Chloroplast-actin filaments decide the direction of chloroplast avoidance movement under strong light in Arabidopsis thaliana

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Title Page

Title: Chloroplast-actin filaments decide the direction of chloroplast avoidance movement under strong light in *Arabidopsis thaliana*

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Abstract (less than 300 words)

Chloroplast-actin (cp-actin) filaments are crucial for light-induced chloroplast movement, and appear in the front region of moving chloroplasts when visualized using GFP-mouse Talin. They are short and thick, exist between a chloroplast and the plasma membrane, and move actively and rapidly compared to cytoplasmic long actin filaments that run through a cell. The average period during which a cp-actin filament was observed at the same position was less than 0.5 sec. The average lengths of the cp-actin filaments calculated from those at the front region of the moving chloroplast and those around the chloroplast periphery after stopping the movement were almost the same, approximately 0.8 μm. Each cp-actin filament is shown as a dotted line consisting of 4-5 dots. The vector sum of cp-actin filaments in a moving chloroplast is parallel to the moving direction of the chloroplast, suggesting that the direction of chloroplast movement is regulated by the vector sum of cp-actin filaments. However, once the chloroplasts stopped moving, the vector sum of the cp-actin filaments around the chloroplast periphery was close to zero, indicating that the direction of movement was undecided. To determine the precise structure

of cp-actin filaments under electron microscopy, Arabidopsis leaves and fern *Adiantum capillus-veneris* gametophytes were frozen using a high-pressure freezer, and observed under electron microscopy. However, no bundled microfilaments were found, suggesting that the cp-actin filaments were unstable even under high-pressure freezing.
Key words: actin filament, avoidance response, chloroplast movement, GFP-mTalin, high pressure freezing

Introduction

Chloroplasts move to a brighter area in a cell under low light conditions to enhance photosynthesis (accumulation response) (Gotoh et al. 2018) but move away from strong light to avoid photo-damage to the chloroplasts (avoidance response) (Kasahara et al. 2002). For movement, an actin-binding protein, CHLOROPLAST UNUSUAL POSITIONING 1 (CHUP1) on the chloroplast outer membrane (Kong et al. in press; Oikawa et al. 2003; 2008; Schmidt von Braun and Schleiff 2008) and chloroplast actin (abbreviated as cp-actin) filaments (Kadota et al. 2009; Kong et al. 2013; Tsuboi and Wada 2012b) at the front region of the moving chloroplasts are crucial. CHUP1 is also involved in chloroplast anchorage to the plasma membrane (Oikawa et al. 2008). The velocity of chloroplast movement was correlated with the difference in the number of cp-actin filaments visualized by GFP-mouse Talin (GFP-mTalin) fluorescence in the front and rear parts of the chloroplast (Kadota et al. 2009; Kong et al. 2013). The difference in cp-actin filament amounts was enhanced by the depolymerization of cp-actin filaments irradiated with strong blue light, but not with weak light (Kong et al. 2013); thus, the speed of the avoidance response was proportional to the light intensity (Kagawa and Wada 2004; Kadota et al. 2009; Tsuboi and Wada 2011; Higa and Wada 2016), but the speed of the accumulation response was almost constant under different light intensities (Kagawa and Wada 1996). All experiments on cp-actin filaments in live cells were performed using GFP-mTalin transgenic plants of *A. thaliana*. The importance of cp-actin filaments in chloroplast movement is evident, and cp-actin filaments are almost parallel to the direction of chloroplast movement in both accumulation and avoidance responses, as observed in the chemically fixed gametophyte cells of the fern *A. capillus-veneris* (Tsuboi and Wada 2012b). However, the mechanism by which the motive force of chloroplast movement can be generated by cp-actin filaments is unknown. To clarify this mechanism, precise analyses of the behavior of cp-actin filaments in live cells and their structural details using electron microscopy are required. Here, the cp-actin filament behavior was analyzed using movie flames acquired by high-speed photography at eight frames per second.

Methods

Plant materials

A. thaliana cultivar Columbia wild-type and its transgenic plants expressing GFP-mTalin that binds to and visualizes actin filaments were cultivated on an agar plate of white's basal salts mixture (Nacalai tesque, Inc.) under 100 μmol m^{-2} s^{-1} fluorescent white light (16 h)/dark (8 h) cycles at 23 ºC for 3-4 weeks. Spores of *A. capillus-veneris* were sterilized with a 1/10 strength solution of sodium hypochlorite

(Nacalai tesque, Inc.) and then, sown on the surface of 0.5% agar (Nacalai tesque, Inc.) medium of White's basal salts mixture (Nacalai tesque, Inc.), incubated for one day in the dark and then cultivated under continuous white light (6 μmol m^{-2} s^{-1}) for several weeks. The resulting gametophytes were used in this study.

