the direction of unilateral red light was changed by 90 degrees and the cells were cultured for 1 d, chloroplast accumulation at the cross-wall disappeared and, instead, the chloroplasts gathered at the cell flanks facing the incident red light (Fig. 1c). No branch formation was observed during this period. These results indicate that the chloroplast relocation movement is induced by red light.

Chloroplast relocation under polarized red and blue light. Red-light-grown protonemata were further cultured for 1 d under polarized red (0.15 W m^{-2}) or blue light (0.11 W m^{-2}) applied vertically from above, with the

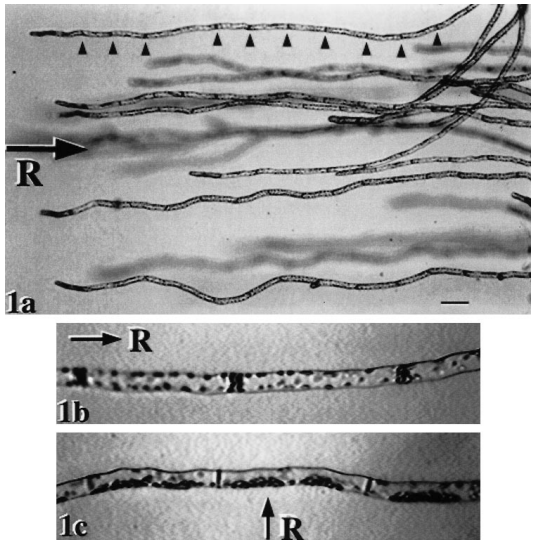

Fig. 1a–c. Red-light-grown protonemata of *Physcomitrella patens*. **a** Protonemata were cultured under unilateral red light of ca. 0.5 W m^{-2} for 5 d. *Arrow* indicates the direction of red light (*R*). *Small arrowheads* show the cross-walls of linearly arranged cells. Bar = 50 μm **b** Close-up of the cells shown in **a**. Note that chloroplasts gather at the cross-wall region facing the red light. **c** Protonemata shown in **a** were further cultured for 1 d after changing the direction of the red light by 90 degrees. Note that chloroplasts are absent in the cross-wall region but gather at the flanks facing the red light

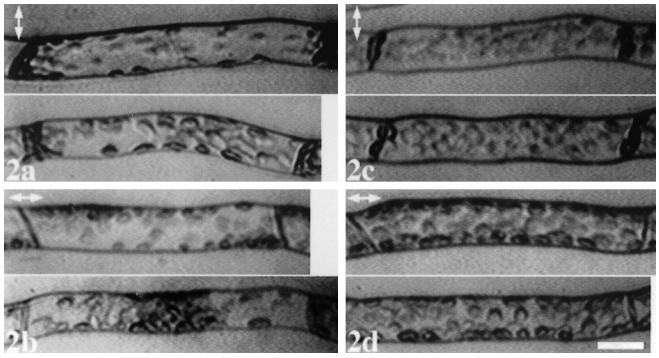

Fig. 2a-d. Chloroplast distribution in protonemal cells of *P. patens* under polarized red and blue light. Red-light-grown cells were irradiated with polarized red (0.15 W m^{-2}; **a**, **b**) or blue (0.11 W m^{-2}; **c**, **d**) light in which the vibration plane of the electrical vector (E-vector; *double-headed arrow*) was perpendicular (**a**, **c**) or parallel (**b**, **d**) to the protonemal axis. Two example cells are presented for each treatment. Note that chloroplasts accumulated at the cross-walls when the E-vector was parallel to the cross-walls (i.e. perpendicular to the cell axis) in both red and blue light. It is also noteworthy that, under these conditions, chloroplasts are absent along the cell flanks in blue light but not in red light. Bar = 20 μm

vibration plane either parallel or perpendicular to the protonemal axis. As shown in Fig. 2, under both red and blue light, accumulation of chloroplasts along the cross-wall was evident when the electrical vector (E-vector) was roughly parallel to the cross-wall (i.e. perpendicular to the protonemal axis). No accumulation was observed when the E-vector was perpendicular to the cross-wall (i.e. parallel to the protonemal axis). Under polarized blue light, dependence of the intracellular distribution of chloroplasts on the E-vector was also evident around the cell flank. Under polarized blue light vibrating perpendicularly to the protonemal axis, chloroplasts avoided the cell flanks (seen from above) while under light vibrating parallel to the axis, chloroplasts located at the cell flanks. This kind of difference in chloroplast distribution was not observed under polarized red light.

