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A Redox-Responsive Pathway for Aerobic Regulation of Photosynthesis Gene Expression in *Rhodobacter sphaeroides* 2.4.1

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To further understand the proposed signal transduction pathway involving the presumed redox proteins RdxBH and cbb_3 cytochrome oxidase in *Rhodobacter sphaeroides* 2.4.1, a series of mutants lacking components of both the Prr two-component activation system and the cbb_3-type cytochrome oxidase or RdxBH were constructed. We report that under highly aerobic conditions, aberrant photosynthesis gene expression and spectral complex formation typical of cbb_3- or RdxBH-deficient mutants were no longer observed when either *prrA* (encoding the response regulator of the Prr system) or *prrB* (encoding the presumed sensor kinase) was also deleted. These double-mutant strains are phenotypically identical to single-mutant PrrA and PrrB strains, suggesting that the signal(s) originating from the cbb_3 terminal oxidase affects downstream *puc* and *puf* operon expression by acting exclusively through the Prr system. When the same double-mutant strains were examined under anaerobic dark dimethyl sulfoxide growth conditions, photosynthesis gene expression was obligatorily linked to the two-component activation system. However, photosynthesis gene expression under the same growth conditions was significantly higher in the cbb_3 mutant strain when compared to that in the wild type. Similarly, under anaerobic photosynthetic conditions the high levels of the oxidized carotenoid, spheroidenone, which accumulate in cbb_3-deficient mutants were nearly restored to normal in a PrrB$^-$ CcoP$^-$ double mutant. This observation, together with previously published results, suggests that the regulation of the CrtA-catalyzed reaction possesses both transcriptional and posttranscriptional regulatory effectors. We propose that the cbb_3 cytochrome oxidase, which by definition can interact with external oxygen, serves to control the activity of the Prr two-component activation system under both aerobic and anaerobic conditions. Although independent from the cbb_3 oxidase, the RdxBH proteins are also required for normal functioning of the Prr two-component activation system and are therefore believed to lie between the cbb_3 oxidase in this oxygen-sensing, redox signaling pathway and the Prr activation system.

The facultative photoheterotroph *Rhodobacter sphaeroides* 2.4.1 displays a remarkable ability to grow under a variety of environmental conditions, being capable of aerobic, anaerobic, and photosynthetic growth and of fixing atmospheric nitrogen and carbon dioxide (15). Significant progress has been made in understanding molecular aspects of the regulatory pathways which make this adaptability possible, and many of the key components involved in the regulation of photosystem formation have been identified. However, much less is known about interactions between the regulatory pathways themselves.

At least four such regulatory pathways in *R. sphaeroides* are known to be responsive to oxygen, with the FnrL protein and the Prr two-component system acting in a positive manner to control the expression of many pigment and light-harvesting-complex genes (4–6, 38), while the repressor PpsR negatively regulates photosynthesis gene (PS gene) expression (9). The TspO regulatory network appears to generate a signal through which a number of pigment biosynthesis genes and the *puc* operon are also negatively regulated (33, 34). Recently, we reported that the membrane-bound RdxBH proteins and the cbb_3 cytochrome oxidase are involved in providing a signal through which aerobic photosynthesis gene expression is repressed. These same redox-active proteins also play a role in governing the relative accumulation of the carotenoids (Crt) spheroidene (SE) and spheroidenone (SO) under anaerobic conditions (24). Because the cbb_3-generated signal normally acts to inhibit PS gene expression under aerobic conditions, it was reasoned that its target is a positive-acting regulatory effector, namely the Prr system.

The genetic region encoding the *prr* locus comprises three genes. The *prrA* gene is predicted to encode a cytoplasmic regulator, while *prrB* appears to encode a membrane-bound histidine kinase. Together, PrrA and PrrB represent a two-component signal transduction system. PrrA mutants are photosynthetically incompetent, while PrrB mutants do grow photosynthetically, but only under high light intensity. It was genetically shown that PrrA can be activated by phosphoryl donors other than PrrB (6, 10). The *prrC* gene is predicted to encode a membrane-associated protein somewhat analogous to a yeast cytochrome oxidase assembly factor (5); however, a specific role for PrrC has not yet been identified, although it does appear to be involved in this signal transduction pathway, as observed when deletions affecting different lengths of *prrC* are used (5). It has previously been proposed that PrrB may directly sense oxygen or the redox state of some other membrane component(s) (5); however, the precise mechanism(s) through which oxygen influences PrrB activity remains to be determined.

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In order to examine the relationship between Prr- and cbb_3-RdxBH-dependent PS gene expression and carotenoid biosynthesis, we have constructed a series of mutants lacking components of the Prr system and either the cbb_3-type cytochrome oxidase or the RdxBH proteins. The production of both light-harvesting complexes and the transcriptional activity of the *puc* and *puf* operons in cbb_3-PRR double mutants have been examined under aerobic and anaerobic conditions. By using a CCOP1PRRB1 double mutant, the accumulation of the carotenoids SE and SO under high-light photosynthetic conditions was also measured. The data presented in this paper indicate that the RdxBH proteins and cbb_3 cytochrome oxidase can be categorized as new members of a complex pathway, represented by the Prr two-component system, which plays a central role in the regulation of PS gene expression and photopigment biosynthesis. Together, these constitute a sensor-signal transduction pathway, positively regulating PS gene expression.

