DIVISION OF PHOTOBIOLOGY (ADJUNCT)

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Plants respond to light as an environmental factor to optimize development and 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 photo-morphogenesis.

I. Chloroplast relocation movement

One of our major subjects is chloroplast photo-relocation movement, which is thought to be one of the simplest model systems to study photomorphogenesis. We use the fern *Adiantum capillus-veneris* and the moss *Physcomitrella patens* as model plants for our cell biological approach since the gametophytes are very sensitive to light and the organization of the cells is very simple. We also use *Arabidopsis* mutants to identify the genes regulating chloroplast photo-relocation movement.

1-1 *Arabidopsis*

Chloroplasts relocate in a cell according to ambient light conditions: accumulation response occurs under low-light condition and avoidance response under high-light condition. In *Arabidopsis thaliana*, the accumulation response is mediated redundantly by two blue light receptors, i.e. phototropins (phot1 and phot2) (Sakai *et al*. 2001) and the avoidance response is mediated only by phot2 (Kagawa *et al*. 2001). We isolated a mutant, *jac1* (*J*-domain protein required for chloroplast *ac*cumulation response 1) which lacks accumulation response under weak light, but shows normal avoidance response under strong light. Positional cloning of *JAC1* revealed that this gene encodes a J-domain protein at its C-terminus similar to clathrin uncoating factor auxilin. In dark-adapted wild type cells, chloroplasts sediment on the cell bottom, but the *jac1* mutant lacks this response. The green fluorescent protein (GFP)-JAC1 fusion protein showed the similar localization pattern to GFP protein at the transient assay with onion epidermal cells, suggesting that JAC1 protein may be a soluble cytosolic protein. The results suggest that JAC1 is one of the essential components of phototropin-mediated chloroplast movement.

1-2 *Mougeotia*

Most plant species from alga to flowering plants utilize blue light for inducing phototropism and chloroplast movement; many ferns, however, as well as some mosses and green alga utilize red as well as blue light for the regulation of these responses, resulting in efficient capture of white light under low light levels. During their evolution, ferns have created a chimeric photoreceptor (phy3 in *Adiantum*) between phytochrome and phototropin enabling them to utilize red light effectively. We have identified two genes resembling *Adiantum* PHY3, NEOCHROME1 and 2 (MsNEO1 and MsNEO2), from the green alga *Mougeotia scalaris*, a plant famous for its phytochrome-dependent chloroplast movement. Like *Adiantum* PHY3, both MsNEO gene products show phytochrome-typical bilin binding and red/far-red reversibility, the difference spectra closely matching the known action spectra of light-induced chloroplast movement in *Mougeotia*. Furthermore both rescue red-light-induced chloroplast movement in *Adiantum* phy3 mutants, indicating functional equivalence. The fern and algal genes seem to have arisen independently in evolution, demonstrating an intriguing example of convergent evolution.

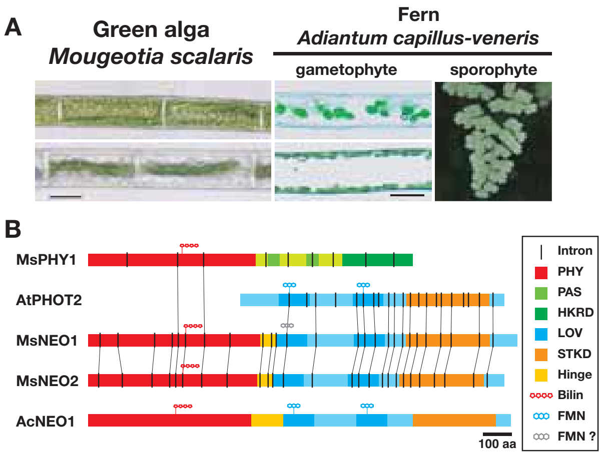

Figure 1. A, Giant chloroplasts of *Mougeotia scalaris* in their face-on (upper panel) and side-on orientation (lower panel) and those of *Adiantum capillus-veneris* protonemal cells in which the chloroplast accumulation to the upper (upper panel) or side wall (lower panel) are induced by polarized red light vibrating parallel or perpendicular to the cell axis irradiated from horizontal direction. Scale bar 20 μm. The right panel shows *A. capillus-veneris* sporophyte. B, The domain structure of fern and algal neochromes and canonical plant phytochrome and phototropin. MsNEO1 LOV1 domain has so far failed to bind FMN in vitro, the other MsNEO LOV domains are unlikely to bind flavins. Introns whose positions are equivalent in each gene are connected with thin lines. PHY: phytochrome photosensory region. HKRD: histidine kinase-related domain. STKD: serine/threonine kinase domain. PHOT: phototropin region.

