Short actin-based mechanism for light-directed chloroplast movement in Arabidopsis

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Organelle movement is essential for proper function of living cells. In plants, these movements generally depend on actin filaments, but the underlying mechanism is unknown. Here, in Arabidopsis, we identify associations of short actin filaments along the chloroplast periphery on the plasma membrane side associated with chloroplast photorelocation and anchoring to the plasma membrane. We have termed these chloroplast-actin filaments (cp-actin filaments). Cp-actin filaments emerge from the chloroplast edge and exhibit rapid turnover. The presence of cp-actin filaments depends on an actin-binding protein, chloroplast unusual positioning1 (CHUP1), localized on the chloroplast envelope. chup1 mutant lacked cp-actin filaments but showed normal cytoplasmic actin filaments. When irradiated with blue light to induce chloroplast movement, cp-actin filaments relocalized to the leading edge of chloroplasts before and during photorelocation and are regulated by 2 phototropins, pho1 and pho2. Our findings suggest that plants evolved a unique actin-based mechanism for organelle movement.

actin filament | chloroplast photorelocation | chloroplast unusual positioning1 (CHUP1) | organelle movement | phototropin

Organellar movement is ubiquitous and essential for basic cellular functions in eukaryotes, including animals, fungi, and plants. Many systems of organelle movement are based on the actin cytoskeleton. Two mechanisms of actin-based organelle movement have been identified, mainly in animals and in yeast (1). One depends on myosin, which binds organelle cargos in its tail domain and transports them by sliding on actin cables (2). The other depends on the Arp2/3 complex, which can nucleate actin filaments and form complex filament arrays. Arp2/3 complex-dependent actin polymerization at the organelle edge (that is, actin “comet tail” formation) generates the motive force to push the organelle (3).

Various organelle movements in plants have been shown to depend on actin filaments by inhibitor studies (4). Among plant organelle movements, chloroplast movement is the best-characterized response, and is therefore a good experimental system for studying the mechanisms of organelle movement (5). Chloroplasts relocate in response to external stimuli, particularly light. Weak light induces a chloroplast accumulation response so that light is captured efficiently for photosynthesis. Strong light induces an avoidance response to evade photodamage (5, 6).

Through molecular genetic analysis using Arabidopsis thaliana, it was demonstrated that 2 phototropin blue light receptors (pho1 and pho2) redundantly mediated the accumulation response, and pho2 alone regulated the avoidance response (7–9). However, the mechanism of light regulation of chloroplast movement remains to be determined.

Except in very rare cases, such as a moss species, most land plant species use actin filaments exclusively (rather than microtubules) for chloroplast movement (5). Anti-actin drugs (but not antimicrotubule drugs) inhibit chloroplast movement in various green plant species (5). Interaction of chloroplasts with actin filaments was observed by actin labeling in various plant species such as a fern Adiantum capillus-veneris (10) and A. thaliana (11) and in vitro cosedimentation assays using isolated spinach chloroplasts (12).

The other actin-based system drives chloroplast movement remains obscure.

In the present study, we performed detailed analyses of actin dynamics during chloroplast photorelocation by live cell imaging of Arabidopsis plants in which actin was visualized by GFP fluorescence labeling techniques using a custom-made microscope equipped with a microbeam irradiation unit to induce precise chloroplast movement.

Results

Chloroplasts Associate with Short Actin Filaments (CP-Actin Filaments) and Their Biased Relocalization Is Coupled to Light-Directed Chloroplast Movements. Using transgenic Arabidopsis plants expressing the GFP-mouse talin fusion protein (13) and tdTomato-fimbrin fusion protein, we analyzed actin dynamics in vivo in leaf petiole or mesophyll cells. Under intermittent GFP excitation (~7.5 × 102 µmol m−2 s−1 for 1 s) at 3-s intervals while chloroplasts were stationary, the fluorescence images revealed the presence of short actin filaments around the chloroplast peripheral region (referred to herein as chloroplast-actin filaments, abbreviated “cp-actin filaments”) (Fig. 1A Inset, and Movie S1 and Movie S2) as well as cytoplasmic actin cables. Cp-actin filaments demonstrated a complex pattern of dynamics, appearing and disappearing very rapidly in a nonsynchronous manner.