MATERIALS AND METHODS

Bacterial strains, plasmids, growth conditions, and DNA manipulations. Strains and plasmids used in this work are described in Table 1. *Escherichia coli* strains were grown at 37°C on LB medium (19) supplemented, when required, with the following antibiotics: tetracycline, 15 μg/ml; ampicillin, 50 μg/ml; kanamycin, 50 μg/ml; streptomycin and spectinomycin, 50 μg/ml each. *R. sphaeroides* 2.4.1 strains were grown at 30°C on Sistrom's medium A (SIS) (1) containing succinate as the carbon source and supplemented as required with the following antibiotics: tetracycline, 1 μg/ml; kanamycin, 25 μg/ml; trimethoprim, 50 μg/ml; streptomycin and spectinomycin, 50 μg/ml each. Chemoheterotrophic cultures were grown aerobically on a rotary shaker or sparged with 30% O_2–69% N_2–1% CO_2. Photosynthetic cultures were grown at high incident light intensity of 50 W/m^2 and sparged with 98% N_2–2% CO_2. Strains grown anaerobically were cultured in SIS supplemented with 0.1% yeast extract in the presence of dimethyl sulfoxide (DMSO).

DNA manipulations and analysis. Standard protocols or manufacturer's instructions were followed to isolate plasmid DNA, as well as for restriction endonuclease, DNA ligase, and other enzymatic treatments of plasmids and DNA fragments. Enzymes were purchased from New England Biolabs, Inc. (Beverly, Mass.), Promega Corp. (Madison, Wis.), United States Biochemical Corp. (Cleveland, Ohio), Boehringer Mannheim Biochemicals (Indianapolis, Ind.), and Bethesda Research Laboratories Life Technologies Inc. (Gaithersburg, Md.).

Conjugation techniques. Plasmids were mobilized in biparental matings from *E. coli* S17-1 strains into *R. sphaeroides* strains as described elsewhere (2).

Construction of *R. sphaeroides* mutants. The *prrA* and *prrB* genes were mutated individually in the RDXB1 and CCOP1 backgrounds. Construction of the RDXB1 and CCOP1 mutations is described elsewhere (24). Plasmids used in the construction of the PRRA2 and PRRB1 strains (5) were again employed in the disruption of the *prrA* and *prrB* genes in CCOP1 and RDXB1. CCOP1 strains contain an ΩTp insertion into the *Pst*I site 584 bp from the start of the structural gene, and RDXB1 strains have an ΩTp cassette in the *Eco*RI site 240 bp from the 5′ end of the structural gene. The PRRA2 and PRRB1 strains contain internal deletions in the respective genes. Thus, PRRA2 contains a 500-bp *Bst*BI-*Pst*I internal deletion in the *prrA* gene, and PRRB1 contains a 1,037-bp *Rsr*II-*Nru*I internal deletion in the *prrB* gene. The genomic structures of the mutants were confirmed by Southern hybridizations, which were performed as previously described (3).

Southern hybridization analysis. Hybridizations were performed according to the protocol described for Quickhyb rapid hybridization solution (Stratagene). DNA probes were oligolabeled with [α-^{32}P]dCTP, using a random primer (New England Biolabs) and the Sequenase enzyme (United States Biochemical) as described elsewhere (28).

Spectral analysis of membrane fractions. Crude cell-free lysates were prepared by sonication for 1 min in ICM buffer (10 mM KPO_4, 1 mM EDTA; pH 7.2) with a Sonifier cell disrupter (Branson Sonic Power Co., Danbury, Conn.) followed by two rounds of centrifugation in a bench top microcentrifuge (CENTRA MP4R; International Equipment Co.) at 13,000 rpm to remove cell debris. Spectra were recorded with a UV 1601PC spectrophotometer (Shimadzu Corp.). The amount of bacteriochorophyll (Bchl) present in the B800-850 and B875 light-harvesting complexes was determined by methods that have been described elsewhere (17, 20). All analyses were performed in duplicate at least twice, and the data presented are the averages of the values obtained. Duplicate values vary by approximately ±15%.

Carotenoid and Bchl analyses. Photopigments were extracted with acetone-methanol (7:2, vol/vol) from cell pellets and quantitated as described previously (1). The acetone-methanol-extracted pigments were then concentrated for high-performance liquid chromatography analysis on a Shimadzu system equipped with an SPD-M10AV diode array detector as described by Yeliseev et al. (32).

