PLANT SIGNALING & BEHAVIOR
2017, VOL. 12, NO. 10, e1370163 (5 pages)
https://doi.org/10.1080/15592324.2017.1370163

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Roles of actin cytoskeleton for regulation of chloroplast anchoring

Yuuki Sakai[†] and Shingo Takagi

Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka, Japan

ABSTRACT

Chloroplasts are known to maintain specific intracellular distribution patterns under specific environmental conditions, enabling the optimal performance of photosynthesis. To this end, chloroplasts are anchored in the cortical cytoplasm. In leaf epidermal cells of aquatic monocot *Vallisneria*, we recently demonstrated that the anchored chloroplasts are rapidly de-anchored upon irradiation with high-intensity blue light and that the process is probably mediated by the blue-light receptor phototropins. Chloroplast de-anchoring is a necessary step rendering the previously anchored chloroplasts mobile to allow their migration. In this article, based on the results obtained in *Vallisneria* together with those in other plant species, we briefly discussed possible modes of regulation of chloroplast anchoring and de-anchoring by actin cytoskeleton. The topics include roles of photoreceptor systems, actin-filament-dependent and -independent chloroplast anchoring, and independence of chloroplast de-anchoring from actomyosin and microtubule systems.

ARTICLE HISTORY
Received 1 August 2017
Accepted 16 August 2017

KEYWORDS
Actin cytoskeleton; chloroplast anchoring; light-induced chloroplast redistribution; *Vallisneria*

Light-induced chloroplast redistribution in *Vallisneria* epidermal cells

The distribution pattern of chloroplasts in photosynthesizing plant cells is under a precise control of environment. Light is one of the most important environmental factors for plant life. Chloroplasts take different distribution patterns under different light conditions to maintain optimal photosynthesis at all times. In general, chloroplasts accumulate into the irradiated region with low-intensity light (accumulation response), while they escape from the irradiated region with high-intensity light (avoidance response).[1,2] It is experimentally demonstrated that the accumulation response enhances photosynthesis under low-intensity light,[1] while the avoidance response prevents photodamages caused by high-intensity light.[3]

The light-induced chloroplast redistribution is observed widely in the plant kingdom, from algae to seed plants, including a submerged aquatic monocot *Vallisneria* (Alismatales Hydrocharitaceae). *Vallisneria* lives in fresh-water rivers and lakes in the subtropical and temperate zones. Leaves of *Vallisneria* have single layer of rectangular parallelepiped-shaped epidermal cells, which atypically harbor mature chloroplasts rather than plastids incapable of photosynthesis, providing an excellent experimental system for light microscopic studies of the chloroplast movement.[4,5] Chloroplasts in the epidermal cells accumulate into the outer periclinal cytoplasm under low-intensity light, whereas they rapidly migrate to the anticlinal cytoplasm upon exposure to high-intensity light. In contrast to most terrestrial plants, in which both responses are induced exclusively by blue light, the accumulation response is induced most effectively by red light, whereas the avoidance response is induced specifically by blue light.[6] Since light-induced chloroplast redistribution in *Vallisneria* is accompanied with dynamic changes in the configuration of actin filaments,[7-10] we have attempted to dissect initial processes of chloroplast redistribution focusing on the roles of actin cytoskeleton.

Immobilization of chloroplasts under low-intensity light

In *Vallisneria* epidermal cells, the distribution pattern of chloroplasts in darkness is determined depending on the light condition immediately before dark treatment.[6] When cells are dark-adapted after exposure to high-intensity white light, which fully induced the avoidance of chloroplasts to the anticlinal cytoplasm, only a small number of chloroplasts are located on the outer periclinal cytoplasm after dark treatment. Those chloroplasts exhibit fine, randomly oriented movement. Long, thin bundles of actin filaments form a loose network over the outer periclinal cytoplasm, apparently not contacting with each chloroplast.[7] The random movement of chloroplasts is accelerated by irradiation with low-intensity red light within a few minutes, producing increased numbers of chloroplasts that migrate between the outer periclinal cytoplasm and the anticlinal cytoplasm.[11] These effects are red/far-red light reversible, probably mediated by phytochromes, which regulate the cytoplasmic motility in these cells.[12] The observations suggest that chloroplasts in *Vallisneria* epidermal cells move only passively; the motile cytoplasmic matrix drives the movement of chloroplasts. This was already pointed out a century ago by Senn,[13] who did pioneering studies on chloroplast movement in a wide variety of plant species, in his famous book "*Die Gestalts- und Lageveränderung der Pflanzen-Chromatophoren* (The Changes in Shape and Position of Plant Chloroplasts)".

