The widely distributed phytochrome photoreceptors carry a bilin chromophore, which is covalently attached to the protein during a lyase reaction. In plant phytochromes, the natural chromophore is coupled to a thioether bond between its ring A ethylidene side chain and a conserved cysteine residue within the so-called GAF domain of the protein. Many bacterial phytochromes carry biliverdin as natural chromophore, which is coupled in a different manner to the protein. In phytochrome Agp1 of \textit{Agrobacterium tumefaciens}, biliverdin is covalently attached to a cysteine residue close to the N terminus (position 20). By testing different natural and synthetic biliverdin derivatives, it was found that the ring A vinyl side chain is used for chromophore attachment. Only those bilins that have ring A vinyl side chain were covalently attached, whereas bilins with an ethylenide or ethyl side chain were bound in a noncovalent manner. Phycocyanobilin, which belongs to the latter group, was however covalently attached to a mutant in which a cysteine was introduced into the GAF domain of Agp1 (position 249). It is proposed that the regions around positions 20 and 249 are in close contact and contribute both to the chromophore pocket. In competition experiments it was found that phycocyanobilin and biliverdin bind with similar strength to the wild type protein. However, in the V249C mutant, phycocyanobilin bound much more strongly than biliverdin. This finding could explain why during phytochrome evolution in cyanobacteria, the chromophore-binding site swapped from the N terminus into the GAF domain.

Phytochrome proteins are biliprotein photoreceptors that were discovered in plants (1) but were recently also found in bacteria (2–4), fungi (5), and slime molds (6). The photocycle of phytochromes has two long-lived forms, the red-absorbing form (Pr), which is synthesized in darkness, and the far red-absorbing form (Pfr). Both forms are reversibly interconverted by light. In general, both forms are stable in darkness, but exceptions are often found. The Pfr form of many plant phytochromes (7) and phytochrome 1 from \textit{Agrobacterium tumefaciens} (here termed Agp1) (8) slowly converts to Pr in darkness. In the case of \textit{Bradyrhizobium} phytochome and \textit{Agrobacterium} phytochrome 2, Pr converts to Pfr in darkness (9, 10). Typically, the phytochrome proteins consist of an N-terminal chromophore module, to which one bilin chromophore is covalently attached, and a C-terminal output module. In prokaryotic phytochromes, the output module is most often a histidine kinase (2, 8), but other C-terminal modules are also possible (9, 10). Almost all plant phytochromes have a histidine-kinase like domain and an additional ~300-amino acid stretch with two PAS domains, which is located between the chromophore module and the histidine-kinase-like domain (11). Within the chromophore module, the GAF domain and a PHY domain, which is located C-terminal of the GAF domain, can be distinguished. The GAF domain is the region most homologous among the phytochrome family. Further N-terminal of this domain, there are 140–200 additional amino acids of lower homology. Within this region, a PAS domain was identified in some phytochrome species (11). The domain structure of \textit{Agrobacterium} phytochrome Agp1 is given in Fig. 1.

Plant phytochromes carry either phycocyanobilin (PΦB) or phycocyanobilin (PCB) as chromophore (12, 13). Both bilins are synthesized from biliverdin (BV) by reduction of the BV ring A double bond and a concomitant conversion of ring A vinyl to ethylenide. For PCB biosynthesis, the ring D vinyl group is further reduced to an ethyl group (14, 15). In plant phytochromes, the chromophore binds covalently to the protein by a thioether bond between the ring A ethylidene side chain of the chromophore (12) and a conserved cysteine within the GAF domain. In cyanobacteria, two types of phytochromes are distinguished with respect to chromophore attachment. In some phytochromes such as Cph1 from \textit{Synechocystis} or CphA of \textit{Calothrix}, the cysteine residue is conserved (2, 16). These phytochromes also attach PCB or PΦB in a covalent manner. Most cyanobacteria synthesize large amounts of PCB as phycobiliprotein compound, and PCB was found as a natural chromophore of Cph1 (17). Other cyanobacterial phytochromes, such as CphB from \textit{Calothrix}, are missing the chromophore-binding cysteine in the GAF domain. Recombinant CphB incorporates PCB to a photoactive adduct, but the attachment is noncovalent, and the bilin can easily be removed from the protein. When a cysteine was introduced at the appropriate site of CphB, the protein bound PCB covalently (16). Phytochromes of other bacteria are all missing the chromophore-binding cysteine in the GAF domain (4, 5, 8). As far as analyzed, these phytochromes incorporate BV as natural chromophore (5, 8),
but recombinant proteins assemble to photoactive adducts also with PCB and PφB (4, 8). For phytochrome Agp1 from A. tumefaciens, it was shown by site-directed mutagenesis that BV is covalently bound to a cysteine residue close to the N terminus of the protein (8). These results have been confirmed by mass spectrometry analyses. This cysteine residue is highly conserved within the group of BV-binding phytochromes and is probably also used as attachment site in other phytochromes of this group. Because BV has a vinyl side chain at ring A, the coupling mechanism must be different between Agp1 and plant phytochromes. It has been proposed that BV binds via its ring D to the protein (8), but this assumption has not been confirmed by chemical studies.