TABLE 1. Strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source or reference
R. sphaeroides		
2.4.1	Wild type	W. Sistrom
RDXB1	*rdxB*::ΩTp^r	24
CCOP1	*ccoP*::ΩTp^r	24
PRRA2	Δ*prrA*::ΩSp^r St^r PS$^-$ RC$^-$ B875$^-$ B800-850$^-$ Crt$^-$	5
PRRB1	Δ*prrB*::ΩSp^r St^r PS$^-$	5
CCOP1PRRA2	*ccoP*::ΩTp^r Δ*prrA*::ΩSp^r St^r	This study
CCOP1PRRB1	*ccoP*::ΩTp^r Δ*prrB*::ΩSp^r St^r	This study
RDXB1PRRA2	*rdxB*::ΩTp^r Δ*prrA*::ΩSp^r St^r	This study
RDXB1PRRB1	*rdxB*::ΩTp^r Δ*prrB*::ΩSp^r St^r	This study
E. coli		
DH5α*phe*	(ϕ80d*lacZ*ΔM15) Δ*lacU169 recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 phe*::Tn*10d*Cm	4
S17-1	Pro$^-$ Res$^-$ Mod$^+$ *recA*; integrated plasmid RP4-Tc::Mu-Km::Tn*7*	29
Plasmids		
pRK415	Mob$^+$ *lacZ*α Tc^r IncP	16
pBS	Ap^r	Stratagene
pHP45Ω	Source of ΩSp^r St^r cassette	26
pUI1680	Derivative of pBS; source of ΩTp^r cassette	J. Eraso
pSUP203	pBR325 Mob$^+$ Ap^r Cm^r Tc^r ColE1	29
pJE1024	pUI1643 derivative containing internal 500-bp *Bst*BI-*Pst*I deletion in *prrA* gene; used in construction of PRRA2 strain	5
pCF200Km	Sp^r St^r Km^r IncQ *puc*::*lacZYA′*	18
pUI1663	Sp^r St^r Km^r IncQ *puf*::*lacZYA′*	6
pUI1621	0.8-kb fragment from cosmid 533 containing the *prrA* gene in pRK415 derivative with ΩSp^r St^r cassette (*Hin*dIII)	4
pUI1649	1.9-kb *Sma*I-*Cla*I fragment containing *prrB* in pRK415	5
pUI1643	4-kb *Bam*HI-*Hin*dIII fragment containing *prrBCA* in pBS	5
pUI1653	pUI1643 derivative containing internal 1,037-bp *Nru*I-*Rsr*II deletion in *prrB* gene; used in construction of PRRB1 strain	5

All values are the results of duplicate assays involving duplicate experiments and vary by approximately ±10% from the mean.

β-Galactosidase assays. *R. sphaeroides* cultures used for the determination of β-galactosidase activity were grown chemoheterotrophically, with sparging with 30% O_2–69% N_2–1% CO_2, to an optical density at 660 nm (OD_{660}) of ~0.15. The assays were performed as described elsewhere (30). Reagent-grade *o*-nitrophenyl-β-D-galactopyranoside, purchased from Sigma Chemical Co., was used as the substrate. The data provided are the averages of at least two separate experiments, each performed in duplicate. Values vary by approximately ±20% from the mean.

Protein determination. Protein concentration in crude cell extracts used for spectral analysis or β-galactosidase activity was measured by the bicinchoninic acid protein assay (Pierce, Rockford, Ill.).

RESULTS

Construction of *prr* mutations in RDXB1 and CCOP1. Mutations in the *prrA* and *prrB* genes and in the *ccoP* and *rdxB*

genes have previously been constructed and analyzed in this laboratory (4, 5, 24). For this study individual deletions of the *prrA* and *prrB* genes were constructed in *R. sphaeroides* RDXB1 and CCOP1 mutant backgrounds as described in Materials and Methods. Disruption of any *prr* gene in the RDXB1 or CCOP1 strains was accompanied by a dramatic reduction in the deep red colony pigmentation of the parental strains, as observed on SIS agar plates in the presence of oxygen. This is similar to earlier observations on the effect of *prr* mutations in an otherwise wild-type strain except that the colony pigmentation of wild-type cells in the presence of oxygen is light red (4, 5), as opposed to the deep red colony pigmentation in the RDXB1 and CCOP1 mutant strains. Thus, colonies with increasingly less pigmentation were generated following deletion of the *prrB* and *prrA* genes, in that order, irrespective of whether these mutations were constructed in the wild-type, RDXB1, or CCOP1 strains. In either case, the final colony pigmentation was strictly dependent upon the status of the appropriate *prr* gene. This was the first indication that mutations in the *prr* genes have a dominant effect, relative to alteration of either the *cco* or *rdxBH* genes, on the regulation of photosystem formation under aerobic conditions. We have previously proposed that the *rdxBHIS* and *ccoNOQP* gene products encode independent members of the same redox signaling pathway (24). Our observations here, that similar phenotypes are associated with either CCOP1PRR or RDXB1PRR double mutants, are consistent with this proposal. In order to be succinct, the remainder of this report will be limited to a description of data obtained from the analysis of CCOP1PRR double mutants. However, although not presented, analysis of key RDXB1PRR double mutants was also performed and confirmed that these mutants behaved identically to the CCOP1PRR double mutants.

