A Singular Bacteriophytochrome Acquired by Lateral Gene Transfer

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Bacteriophytochromes are phytochrome-like proteins that mediate photosensory responses in various bacteria according to their light environment. The genome of the photosynthetic and plant-symbiotic Bradyrhizobium sp. strain ORS278 revealed the presence of a genomic island acquired by lateral transfer harboring a bacteriophytochrome gene, BrBphP3.ORS278, and genes involved in the synthesis of phycocyanobilin and gas vesicles. The corresponding protein BrBphP3.ORS278 is phylogenetically distant from the other (bacterio)phytochromes described thus far and displays a series of unusual properties. It binds phycocyanobilin as a chromophore, a unique feature for a bacteriophytochrome. Moreover, its C-terminal region is short and displays no homology with any known functional domain. Its dark-adapted state absorbs maximally around 610 nm, an unusual wavelength for (bacterio)phytochromes. This form is designated as Po for orange-absorbing form. Upon illumination, a photo-reversible switch occurs between the Po form and a red (670 nm)-absorbing form (Pr), which rapidly back-reacts in the dark. Because of this instability, illumination results in a mixture of the Po and Pr states in proportions that depend on the intensity. These uncommon features suggest that BrBphP3.ORS278 could be fitted to measure light intensity rather than color.

To adapt their growth and development to light environment, plants use a large panel of photoreceptors. Among them, phytochromes utilize bilin as chromophore to control several aspects of morphogenesis like germination, flowering, shade avoidance, chloroplast development, etc. (1–2). Phytochromes respond to red and far-red light via a reversible shift from a red-absorbing form (Pr) to a far-red absorbing form (Pfr). In the last decade, thanks to the accumulating genomic data, it has been recognized that phytochromes are not restricted to plants but are widely distributed among prokaryotes and eukaryotes. This finding has provided new insights into the diversity, the evolution, and the biochemical and structural description of this light sensor family (3–8). A recent phylogenetic analysis shows that the phytochrome superfamily is organized in five different clades: the Phys (plants phytochromes), Cphs (cyanobacterial phytochromes), BphPs (bacteria phytochromes), Fphs (fungi phytochromes), and a cluster of Phy-like sequences (6).

The architecture of most phytochromes is composed of a N-terminal photosensory core domain (PCD) and of a C-terminal module involved in signal transduction. The PCD, comprised of the PAS, GAF, and PHY subdomains, attaches auto-catalytically a linear tetrapyrrole (or bilin). All bilins are derived from a heme molecule, converted to biliverdin IX (BV) by a heme oxygenase. Whereas BphPs use this simplest linear tetrapyrole as a chromophore (9), the chromophores of Phys and Cphs are 3E-phytochromobilin (PfB) and 3Z-physocyanobilin (PCB), respectively, modified from BV by bilin reductases (10). These bilins are covalently bound to a Cys residue located in the N-terminal region for BV and in the GAF domain for PfB and PCB (11, 12). Although little is known about the Fph type, their similarity to BphPs and their biochemical properties are strong indications that BV is also their natural chromophore (13). The Phy-like proteins display an unusual PCD architecture characterized by the absence of PAS and PHY domains and may therefore represent a distinctive group of bilin proteins (6).

The C-terminal region of most BphPs possesses a two-component histidine kinase motif that transfers phosphate to a response regulator (RR) whose gene is often found within the BphP operon (14). However, other output modules, like PAC, RR, or GGDEF and EAL domains have been described (3). The spectral properties of BphPs are also highly disparate. Their dark-adapted ground state can be either the Pfr (bathyBphP) or the Pr form (15, 16), and a short wavelength-absorbing form (Pnr) is photo-induced for RpBphP3 from Rhodopseudomonas (Rps.) palustris (17). Altogether, these results highlight the modularity and the adaptability of the BphP family.

Photosynthetic bradyrhizobia belong to the α-sub-branch of the Proteobacteria family. They have the capacity to form nitro-
gen-fixing nodules on both roots and stems of some aquatic legumes belonging to the genus *Aeschynomene* (18). Besides their life in association with plant, these bacteria develop freely in aquatic environments or in soils. Recently, a bacteriophytochrome (BrBphP) whose gene is located in the photosynthesis gene cluster was shown to play a key role in the control of photosystem synthesis by antagonizing the action of the repressor PpsR (16). The recent sequencing of the genomes of two photosynthetic *Bradyrhizobium* strains (ORS278 and BTAi1) revealed the presence of two other BphPs in each strain.5 One of these is common to both strains, whereas the second is specific to each strain.

