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Review

Recent advances in understanding the molecular mechanism of chloroplast photorelocation movement ☆

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ABSTRACT

Plants are photosynthetic organisms that have evolved unique systems to adapt fluctuating environmental light conditions. In addition to well-known movement responses such as phototropism, stomatal opening, and nastic leaf movements, chloroplast photorelocation movement is one of the essential cellular responses to optimize photosynthetic ability and avoid photodamage. For these adaptations, chloroplasts accumulate at the areas of cells illuminated with low light (called accumulation response), while they scatter from the area illuminated with strong light (called avoidance response). Plant-specific photoreceptors (phototropin, phytochrome, and/or neochrome) mediate these dynamic directional movements in response to incident light position and intensity. Several factors involved in the mechanisms underlying the processes from light perception to actin-based movements have also been identified through molecular genetic approach. This review aims to discuss recent findings in the field relating to how chloroplasts move at molecular levels. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

Plants play a role as the major agent of inorganic carbon fixation into biomass. The photoautotrophic process converts light energy into chemical energy, which occurs in chloroplasts that serve as the photosynthetic factory equipped with photosystems. However, excess light energy is harmful, because it can lead to an overproduction of highly reactive intermediates and by-products that cause photo-oxidative damage and inhibit photosynthesis [1,2]. To protect themselves from the adverse effects of high light intensity, plants have evolved two distinct mechanisms for sensing and responding to light. It involves chloroplast photorelocation to avoid strong light and non-photochemical quenching relying on the conversion and dissipation of the excess excitation energy into heat [3].

The ability of chloroplasts to change their intracellular position in response to light fluctuations was discovered over a century ago [4]. The chloroplast movement is a phenomenon commonly observed in all plant groups from algae to land plants [4–6]. Depending on the plant groups, however, chloroplasts exhibit various positioning in the cells under different light conditions [7–9] (Fig. 1). Chloroplasts dynamically change their positions by monitoring incident light position and intensity [8]: they accumulate to the area illuminated with low-intensity light (accumulation response), while they move away from the area illuminated with high-intensity light within a few tens of minutes (avoidance response). These phenomena were clearly observed when cells were irradiated partially. Blue light is the most effective wavelength to induce these two responses in most plants tested including angiosperms (*Arabidopsis*, spinach, tobacco, etc.) [10,11] and cryptogams (ferns, mosses, liverworts, algae, etc.) [12]. However, red light is also effective for the induction of chloroplast photorelocation movement in some cryptogam plants such as the alga *Mougeotia scalaris*, the fern *Adiantum capillus-veneris*, and the moss *Physcomitrella patens* [5,12,13].

Chloroplast photorelocation responses are strongly correlated with the function of chloroplast: The accumulation response helps to optimize photosynthetic ability [10,14] and the avoidance response aids to reduce photodamage by excess light [15–17]. Plants that carry mutations impairing the chloroplast avoidance are more susceptible to oxidative stress under excess light [11,18,19]. Thus, chloroplast photorelocation movement is essential for plant survival under fluctuating light conditions.

Genetic studies of chloroplast photorelocation movement contributed to the identification of several components involved in signal transduction as well as actin-based movement [20]. The regulation mechanisms of chloroplast photorelocation movement have been progressively elucidated through recent progress in imaging techniques for the visualization of chloroplasts and cytoskeletal components [21–23]. In this review, we

Abbreviations: BDM, 2,3-butanedione monoxime; BFA, Brefeldin A; cp-actin, chloroplast actin; GFP, green fluorescent protein; FMN, flavin mononucleotide; LOV, light, oxygen or voltage; P2C, C-terminal 534–915 amino acid fragment of *Arabidopsis* phototropin 2; P2N, N-terminal 1–533 amino acid fragment of *Arabidopsis* phototropin 2; phot1, phototropin 1; phot2, phototropin 2; TIRF, total internal reflection fluorescence

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Fig. 1. A visible pattern of chloroplast movements drawn by blue light on a young leaf of *Solanum americanum*. Chloroplasts in pale green areas take the high light-induced avoidance position, while those in darker green areas do the low light-induced accumulation position.

summarized recent advances on chloroplast photorelocation responses covering from light perception to actin-based movement.

2. Photoperception for chloroplast photorelocation movement

Plants as sessile organisms have evolved unique systems for sensing and responding to environmental light. Members of both the phototropin and phytochrome families of photoreceptors regulate the sophisticated blue-light- and/or red-light-induced chloroplast relocation movements.

2.1. Blue light receptors: phototropins

Light-driven chloroplast relocation responses are well described in *Arabidopsis thaliana* using photometric and microscopic analyses, or observed by the naked eye (named white and green band assay) [10,23,24]. The blue light-dependent chloroplast movements are mediated by plant specific photoreceptors, phototropins. Phototropins are found in various organisms from unicellular algae to higher plants [7,25]. *Arabidopsis* has two phototropins (phot1 and phot2). phot1 (originally named NPH1) was discovered as a blue light receptor kinase that regulates phototropic response in *Arabidopsis* [26,27]. Later, phot2 as a homolog of phot1 (formerly NPL1) was identified from a mutant defective in high light-induced avoidance response in *Arabidopsis* [28,29].