CONTACT Yuuki Sakai yuukis0110@gmail.com Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, 113-0033, Japan.
[†]Present address: Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

In 30–60 min of exposure to low-intensity red light, chloroplasts on the outer periclinal cytoplasm lose their motility depending on photosynthesis.[7] Concomitantly, the configuration of actin filaments on the outer periclinal cytoplasm changed into a conspicuous honeycomb array, surrounding each chloroplast.[7,8] Since the accelerated migration of chloroplasts from the anticlinal to outer periclinal cytoplasm continues, the number of chloroplasts resided on the outer periclinal cytoplasm increases.[11] This mode of accumulation response is totally different from that reported in other plant species including the moss *Physcomitrella patens,*[14] fern *Adiantum capillus-veneris,*[15] and *Arabidopsis thaliana,*[16] in which chloroplasts exhibit more unidirectional, phototactic movements, driven by short actin filaments associated with the chloroplasts. The mode of chloroplast accumulation in *Vallisneria* is more similar to that reported in the stramenopile alga *Vaucheria sessilis,*[17,18] in which reticulated cytoplasmic fibers, most probably composed of actin filaments, trap chloroplasts in the irradiated region with low-intensity blue light.

Chloroplast anchoring induced by low-intensity light

The chloroplasts, which have lost their motility after exposure to low-intensity red light, become resistant to the centrifugal force, suggesting the occurrence of 'chloroplast anchoring'. We propose that the chloroplast anchoring is an active mechanism to maintain specific distribution patterns of chloroplasts under specific environmental conditions.[19] Chloroplasts are rendered immobile through the mechanism where they should keep their positions to achieve optimal photosynthesis. Chloroplast redistribution in response to environmental fluctuation may entail de-anchoring of previously anchored chloroplasts, migration of chloroplasts out of inappropriate regions, and re-anchoring of chloroplasts at appropriate regions. Although the molecular machinery is still elusive, the magnitude of chloroplast anchoring can be evaluated by applying a centrifugal force to living plant cells, for example, epidermal[8,9,20,22] and mesophyll cells[21] of *Vallisneria, Elodea* or *Egeria,*[22-24] and terrestrial plants[25,26] as well. Tightly anchored chloroplasts resist stronger centrifugal forces, while loosely anchored chloroplasts obey weaker centrifugal forces.

In *Vallisneria* epidermal cells, the resistance of chloroplasts to centrifugal force, provided after irradiation with low-intensity red light, was completely antagonized by treatment with the actin-depolymerizing reagent, which almost completely fragmented the actin filaments around the chloroplasts.[8] On the other hand, when *Vallisneria* epidermal cells are exposed to high-intensity blue light, chloroplasts which migrated from the outer periclinal into the anticlinal cytoplasm become resistant to the centrifugal force, and simultaneously, surrounded by thin actin bundles.[9] Both on the outer periclinal cytoplasm under low-intensity red light[7,8] and the anticlinal cytoplasm under high-intensity blue light,[9] photosynthetic inhibitors impair the normal chloroplast redistribution, the gain in resistance of chloroplasts to centrifugal force, and the reorganization of actin cytoskeleton to be tightly associated with each chloroplast. Consequently, we have concluded that photosynthesis-dependent chloroplast anchoring is the essential event for successful chloroplast redistribution induced by light, and moreover, that the actin cytoskeleton plays critical roles in its regulation. Although a possible involvement of photosynthesis in the regulation of chloroplast positioning has also been suggested in other plant species,[22,27] the mode of involvement is still obscure.[4]

