

Article Addendum

Phytochrome A Function in Red Light Sensing

Keara A. Franklin*

Garry C. Whitelam

Department of Biology; University of Leicester; Leicester, UK

*Correspondence to: Keara A. Franklin; Department of Biology; University of Leicester UK; Tel. +44.116.252.3339; Fax: +44.116.252.2791; Email addresses: kaf5@le.ac.uk, gcw1@le.ac.uk

Original manuscript submitted: 04/12/07
Manuscript accepted: 04/12/07

Previously published online as a *Plant Signaling & Behavior* E-publication:
<http://www.landesbioscience.com/journals/psb/article/4261>

KEY WORDS

phytochrome A, red light, irradiance, hypocotyl, cotyledon, photoprotection

Addendum to:

Phytochrome A is An Irradiance-Dependent Red Light Sensor

Franklin KA, Allen T, Whitelam GC

Plant J 2007; 50:108–17

PMID: 17346261

DOI: 10.1111/j.1365-313X.2007.03036.x

ABSTRACT

Light signals perceived by the phytochrome family of red (R) and far-red (FR) light-absorbing photoreceptors direct plant growth and development throughout their lifecycle. In contrast to other family members, phyA displays rapid light-induced proteolytic degradation upon conversion to the biologically active Pfr form and mediates high irradiance responses to continuous FR. These unique properties together with limited examples of phyA function in R have resulted in an over-simplified portrayal of phyA as a FR sensor which acts predominantly in seed germination and early stages of seedling de-etiolation. In a recent work, published in *The Plant Journal*, we report significant phyA activity in *Arabidopsis thaliana* at high (>100 $\mu\text{molm}^{-2}\text{s}^{-1}$) photon irradiances of R. Under these conditions, we observed retarded degradation of a pool of nuclear-localised phyA, consistent with the phenomenon of photoprotection, and showed *phyBphyCphyDphyE* quadruple null mutants, containing only functional phyA, to de-etiolate and survive to flowering. The photon irradiances used in this study were greater than those routinely used for photomorphogenic analysis in the laboratory but considerably lower than those commonly observed in daylight. In this addendum we present additional analyses of the *phyBphyCphyDphyE* mutant and discuss the possibility that phyA may perform a significant role in the growth and development of daylight-grown plants.

INTRODUCTION

Analyses of mutants, deficient in individual and multiple combinations of phytochromes, have been paramount in elucidating the functions of family members throughout plant development.¹ Phytochrome A is the most abundant phytochrome in etiolated seedlings and performs a fundamental role in seedling de-etiolation.² The rapid decrease in phyA levels upon transfer to light results from both light-mediated turnover of the protein and down-regulation of *PHYA* transcription.^{3,4} A variety of phyA-mediated very low fluence responses (VLFRs) and high irradiances responses (HIRs) to continuous far-red light (FRc) have been characterised but limited examples of phyA functions in R exist in the literature. Modestly increased hypocotyl length, reduced hook opening and reduced cotyledon expansion have all been reported in Rc-grown *phyAphyB* mutants when compared with monogenic *phyB* mutants, suggesting a role for phyA in Rc-mediated de-etiolation.⁵⁻⁷ In addition, phyA has been reported to mediate the R-enhancement of phototropic curvature in blue light⁸ and R-induced positive phototropism in roots.⁹ Recent microarray studies have also shown phyA to be the principle phytochrome regulating rapidly responding genes during early stages of R-mediated de-etiolation.¹⁰ The photon irradiance used in all these studies was, however <50 $\mu\text{molm}^{-2}\text{s}^{-1}$. We observed considerable phyA activity at high (>100 $\mu\text{molm}^{-2}\text{s}^{-1}$) photon irradiances of R. At these photon irradiances we also observed retarded degradation of the protein in wild-type (WT) plants and prolonged epifluorescence of nuclear-localised *phyA::YFP* in transgenic lines.

MUTANTS CONTAINING ONLY FUNCTIONAL phyA DISPLAY CONSIDERABLE DE-ETIOLATION AT HIGH PHOTON IRRADIANCES OF R

Creation of a *phyBphyCphyDphyE* quadruple null mutant enabled the role of phyA in R signalling to be examined in the absence of other phytochromes. The de-etiolation of these plants at a range of different photon irradiances is shown in Figure 1A. At photon irradiances >100 $\mu\text{molm}^{-2}\text{s}^{-1}$ *phyBphyCphyDphyE* mutants displayed significant inhibition

