Phototropin signaling in higher plants: Crosstalk with phosphoinositide pathway and the mechanism of phototropin2 internalization

Research summary

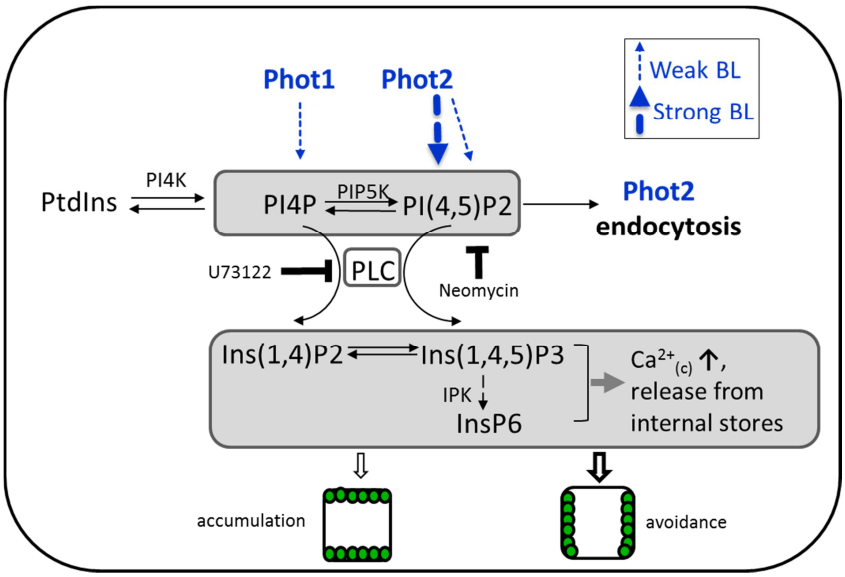
Phototropins are UVA/blue-light membrane receptors in plants, which control a range of responses (phototropism, stomatal opening and chloroplast movements) that serve to promote plant growth and optimize photosynthetic efficiency. The model plant *Arabidopsis thaliana* contains two phototropins, phot1 and phot2. Phosphoinositides are small membrane lipids which have both structural and signaling roles in the cell. The rapid synthesis and degradation of phosphoinositides places them as ideal regulators of cellular processes. Besides their function of mobilizing Ca^{2+} from internal stores via phosphatidylinositol 4,5-bisphosphate metabolism, they are also involved in the control of other cellular events which include rearrangement of cytoskeletal network, membrane trafficking and intracellular signaling. Studies performed in the last decade suggest the significance of phosphoinositide signaling in phototropin-mediated activities, in particular phototropism and stomatal opening. The aim of my thesis was to identify phosphoinositide(s) involved in phototropin-induced chloroplast movements and to study their mode of action in this process. For the latter purpose, the importance of phosphoinositide(s) was investigated in three areas: cytosolic Ca^{2+} signaling, actin network dynamics and blue-light-activated phot2 translocation in the cell. Another key part of the study was devoted to understanding the mechanism and significance of phot2 internalization.

In the first part, the involvement of phosphatidylinositol 4,5-bisphosphate - phospholipase C [PI(4,5)P2-PLC] pathway was analyzed during phototropin-activated chloroplast relocations, in *Arabidopsis* leaves. For this purpose, chemical modifiers of the PLC pathway, namely neomycin and U73122, were infiltrated to the leaves. The inhibitory action of these drugs towards the PI(4,5)P2-PLC pathway was verified using ELISA. Both the agents suppressed the phot2-mediated weak blue light (chloroplast accumulation) and strong blue light (avoidance) responses, without affecting movement responses controlled by phot1. After the application of U73343, an inactive structural analog of U73122, normal chloroplast movements were observed. Effects of the drugs on relocations were tested by photometry and microscopy. The inhibitory action observed with phosphatidylinositol metabolism inhibitors was, at least partly, due to disturbances in light-induced transient cytosolic Ca^{2+} changes. In response to the classical function of PI(4,5)P2 in Ca^{2+} signaling, the cytosolic Ca^{2+} levels were examined in the presence of U73122. Using transgenic *Arabidopsis* WT plants

