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Chloroplast Photorelocation Movement: A Sophisticated Strategy for Chloroplasts to Perform Efficient Photosynthesis

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1. Introduction

Chloroplasts move to weak light so that they can perceive light efficiently (the accumulation response), whereas they escape from strong light to avoid photodamage (the avoidance response) (Fig. 1).

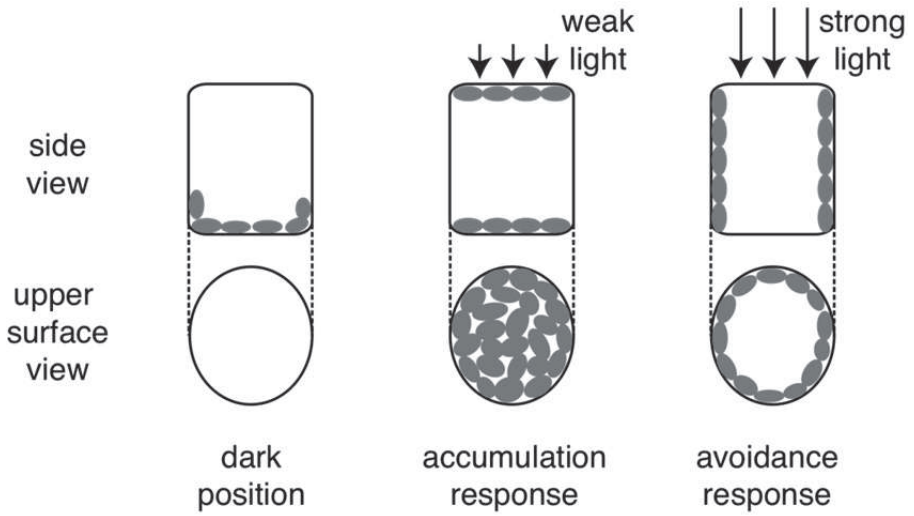

Fig. 1. Typical intracellular distribution pattern of chloroplasts by their photorelocation movement. In darkness, chloroplasts are located on the cell bottom in *Arabidopsis thaliana*. Note that the dark position varies among plant species. Weak light induces the chloroplast accumulation response along the peliclinal walls so that chloroplasts can perceive light efficiently. Strong light induces the chloroplast avoidance response toward the anticlinal walls to reduce photodamage.

The phototropin photoreceptor family of proteins, which includes phototropin (phot) and neochrome (neo), mediate chloroplast photorelocation movement in green plants (reviewed

by Suetsugu & Wada, 2007b, 2009). Phot mediates blue-light-induced chloroplast movement in most green plant species, and neo mediates red-light-induced chloroplast movement in ferns and some green alga (reviewed by Suetsugu & Wada, 2005, 2007a). Like other plant organelle movement responses, chloroplast photorelocation movement depends on actin filaments (reviewed by Wada & Suetsugu, 2004). Detailed physiological and photobiological analyses revealed that chloroplasts could move in any direction without turning or rolling within a short lag time during both the accumulation and the avoidance responses (Tsuboi et al., 2009; Tsuboi & Wada, 2011a). This fact argued that chloroplasts move by utilizing preexisting actin filaments and myosins. However, recent detailed microscopic analyses in the flowering plant *A. thaliana* (Kadota et al., 2009), the fern *Adiantum capillus-veneris* (Tsuboi & Wada, 2011b) and the moss *Physcomitrella patens* (Yamashita et al., 2011) have revealed that short actin filaments around the periphery of chloroplasts (called as cp-actin filaments) but not cytoplasmic actin cables are involved in chloroplast photorelocation movement and in the attachment to the plasma membrane (Fig. 2). Furthermore, chloroplast photorelocation movement was normal in all of the examined multiple-myosin knockout plants and even in myosin mutant plants severely defective in movements of the mitochondria, Golgi bodies, peroxisomes, endoplasmic reticulum and cytoplasm (Suetsugu et al., 2010b). Molecular genetic analyses using *A. thaliana* are of a great benefit to the study of chloroplast movement. First, various molecular factors that regulate cp-actin filament generation and reorganization during chloroplast movement can be identified (Fig. 3). Two phototropins, phot1 and phot2, mediate chloroplast photorelocation movement (Jarillo et al., 2001; Kagawa et al., 2001; Sakai et al., 2001) by reorganizing cp-actin filaments (Kadota et al., 2009, Ichikawa et al., 2011). Two interacting coiled-coil proteins, WEB1 (weak chloroplast movement under blue light 1) and PMI2 (plastid movement impaired 2), regulate the velocity of chloroplast movement via light-induced cp-actin filament reorganization, possibly by suppressing JAC1 (J-domain protein required for chloroplast accumulation response 1) (Kodama et al., 2010, 2011; Luesse et al., 2006; Suetsugu et al., 2005a). A chloroplast outer envelope protein, CHUP1 (chloroplast unusual positioning 1), and two kinesin-like proteins, KAC1 (kinesin-like protein for actin-based chloroplast movement 1) and KAC2, are indispensable for cp-actin filament formation (Kadota et al., 2009; Oikawa et al., 2003; Suetsugu et al., 2010a). CHUP1 and KAC1 showed in vitro F-actin binding activity, and CHUP1 also interacted with G-actin and profilin in vitro (Oikawa et al., 2003; Schmidt von Braun & Schleiff, 2008; Suetsugu et al., 2010a), suggesting the direct involvement of these proteins in cp-actin filament generation and regulation. Most of these components are highly conserved in land plants from bryophytes to angiosperms (reviewed by Suetsugu et al., 2010b), suggesting that cp-actin filament-mediated chloroplast movement may facilitate the explosive evolution of land plants when in a fluctuating, ambient light environment. Second, the availability of mutants deficient in chloroplast movement encouraged us to verify a long-standing hypothesis that chloroplast movement is required for efficient photosynthesis in fluctuating light conditions. Experiments using mutants deficient in avoidance movement showed that the avoidance response is necessary for reducing photodamage under strong light conditions (Kasahara et al., 2002)(Fig. 4a). Some reports have suggested that the avoidance response (i.e. the distribution of chloroplasts on the anticlinal walls) affects CO_2 diffusion by changing the chloroplast surface that is exposed to intercellular air spaces and that the avoidance response in the upper part of the leaf could

facilitate leaf photosynthesis by allowing greater light penetration to lower parts within the leaf (reviewed by Suetsugu & Wada, 2009) (Fig. 4b & 4c). However, these hypotheses are controversial and have not yet been clearly demonstrated experimentally.
In this chapter, we review three topics of chloroplast photorelocation movement: (i) the insights gained from physiological and photobiological analyses, (ii) the molecular mechanism and (iii) the contribution to photosynthesis.

