Both Subunits of the Dimeric Plant Photoreceptor Phytochrome Require Chromophore for Stability of the Far-red Light-absorbing Form*

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The dimeric plant photoreceptor phytochrome is converted from its inactive red light-absorbing form (Pr) into the active far-red light-absorbing form (Pfr) upon light absorption. Dynamics of Pfr generation and of thermal Pfr-to-Pr conversion are of fundamental importance for inducing adequate responses to light signals. Here, we analyzed the role of subunit interactions on spectroscopic properties of dimeric phytochrome A. Using a coexpression system and affinity chromatography, we prepared mixed phytochrome dimers that can incorporate the essential chromophore only in one subunit. We demonstrate that such mixed dimers have unaltered difference spectra. In contrast, dark reversion differed greatly between Pfr-Pfr homodimers and Pfr-Pr heterodimers, the former being about 100-fold more stable. Temperature dependence of reaction rates revealed an additional stabilization of about 4 kcal/mol in homodimers. Consequences of these findings are discussed in relation to the biological function of, and functional diversification between, phytochrome family members.

Phytochromes are photoreceptors that coordinate plant development according to the light environment. Major processes regulated by phytochromes are seed germination, morphological changes of seedlings upon emergence from the soil (detection), growth rates of adult plants, and induction of flowering (1). Studies using mutants revealed that the five phytochromes (phy1A–phyE) in the model plant Arabidopsis have both overlapping and distinct physiological functions (2).

Higher plant phytochromes are homodimers of subunits of about 120 kDa. Each subunit consists of two major domains. The N-terminal half is required for spectral integrity, whereas the C-terminal half mediates dimerization and interactions with signaling partners (3). The C-terminal domain has distant homology to histidine kinases of two component signaling systems. Phytochromes are, however, not histidine kinases but serine/threonine kinases (4). Several substrates for phytochrome kinase activity have been determined, but the involvement of phosphorylation in signal transduction remains to be proven. Phytochrome apoproteins incorporate the linear tetrapyrrol phycobilin, a reaction catalyzed by an intrinsic chromophore lyase activity (3). The chromophore is bound via a thioester bond to a conserved cysteine in the N-terminal domain. Phycocyanobilin (PCB) of cyanobacteria can be incorporated to form a chromoprotein with a hypsochromic shift of about 10 nm and similar kinetic parameters to native phytochromes (5).

The holoprotein can adopt two major conformations: the red-absorbing Pr form and the far red-absorbing Pfr form. Newly synthesized phytochrome is in the biologically inactive Pr form. Upon absorption of light, Pr will be converted into biologically active Pfr. Because the absorption spectra of Pr and Pfr overlap, irradiation with light establishes a photoequilibrium (ϕ), which is highest (−0.8) around 660 nm and drops to 0.03 at 720 nm (6). Beside the light reactions, Pfr also converts thermally back into Pr by a process called dark reversion. Dark reversion has been suggested to attenuate phytochrome signaling because it reduces the amount of active Pfr when no light stimuli are present (7). Importantly, a mutant form of phyB (phyB101) with accelerated dark reversion has greatly reduced signaling abilities (7). The extent and rate of dark reversion vary widely between species and different phytochromes (8, 9). Especially, phyA of monocotyledonous plants appears not to undergo dark reversion at all (10, 11). In this case, like for other light labile phytochromes, signal attenuation is achieved by rapid degradation of the activated Pfr form (12). Using a yeast expression system for domain swapping experiments, it could be shown that the entire molecule is required to prevent spontaneous dark reversion (13). Previously, it was observed that dark reversion is often incomplete; therefore, Brockmann et al. (14) suggested that Pfr-Pfr homodimers are stable and do not undergo dark reversion.

