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# Controlling Organelle Positioning: A Novel Chloroplast Movement Protein

It has been recognized for more than a century that chloroplasts alter their distribution within cells depending on the external light conditions. Senn (1908) documented light-induced chloroplast movement in a number of higher plants and algae, and the phenomenon has long been accepted as a means of optimizing photosynthetic light absorption under changing light conditions. Thus, chloroplasts can be observed to move to positions that maximize photon absorption under low-fluence light (along the periclinal walls parallel to the leaf surface and perpendicular to the incident light) and, conversely, to move to positions that minimize photon absorption under high-fluence light (appressed along the anticlinal walls perpendicular to the leaf surface and parallel to the incident light). The movement away from areas of strong light is believed to offer protection against photooxidative damage. Kasahara et al. (2002) recently showed that Arabidopsis mutants defective in chloroplast high-light avoidance movement are more susceptible than wild-type plants to photoinhibition under high-light conditions, confirming the physiological importance of this phenomenon.

The photoreceptors responsible for light-induced chloroplast movement in higher plants are phototropins, the blue light receptors that also mediate phototropism. In some species of green algae, moss, and fern, chloroplast relocation apparently is controlled by red light as well as blue light, suggesting that phytochrome plays a role in this process (Kagawa and Wada, 2002). Interestingly, the photoreceptor for red light control of chloroplast relocation and phototropism in the fern *Adantium capillus-veneris* was identified recently as phytochrome3, which is a chimera protein of phytochrome and phototropin that has been found only in *Adantium* and a number of other ferns (Kawai et al., 2003). Arabidopsis encodes two phototropins, PHOT1 and PHOT2 (previously known as NPH1 and NPL1, respectively [Briggs et al., 2001]). PHOT1 is the primary photoreceptor that controls phototropism in low-intensity light (Huala et al., 1997), whereas PHOT2 is responsible for the light-avoidance relocation of chloroplasts under high light (Kagawa et al., 2001; Jarillo et al., 2001). Sakai et al. (2001) demonstrated that PHOT1 and PHOT2 actually have overlapping functions in both phototropic and chloroplast relocation responses in a fluence rate–dependent manner. PHOT1 appears to function in response to a broad range of blue light intensities, whereas PHOT2 functions principally in response to high-fluence-rate blue light. Thus, PHOT1 is the primary photoreceptor for chloroplast accumulation movements under low light, but only PHOT2 is responsible for the light avoidance movement in response to high light. PHOT1 also appears to be the primary photoreceptor control-

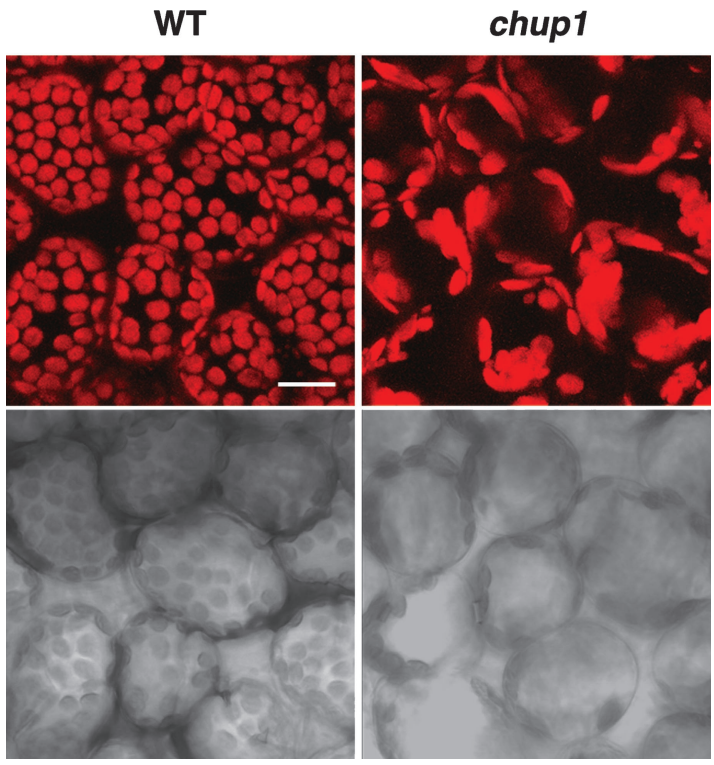


**Figure 1.** Chloroplasts from Wild-Type and *chup1* Mesophyll Cells.

Wild-type chloroplasts localize along the periclinal walls under low light, maximizing the capacity for light absorption, and relocate to become appressed against the anticlinal walls under high light, thus minimizing the potential for photoinhibition. *chup1* chloroplasts remain in relatively fixed positions under high or low light, mainly on the abaxial side of cells. Micrographs of wild-type and *chup1* cells under low light are shown. Autofluorescence from chloroplasts appears red in the top panels, and the bottom panels show light micrograph images. Bar = 20 μm.

