The degradation rate of MKP-1 after activation of $p42^{MAPK}$ or $p44^{MAPK}$ was less than half that in the absence of estradiol. Under the same conditions, the degradation rate of inhibitor of κBα (IκBα) in response to tumor necrosis factor–α was unaltered in ΔRaf-1::ER-expressing cells after addition of estradiol (*18*). Hence, phosphorylation of MKP-1 by $p42^{MAPK}$ or $p44^{MAPK}$ serves to reduce ubiquitin-dependent degradation of the phosphatase. Activation of $p42^{MAPK}$ or $p44^{MAPK}$ therefore regulates MKP-1 protein expression through both an up-regulation of the rate of transcription (*7*) and a reduction in the rate of proteasome-mediated degradation.

The $p42^{MAPK}$ and $p44^{MAPK}$ enzymes have a central role in the capacity of cells to divide in response to growth factors. Activation of $p42^{MAPK}$ and $p44^{MAPK}$ is a prerequisite for cell-cycle reentry (*3*). However, inappropriate or constitutive activation of the $p42^{MAPK}$ or $p44^{MAPK}$ cascade may provoke cellular senescence (*25*). Taken together, these findings illustrate a complex control mechanism designed to limit undesirable long-term activation of $p42^{MAPK}$ and $p44^{MAPK}$ and further demonstrate the importance of regulated protein degradation to the control of cell division processes.

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19. MKP-1 underwent polymerase chain reaction–directed mutagenesis with the following primers: ATG primer, 5′-GCCAGCCATGGTCATGGAAGTG-3′; S364A primer, 5′-GCAGCTGGGAGCGGTCGTAATGGGGCTCTGAAGGTAGC-3′; S359A primer, 5′-GCAGCTGGGAGAGGTCGTAATGGGGGCCTGAAGGTAGC-3′; S359/364A primer, 5′-GCAGCTGGGAGCGGTCGTAATGGGGGCCTGAAGGTAGC-3′; and truncation at amino acid 340 primer, 5′-GTTGAACACGGTGGTGGTGGA-3′. After amplification, DNA was subcloned into pCRII followed by pcDNA3.1(+) in frame with a Myc-epitope tag. All mutations were verified by sequencing.
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26. After MKP-1 immunoprecipitation (*7*), active $p44^{MAPK}$ (10 ng/ml) was added to immunoprecipitates in a final volume of 100 μl containing 50 mM Hepes (pH 7.0), 60 mM NaCl, 60 mM KCl, bovine serum albumin (1 mg/ml), and 5 mM EGTA. Reactions proceeded at 30°C for the times indicated and terminated by addition of 200 μM sodium orthovanadate. A kinase assay to determine $p44^{MAPK}$ activity was then performed (*15*) with PHAS-I as substrate.

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Bacteriophytochromes: Phytochrome-Like Photoreceptors from Nonphotosynthetic Eubacteria

Seth J. Davis,[1] Alexander V. Vener,[2] Richard D. Vierstra[1,2]*

Phytochromes are a family of photoreceptors used by green plants to entrain their development to the light environment. The distribution of these chromoproteins has been expanded beyond photoautotrophs with the discovery of phytochrome-like proteins in the nonphotosynthetic eubacteria *Deinococcus radiodurans* and *Pseudomonas aeruginosa*. Like plant phytochromes, the *D. radiodurans* receptor covalently binds linear tetrapyrroles autocatalytically to generate a photochromic holoprotein. However, the attachment site is distinct, using a histidine to potentially form a Schiff base linkage. Sequence homology and mutational analysis suggest that *D. radiodurans* bacteriophytochrome functions as a light-regulated histidine kinase, which helps protect the bacterium from visible light.