Oxygen regulation of photosynthetic membrane formation in CCOP1PRR mutant strains. Under aerobic conditions the loss of intense coloration in colonies of CCOP1PRR double mutants compared with the deep red coloration of the CCOP1 mutant suggested that the oxygen-insensitive PS gene expression characteristic of the CCOP1 mutant was abolished in strains lacking the Prr two-component system. To investigate this further, membrane fractions from cultures of CCOP1PRRA2 and CCOP1PRRB1, grown in the presence of 30% oxygen to low cell densities to ensure fully oxygenated cultures, were prepared and spectral analysis was performed (Fig. 1A). For comparison, membrane fractions were also prepared from the PRRA2 and PRRB1 mutant strains grown under the same conditions. In the wild-type, PRRA2, and PRRB1 strains, only background levels of the B800-850 and B875 spectral complexes were produced under aerobic conditions. This contrasts with the CCOP1 mutant, which synthesizes substantial levels of both spectral complexes, especially the B875 complex, under these same conditions. Consistent with the observation that CCOP1PRR double-mutant strains have diminished pigmentation, levels of B800-850 and B875 in the double-mutant strains were also dramatically reduced when compared to those in the CCOP1 strain. This was particularly obvious for the CCOP1PRRA2 and CCOP1PRRB1 strains, where B800-850 and B875 levels were reduced to near background levels. These data provided the first direct evidence that oxygen-insensitive production of photosynthetic complexes in the CCOP1 mutant under aerobic growth is dependent upon an intact Prr two-component activation system.

Photosynthetic membrane formation in CCOP1PRR mutants grown anaerobically in the dark. Having examined photosynthetic membrane formation under aerobic conditions, when the synthesis of such membranes is normally repressed, we next examined anaerobic growth conditions, when spectral complexes are normally produced by the wild type. Strains were grown anaerobically in the dark using DMSO as an external electron acceptor. These conditions were chosen because they are gratuitous for photosynthetic membrane formation and mutants lacking the *prrB* or *prrA* gene grow very poorly or not at all under photosynthetic conditions (4, 5).

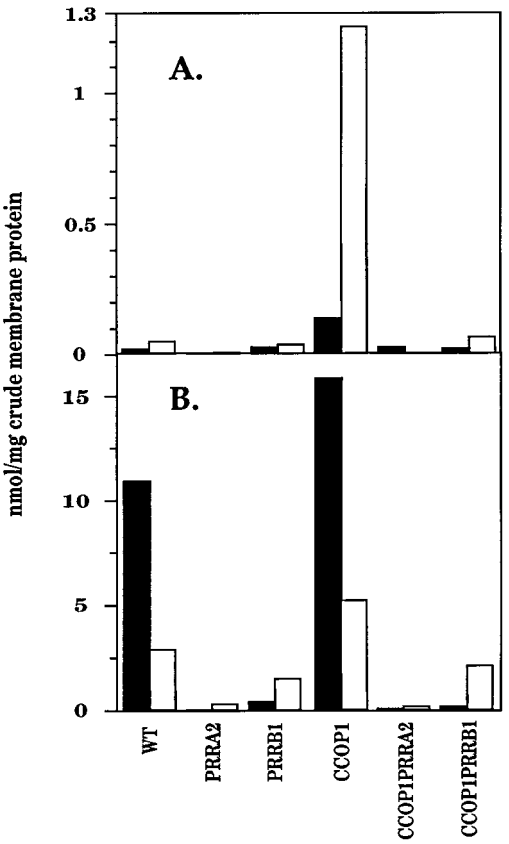

FIG. 1. Spectral complex formation under aerobic (A) and anaerobic (B) conditions of growth. Cells were grown as described in Materials and Methods and harvested at OD_{600} values of approximately 0.2 and 0.5, respectively. The amounts of light-harvesting complexes were expressed as nanomoles of spectral complex per milligram of crude membrane protein. Experiments were performed in duplicate, and standard deviations were ≤15% (Table 2). Filled bars represent LHII (B800-850 light-harvesting complex); open bars represent LHI (B875 light-harvesting complex).