Phototropins have well-conserved structural properties with two LOV (light, oxygen or voltage) domains (LOV1 and LOV2) in their N-terminus and a serine/threonine kinase domain in their C-terminus [25] (Fig. 2). The two phototropins in *Arabidopsis* have highly overlapping functions not only in chloroplast accumulation response but also in phototropism, stomatal opening, and leaf flattening [27–31]. However, phot2 plays specific roles on chloroplast avoidance response [28,29] and leaf morphogenesis [32]. phot1 also induces the avoidance response transiently, but the response is not significant compared to that of phot2 [33]. These distinct functional properties of phototropins are not simply explained because phot1 and phot2 possess intrinsically different properties in many aspects including gene expressions, photochemical properties, and cellular and subcellular localizations (see below).

2.2. Photochemical properties of LOV domain

The LOV domains are widely found not only in plants but also in bacteria, archaea, and fungi, and play a role as the chromophore-binding site to perceive a broad range of UVA to blue light (350–500 nm) via a flavin mononucleotide (FMN) cofactor [34]. The LOV domains undergo a photocycle upon blue light stimulation including transient adduct formation between the chromophore and a conserved cysteine. Of those, two LOV domains (LOV1 and LOV2) of phototropins have been well characterized on their structure and photochemical properties. Core structures and the photochemical reactions of the LOV1 and LOV2 domains are very similar to each other [35]. However, the LOV domains of phot1 differ from those of phot2 in their reaction kinetic properties and relative quantum efficiencies [36].

The LOV domains of phototropins have also important roles in multimer formation [37–39]. The LOV1 domain exists as a dimer in the dark [37,38]. Photochemical analysis using the time-resolved transient grating method further suggested that the dimer of phot1 LOV1 domain associates to form a tetramer upon photoexcitation and the homo-tetramer dissociates into the dimer via the dark recovery process of the chromophore [40]. However, transgenic analysis with the deletion mutant of phot1 LOV1 domain suggested that phot1 LOV1 domain is dispensable to mediate phot1 responses in *Arabidopsis* [41]. By contrast, the activity of phot2 LOV1 domain on the phototropic response

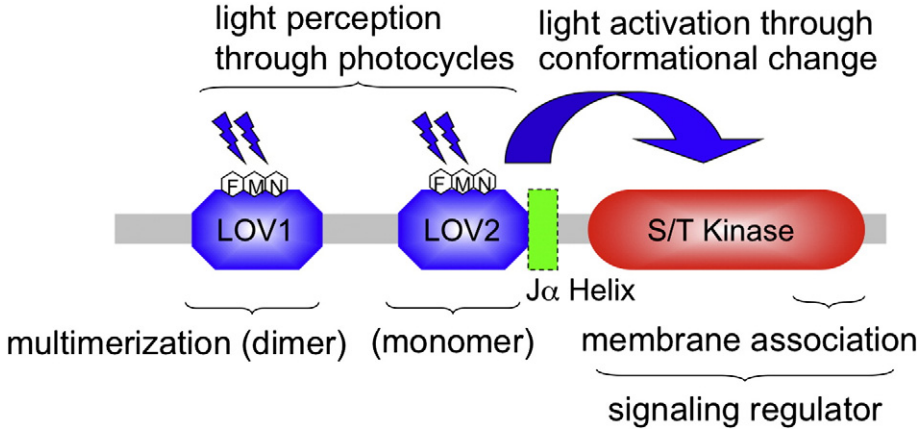

Fig. 2. Schematic illustration of the molecular structure, functional role, and regulation mechanism of phototropins. Phototropins consist of LOV1 and LOV2 as the photosensory domains in their N-terminus and an S/T kinase domain in their C-terminus. The kinase is regulated through the photocycles of LOV domains and conformational changes in LOV2 domain and Jα helix in response to blue light and consequently activates downstream signaling by phosphorylating not-yet-known downstream signaling factor(s). The C-terminus also plays an essential role for the membrane association of phototropin. The details are shown in the text.

and the accumulation response was observed even if the activity was relatively low when the phot2 LOV2 domain was defective to bind the chromophore, indicating that the interdomain interaction between LOV1 and LOV2 domains may be relevant for the signaling process [42]. Hence, the biological significance of the multimer formation of LOV domains remains to be elucidated.

It is widely demonstrated that the LOV2 domains have essential roles in not only the regulation of the kinase but also the physiological responses [42–46]. The photocycle of phot1 LOV2 domain induces conformational changes of the Jα helix situated at the C-terminal side of the LOV2 domain that consequently leads to activation of the kinase [47,48]. Hence, the LOV2 domains have essential roles in the light regulation of phototropins. Taken together, these different photochemical and structural roles of LOV domains may implicate diverse functions in phototropins.