For electron microscopy, the epidermis of the abaxial side of the *A. thaliana* leaf of the gl1 strain was peeled off and a small piece of the leaf was placed on a brass specimen holder (PELCO® freezer hat Type A, Prod#39200, Ted Pella, Inc) with 0.1 M sucrose solution, and chloroplast avoidance movement was induced by strong blue light irradiation for 3 min with a LED panel (ISL-150X150- RHB (CCS Inc.) at 600 μmol m^{-2} s^{-1} measured by LI-250A light meter (LI-Cor) just before high-pressure freezing. The light-treated samples, still immersed in 0.1 M sucrose in the cavity (depth 0.1 mm) of the brass specimen holder, were sandwiched with a second brass specimen holder having no cavity (PELCO® freezer hat Type B, Prod#39201, Ted Pella, Inc) and the sandwiches were immediately cryo-fixed. For *A. capillus-veneris* gametophytes, the procedures for blue light irradiation and high-pressure freezing were almost the same as those described above.

For fluorescence microscopy, *A. thaliana* leaves were cut and evacuated in distilled water using a syringe to remove air from the air space between mesophyll cells. The leaflet was placed on glass slides, filled with distilled water and covered with a cover slip supported with thin and narrow glasses on both sides of the cover slip to make some space thicker than the leaf thickness. The prepared glass slides were sealed with paraffin to prevent tissue dryness during the microscopic observation. These procedures do not affect the sensitivity of chloroplast movement or the behavior of cp-actin filaments.

Fluorescence microscopy

Chloroplast accumulation on the periclinal wall of the uppermost palisade cells, which was induced under the growth condition, and the existence of cp-actin filaments visualized with GFP-mTalin on the chloroplasts were confirmed under a confocal laser-scanning fluorescence microscope Nikon A1. Then, a small rectangular area (36.0 μm x 3.6 μm) of the palisade cell 2-4 μm apart from a chloroplast was irradiated with strong blue light (478.2 nm, Lasor Power 3.0) continuously to induce chloroplast avoidance response. When the chloroplast was detected to move towards the opposite direction from the irradiated area, chlorophyll fluorescence and GFP fluorescence of the cp-actin filaments of the moving chloroplasts were simultaneously recorded under the same strong blue laser light above for several minutes. Each movie frame was constructed by accumulated successive 4 images acquired every 0.25 sec, namely, the first frame was constructed from the first to the 4th images, the second frame from the 2nd to the 5th images, and so on. This procedure, called 'moving accumulation' (or 'walking accumulation'), makes each frame clear and smooth. About ten to twenty cp-actin filaments appeared in the front region of moving chloroplasts (Figures 1a-1c, Supplemental Movies 1-3). Time-lapse images are shown with false-color indicating GFP (green) and chlorophyll (red) fluorescence.

Measurement of cp-actin filament length and its direction

The series of movie frames (8 frames per sec) were projected on a monitor screen as large as possible and the fluorescence of GFP-mTalin binding to a cp-actin filament in several sequential frames were observed repeatedly by changing the frames back and forth, rapidly or slowly to identify the length of the cp-actin filament and its period of life time staying at the same position, then the figures of the cp-actin filaments were traced on a transparent sheet fixed on the monitor screen to measure the length and its direction against the direction of the moving chloroplast.

High-pressure freezing, freeze substitution and resin embedding

Cryofixation of plant tissues was performed using a high-pressure freezer (EM HPM100, Leica Microsystems). The frozen samples were transferred to liquid nitrogen and kept in it until use. Freeze substitution and resin embedding of frozen tissues were performed according to the method described in Murata et al. (2002), with slight modifications. Briefly, the frozen samples were freeze-substituted with 2% (w/v) OsO_4 (Heraeus Chemicals) in acetone (Nacalai tesque, Inc.) at -80°C (more than 2 days), -20°C (1 day), and 4°C (overnight). After samples were rinsed with acetone at 4°C (2 h), they were treated with 5% uranyl acetate (Mitsuwa Chemicals Co., Ltd.) in methanol at 4°C (2 h). The samples were washed with acetone at 4°C, and gradually warmed to room temperature (2-3 h). Samples were removed from the brass specimen holder in acetone solution. They were infiltrated with a graded series of Spurr's resin (Polysciences, Inc.) and then polymerized at 70°C.

