Genetic engineering of phytochrome biosynthesis in bacteria

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Contributed by J. Clark Lagarias, July 19, 2001

The bilin prosthetic groups of the phytochrome photoreceptors and the light-harvesting phycobiliprotein antennae arise from the oxygen-dependent ring opening of heme. Two ferredoxin-dependent enzymes contribute to this conversion: a heme oxygenase and a bilin reductase with discrete double-bond specificity. Using a dual plasmid system, one expressing a truncated cyanobacterial apophytochrome 1, Cph1(N514), and the other expressing a two-gene operon consisting of a heme oxygenase and a bilin reductase, these studies establish the feasibility of producing photoactive phytochromes in any heme-containing cell. Heterologous expression systems for phytochromes not only will facilitate genetic analysis of their assembly, spectrophotometric activity, and biological function, but also might afford the means to regulate gene expression by light in nonplant cells.

metabolic engineering | linear tetrapyrrole biosynthesis | synthetic operon | bilin reductase

Plants, algae, and cyanobacteria, organisms that rely on light as an energy source, possess multiple photoperception and signaling systems to adapt to their light environment (1). The phytochromes are biliprotein photoreceptors that evolved in chlorophyll-containing prokaryotes after the rapid increase in oxygen levels in the early atmosphere (2, 3). Like the light-harvesting phycobiliproteins, phytochromes possess thioether-linked linear tetrapyrrole (bilin) prosthetic groups that enable them to absorb visible light (4). Through their ability to reversibly photoisomerize between two spectrally distinct forms, a red-light-absorbing form (Pr) and a far-red-light-absorbing form (Pfr) of phytochrome, phytochromes function as molecular rheostats to regulate numerous responses to light quality, quantity, duration, and direction (5). Although the biochemical mechanism of phytochrome action is not fully defined, phytochromes are bilin-regulated light-modulated protein kinases that function to mediate gene expression (6, 7).

The bilin prosthetic groups of phytochromes and phycobiliproteins arise from the oxygen-dependent ring opening of heme (8, 9). In the cyanobacterium *Synechocystis* sp. PCC6803, two enzymes contribute to the conversion of heme to 3Z-phycocyanobilin (PCB), the immediate precursor of the chromophores of the phycobiliproteins, phycocyanin and allophycocyanin (8), as well as that of the cyanobacterial phytochromes, Cph1 (10–12) and likely Cph2 (13, 14). These enzymes are heme oxygenase [HO1 (15)] and phycocyanobilin:ferredoxin oxidoreductase [PcyA (16)], which catalyze the ferredoxin-dependent conversions of heme to biliverdin IXα (BV) and BV to PCB, respectively (Fig. 1). Analogous ferredoxin-dependent enzymes mediate the conversion of heme to the phytochrome chromophore precursor, phytochromobilin (PΦB), in plants: a heme oxygenase and a distinct bilin reductase, phytochromobilin:ferredoxin oxidoreductase [phytochromobilin synthase (17, 18)]. In *Arabidopsis thaliana* L., these enzymes are encoded by the *HY1* and *HY2* genes, respectively (19–21). HY1 and HY2 are both plastid-localized enzymes; hence, PΦB must be translocated to the cytoplasm where holophytochrome assembly occurs. It is well established that apophytochromes are bilin lyases that catalyze the formation of a thioether ether linkage to a bilin precursor with an A-ring ethylidene substituent (22, 23).

The cloning of ferredoxin-dependent heme oxygenases and bilin reductases holds great potential for the genetic engineering of phytobilin biosynthesis in any living cell. Such expression systems not only will facilitate genetic analysis of biliprotein assembly, spectrophotometric activity, and biological function of the biliprotein photoreceptors, but also might afford the means to regulate gene expression by light in nonplant cells. The present study was undertaken to test the feasibility of holophytochrome reconstitution in bacteria. The use of a two-plasmid system, one expressing an apophytochrome and the other expressing a dual gene operon containing a heme oxygenase and a bilin reductase, has established the feasibility of producing functional phytochromes in any heme-containing cell.