2.3. Subcellular localizations of phototropins

The subcellular localizations of photoreceptors that were active in controlling the light-induced chloroplast movements were firstly demonstrated by various physiological ways. Partial cell irradiation with a light spot or whole cell irradiation with linearly polarized light suggested that the blue light-absorbing photoreceptor is localized in the outer layer of cytoplasm, most probably in the plasma membrane [6,49]. Chloroplast response to the irradiation of linear polarized light was different from that to unpolarized light both in low or high intensity light responses, suggesting that the photoreceptor molecules are arranged parallel to the plasma membrane. This specific response of chloroplasts to the polarized light occurred only in the visible range above 400 nm but not in the UV range, which was well consistent with the action spectra of the chloroplast positioning [6]. In addition, chloroplast photorelocation responses were also regulated in response to the irradiation of blue light superimposed on linear polarized red and far-red light in *M. scalaris* [50] and *A. capillus-veneris* [51], proposing that the response is mediated by cell membrane-bound phytochrome. Later, this phytochrome was found as the chimeric neochrome in *A. capillus-veneris* [52]. Intriguing chimeric neochrome was also found in *M. scalaris*, although the gene product has not yet been confirmed to control chloroplast rotation in this alga (see details in the next section) [53]. Hence, these physiological analyses with polarized blue or red light have suggested that the photoreceptors that mediate chloroplast photorelocation responses are along the plasma membrane.

Consistent with the previous physiological data, the plasma membrane localization of phototropins was further demonstrated using GFP fusion proteins and biochemical analyses [54–57] (Table 1). Although both phot1 and phot2 are mainly localized to the plasma membrane in the dark, their subcellular localizations under blue light are differently regulated. Some fraction of phot1 is internalized into the cytoplasm from the plasma membrane in response to blue light, via possibly clathrin-mediated endocytosis [54,58,59]. By contrast, some fraction of phot2 associates with the Golgi apparatus in response to blue light [55]. The blue light-induced relocations of phototropins are necessary for their kinase activity [55,58]. Brefeldin A (BFA), an inhibitor of vesicle trafficking, induced accumulations of some fractions of phot1 and phot2 at BFA compartment around the perinuclear region even in darkness [55,60]. The blue light-induced phot1 internalization and phot2 association with Golgi were not affected even in the BFA-treated cells, suggesting that dynamic trafficking of phototropins is regulated in a BFA-insensitive manner [55,60].

Structure and function analysis revealed the important roles of the phot2 C-terminal 534–915 amino acid fragment (P2C) including the entire kinase domain. P2C but not the phot2 N-terminal 1–533 amino acid fragment (P2N) was responsible for the plasma membrane localization and the Golgi association [61,62]. Moreover, the C-terminal 42 amino acids of phot2 following the kinase domain were necessary for the Golgi association, and also for the efficient plasma membrane and chloroplast outer membrane localizations [62]. Functional analysis further suggested that the C-terminal membrane association domain is necessary for the phot2-specific activity that elicits the strong light induced-avoidance response [62].

P2C elicited the phototropin responses without a light stimulus in *Arabidopsis*. That is, chloroplasts were in the avoidance position, stomata opened without light stimulus, phototropic response in hypocotyl was reduced with an inhibition of hypocotyl growth, and cylindrical palisade cell development was promoted in transgenic plants expressing P2C fused to GFP (P2CG) [32,61]. In addition, phot1 and phot2 kinases phosphorylated casein and LOV1-containing N-terminal polypeptide as artificial substrates in vitro, in which the LOV2 domain played an essential role to regulate the kinase activity in a blue light-dependent manner [44,46,63]. Hence, the N-terminal photosensory domain functions as a light switch for the kinase activation through reversible conformational changes between the dark and light states of a photocycle, in which the LOV2 domain functions as a dark-state repressor [43,47,48]. Taken together, the kinase domain has multiple functions not only in activation of downstream signaling but also in subcellular localization (Fig. 2).

Many previous physiological analyses with microbeam irradiations suggest that the photoreceptor for the avoidance response is possibly associated with chloroplasts. Indeed, the avoidance response is induced specifically where the chloroplasts are directly irradiated with high intensity blue light. Consistently, it has been recently demonstrated that both phot1 and phot2 localized at the chloroplast outer membrane. However, phot2 localized more abundantly at the chloroplast outer membrane than phot1 [64]. Thus, the chloroplast outer membrane localization of phototropins is consistent with the physiological aspect of chloroplast photorelocation movement. Phototropins are resistant to high salt concentration but sensitive to alkali ionic strength [57,62,64]. These biochemical features suggest that the C-terminal fragment is likely to be associated with the membranes via ionic interaction rather than covalent bonds.

2.4. Red light photoreceptors in chloroplast photorelocation movement

In addition to phototropin-mediated blue light response, chloroplast photorelocation movement is induced by red light in most species of cryptogam plants. The photoreceptors, phytochrome or neochrome (a chimeric photoreceptor consisting of the N-terminal photosensory domain of phytochrome and the full-length phototropin) [65], are responsible for sensing red light and relay the signal to the chloroplasts in these plant species [12]. In the fern *A. capillus-veneris*, neo1 functions only in the red-light-induced accumulation response, but not in avoidance response [52]. The *NEO1* gene was also found in the green algae *M. scalaris* and successfully rescued the *A. capillus-veneris neo1* mutant phenotype, indicating the functional equivalence [53]. It is an intriguing example of convergent evolution that the chimeric genes have arisen independently.