The phytochrome family of dimeric photoreceptors regulates growth and development by sensing ambient light through the photointerconversion between an inactive red-light (R)–absorbing form and an active far-red-light (FR)–absorbing form (*1*). Although previously thought to be restricted to higher plants, the recent detection of phytochrome-like proteins

in lower plants, algae, cyanobacteria, and purple bacteria suggests that all photosynthetic organisms contain phytochrome (*2*–*4*). The mechanism of action of the higher-plant phytochromes is still unclear, but recent studies suggest that they act as light-regulated protein kinases (*5*). This possibility has been strongly supported by sequence comparisons of the phytochrome-like proteins RcaE and Cph1 from the cyanobacteria *Fremyella diplosiphon* and *Synechocystis* sp. 6803, respectively (*4*, *6*, *7*), and Ppr from the purple bacterium *Rhodospirillum centenum* (*3*). These proteins contain a domain homologous to the chromophore-binding pocket of higher-plant phytochromes attached to a domain common among two-component histidine kinases (*8*). Based on the view that a photosynthetic bacterium is the progenitor of plant chloroplasts, it has been speculated that these prokaryotic genes represent the evolutionary origins of plant phytochromes (*2*, *3*).

Here, we show that phytochrome-like receptors are also present within several nonphotosynthetic organisms, with the discovery of related sequences in the heterotrophic eubacteria *Deinococcus radiodurans* and *Pseudomonas aeruginosa*. These genes, designated *BphP* for bacteriophytochrome photoreceptor, were discovered by scanning genomic databases for coding regions similar to those of phytochromes (*9*). The encoded *D. radiodurans* BphP (*Dr*BphP) and *P. aeruginosa* BphP (*Pa*BphP) proteins are 755 and 728 amino acids, respectively, with an overall amino acid sequence identity of 37% and a similarity of 48% to each other (*10*). Their NH_2-terminal ~500 amino acids are similar to the chromophore-binding region of Cph1, RcaE, Ppr, and plant phytochromes (Fig. 1, A and B). One important distinction is that both BphPs, like Ppr and possibly RcaE, do not contain the positionally conserved cysteine that is considered essential for autocatalytically linking the linear tetrapyrrole chromophore through a thioether bond (*1*, *11*). Like the photosynthetic-bacteria sequences, the COOH-terminal ~250 amino acids of *Dr*BphP and *Pa*BphP are related to histidine kinase domains present in environmental sensors that function as two-component regulators (*8*) (Fig. 1, A and B). This region contains the four conserved motifs that compose the catalytic center, including a positionally conserved histidine that serves as the phosphorylation site (Fig. 1A).

Given the NH_2-terminal sequence homology, we predicted that these BphPs would associate with linear tetrapyrroles to generate R/FR photoreversible chromoproteins. Despite the absence of the conserved cysteine, recombinant *Dr*BphP apoprotein covalently bound phytochromobilin (PΦB) or phycocyanobilin (PCB) in vitro, as demonstrated by zinc-induced autofluorescence of the complex following SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 2A). In fact, the fluorescence intensity of these adducts rivaled that from a similar amount of oat phytochrome A (phyA) assembled in planta (*12*) (Fig. 2A), implying that *Dr*BphP binds chromophore as efficiently as plant phytochromes. Analysis of the PCB-*Dr*BphP holoprotein by electrospray-ionization mass spectrometry (MS) following reversed-phase liquid chromatography (LC) showed that it, like plant phytochromes, bound only one chromophore per polypeptide (*13*). The assembled PCB-*Dr*BphP complex had a mass ~580 daltons greater than that of the apoprotein, a mass difference equal to that of a single PCB chromophore (586 daltons) (Fig. 3, A and B).

Once assembled with either PΦB or PCB, the *Dr*BphP holoprotein became photochromic, capable of repeated photointerconversions between a R- and a FR-absorbing form (*12*) (Fig. 2B). The absorbance-difference spectrum of the holoprotein resembled that of Cph1 (*4*, *6*) and plant phytochromes (*1*, *11*), but with a slight blue shift for the FR maximum [698 nm for *Dr*BphP versus 709 nm for plant phytochrome (*11*) assembled with PCB] (Fig. 2B). A sizable photoreversible difference between 500 and 590 nm (Fig. 2B) was apparent, suggesting that the FR-absorbing form of *Dr*BphP holoprotein also absorbs green light.