Materials and Methods

Plasmid Construction. All molecular cloning experiments used standard protocols and *Escherichia coli* strain DH5α as host (24). All constructs were verified by DNA sequencing. The region corresponding to the N-terminal 514 amino acids of the cyanobacterial phytochrome 1 gene [Cyanobase Locus *slr0473* (25)] from *Synechocystis* sp. PCC6803 was constructed in the bacterial expression vector pBAD-MycHisC (Invitrogen) as follows. The Cph1(N514)-coding region was PCR-amplified with sense primer Pcph1-S1NcoI, 5′-GCACTAGTTAACGAGGGCAAACCATGGCCACCACCGTAC-3′ (restriction sites are underlined in all primers), and antisense primer Pcph1-AS514*Hin*dIII, 5′-GCAAGCTTTTCTTCTGGCTGGCG-3′ using *Synechocystis* sp. PCC6803 genomic DNA as a template. The *Nco*I/*Hin*dIII-restricted PCR product was ligated with a *Nco*I/*Hin*dIII-restricted pBAD-MycHisC vector to yield the bacterial expression plasmid, pBAD-Cph1(N514) (Fig. 2*A*).

A synthetic operon consisting of *Synechocystis ho1* [Cyanobase Locus *sll1184* (25)] and *PcyA* [Cyanobase Locus *slr0116* (25)] coding regions was constructed in the bacterial expression vector pPROLarA122 (CLONTECH) as follows. The entire *ho1* coding region was PCR-amplified with sense primer Pho1-S1KpnI, 5′-ATCGGTACCATGAGTGTCAACTTAGCTTC-3′ and antisense primer Pho1-ASr*Bam*H1, 5′-ATTGGATCCTTTCT*C-CTC*TTTAACTAGCCTTCGGAGGTGGCGA-3′ (antisense synthetic ribosome-binding site italicized) by using *Synechocystis* sp. PCC6803 genomic DNA as template. The 0.7-kbp PCR product was directly cloned into the TA cloning vector pCR2.1 (Invitrogen) to yield plasmid pCR2.1/*ho1-RBS* (not shown). The entire *pcyA* coding region was PCR-amplified by using *Synechocystis* sp. PCC6803 genomic DNA as template with sense primer PpcyA-S1*Bam*H1, 5′-ATCGGATCCATGGCCGT-CACTGATTTAAGT-3′ and antisense primer PpcyA-AS*Xba*I,

Abbreviations: Cph1, cyanobacterial phytochrome 1; IPTG, isopropyl β-D-thiogalactoside; PCB, phycocyanobilin; PΦB, phytochromobilin; Pfr, far-red light-absorbing form of phytochrome; Pr, red-light-absorbing form of phytochrome.

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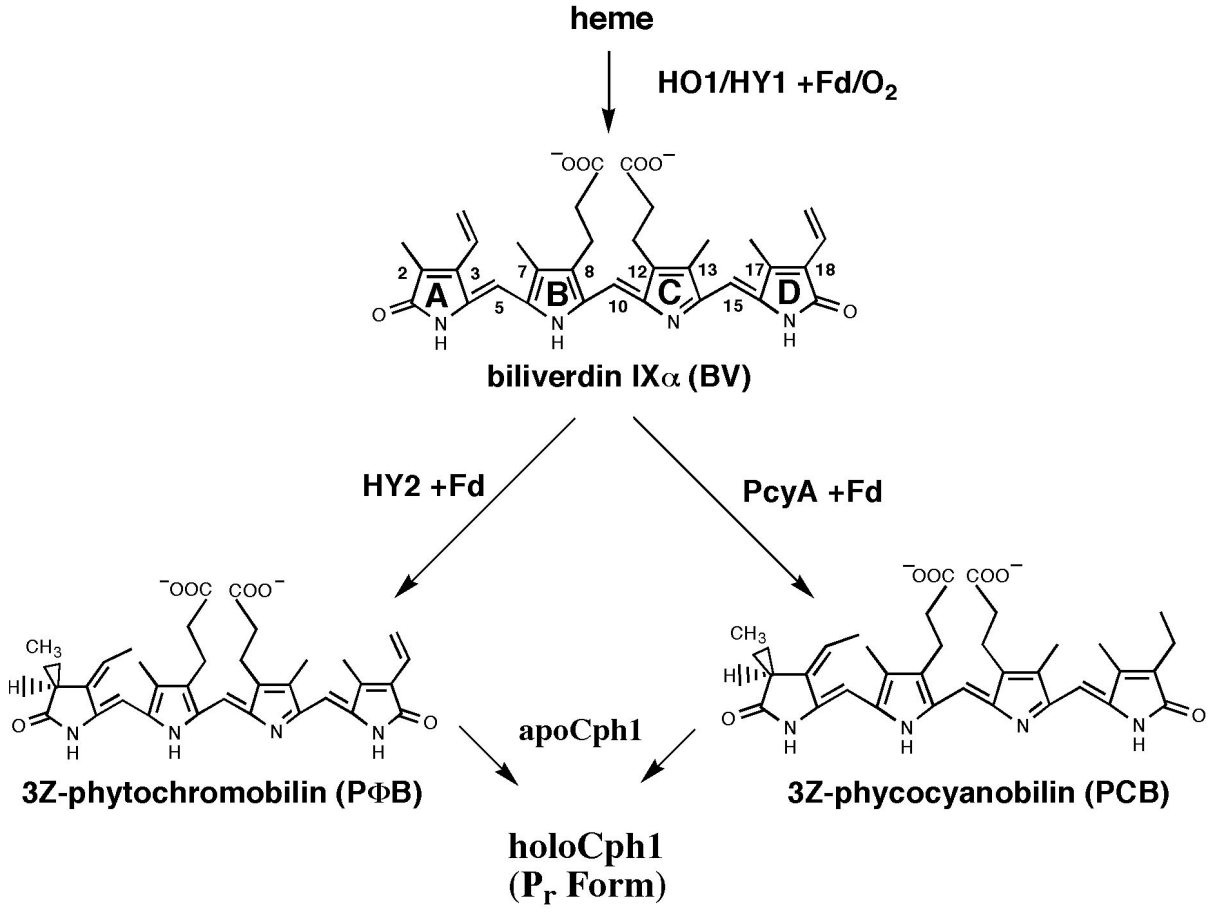