2. From physiological analyses to molecular genetic analyses

Light-induced chloroplast movement (chloroplast photorelocation movement) has fascinated plant biologists since its discovery in the mid-nineteenth century (Böhm, 1856). Comprehensive analyses by Gustav Senn (1875-1945) of chloroplast movement in various plant species revealed the general responses of chloroplasts to light intensity and direction; chloroplasts are distributed at a position that ensures more efficient light absorption under weak light conditions, and they are positioned away from strong light, as if they had escaped (Senn, 1908). In land plant species, which generally bear multiple small chloroplasts in a cell, low light induces chloroplast movement and distribution toward the periclinal walls (the accumulation response), whereas strong light induces chloroplast avoidance toward the anticlinal walls (the avoidance response) (Fig. 1). Blue light is most effective at inducing chloroplast movement in most plant species, but in some cryptogam plant species, red light as well as blue light is effective. This red light effect exhibits red/far-red light reversibility, suggestive of the involvement of a red/far-red light receptor phytochrome. Detailed photobiological analyses, especially by the research groups of Wolfgang Haupt (1921-2005) and Jan Zurzycki (1925-1984), have provided many important insights on putative photoreceptor molecules that regulate chloroplast movement (Haupt, 1999; Zurzycki, 1980). They found that membrane-bound blue light photoreceptors other than phytochrome mediate blue-light-induced chloroplast movement in most plant species and that membrane-bound phytochromes mediate red-light induced chloroplast movement in some cryptogam plants. These predictions were demonstrated by the recent identification of photoreceptor genes in various plant species. The blue light receptor phot mediates blue-light-induced chloroplast movement in various plant species (Jarillo et al., 2001; Kagawa et al., 2001, 2004; Kasahara et al., 2004; Sakai et al., 2001). Neo, the chimeric photoreceptor that is a fusion of phytochrome and phototropin, regulates red-light-induced chloroplast movement in ferns and some green alga (Kawai et al., 2003; Suetsugu et al., 2005b). The photoreceptors are not discussed here; for a comprehensive review, see Suetsugu & Wada, 2005, 2007a, 2007b, 2009. First, we show our attempts to elucidate the mechanism of chloroplast photorelocation movement by detailed photobiological analyses. Second, we review recent molecular biological analyses of chloroplast photorelocation movement. Finally, the contribution of chloroplast movement and positioning to photosynthesis is discussed.

2.1 Elucidation of the mechanism of chloroplast photorelocation movement by detailed photobiological analyses

The underlying processes of chloroplast photorelocation movement can be categorized into three parts: photoperception, signal transduction and the motility system. Most of the photobiological analyses of chloroplast photorelocation movement were performed to

identify the photoreceptor molecules (reviewed by Haupt, 1999; Zurzycki, 1980; Wada et al., 1993). Many pharmacological (i.e. treatment with chemicals and inhibitors) and microscopic (i.e. staining of the cytoskeleton) analyses have provided valuable insights, such as the possible involvement of calcium ions in the signal transduction pathway and the actin filament-dependency of the motility system (reviewed by Suetsugu & Wada, 2007b, 2009). However, the data from pharmacological treatment and microscopic observation of fixed samples should be carefully considered because of possible artifactual results. Thus, we decided to analyze chloroplast relocation movement using detailed physiological and photobiological analyses of the gametophytic cells of a fern *A. capillus-veneris* as a model system (reviewed by Wada, 2007). By changing light conditions, we can easily obtain two types of gametophytes: a filamentous protonemal cell or a two-dimensional prothallus, which is a cell sheet made of a one-cell layer. This fern regulates chloroplast movement by utilizing phot family proteins and actin filaments, like *A. thaliana* (Kadota & Wada, 1992; Kagawa et al., 2004; Tsuboi & Wada, 2011b). Using a microbeam irradiation system, we analyzed chloroplast photorelocation movement in protonemal and phothallial cells and elucidated several aspects of chloroplast movement, especially putative signaling molecules and movement.

2.1.1 Physiological properties of putative signals in chloroplast photorelocation movement

Blue light mediates the influx of calcium ions (Ca^{2+}) into the cytosol and this influx is dependent upon phots in *A. thaliana* and *P. patens* (reviewed by Harada & Shimazaki, 2007). Importantly, a Ca^{2+} chelator inhibited chloroplast movement and external Ca^{2+} ions and Ca^{2+} ionophores changed the distribution of chloroplasts when placed in darkness (reviewed by Suetsugu & Wada, 2009). However, plasma membrane Ca^{2+} channel blockers, which effectively inhibited phot-dependent blue light-mediated Ca^{2+} influx (reviewed by Harada & Shimazaki, 2007), were totally ineffective in suppressing chloroplast photorelocation movement in various plant species (reviewed by Suetsugu & Wada, 2009). Thus, the putative signals that control chloroplast movement remain to be determined. To characterize the properties of these putative signals, chloroplast photorelocation movement was induced by partial cell irradiation with a microbeam irradiator and analyzed in detail (Kagawa & Wada, 1999, 2000; Tsuboi & Wada, 2010a, b).

An open question was whether the signals were different between the accumulation and avoidance responses. When a dark-adapted cell of an *A. capillus-veneris* prothallus (Kagawa & Wada, 1999) and an *A. thaliana* leaf (Kagawa & Wada, 2000) (in this situation, a few chloroplasts were on the upper periclinal walls) were partially irradiated with strong blue light, chloroplasts moved to the irradiated area but could not enter the beam area. Immediately after the light was turned off, the chloroplasts moved into the formerly irradiated area. Similar responses were also found in filamentous protonemal cells in *A. capillus-veneris* (Yatsuhashi et al., 1985) and *P. patens* (Kadota et al., 2000; Sato et al., 2001). These results suggested several characteristics of putative signals (reviewed by Suetsugu & Wada, 2009): (i) Signals for both the accumulation and the avoidance responses are simultaneously generated by strong light. (ii) Signals for the avoidance response function only at the irradiated area whereas those for the accumulation response can be transferred toward chloroplasts when located far from the irradiated area. (iii) Signals for the avoidance response disappear immediately after the light is turned off, whereas those for the

accumulation response are long-lived. (iv) Signals for the avoidance response can be override those for the accumulation response, at least in the irradiated area. Alternatively, it is possible that the signals for the avoidance response can be generated only when chloroplasts are directly irradiated with strong light. In this case, it is likely that the photoreceptor is localized on chloroplasts or that a plasma membrane-localized receptor generates the signals only when it exists in close proximity to the chloroplasts.