Chemically synthesized bilins have been used to characterize the chromophore pocket and to study photoconversion and physiological action of plant phytochromes (18–20). Here we used synthetic BV derivatives to test whether the ring A or ring D vinyl side chain is used for covalent BV attachment in Agp1. We also tested whether PCB binds covalently to the protein if a cysteine residue is introduced in the GAF domain of Agp1. Competition studies with BV and PCB were performed to further characterize the chromophore pocket of Agp1. Our find-

---

FIG. 1. Protein domains of phytochrome Agp1 from A. tumefaciens as given by the PFAM algorithm (www.sanger.ac.uk/Software/Pfam). For each domain, the left and right borders are indicated. The position of chromophore-binding cysteine 20 is indicated by a vertical line. Valine 249 is indicated by another vertical line; this amino acid is homologous to the chromophore-binding cysteine of plant phytochromes. The PAS-like domain (PLD) is identified only in other phytochromes (see text).

FIG. 2. Chromophore assembly with Agp1 and Agp1-V249C. a–g, Agp1 (~10 μM) was mixed with BV and BV derivatives. The abbreviation and chemical structure of the chromophores is given in each panel. The final concentration of BV was 9 μM, and the concentration of the other bilins was ~5 μM. h, the Agp1 V249C mutant (10 μM) was mixed with PCB (~3 μM) and assayed after 60 min. Each panel shows the Pr spectrum after mixing chromophore and protein (solid line), the spectrum after photoconversion by red light (dotted line), and the Pr minus Pfr difference spectrum (dashed line).

---

2 M. Carrascal, J. Abian, N. Michael, G. Rottwinkler, and T. Lamparter, personal communication.
ings have implications for the three-dimensional folding of the protein, the lyase mechanism, and the early evolution of phycobilisomes.

**EXPERIMENTAL PROCEDURES**

**Bilin Chromophores, Synthesis of Bilin Derivatives**—BV was purchased from Frontier Scientific (Carnforth, UK). Phycocyanobilin was extracted from Spirulina sp. with methanol and purified by reverse-phase liquid chromatography (12). The synthesis of P4B was performed as described (22). Synthesis of 3-Et-BV, 18-Et-BV, 3-18-Et-BV, and BV-4 followed published protocols for P4B, PCB, and P4B derivatives (22–25); the experimental details will be published elsewhere. Bilin stock solutions were prepared in methanol. The concentrations of stock solutions were measured spectrophotometrically in 2% HCl and methanol, assuming an extinction coefficient of 30.8 molar−1 cm−1 for BV (26) and 37.9 molar−1 cm−1 for PCB (27) at the absorption maximum in the red region of the spectrum. For 3-Et-BV, 18-Et-BV, 3-18-Et-BV, and BV-4, we used the same value as for BV. In the case of P4B it was assumed that the extinction coefficient is identical to PCB.