EXPERIMENTAL PROCEDURES

Plasmids—Arabidopsis phyA cDNA in pKS (plasmid pRpA7) was kindly provided by P. Quail. For expression in yeast the vectors pAA7 (15) and pAA7b were used. These plasmids allow GAL7 promoter-driven protein expression in yeast based on uracil or leucine auxotrophic selection. The cDNA was subcloned into pAA7 using DraI and SalI, yielding plasmid pAA7a. This cloning strategy eliminated an additional ATG followed by a short open reading frame upstream of the start codon, which caused very low expression levels in yeast (data not shown). Plasmid p-malc2 (New England BioLabs, Beverly, MA) harboring male1, the gene coding for MBP, and pAA7 were used as templates for polymerase chain reaction to generate an in-frame fusion of the MBF cDNA to the 3′ end of the phyA cDNA in pKS (plasmid pAA7A) and in pAA7b (pAbAt3). A mutated phyA-MBP fusion cDNA encoding

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1 The abbreviations used are: phy, phytochrome; PCB, phycocyanobilin; Pr, red light-absorbing form of phytochrome; Pfr, far-red light absorbing form of phytochrome; MBP, maltose-binding protein.

2 L. Hennig; the plasmid will be described elsewhere.
the C323S phyA polypeptide was constructed by polymerase chain reaction. The construct was subcloned into pKS (plasmid pAbA13) and pAA7b (pAbAt4).

**Protein Expression and Purification**—Expression and affinity purification of phyA was carried out as described previously (16). Briefly, yeast *Saccharomyces cerevisiae*, strain KN380 (17) was transformed with appropriate plasmid(s). Cotransformation with pAA7- and pAA7b-derived plasmids allowed coordinated, inducible expression of two polypeptides at similar levels. Single colonies were used to inoculate 100 ml of selective minimal medium (18) and grown for 1 day at 30°C. This culture was diluted with 500 ml of noninducing complete medium (18) and grown overnight at 30°C. An A600 of ∼1, protein expression was induced with 2% (w/v) solid galactose. After an additional 5–8 h of cultivation, the cells were harvested by centrifugation. This procedure usually yielded 0.9–1.2 g of wet weight cells per 100 ml of culture. Cells were resuspended in 2 volumes of Buffer A (100 mM Tris–HCl (pH 7.7), 200 mM NaCl, 1 mM EDTA, 28 mM 2-mercaptoethanol, 4 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, 1 µg/ml antipain) and disrupted in an SLM Aminco French pressure cell with 120 MPa. The extract was centrifuged at 50,000 × g for 30 min at 4°C. Affinity purification on amylose resins (New England Biolabs) was performed according to the manufacturer’s instructions. Briefly, after washing with several volumes of Buffer A, bound material was eluted with 10 mM maltose in Buffer A. Fractions were analyzed by SDS-polyacrylamide gel electrophoresis (19) and Western blotting using MAC3/289 antiserum directed against mustard phyA (kindly provided by B. Thomas, Wellesbourne, Warwick, UK). In this paper, we refer to *Arabidopsis* phyA-PCB holoproteins as PHY, to *Arabidopsis* phyA-MBP fusions assembled with PCB as PHY-MBP, and to the C323S mutant protein, which cannot incorporate PCB, as PHY(C323S)-MBP.

**Assembly with Chromophore and Spectrophotometry**—PCB preparation and assembly were carried out as described in Ref. 16. For spectrophotometric studies, PCB was added to the crude extract from a stock solution in Me2SO to a final concentration of 4 mM and incubated at 0°C under a green safelight for 1 h prior to further analysis.

Difference spectra were recorded using an HP8452A spectrophotometer with the possibility of external irradiation and a temperature-controlled cuvette jacket. Light sources, acquisition of spectra, and data handling were described in Ref. 18. Briefly, phytochrome was converted into Pr by a far-red light pulse (730 nm, 90 s, 6 W/m²), and a blank scan was obtained. Conversion to Pfr was achieved by a pulse of red light (666 nm, 30 s, 50 W m⁻²). The difference spectrum was recorded immediately afterward.

For dark reversion kinetics of purified phytochrome in solution, the absorbance difference, ΔA, between the maximum of the difference spectrum (704 nm) and the isoosbestic point (674 nm) was measured every 30 s after the end of the R pulse using the HP8452A spectrophotometer. Control experiments revealed that the measuring beam did not influence the obtained dark reversion kinetics (data not shown). Alternatively, a custom-built dual wavelength ratiospectrophotometer was used to measure Pfr after incubation of the irradiated cuvettes for various times in a temperature-controlled water bath in the dark. Measurements were carried out at 5°C using CaCO₃ as the scattering agent.

**Protein Quantification**—Concentration of photoreversible phytochrome was calculated from the difference spectra as described (20). Protein concentration was determined by an Amido Black incorporation method (21).