The ability of *Dr*BphP to covalently attach chromophore despite the absence of a cysteine residue at position 259 suggested that *Dr*BphP (and likely *Pa*BphP as well) binds chromophore by a different linkage. To test whether Met^{259} binds chromophore, using its sulfonyl moiety,

[1]Laboratory of Genetics, [2]Cellular and Molecular Biology Program and Department of Horticulture, University of Wisconsin–Madison, 1575 Linden Drive, Madison, WI 53706, USA.

*To whom correspondence should be addressed. E-mail: vierstra@facstaff.wisc.edu

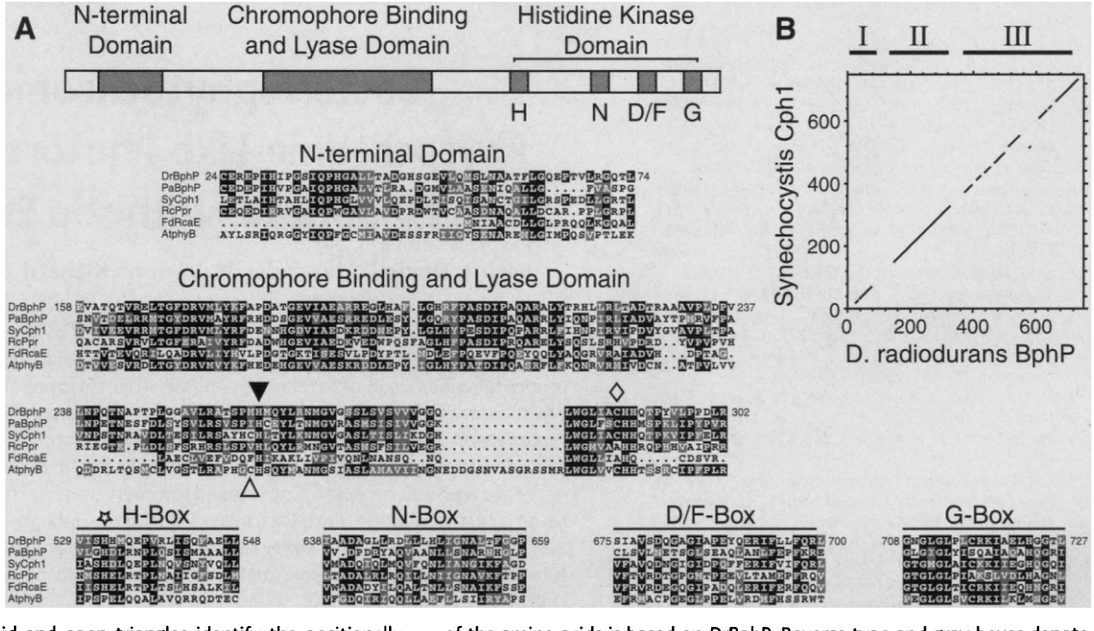

Fig. 1. Relations among phytochromes and phytochrome-like proteins. (**A**) Amino acid sequence alignments of domains conserved among the phytochrome-like proteins of *Dr*BphP, *Pa*BphP, *Synechocystis* Cph1 (GenBank accession number AB001339), *R. centenum* Ppr (GenBank accession number AF064527), *F. diplosiphon* RcaE (GenBank accession number U59741), and the plant phytochrome *Arabidopsis thaliana* phyB (GenBank accession number X17342) (*9*). Boxes H, N, D/F, and G identify catalytic motifs that are common among two-component histidine kinases (*8*); the star identifies the conserved histidine residue essential for phosphate donation. Solid and open triangles identify the positionally conserved His^{260} and Cys^{259} that serve as a chromophore-binding site in *Dr*BphP and in higher-plant phytochromes, respectively. The open diamond locates a second conserved cysteine in *Dr*BphP (Cys^{289}) that is important for spectral integrity but not essential for chromophore attachment. Numbering of the amino acids is based on *Dr*BphP. Reverse type and gray boxes denote identical and similar amino acids, respectively. (**B**) DOTPLOT illustrating three conserved regions in *Dr*BphP as compared to those from *Synechocystis* Cph1. I, an NH_2-terminal domain of unknown function; II, the chromophore-binding pocket; and III, the histidine kinase motif.

