DIVISION OF PHOTOBIOLOGY (ADJUNCT)

Professor (Specially appointed)
WADA, Masamitsu

Associate Professor (Adjunct)
YAMAUCHI, Daisuke

Postdoctoral Fellows	KAGAWA, Takatoshi
	KODAMA,Yutaka
	KONG, Sam-Geun
	SUETSUGU, Noriyuki
	YAMASATO, Akihiro
Visiting Scientist	TSUBOI, Hidenori
Technical Assistants	CHUNG, Hee-Jung
	HIROSE, Mana
	IWATA, Ayumi
	SHIMIZU, Mineko
Secretary	TAKAMATSU, Kaori

Plants respond to light as an environmental factor to optimize growth and development and to regulate other physiological phenomena. Phytochrome (phy) and blue light receptors, such as cryptochrome (cry) and phototropin (phot), are the main photoreceptors for plant photomorphogenesis. The goal of our research is to elucidate the photoperception and signal transduction pathways of photomorphogenesis. One of our major subjects is chloroplast photo-relocation movement, which is mediated by phototropins and is one of the simplest model systems for studying photomorphogenesis. Because the phenomenon is cell autonomous, whole processes from photoperception to chloroplast movement can be accomplished in a single cell without any influence from surrounding neighbor cells. Moreover, gene expression is not involved in the signal transduction pathways, unlike in those of phy- and cry-mediated phenomena. Chloroplast movement is not real plant morphogenesis, but we are studying it because chloroplast movement and photomorphogenesis share the same photoreceptors.

Ⅰ. Chloroplast relocation movement

We use the fern *Adiantum capillus-veneris* as a model plant for our cell-biological and physiological approach to chloroplast movement since the gametophytes are very sensitive to light and the organization of the cells is very simple. We also use *Arabidopsis* mutants as well as wild type plants to identify the genes regulating chloroplast movement and for analyses of the genes' functions.

1-1 Polarity of moving chloroplasts

Chloroplast movement can be induced by whole cell or partial cell irradiation and is easily observed under a microscope. Since the speed of movement is very low, the detection of chloroplast movement during real time observation is not easy, so that chloroplast movement is usually recorded using time lapse movies. The figures obtained every minute were analyzed precisely.

When *A. capillus-veneris* gametophytes were incubated in darkness for two or three days, most chloroplasts moved to the anticlinal wall but only a few chloroplasts could be found on the periclinal wall. Chloroplast movement was induced by partical cell irradiation with sequential microbeams at different areas near the chloroplasts on the periclinal walls. Precise analyses of the chloroplast behavior revealed that chloroplasts can change direction without turning, although a time lag of a few minutes is required for

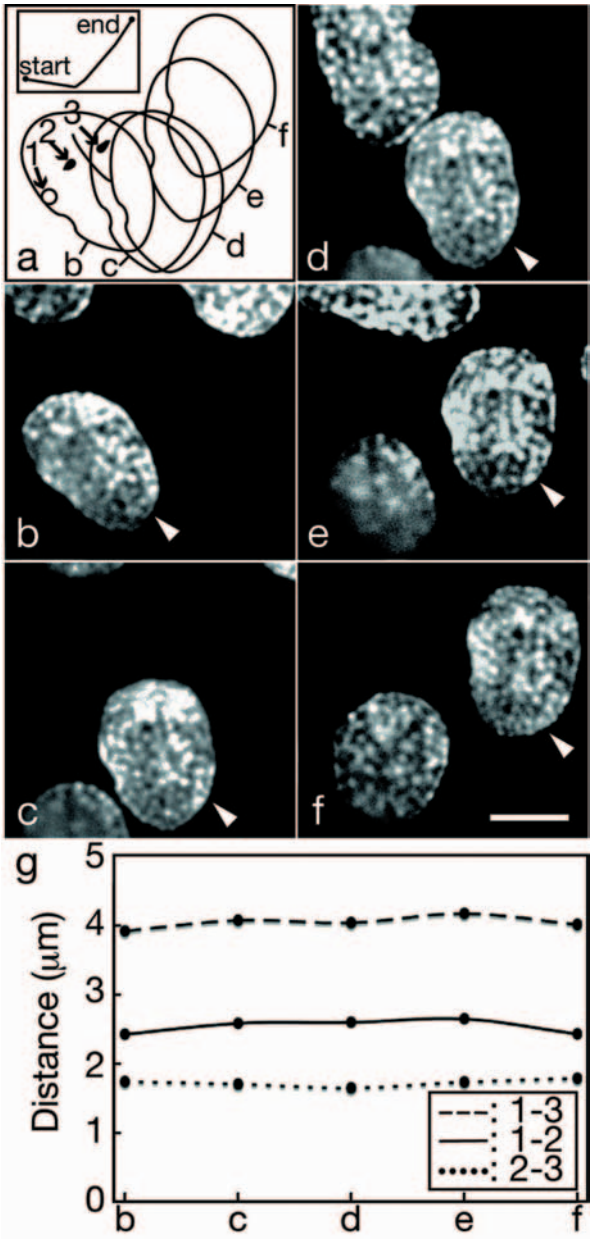

Figure 1. Dark-adapted A. *capillus-veneris* gametophyte was irradiated with red light (4.5 μmol m-2 s-1) to induce chloroplast movement from anticlinal wall to periclinal wall. The change in the distribution pattern of grana stucks during the movement was analyzed every 5 minutes. (a) Outlines of a chloroplast and the path of the center of the chloroplast (inset) during the movement. (b–f) Fluorescent micrographs of chloroplasts taken every 5 minutes. (g) The distances among grana stucks shown as 1, 2 and 3 were plotted (from Journal of Plant Research Tsuboi *et al*. 2009).

