tested here (*P. syringae* BphP) was a more active kinase as the Pfr form. Although the reason for this difference is unknown, the greater kinase activity for the Pfr form of this BphP conforms with the long-held expectation from physiological studies that Pfr is the biologically active form of phytochrome-type photoreceptors[1].

Since our detection of BphPs in *D. radiodurans* and *P. aeruginosa*[4], we have now discovered related sequences in several heterotrophic and photoautotrophic bacteria and in fungi, indicating that this family of photoreceptors is part of a widely used photosensory system. Given this distribution, we predict that BphPs represent the progenitor of phytochromes. Why was BV replaced by PCB and PΦB during the ontogeny of phytochromes? One reason may be reflected by the substantial R shift in the Pr absorbance maximum of the BV–BphP chromoproteins as compared with the Pr absorbance maximum of phytochromes. Whereas phytochromes are perfectly adapted for measuring shade avoidance by photosynthetic organisms because their Pr spectrum closely overlaps that of chlorophylls[1], BV–BphPs are not because their Pr maxima lie at 700 nm. As a consequence, cyanobacteria and plants might have adopted blue-shifted bilins such as PCB and PΦB to detect competition better, and thus enhance photosynthetic light capture. This switch would require an ability to make and bind these bilins, and an ability to prevent coupling of their precursor BV. Such a conversion may have been facilitated in cyanobacteria by their exploitation of PCB in photosynthetic light harvesting[11]. □

Methods

Isolation and detection of BphP from *D. radiodurans*

Cells were grown as described[4], collected by centrifugation, frozen and lysed in 100 mM Tris-HCl (pH 7.0), 140 mM ammonium sulphate, 10 mM EDTA, 50% ethylene glycol, 2 mM phenylmethylsulphonylfluoride. The clarified crude extract was incubated at 4 °C with anti-*Dr* BphP antibodies coupled to Affigel 10 (Pharmacia). Bound protein was eluted with 100 mM glycine (pH 2.5). The eluate was precipitated at −20 °C with eight volumes of acetone plus 0.002% 2-mercaptoethanol, collected by centrifugation, and resuspended in SDS–PAGE sample buffer. We performed immunoblot analysis with Hybond-C Extra membranes (Amersham) blocked with 1×PBS and 10% dried milk, and used horseradish peroxidase linked to goat anti-rabbit immunoglobulins (Kirkegaard and Perry) for detection. Covalently bound bilins were visualized by zinc-induced fluorescence of the complexes subjected to 10% SDS–PAGE[12].

Expression and assembly of BphPs

BphP-coding regions were amplified by polymerase chain reaction from genomic DNA using primers designed to add appropriate restriction sites for expression as His_6-tagged versions in suitable pET expression vectors (Novagen). With the exception of *Dr* BphP, the His_6 domain was appended to the carboxy terminus. For *Dr* BphP, *Nde*I sites were added for insertion into pET28a. For *Ps* BphP, *Pa* BphP, *Dr* BphO, *Syn* HO and *Dr* BphR, *Bam*HI and *Xho*I sites were added for insertion into pET21a. The coding region for the His_6 tag was subsequently removed from each haem oxygenase by appropriate digestion.

Recombinant proteins were expressed at 22 °C in the dark in the *E. coli* strain BL21-CodonPlus (DE3)-RIL (Stratagene) upon induction with 1 mM isopropyl β-D-thiogalactoside. Co-expression of *Dr* BphP with either *Dr* BphO or *Syn* HO was achieved by co-transformation of appropriate plasmids and selection on LB medium containing 50 μg ml^{-1} ampicillin and 30 μg ml^{-1} of kanamycin. We grew the cultures at 37 °C until a roughly 0.4 absorbance at 600 nm was reached, and then induced and incubated them at 22 °C for 4 h. Cells were lysed in 30 mM Tris-HCl (pH 7.8), 300 mM NaCl, 20 mM imidazole. The BphPs were purified from the clarified crude extract by nickel/nitrilotriacetic acid affinity chromatography (Qiagen) using 30 mM Tris-HCl (pH 7.8), 300 mM NaCl, 200 mM imidazole for elution. The eluants were exchanged into 70 mM Tris-HCl (pH 8.0), 1 mM EDTA, 200 mM NaCl, 5% ethylene glycol. For BphPs assembled *in vitro*, the purified apoproteins were incubated for 1 h at 4 °C in a 10-fold molar excess of BV (Porphyrin Products), PCB or PEB (provided by P.-S. Song). Absorbance and difference spectra of BphPs were determined after saturating irradiations with light at 690 and 760 nm. We performed protein kinase reactions as described[9]. Sequences were compared using MACBOXSHADE (Institute of Animal Health, Pirbright, UK) and ClustalW (http://www.ebi.ac.uk/clustalw).

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Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to R.D.V. (e-mail: vierstra@facstaff.wisc.edu). GenBank accession codes for the BphP sequences are AF418994 (*P. fluorescens*), AF418997 (*P. syringae*), AF418995 and AF418996 (*P. putida*), AAK87748 and AAK87910 (*A. tumefaciens*), AJ416905 (*R. leguminosarium*) and AF418998 (*R. sphaerodies*). Incomplete genome sequences are available for *N. crassa* (http://www.genome.wi.mit.edu/annotation/fungi/neurospora) and *A. fumigatus* (http://www.tigr.org/tbd/e2k1/afu1).

erratum

The rhythm of microbial adaptation

Philip Gerrish

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The final paragraph of the Methods was inadvertently omitted.

Analysis of HIV sequences

Eighteen sequences from the c2v3 region of the *env* gene of HIV-1 subtype E were derived from viral samples taken from different infected individuals in Thailand at approximately the same time[26]. Codon alignment and phylogenetic analysis were performed using CLUSTAL[27] and PHYLIP[28]. Twelve of the sequences formed a star phylogeny, suggesting independent evolution, and the founding sequence was determined. Non-synonymous distances were calculated with SNAP[29]. The good agreement between this data and the prediction of equation (2) suggests that non-synonymous evolution in this gene region is mostly adaptive, an observation supported by many other studies of this region. □