In this study, we have carried out a detailed characterization of the specific BphP present in strain ORS278, denoted BrBphP3.ORS278. We give evidence that its gene was acquired by lateral gene transfer together with *bphO* and *pcyA* genes, involved in its chromophore synthesis. This is the first description of a BphP containing phycocyanobilin as chromophore. Furthermore, we show that this new type of BphP possesses a series of unusual photochemical properties, which leads us to propose that it acts as a sensor of light intensity rather than light color.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—*Bradyrhizobium* strain ORS278 was grown in a modified YM agar medium at 35 °C for 7 days (19).

**Cloning, Protein Expression, and Purification**—The BrBphP3.ORS278 gene was amplified by PCR using *Pfu* DNA polymerase (Invitrogen) and the following pairs of primers (restriction sites are underlined): BrBphP3.PstI.pBAD.f 5′-GGACTGAGCCG- AATGAAATACCTTGGCAGTTGATTCTC-3′ and BrBphP3.HindIII.pBAD.r.5′-AGGAAAGCCTGAAATTCCGTCAGAA- TCAACAGGGTTTCTATTTGACATTGTTG-3′, and BrBphP3.HindIII.pBAD.r.5′-AGGAAAGCCTGAAATTCCGTCAGAA- TCAACAGGGTTTCTATTTGACATTGTTG-3′, designed to allow the expression as His6-tagged versions of BrBphP3. ORS278 in pBAD/HisB expression vector (Invitrogen) and the subsequent insertion of the *bphO* and *pcyA* genes. The two last genes were amplified with the two pairs of primers and *pcyA* genes, involved in its chromophore synthesis. This is the first description of a BphP containing phycocyanobilin as chromophore. Furthermore, we show that this new type of BphP possesses a series of unusual photochemical properties, which leads us to propose that it acts as a sensor of light intensity rather than light color.

**Site-directed Mutagenesis**—Single mutations were introduced in BrBphP3.ORS278 using appropriate primers and the QuikChange™ site-directed mutagenesis kit (Stratagene) according to the manufacturer’s recommendations.

**Construction of BrBphP3.ORS278 and gvpA Mutants**—For the construction of the BrBphP3.ORS278 minus mutant, the gene was introduced in the pJQ200mp18 suicide vector (20). A gene fragment was deleted (476-bp Sall) and replaced by the lacZ-kmr cassette of pKOK5 (21). To construct the gvpA minus mutant, the gvpA gene was replaced by the lacZ-kmr cassette of pKOK5. The sequences flanking gvpA were ligated thanks to the following pairs of primers: upgvpA.F 5′-CCGGATCTCGGGT- TAGCCGATGTCGCAAAC-3′ and upgvpA.R 5′-GGATGCG- AATTGCGCTTCAATAGCCACGATTTGAC-3′ and dwgvpA.F 5′-GCTATTGAGGTGCAGATTTGC- ATCTTAACAAAGACTTCTGATCG-3′ and dwgvpA.R 5′-CCGGGCCCGCCGCGGAGATCATGCATTTG-3′ (the primers upgvpA.R and dwgvpA.F contain an overlapping sequence of 27 bp (underlined) to facilitate overlap extension PCR). The ligated product was amplified and introduced into the plQ200-SK plasmid thanks to restriction sites designed in each primer (in bold). The 4.7-kb Sall lacZ-kmr cassette of pKOK5 was then inserted into the Sall site designed in the overlapping sequences of upgvpA.R and dwgvpA.F primers (in italic). These two constructions were introduced and delivered by conjugation into ORS278 strain as described (19).

**Absorbance and Fluorescence Spectra Measurements**—Absorbance and fluorescence spectra of purified BrBphP3. ORS278 were measured as previously described (17). Excitation light was provided by a Luxeon Star/O LXHL-NL98 light-emitting diode (590 nm, half-peak bandwidth 14 nm, 750 µE m⁻² s⁻¹), a He-Ne laser (632.8 nm, 10 milliwatt), or a 24-V quartz lamp filtered through an interference filter (670 nm, 18-nm half-bandwidth).

**Mathematical Treatment**—Under steady-state illumination with a saturating intensity, the fractions of Pr and Po are as shown in Equation 1.

\[
\frac{[Pr]}{K_{ro}} = \frac{[Po]}{K_{ro}} + \frac{K_{ro}}{K_{ro}}
\]

(Eq. 1)

In this Equation, *K*<sub>ro</sub> and *K*<sub>r</sub> are the rate constants, under given illumination conditions, of the Po → Pr and Pr → Po photon conversions, respectively. Saturation requires that these photochemical rate constants are much greater than those for the dark relaxation, which can then be neglected. Expressing spectra as vectors (the *n*th component is the amplitude at the *n*th wavelength; in practice all our spectra were measured with a 1-nm interval), we denote as Pr and Po the spectra of the pure forms Pr and Po. The spectrum Sm of the sample under saturating illumination from some light source labeled *m*, as shown in Equation 2 is thus.

\[
Sm = [Pr] Pr + [Po] Po = \frac{K_{m}^{Pr}}{K_{m}^{Pr} + K_{ro}} Pr + \frac{K_{m}^{Po}}{K_{m}^{Po} + K_{ro}} Po
\]

(Eq. 2)

In this expression, the rate constants *K*<sub>m</sub><sup>Pr</sup> and *K*<sub>m</sub><sup>Po</sup> are products of the number of photons absorbed per unit time and of the probability (*p*<sub>i</sub> or *p*<sub>j</sub>, respectively) that absorption results in successful conversion to the other form. Denoting as Zm the spectrum radiated by source *m*, the absorption rates are the dot products Zm. Pr and Zm. Po and, as shown in Equation 3, one has the following.