Electron microscopy

Serial sections of 2 μm thickness of the tissue were stuck on glass slides, stained with 0.05% toluidine blue (MERK) in 0.1M phosphate buffer (pH7.4) and observed under a light microscope (Figure 3d, Supplemental Figure 1a, b) to predict the possible sites where cp-actin filaments existed, namely between chloroplasts and the plasma membrane under periclinal cell wall, especially the chloroplast positioned close to sidewall of the cell to where chloroplasts move (Kagawa and Wada 1996; Tsuboi and Wada 2012a). When a proper section was found, it was embedded again by putting a resin-filled capsule on the section on a glass slide (Imaichi et al. 2018). Serial sections of 80 nm thickness were cut and stained with saturated uranyl acetate aqueous solution (MERK) for 10 min and Reynolds' lead citrate solution (Reynolds 1963) arranged with lead nitrate (TAAB), sodium citrate (Wako pure chemical), and 1N NaOH (Wako pure chemical) for 10 min, and the predicted positions were observed using electron microscopy (JEOL JEM-1400plus).

Results

Cp-actin filaments fluctuated very actively and rapidly; therefore, most of the cp-actin filaments found in one frame could not be detected at the same position in the next frame taken at 0.125 sec intervals (8 frames per second). New cp-actin filaments suddenly appeared, fragmented, disappeared, or rearranged rapidly (Supplemental Movies 1-3). Cp-actin filaments are involved in chloroplast movement, although the precise mechanisms are not known. Hence, the relationship between the directions of cp-actin

filaments and the directions of moving chloroplasts were investigated (Figures 2a, 2b). Although the direction of each filament was random and not parallel to the moving direction of the chloroplast, the sum of the vectors of each cp-actin filament was parallel to the direction of the moving chloroplast (Figure 2b). When the avoidance movement of chloroplasts ceased, cp-actin filaments appeared around the chloroplast periphery (Figure 1d, Supplemental Movie 4), and the total sum of those cp-actin filaments became zero (Figures 2c, 2d), strongly suggesting the involvement of the direction of cp-actin filaments in the direction of chloroplast movement. However, the average period of each filament remaining at the same position (i.e., the cp-actin filament lifespan) was very short (Figure 3a). On the other hand, quite frequently cp-actin filaments looked as straight and clear short lines with a length of about 0.8 μm (Figure 3b). This might have an important meaning, as the unit length is involved in its function. The thickness of the filaments (predicted from the strength of the GFP fluorescence) varied from thin to thick. The thin filament might be composed of a single cp-actin filament, but the thick filament might be bundles made of several cp-actin filaments. Each cp-actin filament appeared to be composed of 4 to 5 dots (Figure 3c).

The involvement of cp-actin filaments in chloroplast movement is unquestionable, as shown by the above data. However, the precise structure of cp-actin filaments should be determined at the electron microscopy level to clarify the mechanism by which chloroplasts can be induced and the motive force for this is generated. We first tried to determine whether chemical fixation was available to keep the cp-actin structure intact using confocal scanning microscopy. During the observation of cp-actin filaments under confocal microscopy, a small amount of paraformaldehyde solution permeated between the cover slip and the glass slide. Instantaneously, the cp-actin filaments changed their structure and became artefactual shapes. Therefore, we applied high-pressure freezing (HPF) and freeze substitution methods, which succeeded in the quantitative analysis of plant actin filaments in preprophase bands of onion cotyledon epidermal cells (Takeuchi et al. 2016). The timing and the site of ‘cp-actin filaments’ existence are very restricted, that is, several minutes to twenty minutes at latest after the start of strong light irradiation, and the narrow space between chloroplasts and the plasma membrane, especially only in the front region of moving chloroplasts.

Careful procedures were performed repeatedly to obtain cp-actin filaments in the tissue in a plastic block. However, we could not find any cp-actin-like microfilament bundles, although we changed various technical conditions such as the fixation method, materials such as *A. thaliana* leaves and petioles with and without epidermis, wild-type and GFP-mTalin transgenic plants (Figure 3d-h), and fern gametophytes (Supplemental Figure 1). Randomly oriented single microfilaments were observed (Figure 3g), but microfilament bundles could not be found. In the present method, cortical microtubules were well preserved (Figure 3h, Supplemental Figure 1f).