**Purification of Agp1 and the Mutant V249C**—An Escherichia coli expression clone pAG1 in which the gene for Agrobacterium phytochrome Agp1 has been cloned into the pQE12 vector (Qiagen) was used (8). The expressed protein has an N-terminal polyhistidine tag. The V249C mutant was made with a Stratagene (La Jolla, CA) QuickChange site-directed mutagenesis kit according to the manufacturer’s instructions. Details of apoprotein expression and purification are given in earlier publications (8). After Ni2+ affinity chromatography, the protein fractions were pooled, and the protein was precipitated with ammonium sulfate in Tris buffer (50 mM Tris/Cl, 5 mM EDTA, 2 mM dithiothreitol, pH 7.8), and stored at −80 °C. Spectral Characterization and Assay for Covalent Attachment—Spectra were recorded with a Uvikon 931 photometer (Kontron/Biotek, Milano, Italy) at 18 °C. The protein concentration was adjusted to ~10 μg as judged by absorbance at 280 nm. For chromophore assembly, 500 μl of apoprotein was mixed with up to 10 μl of bilin from the stock solution to achieve a final bilin concentration of 4–12 μM. The Pr spectra were usually measured directly after this mixing process. Photoconversion into Pfr was performed in the measuring cuvette by irradiation with a 660-nm light-emitting diode (~50 μmol m−2 s−1) until photoconversion reached an apparent equilibrium as checked by absorbance at 700 nm. The Pr spectra were usually measured directly after this mixing process. Photoconversion into Pfr was performed in the measuring cuvette by irradiation with a 660-nm light-emitting diode (~50 μmol m−2 s−1) until photoconversion reached an apparent equilibrium as checked by absorbance at 700 nm. Thereafter, a second spectrum was recorded. The difference spectra were calculated from both absorption spectra. To test for covalent chromophore-protein interaction, 500 μl of bilin-Ag, Agp1 were mixed with 55 μl of SDS to achieve a final SDS concentration of 1%. In general, SDS was added (0.5–1 min after the chromophore was mixed with Agp1 apoprotein). In the case of the V249C mutant, SDS was added 1 h after start of chromophore assembly. Two minutes after the addition of SDS, a spectrum of the sample was recorded. Thereafter, 500 μl of the same sample were applied to a NAP-5 desalting column (Amersham Biosciences), which had been equilibrated in SDS buffer (1% SDS, 50 mM Tris/Cl, 5 mM EDTA, pH 7.5). Another 1 ml of SDS buffer was applied to the column. The eluted fraction contains protein with covalently bound bilin and is depleted of free bilin. A spectrum of this sample was also recorded. The protein peak at 280 nm was used to normalize both spectra, and from the absorbance in the visible range, it was judged whether chromophore co-elutes with the protein. When covalent chromophore attachment was measured in the competition experiments, only spectra after the NAP-5 separation are shown.

**RESULTS**

The proposed natural chromophore of Agp1 is BV (8). Besides BV, we tested the naturally occurring PCB and P4B, three BV-derivatives with reduced ring A and/or ring D side chains, namely 3-Et-BV, 18-Et-BV, 3-18-Et-BV, and BV-4, in which the ring D vinyl group of the C18 position is exchanged with the methyl group of the C17 position for their interaction with the Agp1 protein. Chromophore assembly results in the formation of the Pr form, which is recognized by unambiguous spectral features; upon incorporation into the protein, the maximum of the red absorption band of the chromophore shifts to approximately 700 nm. At the same time, absorbance in this spectral region increases and exceeds that of the blue absorption band (see Fig. 4a for free BV and Fig. 2a for the BV adduct). This behavior was found for all chromophores tested (Fig. 2, a–g), indicating that they all formed adducts with the Agp1 protein.

In addition, all adducts showed the typical photoconversion into the longer wavelength Pfr form upon irradiation with red light. The maximum of the Pr red absorption band correlated with the number of bilin double bonds that participate in the conjugated π-electron system (Table I). Biliverdin and BV-4 have 13 double bonds, and their adducts showed rather long wavelength absorption maxima at 701 and 698 nm, respectively. Both chromophores with 11 double bonds, PCB and 3-18-Et-BV, gave adducts with the shortest wavelength absorption maxima at 685 and 683 nm, respectively.

| Absorption spectrum (Pr max) | Difference spectrum | Covalent attachment |
|-----------------------------|---------------------|---------------------|
| BV Agp1                     | 701                 | 700                 | 752 52 1.11 Yes |
| 3-Et-BV                     | 695                 | 694                 | 739 45 2.12 No |
| 18-Et-BV                    | 690                 | 688                 | 739 51 0.82 Yes |
| 3-18-Et-BV                  | 683                 | 682                 | 726 44 1.65 No |
| BV-4 Agp1                   | 698                 | 697                 | 740 43 1.18 Yes |
| P4B Agp1                    | 697                 | 696                 | 745 49 1.49 No |
| PCB Agp1                    | 685                 | 683                 | 731 48 1.07 No |
| PCB Agp1-V249C              | 654                 | 652                 | 700 48 1.00 Yes |