General occurrence of actin-filament-dependent chloroplast anchoring

Chloroplasts are frequently associated with actin filaments.[5] Among those reports, disruption of actin cytoskeleton in mesophyll cells of *A. thaliana* by the actin-depolymerizing reagent caused aberrant aggregation of chloroplasts.[28] In living *A. thaliana* leaf cells, Kadota et al.[16] demonstrated that the amount of chloroplast-associated short actin filaments increases when chloroplasts are immobile under low-intensity blue light, while it rapidly decreases upon exposure to high-intensity blue light, which was applied to induce photorelocation movement of the chloroplasts. The dynamic behavior of chloroplast-associated short actin filaments is under the control of blue-light receptor phototropins.[16,29,30] Using mutant plants of *A. thaliana* deficient in chloroplast photorelocation movement, it was revealed that CHLOROPLAST UNUSUAL POSITIONING1 (CHUP1), together with KINESIN-LIKE PROTEIN FOR ACTIN-BASED CHLOROPLAST MOVEMENT1 (KAC1) and KAC2, plays critical roles to organize the chloroplast-associated short actin filaments.[16,30,31] CHUP1 and KACs are equipped with the actin-binding activity *in vitro,*[31,32,33] and moreover, chloroplasts could not keep normal interaction with the cortical cytoplasm without those proteins.[16,34,35]

Takamatsu and Takagi[36] developed a more direct assay system to characterize the chloroplast anchoring. When spinach mesophyll protoplasts were attached onto coverslips and then gently ruptured, the cortical cytoplasm with tightly associated chloroplasts underlying the plasma membrane was exposed on 'plasma-membrane ghosts'. Treatment of the plasma-membrane ghosts with the actin-depolymerizing reagent induces prompt detachment of chloroplasts concomitant with disruption of cortical actin cytoskeleton. These studies, together with the results obtained in *Vallisneria* described above, all suggest the general occurrence of actin-filament-dependent machinery for chloroplast anchoring in plant cells.

Actin-filament-independent chloroplast anchoring in *Vallisneria* epidermal cells

When *Vallisneria* epidermal cells are dark-adapted after exposure to low-intensity white light, which induced chloroplast accumulation into the outer periclinal cytoplasm, a large number of immobile chloroplasts are resided on the outer periclinal cytoplasm, in contrast to the dark adaptation after exposure to high-intensity light[6] (Fig. 1A). Numerous thin bundles of actin filaments are tightly associated with these chloroplasts[10] (Fig. 1B). Do these thin actin bundles contribute to the chloroplast anchoring? To answer this question, we examined the effects of an actin-depolymerizing reagent latrunculin B (LatB). The magnitude of chloroplast anchoring was evaluated, after centrifugation of living cells, as the coverage ratio (Np/Nt); the number of chloroplasts in the centripetal half of the outer