Fig. 1. Biosynthesis of phycocyanobilin and phytochromobilin. Heme oxygenase (HO1) catalyzes the conversion of heme to biliverdin IXα (BV). Subsequently, BV is reduced by phycocyanobilin/ferredoxin oxidoreductase (PcyA) in cyanobacteria or PΦB synthase (HY2) in plants to produce PCB or PΦB, respectively. ApoCph1 is capable of autocatalytically binding either of these chromophores to form a holoCph1 protein in the red-light-absorbing Pr form.

5′-TTATCTAGATTATTGGATAACATCAAATA-3′. The 0.7-kbp PCR product was then cloned into the TA cloning plasmid pCR2.1 to produce plasmid pCR2.1/*pcyA* (not shown). pCR2.1/*pcyA* was restricted with *Bam*HI, and the 0.7 kbp *Bam*HI fragment was ligated with *Bam*HI-restricted pCR2.1/*ho1*-RBS downstream of the *ho1* gene. Plasmid pCR2.1/*ho1-rbs-pcyA* (not shown) with the correct orientation of the *pcyA* insert was identified among the transformants. Plasmid pCR2.1/*ho1*-RBS-*pcyA* was restricted with *Kpn*I and *Not*I, and the 1.4-kbp insert was ligated with *Kpn*I/*Not*I-restricted pPROLarA122 to yield the bilin biosynthetic plasmid pPL-PCB (Fig. 2*B*).

Protein Expression and Purification. *E. coli* strain LMG194 (Invitrogen) was transformed with plasmid pBAD-Cph1(N514) by using standard protocols to produce an ampicillin-resistant apophytochrome-expressing strain. Competent cells of this strain were transformed with plasmid pPL-PCB. Transformants exhibiting both ampicillin and kanamycin resistance were selected. All work with the pPL-PCB plasmid was carried out by using minimal media (RM media, Invitrogen) to repress expression of the bilin biosynthetic operon. One liter of RM media contains 2% (wt/vol) casamino acids, 0.2% (wt/vol) glucose, 1 mM $MgCl_2$, 1× M9 salts. One liter of 10× M9 salts contains 60 g of Na_2HPO_4, 30 g of KH_2PO_4, 5 g of NaCl, and 10 g of NH_4Cl, pH 7.4. *E. coli* strains LMG194 containing both apophytochrome and PCB biosynthetic expression plasmids were grown overnight at 37°C in 1 ml of RM media containing 25 μg/ml of kanamycin and 50 μg/ml of ampicillin. Cultures were transferred to 100 ml of RM media, grown at 37°C to an OD_{600} of ≈0.5, and transferred to 900 ml of Luria–Bertani medium containing 25 μg/ml of kanamycin and 50 μg/ml of ampicillin. Isopropyl β-D-thiogalactoside (IPTG) was added to a final concentration of 1 mM to induce expression of the bilin biosynthetic operon. After incubation for 1 h at 37°C, arabinose was added to a final concentration of 0.002% (wt/vol) both to induce expression of apoCph1 and to hyperinduce the bilin biosynthetic operon. Cultures were grown at 37°C for 4 h, after which cells were collected by centrifugation and resuspended in 20 ml of lysis buffer (50 mM Tris·HCl, pH 8.0/100 mM NaCl/0.05% (vol/vol) Nonidet P-40/2 μg/ml of leupeptin/2 mM benzamidine/2 mM PMSF/1 mM DTT/3 μg/ml of pepstatin A). Cell suspensions were passed once through a French press at 10,000 psi to lyse the cells, and insoluble material was collected by centrifugation. Crude soluble protein extracts were placed on ice at 4°C and examined for holophytochrome expression spectrophotometrically.

