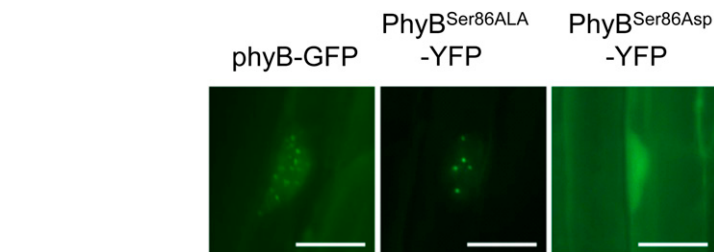


IN BRIEF

Phosphorylation and Dark Reversion of Phytochrome B

Phytochrome B (phyB) is the main light receptor responsible for the shade avoidance response in mature plants. Like other phytochromes (phys), it alternates between the Pfr form and the Pr form. Upon activation by red light (causing the switch to the Pfr conformation), phys interact with various signaling partners and translocate from the cytoplasm to the nucleus, where they are found in structures called photobodies (reviewed in Chen and Chory, 2011). Phytochromes are evolutionarily related to light-regulated His kinases, and the role of phosphorylation in phy signaling has been a matter of much debate. Two phosphatases interact with phyB, suggesting that phosphorylation might be play a role in its activity (Ryu et al., 2005; Phee et al., 2008). New work from **Medzihradzsky et al.** (pages 535–544) shows that phosphorylation of *Arabidopsis thaliana* phyB increases its rate of reversion from the active Pfr form to the inactive Pr form.

Medzihradzsky et al. began by testing whether phyB is indeed phosphorylated in vivo. They found that a phyB-TAP fusion protein purified from transgenic *Arabidopsis* seedlings was phosphorylated in the N-terminal domain, including on Ser-86. They then tested the function of phosphorylation in phyB signaling by mutating Ser-86 to Ala (representing the unphosphorylated state) or Asp (mimicking phosphorylation) and expressing the mutant proteins as fusions in a *phyB* mutant background. The phyB^{Ser86Asp} line showed decreased phyB signaling, especially at low fluences of R light, in terms of hypocotyl growth inhibition, cotyledon expansion, and shade avoidance. By contrast, the phyB^{Ser86Ala} line appeared to have enhanced phyB signaling activity.



Photobody formation in the nucleus requires higher intensity light for phyB^{Ser86Asp}-YFP and lower for phyB^{Ser86Ala}-YFP. Dark-grown *phyB-9* seedlings expressing the indicated proteins were treated with 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ red light for 6 h. Bars = 10 μm . (Reprinted from Medzihradzsky et al. [2013], Figure 3B.)

In fact, the mutations altered virtually every facet of phyB function. Compared with the wild type, lower fluences of red light were able to induce nuclear accumulation and photobody formation of phyB^{Ser86Ala}, whereas higher fluences were necessary for phyB^{Ser86Asp} (see figure). The phyB^{Ser86Asp} mutation also led to reduced interaction with PHYTOCHROME INTERACTING FACTOR3 under nonsaturating light conditions in yeast two-hybrid assays.

In an attempt to explain these myriad effects, the authors examined dark reversion, in which the Pfr form becomes inactive by reverting back to the Pr form. Importantly, they found that the phyB^{Ser86Asp} mutant reverted to the Pr form faster and to a greater extent than did the wild type, whereas phyB^{Ser86Ala} reverted more slowly than the wild type. These changes in dark reversion rate can explain all of the phenotypes caused by the Ser-86 mutations and shed light on the molecular mechanisms of phyB signaling. This thorough study from Medzihradzsky et al. convincingly demonstrates that phosphorylation has a role in phyB function and

that Ser-86 phosphorylation, in particular, is linked with phyB dark reversion.

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