we substituted this residue with alanine, which would block a thioether linkage (*1*, *11*), or with cysteine, the consensus ligation site (*1*), and examined whether these mutant proteins could bind chromophore (*14*). The $Met^{259} \rightarrow Ala^{259}$ (M259A) and the $Met^{259} \rightarrow Cys^{259}$ (M259C) mutants covalently bound PCB to generate R/FR photoreversible chromoproteins, eliminating Met^{259} as the ligation site (Fig. 2, C and D). However, the difference spectra obtained with these mutants were altered from that of wild-type *Dr*BphP, indicating that Met^{259} is necessary for chromoprotein integrity. Another potential chromophore-binding site was Cys^{289}, the only other cysteine conserved in the NH_2-terminal region of most phytochrome and phytochrome-like sequences (Fig. 1A). The $Cys^{289} \rightarrow Ala^{289}$ (C289A) mutant (*14*) also bound PCB covalently to generate a photoreversible chromoprotein but had aberrant spectral properties (Fig. 2, C and D). Collectively, these results show that *Dr*BphP does not use the typical thioether linkage to bind linear tetrapyrroles.

To locate the chromophore-binding site, we subjected the PCB-*Dr*BphP holoprotein to chemical digestion with cyanogen bromide (CNBr) (*13*) and analyzed the resulting fragments by LC/MS. Several molecules with absorbance at 374 nm were resolved by LC (Fig. 3C). Subsequent MS analysis identified these compounds as free PCB, several contaminants, and a single PCB-bound peptide (*10*). The chromopeptide was a singly charged ion with a mass-to-charge ratio (*m/z*) of 805.6 (Fig. 3D), consistent with the expected mass of a CNBr product, PCB-histidyl homoserine lactone, formed from the release of a His-Met chromodipeptide from the holoprotein. Its mass predicted that PCB was bound to histidine through a Schiff base linkage by a dehydration reaction; this linkage would produce an unprotonated ion with a positive charge at the quaternary amine and a calculated *m/z* of 805.5. The Met-His-Met sequence required to generate such a CNBr product appears only once in *Dr*BphP (residues 259 through 261), locating His^{260} as the ligation site.

To confirm that His^{260} is the bilin linkage site, we substituted it with alanine (*14*) and tested whether this mutant protein could bind PCB. Compared to wild-type *Dr*BphP, the chromophore-binding activity of the $His^{260} \rightarrow Ala^{260}$ (H260A) mutant was reduced at least 100-fold (Fig. 3E). His^{260} is positionally conserved in all phytochromes and phytochrome-like proteins (Fig. 1A) and appears to be important for chromophore attachment in plant phytochromes (*11*). For other phytochrome-like proteins that do not contain the adjacent cysteine (*Pa*BphP, Ppr, and RcaE), this histidine residue may serve as the ligation site. For those that have the adjacent cysteine (higher-plant phytochromes and Cph1), this histidine could form a transient Schiff base intermediate with the chromophore, which is then attacked by the sulfur nucleophile of the proximal cysteine to link the chromophore by a thioether bond.