However, it is clear that the signals for the accumulation response are generated by the activation of photoreceptors in the irradiated area and are subsequently transferred toward chloroplasts. If measuring the speed of signal transfer for the accumulation response were possible, we could guess as to what is the putative signal by comparing the speeds between the putative signal and the known signaling molecules. When the chloroplast accumulation response was induced by microbeam irradiation in *A. capillus-veneris* protonemal cells, the onset of the accumulation movement lagged in proportion to the increase in the distance between the irradiated area and the chloroplasts, suggesting that the speed of the signal can be calculated as the lag time before the onset of movement (Tsuboi & Wada, 2010a, b). Similar calculations were also performed in *A. capillus-veneris* prothallial and *A. thaliana* mesophyll cells (Tsuboi & Wada, 2010a, b). These analyses revealed three interesting features of the putative signals. First, in protonemal cells, the speed of the signals in the basal-to-apical directions (about 2.3-2.4 μm min^{-1}) was about three times faster than that in the apical-to-basal direction (about 0.6-0.9 μm min^{-1}). However, the speed of the signals was almost equal in each cell type (about 0.9-1.1 μm min^{-1} in *A. capillus-veneris* prothallial cells and about 0.7 μm min^{-1} in *A. thaliana* mesophyll cells) (Tsuboi & Wada, 2010a, b). This difference in speed could result from the difference in the cell growth pattern of the cells, i.e. polarized (protonemal cells) and diffusive (prothallial and mesophyll cells). Second, in fern gametophytic cells, the speed of the signal and the maximum distance over which the signals could be transferred were almost equal irrespective of the intensity of the red or blue light microbeam, although in this case, more chloroplasts that were located farther away responded under continuous irradiation, compared to those submitted to a 1 min pulse of irradiation (Tsuboi & Wada, 2010a, b). Interestingly, the velocity of chloroplast accumulation movement was constant, regardless of the intensity of the microbeam placed on the prothallial cells (Kagawa & Wada, 1996; Tsuboi & Wada, 2011a). These results suggested that the properties of the signal, such as the speed, the amount and the activity, do not change in proportion to the change of light intensity. However, chloroplasts in the protonemal cells accumulated in the area that had been irradiated by a beam with a higher fluence rate, compared to adjacent areas that had been irradiated with beam of lower fluence rates of blue or red light. This result suggests that the amount or activity of the signal was increased when exposed to a beam with a higher fluence rate (Yatsuhashi et al., 1987; Yatsuhashi, 1996). Third and most importantly, the speed of signals (about 0.6-2.4 μm min^{-1}) was much slower than that caused by calcium ion spiking or waves known to occur in plant and animal systems (about several μm sec^{-1} to 100 μm sec^{-1}) (Tsuboi & Wada, 2010a, b). Furthermore, the signal transfer must not be actomyosin-dependent because the transfer of the signal still occurred when actin filaments were disrupted by treatment with inhibitor (Sato et al., 2001). Collectively, although the signals for chloroplast movement remained to be determined, our detailed physiological analyses will provide the clue to identify the actual signals.

2.1.2 An actin-based motility system deduced by detailed observation of chloroplast movement

For a long time, it was believed that the actomyosin system mediated chloroplast movement in various species. Many analyses using several kinds of techniques (such as inhibitor treatment, immunocytochemistry and observation of the in vivo dynamics of actin filaments) clarified the involvement of actin filaments in chloroplast movement in various plant species (reviewed by Suetsugu & Wada, 2009; Suetsugu et al., 2010b). However, the involvement of myosin motor proteins was still controversial (reviewed by Suetsugu et al., 2010b). If the actomyosin system is involved in chloroplast movement, it is expected that chloroplasts move along long actin cables that preexist or elongate in the direction of movement immediately after light exposure and, thus, that chloroplast movement should be polarized (i.e. parallel to actin cables). However, this was not the case with chloroplast photorelocation movement at least in *A. capillus-veneris* prothallial and *A. thaliana* mesophyll cells (Tsuboi & Wada, 2009, 2011a). Importantly, chloroplasts moved by sliding but not rolling during both the accumulation and the avoidance responses (Tsuboi & Wada, 2009, 2011a), suggesting that chloroplasts moved by attaching one side to the plasma membrane via actin filaments that spanned between the chloroplasts and the plasma membrane. When observed with a microscope, chloroplasts look elliptic (or dumbbell-shaped for dividing chloroplasts) but not completely round. Therefore, it is plausible that chloroplasts keep their long axis in parallel with the moving direction so that they can take the path of least resistance. If that is the case, then they should turn at an angle formed by an imaginary line spanning their long axis and a second imaginary line that connects the center of the chloroplast, at the original position, to the center of the irradiated area. However, chloroplasts were capable of moving in any direction even without turning. Even if chloroplasts turned immediately before or while they moved, the extent of their turning was so small (Tsuboi & Wada, 2009, 2011a). Exceptionally, chloroplasts of Arabidopsis mesophyll cells tended to adjust their short axis to be parallel with the moving direction during the avoidance movement, although they started to move without turning (Tsuboi & Wada, 2011a). Importantly, chloroplasts escaped from strong light by taking the shortest route, suggesting that they are capable of determining the location of the closest area that is out of the strong light (Tsuboi & Wada, 2011a). Moreover, when sequentially irradiated with weak or strong light, chloroplasts could change their moving direction according to the position of subsequent irradiated beam, with a short lag time (Tsuboi & Wada, 2009, 2011a). Collectively, these detailed microscopic analyses argued against the hypothesis that chloroplasts utilize of pre-existing actin filaments for photomovement and suggested that they move using actin filaments that dynamically reorganize in response to light irradiation.

2.2 Conserved molecular mechanism of chloroplast photorelocation movement in land plants

Generally, blue light is most effective in inducing chloroplast photorelocation movement, although red light can also induce the movement in some cryptogam plants (green algae, mosses and ferns). Phot is the blue light receptor for chloroplast movement and also mediates phototropism and stomatal opening (reviewed by Christie, 2007). Phototropins were identified in green plants, from green alga to seed plants, and were shown to regulate blue-light-induced chloroplast movement at least in *A. thaliana*, *A. capillus-veneris* and *P. patens* (reviewed by Suetsugu & Wada, 2005, 2007a, 2007b, 2009). Red-light-induced

chloroplast movement is mediated by neo in several ferns and probably in some green algae (reviewed by Suetsugu & Wada, 2005, 2007a, 2007b, 2009). Regardless of significant advances in photoreceptor identification, the molecular mechanism of signal transduction and the identity of the motility system for chloroplast movement have been obscure. However, molecular genetic analyses using *A. thaliana* have identified several components that regulate chloroplast movement. Furthermore, recent imaging analyses have revealed that a novel actin-based mechanism governs chloroplast photorelocation and positioning. By combining these results, we could imagine the molecular framework of chloroplast photorelocation movement.

