

## Phytochromes A and B: Specificity of photoperception and structure/function analysis of bilin chromophores

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Phytochrome A (phyA) and phytochrome B (phyB) perceive light and adapt to fluctuating circumstances by different manners in terms of effective wavelengths, required fluence and photoreversibility. Action spectra for induction of seed germination and inhibition of hypocotyl elongation using phytochrome mutants of *Arabidopsis* showed major difference. PhyA is the principal photoreceptor for the very low fluence responses and the far-red light-induced high irradiance responses, while phyB controls low fluence response in a red/far-red reversible mode. The structural requirement of their bilin chromophores for photosensory specificity of phyA and phyB was investigated by reconstituting holophytochromes through feeding various synthetic bilins to the following chromophore-deficient mutants: *hyl*, *hyl/phyA* and *hyl/phyB* mutants of *Arabidopsis*. We found that the vinyl side-chain of the D-ring in phytochromobilin interacts with phyA apoprotein. This interaction plays a direct role in mediating the specific photosensory function of phyA. The ethyl side-chain of the D-ring in phycocyanobilin fails to interact with phyA apoprotein, therefore, phyA specific photosensory function is not observed. In contrast, both phytochromobilin and phycocyanobilin interact with phyB apoprotein and induce phyB specific photosensory functions. Structural requirements of the apoproteins and the chromophores for the specific photoperception of phyA and phyB are discussed.

**Key words:** phytochrome, action spectra, chromophore, mutant, *Arabidopsis*, rice

### INTRODUCTION

Physiological and photochemical studies over several decades have shown that presumed phytochrome-mediated responses conform to one of several different response modes. Light responses in photomorphogenesis were classified as either a "low energy reaction" or a "high energy reaction" in accordance to their specific energy requirements [1]. A short pulse of light irradiation induced low energy reactions. These were divided into "low fluence response" (LFR, previously cited as "low irradiance response") and "very low fluence response" (VLFR, previously cited as "very low irradiance response") [2]. The

high energy reaction, which required radiation with relatively high energy for a long period of time was renamed "high irradiance response" (HIR) [3].

*Arabidopsis thaliana* (L.) Heynh. has a phytochrome gene family that is composed of five members (*PHYA* to *PHYE*) [4, 5]. Recent research results have led to the idea that phytochrome A (phyA) and phytochrome B (phyB) have overlapping physiological functions, although these two phytochromes perceive distinct environmental light.

Here we report that phyA and phyB are different in modes of photoperception-based results from the action spectra. Using chemically synthesized phytochromobilin (PΦB), phycocyanobilin (PCB) and various analogs [6], we found the synthetic bilin chromophores effect on the photobiological activities of phyA and phyB *in vivo*.

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## MATERIALS AND METHODS

*Plant materials and light treatment.* The mutant alleles of *A. thaliana* used in this study were *phyA-201*, *phyB-1*, *hy1*, *hy2* and their double mutants. Light-emitting diodes (LEDs) were used to develop a new light irradiation system [7].

*Action spectra.* Fluence-response curves for induction of seed germination and inhibition of hypocotyls elongation were determined using the Okazaki large spectrograph. From these curves, the photon fluence of a light pulse required for 50% induction of germination [8] and for inhibition of hypocotyl elongation [7] at each measured wavelength.

*Synthetic bilins and growth condition.* PCB, P $\Phi$ B and their analogs were chemically synthesized and applied for the two-day-old etiolated seedlings [9].

## RESULTS

*Physiological characteristics of phyA and phyB.* It was

demonstrated conclusively that *phyA* in *A. thaliana* has at least two different modes of photoperception (Fig. 1 and Table 1). Depending on the photoconversion from Pr to Pfr, the first was observed in "photoirreversible" VLFR [8]. Another was observed in "photoreversible" HIR depending on the photoconversion from "Pfr to Pr"[7]. These results suggest that the *phyA*-dependent inhibition of hypocotyl