The Pr/Pfr absorbance ratio was calculated from the absolute absorbance values of the difference spectra at λmax (for Pr) and λmin (for Pfr).
tion bands of the chromophores appear at approximately 380 and 620 nm, respectively. The spectrum of the BV adduct was slightly altered during the column separation in the blue region, an effect that might be explained by conformational alterations during the SDS treatment or by interactions with the column matrix (Fig. 3a). The assay shows, however, that most if not all BV was covalently attached to the protein. In the case of 18-Et-BV, both spectra were almost identical; thus 100% of the chromophore were covalently bound (Fig. 3c). From the spectra of the BV-4 adduct, it can be judged that approximately 90% of the chromophore were covalently attached (Fig. 3e). All three covalently bound chromophores have a ring A vinyl side chain but differ by their ring D side chains. Those chromophores that contain a ring A ethyl or ethyliden side chain, namely 3-Et-BV, 3-18-Et-BV, PCB, and PΦB, were noncovalently bound to the protein. In all those cases, the absorbance in the visible range was very small after the column separation (Fig. 3, b, d, f, and g). Because ~5% of free bilins elute from the desalting columns under the test conditions (8), minor traces of chromophore absorbance might be attributed to co-eluted free bilin.

The noncovalent mode of PCB-protein interaction in Agp1 is comparable with CphB from the cyanobacterium Calothrix. We tested for Agp1 whether the introduction of a cysteine in the GAF domain can also mediate covalent attachment of PCB as was found for CphB (16). The amino acid valine 249, which is homologous to the chromophore-binding cysteine of plant phytochromes, was replaced by cysteine, and the purified protein Agp1-V249C was tested for PCB assembly. The adduct had a Pr absorption maximum of 654 nm and was thus blue-shifted by

![Graphs showing absorbance spectra of different chromophores and their adducts.](image-url)
FIG. 4. Competition experiments with BV and PCB. The chromophore-protein mixings were performed under a green safelight (light-emitting diodes with 505 nm), and the samples were incubated in darkness. a, spectra of free BV and PCB in assay buffer, concentration 12 μM. b, absorption spectrum of Agp1 (10 μM) with PCB (12 μM, solid line) and the same sample immediately after the addition of 12 μM BV (dashed line). c, the same experiment as in b, but the chromophores were added in reverse order. d, the same experiment as in b, but before BV addition, the PCB adduct was photoconverted by red light (dotted line). e, the same experiment as in c, but before the addition of PCB, the BV adduct was photoconverted by red light (dotted line). f, time course of the Pr absorption maxima of the experiments depicted in b–e. The time scale starts with the addition of the second chromophore. The time needed for handling of cuvette and photoconversion of the sample before chromophore addition is not considered. The values for t = 0 are taken from the Pr sample before photoconversion and addition of the second chromophore. g, BV-Agp1 (10 μM) was incubated with or without excess PCB (20 μM) for 7 h. Both samples were then mixed with SDS and subjected to NAP-5 separation.
This shift implies that the ethylidene double bond of PCB, which is part of the \( \pi \)-electron system, is saturated as a result of the formation of a covalent thioether link. Covalent attachment was confirmed by the SDS assay (Fig. 3h).

To see whether two types of chromophores use the same chromophore-binding site, competition experiments were performed with BV and PCB. Agp1 was first mixed with PCB of slight molar excess and then incubated with additional BV (Fig. 4b). In the opposite experiment, the apoprotein was first mixed with BV and then incubated with PCB (Fig. 4c). Addition of the second chromophore increased the absorbance in the red region only slightly, this increase results from the low and broad absorption of free bilin (Fig. 4a). This finding shows that the same pocket is used for both chromophores.

In both competition experiments, the Pr absorption maxima shifted immediately after the addition of the second chromophore (Fig. 4, b and c). A simple superposition of the spectrum of either free chromophore and the Pr absorption spectrum of either adduct does not significantly alter the position of the Pr absorption maximum (data not shown). Hence, the observed shifts show that the incorporated bilin was partially replaced by the added second bilin in both competition experiments. During the subsequent minutes, the shifts increased further (Fig. 4f, closed symbols). After 4 h, the absorption maxima of both mixing experiments reached a constant value of 695 nm (data not shown). Because this value is closer to the absorption maximum of the BV adduct than to the PCB adduct, it seems that BV binds slightly stronger to Agp1 than PCB, but the results clearly show that covalent BV attachment does not lead to an exclusive binding. These findings raised the question whether the covalent BV-Agp1\(^2\) link is lost by the PCB competition. We thus compared covalent BV attachment with and without additional PCB. The spectra that were obtained after SDS-denaturation and column separation were almost identical (Fig. 4g). Therefore, excess PCB does not induce the cleavage of BV from the binding site.