2.2.1 Unique actin-based mechanism for chloroplast movement in land plants

For many years, it was thought that chloroplasts moved along long cytosolic actin cables by myosin motor proteins, similar to the movements of other organelles. However, the aforementioned studies (Tsuboi & Wada, 2009, 2011a) suggested that chloroplasts could utilize an actin-based mechanism that is different from those of other organelles.

To find the actin-based mechanism for chloroplast movement, we utilized Arabidopsis transgenic lines in which actin filaments could be visualized by various fusions of fluorescent proteins and actin binding proteins (such as GFP-talin and tdTomato-fimbrin) and analyzed the behavior of the actin filaments during chloroplast movement using a custom-made microscope and a confocal microscope (Kadota et al., 2009). Although cytoplasmic actin cables and filaments were associated with chloroplasts, they did not change much in response to light irradiation, and their behavior did not associate with directional chloroplast movement. Instead, we found that short actin filaments found around chloroplasts dynamically changed their structure in response to light irradiation and that their dynamics correlated with the direction and speed of chloroplast movement. We have named these actin filaments chloroplast-actin filaments (abbreviated as cp-actin filaments) (Kadota et al., 2009)(Fig. 2). When chloroplasts were stationary, cp-actin filaments were distributed around the chloroplast periphery. In response to strong blue light, cp-actin filaments transiently disappeared within about 30 seconds and then reappeared at the one side of the chloroplasts, which would eventually be the front region of the moving chloroplasts (Fig. 2). We called this pattern of localization of cp-actin filaments at the front region “biased” (Fig. 2). After biased cp-actin filaments were fully formed, chloroplasts moved toward the side where the cp-actin filaments accumulated (Kadota et al., 2009). The generation of biased cp-actin filaments was also found during the accumulation response that had been induced by weak blue light, but this was not accompanied by a transient disappearance of cp-actin filaments, unlike what occurred during the avoidance response (Kadota et al., 2009). Thus, the light-induced generation of biased cp-actin filaments is a prerequisite for both the avoidance and the accumulation responses. Possibly, a transient disappearance of cp-actin filaments induced by strong blue light facilitated an acceleration of chloroplast avoidance movement. As more cp-actin filaments accumulated at the front halves of the chloroplasts, in relation to the rear halves, the velocity of chloroplast avoidance also increased. When irradiated with a higher fluence of blue light, even more cp-actin filaments accumulated at the front halves, and chloroplasts moved even faster (Kadota et al., 2009). Thus, strong light caused a greater difference in the amount of cp-actin filaments at certain locations on the chloroplasts because cp-actin filaments located at the rear halves of the chloroplasts did not increase after transient disappearance. Conversely, weak light could not induce transient disappearance of cp-actin filaments, so a greater difference in

the amount of cp-actin filaments at certain locations was not made. Actually, the velocity of chloroplast accumulation movement was constant irrespective of light intensity (Kagawa & Wada, 1996; Tsuboi & Wada, 2011a). Cp-actin filaments localized at the interface between the chloroplast and the plasma membrane, elongated from the edge of the chloroplast and shortened toward the chloroplast periphery, suggesting that the nucleation site of cp-actin filaments might exist on the chloroplast edge and that the force for chloroplast movement by cp-actin filaments might be generated there (Kadota et al., 2009). Cp-actin filaments mediated the anchoring of chloroplasts to the plasma membrane as well as their directional movement (Kadota et al., 2009). The strong-light-induced disappearance of cp-actin filaments was accompanied by increased chloroplast motility in random directions before avoidance movement, suggestive of the detachment of chloroplasts from the plasma membrane. Conversely, weak blue light induced the increase of cp-actin filaments around the chloroplast periphery and accompanied a decrease in chloroplast motility, likely facilitating chloroplast anchoring to the plasma membrane. In summary, there are three types of blue-light-induced rearrangements of cp-actin filaments that mediate both directional movement and the anchoring of chloroplasts to the plasma membrane: (i) the formation of biased cp-actin filaments during both the accumulation and the avoidance responses; (ii) a strong-blue-light-induced transient disappearance of cp-actin filaments; (iii) a weak-blue-light-induced increase in cp-actin filaments. Importantly, cp-actin filament-mediated chloroplast movement is conserved in a fern, *A. capillus-veneris*, and in a moss, *P. patens* (Tsuboi & Wada, 2011b; Yamashita et al., 2011). Thus, the regulation of chloroplast movement by cp-actin filaments was likely to be utilized during the early stages of land plant evolution.

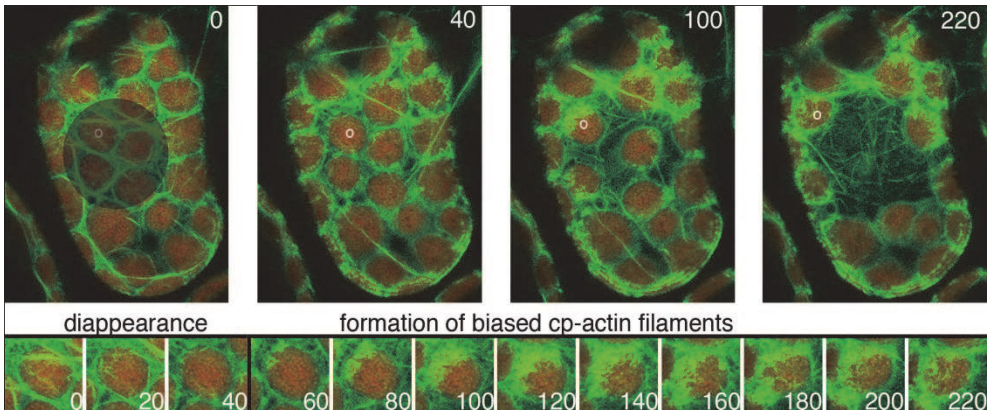

Fig. 2. Light-induced cp-actin filament reorganization during the chloroplast avoidance response. The chloroplast avoidance response was induced by scanning circular regions of interest (diameter 15 μm indicated by a shaded circle) with 2.8 mW of a 458 nm laser and using a confocal microscope (SP5, Leica). Time-lapse images of chloroplast movement and the associated cp-actin filament dynamics were captured at the indicated times (sec). Detailed dynamics of the cp-actin filaments of a chloroplast marked with a white circle are indicated below. After 40 sec of light irradiation, cp-actin filaments disappeared and then reappeared at the front region of the chloroplast, which had moved to the upper left side in this figure via the avoidance response.

