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-intensity (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 ($377 \mu$mol 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 S2 (MOV)
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 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 S7.** This movie shows the normal chloroplast avoidance response and reorganization of cp-actin filaments in the \( \text{arp}3 \) mutant (\( \text{dis}1-1 \)). The avoidance response was induced by continuous irradiation with a blue light microbeam (10 \( \mu \text{m} \) in width) of 377 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).
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 S10 (MOV)
**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 S11 (AVI)**
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 S13. Confocal image of disappearance of cp-actin filaments. This movie is composed of the same photographs used to prepare Fig. 4 B 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 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.