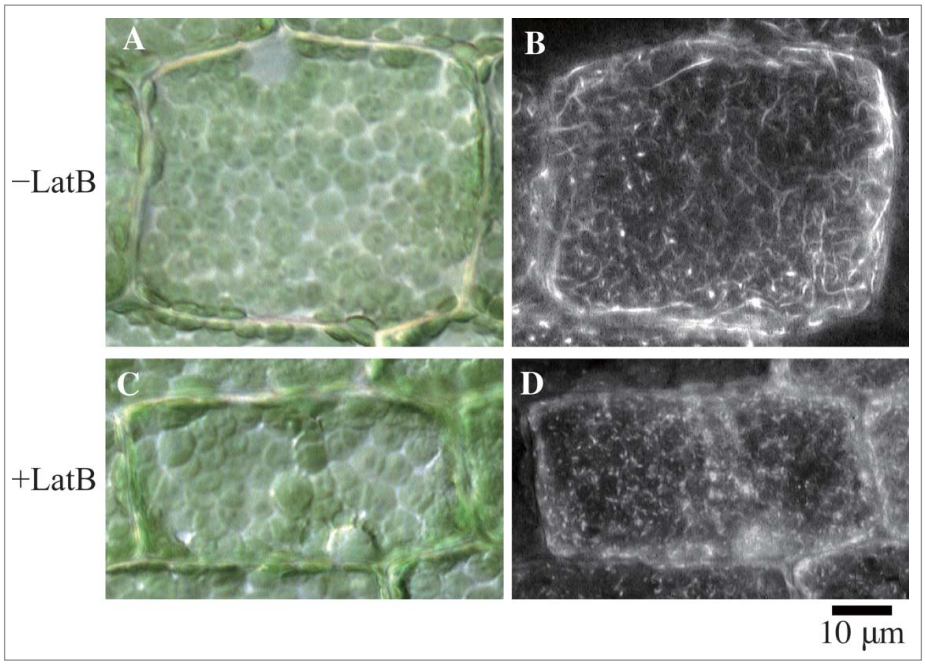

Figure 1. Effects of LatB on the distribution of chloroplasts and the configuration of actin filaments on the outer periclinal cytoplasm of dark-adapted *Vallisneria* epidermal cells. Dark-adapted leaf epidermal cells were treated with (C, D) or without (A, B) 1 μM LatB for 1 hour and stained with Alexa488-phalloidin as described in Sakai and Takagi [ref 9]. The representative fluorescence images of outer periclinal cytoplasm (B, D) are arranged with the bright-field images (A, C). Bar = 10 μm.

periclinal cytoplasm of the cell (Np) divided by the total number of chloroplasts (Nt) observed in the outer periclinal cytoplasm of the cell.[9] When chloroplasts resist the centrifugal force, the coverage ratio is kept at around 0.5, while it becomes lower than 0.5 when chloroplasts obey the centrifugal force.

In the dark-adapted cells, the coverage ratio was kept at around 0.5 at the centrifugal forces under 680 × *g*, and slightly declined from 0.5 at the centrifugal forces over 940 × *g* (Fig. 2A, Dark), indicating that these chloroplasts are strongly anchored. As described below, when the dark-adapted cells were irradiated with high-intensity blue light, the coverage ratio substantially declined even at the centrifugal force of 680 × *g* (Fig. 2A, BL), depicting the chloroplast de-anchoring. After treatment of the dark-adapted cells with 1 μM LatB for one hour, though no apparent change in the distribution pattern of chloroplasts on the outer periclinal cytoplasm could be detectable (Fig. 1C), the whole actin cytoskeleton was completely disrupted and only fragmented, very short actin bundles could be seen (Fig. 1D).

If the chloroplast anchoring depends solely on the thin actin bundles associated with each chloroplast, chloroplasts should no longer resist the centrifugal force after treatment with LatB. Unexpectedly, however, the chloroplasts in LatB-treated cells still resisted the centrifugal force even at 1160 × *g* (Fig. 2B, Dark). Thus disruption of actin cytoskeleton could not mimic the effect of blue light to induce chloroplast de-anchoring, suggesting the occurrence of actin-filament-independent chloroplast anchoring in these cells. As described in the previous section, Dong et al.[8] demonstrated that disruption of actin cytoskeleton impairs chloroplast anchoring during accumulation of chloroplasts into the outer periclinal cytoplasm under