1-3 *Adiantum*

The avoidance movement response in *Adiantum* phot2 deficient mutants can be restored by transient expression of non-mutant AcPHOT2 cDNA, indicating that chloroplast avoidance movement in this fern is mediated by the Acphot2 protein as is the case in Arabidopsis. To know whether Acphot1 functions as a photoreceptor of blue light-induced chloroplast relocation movement, we need either triple mutant or at least three double mutants of Acphot1, Acphot2 and Acphy3, because phy3 might also be one of the blue light receptors in *Adiantum*. We are screening double mutants based on *Acphot2* or *Acphy3* mutant lines. As some double mutants have been obtained, the function of Acphot1 might be clarified soon.

II. Photomorphogenesis

2-1 Branching in *Physcomitrella*

Side branch formation in the moss *Physcomitrella patens* has been shown to be light dependent and cryptochrome 1a and 1b (Ppcry1a and Ppcry1b) were assigned as the blue light receptors for this response (Imaizumi *et al*. 2002). Here we analyzed this phenomenon in detail, and revealed the complex nature of the response where multiple photoreceptors are involved. For branch induction, blue light of a fluence rate higher than 6μmol $m^{-2}s^{-1}$ for period longer than 3 h was required. Number of branches is dependent on fluence rate of blue light but further increased when red light was applied together with the blue light, although red light alone had much less effect. By partial irradiation of a cell, both receptive sites for blue and red light were found to be located around the nucleus.

Both red and blue light determine the position of branches in a way affected by the vibration plane of polarized light. The red light effect was nullified by simultaneous far-red light irradiation, indicating phytochrome involvement. The blue light effect was not found in phototropin disruptants. Thus, dichroic phytochrome and phototropin possibly on the plasma membrane regulate branch position. Together, at least four distinct photoreceptor systems- namely cryptochromes and red light receptors around or in the nucleus, and dichroic phytochrome and phototropin around the cell periphery - are involved in the light induction of side branches in the moss *Physcomitrella patens*.

III. Gene targeting and gene silencing

In order to elucidate the role of genes in *Adiantum* and rice, we have tried to establish new methods for gene targeting in these organisms.

3- 1 Miniature transposable element

Transposable elements constitute a large portion of eukaryotic genomes and contribute to their evolution and diversification. We identified active transposable elements, miniaturePing (mPing), Ping and Pong in rice (Kikuchi *et al*. 2003). The mPing element was identified as the first active MITE from any organism. mPing is a short 430 base pair element with 15 base pair terminal inverted repeats that lacks a transposase. mPing elements are activated in calli derived from another culture and excise efficiently from original sites to reinsert into new loci. *Ping* and *Pong* transposable elements were isolated as putative autonomous elements encoding an IS/PIF/Harbinger superfamily of transposases. We are now trying to detect its transposase function.

3- 2 DNA interference in *Adiantum*

Silencing of gene expression by RNA interference (RNAi) is a useful technique for determining the roles of genes of unknown function in a wide range of organisms. In fern we found a simple method for gene-silencing using DNA fragments homologous to the target gene, called DNAi. It has the advantage of being faster and simpler than current RNAi approaches. To make DNAi a more powerful tool to study function-unknown genes in pteridophytes, expressed sequence tags (ESTs) were obtained from a normalized cDNA library of *Adiantum capillus-veneris* constructed from prothallia grown under white light. Clustering of 10,552 sequences in total resulted in 7,132 non-redundant groups. Of these, 1,608 EST groups were found to be similar to sequences of known function and 1,092 EST groups showed similarities to sequences of unknown function.

Publication List:

Original papers

Suetsugu, N., Kagawa, T., and Wada, M. (2005). An auxilin-like J-domain protein, JAC1, regulates phototropin-mediated chloroplast movement in *Arabidopsis thaliana*. Plant Physiology *139*,151-162.

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Wada, M. (2005). Chloroplast movement. In Light Sensing in Plants, M. Wada, K. Shimazaki, and M. Iino, eds. (Tokyo, Springer-Verlag), pp. 193-199.