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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 one of the simplest model systems to study 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 Velocity of signal transfer

Phototropins (phot1 and phot2) and a neochrome (which is a chimera photoreceptor of phytochrome chromophore binding domain and phototropin) were identified as photoreceptors for chloroplast movement (Kagawa *et al.* 2001, Kawai *et al.* 2003, Kagawa *et al.* 2004); however, a signal transferred from photoreceptors to chloroplasts remained to be clarified. We therefore studied the velocity

of signal transfer using *Adiantum* gametophytes to find a clue to the identity of any possible candidates for the signal. The velocity in a long protonemal cell is confirmed to be different between the signal moving from the base to the tip (approximately $2.3 \mu\text{m min}^{-1}$) and from the tip to the base (approximately $0.6 \mu\text{m min}^{-1}$) of the protonemata both in the red and blue light-induced chloroplast movement. On the other hand, the velocity of signal transfer in two-dimensional gametophyte cells is the same (approximately $0.7 \mu\text{m min}^{-1}$) irrespective of the direction of signal transfer or the wavelength (either red or blue light) that induces chloroplast movement. The velocities are slower than that of cytoplasmic streaming. The velocity of the signal transfer was very slow compared to our expectations, and we do not have any clear idea at the moment what is the signal and what is the mechanism of the transfer.

1-2 Chloroplast movement under cold condition

Chloroplasts movement was induced at about 4°C in *Adiantum* gametophytes. At low temperatures chloroplasts move to the anticlinal walls under either dark or light conditions. The position is similar to those of chloroplasts found in the dark or under strong light. Although the movement was even induced under darkness, it is not dark-induced movement, because the response could be induced in the mutant gametophytes defective of dark-induced chloroplast movement.

II. Photoreceptor functions

2-1 Photoreceptors mediating nuclear movement in the fern *Adiantum*

In gametophyte cells of the fern *Adiantum capillus-veneris*, nuclei as well as chloroplasts change their position according to light conditions (Kagawa and Wada 1993, 1995). Nuclei reside on anticlinal walls in darkness and move to periclinal or anticlinal walls under weak or strong light conditions, respectively (Figure 1).

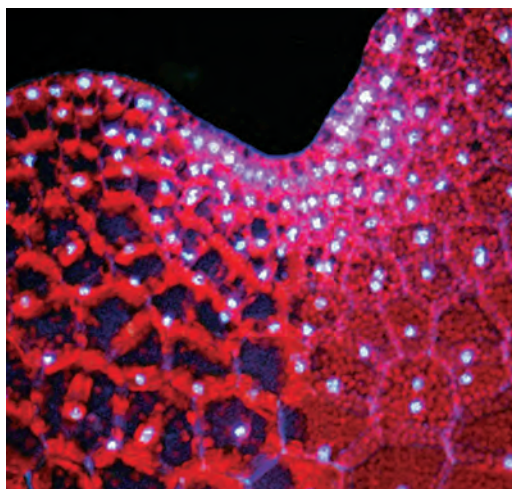


Figure 1. The left half of an *Adiantum* gametophyte cultured under weak white light was irradiated with strong white light to induce nuclear avoidance response and then fixed with glutaraldehyde and stained with DAPI. Nuclei irradiated with strong light moved to the anticlinal walls from the center of cells. The response was mediated by a blue light receptor, phototropin2 (Tsuboi *et al.* 2007).

This year we tried to identify the photoreceptor(s) that mediates nuclear movement in the gametophyte cells using photoreceptor mutants, *neo1* (neochrome1 defective), *phot2* (phototropin2 defective), and *neo1phot2* (Figure 2) and revealed that red and blue light-induced nuclear accumulation movement is mediated by *neo1*, and possibly *phot1* and *phot2*, respectively, and that blue light-induced avoidance movement is mediated by *phot2* (Tsuboi *et al.* 2007).