Crude soluble fractions were dialyzed overnight at 4°C against 2 liters of extraction/wash (EW) buffer (50 mM Tris·HCl, pH 7.0/300 mM NaCl/10% (vol/vol) glycerol/20 mM imidazole/0.05% (vol/vol) Tween-20/1 mM 2-mercaptoethanol), applied to a Talon (CLONTECH) metal-affinity chromatography column (20 ml of bed volume), washed with 80 ml of extraction/wash buffer, and eluted with 2 bed volumes of elution buffer (EW buffer containing 200 mM imidazole). The resulting purified (apo)phytochrome solutions were dialyzed overnight

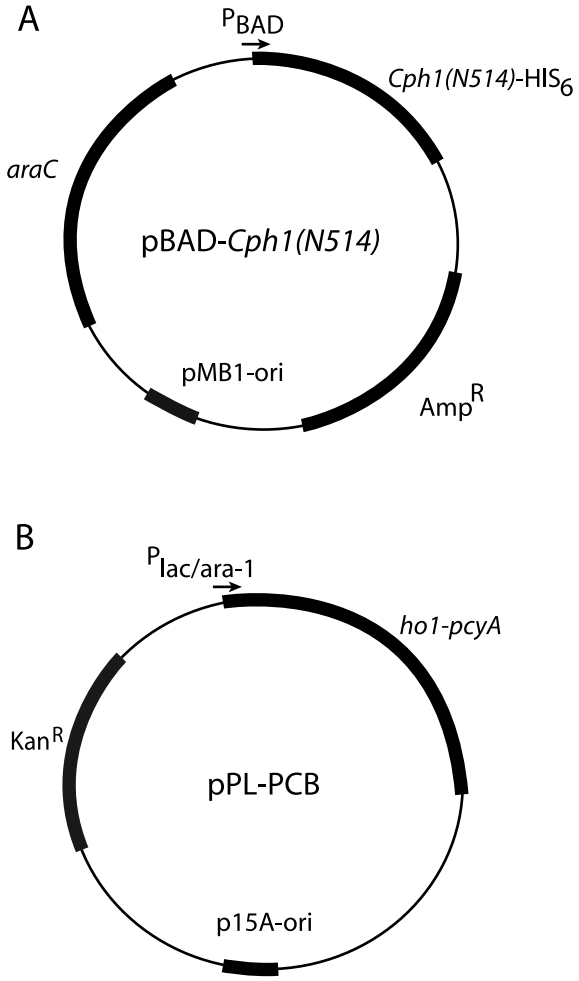

Fig. 2. Expression vectors. Plasmid maps of Cph1 expression construct pBAD-Cph1(N514) (*A*) and the bilin biosynthetic pathway expression construct pPL-PCB (*B*) are shown. A histidine-tagged apophytochrome Cph1(N514) gene under the control of the arabinose-regulated P_{BAD} promoter was cloned into the high-copy pBAD vector that possesses the ampicillin-resistance and *araC* genes, the latter of which encodes the arabinose-regulated repressor. The PCB biosynthetic operon consisting of the synechocystis *ho1* and *pcyA* genes under control of the dual arabinose- and lactose-regulated $P_{lac/ara-1}$ promoter was cloned into the low-copy pPROLar vector, which possesses a kanamycin resistance marker. See *Materials and Methods* for details.

against 2 liters of 10 mM Hepes buffer, pH 7.5, concentrated by using an Amicon ultafiltration cell and desalted with a D-Salt Excellulose Plastic Desalting Column (Pierce).