The data obtained were qualitatively consistent with the aerobic data (Fig. 1B, note change in scale), although as expected the levels of spectral complexes under dark DMSO growth were substantially increased over those in cells grown in oxygen. Levels of the B800-850 and B875 spectral complexes were significantly elevated (individual determinations of spectral complex levels do not vary by more than ±15%) in the CCOP1 strain even when compared to the wild type (1.45- and 1.80-fold, respectively), despite the absence of oxygen. Disruption of the *prrA* or *prrB* gene in either the wild-type or CCOP1 backgrounds led to substantial decreases in the levels of spectral complexes. As observed for these strains when grown aerobically, the effect was most pronounced in PrrA mutants and to a lesser extent in the PrrB mutant. These data also indicate that activation of PS gene expression under anaerobic conditions by the Prr system is a prerequisite, whether in the wild type or in the CCOP1 mutant, for spectral complex formation when the PpsR repressor system is intact (11). The fact that the

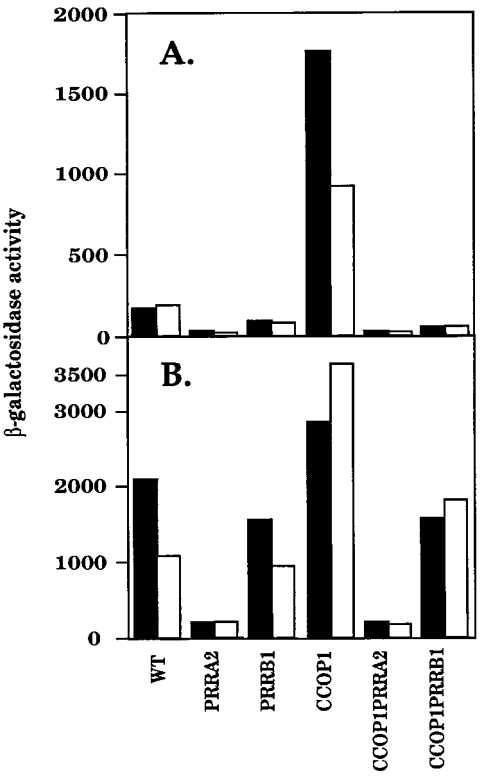

FIG. 2. β-Galactosidase values under aerobic (A) and anaerobic (B) conditions of growth. Strains contain the *puf*::*lacZ* and *puc*::*lacZ* transcriptional fusions in pUI1663 and pCF200Km, respectively, in *trans*. Experiments were performed in duplicate, and standard deviations were ≤20%. Filled bars represent the *puc*::*lacZ* fusion; open bars represent the *puf*::*lacZ* fusion. β-Galactosidase values are expressed in micromoles per minute per milligram of protein.

B800-850 spectral complex is in excess of the B875 complex under anaerobic dark DMSO conditions when compared to the relative proportion of each complex produced aerobically is due to two factors, the hierarchy of spectral complex formation when Bchl is limiting (12, 23) and the relative increased abundance of SO and its effect on B800-850 formation (32).

Analysis of PS gene expression in CCOP1PRR mutants. The absence or reduction in the levels of photosynthetic complexes in the CCOP1PRR mutant strains grown under both aerobic and anaerobic growth conditions when compared to those in the parental CCOP1 strain suggested that at the least, transcription of genes encoding the B800-850 and B875 structural polypeptides (encoded by the *puc* and *puf* operons, respectively) were likely to be affected. Determined with *lacZ* transcriptional fusions, the data for β-galactosidase levels derived from aerobically grown cells for the *puc* and *puf* operons are presented in Fig. 2A and the anaerobic β-galactosidase activities are presented in Fig. 2B. Disruption of the *prrA* gene in either the wild type or CCOP1 strains resulted in below-background levels of *puc* and *puf* operon activity. Mutation of *prrB* was similar in its effect on *puc* and *puf* operon expression as was *prrA* under aerobic conditions, but the mutation of *prrB* was far less effective under anaerobic conditions, which is in keeping with our previous characterization of these genes and their products (5). Thus, the levels of the B800-850 and B875 spectral complexes roughly correlate with *puc* and *puf* operon transcriptional activity, respectively, under either aerobic or anaerobic conditions in the presence of the *cco* mutation, bearing in mind that critical steps in Bchl synthesis are likely subject to other control elements (9, 37). These data provided further evidence for the role of the Prr two-component activation system in the regulation of PS gene expression in response to alterations in the cbb_3 cytochrome oxidase. The relatively high levels of *puc* and *puf* operon expression under anaerobic conditions in the presence of the *prrB* mutation is most likely a reflection of PrrA activation by other phosphoryl donors (6, 10). Finally, it should be noted that the level of both *puc* and *puf* operon expression in the cbb_3 mutant background is significantly higher (~1.36- and 3.40-fold, respectively [±20%]) than that in the wild type even under anaerobic conditions, although the increase is less than that observed in aerobically grown cells (~8.5- and 4.5-fold respectively [±20%]).