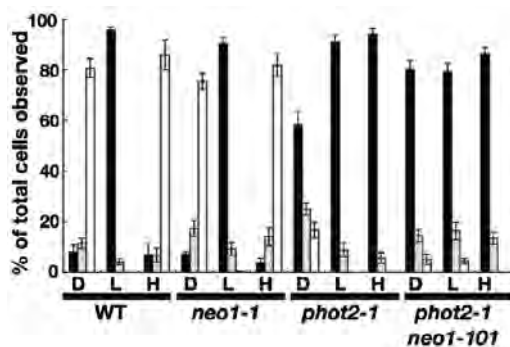


Figure 2. Positioning of nuclei of wild type, *neo1*, *phot2* and *neo1phot2* mutants in the dark and under weak or strong light conditions. Prothalli were dark-adapted for 1 day (D) or irradiated with weak white light (L: 20 - 30 mmol m⁻² s⁻¹) or strong white light (H: about 800 μ mol m⁻² s⁻¹) for 26 hrs. Percentages of nuclei at periclinal, anticlinal and intermediate positions are indicated with black bars, white bars, and gray bars, respectively.

It was also found that *phot2* is necessary for dark positioning of nucleus (Tsuboi *et al.* 2007). It is curious that a photoreceptor is needed for a physiological response in the dark, but it is similar to the case of Arabidopsis chloroplast positioning wherein *phot2* is indispensable in dark positioning. Thus, both the nuclear and chloroplast photorelocation movements may share common photoreceptor systems.

2-2 Aureochrome as a blue light receptor in *Vaucheria*

Vaucheria is a member of the group of stramenopile algae, which includes brown algae and diatoms and shows blue light responses, such as phototaxis, phototropism, photomorphogenesis and chloroplast relocation (Kataoka *et al.* 1975). In collaboration with Professor Kataoka at Tohoku University, we tried to find a blue light receptor(s) that might involve the blue light responses. We cloned two genes containing sequences of one basic-region/leucine-zipper (bZIP) domain and one light-oxygen-voltage (LOV) domain that binds a flavin mononucleotide (FMN). Its bZIP domain binds the target sequence TGACGT. We named them AUREOCHROME1 and 2 (*aureo1* and *aureo2*). RNAi of AUREO2 induces sex organ primordial instead of branches, implicating AUREO2 as a sub-switch to initiate the development of a branch, but not a sex organ. These are the photoreceptors for the blue light-induced branching of *Vaucheria frigida*. AUREO sequences are also found in the genome of the marine diatom but are not in green plants (Takahashi *et al.* 2007).

2-3 Binding proteins to a putative blue light receptor in Arabidopsis

PAS/LOV protein (PLP) is a putative blue light receptor with a PAS domain at its N-terminal region and an LOV domain at its C-terminal region (Crosson *et al.* 2003). PLP interacting proteins were isolated by the yeast two-hybrid system and were studied in collaboration with Dr. Kiyosue at Kagawa University. Those were VITAMIN C DEFECTIVE 2 (VTC2, Jander *et al.* 2002), and VTC2 paralog (VTC2L) and BEL1-LIKE HOMEODOMAIN 10 (BLH10) (Hackbusch *et al.* 2004). The interaction of PLPA with VTC2L was weakened at the intensity of >100 mmole m⁻² s⁻¹ of blue light, while that of PLPB with VTC2L was undetectable at that intensity (Ogura *et al.* 2008).

Publication List

[Original papers]

- Kagawa, T., and Suetsugu, N. (2007). Photometrical analysis with photosensory domains of photoreceptors in green algae. *FEBS Letters* 581, 368-374.
- Takahashi, F., Yamagata, D., Ishikawa, M., Fukamatsu, Y., Ogura, Y., Kasahara, K., Kiyosue, T., Kikuyama, M., Wada, M., and Kataoka, H. (2007). AUREOCHROME, a photoreceptor required for photomorphogenesis in stramenopiles. *Proc. Natl. Acad. Sci. USA* 104, 19625 -19630.
- Tsuboi, H., Suetsugu, N., Kawai-Toyooka, H., and Wada, M. (2007). Phototropins and neochrome1 mediate nuclear movement in the fern *Adiantum capillus-veneris*. *Plant Cell Physiol.* 48, 892-896.

[Review articles]

- Suetsugu, N., and Wada, M. (2007). Phytochrome-dependent photomovement responses mediated by phototropin family proteins in cryptogam plants. *Photochem. Photobiol.* 83, 87-93.
- Suetsugu, N., and Wada, M. (2007). Chloroplast photorelocation movement mediated by phototropin family proteins in green plants. *Biological Chemistry* 388, 927-935.
- Wada, M. (2007). Fern as a model system to study photomorphogenesis. *J. Plant Research* 120, 3-16.