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CHLOROPLAST MOVEMENT

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■ **Abstract** The study of chloroplast movement made a quantum leap at the beginning of the twenty-first century. Research based on reverse-genetic approaches using targeted mutants has brought new concepts to this field. One of the most exciting findings has been the discovery of photoreceptors for both accumulation and avoidance responses in *Arabidopsis* and in the fern *Adiantum*. Evidence for the adaptive advantage of chloroplast avoidance movements in plant survival has also been found. Additional discoveries include mechano-stress-induced chloroplast movement in ferns and mosses, and microtubule-mediated chloroplast movement in the moss *Physcomitrella*. The possible ecological significance of chloroplast movement is discussed in the final part of this review.

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INTRODUCTION

Chloroplasts are the primary photosynthetic apparatus of plants, and their intracellular distribution depends on environmental factors, especially the availability and quality of light. Chloroplast photorelocation movements are classified into two categories: high-fluence-rate responses (movements or arrangements) and low-fluence-rate responses. We prefer the terms avoidance response, or movement, to describe situations where chloroplasts move away from light, and accumulation response to denote situations where chloroplasts move toward light. This terminology holds even if chloroplasts differ in their sensitivity to strong or weak light. For example, the term accumulation directly describes the behavior of chloroplasts during accumulation responses induced by either a pulse or a partial cell irradiation with high-fluence-rate light (Figure 1). Both accumulation and avoidance responses are induced by blue light in all the plants tested to date, but in some plants, such as the green algae *Mougeotia scalaris* and *Mesotaenium caldariorum*, the moss *Physcomitrella patens*, and some ferns such as *Adiantum capillus-veneris*, red light is also involved in the accumulation response (7, 8, 21, 47, 49, 50). Red light is also implicated in the avoidance responses of *P. patens* (12) and *A. capillus-veneris* (51), but these are rather exceptional cases. Chloroplast accumulation responses are thought to maximize photosynthesis, whereas avoidance responses minimize photodamage to chloroplasts (23). The mechanisms underlying chloroplast relocation movements are still not well understood, but it is timely to review recent progress in this field, including persuasive evidence for the adaptive significance of these movements [e.g., (23)] based on analyses at the molecular and cellular levels. In this review, we concentrate on new findings about chloroplast relocation movement made during the past five years. For a more detailed analysis of classic experiments and their results, please refer to earlier reviews (7, 8, 21, 47, 49, 50).

PHOTORECEPTORS CONTROLLING CHLOROPLAST MOVEMENT

The photoreceptors controlling chloroplast photorelocation movement have been studied for many years and, based on action spectra, are predicted to comprise flavins, but no conclusive results have been obtained in the past century of research (7, 8, 21, 47, 49, 50).

Phototropins

Recently, Kagawa et al. (16) screened *Arabidopsis thaliana* mutants defective in the chloroplast avoidance response by a simple method using a narrow slit through which a section of leaf surface was irradiated with high light. The leaves of wild-type plants treated this way developed a pale green band in the irradiated area because of the chloroplast avoidance response, whereas the mutant leaves did not. More than 10 mutant plants were obtained from a screen of