We used a custom-made microscope to observe GFP fluorescence images simultaneously during chloroplast movement induced by microbeam irradiation of specific parts of the cell (Fig. 1A–C and Movie S3). Before irradiation, the velocities of chloroplast movement were approximately zero. When irradiated continuously with a microbeam of high-intensity blue light (377 µmol m−2 s−1), chloroplasts in or near the irradiated area (i.e., numbers 1 and 2 in Fig. 1D, Left Inset) moved transiently at velocities up to 1 µm/min out of the irradiated zone (Fig. 1D), but chloroplasts outside of the

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The authors declare no conflict of interest.

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irradiated area did not move (i.e., numbers 3 and 4 in Fig. 1D, Left Inset; they were ~30 µm or farther away from the beam.). An avoidance response was also induced when blue GFP excitation light was given continuously (Fig. 1E and Movie S4 and Movie S5). Cp-actin filaments transiently disappeared immediately after the high-intensity blue light irradiation [Fig. 1 A–C and E (30 and 39 s)] but gradually reappeared at the leading edge of the chloroplasts [Fig. 1E (63 and 72 s) and F, and Movies S3–S5]. After a full accumulation of cp-actin filaments in the front region of chloroplast (Fig. 1E, 90 s), we observed the chloroplast envelope extending toward the direction of chloroplast movement under both transmission and electron microscopy, although only when the avoidance response was induced (Fig. S1). When the chloroplasts moved out of the beam and subsequently stopped, the cp-actin filaments became nonbiased and redistributed around the entire chloroplast periphery, similar to what was seen before microbeam irradiation (Fig. 1 B and C). Chloroplasts located farther away from the microbeam (such as numbers 3 and 4 in Fig. 1D, Left Inset) neither changed their motility (Fig. 1D) nor showed biased cp-actin filament distribution (Fig. 1D, Right Inset). The appearance of biased cp-actin filaments was also evident for chloroplasts that accumulated toward a low-intensity blue microbeam (3.8 µmol m\(^{-2}\) s\(^{-1}\)) (Fig. S2).

Some of the fluorescence intensity reduction may be due to photobleaching. However, photobleaching is not the main cause for fluorescence reduction in the chloroplast under the high-intensity blue light. First, fluorescence reduction was transiently induced under continuous irradiation with high-intensity blue light, but the fluorescence increased shortly thereafter showing biased distribution (that is the generation

Fig. 1. Biased cp-actin filament relocalization during chloroplast photorelocation movement. (A) Chloroplast avoidance response was induced by a high-intensity blue microbeam (377 µmol m\(^{-2}\) s\(^{-1}\)) (10 µm in width). Arrowheads represent biased cp-actin filaments that were easily seen. (Inset) Nonbiased cp-actin filaments before chloroplast movement. (B and C) Magnified images of dynamics of cp-actin filaments in 2 chloroplasts indicated with arrows in A. Red circles, the position of moving chloroplasts; MB, the microbeam-irradiated area. (D) Movements of 4 chloroplasts during an avoidance response. Two control chloroplasts outside the irradiated area are indicated by cyan and yellow. Two chloroplasts in the irradiated area are indicated by blue and magenta. (Left Inset) shows the chloroplast positions at 5-min intervals after irradiation. Main image shows velocities. (Right Inset) shows the difference of GFP-talin fluorescence between the front and rear halves of the chloroplasts. (E) Reorganization of cp-actin filaments (indicated in black) during avoidance movement induced by continuous blue GFP excitation light irradiation. Numbers, time in seconds after irradiation. Blue and pink circles, the positions where GFP fluorescence intensities were recorded in F. (F) Change in GFP fluorescence intensity at the front and rear of the chloroplast in E. (G) Correlation between biased localization of cp-actin filaments and chloroplast speed during avoidance movements.
of cp-actin filaments) (Fig. 1 and Movie S3 and Movie S4). If the reduced fluorescence is due to photobleaching, fluorescence recovery should not occur during continuous irradiation. Second, there was little, if any, reduction in fluorescence intensity in phot2 or phot1phot2 mutant plants (see Fig. 3 B and C), indicating that the reduced fluorescence intensity in response to high-intensity blue light is a physiologically relevant response.