Absorption Spectrophotometry. Absorption spectra were obtained by using an HP8453 UV-visible spectrophotometer. Phytochrome difference spectra were obtained as described previously (17).

SDS/PAGE and Zinc-Blot Analysis. Protein samples were analyzed by SDS/PAGE by using the Laemmli buffer system (26). After electrophoresis, proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes at 100 V for 60 min. The PVDF membranes were incubated in 1.3 M zinc acetate overnight at 4°C, and the fluorescence was detected by using a Storm 860 Fluorimager in red fluorescence mode (23, 27).

Results

A dual plasmid system was chosen for reconstitution of holophytochrome in *E. coli* cells for several reasons. Apo-

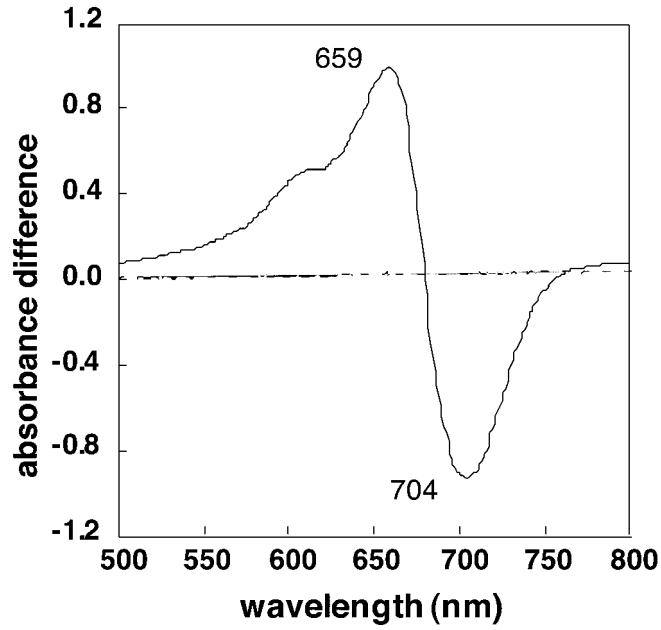

Fig. 3. Crude holophytochrome difference spectra. Normalized holophytochrome difference spectra in crude extracts of the pBAD-Cph1(N514)/pPL-PCB cotransformed LMG194 *E. coli* strain (solid line) and a LMG194 strain containing only the pBAD-Cph1(N514) plasmid (dashed line). Both strains were preinduced with IPTG for 1 h, followed by induction with arabinose, as described in *Materials and Methods*.

phytochrome, i.e., Cph1(N514), was cloned into the high-copy pBAD-MycHisC plasmid vector for robust tightly inducible protein expression. The resulting ampicillin-resistance pBAD/Cph1(N514) plasmid (Fig. 2*A*) places apophytochrome under control of the *Ara* promoter, affording a C-terminal histidine-tagged protein for subsequent affinity purification. In the LMG194 cell line, apophytochrome expression is essentially off until arabinose is added to the growth medium. For the bilin biosynthetic plasmid, a synthetic operon comprised of the *ho1* and *pcyA* coding regions from *Synechocystis* sp. PCC6803 was placed in the vector pPROLarA122 resulting in plasmid pPL-PCB (Fig. 2*B*). A synthetic ribosome-binding site was engineered between the two genes to ensure expression of both pathway enzymes. The pPROLar vector, a low-copy kanamycin-resistance plasmid with a p15A origin of replication, is compatible with the pBAD/Cph1(N514) plasmid within a single cell. This construct places the PCB biosynthetic operon under control of a dual *Ara/Lac* promoter, enabling selective regulation of PCB biosynthesis with the lactose analog IPTG without induction of apophytochrome. Because of their different origins of replications and antibiotic resistance loci, the two plasmids can be maintained in LMG194 cells by continuous selection with both kanamycin and ampicillin.