this. During the movement chloroplasts do not roll but slide, keeping the concave side to the plasma membrane. These chloroplast behaviors are the same under both blue and red light micorobeam irradiation.

1-2 Functional analysis of CHLOROPLAST UNUSUAL POSITIONING 1 protein in chloroplast movement

CHLOROPLAST UNUSUAL POSITIONING 1 (CHUP1) protein has a hydrophobic domain at its N-terminus and localizes on a chloroplast outer envelope. CHUP1 also has an F-actin binding domain and a proline rich region for profilin binding, suggesting the possible role of actin polymerization. The mutant deficient in CHUP1 protein (*chup1*) does not show chloroplast photo-relocation movement, so that chloroplasts sediment at the palisade cell bottom. The function of CHUP1 was studied using the transformants with variously truncated cDNA of *CHUP1* genes in *chup1* background (Oikawa et al 2008). Interestingly, when the CHUP1 N terminus hydrophobic region was replaced with the hydrophobic domain of a chloroplast outer envelope protein 7 (OEP7)-GFP, the chup1 phenotype was complemented, although both amino acid sequences are quite different. The results indicate that the amino acid sequence of CHUP1 N terminus is not important but that its hydrophobic character is essential for the CHUP1 function to attach the functional part of CHUP1 to the chloroplasts.

The coiled-coil region of CHUP1 anchors chloroplasts firmly on the plasma membrane, consistent with the localization of coiled-coil-GFP on the plasma membrane.

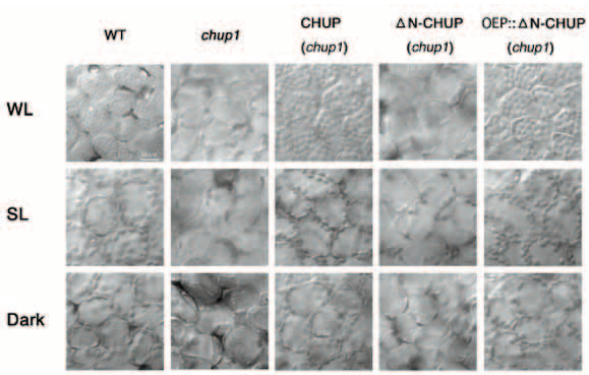

Figure 2. Distribution patterns of chloroplasts in each transgenic line under darkness (Dark), white light (WL), and strong white light (SL) conditions were compared among wild type, *chup1*mutant and the three transgenic plant lines (transferred with a full length *CHUP1* gene (CHUP), with *CHUP1* without N-terminus (ΔN-CHUP), and with *CHUP1* with which CHUP1 N terminus was replaced with a N terminus hydrophobic region of the outer envelope protein 7 (OEP::ΔN-CHUP)), CHUP1 whose N terminus was replaced with OEP7 N terminus rescued *chup1* mutant as *CHUP1* transgenic plants did. The experiments were repeated at least three times. Bar = 10 mm.

Publication List

〔Original papers〕

- Ogura, Y., Komatsu, A., Zikihara, K., Nanjo, T., Tokutomi, S., Wada, M., and Kiyosue, T. (2008). Blue light diminishes interaction of PAS/LOV proteins, putative blue light receptors in Arabidopsis thaliana, with their interacting partners. J. Plant Research *121*, 97 – 105.
- Kodama, Y., Tsuboi, H., Kagawa, T., and Wada, M. (2008). Low temperature-induced chloroplast relocation mediated by a blue light receptor, phototropin 2, in fern gametophytes. J. Plant Research *121*, 441-448.
- Oikawa, K., Yamasato, A., Kong, S.-G., Kasahara, M., Nakai, M., Takahashi, F., Ogura, Y., Kagawa, T., and Wada, M. (2008). Chloroplast outer envelope protein CHUP1 is essential for chloroplast anchorage to the plasma membrane and chloroplast movement. Plant Physiol. *148*, 829-842.

〔Review articles〕

- Wada, M. (2008). Photoresponses in fern gametophytes. In: The Biology and Evolution of Ferns and Lycophytes" Edited by Tom A. Ranker and Christopher H. Haufler, Cambridge Univ. Press. Pp. 3-48.
- Ogura, Y., Tokutomi, S., Wada, M., and Kiyosue, T. (2008). PAS/LOV proteins: A proposed new class of plant blue light receptor. Plant Signaling & Behavior *3*, 966-968.