Although the cytoplasmic actin cables may modulate chloroplast movement, their role in chloroplast movement appears to be minor. Light regulation of cytoplasmic actin cables was examined in WT and phot1phot2 and chup1 mutants to study the involvement of cytoplasmic actin cables in chloroplast movement (Fig. S3). No changes in label intensity were detected before and during microbeam irradiation (both inside and outside of the microbeam).

Even when cytoplasmic actin cables were attached to moving chloroplasts, there was no observed correlation with the direction and/or timing of the chloroplast movement (Fig. 1 and Movie S4). More importantly, phot1phot2 and chup1 mutants that lacked chloroplast photorelocation (9, 13) demonstrated cytoplasmic actin cable dynamics similar to those of WT plants (see Figs. 2G and 3 C and F).

Similarly, visualization of microtubules using a GFP-α tubulin line revealed that microtubule organization did not correlate with chloroplast distribution or photorelocation (Movie S6). Furthermore, depolymerization of microtubules with 5 μM oryzalin had no effect on chloroplast movement, indicating that microtubules are not involved in chloroplast movement. Taken together, these findings suggest that chloroplast movement is correlated with cp-actin filaments and suggest that biased cp-actin filaments may be a prerequisite for generating the motive force required for subsequent chloroplast movement.

When the differences in GFP fluorescence intensities between the front and rear halves of chloroplasts were plotted against the speeds of chloroplast movement, a close correlation between high-intensity blue light and chloroplast motility was evident for both avoidance (Fig. 1 F) and accumulation responses (Fig. S2). The greater the blue light intensity, the greater the speed of chloroplast movement (Fig. 1 G).

Importantly, when we analyzed chloroplast photorelocation and behaviors of cp-actin filaments in Arp2/3 complex-deficient mutants (16), these mutants showed WT responses (Movie S7), indicating that the Arp2/3 complex is not involved in cp-actin filament polymerization and chloroplast movement.

**A Relationship Between Nonbiased Cp-Actin Filaments and Chloroplast Anchoring.** When a wider microbeam (30 μm in width) of high-intensity blue light was continuously applied, chloroplasts inside the beam area remained there for extended periods of time (typically more than 1 h, Movie S8). The amount of cp-actin filaments was reduced during this period (Fig. 2 A and B), and chloroplast movement in random directions increased (Fig. 2C and Fig. S4 A), suggesting that they detached from the plasma membrane. In contrast, under low-intensity blue light, cp-actin filaments increased in number (Fig. 2 D and E), and random motility was reduced (Fig. 2F and Fig. S4B). No apparent changes in cytoplasmic actin cables were observed under these conditions. These results
not disappear under high-intensity light in the
with biased cp-actin filaments. Notably, the cp-actin filaments did not
mutant was highly variable among cells, but chloroplasts
chup1 appeared to be normal (Fig. 2)
detected in the microbeam irradiation (Movie S9). Cp-actin filaments were not
clusters and did not show light-directed movement in response to
avoidance movement but demonstrated an accumulation response (7–9). The
mulation response, and phot2 alone regulates the avoidance re-
Kadota et al. PNAS
Chloroplasts by Regulating Cp-Actin Filaments.

These findings reinforce the hypothesis that the function of cp-actin
filament regulation of the chloroplast.

Chloroplasts. Blue circles indicate lack of transient disappearance of cp-actin filaments in
phot2 mutant under high-intensity blue light (377 µmol m⁻² s⁻¹) were observed every 5 min (A–C). Those
under low-intensity blue light (3.8 µmol m⁻² s⁻¹) were observed every 10 min (D–F). Red circles indicate moving chlo-
roplasts. Blue circles indicate lack of transient disappearance of cp-actin filaments in
phot2 mutant under high-intensity blue light. (G–J) Disappearance of cp-actin filaments after 5
min of high-intensity blue microbeam irradiation (30 µm in width) (G) and increase after 10 min of low-intensity blue microbeam irradiation (the same blue light (H) occurred in the WT but not in the phot1phot2 double mutant. Motility of chlo-
roplasts located inside the microbeam area was determined as the integrated distance traveled for 60 min under high-intensity (I) or low-intensity (J) blue light. Data of G–J are presented as means and SEs derived from 7 independent experiments.