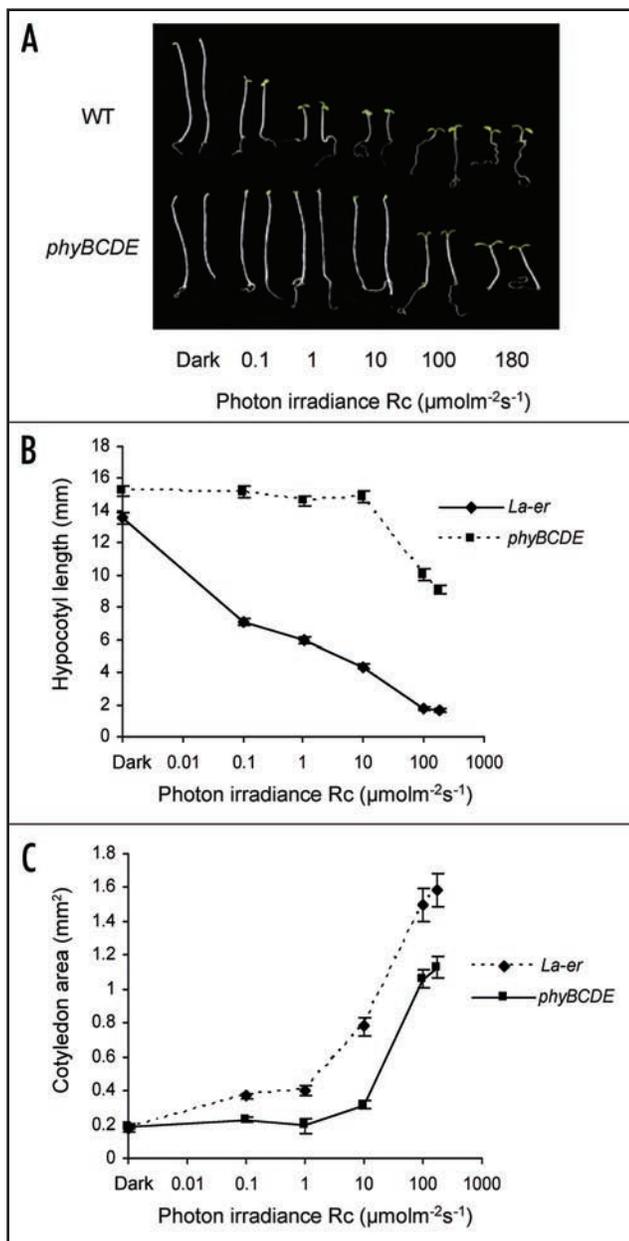


Figure 1. (A) Phenotypes of WT and *phyBphyCphyDphyE* seedlings grown for five days at different photon irradiances of R. (B) Hypocotyl lengths and (C) Cotyledon areas of WT and *phyBphyCphyDphyE* mutants grown for 5 days at different photon irradiances of R. Experimental procedures are described in Franklin et al. 2007.

of hypocotyl elongation (Fig. 1B) and promotion of cotyledon expansion (Fig. 1C). Such observations unequivocally demonstrate the capacity of phyA to promote substantial de-etiolation in R_c in an irradiance-dependent manner.

A SIGNIFICANT ROLE FOR phyA IN DAYLIGHT-GROWN PLANTS?

Phytochrome A activity has been reported throughout the lifecycle of plants. Despite resembling WT plants in continuous white light, *phyA* mutants displayed elongated hypocotyls when grown in light/dark cycles and continuous low R:FR ratio.^{11,12} The phyA-mediated inhibition of hypocotyl growth in low R:FR ratio occurs

at dawn,¹³ when phyA levels are highest, and is thought to 'antagonise' shade avoidance by preventing excessive elongation growth. This response has been shown to be of fundamental importance to seedlings developing under dense, natural vegetational shade. When grown in the field under these conditions, *phyA* mutants displayed extreme hypocotyl elongation, with many failing to establish and dying prematurely.¹⁴ Phytochrome A has also been shown to perform a role in the perception of daylength,² suppression of internode elongation¹⁵ and leaf elongation¹⁶ in mature plants. This study not only demonstrates photoprotection of phyA at high photon irradiances of R (a waveband that maximises Pfr concentration and hence degradation rate) but also provides evidence of significant functional activity for photoprotected phyA. In natural daylight, photoprotection ensures maintenance of a phyA pool, despite the establishment of a relatively high Pfr concentration, conditions which maximise phyA degradation at more modest photon irradiances.^{17,18} Given that photoprotected phyA displays significant biological activity, it is likely that this phytochrome performs a more significant role in development of daylight-grown plants than has previously been considered.

Phytochrome-deficient mutants have proved invaluable in elucidating the functional capacity and redundant interactions of individual family members throughout development. Our understanding of how individual phytochromes functionally interact in WT plants does, however, appear partly dependent on the experimental growth conditions used for analyses. Recently, a small decrease in ambient growth temperature was shown to significantly alter the functional relationships between phytochromes for multiple physiological responses.^{19,20} We have now demonstrated considerable phyA activity in R, using photon irradiances higher than those commonly used for laboratory studies. Given the diverse and fluctuating environmental conditions experienced by plants growing in natural communities, it is likely that further broadening of experimental regimes may reveal additional functional activities for individual phytochromes, thus providing additional insight into the regulatory roles of this important group of plant photoreceptors.

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