Discussion

Cp-actin filaments of *A. thaliana* have been reported twice (Kadota et al. 2009; Kong et al. 2013), revealing that the biased distribution of cp-actin filaments in front and back of a chloroplast determines the

direction of chloroplast movement and its velocity as a mass effect. The higher the cp-actin distribution difference in front and back of chloroplasts, the faster the chloroplast moves. However, the function and the precise structure of each cp-actin filament have not been studied, because cp-actin filaments move very fast. In Kadota et al. (2009), cp-actin filament images by GFP-mTalin were taken every 3 sec at the fastest rate, but usually taken every several minutes. Kong et al. (2013), the images were taken as a several tens second interval. When cp-actin disappearance under strong light was observed under a total internal reflection fluorescence (TIRF) microscope, images were taken every 0.2 sec interval. Here, we acquired the images of cp-actin filaments visualized by GFP-mTalin every 0.125 sec, namely 8 flames per second to trace a single cp-actin filament during chloroplast avoiding movement. Even in this situation, tracing a single cp-actin filament behavior is not easy, meaning that cp-actin filaments move and/or turnover very rapidly.

How the motive force of cp-actin filaments is generated for chloroplast movement is not yet known, but our data (Figure 2b) indicate that the average direction of cp-actin filaments corresponds to the direction of the moving chloroplast. It suggests that cp-actin filaments generate a motive force. Similar data were obtained for *A. capillus-veneris* gametophytes (Tsuboi and Wada 2012b). Compared with our data for *A. thaliana*, the number of cp-actin filaments in *A. capillus-veneris* chloroplasts was low. Less than ten thick cp-actin filaments were shown to be roughly parallel to the direction of chloroplast movement in both accumulation and avoidance responses, although the actin filaments were visualized with Alexa 488 phalloidin in fixed materials (Tsuboi and Wada 2012b). In *A. thaliana* leaves, cp-actin filaments were destroyed quickly when the fixative (paraformaldehyde) was penetrated, suggesting the possibility that the stability of cp-actin filaments to the chemicals is different in *A. capillus-veneris* and *A. thaliana.*

The average length of cp-actin filaments of *A. thaliana* was approximately 0.8 μm (Figure 3B), but in *A. capillus-veneris* it was approximately 5 μm (Tsuboi and Wada 2012b), suggesting the possibility that the number and length of cp-actin filaments on one chloroplast are different in plant groups. The length of cp-actin filaments was calculated in *A. thaliana* leaf cells during depolymerization under strong light (Kong et al. 2013). Before depolymerization, the length of cp-actin filaments in wild-type cells was classified into three categories: 0.2 to 1.0 μm, 1.0 to 1.8 μm, and longer than 1.8 μm. Accordingly, most of the cp-actin filaments in wild-type cells were shorter than 1 μm, with an average length of 0.651 ± 0.193 μm. Even though the conditions were different in both cases, the lengths of the cp-actin filaments were quite similar. Moreover, the cp-actin filaments are considered to consisted of 0.2 μm length sub units (Kong et al. 2013). The cp-actin filaments in this study appeared to consist of 4 to 5 dots (Figure 3c), although we do not know whether a single cp-actin filament consists of several subunits.

Observation under TIRF microscopy suggested that “cp-actin filaments seemed to be composed of short filaments that were connected to each other to create longer actin filaments” (Kong et al. 2013). During our live-cell observation under confocal microscopy (Supplemental Movies 1 to 3), we did not observe severing of cp-actin filaments, probably because, in our system, strong blue light was not

illuminated directly to cp-actin filaments, although we induced avoidance responses by laser scanning next to the rectangular space where cp-actin filaments were observed.

To analyze cp-actin filaments more precisely, we performed electron microscopy observation of them (Figure 3d-h, Supplemental Figure 1). Although microfilament bundles were expected to be candidates for cp-actin filaments, we did not find any such bundles. Possible explanations are as follows: (1) we have not come across the structure because of timing and spatial problems, although the structure is well preserved; (2) the HPF causes the destruction of the cp-actin filaments; and (3) the cp-actin filaments become undetectable because of artifacts induced during the processes of freeze substitution and/or resin embedding, although they are well preserved by the HPF.