**Computational Methods**—Kinetic analysis and presentation of experimental data were performed using SigmaPlot Scientific Graphing System version 4.0 (Jandel Corp., Chicago, IL). Dark reversion kinetics were fitted by nonlinear regression to Equation 1, which was obtained according to the manufacturer’s instructions. Briefly, after washing with several volumes of Buffer A, bound material was eluted with 10 mM maltose in Buffer A. Fractions were analyzed by SDS-polyacrylamide gel electrophoresis (19) and Western blotting using MAC3/289 antiserum directed against mustard phyA (kindly provided by B. Thomas, Wellesbourne, Warwick, UK). In this paper, we refer to *Arabidopsis* phyA-PCB holoproteins as PHY, to *Arabidopsis* phyA-MBP fusions assembled with PCB as PHY-MBP, and to the C323S mutant protein, which cannot incorporate PCB, as PHY(C323S)-MBP.

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**Results**

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**Materials and Methods**

**Experimental Procedures**—The anti-phyA antiserum MAC3/289 was used for detection of PHY. A, PHY-MBP. Lanes are as follows: 1, clarified extract; 2, fraction not binding to amylose; 3, wash; 4–8, fractions eluted with 10 mM maltose. B, PHY. Lanes are as follows: 1, clarified extract; 2, fraction not binding to amylose; 3, wash; 4–6, fractions eluted with 10 mM maltose. C, PHY coexpressed with PHY-MBP. Lanes are as follows: 1, clarified extract; 2, fraction not binding to amylose; 3, elute with 10 mM maltose. D, PHY and PHY-MBP were expressed separately. Extracts were mixed and incubated on ice for 45 min. Lanes are as follows: 1, clarified extract; 2, fraction not binding to amylose; 3, wash; 4, elute with 10 mM maltose. Arrows indicate molecular weights of PHY and PHY-MBP.

**Results**

**Pfr Stability of the Plant Photoreceptor Phytochrome**

Fig. 4A shows that the *Arabidopsis* phyA-MBP fusion (PHY-MBP) heterologously expressed in yeast also bound, and could be affinity-eluted from, amylose. In contrast, heterologously expressed phytochrome without an MBP moiety did not bind amylose (Fig. 1B). To analyze the role of the individual subunits on phytochrome properties, we produced heterodimers of wild-type phytochrome polypeptides and phytochrome bearing a C323S mutation that cannot incorporate the chromophore. Consequently, only one subunit of such heterodimers could adopt the active Pfr form. To this end, we coexpressed PHY and PHY(C323S)-MBP in yeast. Fig. 1C shows that when coexpressed with PHY(C323S)-MBP, PHY could also be eluted from amylose, although in less than stoichiometrical amounts. Obviously, this elute contains PHY(C323S)-MBP, PHY(C323S)-MBP homodimers, and PHY(C323S)-MBP heterodimers. Because the homodimers without a chromophore are spectroscopically inactive, the eluate can be used to characterize the heterodimers in more detail. Alternatively, we expressed PHY and PHY(C323S)-MBP in separate cultures and mixed the crude extracts. After incubating the mixture with amylose, only PHY(C323S)-MBP (but no PHY) could be eluted (Fig. 1D).

**Hallmarks of the structural integrity of phytochromes** are difference spectra. Fig. 2 shows the essentially identical difference spectra of PHY, PHY-MBP in crude extract, affinity-purified PHY-MBP, and PHY PHY(C323S)-MBP heterodimers. The difference spectra have minima at 660 nm, isoosbestic points at 674 nm, and maxima at 704 nm, closely resembling published data (8). In contrast, PHY(C323S)-MBP did not yield any photoreversible signal.
Arabidopsis phyA undergoes considerable dark reversion in living yeast cells (8). Likewise, purified PHY-MBP showed dark reversion in solution (Fig. 3A). Subsequently, we used a different detection system with higher sensitivity and a better signal-to-noise ratio, which is based on a custom-built dual wavelength ratiospectrophotometer. Dark reversion kinetics of PHY-MBP at 25 °C were essentially identical using either assay (Fig. 3, A and B), proceeding with a half-life of 50–60 min and leaving about 40% Pfr after 4 h. In contrast, at 5 °C, no dark reversion of PHY-MBP was detectable within 5 h. Fig. 3C shows dark reversion kinetics of PHY at 5 and 25 °C, which are very similar to those of PHYA-MBP. Using purified PHY PHY(C323S)-MBP heterodimers, which can form only Pfr-Pr heterodimers upon irradiation, we obtained the results shown in Fig. 3D. Strikingly, in this case, a monophasic, fast, and complete dark reversion occurred.

