Regulation of *cab* Gene Expression by Phytochrome

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Light is an important environmental factor in plant growth and development. It provides not only radient energy for photosynthesis, but also signal that makes plants to adapt to the ambient growth conditions. Light is perceived by photoreceptors that include phytochrome, blue light photoreceptor, ultraviolet A (UV-A) photoreceptor, and UV-B photoreceptor. Among these, phytochrome is a principle photoreceptor for plant photomorphogenesis and has been most extensively studied. Its molecular properties and the physiological responses upon light perception are rather well characterized.

Phytochrome is a chromoprotein that is a homodimer of 120 kDa protein. A open tetrapyrrole ring chromophore is covalently attached to it. Phytochrome exists in two interconvertable forms; red light absorbing form (Pr) and far-red light absorbing form (Pfr). The Pfr form is considered as an active form in most cases that elicits physiological responses. The absorption spectra of the two forms overlap in the red region of the spectrum, leading to an equilibrium between the two forms under sun light, called a photostationary state. In addition to the light, other factors such as dark reversion, degradation and synthesis rate also regulate the steady state level of the Pfr form. Recently, phytochrome is known to exist as multiple gene family. The multiple phytochrome species may play different physiological roles in plant

growth regulation, which was not known clearly yet. Generally, PhyA is most likely responsible for type I (photolabile phytochrome-dependent) responses such as de-etiolation, while PhyB for type II (photostable phytochrome-dependent) such as shade-avoidence.

The signal transduction chains of phytochrome that span from the light perception to the physiological responses attract much attentions in this research field but any clue is not known so far. The signal transduction chains have been comprehensively studied in animals and it is considered that plant signal transduction chains may share common events with animal system. Many research has focussed on phytochrome binding partner (phytochrome receptor) that receive Pfr signal and then transmit it to the next signal transducer but failed to identify its existence. The signal transduction events found in animals are also known to be present in plants; GTP-binding protein (G-protein), inositol phospholipid turnover, change in intracellular free $[Ca^{++}]$ in cytosol, membrane potential change and polarization, and ion fluxes across membrane. Much of these events are related with the regulation of protein kinase activity.

Protein phosphorylation and dephosphorylation plays an important roles in the regulation of fundamental cellular metabolism and signal transduction pathways. The involvement of protein phosphorylation in phytochrome signal transduction has been seriously considered, since G-protein, phosphoinositol metabolism and calcium level in cytosol are responded to red light treatment. Moreover, a fraction of phytochrome is phosphoprotein. Phytochrome phosphorylation will have great significance for early step in its signal transduction, if photoconversion could regulate its phosphorylation.

The highly purified phytochrome had kinase activity that phosphorylated itself. Thus, it has been proposed that phytochrome itself is a protein kinase.

However, our results indicate that the kinase activity associated with phytochrome was separable from the purified phytochrome by lowering ionic strength of the medium. We have tried to purify the kinase that phosphorylate phytochrome from the etiolated oat seedlings. A protein kinase of molecular weight of 32,000 has been identified and isolated. In addition to phytochrome, the kinase also phosphorylate several proteins *in vivo*. The enzymatic properties of the kinase have been studied.

Phytochrome controls plant growth in part by regulating gene expression that especially involved in de-etiolation, such as the nuclear-encoded genes for the small subunit of Rubisco (*rbcS*) and the chlorophyll a/b-binding protein of the light harvesting complex (*cab*). Gene expression is regulated by regulatory proteins that bind to the promoter region of the gene. We tried to identify light regulatory elements and their protein binding factors that are engaged in light responsiveness of *cab* gene expression. Nuclear extracts from the leaves of *Arabidopsis thaliana* that received different light/dark treatments were assayed for their binding ability (mobility shift assay) to the light regulatory elements of *A. thaliana cab*1 gene. When *cab* gene expression was suppressed by the dark treatment, a new band that was not seen with the light-grown sample appeared in the light regulatory region. The band represents a negative regulatory factor that represses *cab*1 expression in the dark. The implication of the presence of this factor have been discussed with respect to gene products of the photo-signal transduction *Arabidopsis* mutants.

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