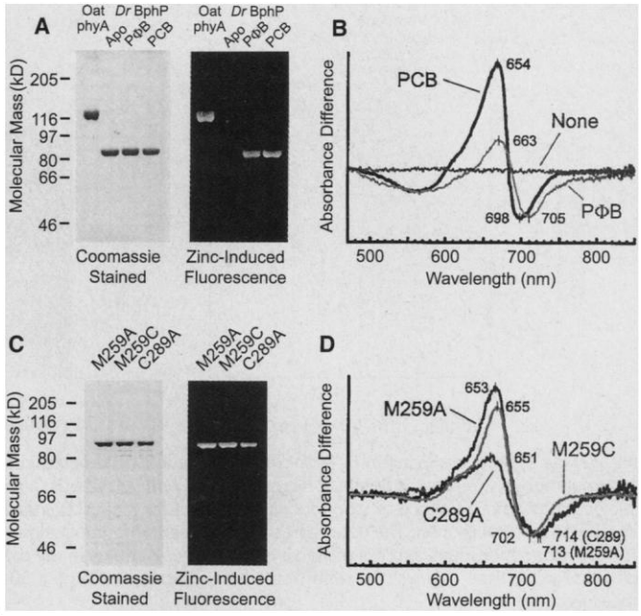

Fig. 2. In vitro assembly and spectral properties of wild-type and mutated versions of *Dr*BphP assembled with PΦB or PCB. (**A** and **C**) The reaction products subjected to SDS-PAGE and stained with Coomassie brilliant blue or analyzed for zinc-induced fluorescence of the bilin chromophore. Oat phyA represents phytochrome assembled in planta and purified from etiolated seedlings (*12*), and Apo represents *Dr*BphP without chromophore addition. (**B** and **D**) Difference spectroscopy of the reaction products following saturating R and FR light. Difference maxima and minima are indicated. All holoproteins in (D) were assembled with PCB.

Immediately downstream of *BphP* in an operon within the *D. radiodurans* genome is a coding region for a 126–amino acid protein with substantial homology to response regulators (*8*) (designated BphR for bacteriophytochrome regulator). Its closest sequence relative (62% similar over the entire length of the polypeptide) is *Synechocystis* Rcp1, the proposed phosphate acceptor of activated Cph1 (*6*). Given its linkage to *Dr*BphP and the homology to Rcp1, we predict that *D. radiodurans* BphR (*Dr*BphR) functions as a phosphate acceptor for the light-activated histidine kinase activity of *Dr*BphP, which in turn initiates its associated light-signaling pathway.

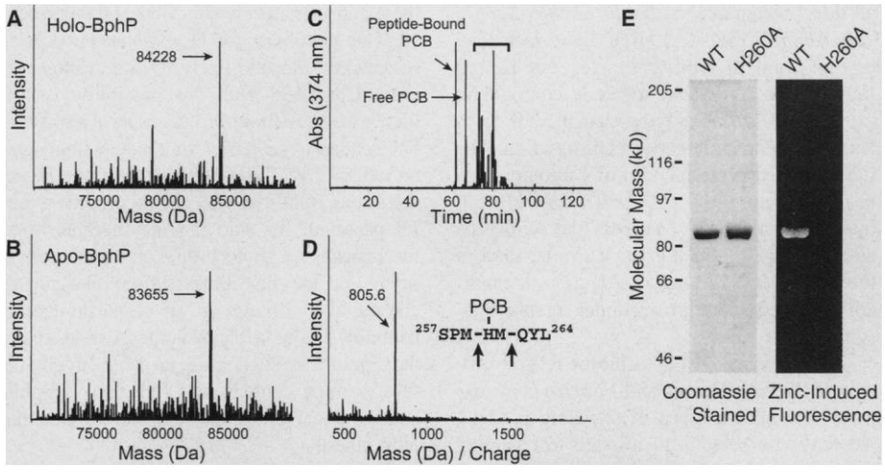

Fig. 3. Characterization of the *Dr*BphP holoprotein assembled with PCB. Molecular-mass determination of (**A**) Holo-*Dr*BphP and (**B**) Apo-*Dr*BphP by LC/MS. The masses indicated with arrowheads were calculated from multiple ion species of the corresponding proteins. (**C**) The LC absorption profile at 374 nm of the *Dr*BphP-PCB holoprotein after cleavage with CNBr. The peaks containing the chromopeptide and free PCB released from the peptide during CNBr treatment are indicated. The peaks under the square bracket are contaminants present in the PCB preparation used to assemble Holo-*Dr*BphP (*10*). Covalent binding of PCB to *Dr*BphP was selective and specific, because there was no apparent binding of other ultraviolet-absorbing contaminants (*10*). (**D**) The MS spectrum showing the chromopeptide ion (arrowhead) identified it as a PCB-histidyl-homoserine lactone predicted to be released from the shown amino acid sequence (S, Ser; P, Pro; M, Met; H, His; Q, Gln; Y, Tyr; and L, Leu). (**E**) Mutation of His^{260} blocks chromophore attachment. Wild-type (WT) and H260A versions of the *Dr*BphP apoprotein were incubated with PCB and analyzed as in Fig. 2.