CHUP1 was identified through molecular genetic analysis of
chup1, a mutant with impaired chloroplast movement and posi-
tioning (6, 13). In the chup1 mutant, chloroplasts aggregated in
clusters and did not show light-directed movement in response to
microbeam irradiation (Movie S9). Cp-actin filaments were not
detected in the chup1 mutant, although cytoplasmic actin cables
appeared to be normal (Fig. 2G) (13). Chloroplast motility in the
chup1 mutant was highly variable among cells, but chloroplasts
moved rapidly via cytoplasmic streaming in many cells (Fig. 2H, Fig.
S5, and Movie S9), a behavior not observed in WT cells (Fig. 2H).
These findings reinforce the hypothesis that the function of cp-actin
filaments is to connect chloroplasts to the plasma membrane. These
findings suggest that CHUP1 may possibly function in cp-actin
filament regulation of the chloroplast.

Phototropins Mediate Directional Photomovement and Anchoring of
Chloroplasts by Regulating Cp-Actin Filaments. Two phototropin blue
light receptors (phot1 and phot2) redundantly mediate the accumu-
lation response, and phot2 alone regulates the avoidance re-
response (7–9). The phot1 mutant cells exhibited both accumulation and avoidance movement, and cp-actin filament dynamics were
similar to WT (Fig. 3 A and D). phot2 mutant plants lacked
avoidance movement but demonstrated an accumulation response
under high-intensity light (Fig. 3B) and low-intensity light (Fig. 3E)
with biased cp-actin filaments. Notably, the cp-actin filaments did not disappear under high-intensity light in the phot2 mutant (Fig.
3B, blue circles). Therefore, phot2 mediates transient cp-actin filament disappearance in response to strong blue light. Neither photorelocation movements nor motility changes were induced in the
phot1phot2 double mutant (Fig. 3C, F, I, J, and Fig. S5). Likewise, no initial loss in cp-actin filaments nor biased cp-actin
filaments were observed in the microbeam experiments in the
phot1phot2 double mutant (Fig. 3 C, F–H, and Fig. S5). These
results further support the role of cp-actin filament reorganization in chloroplast movement and show that phototropin function provides a directional cue for cp-actin reorganization.

Cp-Actin Filaments Localize at the Interface Between the Chloroplast
and the Plasma Membrane and Are Generated from the Edge of
Chloroplasts. To study the precise localization and possible poly-
merization dynamics of cp-actin filaments, we developed a proto-
 plast system in which both accumulation and avoidance responses
could be induced (Movie S10). Three-dimensional confocal mi-
croscopy analyses of GFP-talin fluorescence revealed that cp-actin
filaments are only localized at the interface between the chloroplast and the plasma membrane, irrespective of biased or nonbiased
distribution (Fig. 4, Movie S11, and Movie S12).

For precise analyses of behavior of cp-actin filaments using movies, photographs of a whole or part of a protoplast were obtained every 0.2 to 0.5 s under continuous irradiation with a laser-scanning beam for GFP image acquisition (1 mW by Diode 488–100; Carl Zeiss). If part of a protoplast was irradiated with the laser beam, the chloroplasts next to the beam-irradiated area showed a biased distribution of cp-actin filaments at the side of the
chloroplast opposite to the beam (Fig. 4B and Movie S13). Cp-actin filaments were clearly observed at the beginning of microscopic examination (Fig. 4B) but gradually disappeared within 1 min (Movie S13). Cp-actin filaments shortened during this process toward the chloroplast periphery and finally disappeared at the chloroplast edge (Fig. 4B and D-b1, and D-b2). Cp-actin filaments initially disappeared when the intensity of the laser beam was reduced (0.4 mW), but they reappeared after 30 s to 1 min at the same positions (Fig. 4C and D-c1 and Movie S14). Appearances of cp-actin filaments and disappearances with respect to the chloroplast edge were clearly observed in the kymographs (Fig. 4E). The appearances and disappearances were repeatedly observed within a few minutes at the same positions (Fig. 4F, arrowheads) when protoplasts were treated with dark and strong light cycles (Fig. 4F). This finding indicates that cp-actin assembly sites exist on the chloroplast edge and have a rather long half-life (Fig. 4C and F). Cp-actin filaments disappears (or shortens) centrifugally (Fig. 4D), suggesting that cp-actin filaments may have a polarity with respect to the edge of the chloroplast.