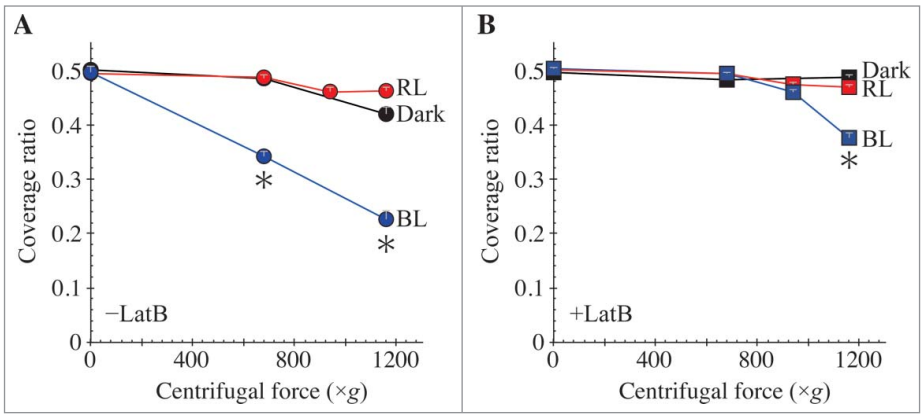

Figure 2. Effects of LatB on chloroplast anchoring and de-anchoring in *Vallisneria* epidermal cells under different light conditions. Dark-adapted cells were treated with (B) or without (A) 1 μM LatB for 1 hour, and then centrifuged before (Dark) and after irradiation with blue light (BL; 470 nm, 70 μmol m^{-2} s^{-1}) or red light (RL; 660 nm, 70 μmol m^{-2} s^{-1}) for 5 min. The coverage ratio (Np/Nt) was evaluated as described in Sakai et al.[37] and plotted against the centrifugal force. The vertical bar on each point indicates the standard error. The asterisks mean that the values were significantly different from those obtained in dark-adapted cells (black symbols) ($p < 0.01$ with Student's *t*-test). n = 50–122.

low-intensity red light. Consequently, we can assume that thin bundles of actin filaments transiently capture mobile chloroplasts through the light-dependent reorganization, and that the anchored state of chloroplasts is maintained during dark adaptation through an actin-filament-independent mechanism.

Possible involvement of actin cytoskeleton in chloroplast de-anchoring induced by high-intensity blue light

Recently, we demonstrated that high-intensity blue light specifically and rapidly induces chloroplast de-anchoring in the dark-adapted *Vallisneria* epidermal cells[37] (Fig. 2A, BL), and proposed that this 1-min-order response, most probably mediated by the blue-light-receptor phototropins, is an initial process of chloroplast avoidance response. High-intensity red light never induces such a response[37] (Fig. 2A, RL). Does the actin cytoskeleton play any roles in the blue-light-induced chloroplast de-anchoring? When the LatB-treated cells after dark adaptation were irradiated with high-intensity blue light (480 nm, 70 μmol m^{-2} s^{-1}) for 5 min, the coverage ratio was kept at around 0.5 at the centrifugal forces under 680 × *g*, declined from 0.5 at 940 × *g*, as in non-irradiated cells after LatB treatment, and to the value significantly lower than that in the non-irradiated cells at 1160 × *g* ($p < 0.01$, Fig. 2B, BL and Dark), indicative of the chloroplast de-anchoring. However, the coverage ratio in the blue-light-irradiated cells in the presence of LatB at 1160 × *g* (0.38 ± 0.01) (Fig. 2B, BL) is significantly higher than that in the blue-light-irradiated cells in the absence of LatB (0.23 ± 0.02) (Fig. 2A, BL) ($p < 0.01$). Consequently, in the LatB-treated cells, blue light can only partially induce chloroplast de-anchoring, suggesting that intact actin cytoskeleton is required to fulfill the chloroplast de-anchoring induced by high-intensity blue light.

Assuming the involvement of actin cytoskeleton, we next asked whether chloroplast de-anchoring needs the activity of actomyosin system. A general inhibitor for ATPase activity of myosins, 2,3-butanedione 2-monoxime (BDM) suppresses the light-induced accumulation response of chloroplasts in *A. thaliana*.[38] In epidermal cells of *Vallisneria*, even in the presence of 100 μM BDM, the coverage ratios before and after blue-light irradiation were similar to those in the control cells (Fig. 3, Control and +BDM), suggesting that the chloroplast de-anchoring proceeds without any motive force generated by the actomyosin system. On the other hand, chloroplast movement with the cytoplasmic streaming in *Vallisneria* is reversibly inhibited by BDM.[39] This is also seen in the case of spinach. Although BDM reversibly inhibits light-induced chloroplast movement in palisade cells (Miyawaki, personal communication), a treatment of the plasma-membrane ghosts with ATP never promotes detachment of chloroplasts from the cortical cytoplasm (Takamatsu, personal communication). These results indicated that, in the whole process of light-induced chloroplast redistribution, the chloroplast de-anchoring and chloroplast migration are separable process, while both are regulated by the actin cytoskeleton.