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5 A. Vermeglio and E. Giraud, unpublished data.
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\[
\kappa^m = \rho_x Zm \cdot Po \\
\kappa^p = \rho_x Zm \cdot Pr
\]

Thus, as shown in Equation 4,

\[
Sm = Pr \frac{\eta Zm \cdot Po}{Zm \cdot Pr + \eta Zm \cdot Po} + Po \frac{Zm \cdot Pr}{Zm \cdot Pr + \eta Zm \cdot Po}
\]

(Eq. 4)

where \( \eta = \frac{\rho_x}{\rho_p} \) is the ratio of the quantum efficiencies.

In the experiment illustrated by Fig. 5A, one measures three spectra, \( S_1, S_2, S_3 \), corresponding to illumination by sources of known spectra, \( Z_1, Z_2, Z_3 \). The \( Pr \) and \( Po \) spectra are unknowns to be determined, together with the ratio of quantum yields \( \rho \). Three \( Sm \) spectra (at least) are necessary in this case, whereas if \( \eta \) were known, only two spectra would suffice. We thus have a system of three vector equations such as Equation 4, with \( m = 1, 2, 3 \). Because the \( Sm \) are linear combinations of \( Pr \) and \( Po \), the latter are instances of the vector family as shown in Equation 5

\[
V(x) = S_1 + x(S_1 - S_2)
\]

(Eq. 5)

where \( x \) is a scalar. The choice of the particular vectors \( S_1 \) and \( S_2 \) out of the three \( Sm \) is arbitrary; in practice, it is reasonable to pick the two most different spectra. Now, considering Equation 5, there are two particular values of \( x \), say \( x_0 \) and \( x_r \), for which \( V(x_0) = Po \) and \( V(x_r) = Pr \). One has thus a system of three vector equations to be solved for three scalar unknowns, \( x_0, x_r, \) and \( \eta \). The generic equation is \( Sm = Sm' = 0 \) (a vector with norm 0), where \( Sm' \) is obtained from Equation 4, replacing \( Po \) and \( Pr \) by \( V(x_0) \) and \( V(x_r) \), respectively, as shown in Equation 6.

\[
Sm' = V(x_0) \frac{\eta Zm \cdot V(x_0)}{Zm \cdot V(x_0) + \eta Zm \cdot V(x_0)}
\]

+ \[
V(x_r) \frac{Zm \cdot V(x_r)}{Zm \cdot V(x_r) + \eta Zm \cdot V(x_0)}
\]

(Eq. 6)

Because the \( S \) and \( Z \) are (noisy) experimental spectra, we are not looking for an exact solution but rather for a non-linear least square procedure (e.g. Levenberg-Marquardt) minimizing the sum of Euclidian norms \( N(x_0, x_r, \eta) = |S_1 - S_1'| + |S_2 - S_2'| + |S_3 - S_3'| \). This is easily handled by the mathematical software Mathcad 12 from Mathsoft Inc.

RESULTS

BrBphP3.ORS278, a BphP Acquired by Horizontal Gene Transfer—The BrBphP3 gene from strain ORS278 encodes a 620-amino acid protein that displays a weak identity (27%) with the Cph-like protein AphA from Anabaena sp. strain PCC 7120 and with the BphP from Pseudomonas fluorescens strain Pf-5. Prediction of the protein architecture using Pfam or SMART tools permits identification of a classical PCD with PAS-GAF-phy subdomains, supporting its classification as phytochrome. However, unlike all the phytochromes described thus far, the C-terminal region is very short and displays no homology with any known functional domain (Fig. 1A). Furthermore, a phylogenetic analysis (Fig. 1C) using the GAF domain (or the entire PCD module, supplemental Fig. S1) indicates that this protein is distant from the five clades that constitute the phytochrome superfamily (6). All these data indicate an unorthodox character for BrBphP3.ORS278 and raise the question of its origin and function.

This is strengthened by the fact that the BrBphP3.ORS278 gene is found in a region of \( \approx 91 \) kb that displays all the hallmark features of a horizontal acquisition island. Indeed, this region is characterized by an atypical \( G + C \) content (62.8% compared with the average value 65.4% of the genome), a RNA insertion site, a distinct codon usage, and the presence of integrase and trA genes known to be involved in integration and conjugal transfer of DNA mobile elements, respectively. These features, together with the absence of a homologue gene in the closely related species (Bradyrhizobium sp. BTAi1, B. japonicum, or Rps. palustris), indicate that BrBphP3.ORS278 was acquired by lateral gene transfer.