2.2.2 Molecular components regulating the generation and/or reorganization of cp-actin filaments

We identified various Arabidopsis mutants deficient in chloroplast photorelocation movement (Kagawa et al., 2001; Kodama et al., 2010; Oikawa et al., 2003; Suetsugu et al., 2005, 2010a), and thus, the analyses of cp-actin filament behavior in these mutants have shed light on the molecular mechanism of cp-actin filament-mediated chloroplast movement (Kadota et al., 2009; Kodama et al., 2010; Suetsugu et al., 2010a; Ichikawa et al., 2011)(Fig.3).

Phototropin is a blue light receptor bearing two photosensory LOV (light, oxygen and voltage) domains at its N-terminus and a C-terminal serine/threonine kinase domain (reviewed by Christie, 2007). In *A. thaliana*, phot1 and phot2 redundantly mediated the chloroplast accumulation response (Sakai et al., 2001), and phot2 alone regulated the avoidance response (Kagawa et al., 2001; Jarillo et al., 2001). In *phot1phot2* double mutant plants, which are completely defective in chloroplast photorelocation movement (Sakai et al., 2001), blue-light-induced cp-actin filament reorganization did not occur, indicating that phototropins mediated chloroplast movement via the regulation of cp-actin filaments (Kadota et al., 2009; Ichikawa et al., 2011). The *phot1phot2* double mutant plants also did not change their amounts of cp-actin filaments in response to both weak and strong blue light and thus showed no light-induced motility changes (Kadota et al., 2009). This outcome indicated that phototropins mediated anchoring of the chloroplast to the plasma membrane via regulation of the amounts of cp-actin filaments. The strong-blue-light-induced transient disappearance of cp-actin filaments did not occurr at all in *phot2* mutant plants, which were impaired in the avoidance response. However, they showed normal biased cp-actin filament formation during the accumulation response (Kadota et al., 2009; Ichikawa et al., 2011), indicating that phot2 mediated the strong-blue-light-induced transient disappearance of cp-actin filaments (Fig.3) and that this reorganization of cp-actin filaments could be a prerequisite for the avoidance response. In *phot1* mutant plants, chloroplast photorelocation movement was only slightly impaired in the accumulation response (Kagawa & Wada, 2000), and therefore light-induced reorganization of cp-actin filaments in these plants was mostly normal (Kadota et al., 2009). However, in response to strong blue light, the onset of biased cp-actin formation and the avoidance movement in *phot1* mutants occurred earlier than in wild-type plants (Ichikawa et al., 2011), suggesting a small inhibition of cp-actin filament reorganization by phot1 during the avoidance movement.

JAC1 has a J-domain at the C-terminus and is similar to a clathrin uncoating factor, auxilin (Suetsugu et al., 2005). The J-domain of JAC1 is necessary for JAC1 function and the crystal structure showed high similarity between that domain and that of the bovine auxilin J-domain (Takano et al., 2010; Suetsugu et al., 2010c). *jac1* mutant plants were completely defective in the accumulation response but retain the avoidance response (Suetsugu et al., 2005). In response to weak blue light, the reorganization of cp-actin filaments did not occur in most chloroplasts of *jac1* mutant plants, but a few chloroplasts that avoided weak light formed biased cp-actin filaments (Ichikawa et al., 2011), indicating that JAC1 is essential for the reorganization of cp-actin filaments during the accumulation response but not for biased cp-actin filament formation (Fig. 3). Interestingly, in *jac1* mutant plants, whole cell irradiation with strong blue light did not induce the disappearance and subsequent biased localization of cp-actin filaments and thus the avoidance movement did not occur. However, when part of a cell was irradiated, chloroplasts that were close to the beam edge showed the avoidance movement with biased cp-actin filament formation, although cp-actin filaments on chloroplasts inside the

beam did not disappear and their motility did not increase (Ichikawa et al., 2011). These results indicate that JAC1 is essential for an efficient chloroplast avoidance response by regulating the disappearance of cp-actin filaments (Fig. 3).

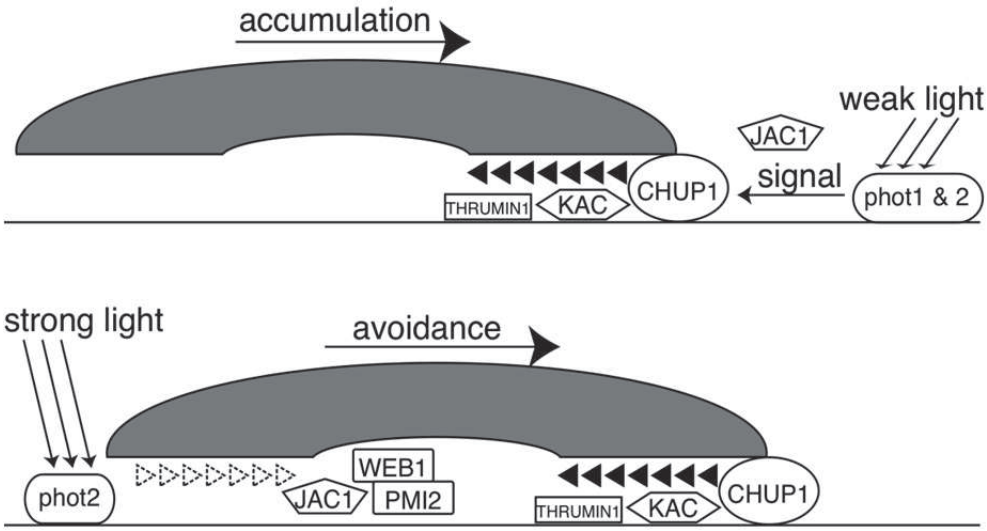

Fig. 3. A schematic model of cp-actin filament-mediated chloroplast movement. Weak light activates both phot1 and phot2, which are localized on the plasma membrane and subsequently generate an as yet unidentified signal that initiates the chloroplast accumulation response. JAC1 may be involved in signal generation, transport and/or perception. The signal activates a cp-actin filament nucleation complex, which is localized at the chloroplast edge, resulting in the polymerization of cp-actin filaments at the leading edge of the chloroplasts (black arrowheads indicate G-actins). CHUP1 could be the nucleation factor, and KAC proteins could be involved in cp-actin filament nucleation and/or maintenance. THRUMIN1 may interact with cp-actin filaments because THRUMIN1-YFP fusion protein decorated actin filaments in vivo. Strong-light-induced cp-actin filament disappearance (indicated by broken-lined arrowheads) is mediated by phot2, JAC1 and WEB1/PMI2. After disappearance, cp-actin filaments reappeared at the leading edge, and the chloroplasts escaped from the strong light.