Finally, we ascertain a possible involvement of another cytoskeletal component microtubule in the blue-light-induced chloroplast de-anchoring. In the presence or absence of a microtubule-disrupting reagent oryzalin, there was no statistically significant difference in the coverage ratios before and after blue-light irradiation (Fig. 3, Control and +Oryz). Thus the chloroplast de-anchoring seems to be independent of microtubule cytoskeleton. This finding is consistent with that in spinach; microtubule depolymerization never induced chloroplast detachment in the plasma-membrane ghost assay.[36] As far as we know, a possible role of microtubule cytoskeleton in chloroplast positioning was suggested only in Chenopodiaceae.[40]

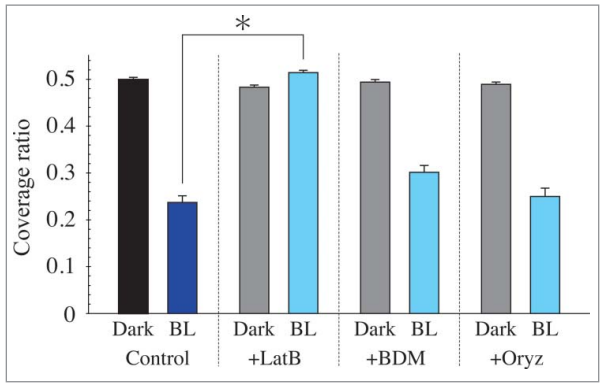

Figure 3. Effects of cytoskeletal inhibitors on the blue-light-induced chloroplast de-anchoring in *Vallisneria* epidermal cells. Dark-adapted cells were treated for 1 hour with 1 μM LatB, 100 mM BDM, or 100 μM oryzalin, respectively, and then centrifuged at 680 × *g* before (Dark) and after irradiation with blue light (BL; 470 nm, 70 μmol m^{-2} s^{-1}) for 5 min. The coverage ratio (Np/Nt) was evaluated as described in Sakai et al.[37] The vertical bar on each column indicates the standard error. The asterisk means that the value was significantly different from that obtained in control cells without inhibitor treatment ($p < 0.01$ with Student's *t*-test). n = 36–66.

Taken together, in *Vallisneria* epidermal cells, previously anchored chloroplasts are de-anchored by exposure to high-intensity blue light through a phototropin-mediated process, and re-anchored in a photosynthesis-dependent manner either in the periclinal or anticlinal cytoplasm rendered to be exposed to low-intensity light. The actin cytoskeleton is intimately involved in both processes, though the detailed mechanisms have not yet been clarified. Separately, while its characteristics have neither been totally unraveled, an actin-filament-independent mechanism functions to maintain the anchored state of chloroplasts during dark adaptation. To explore components involved in the chloroplast anchoring and de-anchoring in *Vallisneria*, molecular genetic approaches are required in addition to further physiological studies. Recently, the genome of a marine monocot *Zostera marina* (L.) (Alismatales Zosteraceae) was fully sequenced, demonstrating the similarities and differences in morphological and metabolic features between aquatic and terrestrial angiosperms.[41] Such genetic information will powerfully support future investigation to understand the regulatory mechanism for chloroplast anchoring in a variety of plant species.

Acknowledgments

We thank Hideyasu Takamatsu and Nana Miyawaki for sharing their unpublished results.

References

1. Zurzycki J. Chloroplasts arrangement as a factor in photosynthesis. Acta Soc Bot Pol. 1955;24:27–63.
2. Wada M, Kagawa T, Sato Y. Chloroplast movement. Annu Rev Plant Biol. 2003;54:455–68.

