PL-5

PHYTOCHROME-SPECIFIC TYPE 5 PHOSPHATASE CONTROLS LIGHT SIGNAL FLUX BY ENHANCING PHYTOCHROME STABILITY AND AFFINITY FOR A SIGNAL TRANSDUCER.

Jong Sang Ryu, Jeong-Il Kim, Tim Kunkel, Byung Chul Kim, Dae Shik Cho, Sung Hyun Hong, Seong-Hee Kim, Aurora Pias Fernndez, Yumi Kim, Jose M. Alonso, Joseph R. Ecker, Ferenc Nagy, Pyung Ok Lim, Pill-Soon Song, Eberhard Schfer, and Hong Gil Nam

Biological light signaling system, in addition to having the information flow pathways to downstream transducers, should control the flux of information. The early flow pathways of phytochrome-mediated light signaling were revealed by analyzing the downstream transducers mostly identified by yeast two-hybrid screening of phytochrome-interacting components; light signaling initiated by photo-conversion of phytochromes is transmitted to the early signal transducers such as PIF3 and NDPK2 through preferential binding of these molecules to the Pfr-phytochromes. Although control of the information flux in phytochrome-mediated photoperception may occur at various steps of the pathways, the earliest control appears to be conducted at the photoreceptor level. Based on the unique characteristics of phytochromes, nucleocytoplasmic partitioning, light-dependent degradation, or regulation of phosphorylation state can be suggested as the ways to effectively control the light information flux.

PAPP5 was isolated in a yeast two-hybrid screening of *Arabidopsis* cDNA clones, employing the *Arabidopsis* phyA as the bait. While PAPP5 was initially isolated as a phyA-interacting protein, it binds to both *Arabidopsis* phyA and phyB *in vitro*. The PAPP5-binding region of *Arabidopsis* phyA was subsequently identified within the C-terminal 253 amino acids. PAPP5 also exhibited their relative binding affinity for the two photoconvertible forms, Pr and Pfr approximately 40% less binding to the Pr form than to the Pfr form of oat phyA.

The deduced peptide sequence of PAPP5 shows a high similarity to those of known type 5 protein phosphatases (PP5s) with a three tetratricopeptide repeat (TPR) domain in its N-terminal region and the highly conserved signature motifs of the type 2A serine/threonine protein phosphatase (PP2Ac) in

its C-terminal region. TPR domain is both necessary and sufficient for the interaction between PAPP5 and phytochromes. The phosphatase activity of PAPP5 was stimulated by polyunsaturated long-chain fatty acids such as arachidonic acid (AA), whereas the PP2Ac domain alone lacking the TPR domain showed AA-independent phosphatase activity, indicating autoinhibitory property of the TPR domain.

The subcellular localization of PAPP5 followed that of phytochromes, being localized in the cytoplasm in darkness and in the nucleus in light. Noticeably, the light-dependent nuclear localization and nuclear speckle formation of PAPP5 depended on the presence of phytochrome, supporting that PAPP5 interacts with phytochromes *in vivo*. PAPP5 positively modulates various light-dependent processes mediated by both phyA and phyB. It should be noted that the degree of modulation of the light responsiveness by PAPP5 is variable depending on light regimes. The response modulated by PAPP5 is more pronounced with pulsed light responses than with prolonged light responses.

PAPP5 can effectively dephosphorylate all of the phospho-serine residues of the Pfr form of oat phyAin *in vitro* and *in vivo*. In contrast, PAPP5 only slightly dephosphorylated the Pr form, indicating that PAPP5-mediated dephosphorylation of phytochromes is dependent on the spectral forms. Recruitment of phytochromes to PAPP5 through the TPR domain was necessary for effective dephosphorylation of phytochromes. These results together with photophysiological observations implied that the alteration of PAPP5-mediated dephosphorylation of biologically active Pfr-phytochromes is responsible for the altered photoresponsiveness.

Light signal perceived by phytochromes is transmitted through several downstream signal transducers that physically bind to phytochromes. When the phosphorylated Pfr-oat phyA were incubated with PAPP5, its binding affinity for NDPK2, a positive downstream transducer, was increased, whereas no noticeable effect was detected in the Pr form. Okadaic acid, an inhibitor of the phosphatase activity, to the reaction mixture negated the PAPP5-mediated effect on NDPK2 binding to phytochromes, indicating that the phosphatase activity is responsible for the altered binding affinity. It was also found that altered NDPK2 binding to Pfr-phytochromes is derived from PAPP5-mediated dephosphorylation of the serineresidue(s) in the hinge region of phytochromes. In addition, the stability of Pfr-phytochrome is increased by PAPP5. The results presented here indicated that the observed effect of PAPP5 on phytochrome stability in *Arabidopsis* is mainly dueto dephosphorylation of serine residue(s)

other than the residue(s) in the hinge region of phytochromes and is thus possibly due to dephosphorylation in the N-terminal extension.

Taken together, our results reveal important mechanisms for the role and regulation of phosphorylation status of phytochromes in photo-signaling; 1) the phosphorylation state of phytochromes is reduced by a type 5 protein phosphatase (PAPP5) that specifically dephosphorylates the biologically active Pfr form; 2) photoresponsiveness is correlated with the phosphatase activity of PAPP5 that is a positive regulatory component in plant photo-signaling. Thus, phosphorylation of Pfr-phytochromes by autophosphorylation and phytochrome-associated kinase(s) is a signal attenuation mechanismthat is counteracted specifically by the phosphatase activity of PAPP5; 3) dephosphorylation of the serine residue(s) in the hinge region of Pfr-phytochromes by PAPP5 results in enhanced affinity of phytochromes for the positive signal transducer, NDPK2, which in turn is positively correlated with photoresponses; 4) dephosphorylation of the serine residue(s) in the N-terminal extension by PAPP5 results in enhanced stability of phytochromes in a Pfr-form preferential manner, leading to enhanced photoresponsiveness. We, thus, propose a model in which the counter-action of the pair of phytochrome autophosphorylation/phytochrome-associated kinase(s) activity and Pfr form-specific phosphatase activity of PAPP5 provides a tuning mechanism that finely controls the flux of light information to downstream photoresponses. This control is mediated through regulation of phytochrome stability and affinity for downstream signal transducers such as NDPK.