Other genes of interesting function are present in this genomic island at the vicinity of BrBphP3.ORS278: (i) a gene encoding a heme oxygenase designated bphO, (ii) a pcyA gene encoding a PCB ferredoxin oxidoreductase, and (iii) a hemA gene encoding a 5-aminolevulinate synthase, the first enzyme of the heme biosynthesis pathway (Fig. 1B). These proteins are key enzymes for the synthesis of PCB, the chromophore of Cphs. BrBphP3.ORS278 is also flanked by genes encoding two putative regulatory proteins (a transcriptional factor of the LuxR family and an anti-anti-I factor) (Fig. 1B). A cluster of gvp genes encoding gas vesicle proteins is also found in the same genomic island (Fig. 1B). Gas vesicles are widely distributed among prokaryotes from aquatic habitats; in particular, they enable cyanobacteria to float up toward the light (22).

BrBphP3.ORS278 Binds PCB as Chromophore—The presence of a pcyA gene in the vicinity of BrBphP3.ORS278 is a strong clue that the native chromophore of BrBphP3.ORS278 is PCB. To test this hypothesis, we overproduced, in E. coli, recombinant BrBphP3.ORS278 proteins using two different constructs. In the first construct, the BrBphP3.ORS278 gene was cloned in the pBAD-HisB expression vector and transformed into an E. coli strain containing the pPL-PCB plasmid. This plasmid, constructed by Gambetta and Lagarias (23), harbors ho1 and pcyA from Synechocystis sp. PCC6803 for the in vivo reconstitution of the holoCph1 with PCB as chromophore. In the second construct, the bphO and pcyA genes found at the vicinity of BrBphP3.ORS278 were added to the previous pBAD-HisB::BrBphP3.ORS278 plasmid together with synthetic ribosome binding sites to ensure their expression. Both E. coli strains produced large amounts of recombinant proteins. The corresponding purified proteins were blue and presented identical spectral properties (Fig. 2B), indicating that pcyA and bphO genes found in the genomic island are functional and that BrBphP3.ORS278 binds a bilin (i.e. PCB). To gain further evidence supporting that the pcyA gene is involved in the modification of the bilin, we cloned the same synthetic operon but omitted the pcyA gene. In this case, the corresponding E. coli cell pellet was light green and the holo-BrBphP3.ORS278 protein was expressed in low amount and as inclusion bodies (data not shown). This indicates that BrBphP3.ORS278 could bind either BV or PCB as a chromophore but that a correct conformational state for the protein is obtained only with PCB.
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Because in phytochromes characterized thus far, BV binds to a Cys located in the N-terminal extremity whereas PCB binds to a Cys located in the GAF domain (11, 12), we investigated the PCB binding site by mutating the Cys residues (Cys-7 and Cys-16) of the N-terminal region and the Cys residue (Cys-260) located in the GAF domain (Fig. 2A). Once purified, these three mutated proteins turned out to be colored (data not shown). However, as revealed by zinc-induced autofluorescence (Fig. 2B, inset), the C260S protein failed to bind PCB covalently, at variance with the other mutants. Thus, the covalent binding of BrBphP3.ORS278 chromoprotein implies specifically Cys-260, which is the attachment site of PCB in cyanobacterial phytochromes (12, 24).

This is several orders of magnitude faster than reported for any phytochrome or BphP studied so far, in which dark recoveries take place in time ranges spanning from minutes to days (15, 24). As made clear below, to three reasons: (i) the speed of the back-reaction to the dark-adapted state, (ii) the spectral overlap between the Po and Pr forms, and (iii) their unequal photochemical efficiencies.

The occurrence of a fast back-reaction is illustrated in Fig. 3A showing the kinetics of the absorption changes at 670 nm during and after a 3-s pulse of 590 nm light (Fig. 2B, red line), a partial bleaching of the 610-nm band was observed, accompanied by the appearance of a long wavelength band ~670 nm, indicative of a red form denoted Pr. However, despite the relatively high illumination intensity used (500 \( \mu \text{E m}^{-2} \text{s}^{-1} \)), only a small fraction of the Po form was photo-transformed. This is due, as made clear below, to three reasons: (i) the speed of the back-reaction to the dark-adapted state, (ii) the spectral overlap between the Po and Pr forms, and (iii) their unequal photochemical efficiencies.

