DIVISION OF PHOTOBIOLOGY (ADJUNCT)

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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 thought to be one of the simplest model systems to study photomorphogenesis. Because the phenomenon is cell autonomous, whole processes from photo-perception 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 it is the reason that chloroplast movement and photomorphogenesis share the same photoreceptors.

I. Chloroplast relocation movement

We use the fern *Adiantum capillus-veneris* and the moss *Physcomitrella patens* as model plants 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 photo-relocation movement and for analyses of the genesí functions.

1-1 Velocity of signal transfer

Phototropins (phot1 and phot2, and 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 remained to be clarified. We therefore studied the velocity of signal transfer to find a clue to the identity of any possible candidates for the signal using long single cells of *Adiantum* protonemata. The velocity is different when the signal moves from the base to the tip (approximately 2.3 μm min^{-1}) and from the tip to the base (approximately 0.6 μm min^{-1}) of the protonemata. The reason for the difference is not clear but the cytoplasmic and/or water flow from the base to the tip to support tip growth of protonemata is the most plausible explanation.

II. Photoreceptor functions

2-1 Acneo1 as a blue light receptor

Fern phytochrome3 (phy3) (renamed *Ac*neochrome1 (*Ac*neo1)) is a chimera photoreceptor that consists of a phytochrome chromophore binding domain and a full-length phototropin (Nozue *et al.* 1998), so that it had been thought that it might be able to absorb red as well as

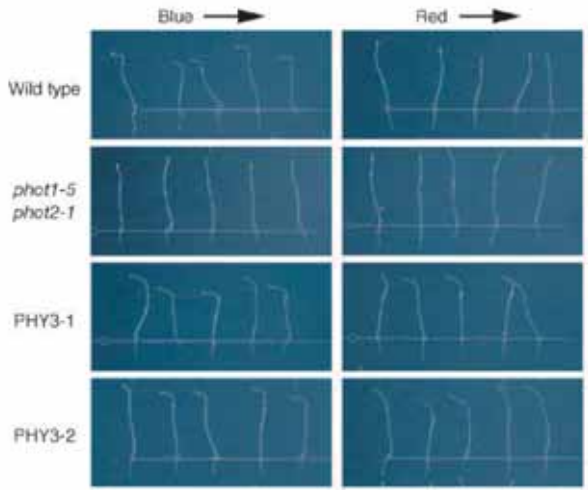

Figure 1. Phototropic response of *Arabidopsis* seedlings. *Ac*neo1 can mediate both red and blue light-induced phototropic response in *Ac*neo1 transgenic *Arabidoposis* seedlings of *phot1phot2* background. Blue and red lights were given from left hand side to the plants cultivated 3 days under darkness. Wild type plants show phototropic response in blue light but not in red light. *phot1phot2* mutant plants do not show phototropism either under red or blue light. Two independent transgenic lines phy3-1 and phy3-2 show tropistic response under both red and blue light. (see Kanegae *et al.* 2006 for more details)

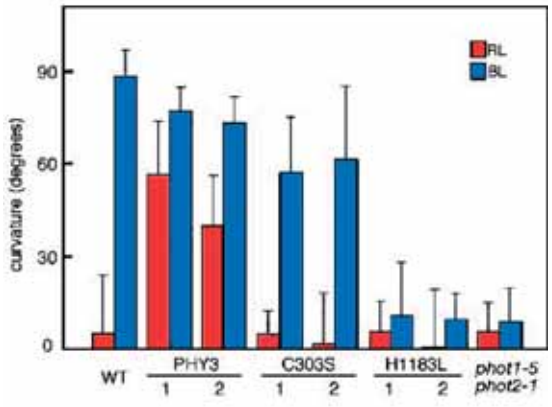

Figure 2. *Ac*neo1 can function as a blue light receptor. Even when phytochrome chromophore does not bind in C303S mutant phototropic response was observed under blue light. The blue light response transferred through kinase domain was shown in H183L mutant plants. (see Kanegae *et al.* 2006 for more details)

blue light. However, no evidence of red and blue light-absorption by *Ac*neo1 has ever been shown. In collaboration with Dr. Kanegae (Tokyo Metropolitan University) we recently demonstrated that *Ac*neo1 can absorb red as well as blue light; we also showed that red and blue light can function synergistically when given simultaneously, resulting in increased sensitivity ten times greater than when red or blue light is given independently (Kanegae *et al*. 2006). In these experiments the degree of phototropic response, but not chloroplast movement, was measured in order to evaluate the sensitivity to light in *Arabidopsis phot1phot2* double mutants transformed with *Ac*neo1.