An alternative means to produce high amounts of Pfr-Pr heterodimers is irradiation with light of longer wavelengths, e.g. around 700 nm. For the blue-shifted PCB-phyA holoprotein, we determined $\varphi$ as 0.11 at 701 nm (data not shown), corresponding to 9.8% Pfr-Pr heterodimers and only 1.2% Pfr-Pfr homodimers. Fig. 4A shows dark reversion kinetics observed after irradiation of PHY with light of 701 nm. As for the PHY PHY(C323S)-MBP heterodimers, a fast and nearly complete dark reversion was observed.

We conclude from the results of Figs. 3 and 4A that Pfr-Pr heterodimers undergo a fast dark reversion, whereas Pfr-Pfr homodimers are stabilized. Therefore, dark reversion kinetics can be described quantitatively with two rate constants, $k_{DR,1}$ and $k_{DR,2}$ (Fig. 4B). The experimental results of Figs. 3 and 4, as well as previously published data (8, 10), were analyzed by nonlinear regression using Equation 1. Table II contains the determined rate constants. Concerning Arabidopsis phyA, purified PHY-MBP behaves very similar to PHY in yeast extracts. Almost no dark reversion was detectable at 5 °C; at 25 °C Pfr-Pfr homodimers reverted with a half-life of about 6 h, whereas Pfr-Pr heterodimers reverted with a half-life of 30–40 min. Furthermore, both assay systems yielded similar results for purified PHY-MBP. Likewise, the rate constants of dark reversion after irradiation with 701 nm matched the other results closely. Moreover, purified PHY PHY(C323S)-MBP heterodimers were stable at 5 °C but reverted rapidly at 25 °C ($t_{1/2}$ = 40 min). Re-analysis of published data revealed that other

**Table I**

| Purification of Arabidopsis PHYA from S. cerevisiae |
|---------------------------------------------------|
| Protein expression and purification was performed as described under “Experimental Procedures.” |

|                      | PHY-MBP |                     |                     |                  |
|----------------------|---------|---------------------|---------------------|------------------|
|                      | µg      | Yield   | Total protein | Purity index | Purification |
|                      | µg      | %       | mg          | index         | fold         |
| Supernatant          | 51      | 100     | 132         | 0.39          |              |
| Affinity-bound       | 25      | 41      | ND          |               |              |
| Affinity-eluted      | 2.5     | 5       | 0.114       | 22            | 56           |

|                      | PHY     |                     |                     |                  |
|----------------------|---------|---------------------|---------------------|------------------|
|                      | µg      | Yield   | Total protein | Purity index | Purification |
|                      | µg      | %       | mg          | index         | fold         |
| Supernatant          | 54      | 100     | 132         | 0.41          |              |
| Affinity-bound       | 8       | 20      | ND          |               |              |
| Affinity-eluted      | 0       | 0       | 0.103       | 0             | 0            |

|                      | PHY(C323S)-MBP + PHY |                     |                     |                  |
|----------------------|----------------------|---------------------|---------------------|------------------|
|                      | µg      | Yield   | Total protein | Purity index | Purification |
|                      | µg      | %       | mg          | index         | fold         |
| Supernatant          | 32      | 100     | 90          | 0.36          |              |
| Affinity-bound       | 5       | 16      | ND          |               |              |
| Affinity-eluted      | 2.4     | 8       | 0.118       | 20            | 56           |

* Phytochrome was quantified by difference spectroscopy as described under “Experimental Procedures.”

* Purity index values are expressed as the ratio of phytochrome concentration (µg/ml) to protein concentration (mg/ml).

* The affinity-bound fraction was determined by spectrophotometric quantification of phytochrome that did not bind to the column.

* Not determined.

![Fig. 2. Difference spectra of phyA-MBP fusion proteins expressed in yeast.](image)
phytochromes from Arabidopsis and tobacco also show a much slower dark reversion of Pfr-Pfr homodimers than Pfr-Pr heterodimers (Table II).