Table 1
Key players in chloroplast photorelocation movement discussed in this review.

Step	Factors	Function	Localization	Refs
Perception of light signal	phot1	Ac	PM, cytosol, chloroplast outer membrane	[54,64]
	phot2	Ac, Av	PM, Golgi, chloroplast outer membrane	[55,64]
Signal transduction	PMI1	Ac, Av		[77]
	PMI2	Av	Cytosol	[79]
	WEB1	Av	PM	[79]
	JAC1	Ac	Cytosol	[76]
Cp-actin regulation	CHUP1	Ac, Av, An	Chloroplast outer membrane	[80,98,99]
	THRUMIN1	Ac, Av	PM	[22,82]
	KAC1, KAC2	Ac, Av, An	Membrane, cytosol	[81,97]

Ac, accumulation response; Av, avoidance response; An, anchoring; PM, plasma membrane.

Red light as well as blue light induces both accumulation and avoidance responses in the moss *Physcomitrella patens*. *P. patens* has four phototropin genes (*PHOTA1*, *PHOTA2*, *PHOTB1*, *and PHOTB2*) that are classified into two groups (*PHOTA and PHOTB*) on the basis of their deduced amino acid sequences. These genes are responsible for blue light-induced chloroplast movements. Although phytochromes are the primary photoreceptors for red light-induced chloroplast movements in *P. patens*, phototropins also function in red light signaling as downstream components of phytochromes [36]. Consistent with these physiological interactions, a subpopulation of phytochrome at the active Pfr form associates physically with phototropins at the plasma membrane [66]. Hence, the light-signaling complex of phytochrome–phototropin at the plasma membrane provides a functional explanation for the evolution of the chimeric photoreceptor, neochrome.

Red light does not induce chloroplast photorelocation movement in *Arabidopsis* [10,24,33], although phytochrome involvement in tropic response is well established. Red light pretreatment induces the retention of phot1-GFP on the plasma membrane that may account for the red light-induced increase in phot1 activity [67]. However, phytochromes are likely to modulate chloroplast photorelocation movements indirectly via increasing cytoplasmic motility [24]. Additionally, multiple forms of interaction such as gene expression and protein stability also influence the blue light-induced movement in *Arabidopsis* (see below).

Chloroplast movement is also induced in mechanical stimulation. Mechanical stimulation-dependent chloroplast movement was found in protonemal cells of the fern *A. capillus-veneris* [68,69] and the moss *P. patens* [70]. Photosynthesis-dependent but nondirectional movement also contributes to the light positioning of chloroplasts in addition to the light-directional movement in *A. capillus-veneris* [71]. Actin and/or microtubule drugs inhibited effectively both light-dependent and light-independent chloroplast movements, suggesting that the motile systems are likely the same to each other.

2.5. Difference in expression patterns and levels

In accordance with different sensitivities of phot1 and phot2 at cellular levels, the genes of both proteins exhibit different expression patterns. Blue and red lights affect the gene expression of phototropins. *PHOT1* is downregulated under the controls of cryptochrome 1 and phytochrome B and *PHOT2* is upregulated under the controls of cryptochromes and phytochrome A in *Arabidopsis* [72]. The phot1 amount is much higher than that of phot2 in etiolated seedlings: phot1 is expressed at whole seedlings but phot2 is highly expressed in aerial parts of the seedlings and green tissues [54,55,73,74]. In addition, the expression of *PHOT2* increased 3 fold by blue and red light treatments in the rosette leaves of *Arabidopsis*, but that of *PHOT1* was slightly downregulated by the light [72]. Consistently, the amount of phot2 is also approximately 2–4 times higher than that of phot1 not only in the rosette leaves but also in the chloroplast outer envelope [64].

Most physiological studies on chloroplast photorelocation movement have been carried out in mesophyll cells of rosette leaves. However, chloroplasts in different tissues of *Arabidopsis* such as petioles also move similarly without any difference [21]. However, amyloplasts have not exhibited light-induced relocation responses in root cells, although several studies have shown amyloplast movement in response to gravity in those cells, in some cases along actin cables [75]. This kind of discrepancy would have resulted from spatial expression and/or interaction of the corresponding factors functioning in chloroplast photorelocation movement.

3. Signal transduction

Several downstream signaling components have been identified through genetic analysis of *Arabidopsis* mutants that were specifically defective in chloroplast photorelocation movement [20]. Those are (i) the possible downstream signaling components: J-domain Protein Required for Chloroplast Accumulation Response1 (JAC1) [76], Plastid Movement Impaired 1 (PMI1) [77], PMI2 [78], Weak Chloroplast Movement under Blue Light 1 (WEB1) [79], (ii) the components for actin-based movement: chloroplast-actin (cp-actin) filaments [21], Chloroplast Unusual Positioning 1 (CHUP1) [21,80], Kinesin-like Protein for Actin-Based Chloroplast Movement 1, 2 (KAC1, 2) [81], and THRUMIN1 [22,82]. These findings provide new insights into the molecular mechanism underlying the entire signaling process. The signaling process of the chloroplast photorelocation movement is composed of the following three parts: photoperception, signal transduction, and chloroplast movement. The signal cascade is initiated from the photoreceptor autophosphorylation in response to blue light [41,83,84].