In initial experiments, cotransformants grown in minimal media were induced with both IPTG and arabinose, whereupon the cell lines turned deep blue-green within 4 h. Strains harboring the single plasmid, pBAD-Cph1(N514) or pPL-PCB, displayed no visible color change. Crude extracts from cotransformed and single plasmid-containing strains were analyzed for the presence of functional photoconvertible holophytochrome spectrophotometrically. The cotransformed strain exhibited a far-red-minus-red difference spectrum characteristic of photoactive holophytochrome (Fig. 3). Extracts from pBAD-Cph1(N514)/pPL-PCB cells exhibited difference maxima and minima at 659 and 704 nm, characteristic of the Cph1-PCB adduct of Cph1 (12). Phytochrome difference spectra were not

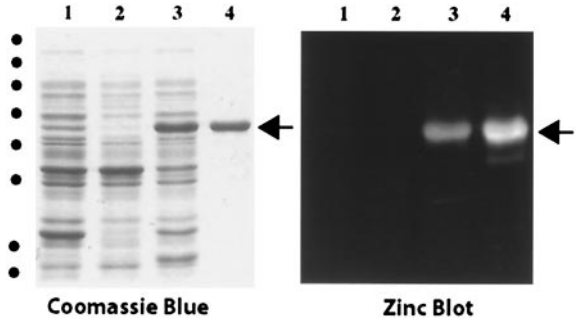

Fig. 4. SDS/PAGE and zinc-blot analysis. SDS/PAGE of crude protein extracts and affinity-purified phytochrome expressed in various plasmid-containing *E. coli* LMG194 strains. The gel was visualized by Coomassie staining (*Left*) and zinc-blot analysis (*Right*). The prominent band at 59.8 kDa (arrow) corresponds with the calculated molecular mass of Cph1(N514). Lane 1, strain harboring pBAD-Cph1(N514) only. Lane 2, strain harboring pPL-PCB only. Lane 3, strain harboring pBAD-Cph1(N514) and pPL-PCB. Lane 4, affinity-purified Cph1(N514)-PCB adduct. Molecular masses of the protein standards (i.e., 172.6, 111.4, 79.6, 61.3, 49, 36.4, 24.7, 19.2 kDa) are shown (*Left*).

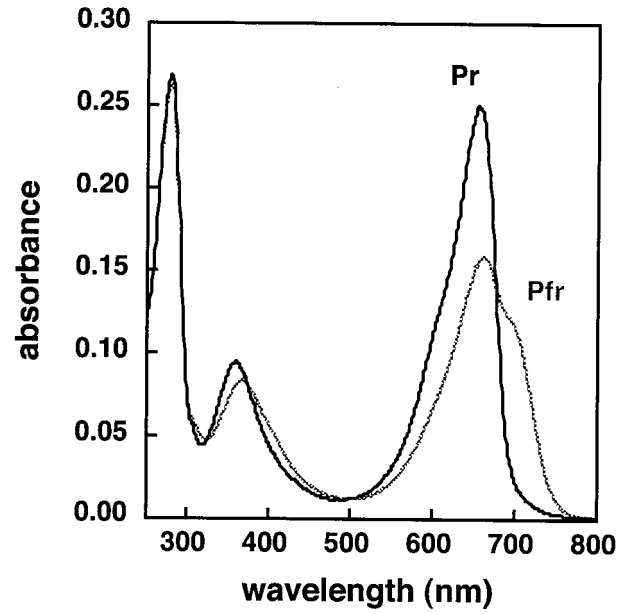

Fig. 5. Purified Cph1(N514)-PCB adduct red and far-red absorption spectra. Absorption spectra of purified Cph1(N514)-PCB adduct after saturating irradiation with red light (dashed line, Pfr form) and far-red light (solid line, Pr form).

detectable in extracts from similarly induced cells containing only one of the two plasmids (Fig. 3).

To ascertain that a covalent bilin adduct of Cph1(N514) had been produced, SDS/PAGE and zinc-blot analyses were performed (Fig. 4). The presence of a prominent Coomassie blue-stained band at 59.8 kDa was observed in crude extracts from IPTG- and arabinose-induced strains containing pBAD-Cph1(N514), consistent with the calculated molecular mass of the Cph1(N514) protein (Fig. 4, lanes 1 and 3). This band was missing in a cell line containing pPL-PCB only (Fig. 4, lane 2). Zinc-blot analysis indicated that the Cph1(N514)/pPL-PCB strain produced Cph1(N514) protein containing a covalently attached bilin chromophore based on the presence of an orange fluorescent band at 59.8 kDa (Fig. 4, lane 3). Indeed, strains harboring only pBAD-Cph1(N514) or pPL-PCB revealed no fluorescence on the zinc blot (Fig. 4, lanes 1 and 2). Taken together, these data indicate that the *Synechocystis* heme oxygenase and bilin reductase are both enzymatically active in *E. coli* cells.

