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Plants use light as an environmental factor which controls their development as well as their other physiological phenomena. Phytochrome and blue light receptors, such as cryptochrome and phototropin (nph1), are the main photoreceptors for plant photomorphogenesis. The goal of our research is to clarify the signal transduction pathways of photomorphogenesis. One of our major subjects is chloroplast photo-relocation movement which is thought to be one of the simplest phenomena in this field. We use the fern *Adiantum capillus-veneris* and the moss *Physcomitrella patens* as model plants for our cell biological approach not only because the gametophytes are very sensitive to light, but also because the organization of the cells is very simple. We also use *Arabidopsis* mutants to clarify the genes regulating chloroplast photo-relocation movement.

I. Cloning and characterization of blue-light photoreceptors

We have described many blue-light induced photomorphological responses in gametophytes of the fern *Adiantum capillus-veneris*. As the first step in understanding the molecular mechanisms of these blue-light responses, we are cloning and sequencing the genes of blue light receptors, and are studying intracellular distributions of the gene products and their function in *Adiantum* and *Physcomitrella.*

1- 1 Cryptochromes

We identified two cryptochrome genes from *Physcomitrella patens*, designated *CRY1a* and *CRY1b* genes, and made single and double disrupants of these genes using gene targeting by means of homologous recombination. Using these disruptants, it was revealed that blue light signals via cryptochromes inhibited the transition of cell types of the moss protonema from chloronema to caulonema, but induced side-branching of the protonema. Gametophore induction and its growth and development were also regulated.

1- 2 Phototropin

Phototropin is another blue light photoreceptor isolated recently in higher plants, and is a flavin binding protein with light sensitive protein kinase activity. A cDNA of *Adiantum NPL1*, a homologue of phototropin has been sequenced. The complete cDNA clone is 3492 bp in length and encodes a protein of 1092 amino acids.

II. Chloroplast relocation

2- 1 *Arabidopsis*

Chloroplasts accumulate at the cell surface under weak light and escape from the cell surface to the anticlinal wall under strong light to optimize photosynthesis. The mechanism of chloroplast relocation, however, is not known. We screened several mutants from T-DNA tagging lines as well as EMS lines of *Arabidopsis*. Gene analysis of several mutants defective in chloroplast relocation movement showed that npl1 is the blue light receptor for the avoidance response under strong light. Both nph1 and npl1 are the blue light receptors for the chloroplast accumulation response under weak light. Gene analysis of mutants defective in accumulation response are also under way.

2- 2 *Adiantum*

Adiantum phytochrome3 (PHY3) is a unique kimeric protein with a phytochrome structure in the N-terminal half and a phototropin structure in the C-terminal half. Heavy-ion-beam- or EMS-induced mutants of *Adiantum* which do not show phototropic response and chloroplast photorelocation movement under red light were revealed to lack the *PHY3* gene or to have a mutation in the *PHY3* gene. It was confirmed by gene silencing that phy3 is the photoreceptor mediating red light-induced tropic response and chloroplast photorelocation movement. However, since these phenomena induced by blue light are normal in these mutants, it is still unknown whether phy3 might or might not absorb blue light as a photoreceptor.

Publication List:

Imaizumi, T., T. Kanegae and M. Wada (2000) Cryptochrome nucleocytoplasmic distribution and gene expression are regulated by light quality in the fern *Adiantum capillus-veneris*. *Plant Cell* **12**: 81-96.

Kagawa, T. and M. Wada (2000) Blue light-induced chloroplast relocation in *Arabidopsis thaliana* as analyzed by microbeam irradiation. *Plant Cell Physiol.* 41: 84-93.

Kiyosue, T. and M. Wada (2000) LKP1 (LOV kelch protein 1): a factor involved in the regulation of flowering time in *Arabidopsis*. *Plant Journal* **23**: 807-815.