Discussion

Myosin-dependent transport along cytoplasmic actin cables and Arp2/3 complex-dependent comet-tailed movement are the prevailing mechanisms for actin-based organelle movement in animals and yeast (1). Like animals and yeast, plants have multiple myosins (17) and a full complement of Arp2/3 complex components (18). Recent comprehensive analyses of myosin mutant lines suggest that myosins are not involved in chloroplast photorelocation movement (19, 20), and we showed that mutants defective in Arp2/3 genes retained normal chloroplast photorelocation movement. Although redundant functions of multiple myosin isoforms and other actin-based mechanism cannot be excluded, the cp-actin filament-mediated chloroplast movement in plant cells reported here may represent an actin-based movement machinery that is distinct from those identified to date in other organelles. Note that the components of chloroplast movement identified are all plant-specific (5), and we showed here that the photoreceptor phototropin and an actin-binding protein CHUP1 played a pivotal role in mediating cp-actin filament regulation during chloroplast photorelocation movement.

Weak light induces chloroplast accumulation response, so that chloroplasts move toward and accumulate in the irradiated area. In chloroplasts outside the irradiated area, cp-actin filament reorganization was induced, and biased cp-actin filaments were formed on the front of accumulating chloroplasts toward the irradiated area (Fig. S2). Thus, long-distance directional signals caused cp-actin filament reorganization on chloroplasts outside of the irradiated area. The biased cp-actin filaments induced by weak blue light for the accumulation response were formed in both the phot1 and phot2 mutants, but not in the phot1phot2 double mutant, indicating that both phot1 and phot2 can produce the directional signal necessary for cp-actin filament reorganizations during the accumulation response (Fig. 3). In response to strong light irradiation in WT plants, cp-actin filaments on irradiated chloroplasts disappeared, and chloroplasts demonstrated increased motility in random directions (Figs. 1 and 2A–C). This disappearance was transient, but it continued until immediately before the chloroplasts began to avoid from the strong light. The speed of chloroplast movement during the avoidance response depended on light intensity and phot2 abundance (21). In this study, we also showed that the differences in the amount of cp-actin filaments at the front and the rear of chloroplasts were also dependent on light intensity. This relationship between the bias of cp-actin filaments and the rate of chloroplast movement provides strong support for a model in which blue light intensity determines the speed of directional chloroplast movement by regulating the difference in the amount of cp-actin filaments between the front and the rear of the chloroplasts.

Our results also shed light on an important role of cp-actin filaments in the stationary phase as well as during the movement of chloroplasts. Weak blue light induced an increase in cp-actin filaments on the entire periphery of chloroplasts in the irradiated area and this increase depended on both phot1 and phot2 (Fig. 3). This increase in cp-actin filaments under low light was associated with the decrease of chloroplast motility, and this decrease did not occur in phot1phot2 (Fig. 3). Moreover, in a chup1 mutant that lacks cp-actin filaments, chloroplasts were aggregated and moved rapidly via cytoplasmic streaming (Fig. 2G and H). Finally, confocal microscopy revealed that cp-actin filaments were present at the plasma membrane side of chloroplasts (which is obvious from chloroplast shape) both in nonbiased and biased cp-actin filaments (Movie S10 and Movie S11, respectively). (B) Disappearance of cp-actin filaments under a high-intensity laser beam (1 mW) for GFP excitation (Movies S13). (C) Disappearance followed by reappearance of cp-actin filaments under a low-intensity laser beam (0.4 mW) (Movie S14). (D) Cp-actin filament images taken every 3 s (b1 and b2) or 10 s (c1) in rectangles B and C (see Movie S14) (E) Kymographs representing cp-actin disappearance (made of 120 photographs taken every 0.5 s on the white lines in b1 and b2 of B) and reappearance (made of 180 photographs taken every 1 s on the white lines in c1 and c2 of C) at the chloroplast edge. Arrows show chloroplast edges. The outside of the chloroplast is on the left. (F) Repeated appearance of cp-actin filaments at the same points of the chloroplast edge (shown with arrowheads). A protoplast was repeatedly observed by fluorescence microscopy for 30 s followed by 1–2-min incubation in the dark. Photographs were taken at the beginning (1, 3, 5, and 7) and ending (2, 4, 6, and 8) of the observation period. A 30-s observation caused cp-actin filament disappearance and a 1–2 min dark treatment allowed the recovery.
microscopic analyses showed that cp-actin filaments only polymerized and localized at the interface between the chloroplast and the plasma membrane (Fig. 4). All these results strongly suggest a function of cp-actin filaments on chloroplast anchoring to the plasma membrane. Note that CHUP1 was localized on the outer envelope of the chloroplast and bound not only to F-actin but also to G-actin or profilin in vitro (13–15), suggesting that CHUP1 may possibly function in cp-actin filament regulation at the chloroplast envelope. Further analyses of cp-actin filaments will be required to understand how cp-actin filaments generate the motive force for chloroplast movement.