Because cryo-fixation can eliminate many artifactual damages that occur by chemical fixation methods, several approaches to electron microscopy studies of plant cytoskeletons using cryo-freezing methods in combination with freeze substitution have been developed. Configurations of plant microfilaments that reflect known plant actin structures have been reported using several cryo-freezing methods such as plunge freezing (Tiwari et al. 1984; Tiwari and Polito 1988; Lancelle et al. 1986, 1987; Tang et al. 1989; Lancelle and Hepler 1991, 1992), slam freezing (Kakimoto and Shibaoka 1988), propane-jet freezing (Ding et al. 1991a, c), and high-pressure freezing (HPF) (Lichtscheidl et al. 1990; Ding et al. 1991b, 1992; Murata et al. 2002; Kanzawa et al. 2006; Takeuchi et al. 2016). Among the various freezing methods, HPF is the most suitable freezing method for our purposes because of the thickness of the samples (Dahl and Staehelin 1989). The freeze-substitution method used in this study has been reported to be useful for the study of cortical microtubule and microfilament behavior in tobacco root tips and onion cotyledon epidermis (Murata et al. 2002; Takeuchi et al. 2016). In the present study, we observed cortical microtubules (Figure 3h, Supplemental Figure 1f) and microfilaments (Figure 3g), suggesting that this method is suitable for observation of cp-actin filaments. HPF is better than other freezing methods for the preservation of single microfilaments, but it has been reported that microfilament bundles are often disturbed under HPF (Ding et al. 1992). Microfilament bundles have been reported in some cells by HPF (tobacco leaf and root tips in Ding et al. 1991b, 1992, motor cells of mimosa leaf in Kanzawa et al. 2006), suggesting that these actin bundles are relatively stable, and bundled microfilaments remain by HPF. Thus, cp-actin filaments may be labile. HPF and/or subsequent specimen preparation procedures may affect labile cp-actin filaments, and bundled microfilaments could not be observed. There is a possibility that some randomly oriented single microfilaments (Figure 3g) are remnants of deformed cp-actin filaments.

Figure legend

Figure 1. (a-c) Cp-actin filaments during the avoidance movement of chloroplast 1-3 in palisade cells of *A. thaliana* leaves. Movie files of these chloroplasts (Supplemental Movies 1-3, respectively) show the typical behavior of chloroplast actin (cp-actin) filaments during the upward chloroplast avoidance response. In the avoidance response, cp-actin filaments were localized at the front region of the moving chloroplast but not in the rear part. Some long and thin cytoplasmic actin filaments, thinner than the cp-

actin filaments, were clearly observed along the plasma membrane outside the chloroplast localization area. The center of the chloroplast frequently lacks thylakoids, so that it looks as if vacuolated. Note: in (b), cp-actin filaments are usually seen at the chloroplast periphery, but in this case, many cp-actin filaments were observed even at the central area of the moving chloroplast. Note in (c), long and thin cytoplasmic actin filaments could be seen between the chloroplast and the plasma membrane. (d) Cp-actin filaments of the chloroplast remaining at the same location after the avoidance response. Cp-actin filaments appeared in the entire area of the plasma membrane side of the chloroplast and fluctuated rapidly, although the chloroplasts remained at the same position. Movie file of this chloroplast is provided in Supplemental Movie 4. Other details are the same as in Figure 1a-1c). Green: actin filaments visualized with GFP-mouse talin (GFP-mTalin). Red: chloroplasts with chlorophyll autofluorescence. Bars are 1 μm. The time (second) of image acquisition is shown in the upper left corner of each image.

Figure 2. Direction of cp-actin filaments during or after avoidance response in palisade cells of *A. thaliana* leaves. (a) Microscopic image of the last frame of the movie of chloroplast 2 and traces of cp-actin filaments of the chloroplast during the avoidance movement. Dotted line: chloroplast position in the first frame of the movie. Blue lines: all of cp-actin filaments observed in movie. White arrow: moving direction of the chloroplast. The other details are the same as those shown in Figure 1. (b) Histogram of the directional frequency of cp-actin filaments in relation to the direction of avoiding chloroplasts observed in chloroplast 2 (Figure 1b) and 3 (Figure 1c). Schematic drawing: red line shows the edge of chloroplast, black arrow shows the direction of moving chloroplast, and green line shows the chloroplast actin filament. Cleary detected cp-actin filaments were traced on a monitor screen, and the angles between the direction of the cp-actin filaments and the direction of the chloroplast movement were measured from the traces. The total number of cp-actin filaments was 156. The velocity of chloroplast movement in chloroplast 2 was 1.1 μm/min, and in chloroplast 3 was 0.8μm/min. (c) Microscopic image of the last frame of the movie of chloroplast 4 and the traces of cp-actin filaments in the remaining chloroplast. The white arrow shows the direction of the movement of the chloroplast before stopping. Other details are the same as those shown in Figure 2a. (d) Histogram of the directional frequency of the cp-actin filaments of chloroplast 4 after the avoidance response was stopped. The total number of cp-actin filaments was 170. Other details are the same as those shown in Figure 2b.