Microbeam irradiation with red and blue light. Partial irradiation of protonemal cells was conducted using red or blue microbeams (20 μm in diameter) at various fluence rates. Cells were irradiated continuously for 2 h. In both light qualities, accumulation of chloroplasts in the beam region was evident with low fluence rates, while with high fluence rates, chloroplasts avoided the beam, accumulating outside the beam (Fig. 3). Dependence of the low-fluence-rate response (LFR; accumulation response) and the high-fluence-rate response (HFR; avoidance response) on the fluence rate used is shown in Table 1.

Cancellation of red-light-induced but not blue-light-induced chloroplast relocation by simultaneous far-red-light irradiation. To examine the involvement of phytochrome in the red-light- and blue-light-induced LFR and HFR, far-red light of 242 W m^{-2} was simultaneously applied as a background irradiation together with an inductive red or blue microbeam. As shown in Fig. 4, both

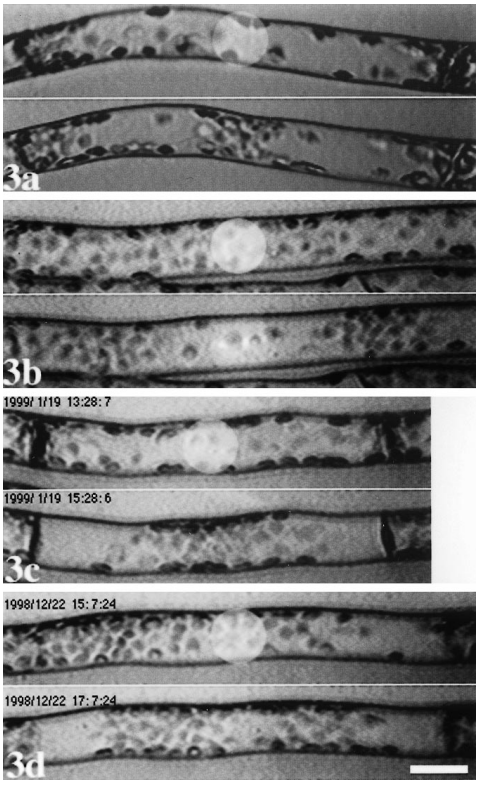

Fig. 3a–d. Chloroplast relocation movements induced by microbeam irradiation with red and blue light. Each panel consists of an upper and a lower picture, which represent the same cell before and after 2 h irradiation, respectively. **a** 1 W m^{-2} red light; **b** 600 W m^{-2} red light; **c** 13.8 W m^{-2} blue light; **d** 285 W m^{-2} blue light. Note the accumulation and the avoidance responses of chloroplasts in low and high fluence rates, respectively. In the case of the avoidance response, it is also noteworthy that chloroplasts gather outside the beam (loose aggregation of chloroplasts far from the beam in **b** and tight aggregation just outside the beam in **d**). 20 μm

Table 1. Fluence-rate dependence of the low-fluence-rate response (LFR; accumulation) and the high-fluence-rate response (HFR; avoidance) in red-light- and blue-light-induced chloroplast relocation. The protonemal cell was irradiated for 2 h with a red or blue microbeam of various fluence rates

Fluence rate (W m^{-2})	Response	
	Red	Blue
0.003		No[a]
0.010	No[a]	Weak LFR
0.10	LFR	LFR
1.0	LFR	LFR
1.8	LFR	
2.9		LFR
6.0	LFR	
10		LFR
18	Weak LFR	
28.5		LFR
60	HFR	
85.5		Weak LFR
285		HFR
600	HFR	