These analyses show that the Cph1(N514) protein levels were considerably greater in the cell line possessing the bilin biosynthetic operon compared with Cph1(N514)-expressing cells lacking this operon (Fig. 4, compare lanes 1 and 3). This result suggests that bilin binding to apoCph1(N514) stabilized the recombinant phytochrome protein. To test this hypothesis, we predicted that induction of the bilin biosynthetic operons in pPL-PCB with IPTG before the arabinose-dependent induction of apophytochrome would increase the yield of phytochrome. Indeed, preinduction of bilin biosynthesis did increase the amount of Cph1(N514) protein accumulation compared with coinduction (data not shown). Using this preinduction protocol, the pBAD-Cph1(N514)/pPL-PCB strain resulted in an ≈10-fold increase in recombinant protein yield compared with a strain expressing Cph1(N514) only (Table 1). Because no photoactive phytochrome could be detected in extracts from the latter, the amount of Cph1(N514) protein in crude extracts from this strain was determined spectrophotometrically after addition of saturating amounts of PCB to the homogenization buffer (Table 1, +PCB). A similar incubation of the pBAD-Cph1(N514)/pPL-PCB strain led to no increase in photoactive Cph1(N514), thus documenting that all of the apoCph1(N514) had assembled with PCB (Table 1; compare −PCB and +PCB for pBAD-Cph1(N514)/pPL-PCB).

Using the preinduction conditions described above, the PCB-producing strain routinely yielded in excess of 65 mg of the Cph1(N514)-PCB adduct per liter of culture in the crude extract. Moreover, this holophytochrome is histidine-tagged, enabling rapid purification of large amounts of the photoreceptor (Table 1). SDS/PAGE and zinc-blot analysis of the purified Cph1(N514)-PCB adduct shown in Fig. 4 (lane 4) documents the homogeneity of these preparations and the presence of a covalently attached bilin. The Pr and Pfr absorption spectra of this preparation after saturating irradiation with far-red and red light are shown in Fig. 5. As shown in Table 2, the spectral properties of the Cph1(N514)-PCB adduct are very similar to those of full length Cph1 reported by other laboratories. The high specific

Table 1. Expression summary

	Crude, mg		Purified, mg			
Sample	−PCB	+PCB	−PCB	+PCB	% yield	SAR
pBAD-Cph1(N514) only	(0)	5.9	(0)	ND	ND	ND
pPL-PCB only	0	0	ND	ND	ND	ND
pBAD-Cph1(N514)/pPL-PCB	68	68	54.3	54.3	79.8	0.93

Summary of crude and purified yields from various strains. Yields were determined via ΔΔA (from phytochrome difference spectra) and reflect 1-liter cultures grown as described. +PCB samples were conjugated with 16 μM PCB at room temperature for 1 hour. The specific absorbance ratio (SAR) is defined as the ratio of the absorbance at 655 nm to that at 280 nm for the far-red irradiated (Pr-form) protein solution. ND, not determined.

Table 2. Comparative spectroscopic data for recombinant Cph1

Spectra	Cph1(N514)-PCB	Cph1(FL)-PCB
Absorbance		
Pr λ_{max}	656 nm	656 nm (29)
		658 nm (28, 36)
Pfr λ_{max}	703 nm	703 nm (29)
		702 nm (28, 36)
SAR	0.93	0.55–0.60 (28)
		0.87 (29)
Pfr-A λ_{max}/Pr-A λ_{max}	0.474	0.523 (29)
Difference		
$\lambda(\Delta A_{max})$	653 nm	654 nm (12)
		655 nm (29)
		655 nm (28)
$\lambda(\Delta A_{min})$	703 nm	706 nm (12)
		703 nm (29)
		708 nm (28)
$\Delta A_{max}/\Delta A_{min}$	1.00	1.028 (29)

The spectroscopic properties of purified PCB-adducts, Cph1(N514) described in this study and full-length Cph1(FL) from other laboratories are shown below. Included are absorbance maxima (λ_{max}) of Pr and Pfr forms, specific absorbance ratio (SAR), the ratio of absorbance maxima of the Pfr and Pr forms (Pfr-A λ_{max}/Pr-A λ_{max}), wavelength values of absorbance difference maximum and minima [$\lambda(\Delta A_{max})$ and $\lambda(\Delta A_{min})$, respectively], and the absorbance change ratio-the ratio of absorbance change at the Pr absorbance maximum to the absorbance change at the Pfr absorption maximum upon photoconversion ($\Delta A_{max}/\Delta A_{min}$).

absorption ratio of holoCph1(N514) further supports the conclusion that the recombinant phytochrome is fully assembled.