The occurrence of a fast back-reaction is illustrated in Fig. 3A showing the kinetics of the absorption changes at 670 nm during and after a 3-s pulse of 590 nm light of variable intensity. The half-time for the dark recovery is ~460 ms at 25 °C.
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Amino acid sequence alignment and absorption spectra of \( BrBphP3.ORS278 \). A, amino acid sequence alignment of the N-terminal extremity and subregion of the GAF domain of various phytochromes. Conserved residues by more than 50 and 90% are highlighted in gray and black, respectively. Open and closed triangles indicate the Cys residue used as BV or PCB binding sites, respectively. For the upper part, the gel was stained by Coomassie Blue.

The Two Spectral Forms of \( BrBphP3.ORS278 \) and Their Dark Equilibrium—The dark-adapted \( BrBphP3.ORS278 \) is clearly predominantly in the Po form. Although this gives a good approximation for the Po spectrum, things are different for the other form because, as mentioned earlier, we have no way of obtaining a total photoconversion. We designed a procedure that allows the determination of the spectra of Po and Pr and also of the relative photoconversion efficiencies of the two forms. The mathematical aspects are described under "Experimental Procedures."

This procedure was applied to the data shown in Fig. 5A. The sample was equilibrated at 4 °C to slow down the decay to the dark-adapted state, thus facilitating light saturation, which was checked by using neutral density filters. The three experimental spectra (green) were obtained with illuminations centered either in the Po region, or close to the isosbestic wavelength, or in the Pr region (gray spectra). The orange and red spectra are those of the pure forms, Po (peaking at 613 nm) and Pr (peaking at 672 nm), resulting from the mathematical treatment of the data. Interestingly, for the ratio of quantum yields of the two forms, \( \eta = \rho_o / \rho_r \), one obtains 0.31, meaning that the photoconversion efficiency of Pr to Po is ~3-fold larger than the reverse process. The reconstituted spectra \( Sm' \) (Equation 6) are superimposable to the corresponding experimental spectra. The solution is unique and well defined, both with respect to the Po and Pr spectra and to the relative yield \( \eta \); in particular, the quality of the fit became clearly unacceptable when imposing equal efficiencies of the two forms (\( \eta = 1 \)).

Effect of Temperature on the Dark Equilibrium between the Po and Pr Forms—Unexpectedly, we noticed a clear effect of temperature on the dark-adapted spectrum of \( BrBphP3.ORS278 \). When lowering the temperature, one observes an increase of the 670-nm band, accompanied by a decrease in the 610-nm region (Fig. 5B). This is suggestive of a temperature-dependent equilibrium between the Pr and Po forms. This view was confirmed by measuring the extents of the light-induced changes caused by a saturating illumination either in the Pr or Po band. When lowering the temperature, the extent of the Po → Pr photoconversion induced by 590 nm light decreased, while the extent of the Pr → Po photo-

fitted by the function expressing the competition between a photochemical process and a dark back-reaction. Even with the highest light intensity used in this experiment (666 \( \mu \)E m\(^{-2} \) s\(^{-1} \)), we still could not fully saturate the absorption change.

The temperature dependence of the dark recovery is examined in Fig. 3C, from 40 °C (where \( t_{1/2} \approx 215 \) ms) down to ~11 °C (\( t_{1/2} \sim 30 \) s). The kinetics are roughly monoexponential. Assuming that Eyring’s transition state theory is applicable to this process, one can estimate the enthalpy and entropy of the transition state \( P# \) (with respect to state Pr) from an Eyring’s plot, ln(\( k/T \)) versus 1/\( T \) (Fig. 3D). The linear regression yields \( \Delta H# = -15 \) kcal mol\(^{-1} \) and \( \Delta S# = 7.6 \) cal mol\(^{-1} \) K\(^{-1} \). We took advantage of the slower back-reaction at low temperature to demonstrate that, once formed, the Pr state can be photo-converted back to Po (Fig. 4). At the low temperature we used (~11 °C), a first illumination with 590 nm light induced the Pr form, which then returned slowly to Po in the dark. When the protein was submitted to a second illumination with 680 nm light preferentially absorbed by the Pr form, a fast increase of the 600-nm absorption was observed, expressing the formation of Po. \( BrBphP3.ORS278 \) presents therefore a photo-reversible shift between the Po and Pr forms and behaves, in this respect, like a “classical” (bacterio)phytochrome.
Po spectrum becomes significantly shifted toward shorter wavelengths when increasing the temperature. The Pr spectrum, however, shows no significant shift with temperature (as seen from light-induced spectra; data not shown). We thus used the 680-nm region in the spectra of Fig. 5B to estimate the relative fractions of Po and Pr. The deduced equilibrium constant of the equilibrium in the dark varies from $[\text{Pr}] / [\text{Po}] \sim 0.02$ at 30°C to $0.27$ at 0°C. The theoretical dependence of $K_{eq}$ on $T$ is given by $K_{eq} = \exp(-\Delta H_0/RT + \Delta S_0/R)$, where $\Delta H_0$ and $\Delta S_0$ are the enthalpy and entropy differences, respectively, between Pr and Po. This relation predicts a linear dependence of $\ln(K_{eq})$ versus $1/T$ (van’t Hoff plot) (Fig. 5C). The estimates of $\Delta H_0$ and $\Delta S_0$ obtained from the fit are but a rough approximation because of the limited $T$ and $K$ range. The decrease of $K_{eq}$ when lowering $T$ implies that the Pr form has both the lowest enthalpy and entropy, so that the stable form is Pr at low $T$ and Po at high $T$. The temperature where the inversion occurs ($K_{eq} = 1$) is predicted around $-12°C$.