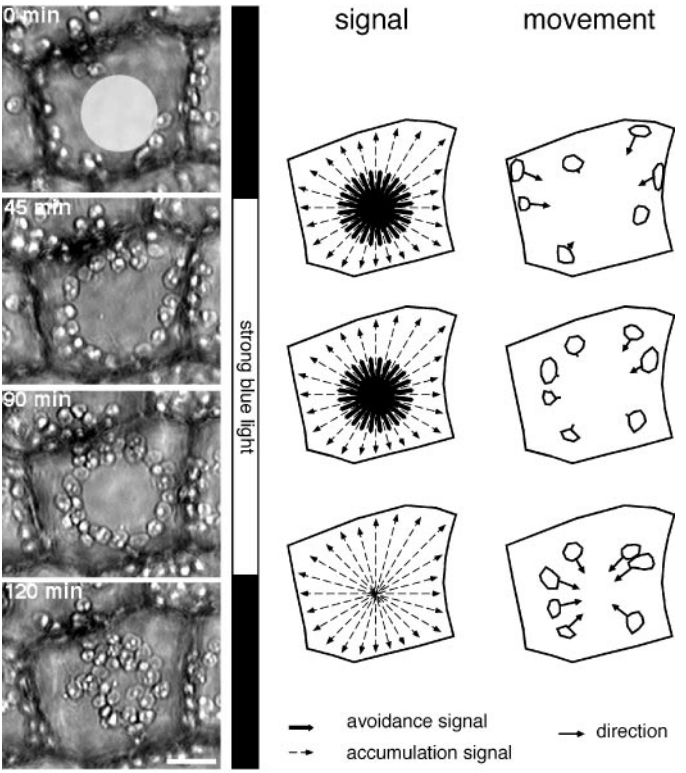

Figure 1 Photorelocation movement induced by a microbeam irradiation in *Adiantum* prothallus and schematic illustrations showing signal transfer and movement of some chloroplasts. A dark-adapted prothallus (0 min), in which chloroplasts move to anticlinal cell walls, was irradiated with a microbeam of strong blue light shown as a white round area at the center of a cell. Chloroplasts start toward the beam-irradiated area after strong light irradiation (45 min) but cannot enter into the beam because of strong light (90 min). When the microbeam is switched off, chloroplasts enter the beam irradiated area (120 min). This experiment shows that signals of accumulation can transfer a long distance, but those for avoidance response cannot, and that an accumulation signal has a longer life than an avoidance signal.

approximately 100,000 ethylmethane sulfonate (EMS)-mutagenized F2 seeds and 15,000 T-DNA-tagged lines. In most mutant lines, the nonphototropic hypocotyl-1 like-1 (*NPL1*) gene was defective. A number of T-DNA-tagged lines defective in *NPL1* were also obtained using reverse genetics, and these mutant lines also lacked the avoidance response (11, 16). NPL1 is a paralog of nonphototropic hypocotyl-1 (NPH1), which was identified as a blue light receptor mediating the phototropic response of *A. thaliana* hypocotyls under low light (10). Using the *npl1nph1* double

mutant, Sakai et al. (34) found that both NPH1 and NPL1 are blue light receptors mediating the phototropic response under high light. Subsequently, NPH1 and NPL1 were renamed phototropin1 (PHOT1) and phototropin2 (PHOT2) as photoreceptors mediating phototropism (3). Phototropins are protein kinases activated by blue light absorbed by flavin mononucleotide (FMN) attached to two light, oxygen, or voltage (LOV) domains (LOV1 and LOV2) at the N-terminus (4) (Figure 2).

The *phot1* null mutant (*nph1-5*) showed a slightly reduced light sensitivity for accumulation response (20), suggesting its involvement in this response. Sakai et al. (34) studied these photoresponses in *phot1* and *phot2* double-mutant plants. Observation and recording of individual chloroplasts' movement in mesophyll cells were performed under a microscope using a microbeam irradiator described elsewhere (48). The double mutant did not show any detectable chloroplast relocation movement when regions of cells were irradiated using a microbeam of low or high blue light ranging from 0.01 to 100 μmol·m^{-2}·s^{-1}. Because both accumulation and avoidance movements are absent in the double mutant, phot1 and phot2 function redundantly in chloroplast accumulation movements, although only phot2 is involved in avoidance movements. It is now clear that phot1 mediates only the accumulation response even under high-fluence-rate blue light. In contrast, phot2 mediates accumulation movements at low-fluence rates but avoidance movements at high-fluence rates. It is interesting that phot2 can switch its function in a fluence rate–dependent manner.