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ling phototropic responses, but PHOT2 contributes to this response under high-intensity light (reviewed by Kagawa and Wada, 2002).

Although we are beginning to have a good understanding of the photoreceptors involved in light perception in the control of chloroplast relocation, almost nothing is known about the downstream components directly responsible for movement. In this issue of *The Plant Cell*, **Oikawa et al. (pages 2805–2815)** characterize another component controlling light-induced chloroplast movements, based on analysis of multiple Arabidopsis mutant alleles called *chloroplast unusual positioning1* (*chup1*) (Figure 1). *CHUP1* encodes an actin binding protein that is required for chloroplast relocation movements. The mutant plants fail to show chloroplast accumulation or light-avoidance movements in response to changing light conditions and instead remain in a relatively fixed position predominantly on the abaxial side of mesophyll cells. The *chup1* mutation was associated specifically with chloroplast movement, because the movement of other organelles—mitochondria and peroxisomes—was not affected similarly: because peroxisomes localize near chloroplasts, they accumulated abnormally along with the chloroplasts in mutant mesophyll cells, but they moved actively, as in wild-type cells, in mutant epidermal cells that lacked chloroplasts. The mutant plants were not impaired in phototropic responses. Therefore, the protein appears to act downstream of phototropins specifically in the chloroplast relocation response pathway.

The authors identified six different *chup1* alleles. The *chup1-1* allele was identified and cloned from a T-DNA insertional mutant population, and five other alleles were identified from an ethyl methanesulfonate–mutagenized population. All mutant alleles were found to lie within the *CHUP1* gene, and complementation tests confirmed that this gene was responsible for the mutant phenotype. *CHUP1* was found to encode a deduced protein of 1004 amino acid residues with a predicted mass of 112 kD that contains several known protein domains, including an actin binding domain, a coiled-coil region, Pro-rich motifs, Leu zipper motifs, and an N-terminal hydrophobic region. No other protein sequences encoded in the Arabidopsis genome were found that were similar to the full-length CHUP1 sequence, although three genes of unknown function encode protein sequences highly similar to a portion of the Pro-rich C-terminal region.

Results of numerous studies (Kandasamy and Meagher, 1999, and others cited by Oikawa et al.) suggest that the actin-based cytoskeleton plays a major role in the positioning and movement of chloroplasts and other organelles. The presence of an actin binding domain led to the hypothesis that CHUP1 functions in the interaction of chloroplasts with the actin-based cytoskeleton. An in vitro actin binding assay using a truncated form of CHUP1 containing the actin binding domain showed that this domain is capable of binding F-actin. Gene expression analysis showed that *CHUP1* is expressed in leaves, stems, and flowers—all tissues that contain chloroplasts—but not in roots. The N-terminal hydrophobic region was hypothesized to play a role in membrane anchoring, and ectopic expression of a green fluorescent protein (GFP) fusion with the CHUP1 N terminus in leaves showed strong fluorescence outlining the chloroplasts, consistent with targeting to the chloroplast outer envelope. Actin filaments were examined in wild-type and *chup1* plants using an actin cytoskeleton marker (mouse talin)–GFP fusion and appeared unaltered in the mutant. Thus, it is logical to hypothesize that CHUP1 functions in anchoring chloroplasts to the actin-based cytoskeleton.

The presence of numerous other motifs suggests that interaction with other factors plays an important role in CHUP1 function and raises a number of interesting mechanistic possibilities for the involvement of CHUP1 in chloroplast motility. The CHUP1 Pro-rich motif is most similar to a domain identified as a profilin binding motif called PRM1 (Holt and Koffer, 2001). Proteins that contain PRM1 recruit profilactin (profilin bound to the actin monomers) to the plasma membrane and facilitate actin polymerization. Many of these PRM1-containing proteins participate in the delivery of actin monomers to specific cellular locations where actin is involved in cell motility (i.e., the formation of membrane protrusions or the propulsion of bacteria inside host cells). Although there was no apparent difference in actin structure between wild-type and *chup1* cells based on the GFP-talin marker, it is possible that CHUP1 affects actin structure in a more subtle manner not detected by this technique, and other methods of examining actin fine structure might reveal critical differences between the wild type and the mutant. CHUP1 also contains a predicted coiled-coil region, a domain often found to mediate protein–protein interactions, to build filaments and other macroscopic structures, or to function in membrane anchoring. The coiled-coil configuration creates a highly versatile protein-folding and subunit-oligomerization motif. Coiled-coil domains often are found in cytoskeleton structural and motor proteins and other proteins involved in movement processes (e.g., cell adhesion and endocytosis) and in molecular recognition and signal transduction proteins (Burkhard et al., 2001). In addition, CHUP1 contains two Leu zipper motifs that could function in homodimerization or heterodimerization. Further study of CHUP1, including in situ localization, protein domain analysis, and yeast two-hybrid interaction assays, should help to define its function, identify interacting partners, and reveal additional information regarding organelle movement and the dynamic nature of the plant cytoskeleton.

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