We also tested for chromophore competition after photocconversion. When the PCB Agp1 adduct was irradiated with red light to convert most of the phytochrome into the Pfr form and BV was added to the sample, a new Pr band appeared at 701 nm. In this case, BV clearly competed with PCB of the Pfr adduct, because the rise of new BV-Pr was accompanied by a reduction of the Pfr absorption band at \( \text{730 nm} \) (Fig. 4d). Qualitatively the same result was obtained when the BV adduct was converted to Pfr and PCB was added to the sample (Fig. 4e). The shift of the absorption maximum that was observed subsequently (Fig. 4f, open symbols) is explained by dark reversion of residual Pfr.

Competition experiments with BV and PCB were also performed with the V249C mutant, which has one covalent binding site for either chromophore. When the protein was first assembled with PCB, and BV was added thereafter, the position of the Pr peak remained unchanged (Fig. 4h). At approximately 700 nm, the position of the BV Pr adduct, a weak shoulder was formed that increased slightly during the subsequent hours (shown for \( t = 5 \) h in Fig. 4h). These data show that PCB is hardly replaced by BV in the mutant protein. When V249C was first assembled with BV, and PCB was added thereafter, the replacement of BV by PCB was clearly visible. Immediately after PCB addition, the Pr absorption maximum shifted to lower wavelengths, and a shoulder appeared at \( \text{650 nm} \), the position of the PCB Pr adduct. After 5 h a prominent peak was observed at 650 nm that rose at the expense of the 700 nm peak (Fig. 4i). Both competition experiments show that (i) PCB binds much stronger to the mutant protein than BV and (ii) PCB binds stronger to the mutant than to the wild type protein. Because in the mutant, either of both chromophores can form a covalent link with the protein, two different chromophores can become covalently bound to one protein unit, as is evident from the assembly spectra of Fig. 4i. This assumption was confirmed by SDS assays (Fig. 4j). After SDS treatment and column separation, the sample in which Agp1-V249C was first assembled with BV and then with PCB had higher chromophore absorbance values than the sample that was assembled with BV alone.

**DISCUSSION**

In plant phytochromes, the phytochromobilin chromophore is bound to a conserved cysteine within the GAF domain (12). Covalent BV attachment has recently been shown for several bacterial phytochromes (5, 8), but it was unclear which BV side group is used as attachment site. The present work approaches this question for Agrobacterium phytochrome Agp1 by using different bilin derivatives. Whereas all tested chromophores formed spectrally integer adducts that showed the typical photocconversion into the Pfr form, only those bilins that have a ring A vinyl side chain were covalently attached to the protein. We therefore propose that the ring A vinyl group forms a thioether bond with the cysteine 20 residue of Agp1.

In general, the ratio between the Pr and the Pfr absorbance was low when the ring A vinyl side chain was present in the chromophore. One can thus conclude that a covalently linked chromophore gives a higher relative extinction coefficient for the Pfr form of Agp1. This assumption agrees with an earlier study, in which covalent BV attachment was blocked by cysteine-reactive agents or by mutation of the chromophore-binding cysteine (8). Although the photocycle is possible with noncovalently attached chromophores, full formation of Pfr seems to be dependent on covalent attachment, probably because only a tight coupling can confer forces required to guide the chromophore into the correct conformation.

