Red Light-Induced Phytochrome Relocation into the Nucleus in *Adiantum capillus-veneris*

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ABSTRACT Phytochromes in seed plants are known to move into nuclei in a red light-dependent manner with or without interacting factors. Here, we show phytochrome relocation to the nuclear region in phytochrome-dependent *Adiantum capillus-veneris* spore germination by partial spore-irradiation experiments. The nuclear or non-nuclear region of imbibed spores was irradiated with a microbeam of red and/or far-red light and the localization of phytochrome involved in spore germination was estimated from the germination rate. The phytochrome for spore germination existed throughout whole spore under darkness after imbibition, but gradually migrated to the nuclear region following red light irradiation. Intracellular distribution of PHY–GUS fusion proteins expressed in germinated spores by particle bombardment showed the migration of *Ac*phy2, but not *Ac*phy1, into nucleus in a red light-dependent manner, suggesting that *Ac*phy2 is the photoreceptor for fern spore germination.

Key words: *Adiantum capillus-veneris*; far-red light; microbeam; phytochrome; red light; spore germination.

INTRODUCTION

It has long been known that seed germination is promoted by red light (R) and inhibited by far-red light (FR) since Borthwick and his coworkers' (1952) research with Grand Rapids lettuce seeds. Almost 100% of the seeds that received a short R pulse germinated, but the R effect was fully reversed by a following short FR treatment. The R and FR effects were repeatedly reversible, as a hallmark of phytochrome involvement. The light dependency of fern spore germination was also well documented. R in the 650–670-nm region most effectively induced spore germination, whereas subsequent irradiation with FR at 733–750 nm, far ultra violet light at 260 nm, near ultra violet light (UV) at 380 nm, and blue (B) at 440 nm inhibited the R-induced germination in *Pteris vittata* (Sugai and Furuya, 1967; Sugai, 1971; Sugai et al., 1984; Sugai and Furuya, 1990). The inhibition of R-induced spore germination by UV and B was also shown in *Adiantum capillus-veneris* L. (Sugai and Furuya, 1985). In *Arabidopsis thaliana*, *PHYA* and *PHYB* among five phytochrome genes (*PHYA–E*) play the main roles in seed germination (Sharrock and Quail, 1989; Clack et al., 1994). PhyA mediates seed germination at a very low fluence of monochromatic light ranging from 300 to 780 nm, and this phyA-dependent response is not reversible by FR. Seed germination regulated by phyB is photoreversible by alternate irradiations with either 540–690 or 695–780 nm under the fluence rates of 0.01–1 μmol m^{-2} (Shinomura et al., 1996). Interestingly, phyE is required for germination under continuous FR irradiation (Hennig et al., 2002). Phytochromes are mainly localized in the cytosol but partially diffused in a nucleus under dark conditions, and transported into nucleus under light and formed speckles (Yamaguchi et al., 1999; Gil et al., 2000; Hisada et al., 2000; Kim et al., 2000; Kircher et al., 2002; Oka et al., 2008). A single, brief (~5 min) FR, R, or B pulse, or white light induced nuclear import of a phyA::GFP fusion protein within a few minutes after the inductive light pulse in etiolated seedlings of *A. thaliana* (Kim et al., 2000; Kircher et al., 2002). Nuclear import of a phyB::GFP fusion protein in etiolated seedlings was, however, insensitive to single red, blue, and far-red pulse, although it was induced by continuous R irradiation (Gil et al., 2000; Kircher et al., 2002). Although light induced phyB translocation into the nucleus, the phyB import was at least one order of magnitude slower than that of phyA and reached its maximum level after 6 h (Kircher et al., 2002). Considering the difference of

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doi: 10.1093/mp/ssr119
Received 20 October 2011; accepted 11 December 2011

transport speed into the nuclei between the phyA and phyB, very fast accumulation of phyA in nuclei should require an efficient active transport system for the molecule to be moved into the nucleus. It was reported that the nuclear accumulation of phyA required at least two small, plant-specific proteins: FAR RED ELONGATED HYPOCOTYL 1 (FHY1) and its homologue FHY1 LIKE (FHL) (Hiltbrunner et al., 2005; Zhou et al., 2005). *At*FHY1 was sufficient for *At*phyA transport into nucleus in isolated nuclei of *Acetabularia acetabulum* (Pfeiffer et al., 2009). FHY1 and FHL were shown to interact with phyA and to be positive regulators of phyA signaling during deetiolation (Desnos et al., 2001; Zhou et al., 2005). Recently, it was shown in *fhy1/fhl* double mutant plants that the light-induced nuclear accumulation of phyA was completely abolished, suggesting an important role of these two proteins in the nuclear-transport mechanism for phyA (Hiltbrunner et al., 2006). Other components of physical interaction of phytochrome are a small subset of constitutively nuclear, basic helix–loop–helix (bHLH) transcription factors, named Phytochrome-Interacting Factors (PIFs) (Castillon et al., 2007). The PIF proteins was initially identified by a yeast two-hybrid screen as phyB-interacting proteins, and subsequently shown to bind phytochrome, in a photoreversible fashion, to the Pfr form of both phyA and phyB (Ni et al., 1999; Shimizu-Sato et al., 2002).