Discussion

These studies document the genetic engineering of the bacterium *E. coli* to produce a photoactive holophytochrome *in situ* through coexpression of a cyanobacterial apophytochrome gene and a synthetic operon consisting of ferredoxin-dependent heme oxygenase and bilin reductase genes. This work thus establishes that both classes of enzymes are functional in *E. coli* cells, and that a suitable reductant is present to support both heme oxygenase and bilin reductase activities. A similar result was observed with Cph1(N514)-expressing cells that had been cotransformed with a synthetic operon comprised of the *Synechocystis ho1* and phytochromobilin synthase (*HY2*) from *Arabidopsis*; however, in this case, the holoCph1(N514) difference spectrum was red-shifted, reflecting covalent attachment of PΦB (data not shown). The presence of excess apoprotein in this PΦB-producing strain argues that bilin is limiting in these cells, implicating a higher activity/expression of the cyanobacterial bilin reductase in the bacterial host.

Coproduction of phytobilin and apophytochrome led to a significant increase in phytochrome accumulation. This increase appears to be caused by an increased stability of holophytochrome, because 10-fold less apophytochrome accumulates in similarly induced cells lacking a bilin biosynthetic plasmid. This increase was especially true when the bilin biosynthetic operon was induced before the induction of apophytochrome. This result suggests that covalent attachment of bilin to the nascent apophytochrome can occur cotranslationally, thereby preventing the misfolding of the recombinant polypeptide. The stabilizing influence of bilin is striking for the PCB-producing pBAD-Cph1(N514)/pPL-PCB strain from which >65 mg of photoactive phytochrome could be recovered in crude extracts of a 1-liter culture. This increased expression has enabled the purification of >50 mg of Cph1(N514)-PCB adduct per liter of culture. The spectroscopic properties of the purified Cph1(N514)-PCB adduct are consonant with those of full length recombinant Cph1 described by other laboratories (28, 29). The stabilizing influence of *in vivo* bilin production has also enabled us to significantly increase the yield of full length photoactive oat holophytochrome in *E. coli* compared with previously studies [(30); data not shown].

The ability to produce large amounts of holophytochrome in cells not only will facilitate three-dimensional structural studies on this important photoreceptor family but also should prove useful for genetic analysis of the structural basis of phytochrome photoactivity and function. By screening a library of apophytochrome mutants expressed in a PCB- or PΦB-producing bacterial strain, it should be possible to identify residues within the apoprotein that are important for phytochrome photoactivity. Mutation of residues that inhibit phototransformation is expected to increase phytochrome's weak fluorescence. These mutants could be readily screened by using both colony-based and flow cytometric methodologies. Mutations that alter the spectral properties of the Pr and/or Pfr form also could be identified by difference digital imaging spectroscopy (31, 32). Such studies are expected to provide a wealth of information with regard to phytochrome photochemistry. In principle, phytobilin biosynthesis also could be reconstituted in yeast or mammalian cells, assuming that these eukaryotes possess reductants that can substitute for bacterial ferredoxins. Through coexpression of a suitably engineered plant apophytochrome, such cell lines may prove useful for reconstitution of a phytochrome-signaling pathway or, alternatively, for engineering of phytochrome to regulate gene expression by light.

A similar approach could be used with different combinations of heme oxygenases and bilin reductases to engineer bacterial cells to produce any number of biliproteins, including phycobiliproteins and phytofluors (33, 34). The accompanying paper (35) documents that this approach is true for the phycobiliprotein, C-phycocyanin. Coexpression of apophytochromes with bilin reductases involved in the production of phycoerythrobilin, the natural chromophore precursor of phycoerythrin, is expected to yield phytofluors—intensely fluorescent phycoerythrobilin adducts of apophytochrome (34). Phytochrome, and potentially other phytochrome-related biliproteins, make up some of the most critical light-signaling systems of oxygenic photosynthetic organisms (36). It is our hope that this technology leads to an advancement of understanding within these areas of study.

We thank Nicole Frankenberg and Beronda Montgomery for their invaluable help and direction. This work was supported by Clontech Laboratories, Inc. Science is not about who discovered what first, but more simply about what is discovered, and how we use this new knowledge wisely.

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