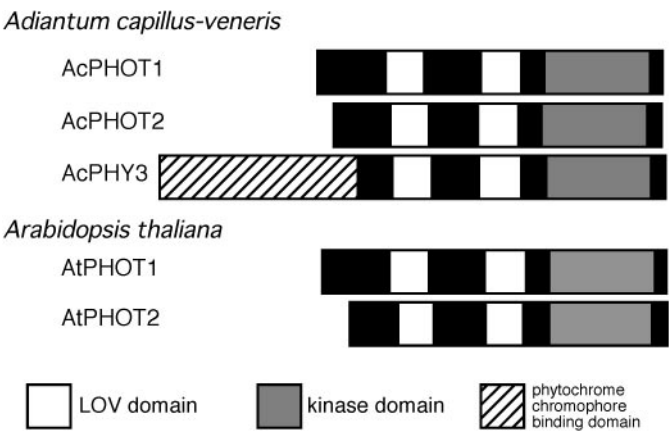

Figure 2 Schematic illustrations of phototropin family proteins in *Arabidopsis* and *Adiantum*. Phototropins have photoactivated protein kinase domain in the C-terminus and two LOV domains in the N-terminus to which FMNs bind under certain light conditions. *Adiantum* phy3 is a chimera protein with a phytochrome chromophore binding domain in the N-terminus and full-length phototropin in the C terminus. See text for details.

The *phot1phot2* double-mutant plants were used for further studies of stomatal opening (25), and both phot1 and phot2 were found to function redundantly as the photoreceptors for blue light–induced stomatal opening.

A Phytochrome with a Phototropin Sequence

In the gametophytes of the ferns *A. capillus-veneris* and *Dryopteris sparsa*, chloroplast relocation movements and phototropism are induced by red light as well as blue light. These red light effects must be mediated by phytochrome because they are reversible by red/far-red light (9, 15, 17, 18, 51, 52). Physiological analyses of more than 10 *A. capillus-veneris rap* (red light aphototropic) mutant plants in which the red light–induced phototropic response was defective revealed that all these mutant plants were also lacking in red light–induced chloroplast movement, indicating that both responses are mediated by a common photoreceptor (14).

A. capillis-veneris sequences encoding phytochrome have been cloned, and at least three genes, *AcPHY1*, *AcPHY2*, and *AcPHY3*, have been characterized (29–31). The deduced amino acid sequences of the *AcPHY1* and *AcPHY2* genes are similar to conventional phytochromes, but *AcPHY3* has a unique sequence: Whereas the N-terminal portion of the gene is similar to phytochrome and has 52% homology to *A. thaliana* PHYA, the C-terminal portion resembles almost the full-length sequence of phototropin and has 57% homology to *A. thaliana* PHOT1 (30) (Figure 2). *AcPHY3* expressed in yeast showed a typical difference spectrum exhibiting red/far-red reversibility when phycocyanobilin was added to the *AcPHY3* solution as a chromophore (30). Spectroscopic analyses of the LOV2 domain of *AcPHY3* expressed in *Escherichia coli* revealed that the *A. capillus-veneris* LOV2 domain binds a FMN (6). These results suggest that *AcPHY3* has an ability to absorb both red/far-red light and blue light and mediates both phytochrome and phototropin responses, especially in chloroplast movement and phototropism.

Kawai et al. (24) characterized some of the *rap* mutants and found that all five mutant lines tested had a mutation in the *AcPHY3* sequence. Moreover, transient complementation of the mutants by particle bombardment introduction of the wild-type *AcPHY3* gene attached to the cauliflower mosaic virus 35S promoter and a NOS terminator restored red light–induced chloroplast relocation movement (24). These results suggest that *AcPHY3* is the photoreceptor mediating red light–induced chloroplast photorelocation movement. *PHY3*-like gene sequences have also been found in other ferns (24).

Interpretation of the role of *AcPHY3* in blue light perception remains problematic. Like *A. thaliana*, *A. capillus-veneris* has *Ac*phot1 and *Ac*phot2 blue light receptors, and *Ac*phot2 has also mediated the chloroplast avoidance response by mutant analyses and rescue experiments using *Acphot2* mutant plants (T. Kagawa, unpublished data). However, the identity of the photoreceptor(s) mediating blue light–induced accumulation response in *A. capillus-veneris* is still unknown. Because mutants deficient in *AcPHY3* and *AcPHOT2* show normal accumulation response, they cannot be the only photoreceptors responsible for

chloroplast accumulation response, although they are among the candidates. By analogy with *A. thaliana*, both *Ac*phot1 and *Ac*phot2 may be the photoreceptors, but they may function redundantly. Moreover, *Ac*phy3 is also a strong candidate as the third blue light photoreceptor for *A. capillus-veneris*.