Subsequently, we obtained dark reversion kinetics of PHY at temperatures between 5 and 30 °C (data not shown). Fig. 5 shows an Eyring plot using the resulting rate constants $k_{DR,1}$ and $k_{DR,2}$. The data reveal a strong temperature dependence of the reactions. Activation enthalpies are 22.2 and 17.8 kcal/mol for Pfr-Pfr homodimers and Pfr-Pr heterodimers, respectively. These values correspond to a 3–4-fold increase of dark reversion rates with a temperature increase of 10 °C.

**DISCUSSION**

The role of dimerization in the biological functions of phytochromes has been controversial for several years (6, 14, 23). Main arguments in this debate were based on indirect, physiological data of whole plants. Here, we chose a direct, biochemical approach to analyze the influence of dimerization on phytochrome dynamics. We used MBP fusions for affinity purification of Arabidopsis phyA from yeast extracts, a system that was previously described for tobacco phyB (16). Thus, both phyA- and phyB-like phytochromes can be purified as MBP fusions. For Arabidopsis phyA, yield and purification were slightly lower than reported for tobacco phyB. However, phyA nonfused to MBP (PHY) does not bind to amylose resins at all (Fig. 1, Table I). The system therefore is well suited for copurification experiments. Indeed, PHY can be detected in amylose resin eluates when coexpressed with PHY-MBP. This observation was confirmed by spectroscopy when photoreversible PHY and PHY(C323S)-MBP were coexpressed and copurified. Interestingly, when PHY and PHY-MBP were expressed separately, and the mixed extracts were incubated with amylose, only PHY-MBP could be affinity-eluted. Therefore, the exchange of subunits between phytochrome dimers appears to be very slow. Possibly, dimerization occurs cotranslationally, leading to large fractions of homodimers. Data of our group suggest that instead of the expected 50%, only 10–20% of phyA is present as mixed dimers.3 Nonetheless, this fraction is large enough to allow spectroscopic analysis.

The MBP moiety was fused to the C terminus of phyA, whereas the spectral integrity is determined by its N terminus. Therefore, in agreement with other observations (16, 24), the difference spectrum was not altered by fusion of Arabidopsis phyA to MBP and affinity purification (Fig. 2). More important,
PHY PHY(C323S)-MBP heterodimers also had an unaltered difference spectrum. Because interactions of the C terminus with the N terminus differ considerably between Pr and Pfr (25), our results indicate that interactions between the sub-units are only of minor importance for absorbance maxima of Arabidopsis phyA. The role of intra-subunit interactions varies. The difference spectra of monomeric, N-terminal fragments are blue-shifted for pea phyA but are unaltered for oat phyA and potato phyB (14, 25).

Dark reversion in vitro apparently depends strongly on sample integrity and conditions (26). The two assay systems for dark reversion applied in this study yielded identical results, largely excluding artifacts in the measurements. Moreover, several independent sample preparations yielded similar results (data not shown). Fusion to MBP and affinity purification did not alter the dark reversion of phyA when compared with PHY in crude yeast extracts (Fig. 3). In contrast, analysis of PHY PHY(C323S)-MBP heterodimers revealed a fast, monophasic, and complete dark reversion. Consequently, the retarded dark reversion of a large fraction of PHY is due to its ability to form Pfr-Pfr homodimers. Apparently, additional inter-subunit contacts are formed in such homodimers, leading to a much higher thermal stability. After irradiation with 701 nm, which forms mainly Pfr-Pr homodimers, PHY also underwent a fast and nearly complete dark reversion, confirming our conclusions.

Analysis of reaction kinetics of dimeric phytochromes (this work and re-evaluation of data from previous reports (8, 10)) demonstrated that in cases in which dark reversion occurred, Pfr-Pr heterodimers of all phytochromes reverted much faster than Pfr-Pfr homodimers (Table II). Interestingly, Arabidopsis phyA rapidly reverted from Pfr to Pr when assayed in living cells at 4 °C (8). In contrast, no detectable dark reversion of extracted or purified Arabidopsis phyA occurred during 12 h at this temperature. We got identical results as reported previously (8) when assaying both PHY and a PHY-S-Tag (Novagen, Madison, WI) fusion in living yeast cells (data not shown). Consequently, dark reversion of Arabidopsis phytochrome is facilitated in yeast cells. Because this acceleration was not observed in crude extracts, the yeast factor responsible must depend on intact cellular metabolism and/or is extremely protease-sensitive. The former alternative is favored by our observation that phytochrome was occasionally completely stable for several hours even in living yeast cells (data not shown). Dark reversion of phytochrome is controlled by intramolecular properties; additionally, it appears to be modulated by external factors in plants (11, 13). Our data demonstrate that some of these factors are also present in yeast and are not exclusively specific to plants. It would be interesting to test whether chaperone-like activities are involved in the acceleration of conformational changes connected to dark reversion.