3. Kasahara M, Kagawa T, Oikawa K, Suetsugu N, Miyao M, Wada M. Chloroplast avoidance movement reduces photodamage in plants. Nature. 2002;420:829–32.
4. Banaś AK, Aggarwal C, Labuz J, Sztatelman O, Gabryś H. Blue light signaling in chloroplast movements. J Exp Bot. 2012;63:1559–74.
5. Takagi S. Actin-based photo-orientation movement of chloroplasts in plant cells. J Exp Biol. 2003;206:1963–9.
6. Izutani Y, Takagi S, Nagai R. Orientation movements of chloroplasts in Vallisneria epidermal cells: Different effects of light at low- and high-fluence rate. Photochem Photobiol. 1990;51:105–11.
7. Dong X-J, Ryu J-H, Takagi S, Nagai R. Dynamic changes in the organization of microfilaments associated with the photocontrolled motility of chloroplasts in epidermal cells of *Vallisneria*. Protoplasma. 1996;195:18–24.
8. Dong X-J, Nagai R, Takagi S. Microfilaments anchor chloroplasts along the outer periclinal wall in *Vallisneria* epidermal cells through cooperation of Pfr and photosynthesis. Plant Cell Physiol. 1998;39:1299–306.
9. Sakai Y, Takagi S. Reorganized actin filaments anchor chloroplasts along the anticlinal walls of *Vallisneria* epidermal cells under high-intensity blue light. Planta. 2005;221:823–30.
10. Sakurai N, Domoto K, Takagi S. Blue-light-induced reorganization of the actin cytoskeleton and the avoidance response of chloroplasts in epidermal cells of *Vallisneria gigantea*. Planta. 2005;221:66–74.
11. Dong X-J, Takagi S, Nagai R. Regulation of the orientation movement of chloroplasts in epidermal cells of *Vallisneria*: Cooperation of phytochrome with photosynthetic pigment under low-fluence-rate light. Planta. 1995;197:257–63.
12. Takagi S, Kong S-G, Mineyuki Y, Furuya M. Regulation of actin-dependent cytoplasmic motility by type II phytochrome occurs within seconds in *Vallisneria gigantea* epidermal cells. Plant Cell. 2003;15:331–45.
13. Senn G. Die Gestalts- und Lageveränderung der Pflanzen-Chromatophoren. Leipzig: Verlag von Wilhelm Engelmann; 1908.
14. Yamashita H, Sato Y, Kanegae T, Kagawa T, Wada M, Kadota A. Chloroplast actin filaments organize meshwork on the photorelocated chloroplasts in the moss *Physcomitrella patens*. Planta. 2011;233:357–68.
15. Tsuboi H, Wada M. Distribution pattern changes of actin filaments during chloroplast movement in *Adiantum capillus-veneris*. J Plant Res. 2012;125:417–28.
16. Kadota A, Yamada N, Suetsugu N, Hirose M, Saito C, Shoda K, Ichikawa S, Kagawa T, Nakano A, Wada M. Short actin-based mechanism for light-directed chloroplast movement in *Arabidopsis*. Proc Natl Acad Sci USA. 2009;106:13106–11.
17. Blatt MR, Briggs WR. Blue-light-induced cortical fiber reticulation concomitant with chloroplast aggregation in the alga *Vaucheria sessilis*. Planta. 1980;147:355–62.
18. Blatt MR, Wessells NK, Briggs WR. Actin and cortical fiber reticulation in the siphonaceous alga *Vaucheria sessilis*. Planta. 1980;147:363–75.
19. Takagi S, Takamatsu H, Sakurai-Ozato N. Chloroplast anchoring: Its implications for the regulation of intracellular chloroplast distribution. J Exp Bot. 2009;60:3301–10.
20. Takagi S, Kamitsubo E, Nagai R. Light-induced changes in the behavior of chloroplast under centrifugation in *Vallisneria* epidermal cells. J Plant Physiol. 1991;138:257–62.
21. Takagi S, Kamitsubo E, Nagai R. Visualization of a rapid, red/far-red light-dependent reaction by centrifuge microscopy. Protoplasma. 1992;168:153–8.
22. Seitz K. Light induced changes in the centrifugability of chloroplasts mediated by an irradiance dependent interaction of respiratory and photosynthetic processes. In: Senger H, editor. The blue light syndrome. Berlin, Heidelberg: Springer-Verlag; 1980. p. 637–42.
