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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 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 gametophytes as a model plant 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 for mutant screening to clarify the genes regulating chloroplast photo-relocation. We have begun to use Physcomitrella patens as a model system for which gene targeting is available.

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 various blue-light responses in Adiantum, we are working on the cloning and sequencing of the genes of blue light receptors and on the gene expression and intracellular distributions of the gene products.

1- 1 Cryptochromes

We identified two different genes, designated Adiantum cryptochrome4 and 5 (CRY4 and 5), from a genomic DNA library, and found stage specific and light dependent gene expression of the five CRY genes (two newly isolated and three previously isolated genes) in Adiantum. The expression of CRY4 and CRY5 is regulated by light and is under phytochrome regulation. The intracellular distribution of reporter GUS-CRY fusion proteins indicates that GUS-CRY3 and GUS-CRY4 localize in gametophyte nuclei. The nuclear localization of GUS-CRY3 is regulated in a light-dependent manner (Imaizumi et al, Plant Cell 12: 81-96, 2000).

Cryptochrome genes of Physcomitrella patens have also been identified.

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 phototropin has been sequenced. The complete cDNA clone is 3492 bp in length and encodes a protein of 1092 amino acids. Southern blot analysis showed that Adiantum phototropin is likely to be a single copy gene. RT-PCR analysis revealed that it was expressed in various developmental stages (imbibed spores, protonemata, dark grown young leaves, and light grown young leaves). The chromophore attached to LOV (Light, Oxygen, or Voltage) domains was flavin mononucleotide (FMN).

We also cloned and sequenced two cDNAs and respective genomic DNAs of

phototropin from Physcomitrella patens.

1- 3 Adiantum Phytochrome 3

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. From the analytical study of fusion protein expressed in E. coli, LOV domains of PHY3 also bind with a FMN, suggesting that phy3 absorbs blue light as well as red/far-red light.

II. Chloroplast relocation

2- 1 Arabidopsis

Chloroplasts accumulate at the cell surface under weak light and escape from strong light to optimize photosynthesis. The mechanism of chloroplast relocation, however, is not known. We studied light induced-chloroplast relocation in leaves of Arabidopsis mutants such as cry1, cry2, cry1cry2 and nph1 and found that the chloroplast relocation movement was normal in these mutants, meaning that another (i.e. a new) blue light receptor for chloroplast relocation must exist. To find the photoreceptor for blue light-induced chloroplast relocation, we screened several mutants from T-DNA tagging lines as well as EMS lines of Arabidopsis. The identification of the mutated genes of Arabidopsis mutants is now in progress.

2- 2 Adiantum

A heavy-ion-beam induced deletion mutant of Adiantum which does not show phototropic response and chloroplast photorelocation movement under red light was revealed to lack the PHY3 gene by genomic PCR and Southern blot analysis. It is suggested that phy3 is the photoreceptor mediating red light-induced tropic response and chloroplast photorelocation movement. However, these phenomena induced by blue light are normal, meaning that phy3 might or might not absorb blue light. In the former case, Adiantum phototropin may also work on blue light-induced response as phy3 does.

Publication List:

Christie, J.M., M. Salomon, K. Nozue, M. Wada and W.R. Briggs (1999) LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): Binding sites for the chromophore flavin mononucleotide. Proc. Natl. Acad. Sci. USA 96: 8779-8783.

Imaizumi, T., T. Kiyosue, T. Kanegae and M. Wada (1999) Cloning of the cDNA encoding the blue-light photoreceptor (cryptochrome) from the moss *Physcomitrella patens* (Accession No. AB027528) (PGR99-110) Plant Physiol. 120: 1205.

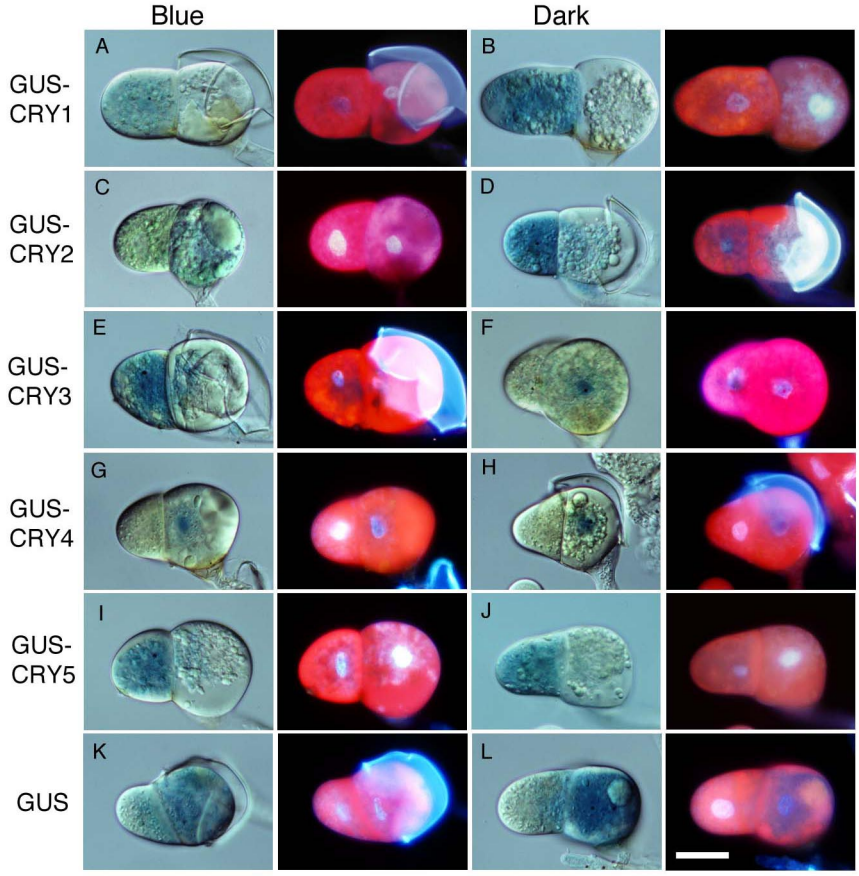

FIG. Representative Images of the Intracellular Distribution of GUS-CRY Fusion Proteins in Germinating Cells of *Adiantum*.
Intracellular distribution of GUS-CRY fusion proteins under different light conditions. GUS-CRY1 (A and B), GUS-CRY2 (C and D), GUS-CRY3 (E and F), GUS-CRY4 (G and H), GUS-CRY5 (I and J), GUS (K and L). Two-celled protonemata expressing various GUS-CRY fusion proteins were incubated under blue light (A, C, E, G, I, and K) or in the dark (B, D, F, H, J, and L) for 16 hr and stained under the same light conditions. The cells showing GUS activity were photographed using Nomarski optics (left panels) and fluorescence micrographs show the position of the nuclei in the same cells stained with 4',6-diamidino-2-phenylindole (right panels). Note that under fluorescence, chlorophyll autofluoresces red and spore coats appear bluish white. The bar shown in L represents 20 µm for all panels.