Recently, we identified two coiled-coil proteins, WEB1 and PMI2, as factors that regulate light-induced cp-actin filament reorganization (Kodama et al., 2010; Luesse et al., 2006). WEB1 and PMI2 belong to a coiled-coil protein family that contains a DUF827 (Domain of Unknown Function 827) domain (Kodama et al., 2010, 2011). WEB1 and PMI2 interacted with each other in yeast and plant cells, and WEB1 showed self-interaction activity, forming large complexes in plant cells, indicating that both WEB1 and PMI2 have protein-protein interaction activity (Kodama et al., 2010). Both *web1* and *pmi2* mutant plants showed severe defects in the avoidance response and slight defects in the accumulation response. Because the phenotypes of *web1pmi2* double mutant plants were very similar to those of *web1* and *pmi2* single-mutant plants, it was concluded that WEB1 and PMI2 probably function in the same pathway, possibly as a complex (Kodama et al., 2010, 2011). Because these mutants

were severely impaired in the strong-light-induced disappearance and subsequent biased localization of cp-actin filaments, it was concluded that the mutant phenotypes observed were a result of the impairment in cp-actin filament reorganization (Kodama et al., 2010)(Fig. 3). The defective avoidance response phenotype in these mutants was suppressed by a *jac1* mutation, suggesting a role for WEB1 and PMI2 in suppressing JAC1 activity, which regulates the accumulation response under high light conditions (Kodama et al., 2010). Given that the strong-light-induced reorganization of cp-actin filaments was severely impaired in *web1*, *pmi2* and *jac1* mutant plants (Kodama et al., 2010; Ichikawa et al., 2011), it is possible that WEB1/PMI2 and JAC1 cooperatively mediate the strong-light-induced reorganization of cp-actin filaments, although the detailed molecular mechanism remains to be determined.

Currently, two types of proteins, CHUP1 and KAC (KAC1 and KAC2), were identified as the factors necessary for the existence of cp-actin filaments, possibly serving as nucleators and/or stabilizers of cp-actin filaments (reviewed by Suetsugu et al., 2010b). CHUP1 is a multi-domain protein that bears an N-terminal hydrophobic region, a coiled-coil region, an F-actin-binding domain, a proline-rich region and a highly conserved C-terminal region (Oikawa et al., 2003). The hydrophobic region is essential for the localization of chloroplast outer envelope (Oikawa et al., 2003, 2008; Schmidt von Braun & Schleiff, 2008) and the coiled-coil region confer the ability of the protein to dimerize in vitro (Lehmann et al., 2011). The actin-binding domain was capable of interacting with F-actin in vitro (Oikawa et al., 2003), and the proline-rich region might serve as the profilin-interacting domain (Schmidt von Braun & Schleiff, 2008). KAC proteins belong to a microtubule motor kinesin-14 subfamily, but their motor and microtubule-binding activities have not yet been detected (Suetsugu et al., 2010a). A subset of KAC proteins was associated with the plasma membrane and chloroplast envelope although the bulk of the KAC proteins were found as soluble proteins (Suetsugu et al., 2010a). Both *chup1* and *kac1kac2* double mutant plants completely lacked cp-actin filaments but retained the normal cytosolic actin filament structure, indicating that the CHUP1 and KAC proteins are essential for cp-actin filament formation and/or maintenance (Kadota et al., 2009; Suetsugu et al., 2010a)(Fig. 3). Importantly, both mutants showed no chloroplast photorelocation movement and defects in the anchoring of chloroplasts to the plasma membrane. This result reinforced the notion that cp-actin filaments mediate photorelocation and the anchoring of chloroplasts to the plasma membrane. In *kac1* single mutant plants, significantly fewer amounts of cp-actin filaments were observed, the accumulation response was severely impaired and the velocity of the avoidance movement was much slower compared to wild-type plants (Suetsugu et al., 2010a). Thus, the amount of KAC proteins is an important factor that determines the chloroplast velocity by regulating the amounts of cp-actin filaments.

Although PMI1 and THRUMIN1 were identified through the analyses of mutants deficient in chloroplast photorelocation movement (DeBlasio et al., 2005; Whippo et al., 2011), their involvement in cp-actin filament regulation is unknown. Because THRUMIN1 has an actin bundling activity (Whippo et al., 2011), analyses of mutants deficient in these factors will reveal a more detailed framework of cp-actin filament-dependent chloroplast movement.

Three essential genes for cp-actin filament-mediated chloroplast movement, *PHOT*, *CHUP1* and *KAC*, are found in the genome of a liverwort, *Marchantia polymorpha*, and a moss, *P. patens*. Because cp-actin filament-mediated chloroplast movement is found in *P. patens*

(Yamashita et al., 2011), the molecular mechanism of cp-actin filament-mediated chloroplast movement must have evolved early in land plant evolution.

2.3 Contribution of chloroplast photorelocation movement to photosynthesis

The intracellular distribution of chloroplasts is essential for the promotion of photosynthetic performance. For example, the chloroplast distribution in bundle sheath cells of C4 plants may be necessary for efficient C4 photosynthesis because it controlled CO_2 diffusion and/or facilitated the metabolite exchange between mesophyll and bundle sheath cells (reviewed by von Caemmerer & Furbank, 2003). The chloroplast accumulation response could play an important role in efficient light capture under weak light conditions, although it has not been demonstrated experimentally (Zurzycki, 1955). The avoidance response is required for chloroplats to escape from photodamage under excess light conditions (Fig. 4a); however, two other hypotheses exist that could also explain the ecological advantages of chloroplast distribution on anticlinal walls by the avoidance response (Fig. 4b & 4c).