Materials and Methods

Plant Material and Growth. Arabidopsis plants were grown in plastic dishes or pots under light/dark regimes at 23 °C. Light was provided by three 20-W white fluorescent tubes (FL20SW; Toshiba Lighting & Technology). The GFP-talin-expressing WT line (Col gt1) was the same as described in ref. 13. The GFP-talin-expressing lines were obtained in mutant backgrounds by genetic crossing. A tdf tomato-fimbriin-expressing line was generated by transforming a construct containing tdf tomato fused to the N-terminal region of an actin binding domain expressing lines were obtained in mutant backgrounds by genetic crossing. A pots under light/dark regimes at 23 °C. Light was provided by three 20-W white

Kadota et al. PNAS | August 4, 2009 | vol. 106 | no. 31 | 13111

Confocal Microscopic Analyses of Cp-Actin Dynamics in Mesophyll Protoplasts. Mesophyll cell protoplasts were prepared from rosette leaves of 4-week-old WT plants treated with digestion medium (pH 5.7) containing 1% (wt/vol) cellulase R-10 (Yakult Honsya), 0.1% pectolyase Y-23 (Kyowa Chemical Products), 0.2% BSA, 20 mM KCl, 0.4 M mannitol, 10 mM CaCl2, and 20 mM Mes. Samples were digested by shaking (15 rpm) at 28 °C for 10 min and an additional 3 times (5 min each) with fresh digestion medium. Isolated protoplasts were washed 3 times with White's mineral salt solution (24) and incubated overnight at 4 °C before use. For microscopic observation, protoplasts were attached to poly-L-lysine-coated coverslips and mounted in White's mineral salt solution. Fig. 4A used a custom-made high-speed 3-dimensional confocal microscope (see ref. 25 for details). Fig. 4 B–E used a confocal microscope (LSM LIVE; Carl Zeiss), and Fig. 4F used a fluorescence microscope (Imager Z1; Carl Zeiss).