Figure 3. (a) Histogram of the life span of cp-actin filament in palisade cells of *A. thaliana* leaves. Life span was calculated as the duration for which a cp-actin filament was observed in the same area. Thirty-two cp-actin filaments were observed. The cp-actin life span was mostly less than 1 second. (b) Histogram of cp-actin filament length during and after the avoidance response obtained from 72 cp-actin filaments in chloroplasts 2, 84 in chloroplast 3, and 170 in chloroplast 4, respectively. The average length in each chloroplast was 0.80 ± 0.12, 0.82 ± 0.11, 0.76 ± 0.14, respectively. Letters indicate statistically significant differences ($p < 0.05$) determined by one-way analysis of variance (ANOVA), followed by Tukey's test. (c) Microscopic image showing cp-actin filaments composed of small dots. The

photograph is the first frame of the movie of the chloroplast 4. A group of 3-5 white arrows indicates the dotted structure of each cp-actin filament. (d-h) Ultrastructural analysis of light-induced chloroplast movements in *A. thaliana* leaves by high-pressure freezing. (d) A light micrograph of a 2 μm-thick section stained with toluidine blue. The region encircled by a dotted white rectangle contains cytoplasm showing chloroplasts near the plasma membrane, where cp-actin filaments should be localized. The sections were embedded again and 80 nm-thick serial sections were cut for electron microscopy. (e) Electron micrographs of an ultrathin section of the region indicated by the white rectangle in (d). (f and g) Higher magnification pictures showing the rectangular areas in (e) and (f), respectively. Single microfilaments (arrow) are seen in the cytoplasm between the cell wall and the chloroplast in (g). (h) Electron micrograph of an *A. thaliana* leaf cell showing the cortical microtubules (arrowhead). Bars in (d), (e), (f), (g), and (h) indicate 50 μm, 5 μm, 500 nm, 100 nm, and 200 nm, respectively.

Supplemental Figure 1. Ultrastructural analysis of light-induced chloroplast movements in gametophytes of *A. capillus-veneris*. Chloroplast movement in *A. capillus-veneris* gametophytes was induced by strong blue light irradiation for 10 min. (a and b) Light micrographs of 2 μm-thick section stained with toluidine blue. (b) High-magnification image of the region indicated by the arrow in (a). The region indicated by arrows contains the cytoplasm between the chloroplasts and the plasma membrane, where cp-actin filaments may be localized. The section was embedded again, and 80 nm-thick serial sections were cut for electron microscopy. (c) Electron micrograph of the region indicated by the white arrow in (b). (d and e) High-magnification images showing the rectangular areas in (c) and (d). Microfilament-like structures were not observed in the cytoplasm between the cell wall and the chloroplast. (f) Electron micrograph of an *A. capillus-veneris* gametophyte cell showing the cortical microtubules (arrowheads). Bars in (a), (b), (c), (d), (e), and (f) indicate 100 μm, 10 μm, 2 μm, 500 nm, 100 nm, and 200 nm, respectively.

Supplemental movie1
Supplemental movie2
Supplemental movie3
Supplemental movie4

Headings: Chloroplast actin filaments

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Author contribution

MW conceived the study, MW and YM designed the study, MW, TH and KK performed the live imaging experiments, MW, TH, YM, TN, NM, SS, YN and AM performed the electron microscopy experiments, MW and YM analyzed the data, MW and YM wrote the manuscript.