[a]No response

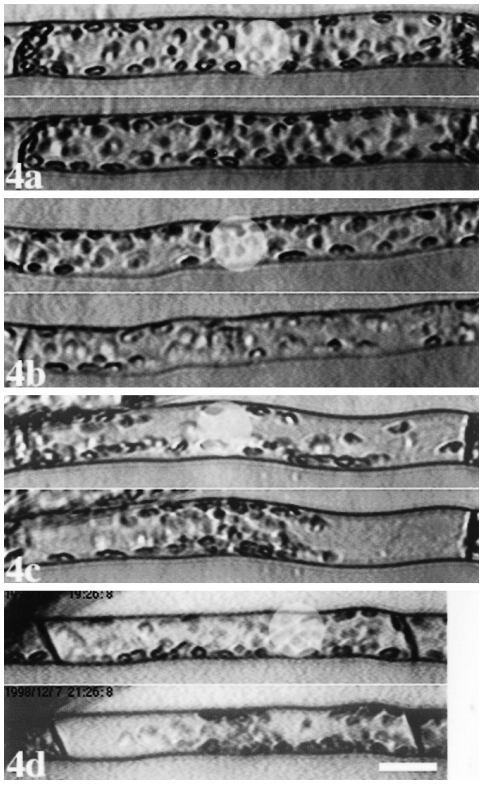

Fig. 4a–d. Effect of simultaneous far-red background irradiation on the red- and blue-microbeam-induced chloroplast relocation. Each panel consists of an upper and lower picture, which represent the same cell before and after 2 h irradiation, respectively. Far-red light of 242 W m^{-2} was applied as background irradiation together with a red or blue microbeam. **a** 1 W m^{-2} red light; **b** 600 W m^{-2} red light; **c** 13.8 W m^{-2} blue light; **d** 285 W m^{-2} blue light. Note that the red-light responses but not the blue-light responses are cancelled by the background far-red light. Bar = 20 μm

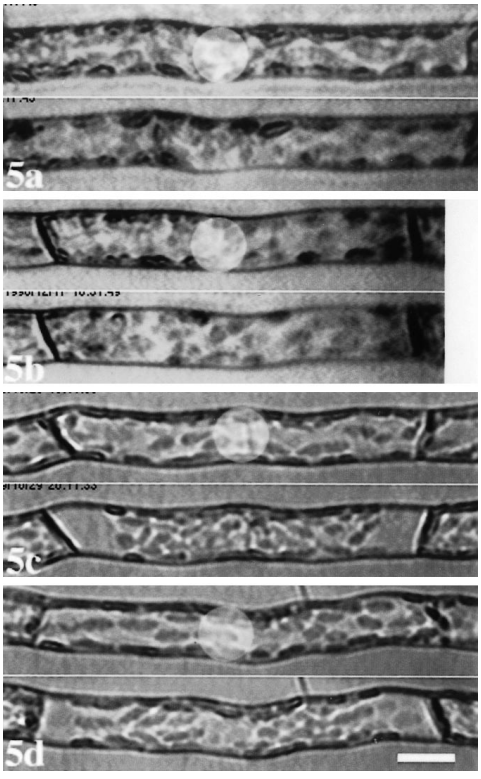

Fig. 5a–d. Disappearance of red-light-induced chloroplast relocation in *P. patens* protonemata cultured under white light for 2 d. Each panel consists of and upper and lower picture, which represent the same cell before and after 2 h irradiation, respectively. **a** 1 W m^{-2} red light; **b** 600 W m^{-2} red light; **c** 13.5 W m^{-2} blue light; **d** 246 W m^{-2} blue light. Note the red-light responses but not the blue-light responses are lost after culture under white light. Bar = 20 μm

LFR and HFR induced by red light were cancelled by the simultaneous far-red background irradiation. Blue-light-induced responses, however, were not affected at all by the treatment. Background far-red light alone had no effect on chloroplast distribution in the cell (data not shown).

Disappearance of red-light-induced chloroplast relocation after culture under white light. Microbeam-induced chloroplast relocation movement was further investigated in the cells which were precultured under red light in the same way as above and then further cultured under white light (ca. 6 W m^{-2}) for 2 d. Basal cells grown during the red-light culture period were used for experiments. No red-light-induced LFR or HFR was observed in these cells, while blue-light-induced responses were evident (Fig. 5), showing that the red-light responses were specifically lost during the 2-d culture under white light.