Fluorescence Properties of BrBphP3.ORS278—Fig. 6A shows the excitation (continuous line) and emission (dotted line) spectra for a suspension of BrBphP3.ORS278 recorded at room temperature. The excitation spectrum is very similar to the absorption of the Po form of BrBphP3.ORS278, except that the long wavelength band peaks at slightly longer wavelength because of uncorrected instrumental distortion in the red part of the spectrum. A clear demonstration that the Po form is markedly more fluorescent than the photo-induced form Pr is exemplified by the light-induced fluorescence changes (Fig. 6B). These changes, recorded at 4°C for a suspension of BrBphP3.ORS278 subjected to two 6-s pulses of 590 nm light, imply that the formation of state Pr induces an important decrease in the fluorescence yield. The fluorescence properties of BrBphP3.ORS278 do not support a simple identification of its light-induced state with the Pr state of classical phytochromes, despite the similar spectral position. In the latter, Pr is the fluorescent state, whereas the fluorescence is quenched in the far-red form, Prf (25). In BrBphP3.ORS278 the Po form is fluorescent, whereas the light-induced Pr state emits no or little fluorescence. In this respect, BrBphP3.ORS278 behaves as other phytochromes, where the short wavelength form is the only fluorescent one.

**Attempts to Determine the Role of BrBphP3.ORS278**—Because of its localization at the vicinity of gvp genes,
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**FIGURE 5. Spectral deconvolution of the Po and Pr forms and temperature dependence of their dark equilibrium.** A, the three green curves are (from smaller to larger in the 600-nm region) the steady-state spectra recorded at 4 °C under saturating illuminations centered at 590, 633, and 680 nm, respectively. The spectral shapes of these illuminations are shown as gray bands. The orange and red spectra are those of the Po and Pr states computed as explained under “Experimental Procedures.” B, spectra recorded from 0 to 35 °C (the arrows indicate the evolution when increasing T). C, a van’t Hoff plot $\ln(K_{eq})$ versus $1/T$, where $K_{eq}$ is the equilibrium constant $[P_{H}][P_{O}]$ in the dark-adapted state, determined from the contribution of the Pr form to the spectra in panel B in the 680-nm region. The line is a regression whose parameters correspond to $\Delta H_{0} = -11$ kcal mol$^{-1}$ and $\Delta S_{0} = -43$ cal mol$^{-1}$ K$^{-1}$ (pertaining to the energy differences Pr $\rightarrow$ Po). The inset shows the free energy profile for the Pr $\rightarrow$ Po transition, compiling the present estimates of $\Delta H_{0}$ and $\Delta S_{0}$, with the information on the transition state Pr obtained in Fig. 3D. The gray squares and blue arrows show the magnitude and direction of the entropic contribution $-T\Delta S$, for $T = 300$ K. The vertical scale is given in $k_{B}T$ units.

**FIGURE 6. Fluorescence properties of BrBphP3.ORS278.** A, excitation (continuous line) and emission (dotted line) for a suspension of BrBphP3.ORS278 at room temperature. For the excitation spectrum, the fluorescence was collected at 732 nm. Recording of the emission spectrum was performed under 355 nm excitation. B, light-induced fluorescence changes recorded at 4 °C for a suspension of BrBphP3.ORS278 subjected to two 6-s pulses of 590 nm light provided by a light-emitting diode.

BrBphP3.ORS278 might be involved in the control of the synthesis of gas vesicles. The photosynthetic activity of *Bradyrhizobium* ORS278 requires both light and strictly aerobic conditions, a particularity shared with the aerobic anoxygenic photosynthetic bacteria (18). One possibility is therefore that BrBphP3.ORS278 is involved in the regulation of gas vesicle synthesis by allowing the bacteria to reach the appropriate ecological niche in oxygen tension and light intensity for optimal growth. Such a situation is encountered in *Halobacteria*, for example, where the synthesis of gas vesicles is known to be under the control of various environmental factors, including light and oxygen tension (26, 27). Several attempts were made to test such a hypothesis by cultivating *Bradyrhizobium* ORS278 with various growth media and under different light conditions and/or oxygen tension. However, in all these assays we failed to reveal a buoyancy phenotype. We also constructed deletion mutants in *BrBphP3.ORS278* and *gvpA* and examined their phenotypes under various growth conditions. No obvious differences could be observed between the wild type and the mutants concerning, for example, growth rate, photosynthetic activity, etc. Similarly, the use of a lacZ reporter gene did not permit identification of specific conditions for which *gvpA* is expressed. In contrast, a low but significant β-galactosidase activity (~50 Miller units) was observed for the various conditions tested, indicating that *BrBphP3.ORS278* is constitutively expressed.