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436
437

Fig. 1

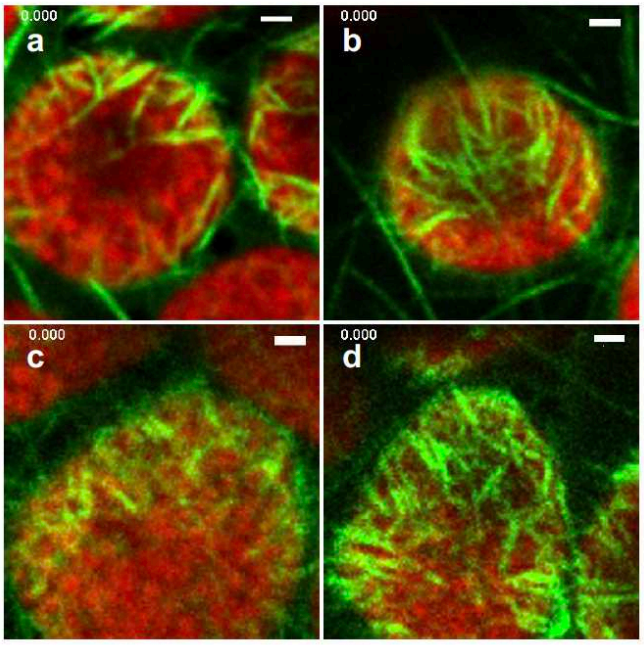

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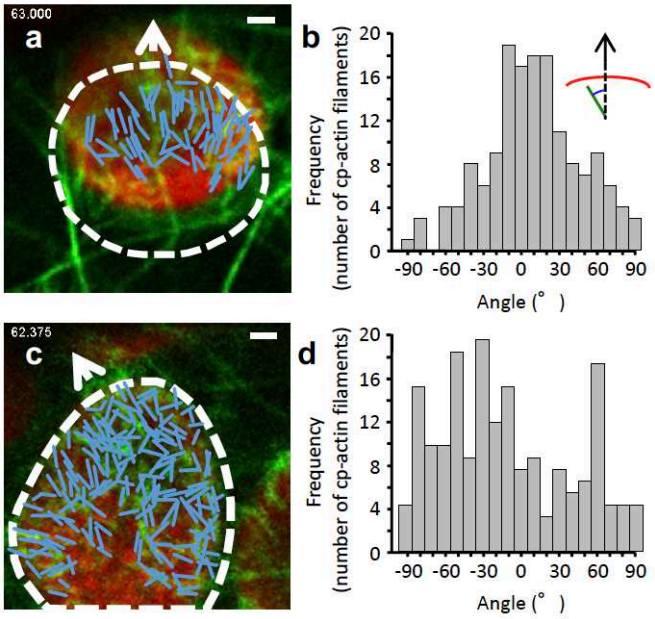
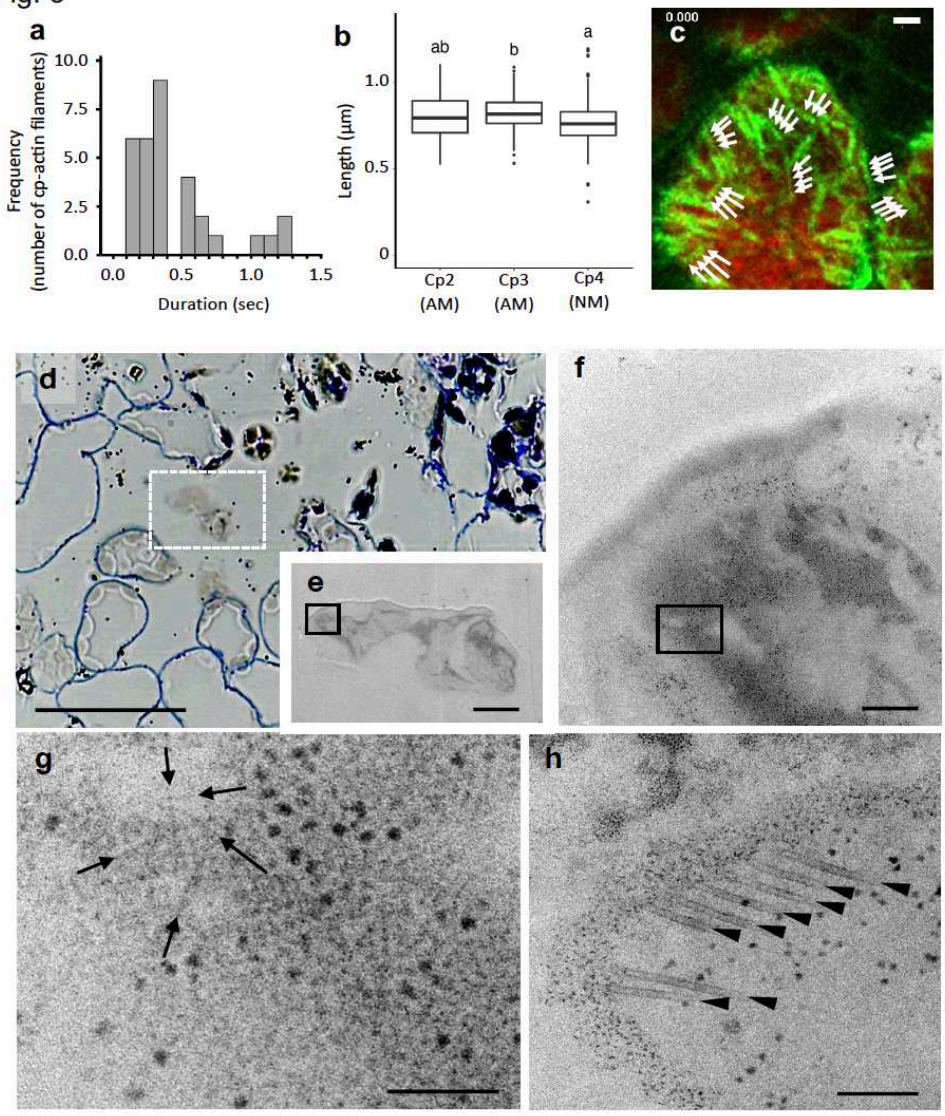
Fig. 2