2.3.1 Promotion of light penetration to deeper leaf cell layer by the avoidance response

In the leaves of *Oxalis*, *Marah* and *Cyrtomium*, changes in leaf absorptance due to chloroplast movement positively correlated with changes in fluorescence emission; in particular, changes in fluorescence emissions increased during the avoidance response induced by strong blue light, whereas they decreased during subsequent relaxation in red light (Brugnoli & Björkman, 1992). Considering that leaves consist of multiple layers of photosynthetic cells and that the efficiency of net leaf photosynthesis depends on the efficient light utilization of chloroplasts in all cell layers, it is reasonable to conclude that chloroplast distribution to anticlinal cell walls, as a result of the avoidance response in the upper cell layer (or palisade layer), could facilitate the penetration of the incident light to a deeper cell layer (or sponge layer)(Fig. 4b). Light transmittance through the palisade layer was greater in high light-irradiated *Alocasia* leaves than in dark-adapted leaves. However, the difference in light transmittance through the spongy layer was not significant between high light- and dark-adapted leaves, indicating that chloroplast positioning on the anticlinal walls by the avoidance response in the palisade layer could facilitate light penetration to a deeper layer (Gorton et al., 1999). In *Tradescantia* leaves, which consist of three mesophyll cell layers (the first is a palisade layer, and the second and third are sponge layers), the chloroplasts in the second layer did not move to the anticlinal walls when irradiated with strong light of 100 μmol m^{-2} s^{-1} from either the adaxial or the abaxial side. However, by abaxial-side irradiation, chloroplasts in the third layer were positioned on the anticlinal walls by way of the avoidance response (Terashima & Hikosaka, 1995). These results suggest that the avoidance response in the surface mesophyll layer facilitates light capture in the deeper cell layers, resulting in a net increase of whole leaf photosynthesis. However, this hypothetical role of the avoidance movement in the enhancement of light penetration to the deeper cell layers has not yet been demonstrated conclusively.

2.3.2 Influence of the chloroplast avoidance response on CO_2 diffusion between air spaces and mesophyll cells

The diffusion path length of CO_2 from intercellular air spaces to the chloroplast stroma must be short so that mesophyll chloroplasts can efficiently utilize CO_2 from those air spaces.

Thus, chloroplasts should be located on the cell wall facing air spaces. Mesophyll cell chloroplasts tended to be located along intercellular air spaces in various plant species (Senn, 1908; Psaras et al., 1996) (Fig. 4c). Senn (1908) hypothesized that this positioning might result from the chemotaxis of chloroplasts to the CO_2 in air spaces. Using different approaches and plant species, three groups examined whether chloroplast photorelocation movement, especially the avoidance response, influenced CO_2 diffusion in leaves (Gorton et al., 2003; Loreto et al., 2009; Tholen et al., 2008). One research group hypothesized that chloroplast distribution on the anticlinal walls by the avoidance response facilitated CO_2 utilization by shortening the CO_2 diffusion path length from air spaces to the mesophyll chloroplasts (Gorton et al., 2003). This group measured oxygen diffusion times using pulsed photoacoustics (as a substitution for CO_2 diffusion) between control and strong light-irradiated *Alocasia* leaf samples, but they could not find any differences in CO_2 diffusion rates between the two samples (Gorton et al., 2003). Additionally, they could found no difference in the distance between the centers of the chloroplasts and the closest air spaces between two samples (Gorton et al., 2003). Another group found that blue light rapidly reduced CO_2 diffusion from intercellular air spaces to the chloroplasts in both *Nicotiana* and *Platanus* leaves and that this reduction was completed before chloroplasts finished the avoidance movement to the aniticlinal walls (Loreto et al., 2009). Importantly, the blue-light-induced reduction of CO_2 diffusion was still normal in samples treated with an anti-actin inhibitor cytochalasin, which completely inhibits chloroplast movement (Loreto et al., 2009). Thus, results by two independent groups indicated that chloroplast movement did not significantly change the efficiency of CO_2 diffusion from intercellular air spaces to the chloroplasts. However, the results by a third group suggested that the avoidance response reduced the CO_2 diffusion rate rather than increased it (Tholen et al., 2008). In *Arabidopsis* wild-type plants, the surface area of chloroplasts facing air spaces was reduced after the induction of the chloroplast avoidance response and resulted in the reduction of CO_2 diffusion. However, these reductions were not found in *phot2* and *chup1* mutant plants or in cytochalasin-treated plants (Tholen et al., 2008). Compared to wild type, the surface area of chloroplasts that faced air spaces and the rate of CO_2 diffusion were constitutively lower in *chup1* mutant plants because of aberrant positioning of their chloroplasts (Tholen et al., 2008). Collectively, these results suggested that the chloroplast avoidance response was not involved in CO_2 diffusion or that it possibly decreased, rather than increased, the diffusion rate. However, these three groups examined the contribution of chloroplast movement to CO_2 diffusion using different plant species and techniques: pulsed photoacoustics (Gorton et al., 2003), a chlorophyll fluorescence-based method (Loreto et al., 2009) and a carbon isotope discrimination method (Tholen et al., 2008). The examination of one plant species using different techniques and/or that of various plant species by one technique are required to uncover whether chloroplast photorelocation movement influences CO_2 diffusion.

2.3.3 Chloroplast avoidance response is essential for protection against photodamage by strong light

The two aforementioned hypotheses on the roles of the avoidance response are applicable only in multilayered leaf tissue and not in gametophytic cells that have single cell layers or in the filamentous structures of fern, moss, liverwort and green alga. During the early period of land plant evolution, plants were exposed directly to sunlight until seed plants eventually dominated terrestrial ecosystems and formed a dense canopy. Thus, it is plausible that the main role of the chloroplast avoidance response is to prevent chloroplasts

from photodamage caused by strong light (Zurzycki, 1957) (Fig. 4a). Shade plants (such as *Oxalis oregana* and *Tradesdantia albiflora*) showed a greater avoidance response than that of non-shade plants (such as sunflower and pea) (Brugnoli & Björkman, 1992; Park et al., 1996), partly explaining why *T. albiflora* was more tolerant of strong light stress than pea plants (Park et al., 1996). Furthermore, cytochalasin-treated *Platanus* leaves, whose chloroplast movements were inhibited by the drug, but not untreated leaves showed a strong inhibition of photochemical efficiency (Loreto et al., 2009). Clumping of chloroplasts in succulent plants (Kondo et al., 2004) and the aggregative movement of C4 mesophyll chloroplasts (Yamada et al., 2009) were induced by drought stress in a light-dependent fashion, and these