**DISCUSSION**

We have described the properties of a new type of bacteriophytochrome present in the photosynthetic strain *Bradyrhizobium* sp. ORS278. Its gene is located in a horizontally acquired genomic island that displays hallmarks suggestive of recent gene transfer. To our knowledge this is the first clear evidence for the acquisition of a phytochrome gene by a prokaryote through lateral transfer. The presence of a *pcyA* gene, identified thus far only in cyanobacteria (28), may appear as a strong clue for a cyanobacterial origin of this genomic island. However, phylogenetic analyses (Fig. 1C and supplemental Fig. S1) indicate that this protein is distant from the five clades of the phytochrome superfamily, including Cphs (6). In addition, phylogenetic analysis using other genes (*bphO*, *hemA*, and *gvpA*)

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*E. Giraud, unpublished results.*
identified in this island does not reveal any obvious relationship either with the cyanobacteria phylum or another bacterial phylum (supplemental Figs. S2–S4). As a whole, these results do not confirm a cyanobacterial source for this genomic island and leave open the question of its origin.

Despite the co-localization of BrBphP3.ORS278 and gvpA genes in this genomic island, we could not establish a functional relationship between them. The gvp genes are likely to be functional because they are not fragmentated as is often found for remnant genes. The absence of gas vesicle synthesis in various assays may therefore reflect the intrinsic difficulty of reconstituting the physiological conditions where this function is activated. Indeed, such a difficulty is not unprecedented: in Bacillus megaterium, all the genes necessary for the synthesis of gas vesicles are functional when expressed in E. coli but nevertheless they have not been observed in this species (29).

BrBphP3.ORS278 has unique features among all members of the phytochrome superfamily studied thus far: (i) the short wavelength of its dark-adapted form Po, (ii) its very fast recovery to the stable state after illumination, (iii) the temperature dependence of the dark equilibrium between its two spectral forms, and (iv) no homology with any known functional domain for its C-terminal part. One may, however, consider BrBphP3.ORS278 as a bona fide phytochrome because it presents a clear photo-reversible conversion between two distinct states (Fig. 4). The two genes encoding putative regulatory proteins found at the vicinity of BrBphP3.ORS278 are possibly involved in the light signal transduction pathway. This may represent a new type of mechanism because the C-terminal region does not possess a "classical" output module.

Although zinc-induced autofluorescence clearly demonstrated that PCB is covalently bound via Cys-260, the absorption spectrum of the stable Po form resembles that of the free PCB (Fig. 5A). This may be related to the free energy profile emerging from the present study (Fig. 5C), which throws an interesting light on the unusual spectral properties of this BphP. The Po state is massively stabilized by a large entropic contribution. The entropy of the Po form implies a somewhat disordered structure of the protein, which would suggest that the chromophore is subjected to little constraints. This may account for its solution-like spectrum, i.e. possibly for a C15-Z,syn conformation. On the other hand, the absorption spectrum of the light-induced form resembles that of the Pr form of BphPs (Fig. 5A), where the chromophore has been shown to be in the C15-Z,anti conformation (30). One should note, however, that besides the isomerization structure, the protonation state of the chromophore is also predicted to have a strong effect on the spectral properties (31). Furthermore, the fluorescence properties of BrBphP3.ORS278 do not support a simple identification of its light-induced state with the Pr state of classical phytochromes. Indeed, we observed (Fig. 6) that the Po form of BrBphP3.ORS278 was fluorescent, whereas the fluorescence from the light-induced Pr state was undetectable. Therefore, despite the spectral similarity, the Pr form of BrBphP3.ORS278 differs from the "classical" Pr state of phytochromes, which is fluorescent.

A high light intensity is required to photoconvert a significant amount of BrBphP3.ORS278 (Fig. 3B). As previously mentioned, this is because of the fast back-reaction, of the spectral overlap between the two Po and Pr forms, and of the higher quantum yield of photoconversion of the Pr form with respect to the Po form. These features make the Po/Pr composition of the steady state reached in the light very dependent on the intensity, precisely in the range expected for physiological illumination conditions. The half-saturation occurs around 160 \(\mu E\) m\(^{-2}\) s\(^{-1}\), to be compared with the full intensity sunlight (~2,000 \(\mu E\) m\(^{-2}\) s\(^{-1}\)) where BrBphP3.ORS278 would be 92% saturated. From this property, we surmise that BrBphP3.ORS278 evolved as sensor of light intensity rather than light color, at variance with the (bacterio)phytochromes characterized thus far.