Complementation of CCOP1PRR mutants. Plasmids bearing the individual *prrA* and *prrB* genes were introduced in *trans* to complement the CCOP1PRRA2 and CCOP1PRRB1 mutants, respectively. Complementation, which was defined as the restoration of B800-850 and B875 to levels similar to those of either the wild type or CCOP1 mutant strain containing the same complementing plasmid, was performed under both aerobic and anaerobic growth conditions (Table 2). We reasoned that if the *prr* genes in *trans* could restore production of spectral complexes to the various mutant strains, this would further

TABLE 2. Spectral complex levels of *R. sphaeroides* complemented with *prr* genes

Strain	Spectral complex level in strain complemented with[a]:											
	pRK415[b]				*prrA*[c]				*prrB*[c]			
	$+O_2$		$-O_2$		$+O_2$		$-O_2$		$+O_2$		$-O_2$	
	LHII	LHI	LHII	LHI	LHII	LHI	LHII	LHI	LHII	LHI	LHII	LHI
2.4.1	0.02	0.05	10.9	2.9	0.16	1.46	15.2	4.4	0.12	0.44	3.6	1.6
PRRA2	<0.01	0.0	0.04	0.3	0.11	1.08	5.7	1.9	—	—	—	—
PRRB1	0.03	0.04	0.4	1.5	—[d]	—	—	—	0.05	0.38	13.7	3.9
CCOP1	0.13	1.25	15.8	5.2	0.22	1.32	20.0	4.9	0.21	1.52	12.8	4.0
CCOP1PRRA2	0.02	0.0	0.06	0.16	0.17	1.13	10.2	2.9	—	—	—	—
CCOP1PRRB1	0.02	0.06	0.15	2.1	—	—	—	—	0.18	1.28	6.4	2.6

[a] Cells were grown chemoheterotrophically ($+O_2$) and sparged with a mixture of 69% N_2, 30% O_2, and 1% CO_2 and anaerobically in the dark with DMSO as the electron acceptor ($-O_2$). The amounts of light-harvesting complexes were determined as described previously (17) and expressed as nanomoles of spectral complex per milligram of crude membrane protein. Experiments were performed in duplicate, and standard deviations were ≤15%. LHI and LHII are the light-harvesting complexes B875 and B800-850, respectively.

[b] pRK415 is the vector containing the complementing *prr* genes.

[c] Complementing *prrA* and *prrB* genes.

[d] —, not determined.

TABLE 3. Spectral complex and Crt composition of *R. sphaeroides* 2.4.1 grown under high-light photosynthetic conditions

Strain[a]	Amt (nmol/mg of protein)[b]			SO:SE
	B800-850	B875	Crt	
2.4.1	2.97 ± 0.26	4.19 ± 0.78	81.8 ± 10.6	1.16
CCOP1	2.35 ± 0.26	3.25 ± 0.29	66.0 ± 8.0	35.70
PRRB1	0.19 ± 0.02	1.88 ± 0.24	43.0 ± 1.8	0.84
CCOP1PRRB1	0.24 ± 0.07	1.91 ± 0.33	33.0 ± 2.8	2.56

[a] Strains were grown at high light intensity (60 W/m^2), with sparging with 98% N_2–2% CO_2, to an OD_{600} of 0.1 to 0.2.

[b] The levels of B800-850 and B875 spectral complexes were determined as described previously (17). Values are the averages of at least two independent determinations, and standard deviations from the mean are provided.

reveal the obligatory nature of the Prr two-component activation system in the cbb_3 sensory transduction pathway. Introduction of these plasmids into the wild-type strain led to the production of spectral complexes, albeit at low levels, under aerobic conditions, with *prrA* in *trans* having the greatest effect followed by *prrB* (Table 2). Similarly, introduction of these plasmids into CCOP1 also resulted in spectral complex levels under aerobic conditions similar to those obtained with the *cco* mutation alone. This is consistent with our previous analysis (24) of the CCOP1 and RDXB1 mutants grown aerobically, as well as of the wild type, which indicated that it is the limitation of Bchl levels (12, 23, 32) which ultimately governs the formation of spectral complexes in these strains, even though *puc* and *puf* operon expression is at or near maximal levels. Under anaerobic conditions the introduction of the *prrA* gene also led to enhanced production of spectral complexes in both the wild-type and CCOP1 strains (Table 2). In each case *prrA* and *prrB* could restore spectral complex production to the double-mutant strains.

Crt composition in the CCOP1PRR double mutants. Under anoxygenic photosynthetic (medium light) and diazotrophic growth conditions, CCOP1 mutants accumulate high levels of the oxidized carotenoid SO, which in the wild type accumulates primarily under aerobic and semiaerobic growth conditions (24). Further, the effect of the *ccoP* mutation on SO accumulation appears to derive in part from the altered posttranscriptional regulation of carotenoid biosynthesis (24). In order to investigate the effect of *prr* mutations on SO accumulation in the CCOP1 background, we chose to examine photopigments extracted from the CCOP1PRRB1 mutant grown under high-light photosynthetic growth conditions. Recall that *prrB* mutations produce sufficient levels of spectral complexes to allow such mutants to grow photosynthetically under high light (6). Mutations in the *prrA* gene render strains photosynthetically incompetent irrespective of light intensity. The high-light photosynthetic cultures were sparged with a gas mixture of 98% N_2–2% CO_2 to ensure the complete absence of oxygen. Measurement of the relative amounts of SE and SO in the photopigments was determined as described previously (32).