23. Virgin HI. Further studies of the action spectrum for light-induced changes in the protoplasmic viscosity of *Elodea densa*. Physiol Plant. 1954;7:343–53.
24. Tominaga Y, Kuchitsu K, Katsuhara M, Tazawa M, Miyachi S. Cytoplasmic alkalization and cytoplasmic streaming induced by light and histidine in leaf cells of *Egeria densa*: *In vivo* ^{31}P-NMR study. Plant Cell Physiol. 1991;32:261–8.
25. Ståhlfelt MG. The protoplasmic viscosity of terrestrial plants and its sensitivity to light. Protoplasma. 1955;55:285–92.
26. Virgin HI. Effects of red, far-red and blue light on the viscosity of the cytoplasm of wheat leaf cells. Physiol Plant. 1987;70:203–8.
27. Sugiyama Y, Kadota A. Photosynthesis-dependent but nechrome1-independent light positioning of chloroplasts and nuclei in the fern *Adiantum capillus-veneris*. Plant Physiol. 2011;155:1205–13.
28. Kandasamy MK, Meagher RB. Actin-organelle interaction: Association with chloroplast in *Arabidopsis* leaf mesophyll cells. Cell Motil Cytoskeleton. 1999;44:110–8.[https://doi.org/10.1002/(SICI)1097-0169(199910)44:2%3c110::AID-CM3%3e3.0.CO;2-O]
29. Ichikawa S, Yamada N, Suetsugu N, Wada M, Kadota A. Red light, phot1, and JAC1 modulate phot2-dependent reorganization of chloroplast actin filaments and chloroplast avoidance movement. Plant Cell Physiol. 2011;52:1422–32.
30. Kong S-G, Arai Y, Suetsugu N, Yanagida T, Wada M. Rapid severing and motility of chloroplast-actin filaments are required for the chloroplast avoidance response in *Arabidopsis*. Plant Cell. 2013;25:572–90.
31. Suetsugu N, Yamada N, Kagawa T, Yonekura H, Uyeda TQP, Kadota A, Wada M. Two kinesin-like proteins mediate actin-based chloroplast movement in Arabidopsis thaliana. Proc Natl Acad Sci USA. 2010;107:8860–5.
32. Oikawa K, Yamasato A, Kong S-G, Kasahara M, Nakai M, Takahashi F, Ogura Y, Kagawa T, Wada M. Chloroplast outer envelope protein CHUP1 is essential for chloroplast anchorage to the plasma membrane and chloroplast movement. Plant Physiol. 2008;148:829–42.
33. Schmidt von Braun S, Schleiff E. The chloroplast outer membrane protein CHUP1 interacts with actin and profilin. Planta. 2008;227:1151–9.
34. Oikawa K, Kasahara M, Kiyosue T, Kagawa T, Suetsugu N, Takahashi F, Kanegae T, Niwa Y, Kadota A, Wada M. Chloroplast unusual positioning1 is essential for proper chloroplast positioning. Plant Cell. 2003;15:2805–15.
35. Suetsugu N, Sato Y, Tsuboi H, Kasahara M, Imaizumi T, Kagawa T, Hiwatashi Y, Hasebe M, Wada M. The KAC family of kinesin-like proteins is essential for the association of chloroplasts with the plasma membrane in land plants. Plant Cell Physiol. 2012;53:1854–65.
36. Takamatsu H, Takagi S. Actin-dependent chloroplast anchoring is regulated by Ca^{2+}–calmodulin in spinach mesophyll cells. Plant Cell Physiol. 2011;52:1973–82.
37. Sakai Y, Inoue S-I, Harada A, Shimazaki K-I, Takagi S. Blue-light-induced rapid chloroplast de-anchoring in *Vallisneria* epidermal cells. J Integr Plant Biol. 2015;57:93–105.
38. Paves H, Truve E. Myosin inhibitors block accumulation movement of chloroplasts in *Arabidopsis thaliana* leaf cells. Protoplasma. 2007;230:165–9.
39. Takagi S, Hayashi T, Ryu J-H, Nakanishi Y. Cell-wall-dependent organization of actin cytoskeleton in *Vallisneria* mesophyll cells. Plant Morphol. 2001;13:11–20.
40. Chuong SDX, Franceschi VR, Edwards GE. The cytoskeleton maintains organelle partitioning required for single-cell C_4 photosynthesis in Chenopodiaceae species. Plant Cell. 2006;18:2207–23.
41. Olsen JL, Rouzé P, Verhelst B, Lin YC, Bayer T, Collen J, Dattolo E, De Paoli E, Dittami S, Maumus F, et al. The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. Nature. 2015;530:331–5.