3.1. Downstream signal transduction

Chloroplasts are differently localized in the cells under different light conditions. Chloroplasts are located on the cell bottom or at the anticlinal walls in dark-adapted cells. When a part of the cell was irradiated with a microbeam of high intensity blue light, the chloroplasts moved towards the irradiated area after some lag time showing accumulation response, but stopped at the edge of the microbeam and did not enter into the irradiated area. As soon as the microbeam was turned off, the chloroplasts rushed into the previously irradiated area [24,85,86]. These chloroplast behaviors suggest that the signals for both accumulation and avoidance responses are generated in the same place at the same time by high intensity light. Furthermore, the moving chloroplasts are continuously monitoring the intensity and direction of signals that are released from the photoreceptors to reach the appropriate position [87]. However, the signaling mechanism for the accumulation and avoidance responses might be differently regulated (see below).

Chloroplasts do not have any polarity in the sense of moving direction, although they may have a polarity for development and division, so that they can move along their short or long axis to any direction without rotation and rolling [88,89]. The velocity of chloroplast avoidance response is a range of 1–2 μm min^{-1}, which is dependent on the fluence rate of blue light and the level of phot2 in *Arabidopsis* [73,90]. Conversely, the velocity of the accumulation response is almost constant regardless of the fluence rates of red or blue light around 0.3 μm min^{-1} in the prothallial cells of the fern *A. capillus-veneris* and around 1 μm min^{-1} in the palisade cells of *Arabidopsis* [85,88].

The speed of signal transfer was carefully analyzed in the fern protonemal cells by measuring the lag time from a pulse microbeam irradiation to the onset of chloroplast movement and the distance of chloroplast position from the microbeam-irradiated area [91]. The deduced signaling speeds were about 0.7 μm min^{-1} in the apical-to-basal direction and about 2.3 μm min^{-1} in the basal-to-apical direction, indicating that the speed of signal transfer is influenced by cell polarity. It is interesting but hard to understand that there is a tendency that protonemal chloroplasts positioned farther from the microbeam moved faster irrespective of light conditions [91]. In addition, the speeds of signal transfer in dark-adapted prothallial cells and in *Arabidopsis* palisade cells whose polarities are not clear were about 1.0 μm min^{-1} and about 0.7 μm min^{-1}, respectively [91]. Although the speed of signal transfer is slightly different among cell types or plant species, the difference is not so big and within one order. Some different speeds that were found in different cell types might be caused by differential flow rates of cytoplasmic streaming. Together, the mechanism of signal transfer may be conserved in plants.

The accumulation response is probably mediated by a signal released by photoreceptors via a not-yet-known long distance signaling cascade. By contrast, the avoidance response is observed with a lag time of several minutes when chloroplasts are irradiated directly with strong light [24,85]. Thus, the light perception for chloroplast avoidance response is possibly mediated by the photoreceptors localized on the chloroplasts or the plasma membrane underneath the chloroplasts. Since phot2 is more abundant than phot1 in the chloroplast and the

plasma membrane in the leaf cells, phot2 could play a primary role in mediating the avoidance response signal that should be proximately associated with chloroplasts and/or the plasma membrane [64]. It is an intriguing possibility that phototropin could phosphorylate their substrates at different subcellular localizations. Further analysis is necessary to identify substrates of phototropin kinase.

Studies using inhibitors such as calcium channels or lipid metabolism have suggested that cytosolic Ca^{2+} and phosphatidylinositol (Ptdlns) lipid as key signaling mediators are involved in phototropin-mediated responses including phototropism, stomatal opening, and chloroplast movement [92]. phot1 is responsible for calcium channel in the plasma membrane, while phot2 functions in the regulation of cytosolic calcium [56]. However, how the signaling mediators are organized spatiotemporally to regulate cellular motility and polarity remains to be clarified. The phot-induced phosphoinositide (PI) metabolism has been shown to be essential for stomatal opening and phototropism. Aggarwal et al. [93] have recently decoded the role of PIs in the regulation of phot1- and phot2-mediated chloroplast movements in *Arabidopsis*. The inhibition of the Ptdlns(4,5)-bisphosphate [PI(4,5)P_2]-phospholipase C (PLC) pathway, using neomycin and U73122, suppressed specifically the phot2-mediated chloroplast accumulation and avoidance responses but not the phot1-mediated chloroplast movement [93]. The inactivation of PI3-kinase and PI4-kinase by wortmannin and LY294002, severely affected the weak blue-light-activated accumulation response but had little effect on the avoidance response. These effects of inhibitors are partly derived due to a disturbance in Ca^{2+}(cytosol) signaling, suggesting the involvements of PIs that modulate cytosolic Ca^{2+} signaling for chloroplast movements [93].

Although no phototropin substrate has been demonstrated in chloroplast photorelocation movement yet, several proteomic analyses suggest an intriguing possibility that all of these components are phosphorylated in *Arabidopsis* cells [20]. The downstream signaling components exhibit different subcellular localizations (Table 1), and their biochemical functions remain unknown. Nevertheless, the blue light-induced signal transduction in chloroplast photorelocation movement should be initiated by phosphorylation of the downstream components via phototropins. Diverse subcellular localizations of phototropins imply their spatial and precise regulation for the downstream components.