Other Plausible Photoreceptors

Tlalka et al. (45) investigated the possibility that zeaxanthin is the photoreceptor mediating chloroplast movement in *Lemna trisulca*, as has been proposed for stomatal opening (53) and phototropic responses (33). They found a parallel increase of zeaxanthin and chloroplast movement under high levels of blue light. They concluded, however, that "the absolute level of zeaxanthin *per se* does not appear to correlate with the rate or direction of chloroplast movements" (Reference 45, p. 455). Considering that the photoreceptors for chloroplast photorelocation movement in *A. thaliana* are phot1 and phot2, the main photoreceptor(s) for *L. trisulca* must be phototropin(s) and not zeaxanthin, as has also been shown for stomatal opening in *A. thaliana* (25). However, the hypothesis of Tlalka et al. (45) that zeaxanthin, which is located within the thylakoids and chloroplast outer membrane (see Reference 45), constitutes part of the photoperception system associated with individual chloroplasts is still possible for many reasons. First, the avoidance response occurs only among chloroplasts within an irradiated area, although one photoreceptor, phot2, must exist at or close to the plasma membrane. Second, unlike the accumulation response, the signal for the avoidance response cannot be transferred long distances from the edge of the irradiated area (19).

DOWNSTREAM FROM THE PHOTORECEPTORS

The precise signal(s) transferred from the photoreceptors to the chloroplasts is not yet known, but interesting information has been obtained by partial cell irradiation using a microbeam (19). When a small area of a dark-adapted cell was continuously irradiated with high-intensity blue light, the chloroplasts around the anticlinal wall started to move toward the beam, but they did not enter the beam (Figure 1). Once the blue light was removed, the chloroplasts moved inside the area that had been irradiated. These results suggest that (*a*) high-fluence-rate blue light generates both accumulation and avoidance signals; (*b*) the signal for accumulation movements travels a relatively long distance, but the one for avoidance movements stays within the irradiated area; (*c*) the signal for avoidance movements is dominant over that for accumulation movements when irradiated; and (*d*) the duration of the signal for accumulation responses is longer than that for avoidance movements. Despite this progress, complete characterization of these signals is still a long way off.

Calcium Ions

It is not yet known whether accumulation and avoidance movements share the same signal, although they do, in part, share a photoreceptor. If they do share the same

signaling pathway, different concentrations of signals must be involved. Calcium ions have been considered a candidate signal for transfer from the photoreceptors to the chloroplasts, although negative data have also been reported [see review by Wada & Kagawa (49)]. Baum et al. (2) studied blue light–induced $[Ca^{2+}]_c$ elevation in plants transformed with an aequorin gene. The level of transient $[Ca^{2+}]_c$ was increased by blue light irradiation in wild-type plants and in *cry1* and *cry2* mutant plants, which have additional blue light receptors, but was much reduced in *phot1* mutant plants. In electrophysiological studies, Stoelzle et al. (42) showed that Ca^{2+} influx was mediated by phototropins. In mesophyll cells of wild-type and *cry1 cry2* double-mutant plants, the phototropin-activated, calcium-permeable channel (PACC) was opened and Ca^{2+} influx was induced by blue light irradiation, while the response was completely abolished in *phot1phot2* double mutants. This suggests that the primary mechanism underlying phototropin response is Ca^{2+} influx. Furthermore, the artificial increase of $[Ca^{2+}]_c$ induced using the ionophore A23187 alters chloroplast positioning in *L. trisulca* and *M. scalaris* (41, 43, 44). Given the above results, Ca^{2+} has been considered the prime candidate for the signal connecting photoreceptors and chloroplasts. However, there is one discrepancy in connecting Ca^{2+} influx and chloroplast photorelocation. The transient increase of $[Ca^{2+}]_c$ and Ca^{2+} influx induced by blue light was sensitive to low concentrations (100 μM to 3 mM) of La^{3+}, a plasma membrane Ca^{2+} channel blocker, but the same or even higher concentrations (up to 10 mM) of La^{3+} failed to inhibit chloroplast movement induced by red and blue light (36, 38, 39, 44).