63.000
a
b
Frequency
(number of cp-actin filaments)
20
16
12
8
4
0
-90 -60 -30 0 30 60 90
Angle (°)
62.375
c
d
Frequency
(number of cp-actin filaments)
20
16
12
8
4
0
-90 -60 -30 0 30 60 90
Angle (°)

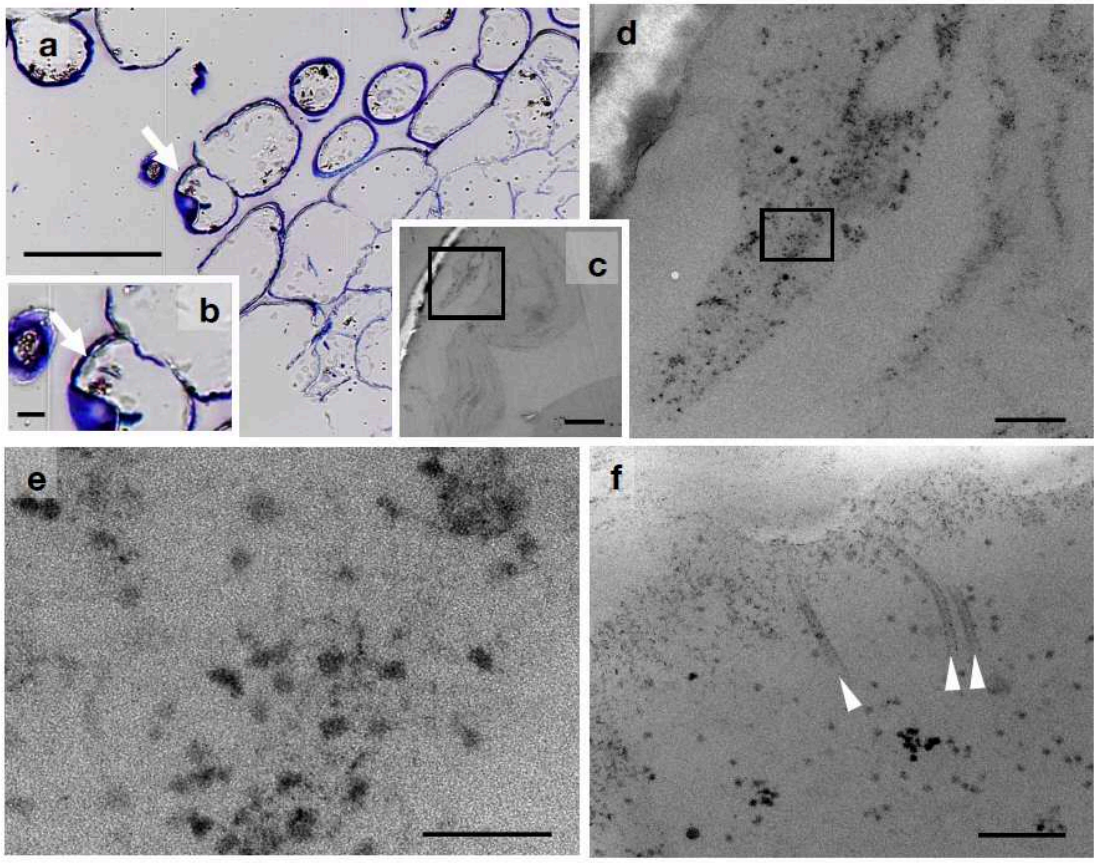
440
441

Fig. 3


a
Frequency (number of cp-actin filaments)
10.0
7.5
5.0
2.5
0.0
0.0
0.5
1.0
1.5
Duration (sec)
b
Length (µm)
1.0
0.5
0
ab
b
a
Cp2 (AM)
Cp3 (AM)
Cp4 (NM)
c
0.000
d
e
f
g
h

442

Fig. S1



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