2-2 Photoreceptors in fern stomata opening

Photoresponse of fern stomata opening was studied in collaboration with Drs. Doi and Shimazaki of Kyushu University. Fern stomata open under red light but not in blue light, although in seed plants stomata opening is redundantly mediated by blue light receptors phot1 and phot2 (Kinoshita *et al*. 2001). Therefore, *Ac*neo1 is a good candidate for this response because of its chimera structure made of red light receptor phytochrome and phototropin. Contrary to our expectations, *rap2* mutant lines deficient in *Ac*neo1 still showed red light-mediated stomata opening, indicating that the stomata opening in ferns is not mediated by phototropin family proteins but controlled by mechanisms completely different from those of seed plants. It is curious that blue light does not mediate fern stomata opening although phototropins are expressed in the fern guard cells.

III. Photomorphogenesis

3-1 Phototropism of Adiantum *rhizoid*

Phototropic responses in seed plants are induced by blue light and mediated by blue light receptor phototropins. In many cryptogam plants, including the ferns and mosses, however, red as well as blue light is effective in inducing a positive phototropic response in protonemal cells. In *A. capillus-veneris*, both protonemata and sporophytes show red light-induced phototropism and the photoreceptor for this response is *Ac*neo1 (Kawai *et al*. 2003). Because nothing precise has been reported so far concerning phototropism of fern rhizoid cells, we analyzed negative phototropism of *A. capillus-veneris* rhizoid cells. Mutants defective of *Ac*neo1 lacked red light-induced negative phototropism, indicating that under red light, *Ac*neo1 mediates negative phototropism in rhizoid cells, in contrast to its role in regulating positive phototropism in protonemal cells. *Acneo1* mutants were also partially deficient in blue light-induced negative phototropism in rhizoid, suggesting that *Ac*neo1, in conjunction with phototropin, redundantly mediates the blue light response.

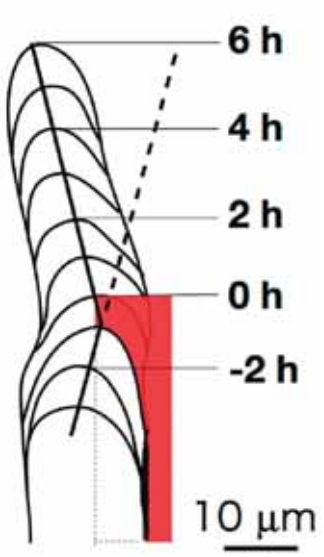

Figure 3. A half side of *Adiantum* rhizoid was irradiated with red microbeam light (5.5μmol m^{-2} s^{-1}. 1min) as shown in red rectangular at hour 0. Rhizoidal images were traced every 1 hour to follow the growth direction. The dotted line shows the original direction of rhizoid growth and the solid line shows the new direction. Note that the rhizoid showed negative phototropism. (see Tsuboi *et al*. 2006 for more details)

Original papers

Doi, M., Wada, M., and Shimazaki, K. (2006). The fern *Adiantum capillus-veneris* lacks stomatal responses to blue light. Plant Cell Physiol. *47*, 748-755.

Tsuboi, H., Suetsugu, N., and Wada, M. (2006). Negative phototropic response of rhizoid cells in the fern *Adiantum capillus-veneris*. J. Plant Res. *119,* 505-512.

Kanegae, T., Hayashida, E., Kuramoto, C., and Wada, M. (2006). A single chromoprotein with triple chromophores acts as both a phytochrome and a phototropin. Proc. Natl. Acad. Sci. USA *103*, 17997-18001.

Review articles

Kanegae, T., and Wada, M. (2006). Photomorphogenesis of Ferns. In Photomorphogenesis in Plants 3rd Edition. E. Sch%fer, and F. Nagy, eds. (Dordrecht, Kluwer Academic Publishers), pp. 515-536.