4. Actin-based dynamic movement

Similar to the main subcellular organelles, such as nucleus, mitochondria, endoplasmic reticulum (ER), Golgi and other compartments, chloroplasts utilize two different kinds of cytoskeletons, actin filaments and microtubules, for their dynamic movement and positioning [20,70,94]. In *Arabidopsis*, actin filaments are predominantly utilized for chloroplast movement [20]. The utility of fluorescent proteins tagged to actin-binding proteins in *Arabidopsis* has shed new light on the molecular mechanism of chloroplast photorelocation movement. The advent of live cell imaging and fluorescent probes has recently confirmed the highly dynamic actin-based chloroplast photorelocation movement. The most striking finding in this field is the novel cp-actin filaments that have distinct structure and dynamics from cytoplasmic actin cables [21,22].

4.1. Chloroplast anchoring/positioning

For performing their proper function in a cell, chloroplasts should move to and fix themselves at the appropriate positions in response to environmental light conditions. Consequently, the chloroplast de-anchoring from and re-anchoring to the plasma membrane (or cortical cytoplasm) should be reversibly regulated for chloroplast movement and positioning, respectively [95]. Because the high intensity of blue light effectively induced chloroplast de-anchoring via the actin depolymerization [96], that should be mainly mediated by phot2. However, treatment of actin inhibitors such as cytochalasin D and latrunculin B effectively disrupted chloroplast movement but not positioning in living cells, suggesting the involvement of other factors in addition to the actin filaments in the chloroplast anchoring. Calcium treatment induced chloroplast de-anchoring from the plasma membrane and calmodulin (CaM) inhibitor blocked this process [96]. Thus, calcium and calcium responding CaMs are likely involved in the downstream signaling of actin-dependent chloroplast anchoring.

The chloroplasts in *chup1* and *kac1kac2* mutant cells accumulated at the bottom of the cells around the nucleus [80,81,97], indicating that these proteins are essential for the proper chloroplast anchoring. CHUP1 is localized to the chloroplast outer envelope via the N-terminus hydrophobic region [80,98]. The coiled-coil region of CHUP1 is responsible for the plasma membrane anchorage [98], so that CHUP1 is like a bridge anchoring chloroplasts to the plasma membrane. Importantly, CHUP1 has the ability to bind G- and F-actin and profilin [80,99]. In *chup1* mutant, cp-actin filaments were not observed on the chloroplasts [21,22]. KACs are kinesin-like proteins that are essential for chloroplast anchoring [81,97]. KAC motor domains are similar to those of the kinesin-14 subfamily but do not possess detectable microtubule binding activity. By contrast, the C-terminal domain of KAC1 could bind to actin filaments in vitro, suggesting that KACs function in chloroplast movement via cp-actin filaments rather than microtubules [81]. Thus, both CHUP1 and KAC proteins are essential to the chloroplast anchoring to the plasma membrane, for which their interaction(s) with a membrane protein(s) might be necessary to anchor chloroplast to the plasma membrane.

4.2. Cp-actin-based chloroplast movement

Chloroplasts attached to the plasma membrane move to the preferable sites in a cell depending on the fluctuating environmental conditions by monitoring the direction of incident light and its intensity. Actin filaments (and microtubules in the moss *Physcomitrella*) are involved in the regulation of chloroplast movement. In accord with the dynamic chloroplast movement responding to blue light, actin filaments between the chloroplast envelope and the plasma membrane are dramatically rearranged. The association of actin filaments with chloroplasts has been reported in many plant species [9,20,94]. The actin filament association to the isolated chloroplasts was also demonstrated in spinach [95,96].

Cp-actin filaments are novel actin structures that function in chloroplast movement [21]. The dynamic rearrangements of cp-actin filaments are essential for the light-driven chloroplast movement [21,22]. The features of cp-actin filaments are quite different from those of cortical actin filaments in unit length, shape, behavior and regulation mechanism [21,22]. Cp-actin filaments are localized specifically to the space between the chloroplast and the plasma membrane and are essential for the chloroplast anchoring and the chloroplast movement (Fig. 3). When chloroplasts are stationary without movement under a constant light condition, cp-actin filaments are distributed evenly around the chloroplast periphery, which should play a role in chloroplast anchoring to the plasma membrane.

Cp-actin filaments are dominantly depolymerized and disappear under strong light conditions, while they are intensively polymerized at the front part of the chloroplasts during high light-induced avoidance response (Fig. 3). Rapid disappearance of cp-actin filaments under strong light conditions plays a tremendous role for establishing a big difference in cp-actin distribution between the front and rear parts of chloroplasts in a short period. Under low light-induced accumulation response, rearrangement of cp-actin filament is not drastic but only a small amount of cp-actin filaments are accumulated on the front side. As a result, there is a strong correlation between the light intensity and the movement speed, because the disappearance of cp-actin filaments is dependent on blue light intensity [21,22].