Thus, the phytochrome property of translocation from cytoplasm to nuclei in *Arabidopsis* has been well established. Moreover, the functions of phytochrome-interacting factors such as FHY1 and FHL or PIFs were resolved at the molecular level. However, the precise experiments showing translocation of functional phytochrome from cytoplasm to nuclei has not yet been performed at cellular level. Here, we applied the partial cell irradiation technique (Wada and Furuya, 1978; Wada, 2008; Tsuboi and Wada, 2011a, 2011b, 2011c) on the spore germination of *A. capillus-veneris* to determine whether physiologically functional phytochromes migrate towards the nuclear region and move into nuclei. Whether *Adiantum* phy1, phy2, or both mediates the spore germination was also investigated.

RESULTS

It has been known that R-induced fern spore germination obeys the reciprocity law (Sugai and Furuya, 1967, 1985). Hence, to know the fluence for the maximum germination rate in *A. capillus-veneris* spores, the fluence–response relationship was obtained with the constant fluence rate of 4.2 W m^{-2} of R and different irradiation periods for 3–240 s by whole spore irradiation with a microbeam (255 × 440 μm). Germination rate was determined at 5 d after the light irradiation (Figure 1A). Regardless of the length of the irradiation up to 60 s, the germination rate was proportional to the total fluence. As the spore germination rate showed the saturation at 60% with 252 J m^{-2} (4.2 W m^{-2} for 60 s: Figure 1A, arrow), this fluence was used for the following experiments.

A fluence–response curve for FR reversibility on the R-induced spore germination was obtained (Figure 1B). Immediately after

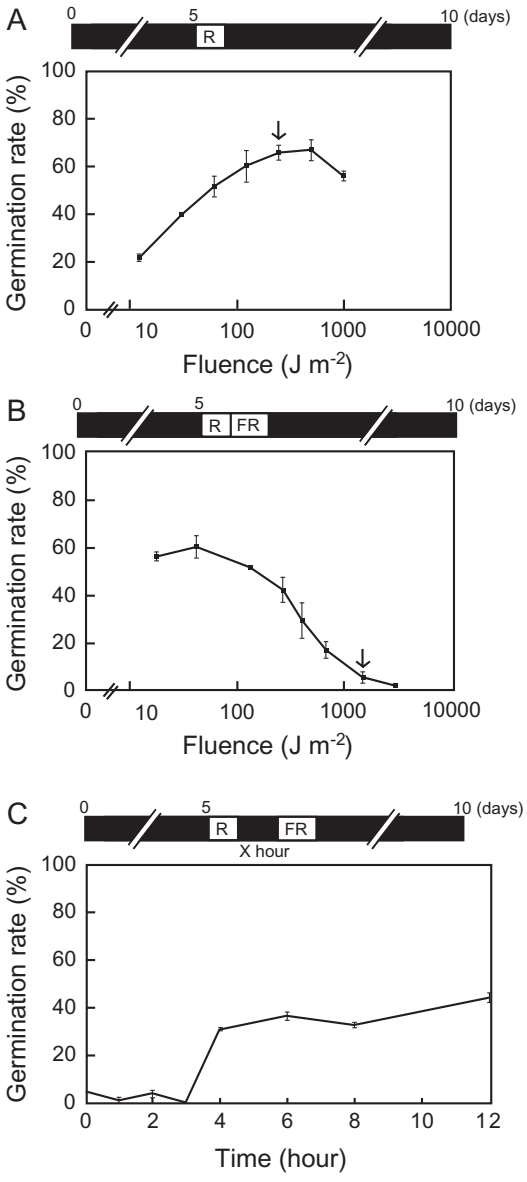

Figure 1. Fluence–Response Relationship for Spore Germination.

(A) Spores imbibed for 5 d under darkness were irradiated with a R microbeam (255 × 440 μm) with 4.2 W m^{-2} for various periods of time (from 3 to 240 s). Arrow indicates the fluence (252 J m^{-2}) used for the following experiments.

(B) Fluence–response relationship for FR reversibility on the R-induced spore germination. Whole spore was irradiated with 4.2 W m^{-2} of R microbeam (255 × 440 μm) followed by an FR microbeam (255 × 440 μm) irradiation for various periods of time (from 3 to 480 s). Arrow indicates the fluence (1440 J m^{-2}) used for the following experiments.

(C) Escape response from FR reversibility in R-induced spore germination. Spores were irradiated with an R microbeam (255 × 440μm; 4.2 W m^{-2} for 60 s) and then incubated in the dark for various periods of time from 0 to 12 h. Then, the spores were irradiated with an FR microbeam (255 × 440 μm; 6.0 W m^{-2} for 240 s) and incubated in the dark for 5 d. Each point represents the mean ± SE determined from two or three experiments. More than 100 spores were counted in each experiment.