Discussion

The present study clearly demonstrates that both phytochrome and the blue-light receptor are responsible for intracellular chloroplast photorelocation in the moss *Physcomitrella patens*. In both red- and blue-light responses, action dichroism was shown under irradiation with polarized light, indicating that dichroic photoreceptors mediate these responses. This is the first report showing dichroic phytochrome-induced chloroplast photorelocation in moss cells, although the involvement of a dichroic blue-light receptor in the response was well studied in *Funaria hygrometrica* by Zurzycki (1967).

Changes in chloroplast distribution in response to the polarization plane of the light, namely, action dichroism, were observed along the cross-walls of *P. patens* cells. Chloroplast accumulation along the cross-wall was seen when the E-vector was parallel to the cross-wall but not when it was perpendicular. Thus the absorption dipole moment is likely to be aligned parallel to the plane of the cross-wall in both photoreceptors, this being consistent with the orientation of these receptors in the fern cells (Etzold 1965; Kadota et al. 1982). In polarized blue light but not in polarized red light, dependence of intracellular chloroplast distribution on the polarization plane was also found along the cell flanks. In fern cells, the latter phenomenon is observed in both red and blue light. Assuming the surface-parallel orientation of photoreceptors along the periphery of cylindrical cells,

probably on the plasma membrane, the difference in chloroplast distribution under different vibration planes was considered to reflect a degree of difference in light absorption between the front (and the rear) region, i.e. the mid area of a cell seen from the direction of incident light, and the flank region (Haupt and Scheuerlein 1990; Yatsuhashi et al. 1985). The apparent lack of this phenomenon in red-light-induced chloroplast relocation of *Physcomitrella* may indicate that the dipole moment of phytochrome is not so strictly confined to the plane of the plasma membrane that the difference in light absorption generated between the front (and the rear) and the flanks was enough to induce chloroplast relocation from the flanks to the front (and the rear) region of the cell.

Depending on the fluence rate used, both LFR and HFR could be induced in *P. patens* by microbeam irradiation with red and blue light. These are just the same results as found in protonemal cells of the fern *Adiantum* (Yatsuhashi et al. 1985). The fluence rates for the transition from LFR to HFR, however, are very different in the two species. In *Adiantum* cells, blue light of 1 W m^{-2} induces accumulation in response to the beam (LFR) whereas that of 10 W m^{-2} induces an avoidance response (HFR). In the present study on *Physcomitrella*, the latter condition still induced an LHR while an HFR was evident with an irradiation of 285 W m^{-2} blue light. Further, red light of 60 W m^{-2} was enough to induce an HFR in *P. patens* cells while in *Adiantum* cells red light of as high as 470 W m^{-2} was necessary for an HFR.

We used red-light-grown protonemata as an experimental material since we noticed the parallel occurrence of red-light-induced chloroplast relocation and red-light phototropism in fern protonemal cells (Kadota et al. 1989; Kadota and Wada 1999). In fact, our initial trial using white-light-grown protonemata failed to demonstrate the red-light-induced chloroplast relocation. Protonemata of *Physcomitrella* precultured under unilateral red light showed clear phototropism towards red light. The protonemata were rarely branched and the cells had the mixed features of a chloronema and a caulonema, i.e. they had many chloroplasts (typical of a chloronemal cell) and the cross-walls were sometimes perpendicular (typical of a chloronema) and sometimes oblique to the protonemal axis (typical of a caulonema). The phytochrome-mediated LFR and HFR, but not the blue-light responses, were found to be lost after transfer to white light, although there was no apparent change in cell morphology during the period (many branches were induced in the cells of the apical region but no such branching was induced in the basal cells used for the present study). This fact may well explain the lack of knowledge of the red-light effect because most previous work was conducted on cells grown under white light. The white-light effect may be due to the spectral region other than that of red light, probably that of blue-light. It is likely that an essential part of the signal transduction leading to the red-light response is lost under the white light. Since the blue-light response survives, the missing part should be specific to the phytochrome signaling pathway, including the phytochrome molecule itself. In the fern system, as mentioned in the *Introduction*, it is suggested that the same phytochrome species and the same early signal transduction steps are shared in both chloroplast photorelocation and phototropism (Kadota et al. 1989; Kadota and Wada 1999). This situation may be also possible in the moss protonemata, as revealed in the present study. Further studies, however, should be done to clarify this point.