Total internal reflection fluorescence (TIRF) microscopic analysis on the disappearance of cp-actin filaments revealed their unique

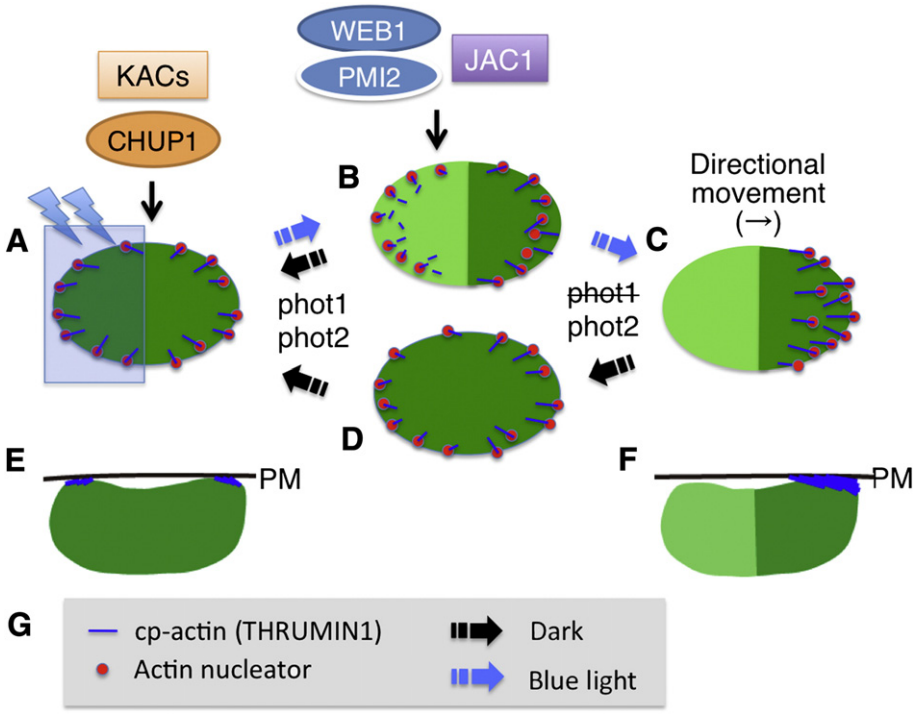

Fig. 3. Schematic representation of cp-actin-based chloroplast movement. (A) to (D) Cp-actin filament dynamics during the avoidance response induced by microbeam irradiation with strong blue light at the left half of the chloroplast as shown in (A). The cp-actin filament (blue lines) is shown with a cp-actin nucleator (red circles) that should be located on the chloroplast envelope. The cp-actin filaments rapidly disappear (C) through rapid severing and random movement in the rear region under strong blue light (B), which is primarily regulated by phot2 (A to C). By contrast, the cp-actin filaments are newly polymerized at the front region and become more abundant than before (compare A and C). The asymmetric distribution of cp-actin filaments drives directional movement (C). After avoiding the strong light-irradiated area or during dark adaptation, cp-actin filaments are rearranged on the whole region of chloroplast periphery (D to A). CHUP1 and KACs are essential for the existence and/or the maintenance of cp-actin filaments, but WEB1/PMI2 and JAC1 are likely to function in disappearance of cp-actin filaments. The details are shown in the text. (E) and (F) Cp-actin filaments on side-viewed chloroplast. The chloroplasts (E) and (F) correspond to chloroplasts (A) and (C), respectively. Note that cp-actin filaments are specifically present at the interface between the plasma membrane and chloroplast envelope. (G) Legends for a cp-actin filament, an actin nucleator, and light conditions.

regulation and dynamics. The cortical actin filaments were abundantly observed in long, smooth, and straight shapes. The actin filaments were dynamically remodeled through rapid growth, severing activity, and rapid depolymerization in the *Arabidopsis* cortical array [100]. Similar actin filament dynamics were confirmed in *chup1* mutant cells. By contrast, cp-actin filaments were rapidly severed to short actin fragments under strong blue light instead of depolymerization at their ends. The short, fragmented actin filaments were generally long-lived and gradually disappeared with random motility from the chloroplast periphery. These dynamic reorganizations of cp-actin filaments were regulated by phot2 [22]. Hence, rapid severing and random motility of cp-actin filaments are essential for the disappearance of cp-actin filaments. Together, the blue light-dependent dynamics of cp-actin filaments are an essential response for the rapid and directional movements of chloroplasts (Fig. 3).

Chloroplasts exhibit rapid movement towards the nearest anticlinal wall when whole cells are irradiated with high intensity blue light, but when a small blue light spot (e.g. 10 μm in diameter) is positioned at a part of the chloroplast, the chloroplast escapes from the light beam promptly towards the non-irradiated side [21,22,24]. These light-dependent movements are well consistent with the reorganizations of cp-actin filaments. Hence, it is obvious that cp-actin filaments are indispensable in blue light-induced chloroplast movement. Moreover, the existence of cp-actin filaments has widely been demonstrated in land plants such as fern, moss and *Arabidopsis* [21,22,101,102]. In addition, the components of core machinery to maintain cp-actin filaments such as CHUP1 and KAC are highly conserved in land plants [97,103]. Therefore, the scheme of cp-actin-based chloroplast movement is broadly conserved in land plants as a general mechanism.