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Fig. S1. Dynamics of cp-actin filaments and extension of chloroplast envelope during chloroplast photomovement. (A) Envelope extension during movement was observed under transmission (Upper) and fluorescence (Lower) microscopy. Arrowheads and arrows indicate envelope extension and the direction of chloroplast motion, respectively. The chloroplast exhibited an avoidance response induced by microbeam irradiation of its left side with high-intensity blue light (377 µmol m⁻² s⁻¹). *Debris from the cell wall of an adjacent cell. (B and C) Electron micrographs of chloroplasts, with a higher magnification shown in the boxed area. (B) Chloroplast before irradiation. Mt, mitochondria. (C) Chloroplast observed 5 min after high-intensity light irradiation. Note the envelope extension at the right edge of the chloroplast.
Fig. S2. Biased relocalization of cp-actin filaments during microbeam-induced chloroplast accumulation movement. (A) The accumulation response was induced by continuous irradiation with a microbeam (10 µm in width) of low-intensity blue light (3.8 µmol m⁻² s⁻¹). Actin filament dynamics were observed every 10 min. Arrowheads indicate biased cp-actin localization at the front of moving chloroplasts. Also note the increase in cp-actin filaments on chloroplasts in the microbeam area. (B) Magnified images of the chloroplast marked with an * in A. (C) Speed (determined as the distance traveled every 10 min) was plotted before and after irradiation of chloroplasts located near and far away from the microbeam area. Accumulation movement was induced by continuous irradiation with a microbeam (MB) of low-intensity blue light (3.8 µmol m⁻² s⁻¹, 10 µm in width). Numbers indicate individual chloroplasts located near (1–3) and far away (4 and 5) from the beam area at the beginning of irradiation. Chloroplasts (1–3) that showed the accumulation movement exhibited a transient increase in speed. (Inset) Cp-actin localization as determined by the difference in fluorescence intensity between the front and rear half of the chloroplast was plotted before and after microbeam irradiation. Biased relocalization of cp-actin filaments after microbeam irradiation was only evident for chloroplasts showing accumulation movement. (D) Correlation between biased localization of cp-actin filaments and chloroplast speed during accumulation movement.
Fig. S3. Abundant cytoplasmic actin cables before and after high-intensity blue microbeam irradiation, as revealed by measuring GFP-talin fluorescence intensity. Fluorescence intensity of a spot (5 μm in diameter) located in, to the left, or to the right of the microbeam area was measured in WT, phot1phot2, and chup1 mutants. The spots did not include chloroplasts during this period. Cells were irradiated with a microbeam (10 μm in width) of 377 μmol m⁻² s⁻¹ blue light. No apparent change in the abundance of cytoplasmic actin cables associated with blue microbeam irradiation was detected.
Fig. S4. Chloroplast motility under high- and low-intensity blue light irradiation. Cells were continuously irradiated with a 30-μm-wide microbeam of high-(A) or low-intensity (B) blue light (377 and 3.8 μmol m⁻² s⁻¹, respectively). Movement paths of chloroplasts located inside or outside the microbeam area, but not demonstrating avoidance or accumulation movement, were acquired either every 5 min (high-intensity light condition) or every 10 min (low-intensity light condition) for a total of 60 min. Chloroplasts displayed movement in random directions. Motility was increased under high-intensity light but decreased under low-intensity light.
Fig. S5. Correlation between biased cp-actin filaments and chloroplast movement. Biased localization of cp-actin filaments (difference in fluorescence intensity between the front and rear half of the chloroplast) and regulation of chloroplast speed under high-intensity blue light (377 μmol m⁻² s⁻¹) did not occur in chup1 (blue symbols) and phot1phot2 (red symbols) mutant. Data obtained for WT (green symbols) were the same as in Fig. 1G. Note that chup1 chloroplasts showed high motility regardless of the small difference in fluorescence between the front and rear of the chloroplast because no cp-actin filaments are present. An apparently large difference in fluorescence in chup1 (blue triangles in the plot) was caused by the association of cytoplasmic thick actin cables to one side of the chloroplasts. At least 3 cells were examined for each mutant and are indicated by different symbols.
Movie S1. This movie shows the dynamics of cp-actin filaments on stationary chloroplasts. Images were recorded every 3 s with intermittent excitation light and show rapid turnover of cp-actin filaments that are located mostly at the chloroplast periphery.
**Movie S2.** This movie shows the dynamics of cp-actin filaments on stationary chloroplasts in transgenic Arabidopsis plants expressing tdTomato-fimbrin. Images were recorded every 3 s with intermittent excitation light. Note that the actin dynamics are similar to those observed in GFP-talin transgenics (Movie S1).
Movie S3. This movie shows reorganization of cp-actin filaments during chloroplast avoidance movement that is induced by microbeam irradiation with high-intensity blue light (377 μmol m⁻² s⁻¹). Note the biased localization of cp-actin filaments to the front of moving chloroplasts. Chloroplasts in the microbeam area show various delays before demonstrating avoidance movement. While in the microbeam area, the cp-actin filaments on the chloroplasts disappeared. The analytical data obtained from this movie are presented in Fig. 1. Green shows GFP-talin fluorescence and red shows chlorophyll fluorescence in the chloroplast. The area irradiated with the microbeam is indicated by a blue color.
Movie S4. This movie shows the dynamics of cp-actin filaments during chloroplast avoidance movement. The initial and final positions of each chloroplast during the movement are indicated at the beginning of the movie. Images were recorded every 3 s under continuous blue excitation light. Transient disappearance of cp-actin filaments and their biased relocalization to the front of the moving chloroplast are evident. Note that chloroplasts move independently of cytoplasmic actin cables.