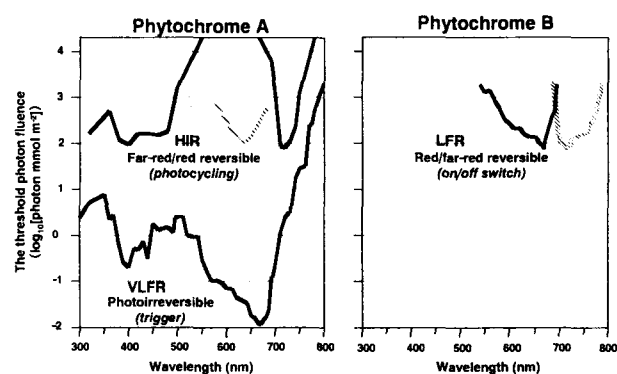


Figure 1. Action spectra for phytochrome-mediated responses in *Arabidopsis thaliana*.

Table 1. Overview of physiological characteristics of phytochrome A and phytochrome B for regulation of LFR, VLFR and HIR in *Arabidopsis thaliana*.

Corresponding response and phytochrome species	Inhibition of hypocotyl elongation		Induction of seed germination	
	FR-HIR	R-HIR	VLFR	LFR
	<i>phyA</i>	<i>phyB</i>	<i>phyA</i>	<i>phyB</i>
Effective light exposure (Maximum intervals of pulsed light)	IPLS * (3 min <sup>[7]</sup> ) or C **	IPLS * (4 h <sup>[10]</sup> ) or C **	Single pulse irradiation	Single pulse irradiation
The Bunsen-Roscoe reciprocity law	Valid <sup>[7]</sup>	Valid <sup>[10]</sup>	Valid <sup>[8]</sup>	Valid <sup>[8]</sup>
Effective range of wavelength (nm)				
Inducing response	320-500/700-750 <sup>[7]</sup>	320-700 <sup>4)</sup>	300-770 <sup>[8]</sup>	540-690 <sup>[8]</sup>
Reversible response	660 *** <sup>[7]</sup>	750 *** <sup>[7] [10]</sup>	Not observed <sup>[8]</sup>	695-780 <sup>[8]</sup>
Photo-reversibility	FR/R reversible <sup>[7]</sup>	R/FR reversible <sup>[10]</sup>	Not observed <sup>[8]</sup>	R/FR reversible <sup>[7]</sup>
The threshold photon fluence ( $\mu$ mol m <sup>-2</sup> )				
400 nm	5 <sup>[7]</sup>	ND #	0.2 <sup>[8]</sup>	2,000 <sup>[8]</sup>
667 nm	less than 700 <sup>[7]</sup>	ND #	0.001 <sup>[8]</sup>	30 <sup>[8]</sup>
726 nm	70 <sup>[7]</sup>	ND #	0.3 <sup>[8]</sup>	40 <sup>[8]</sup>

\*IPLS, intermittent irradiation with pulses; \*\*C, continuous irradiation; \*\*\*The wavelength implies the peak emission of the broad banded light sources; # ND, not determined yet.

elongation may represent a specialized branch of the FR/R sensing pathway. This signaling pathway may have unique transduction components that are not shared by the other photoreceptors or with unique localization of transduction components.

**Chromophore structure and phyA function.** To investigate the role of phytochromobilin (PΦB) and phycocyanobilin (PCB) in phyA specific response we measured the hypocotyl lengths of *hy2* seedlings with either PΦB or PCB by irradiation with intermittent FR light. The PΦB-treated *hy2* seedlings showed significant different growth patterns in terms of hypocotyl length, cotyledon opening when compared to PCB-treated *hy2* seedlings (Fig. 2). The PΦB-treated *hy2* seedlings possessed hypocotyl lengths as short as WT seedlings [9]. PCB-treated seedlings were indistinguishable from the dark grown controls (Fig. 2). These results showed that the phyA-mediated-responses occurred only when PΦB was supplied.