However, experiments by Sato et al. again implicate Ca^{2+} as the signal (36) (Figure 3). Mechanical stress, as well as light, can induce chloroplast movement (35). When a region of an *A. capillus-veneris* protonemal cell is briefly touched with a glass rod (e.g., for 1 min), the chloroplasts around the contact site move away thereafter, a response termed mechano-relocation movement. This mechano-relocation movement is inhibited by 10-μM Gd^{3+} (a stretch-activated channel blocker) or 100-μM La^{3+}. In contrast, both red and blue light–induced accumulation responses and blue light–induced avoidance responses are normal in the same cells in the same medium (Figure 3). Similar results were obtained for the moss *P. patens* (38). These results suggest that mechanical stress–induced chloroplast movement requires Ca^{2+} influx through the plasma membrane, but photorelocation movements do not, or at least not at the same level of Ca^{2+} influx required through the plasma membrane (36, 38). The chloroplast mechano-avoidance response can be induced under all photorelocation movement conditions, indicating that it is dominant to photomovement (36).

Tlalka & Fricker (43) concluded that "although proper regulation of $[Ca^{2+}]_{cyt}$ homeostasis is critical for both strong blue light (SBL) and weak blue light (WBL) responses to take place, additional factors may be required to specify the direction of chloroplast movement." (Reference, 43, p. 470). They also commented that the "results . . . underline the importance of intracellular Ca^{2+} stores in the blue light responses, but do not provide any clues as to how the direction of movement is controlled." (Reference 43, p. 469). Given the attenuation of the blue light–induced

transient increase of cytosolic Ca^{2+} level in *phot1* mutant plants (2), the complete abolition of blue light–induced Ca^{2+} influx through the plasma membrane in *phot1phot2* double-mutant plants (42), and the effect of Ca^{2+} in the mechano-movement of chloroplasts, Ca^{2+} will likely play a role on chloroplast relocation movement. However, a coherent explanation for these results that accounts for the apparent discrepancies enumerated above is not currently possible.

Cytoskeleton

The signals induced by light and mechanical stress must ultimately be transferred to cytoskeletal components to result in chloroplast movement. There is a long history of using cytoskeletal inhibitors, such as the actin depolymerizing agent cytochalasin B and D, the cross-linker of the actin subunit in F-actin m-maleimidobenzoic acid *N*-hydroxysccinimide ester (MBS), and the myosin ATPase inhibitor sulfhydryl group reagent *N*-ethylmaleimide (NEM), to study the motile system responsible for chloroplast photomovement in plant cells, such as in *L. trisulca* (28, 44) and in *A. capillus-veneris* (13). These studies suggest the involvement of actin filaments, but not microtubules. Using immunofloures-cence staining in their work on the structural relationship between chloroplasts and the cytoskeleton in *A. thaliana*, Kandasamy & Meagher (22) also indicated that chloroplasts move along actin filaments. The mechano-avoidance response in *A. capillus-veneris* is also dependent on actin filaments (35).

Recently, however, both microtubules and actin filaments were found to be involved in chloroplast relocation movement in the moss *P. patens* (37) (Figure 3). Analyses of chloroplast photorelocation movement were performed under various light conditions with or without 10-μM Cremart; microtubule depolymerizing agent; and/or 0.1-mM cytochalasin B, an actin depolymerizing agent. Both red light–induced accumulation and avoidance movement were blocked by Cremart, but not by cytochalasin B. In contrast, blue light–induced accumulation and avoidance movement were not blocked unless cells were treated simultaneously with both inhibitors. Based on these observations, we propose that phytochrome signals are transferred to the microtubule motile system, whereas blue light signals are transmitted to both actin filaments and the microtubule system.

The rates of chloroplast movement have been calculated for *A. capillus-veneris* (18), *A. thaliana* (20), and *P. patens* (37). To do this, *A. capillus-veneris* gametophytes and *A. thaliana* leaf cells were pretreated in darkness overnight to allow chloroplast movement to the anticlinal wall (the dark position), and then the central portion of a cell was irradiated with a blue microbeam 10 μm in diameter. In a cell comprising two-dimensional gametophytes of *A. capillus-veneris*, the rate of movement was constant at approximately 0.3 μm·min^{-1} under both red light and blue light of fluences between 10 and 1000 Jm^{-2} (18). In the blue light–induced accumulation response of *P. patens*, the rate of actin filament–dependent chloroplast movement is similar to that of *A. capillus-veneris*, whereas that of microtubule-dependent movement is more than five times faster

($\sim$2.5 μm·min^{-1}) (37). In *A. thaliana*, the rate of accumulation movement induced by a blue microbeam in mesophyll cells was slow, less than 0.09 μm·min^{-1}, but when red light was given to whole cells as background irradiation, the rate was much accelerated, up to 0.4 μm·min^{-1} (19). The red light effect is not well understood, but increased cytoplasmic motility is clearly involved.