Dark reversion rates of Pfr-Pr homodimers are about one order of magnitude lower than those of Pfr-Pr heterodimers (Table II). Whereas Arabidopsis and tobacco phyB Pfr-Pr heterodimers revert extremely rapidly (t1⁄2 = 1–4 min at 4 °C), Pfr-Pr heterodimers of phyA revert much slower (t1⁄2 = 20 min). Heterodimers of rice phyA are even more stable. In contrast, Pfr-Pr homodimers revert with half-lives of at least several hours for all analyzed phytochrome molecules. To evaluate

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**Table II**

Rate constants of dark reversion for various phytochromes

| Phytochrome | Sample | Assay | Temperature | kDR,1 | kDR,2 | kDR,1/kDR,2 | Ref. |
|-------------|--------|------|-------------|-------|-------|-------------|-----|
| ArPHYA-MBP  | Purified | a    | 5 °C        | >12 h | >12 h | n.d.        | This work |
| ArPHYA-MBP  | Purified | c    | 25 °C       | 8.2 h | 44 min | 11          | This work |
| ArPHYA-MBP  | Purified | a    | 25 °C       | 5.7 h | 30 min | 11          | This work |
| Heterodimers| Purified | a    | 5 °C        | >12 h | >12 h | n.d.        | This work |
| Heterodimers| Purified | c    | 25 °C       | >12 h | >12 h | n.d.        | This work |
| ArPHYA      | Yeast extract | a   | 5 °C        | >12 h | >12 h | n.d.        | This work |
| ArPHYA      | Yeast extract | a   | 25 °C       | 5.5 h | 41 min | 8           | This work |
| ArPHYA      | Yeast extract | a   | 25 °C       | 5.4 h | 33 min | 10          | This work |
| ArPHYA      | Yeast cells | a    | 4 °C        | >12 h | 3 min  | >240        | 8   |
| ArPHYB      | Yeast cells | a    | 4 °C        | 3.6 h | 5 min  | 43          | 8   |
| ArPHYC      | Yeast cells | a    | 4 °C        | >12 h | 10 min | >72         | 8   |
| ArPHYC      | Yeast cells | a    | 4 °C        | 4.5 h | 1 min  | 270         | 13  |
| NtPHYB-MBP  | Purified | c    | 25 °C       | 6.5 h | 4 min  | 96          | 1   |
| OsPHYA      | Yeast cells | a   | 4 °C        | >12 h | >12 h | n.d.        | 13  |

a: Dual-wavelength ratio spectrophotometer (irradiation with 660 nm).

b: Not determined.

c: HP8452A spectrophotometer (irradiation with 660 nm).

d: Dual-wavelength ratio spectrophotometer (irradiation with 701 nm).

L. Henning and E. Schäfer, unpublished data.
relative stabilities of hetero- and homodimers, we calculated the ratio of $k_{DR,1}$ to $k_{DR,2}$ (Table II). This ratio is 100–300 for B-type phytochromes, 10–20 for phyA, and has intermediate values for phyC and phyE. Therefore, Pfr-Pr heterodimers are relatively short-lived in the evolutionarily ancient phyB, whereas they are considerably stabilized in the more recently evolved phyA (27). Because rice phyA, which displays barely any dark reversion, is further stabilized compared with Arabidopsis phyA, the stability of Pfr-Pr heterodimers has been enhanced selectively during evolution. Intriguingly, physiological studies indicated that the biological activity of Pfr-Pr heterodimers is closely connected to phyA. In contrast, phyB has a strong temperature dependence in bovine rhodopsin but might be stabilizing to phytochrome. Importantly, indications of control of dark reversion by intramolecular and extramolecular mechanisms evolved differentially for individual phytochromes depending on their biological functions.

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