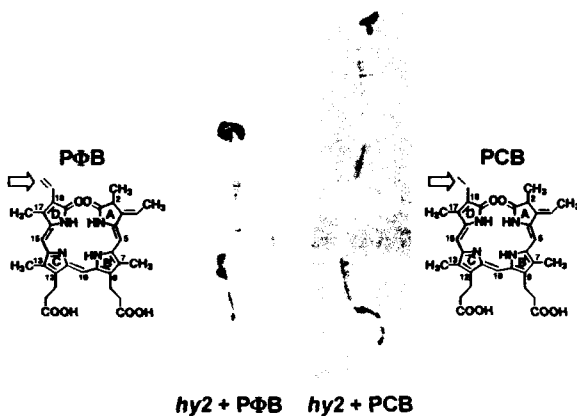


Figure 2. Effects of PΦB and PCB on *hy2* growth response by irradiation with FR light.

**Chromophore structure and phyB function.** We next examined the effect of exogenously supplied PCB analogs on the hypocotyl growth of *hy2* mutant seedlings that were grown under intermittent R or FR light irradiation (Fig. 3). These analogs were substituted from PCB at the C18 position of the bilin D-ring with a saturated alkyl group such as *n*-propyl (Analog 16), *n*-pentyl (Analog 17) or *n*-octyl (Analog 18)(Fig. 3). Although all analogs were effective to the phyB responsiveness in *hy2* seedlings, the effectiveness of R varied (Fig. 3). R/FR reversibility on hypocotyl

elongation decreased according to the increasing length of the side-chains (Fig. 3).

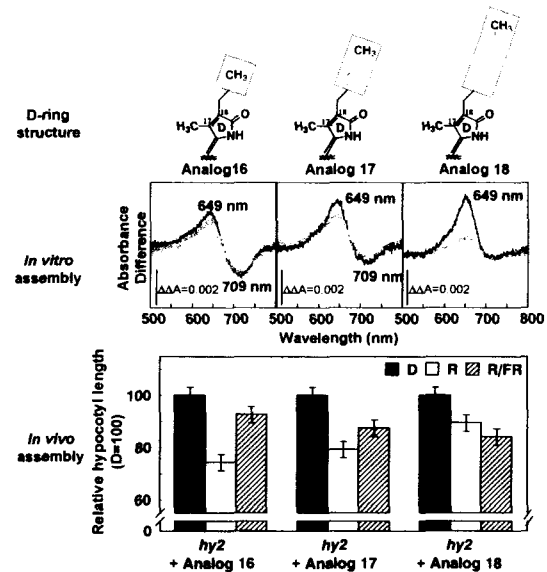


Figure 3. Effect of chromophore structure (the side-chain of the PCB D-ring) on phyB-mediated inhibition of hypocotyl elongation in *hy2* seedlings.

## DISCUSSION

**Photoperception of phytochrome species.** Both phyA and phyB are essentially important for the *A. thaliana* development and life cycle from seed germination to flowering. Recently, several papers have been published addressing function of PhyC, PhyD and PhyE. These phytochromes also have overlapping physiological functions as observed in PhyA and PhyB, but they play only minor roles in the presence of PhyB [11]. Interestingly, in rice *phyA* mutants, FR light induced *RBCS* and *CAB* gene [12]. This result suggests that photoreceptor(s) other than phyA (probably phyC) can mediate the expression of these genes in response to irradiation with FR light in etiolated rice seedlings [12].

**Phytochrome function and chromophore structure.** The structure of PΦB is closely related to PCB. In addition, the photochemical properties and the molar absorption coefficients of PΦB- and PCB-adducts of oat PHYA are

quite similar [13]. The loss of phyA-mediated response to FR in PCB-supplied seedlings (Fig.2) might be caused by the bilin structure modification. The double bond in the vinyl side chain of the PΦB D-ring was crucial for the photosensory function of phyA. We can speculate that some amino acid(s) in the N-terminal domain of PHYA interact directly and would therefore carry out an essential role for direct interaction with this vinyl side chain. One candidate for such an amino acid in PHYA is Ile 80 [9] that preferentially interacts with the vinyl group of bilin D-ring in a qualitative model [14]. The other amino acid(s) of PHYA would also directly interact with the vinyl group of the D-ring [9]. PHYB seems to be more flexible than PHYA in chromophore compatibility, because phyB accepts both PΦB and PCB as a functional chromophore for induction of physiological response.

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