In addition to the essential functions of CHUP1 and KACs in the maintenance and/or regulations of cp-actin filaments, the downstream components of chloroplast photorelocation movement such as PMI2, WEB1, JAC1 are also involved in the regulation of cp-actin filaments [79,104]. THRUMIN1, an actin bundling factor also functions in the regulation of cp-actin filaments [22,82]. The functions of these components are not yet clarified, however, these factors should cooperate intimately for dynamic reorganizations of cp-actin filaments (Fig. 3). Our recent research [22] on cp-actin filaments using various actin probes in various intact cells clearly demonstrated that all actin probes were useful to observe dynamics and organizations of cp-actin filaments. THRUMIN1 preferably exhibited the same structure and dynamics of the cp-actin filaments [22]. In addition, cp-actin filaments were more effectively visualized using mouse Talin than fABD2 which exhibits strong affinity to cortical actin filaments. Together, the actin probes might have distinct binding affinities to cp-actin filaments.

4.3. Cp-actin-based motive force generating system

Recent findings on the role of myosins in plants suggest that myosins function in actin dynamics as well as various organelle movements [105]. As is well known in animal cells, myosins function in the organelle movements of the endoplasmic reticulum (ER), peroxisomes, mitochondria, and the Golgi apparatus in plants which are demonstrated through molecular genetic analysis with multiple myosin mutants [106–108]. Subcellular localizations of *Arabidopsis* myosins have also supported their possible functions in chloroplast photorelocation movement [109–111]. However, the double, triple, and quadruple mutants of myosin VIII and/or XI families were normal in chloroplast photorelocation movement even though they exhibited severe retardation of organelle movement and morphological defects [108,112].

Myosin-dependent organelle movements are distinct from the cp-actin-dependent chloroplast movements in several issues. Organelles,

in general (excluding chloroplasts), do not simply move at the same speed in the same direction. They frequently stop and restart, and occasionally switch the moving direction. The movement speed reaches up to 7–8 μm sec^{-1}. The motions and speeds of light-induced chloroplast movements are distinct from those of other organelles as mentioned above. Irrespective of the results obtained by mutant analyses and physiological aspects, pharmaceutical analysis with 2,3-butanedione monoxime (BDM) raises a different aspect on the myosin function in chloroplast movement. The high doses of BDM (50–100 mM) inhibited the chloroplast photorelocation movement besides the myosin-dependent organelle movement [22,113]. Moreover, cp-actin dynamics (random motility and severing activity) were significantly interfered with by BDM treatment [22]. Therefore, we could not exclude the possibility that BDM interferes with cp-actin dynamics due to the inhibition of myosin activity [114]. This discrepancy should be figured out in the near future.

5. Concluding remarks and future perspectives

Over the past decade, great advancement has been made in understanding the molecular factors that potentially function in the signal transduction from light perception to cp-actin-based chloroplast movement through various studies of physiology, molecular genetics and cell biology. However, we are still halfway to understanding the signal transduction pathways. One overarching theme is that there still appear more questions than answers relating to the molecular function and regulation of downstream factors in chloroplast photorelocation movement, especially we do not know how signal transduction is turned on. Thus, we should also continue to elucidate the molecular functions of downstream signaling factors, yet their mechanisms still remain to be clarified. We also have very limited knowledge of how motive force for the chloroplast movement is generated. The motive force generation from rapid polymerization of cp-actin filaments should be a fascinating novel model for the chloroplast movement. It should be empirically demonstrated in the near future.

It should be the most important question how CHUP1 perceives the signals released by photoreceptors and activates the actin polymerization. That is, it is essential to study the mechanisms underlying the CHUP1-dependent cp-actin filament dynamics to understand the molecular mechanism of chloroplast photorelocation movement. However, we meet various limits to observing a dynamic system of cp-actin filaments at molecular level in a live cell. Indeed, microscopic analyses using fluorescent proteins in *Arabidopsis* meet some limitations as follows: 1. Overlapping of the excitation wavelength of fluorescent proteins (CFP, GFP) and the absorption wavelength of phototropins (320–500 nm). 2. No applicability of TIRF microscopy because of an epidermal cell layer between cover glass and the optic specimen of mesophyll cell layer. 3. Limitation of simultaneous observation of some fluorescent probes tagged to protein factors because of strong autofluorescence of plant cells. 4. Detrimental effects of actin probes on acin dynamics by using general actin-binding proteins to visualize cp-actin filaments in live plant cells. It is also difficult to visualize a polymerization process of cp-actin dynamics particularly at high temporal and spatial resolutions. Together, future research on the molecular mechanism of chloroplast movement tackles new challenges. Therefore, it is necessary to create new experiment systems to analyze chloroplast photorelocation movement at the molecular level. In vitro reconstruction of chloroplast photorelocation movement would be a good example for the analysis. Protein and genetic interaction maps should be prerequisite to reveal the overall physical and functional landscapes of chloroplast photorelocation movement.

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