In conclusion, we have presented evidence showing that Bradyrhizobium sp. ORS278 acquired by lateral gene transfer a new type of bacteriophytochrome. The unusual properties of BrBphP3.ORS278, in particular its capability of measuring light intensity and its extended absorption range toward short wavelengths, reveal a remarkable adaptability for this family of sensors. This discovery is an additional demonstration of the high diversity among the phytochrome superfamily. In the prospect opened by Wagner and et al. (7) it is to be hoped that the resolution of three-dimensional structures of different chromo-proteins of the phytochrome superfamily will throw light on the molecular basis for this photochemical diversity.

REFERENCES
1. Quail, P. H., Boylan, M. T., Parks, B. M., Short, T. W., Xu, Y., and Wagner, D. (1995) Science 268, 675–680
2. Smith, H. (2000) Nature 407, 585–591
3. Rockwell, N. C., Su, Y. S., and Lagarias, J. C. (2006) Annu. Rev. Plant Biol. 57, 837–858
4. Hughes, I., Lamparter, T., Mittmann, F., Hartmann, E., Gärtner, W., Wilde, A., and Börner, T. (1997) Nature 386, 663
5. Davis, S. J., Vener, A. V., and Vierstra, R. D. (1999) Science 286, 2517–2520
6. Karniol, B., Wagner, J. R., Walker, J. M., and Vierstra, R. D. (2005) Biochem. J. 392, 103–116
7. Wagner, J. R., Brunzelle, J. S., Forest, K. T., and Vierstra, R. D. (2005) Nature 438, 325–331
8. Evans, K., Grossmann, G., Fordham-Skelton, A. P., and Papiz, M. Z. (2006) J. Mol. Biol. 364, 655–666
9. Bhoo, S. H., Davis, S. J., Walker, J., Karniol, B., and Vierstra, R. D. (2001) Nature 414, 776–779
10. Frankenberger, N., Mukougwa, K., Kohchi, T., and Lagarias, J. C. (2001) Plant Cell 13, 965–978
11. Lamparter, T., Carrascal, M., Michael, N., Martinez, E., Rottwinkel, G., and Abian, J. (2004) Biochemistry 43, 3659–3669
12. Lamparter, T. (2006) FEBS Lett. 573, 1–5
13. Flosshich, A. C., Noh, B., Vierstra, R. D., Loros, J., and Dunlap, J. C. (2005) Eukaryot. Cell 4, 2140–2152
14. Yeh, K. C., Wu, S. H., Murphy, J. T., and Lagarias, J. C. (1997) Science 277, 1505–1508
15. Karniol, B., and Vierstra, R. D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2807–2812
16. Giraud, E., Fardoux, J., Fournier, N., Hannibal, L., Genty, B., Bouyer, P., Dreyfus, B., and Verméglio, A. (2002) Nature 417, 202–205
17. Giraud, E., Zappa, S., Vuillet, L., Adriano, J.-M., Hannibal, L., Fardoux, J., Berthomieu, C., Bouyer, P., Pignol, D., and Verméglio, A. (2005) J. Biol. Chem. 280, 32389–32397
18. Giraud, E., and Fleischman, D. (2004) Photosynth. Res. 82, 115–130
19. Giraud, E., Hannibal, L., Fardoux, J., Verméglio, A., and Dreyfus, B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14795–14800
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20. Quandt, J., and Hynes, M. F. (1993) *Gene* **127**, 15–21
21. Kokotek, W., and Lotz, W. (1989) *Gene* **84**, 467–471
22. Walsby, A. E. (1994) *Microbiol. Rev.* **58**, 94–114
23. Gambetta, G. A., and Lagarias, J. C. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 10566–10571
24. Lamparter, T., Michael, N., Mittmann, F., and Esteban, B. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 11628–11633
25. Sineshchekov, V. A. (1995) *Biochim. Biophys. Acta* **1228**, 125–164
26. Pfeifer, F., Gregor, D., Hofacker, A., Plosser, P., and Zimmermann, P. (2002) *J. Mol. Microbiol. Biotechnol.* **4**, 175–181
27. Damerval, T., Guglielmi, G., Houmard, J., and Tandeau de Marsac, N. (1991) *Plant Cell* **3**, 191–201
28. Frankenberg, N., and Lagarias, J. C. (2003) *J. Biol. Chem.* **278**, 9219–9226
29. Li, N., and Cannon, M. C. (1998) *J. Bacteriol.* **180**, 2450–2458
30. Inomata, K., Hammam, M. A. S., Kinoshita, H., Murata, Y., Khawn, H., Noack, S., Michael, N., and Lamparter, T. (2005) *J. Biol. Chem.* **280**, 24491–24497
31. Göller, A., Strehlow, D., and Hermann, G. (2005) *ChemPhysChem.* **6**, 1259–1268