Movie S4 (MOV)
Movie S5. This movie shows the dynamics of cp-actin filaments during chloroplast avoidance movement. Images were recorded every 3 s under continuous blue excitation light. This movie was analyzed in Fig. 1 E and F. Cp-actin filaments are shown as black lines using an inverse look-up table.

Movie S5 (AVI).
Movie S6. This movie shows microtubule dynamics during chloroplast avoidance movement induced by microbeam irradiation with high-intensity blue light (377 μmol m⁻² s⁻¹). No change in microtubule organization that could be responsible for chloroplast movement was detected. Cytoplasmic streaming, as detected by the movement of small vesicles, was not affected by microbeam irradiation, indicating that directional chloroplast movement occurs independent of cytoplasmic streaming.

Movie S6 (MOV)
Movie S7. This movie shows the normal chloroplast avoidance response and reorganization of cp-actin filaments in the arp3 mutant (dis1-1). The avoidance response was induced by continuous irradiation with a blue light microbeam (10 μm in width) of 377 μmol m$^{-2}$ s$^{-1}$.

Movie S7 (AVI).
Movie S8. This movie shows the increased motility of chloroplasts that have lost cp-actin filaments. When a wide microbeam (30 μm in width) of high-intensity blue light (377 μmol m⁻² s⁻¹) was applied, chloroplasts in the beam area remained within this area for extended periods of time. During this period, cp-actin filaments disappeared and chloroplasts showed increased motility in random directions. Analyses of this movie are presented in Fig. 2 A–C and Fig. S4A.
Movie S9. This movie shows the lack of cp-actin filaments and the increased motility of chup1 chloroplasts. Cp-actin filaments are absent, and chloroplasts show surprisingly rapid movement in the chup1 mutant, suggesting that they are detached from the plasma membrane and move by cytoplasmic streaming. Chloroplasts in the chup1 mutant do not respond to microbeam irradiation (377 μmol m⁻² s⁻¹).

Movie S9 (MOV)
Movie S10. This movie shows chloroplast photorelocation in a protoplast of mesophyll cells. The avoidance response was induced by continuous irradiation with a blue light microbeam (10 μm in diameter at the beginning and then 25 μm after the first flash of light, 98.5 μmol m⁻² s⁻¹). The microbeam was switched off after the second flash of light to induce the accumulation response. Photographs were taken every 1 min under red light.
Movie S11. Three-dimensional distribution patterns of cp-actin filaments are shown as rotating images. Shown are nonbiased cp-actin filaments. Note that cp-actin filaments are localized to one side of the chloroplasts, the side facing plasma membrane.
Movie S12. Three-dimensional distribution patterns of cp-actin filaments are shown as rotating images. Shown are biased cp-actin filaments. Note that cp-actin filaments are localized to one side of the chloroplasts, the side facing plasma membrane.

Movie S12 (AVI)
Movie S13. Confocal image of disappearance of cp-actin filaments. This movie is composed of the same photographs used to prepare Fig. 4B and D-b1, D-b2, E-b1, and E-b2. Disappearance of cp-actin filaments was induced by a laser beam of 488 nm at 1 mW used for GFP image acquisition. A part of a protoplast was irradiated for a short period (~1 min) with a continuous laser beam, and chloroplasts at the nonirradiated area were then observed. Cp-actin filaments were clearly evident on the chloroplast peripheral region farthest from the irradiated region, followed by its gradual disappearance. Photographs were acquired every 0.5 s. Chlorophyll autofluorescence was observed at 532 nm and 0.075 mW.

Movie S13 (AVI)
Movie S14. Confocal images of disappearance and reappearance of cp-actin filaments. This movie is composed of the same photographs used to prepare Fig. 4 C and D-c1, E-c1, and E-c2. Dynamics of cp-actin filaments were induced by the 488-nm, 0.4-mW laser beam used for GFP image acquisition. Cp-actin filaments were observed upon initiation of laser beam irradiation, disappeared within 1 min, and then reappeared in the same area after 30 s to 1 min. Chlorophyll autofluorescence was observed at 532 nm and 0.075 mW.

Movie S14 (AVI)