During the evolution of phytochromes, there was a switch from BV to PCB/PhB chromophores that took place in the group of cyanobacteria. Biliverdin-binding phytochromes have a conserved cysteine close to the N terminus, which at least in Agp1 is used as a chromophore attachment site; the other species have a conserved chromophore-binding cysteine in the GAF domain. The switch between both binding sites was, however, not difficult, as shown by mutant studies; in two different BV-binding phytochromes, Calothrix CphB (16) and Agrobacterium Agp1 (this work), the introduction of a cysteine in the GAF domain was sufficient to achieve covalent PCB attachment. Each phytochrome monomer has only one chromophore pocket, as shown here by competition experiments for Agp1 (Fig. 4). Because BV binds with its ring A vinyl side chain to Cys\(^{20}\) of Agp1, and PCB binds with its ring A ethylidene side chain to Cys\(^{240}\) of Agp1-V249C, the protein must be folded in as in Fig. 3. The spectra are normalized at 280 nm. \( h \), the V249C mutant of Agp1 (10 \( \mu \)M) was first mixed with 12 \( \mu \)M PCB. After completion of assembly (solid line), 12 \( \mu \)M BV were added to the sample, which was measured immediately thereafter (dotted line) and 5 h later (dashed line). \( i \), as for \( h \), but chromophores were added in reverse order, \( j \), Agp1 V249C (10 \( \mu \)M) was assembled with 12 \( \mu \)M PCB (solid line), 12 \( \mu \)M BV (dotted line), or BV followed by PCB (dashed line). The samples were incubated for 20 h, mixed with SDS, and subjected to NAP-5 separation. The spectra are normalized at 280 nm.
such a way that amino acid 249 comes very close to Cys\textsuperscript{20} of Agp1. It is thus obvious that both corresponding regions of the protein contribute to the same part of the chromophore pocket. Quite interestingly, a PAS domain is identified by secondary structure prediction program in the \textit{N} terminus of two bacterial phytochromes, Cph1 and DrBphP \cite{11}. The PAS-homologous part of Agp1 stretches from Pro\textsuperscript{24} to Trp\textsuperscript{82} (Fig. 1) and thus starts just behind the chromophore-binding Cys\textsuperscript{20} of Agp1. In oat phytochrome A, the PAS-homologous part covers the region around amino acid 80, which is critical for chromophore attachment \cite{28}. PAS domains often serve as protein/protein contact sites \cite{29,30}, but in Fxl\textit{L} \cite{31}, photoactive yellow protein \cite{31}, and phototropin \cite{32}, this domain mediates chromophore binding. The PAS-like domain in the \textit{N} terminus of phytochromes could thus be part of the chromophore pocket. It could even be possible that in more ancient phytochromes, this domain formed a chromophore pocket by its own and that the GAF domain functioned as lyase, which catalyzes covalent chromophore attachment.

The competition studies point to an interesting explanation for why the chromophore binding site could have swapped from the \textit{N} terminus into the GAF domain. Although covalently bound to Agp1, BV was partially removed from the chromophore pocket by PCB. The competition by PCB was even stronger when the BV adduct was converted into the Pfr form. High levels of free bilin would thus significantly disturb the photocycle of Agp1-like phytochromes. As soon as the host cell produces PCB, as cyanobacteria do, the possibility exists to use this bilin as a phytochrome chromophore. Phycocyanobilin has produced PCB, as cyanobacteria do, the possibility exists to use.

\section*{Acknowledgments}
We thank Maarten Heyn, Harald Otto, and Berthold Borucki for helpful discussions.

\textbf{REFERENCES}

1. Butler, W. L., Norris, K. H., Siegelman, H. W., and Hendricks, S. B. (1959) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{45}, 1703–1708

2. Lamparter, T., Mittmann, F., Gärtner, W., Borner, T., Hartmann, E., and Hughes, J. (1997) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{94}, 11792–11797

3. Yeh, K. C., Wu, S. H., Murphy, J. T., and Lagarias, J. C. (1997) \textit{Science} \textbf{277}, 1505–1508

4. Davis, S. J., Vener, A. V., and Vierstra, R. D. (1999) \textit{Science} \textbf{286}, 2517–2520

5. Bhoo, S. H., Davis, S. J., Walker, J., Karniol, B., and Vierstra, R. D. (2001) \textit{Nature} \textbf{414}, 776–779

6. Starostzik, C., and Marwan, W. (1995) \textit{FEBS Lett.} \textbf{370}, 146–148

7. Mancini, A. (1994) in \textit{Photomorphogenesis of Plants} (Kendrick, R. E., and Kronenberg, G. H. M., eds) 2nd Ed., pp. 211–269, Kluver Academic Publishers, Dordrecht, The Netherlands

8. Lamparter, T., Michael, N., Mittmann, F., and Esteban, B. (2002) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{99}, 11628–11633