The data (Table 3) revealed that the carotenoid composition of the CCOP1 mutant is almost exclusively SO relative to SE. This is consistent with the previous analysis of this strain grown diazotrophically and photosynthetically (medium light intensity) (24). The almost exclusive accumulation of SO in the CCOP1 mutant compares with the accumulation of approximately equal levels of SE and SO in the wild-type strain grown under similar conditions. As in the wild type, the levels of SE and SO in the PRRB1 single mutant were also approximately equal, suggesting that mutation in the *prrB* gene alone does not significantly affect the relative levels of SO accumulation. In the CCOP1PRRB1 mutant, the Crt composition was only slightly altered when compared to that in the wild-type strain. Although SO remained the predominant Crt in the double mutant, very significant levels of SE were also produced. Therefore, we must assume that PrrB plays some role in *crtA* gene expression. Although the total levels of Crt accumulated in the PrrB mutant strains are lower than those in either the wild type or cbb_3 mutant, which are similar, the distribution of SE and SO are fully consistent with the Prr system affecting the expression of the *crtA* gene and functioning through the cbb_3-RdxBH redox carriers.

DISCUSSION

PS gene expression in *R. sphaeroides* 2.4.1 is dependent upon oxygen levels and light intensity. Expression of PS genes is minimal under high-oxygen conditions (~30% O_2) but increases at low oxygen levels (~2% O_2), and it reaches full expression under anaerobic conditions under low light intensity (17, 36). Not only is the concentration of oxygen in the surrounding environment the key regulatory parameter governing the expression of PS genes in *R. sphaeroides* 2.4.1, it additionally dictates the form and abundance of the terminal oxidase used, depending on the particular oxygen concentration. When *R. sphaeroides* grows under highly oxygenated conditions, the (low-oxygen-affinity) cytochrome aa_3 oxidase is the predominantly used cytochrome *c* oxidase (13), although under these same conditions, but to a much lesser extent, *R. sphaeroides* can also use the cbb_3 oxidase (8). The cbb_3 (high-oxygen-affinity) oxidase activity, nevertheless, is dominant when cells grow under microaerobic conditions (≤2% O_2), during which its expression is maximal. The cellular expression of the *ccoNOQP* operon, studied using *lacZ* transcriptional fusions, shows a 90% reduction in *cco* expression under high oxygen (30%) relative to that under low oxygen (2%), and this enhanced expression is FnrL dependent (22). In this respect, the *ccoNOQP* operon, which encodes the cbb_3 oxidase, resembles the *cyd* operon in *E. coli*, which encodes the (high-oxygen-affinity) cytochrome *d* oxidase and whose expression is also maximal under microaerobic conditions (31). Thus, like *E. coli*, *R. sphaeroides* varies the synthesis of its respiratory enzymes in response to oxygen availability. In addition to two cytochrome *c* oxidases, genes for two quinol oxidases have recently been identified in *R. sphaeroides* (21). They are predicted to be responsible for allowing an *R. sphaeroides* cytochrome *bc1* complex mutant to grow under aerobic conditions (35), since *bc1* is believed to be the obligatory electron donor to both the aa_3 and cbb_3 oxidases in *R. sphaeroides* under aerobiosis.

We have reported previously that mutation of the *ccoNOQP* operon leads to high-level PS gene expression, as well as spectral complex formation, under highly aerobic growth conditions (24), when the cells are primarily dependent upon the cytochrome aa_3 terminal oxidase for aerobic growth (13). Because such cells are still subject to light-dependent regulation of PS gene expression when grown anaerobically, it was reasonable to conclude that the PpsR repressor-AppA antirepressor system was functioning normally (11). This was also borne out by the fact that the PpsR target gene, *bchF*, was not affected in the *cco* background (24). On the other hand, previous studies in our laboratory (4, 5) revealed that changes in the histidine kinase-phosphatase PrrB, or extra copies of the response regulator PrrA, which together positively regulate PS gene expression, could lead to PS gene expression and spectral complex formation under highly aerobic conditions. Thus, it was reasonable to conclude that there was likely to exist a

relationship between the redox-responsive cbb_3-RdxBH and the Prr activation system.

The data presented here clearly show that under aerobic growth conditions, both PS gene expression and spectral complex formation in the cbb_3 mutant background are under the control of the Prr regulatory system. This two-component system is normally unable to stimulate PS gene expression in the presence of a functional cbb_3-RdxBH, and therefore, Prr activation lies downstream of, but in the same pathway as, the cbb_3-RdxBH redox carriers. At this point we cannot conclude that there exists a direct physical interaction between cbb_3-RdxBH and the membrane-localized PrrB, although a functional interaction seems clear. Therefore, we propose that cbb_3 is the primary element, or sensor of oxygen, in this regulatory pathway through its interaction with oxygen and that whatever "signal" is generated can be transmitted, we presume, to RdxBH. Alteration of RdxBH also results in aerobic expression of PS genes and spectral complex formation without affecting the activity of cbb_3 (24). We presume RdxBH to lie downstream of cbb_3 but upstream of Prr since the results observed here are similar regardless of whether cbb_3 or RdxBH mutants are studied. The signal coming through the cbb_3-RdxBH sensor-signal generator will either inhibit the kinase activity of PrrB or increase its phosphatase activity or both, resulting in the lack of activation of PrrA under aerobic conditions. The complementation results unmistakenly place the Prr system as an obligatory intermediate in the cbb_3-RdxBH redox sensing-signaling pathway.