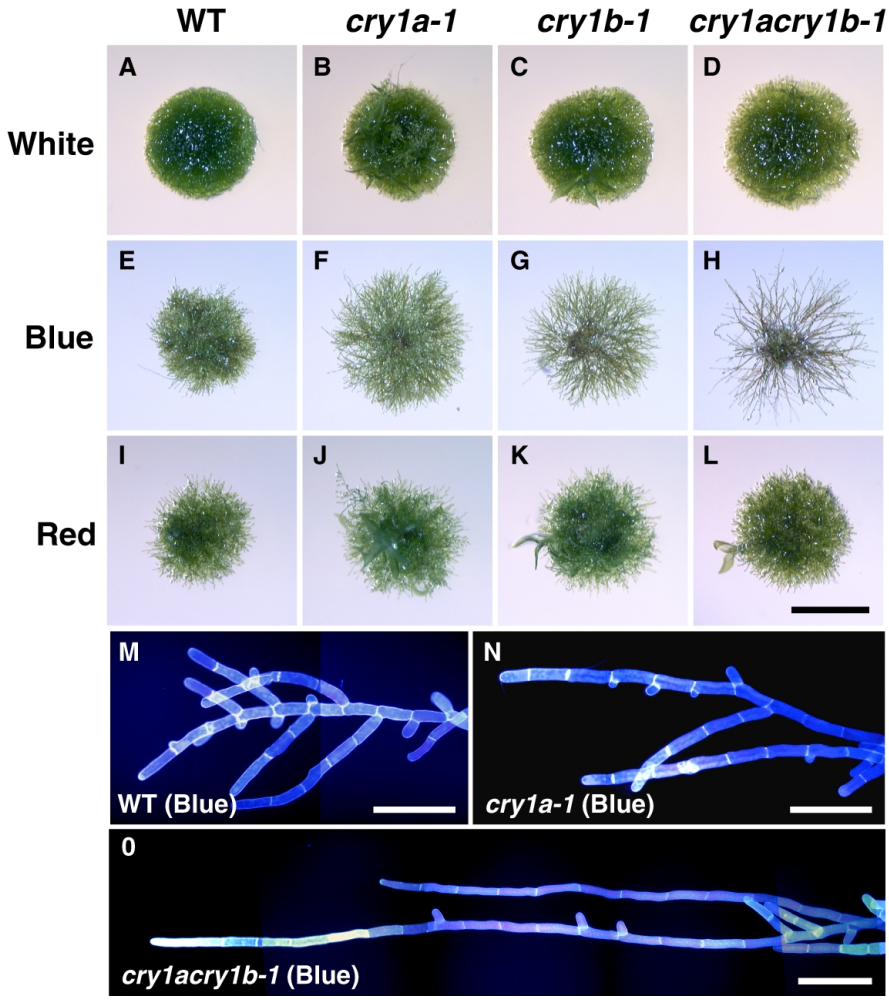

Figure. Protonemal Colonies of the Cryptochrome Disruptants under Different Light Conditions.
The protonemal colonies (ca. 1 mm in diameter) of wild type, *cry1a*, *cry1b*, and *cry1acry1b* strains were inoculated on the agar plates. These plates were incubated under continuous white, blue, or red light for 10 days. Representative colony images of one of each cryptochrome disruptant were shown. The colonies are the following strains: (A, E, and I) the wild type (WT); (B, F, and J) *cry1a-1*; (C, G, and K) *cry1b-1*; and (D, H, and L) *cry1acry1b-1*. The light conditions are given: (A-D) white light; (E-H) blue light; and (I-J) red light.
The blue-light grown protonemata are shown in M-O. These are (M) wild type; (N) *cry1a-1*; and (O) *cry1acry1b-1*. The protonema cell walls were stained with a drop of 0.1% (v/v) Calcofluor White to help distinguish each cell. The appearance of protonemata of *cry1b* strains was similar to that of *cry1a* strain protonemata.
The bar in L represents 2 mm for the panels A-L, and the bars in M-O represents 200 μm.