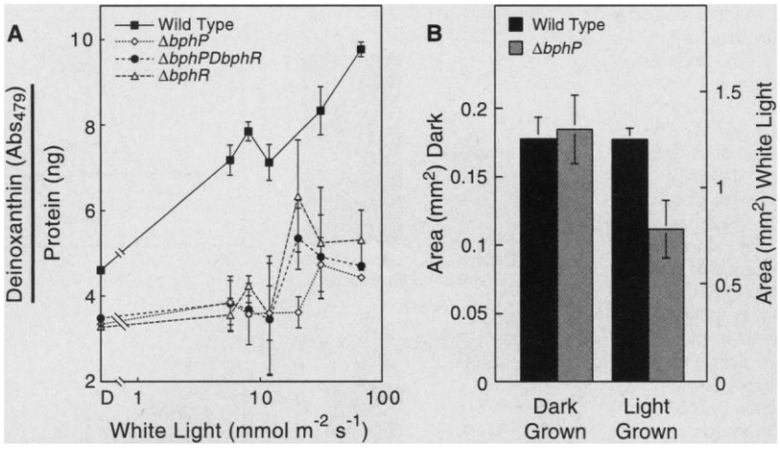

Fig. 4. Role of BphP and BphR in *D. radiodurans* carotenoid accumulation and growth. **(A)** Deinoxanthin accumulation in wild-type, Δ*bphP*Δ*bphR*, Δ*bphP*, or Δ*bphR* strains of *D. radiodurans* kept in the dark or subjected to various fluences of continuous white light for 4 days. Deinoxanthin content was expressed in relation to total protein. The data represent the mean of four experiments (error bars indicate ±SD). **(B)** Growth of individual colonies of wild-type and Δ*bphP* strains in the dark or in ~350 μmol m^{-2} s^{-1} of white light. After 4 days, the area (error bars indicate ±SD) of ~50 representative colonies was measured.

Because phytochromes and phytochrome-like proteins regulate pigmentation (*3*, *7*, *15*), it was likely that the BphP/BphR system also regulates pigment synthesis in *D. radiodurans*, the most abundant of which is the carotenoid deinoxanthin (*16*). To examine this, we generated null mutations in the *BphP/BphR* operon by homologous-gene replacement and examined the photoresponses of the cultures (*17*). Wild-type bacteria accumulated higher amounts of deinoxanthin when grown under increasing fluence rates of white light. However, this induction was severely repressed in all three mutant strains, Δ*bphP*Δ*bphR*, Δ*bphP*, and Δ*bphR* (Fig. 4A). Red light increased pigmentation in wild-type cells, but far-red light had no observable effect in comparison to darkness (*10*), implying that the Pfr form was the biologically active conformation. The light-induced accumulation of carotenoid appears to help protect *D. radiodurans* from intense visible light. Whereas the wild type and the Δ*bphP* mutant grew at similar rates in the dark, colony growth of Δ*bphP* was markedly reduced when grown under intense light (Fig. 4B).

The presence of phytochrome-related proteins in *D. radiodurans* and *P. aeruginosa* expands the known types of signaling used by heterotrophic bacteria to respond to changing light. Although *Pa*BphP was not directly tested here, we presume that its photochemical properties and mechanism of action are similar to that of *Dr*BphP, given their extensive sequence homology within both the chromophore-binding pocket and the histidine kinase domain (Fig. 1A). The *Dr*BphP photoreceptor is similar but photochemically distinct from plant phytochromes, having blue-shifted absorbance spectra and using a different linkage to bind chromophore, which involves a histidine rather than a cysteine to potentially form a Schiff base–type bond. The identity of the natural *Dr*BphP chromophore is unknown, but its similarity to PCB is likely.