We further suggest that as oxygen becomes limiting, increased expression of *ccoNOQP*, requiring FnrL (22) and, we presume, *rdxBH*, leads to increased levels of the cognate redox carriers. Increased levels of these redox carriers, as oxygen concentrations diminish, will lead to both continued respiratory growth and a gradual diminution, we imagine, in the "strength" of the signal which negatively affects PrrB. Decreasing oxygen levels would normally result in a loss of that signal, but increased cbb_3, etc., moderate that loss so that PS gene activation is gradual and not abrupt.

In the absence of cbb_3, expression of the *puf* and *puc* operons under aerobic conditions is near maximal levels (compare Fig. 2A and B), while the levels of spectral complexes under aerobic conditions do not reflect the levels of *puf* and *puc* operon expression (compare Fig. 1A and B). This paradox is resolved if we consider that Bchl levels continue to remain limiting in the cbb_3 mutant under aerobic as opposed to anaerobic conditions. This limitation could reflect either the activity of the PpsR repressor (9) or the low-level expression of specific genes which may require FnrL for their activation and which we believe are involved in tetrapyrrole and Bchl biosynthesis (37) or both.

These data also reveal that in the absence of an intact cbb_3-RdxBH, the Prr pathway is effective, even under anaerobic conditions, in leading to increased *puc* and *puf* operon expression relative to that in the wild type (Fig. 2) and that this enhanced expression of *puc* and *puf* is through PrrA. As we have observed previously (24), and now again (also see below), cbb_3-RdxBH must possess some form of activity (presumably as redox carriers) even in the absence of oxygen. That is, there must be electron flow through cbb_3 under anaerobic conditions, but the ultimate acceptor remains unknown. This is in line with the observation that *ccoNOQP* is expressed in photosynthetic growth to a level about twofold greater than that expressed in the presence of high oxygen (22). What is equally striking about these results is that the PpsR repressor-AppA antirepressor system (11) does not appear to play a major role in *puc* and *puf* operon expression when the signal that presumably keeps PrrB "inactive" is removed.

These data further support our earlier findings that PrrA can be activated through the intervention of heterologous signal transduction pathways (6), particularly under anaerobic conditions (compare Fig. 2A and B). This observation most likely relates to the role of PrrA in those activities which are derivative of the photosynthetic lifestyle, e.g., the uptake hydrogenase (10), CO_2 fixation, N_2 fixation (15), and presumably others. By inference, these results suggest that the cbb_3-RdxBH oxygen sensor-signal generator also plays a role in the expression of these "dependent" metabolic pathways. However, these pathways, e.g., N_2 fixation, may possess additional regulatory elements, as does PS gene expression, such as PpsR or FnrL.

We should return to the question of how the cbb_3-RdxBH sensor-signal generator pair communicates with PrrB, which we presume to be the responsive element in this activation pathway. One possibility is through some membrane-localized redox flux. In this sense, PrrB resembles more the sensor histidine kinase-phosphatase ArcB in *E. coli*, which has been proposed to sense oxygen by the level of either an electron transport component in reduced form or another compound linked to the process by a redox reaction (14). Thus, photosynthesis and respiration might emerge as being coordinately regulated, and might share common components, as has been previously suggested (27). Another possibility is through the PrrC protein; the presence of extra copies of the *prrC* gene actually results in modest inhibition of PS gene expression (5). A third possibility is through some element(s) of the cbb_3 terminal oxidase, e.g., the *Q* gene product, whose absence has no apparent effect on cbb_3 oxidase activity (25), or through some other, as yet unidentified activity or component. Interestingly, the *Rhodobacter capsulatus* equivalent of PrrC, namely SenC, when inactivated, has an effect upon cbb_3 activity, which is not true in *R. sphaeroides* (7).

In this report (Table 3) and elsewhere (33), the data suggest that *crtA* gene expression is regulated at the transcriptional level through the cbb_3-RdxBH–Prr pathway. However, earlier results (24) also revealed that *crtA* activity was also likely to be under posttranscriptional control. It was shown that in the cbb_3 mutant strain the level of SE decreased by a factor of 3, but a *crtA*::*lacZ* fusion showed only an ~55% increase in *lacZ* expression in the cbb_3 mutant relative to that in the wild type.

The tools appear to be at hand to address all of these questions, and it is apparent that PS gene regulation is inextricably linked to numerous dependent pathways which together constitute the photosynthesis lifestyle.

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