The function of actin filaments (and microtubules in the case of *P. patens*) during chloroplast movement has not yet been fully characterized. It is not known whether chloroplasts are simply carried along a ready-made actin network system or whether they use newly polymerized actin filaments after the direction of movement has been determined. From a structural analysis of chloroplasts and actin filaments, Kandasamy & Meagher (22) proposed that "using motor molecules, some chloroplasts migrate along the actin cables directly, while others are pulled along the cables by the fine actin filaments." (Reference 22, p. 110). However, considering that a chloroplast moves in any direction when a small microbeam (a few micrometers in diameter) is focused anywhere near it (E. Matsumoto & M. Wada, unpublished data) the newly polymerized filament hypothesis remains to be validated. Now that green fluorescent protein (GFP)- or red fluorescent protein (RFP)-tagged actin binding proteins, such as GFP-talin (27) are available, the behavior of actin filaments during movement can readily be observed and should provide important clues regarding the mechanisms underlying movement.

ECOLOGICAL SIGNIFICANCE OF CHLOROPLAST MOVEMENT

Augustynowicz & Gabrys (1) considered the ecological significance of chloroplast movements under natural conditions, using mature leaves of several fern species living in different ecological niches. *A. capillus-veneris* and *Pteris cretica* had a high degree of environmental flexibility and showed many sensitive chloroplast photorelocation movements (avoidance and accumulation) under high and low blue light, respectively.

In *A. capillus-veneris*, accumulation response occurs under red light. On the other hand, *Adiantum caudatum* requires high light for growth and undergoes almost no photorelocation movement, whereas *Adiantum diaphanum*, a shade-loving species, undergoes only weak photorelocation movements. These results indicate that chloroplast relocation movement is necessary or effective only for plants growing in environments where light intensities fluctuate greatly, but it is not as important for plants living in rather constant light conditions, or even in direct sunshine.

Trojan & Gabrys (46) have studied the relationship between chloroplast distribution and light conditions during plant growth. They cultured *A. thaliana* under different light conditions, e.g., weak light close to the compensation point for photosynthesis and light levels similar to those found in the natural environment but not sufficient to cause photodamage. Plants were irradiated with high or low light or kept in darkness, and the distribution patterns of chloroplasts were compared. In

darkness, chloroplasts in the plants grown under weak light remain close to the periclinal walls where they can then respond quickly to any added light. On the other hand, the chloroplasts in plants grown under high light stay close to the anticlinal walls, probably because light is not a limiting factor. In contrast, in plants grown under low or high light, high-light levels cause chloroplasts to distribute along the anticlinal wall. Thus, the avoidance response from high light appears to be of prime importance and dominates any other orientation behavior of chloroplasts.

Park et al. (32) studied the photoinactivation of photosystem II (PSII) under high-fluence light conditions in relation to chloroplast movement in the facultative shade plant *Tradescantia albiflora* and in comparison to that of *Pisum sativum*. The photosystem II (PSII) of *T. albifolia* showed greater resistance to light stress than that of the pea, although not owing to differences in PSII antenna size or the index of susceptibility of PSII to light stress. Light transmittance (a measure of chloroplast movement) was greater in *T. albifolia* than in *P. sativum*. Park et al. suggest that "chloroplast movement in shade plants . . . may be a better strategy for coping with the fluctuating light environment within the canopy than D1 repair, which is a more energy-consuming process" (Reference 32, p. 874). It seems reasonable for plants under the canopy to use chloroplast movement to maximize photosynthesis because spotted light coming through the canopy is weak and transient owing to the sun's movement.