Given that *D. radiodurans* and *P. aeruginosa* are distantly related to each other and to photosynthetic bacteria (*10*), these BphPs broaden our current view of phytochrome evolution. Because the other nonphotosynthetic bacteria whose genome sequences are available do not contain phytochrome-related genes (*10*), bacteriophytochromes are not universally distributed, thus raising the question as to how these eubacteria evolved or obtained this receptor. One possibility for *D. radiodurans* is horizontal gene transfer, likely from a cyanobacterium. Consistent with this possibility is that numerous *D. radiodurans* genes have a substantial sequence similarity to those within cyanobacteria (*18*). Whatever its origin, the *D. radiodurans* BphP pathway should provide a useful paradigm for studying the mechanism or mechanisms of phytochrome action because it offers for the first time a simplified phytochrome-like response in an organism that is naturally devoid of photosynthesis. In this case, the signal transduction chain could involve as few as three components: *Dr*BphP, *Dr*BphR, and the target genes that regulate deinoxanthin biosynthesis.

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12. Oat phyA was purified as described [R. D. Vierstra and P. H. Quail, *Biochemistry* **22**, 2498 (1983)]. *DrBphP* was amplified by polymerase chain reaction (PCR) from genomic DNA and ligated into pET28a$^+$ (Novagen, Madison, WI) for expression in *Escherichia coli* with an NH_2-terminal polyhistidine tag, and the recombinant protein was purified by nickel-chelate chromatography (Qiagen, Valencia, CA). Holo-*Dr*BphP was assembled with PCB and PΦB as described for Cph1 (*4*, *6*). Adducts were visualized by zinc-induced fluorescence of the chromoprotein following denaturing SDS-PAGE (*11*). Difference spectra were obtained after saturating R (660 nm) and FR (730 nm) irradiations (*4*).
13. We analyzed proteins and peptides using the API 365 LC/MS system (Perkin-Elmer, Norwalk, CT) with a C18 column (5 μm, 1.0 mm by 150 mm) (Vydac, Hesperia, CA) and a 0 to 95% water-acetonitrile gradient in 0.05% trifluoroacetic acid for LC. Peptide hydrolysis was accomplished by incubating the *Dr*BphP holoprotein for 16 hours in the dark at ~20°C in 70% formic acid with a 200-fold molar excess of CNBr over methionine.
14. Site-directed mutations were introduced by Quikchange (Stratagene, La Jolla, CA) for *DrBphP* (M259A) and by overlap-extension PCR for *DrBphP* (M259C), *DrBphP* (C289A), and *DrBphP* (H260A).
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17. *Deinococcus radiodurans* R1 was grown at 30°C on carotenoid-free media prepared with solid complete yeast–synthetic media (Difco, Detroit, MI) supplemented with tryptone (5 g/liter) and glucose (1 g/liter). To create vectors to disrupt *DrBphP*, *DrBphR*, or both, we generated a targeting plasmid (pFSI31) by inserting the Stu I–Fsp I fragment containing the chloroamphenicol acetyltransferase gene from pI3 (*19*) into the Eco RV site of pBS SK$^+$ (Stratagene). Sequences flanking *DrBphP*, *DrBphR*, or both were amplified by PCR from *D. radiodurans* DNA and introduced into pFSI31 so that, after homologous integration, the respective loci would be exactly replaced by the chloroamphenicol-resistance marker. Transformation and mutant identification was according to (*19*), and mutants were identified and confirmed by Southern blotting (*10*). Cells were irradiated with varying fluences of white light provided by a sodium lamp supplemented with fluorescent lighting. Deinoxanthin was measured spectrophotometrically following methanol extraction, as described (*19*).
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20. We thank K. Minton for supplying *D. radiodurans* R1 and the pI3 plasmid, O. White for discussions on the *D. radiodurans* sequence, The Institute for Genomic Research and the *Pseudomonas* Genome Project for distributing the genomic sequences described here, S. H. Bhoo and P.-S. Song for providing PCB and PΦB, and A. Harms for assistance with MS. We dedicate this paper to the memory of Rudy J. Wodzinski. This work was supported by an NIH predoctoral fellowship (S.J.D.) and U.S. Department of Energy grant DE-FG02-88ER-13968 (R.D.V.).

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