(expressing cytosolic aequorin), it was shown that U73122 suppresses the blue-light-induced transient Ca^{2+} rise in the leaves. The details of this work are published in PlosOne (Aggarwal et al., 2013). Besides Ca^{2+} signaling, phosphoinositides are key elements regulating actin-cytoskeleton dynamics in the cell. Since actin filaments provides tracks for chloroplast movements in the cell, the effect of neomycin was studied on actin rearrangement in mesophyll cells of *Nicotiana tabacum*. Pretreatment with neomycin, followed by staining of F-actin using alexa-fluor phallodin showed no obvious effect on the actin network arrangement under dark conditions or after blue light irradiation when compared to control samples. Lastly, the effect of neomycin was studied on phot2 translocation in the cell. PI(4,5)P2 is known to participate in formation of clathrin-coated vesicles and thereby mediate clathrin-dependent endocytosis. It has been previously reported that plasma membrane phot2 shows cytosolic punctuate structures upon blue light irradiation. The punctuate structures were shown to co-localize with the Golgi apparatus. Treatment with neomycin stopped the formation of punctuate structures, indicating the participation of PI(4,5)P2 in phot2 internalization. A working model of PI action consistent with the data is presented in **figure 1**.

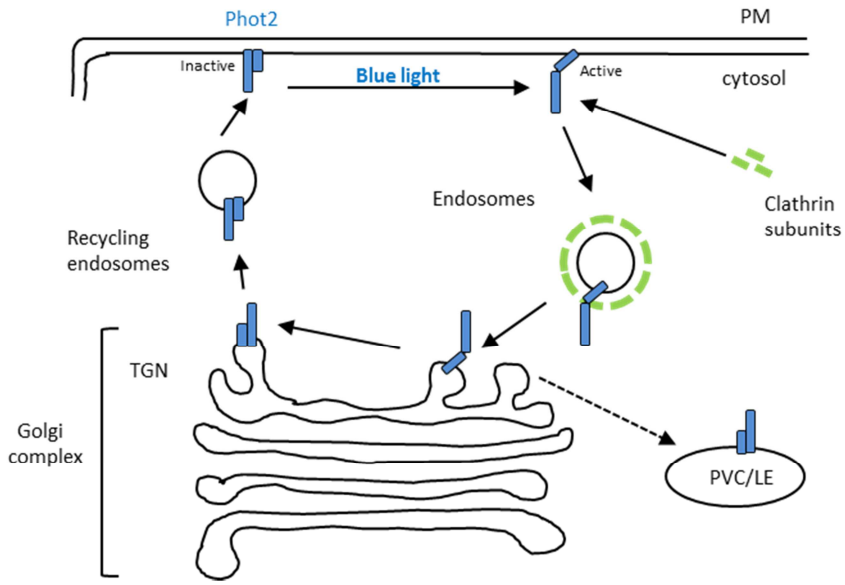
The second part of the study was focused on the phot2 intracellular trafficking. Endocytosis is an essential process in plants which is responsible for internalization of receptors and their subsequent degradation or recycling. Different approaches were examined to understand the mechanism of phot2 internalization. Using bimolecular fluorescence complementation and co-immunoprecipitation assays, strong association of phot2 with the clathrin light chain was determined at the plasma membrane and endosomal structures. Tyrphostin 23, a drug which interferes with receptor recruitment into the clathrin pathway, blocked phot2 endocytosis, confirming the clathrin-mediated internalization of phot2. Furthermore, disruption of the Golgi complex with a vesicle trafficking inhibitor brefeldin A resulted in phot2-containing 'BFA-compartments' in *Nicotiana benthamiana* protoplasts. Using specific endosomal compartment markers blue-light-activated phot2 was shown to travel from the plasma membrane to early endosome/trans-Golgi network vesicles with a very small fraction going for late endosomal pathway. These data were supported by turnover studies which showed a high stability of the phot2 in the presence of strong blue light with a half-life of ~ 5-7 h. In addition, the obtained results suggested that the internalized phot2 most likely recycles back to the cell surface. Western blot studies indicated that a possible reason for phot2 internalization and recycling is the de-phosphorylation of the blue-light-activated receptor. A model representing the route of phot2 trafficking is shown in **figure 2**.

Figure 1



** BL (blue light), $Ca^{2+}_{(c)}$ (cytosolic Ca^{2+})

Figure 2



** TGN (trans-Golgi network), PM (plasma membrane), PVC/LE (pre-vacuolar compartment/late-endosome)