Until recently, there was no clear evidence for the adaptive value of chloroplast movement because chloroplast movement mutants were not available. Kasahara et al. (23) studied the importance of chloroplast avoidance movement using two different kinds of mutants, *phot2* (a photoreceptor mutant) and *chup1* (a mutant deficient in an actin-binding protein and possibly a mutant of the motile system in general). Chloroplast avoidance movement was not observed in the leaves of these mutants. When mutant plants were cultured under low-fluence-rate white light (100 μmol·m^{-2}·s^{-1}) and were then transferred to continuous high-fluence-rate light (1400 μmol·m^{-2}·s^{-1}), the mature leaves bleached and then became necrotic. The mechanism for this response is unknown, but the photodamage was severe and terminal, indicating that the avoidance response plays a significant role in protecting the photosynthetic apparatus from high-light damage.

Finally, it would be especially valuable to understand the mechanism of repositioning of chloroplasts under darkness from the periclinal to the anticlinal cell walls. Senn described this well-known phenomenon in his classic text published in 1908 (40). The diurnal rhythm of this phenomenon has been studied in green algae, including *Ulva lactuca* and *Aceptabularia mediterranea* (5, 26); however, its physiological and ecological meaning has not been clarified. Senn performed some experiments on this phenomenon and concluded that the attraction effect of anticlinal walls in darkness, that is, Apostrophe, is caused by chemotactic agents. All salts of the soil (e.g., sulphates) and organic migrating compounds are involved. At their migration from cell to cell they flow particularly through the anticlinal walls and are there in a higher concentration. If there are very high concentrations of these agents, they diffuse also to the outer cell walls and accordingly there is

less Apostrophe in darkness. The best Apostrophe can be seen in cells that are hungry. Recently we isolated *A. capillus-veneris* mutants that do not show dark positioning of chloroplasts (M. Wada & K. Motoyama, unpublished data). Further study of these mutants may reveal whether they are still hungry or always full.

PERSPECTIVES

The photorelocation movement of chloroplasts is now amenable to the study of mutants, and with this approach, investigators might be able to rapidly reveal all of the components involved in the signal transduction pathways. In addition, molecular and cellular biology techniques have made it almost possible to see the intracellular distribution and/or behavior of these components, including the cytoskeleton and even the ions involved. Soon we will understand this phenomenon at the molecular level, from the functional domains of the photoreceptor proteins and the various components of the signal transduction pathways to their constituent protein-protein interactions.

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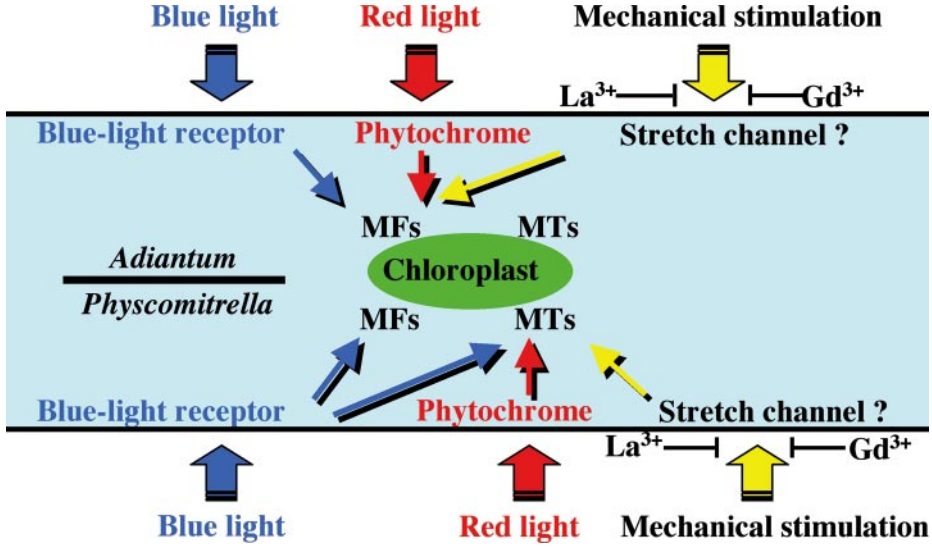

Figure 3 A model for signaling pathway of chloroplast photo- and mechano-relocation movement in *Adiantum* and *Physcomitrella*. Mts: microtubules; MFs: actin microfilaments.

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