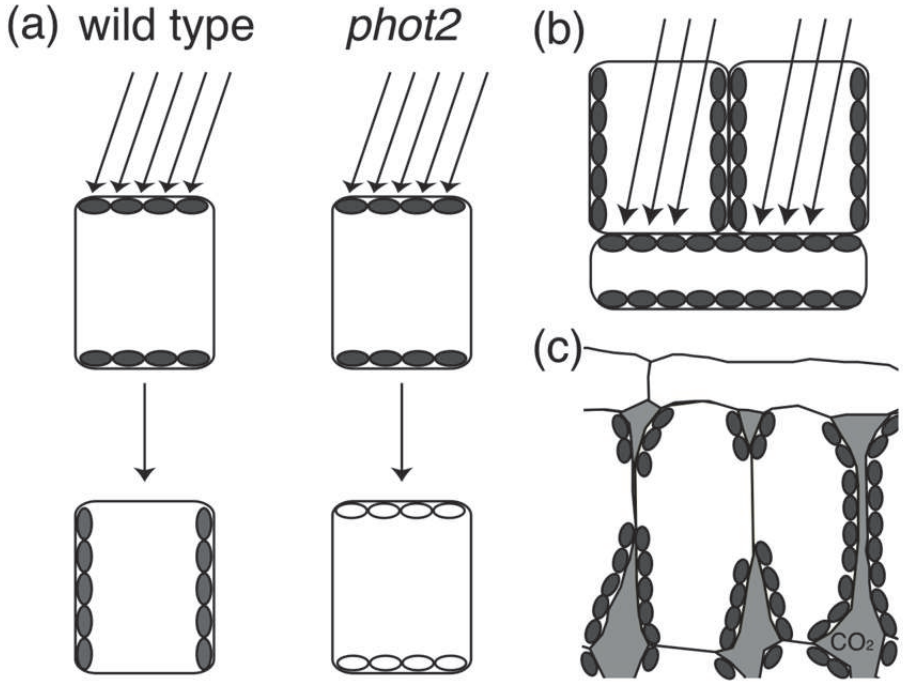

Fig. 4. Three hypotheses for the ecological significance of the chloroplast avoidance response: (a) Protection from photodamage. Chloroplasts escape from strong light and distribute along the anticlinal walls so that they do not directly perceive excess light energy, which could cause photodamage. As a result, plants can tolerate strong light stress. Mutants deficient in the avoidance response, such as Arabidopsis *phot2* mutants, cannot survive under the strong light conditions because their chloroplasts are directly exposed to extremely strong light and are therefore severely damaged and die. (b) Promotion of light penetration in leaves. Chloroplasts distributed along the anticlinal walls in the upper cell layer facilitate light penetration to deeper cell layers. Consequently, light perception and thus photosynthesis in the deeper cell layers increases. (c) Modulation of CO_2 diffusion from intercellular air spaces to the chloroplast. The chloroplast avoidance response can change the total chloroplast surface area facing airspace and thus the efficiency of CO_2 diffusion from intercellular air spaces to the chloroplast may increase.

responses were implicated in the protection from photodamage in plants that inhabit tropical and/or dry areas. Experiments using *Arabidopsis* wild-type and mutant plants definitely demonstrated that chloroplast avoidance movement is essential for the protection of plants from photodamage by strong light (Kasahara et al., 2002). Leaf transmittance in wild type and *phot1* mutant plants increased as light intensity was increased to about five-fold of the initial value (about 500 μmol m^{-2} s^{-1} of white light). However, little change in leaf transmittance occurred in *phot2* and *chup1* mutant leaves, even at 2000 μmol m^{-2} s^{-1} of white light, indicating that *phot2* and *chup1* mutant plants are defective in the avoidance response under a wide range of light intensity. When low-light-acclimated plants were shifted to a strong light condition, the leaves of *phot2* and *chup1* mutant plants were bleached after 10 h and were severely necrotic after 22 h. However, wild-type and *phot1* plants did not show leaf necrosis even after 31 h. When the change in the chlorophyll fluorescence parameter F_v/F_m (representing the maximal quantum yield of photosystem II photochemistry) was analyzed during strong light treatment, the F_v/F_m value in wild-type and *phot1* plants steeply declined to about 80% of the initial value within 1 h and then gradually decreased and finally reached about 70% of the initial value in 5 h. However, the F_v/F_m values in *phot2* and *chup1* mutant plants declined more rapidly than in wild type and consequently reached about 50% of the initial value in 5 h. In *phot2* mutants, the extent of the decrease of the F_v/F_m value after 1 h of light treatment was larger at all examined light intensities than that of wild-type plants. Furthermore, the F_v/F_m value in *phot2* mutant plants did not fully recover after 6 h, whereas the F_v/F_m values in wild-type plants almost fully recovered after 3 h under low light. Collectively, these results indicate that *phot2* and *chup1* mutant plants are highly susceptible to strong light stress and their photosystem IIs are much less tolerant of light stress resulting in leaf necrosis. Note that *phot2* and *chup1* mutant plants were normal in chlorophyll content, chlorophyll fluorescence parameters, antioxidant contents and the activities of reactive oxygen-scavenging enzymes. *phot2* mutant plants showed a slight defect in stomatal opening (Kinoshita et al., 2001), but this defect was less than that in *phot1* mutant plants and was negligible in the strong light conditions used by Kasahara et al. Recently, another research group confirmed that photosystem II of *phot2* mutant plants was more susceptible to strong light (Sztatelman et al., 2010). Overall, we conclude that chloroplast avoidance movement is indispensable for plant survival under strong light conditions.

3. Conclusion

Although chloroplast photorelocation movement has been extensively studied by many researchers, we still cannot accurately explain the molecular mechanism of chloroplast photorelocation movement. Some unanswered questions remain: what is the signal for the chloroplast accumulation response?; what protein(s) nucleate cp-actin filaments?; and how do cp-actin filaments generate the motive force for chloroplast movement? To answer these questions, chloroplast movement must be analyzed by combining various approaches: physiology, molecular biology, proteomics, crystallography and imaging techniques. Chloroplast movement under natural light conditions must also be examined because natural light is usually much more severe and always fluctuates, compared to laboratory conditions. Because various mutants deficient in chloroplast movement are available, the growth of these mutant plants under natural conditions must be analyzed, and the ecological significance of chloroplast photorelocation movement must be verified.

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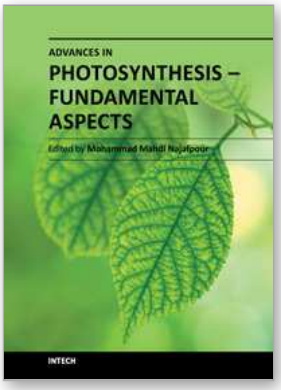

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Photosynthesis is one of the most important reactions on Earth. It is a scientific field that is the topic of many research groups. This book is aimed at providing the fundamental aspects of photosynthesis, and the results collected from different research groups. There are three sections in this book: light and photosynthesis, the path of carbon in photosynthesis, and special topics in photosynthesis. In each section important topics in the subject are discussed and (or) reviewed by experts in each book chapter.

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