9. Giraud, E., Fardoux, J., Fourrier, N., Hannibal, L., Genty, B., Bouyer, P., Dreyfus, B., and Vermeglio, A. (2002) \textit{Nature} \textbf{417}, 202–205

10. Karmiol, B., and Vierstra, R. D. (2003) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{100}, 2807–2812

11. Montgomery, B. L., and Lagarias, J. C. (2002) \textit{Trends Plant Sci.} \textbf{7}, 357–366

12. Ridgeley, W., and Thummler, F. (1994) in \textit{Photomorphogenesis of Plants} (Kendrick, R. E., and Kronenberg, G. H. M., eds) 2nd Ed., pp. 51–69, Kluver Academic Publishers, Dordrecht, The Netherlands

13. Wu, S. H., McDowell, M. T., and Lagarias, J. C. (1997) \textit{J. Biol. Chem.} \textbf{272}, 25700–25705

14. Kohchi, T., Mukougawa, K., Frankenberg, N., Masuda, M., Yokota, A., and Lagarias, J. C. (2001) \textit{Plant Cell} \textbf{13}, 425–436

15. Frankenberg, N., Mukougawa, K., Kohchi, T., and Lagarias, J. C. (2001) \textit{Plant Cell} \textbf{13}, 965–978

16. Jorisissen, H. J., Quest, B., Remberg, A., Coursin, T., Braslavsky, S. E., Schaffner, K., Tandeau de Marsac, N., and Gartner, W. (2002) \textit{Eur. J. Biochem.} \textbf{269}, 2662–2671

17. Hübschmann, T., Borner, T., Hartmann, E., and Lamparter, T. (2001) \textit{Eur. J. Biochem.} \textbf{266}, 2055–2063

18. Hanzawa, H., Shinomura, T., Inomata, K., Kakiuchi, T., Kinoshita, H., Wada, K., and Furuya, M. (2002) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{99}, 4725–4729

19. Hanzawa, H., Inomata, K., Kinoshita, H., Kakiuchi, T., Jayasundera, K. P., Sawamoto, D., Ohta, A., Uchida, K., Wada, K., and Furuya, M. (2001) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{98}, 3612–3617

20. Lindner, I., Knipp, B., Braslavsky, S. E., Gartner, W., and Schaffner, K. (1998) \textit{Angew. Chem. Int. Ed. Engl.} \textbf{37}, 1845–1846

21. Lamparter, T., Esteban, B., and Hughes, J. (2001) \textit{Eur. J. Biochem.} \textbf{268}, 4720–4730

22. Kakiuchi, T., Kinoshita, H., and Inomata, K. (1999) \textit{Synlett} \textbf{SI}, 901–904

23. Kakiuchi, T., Kato, H., Jaysundera, K. P., Higashi, T., Watabe, K., Sawamoto, D., Kinoshita, H., and Inomata, K. (1998) \textit{Chem. Lett.} 1001–1002

24. Takeda, S., Jayasundera, K. P., Kakiuchi, T., Kinoshita, H., and Inomata, K. (2001) \textit{Chem. Lett.} \textbf{590}–591

25. Sawamoto, D., and Inomata, K. (2001) \textit{Chem. Lett.} \textbf{588}–589

26. McDonagh, A. F. (1979) in \textit{The Porphyrins} (Dolphin, D., ed) pp. 293–491, Academic Press, New York

27. Cole, W. J., Chapman, D. J., and Siegelman, H. W. (1967) \textit{J. Am. Chem. Soc.} \textbf{89}, 3642–3645

28. Bhoo, S. H., Hirano, T., Jeong, H. Y., Lee, J. G., Furuya, M., and Song, P.-S. (1997) \textit{J. Am. Chem. Soc.} \textbf{119}, 11717–11718

29. Huang, Z.-J., Ederly, I., and Rosbash, M. (1993) \textit{Nature} \textbf{364}, 259–262

30. Zhu, Y., Pepperman, J. M., Fairchild, C. D., and Quail, P. H. (2000) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{97}, 13419–13424

31. Gong, W., Hao, B., Mansy, S. S., Gonzalez, G., Gilles-Gonzalez, M. A., and Chan, M. K. (1998) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{95}, 15177–15182

32. Christie, J. M., Salomon, M., Nozue, K., Wada, M., and Briggs, W. R. (1999) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{96}, 8779–8783