We thank Dr. Hasebe, M. (NIBB, Okazaki, Japan) for the gift of *Physcomitrella patens*. We also thank Dr. Jo Ann Banks (Purdue University, West Lafayette, Ind., USA) for careful reading of the manuscript. This work was carried out under the NIBB Cooperative Research Program (98-162) and was partly supported by a Grant-in-Aid for Scientific Research (C, No. 11640651) to A.K., a Grant-in-Aid for International Scientific Research (Joint Research, No. 10044214), a Grant-in-Aid for Scientific Research (B, 09440270) and PROBRAIN to M.W.

References

Ashton NW, Cove DJ (1977) The isolation and preliminary characterisation of auxotrophic and analogue resistant mutants of the moss, *Physcomitrella patens*. Mol Gen Genet 154: 87–95

Bittisnich D, Williamson RE (1985) Control by phytochrome of extension growth and polarotropism in chloronemata of *Funaria hygrometrica*. Photochem Photobiol 42: 429–436

Briggs WR, Liscum E (1997) The role of mutants in the search for the photoreceptor for phototropism in higher plants. Plant Cell Environ 20: 768–772

Bünning E, Etzold H (1958) Über die Wirkung von polarisierten Licht auf keimende Sporen von Pilzen, Moosen und Farnen. Ber Dtsch Bot Ges 71: 304–306

Cove DJ, Schild A, Ashton NW, Hartmann E (1978) Genetic and physiological studies of the effect of light on the development of the moss, *Physcomitrella patens*. Photochem Photobiol 27: 249–254

Davis BD (1975) Bending growth in fern gametophyte protonema. Plant Cell Physiol 16: 537–541

Etzold H (1965) Der Polarotropismus und Phototropismus der Chloronemen von *Dryopteris filix mas* (L.) Schott. Planta 64: 254–280

Hartmann E, Kliugenberg B, Bauer L (1983) Phytochrome-mediated phototropism in protonemata of the moss *Ceratodon purpureus* Brid. Photochem Photobiol 38: 599–603

Haupt W, Scheuerlein R (1990) Chloroplast movement. Plant Cell Environ 13: 595–614

Jenkins GI, Cove DJ (1983) Phototropism and polarotropism of primary chloronemata of the moss *Physcomitrella patens*: responses of the wild-type. Planta 158: 357–364

Kadota A, Wada M (1999) Red light-aphototropic (rap) mutants lack red light-induced chloroplast relocation movement in the fern *Adiantum capillus-veneris*. Plant Cell Physiol 40: 238–247

Kadota A, Wada M, Furuya M (1982) Phytochrome-mediated phototropism and different dichroic orientation of Pr and Pfr in protonemata of the fern *Adiantum capillus-veneris* L. Photochem Photobiol 35: 533–536

Kadota A, Kohyama I, Wada M (1989) Polarotropism and photomovement of chloroplasts in the protonemata of the ferns *Pteris* and *Adiantum*: evidence for the possible lack of

dichroic phytochrome in *Pteris*. Plant Cell Physiol 30: 523–531

Kagawa T, Lamparter T, Hartmann E, Wada M (1997) Phytochrome-mediated branch formation in protonemata of the moss *Ceratodon purpureus*. J Plant Res 110: 363–370

Miller JH, Greany RH (1974) Determination of rhizoid orientation by light and darkness in germinating spores of *Onoclea sensibilis*. Am J Bot 61: 296–302

Nakazato T, Kadota A, Wada M (1999) Photoinduction of spore germination in *Marchantia polymorpha* L. is mediated by photosynthesis. Plant Cell Physiol 40: 1014–1020

Wada M, Kadota A (1989) Photomorphogenesis in lower green plants. Annu Rev Plant Physiol Plant Mol Biol 40: 169–191

Wada M, Grolig F, Haupt W (1993) Light-oriented chloroplasts positioning. Contribution to progress in photobiology. J Photochem Photobiol 17: 3–25

Yatsuhashi H, Kadota A, Wada M (1985) Blue- and red-light action in photoorientation of chloroplasts in *Adiantum* protonemata. Planta 165: 43–50

Zurzycki J (1967) Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*. I. Action spectrum. Acta Soc Bot Pol 36: 133–142