From Redox Flow to Gene Regulation: Role of the PrrC Protein of *Rhodobacter sphaeroides* 2.4.1[†]

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ABSTRACT: Activation of photosynthesis (PS) gene expression by the PrrBA two-component activation system in *Rhodobacter sphaeroides* 2.4.1 results from the interruption of an inhibitory signal originating from the cbb_3 cytochrome *c* oxidase via its interaction with oxygen, in conjunction with the Rdx redox proteins. The CcoQ protein, encoded by the *ccoNOQP* operon, which encodes the cbb_3 cytochrome *c* oxidase, was shown to act as a "transponder" that conveys the signal derived from reductant flow through cbb_3 to oxygen, to the Prr system. To further define the elements comprising this signal transduction pathway we considered the *prrC* gene product, which to date possessed no definable role in this signal transduction pathway despite its being part of the *prrBCA* gene cluster. Similar to mutations in cbb_3 and *rdx*, suitably constructed *prrC* deletion mutations lead to PS gene expression in the presence of high oxygen. Unlike mutations that remove cbb_3 terminal oxidase activity or Rdx function, the PrrC deletion mutant shows no effect upon cbb_3 activity, nor does it affect the ratio of the carotenoid (Crt) spheroidene (SE) to spheroidenone (SO). Thus, the PrrC deletion mutant behaves identically to the CcoQ deletion mutant. Taking these and previous results together, we suggest that PrrC is located upstream of the two-component PrrBA activation system in the signal transduction pathway but downstream of the cbb_3 cytochrome *c* oxidase and its "transponder" CcoQ. The PrrC deletion mutant was also shown to lead to an increase in the DorA protein under aerobic conditions as was shown earlier for the cbb_3 mutant. Finally, PrrC is a member of a highly conserved family of proteins found in both prokaryotes and eukaryotes, and this appears to be the first instance in which a direct regulatory role has been ascribed to a member of this protein family.

Rhodobacter sphaeroides 2.4.1 is a purple non-sulfur facultative photoheterotroph that displays a remarkable ability to live under a variety of environmental conditions. It can grow aerobically and anaerobically by respiration, fermentatively, and it can also grow photoautotrophically and photoheterotrophically under anaerobic conditions in the light. While growing aerobically *R. sphaeroides* possesses two cytochrome *c* oxidases (*1*−*3*), the abundant aa_3 oxidase, which has a low affinity for oxygen, and the less abundant cbb_3 oxidase, which has a high affinity for oxygen and is presumed to function primarily under semiaerobic conditions (*4*). In addition, *R. sphaeroides* has also been shown to possess the genes encoding at least two quinol oxidases (Mouncey, Gak, Choudhary, and Kaplan, manuscript in preparation).

When oxygen tensions fall below ∼3% (semiaerobic conditions), the photosynthetic apparatus or intracytoplasmic membrane (ICM) is induced and arises as invaginations from the cytoplasmic membrane (*6*). The ICM contains a reaction center (RC) and two light-harvesting (LH) pigment−protein complexes, the B800−850 and the B875. Thus oxygen is the primary regulator of ICM presence. In addition, the intensity of light incident to the culture is also a regulator of photosystem formation when cells are growing photosynthetically (*6*, *7*).

Extensive analyses in this and other laboratories have identified a number of regulatory components involved in photosystem formation, namely, (1) the PrrBA two-component activation system, (2) the AppA−PpsR antirepressor/repressor system, (3) the outer-membrane-localized TspO protein, and (4) FnrL (*7*, *8*). Null mutations in *prrA* (*9*), *fnrL* (*10*), and *appA* (*11*) render *R. sphaeroides* either unable to grow photosynthetically or otherwise severely compromised under photosynthetic conditions. Null mutations in *ppsR* (*12*) and some mutations in *prrB*, like PrrB78 (*13*), turn on PS gene expression, under aerobic conditions. In addition, and most importantly, mutations in the *ccoNOQP* operon, encoding the cbb_3 cytochrome *c* oxidase (*14*), and the downstream *rdxBHIS* operon (*14*) (Roh and Kaplan, manuscript in preparation) or in genes encoding constituent elements of the electron transport chain (Oh and Kaplan, manuscript in preparation) lead to synthesis of the ICM under aerobic conditions. To our knowledge this is the first detailed demonstration of an electron transport chain and associated downstream elements directly regulating gene expression.

With remarkable insight at the time, Cohen-Bazire et al. (*15*) proposed that the redox state of the electron transport chain might ultimately control the response of photoheterotrophs to both oxygen and light. The first experimental

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Table 1: Bacteria and Plasmids

strain	genotype/phenotype[a]	source
E. coli DH5α*phe*	F$^-$ *ϕ*80d*lac*ZΔM15 Δ(*lacZYA*−*argF*)U169 *recA1 endA1 hsdR17* (r_K^-, m_K^+) *supE44 λ*$^-$ *thi-1 gyrA relA1 phe*::Tn10dCm	*9*
	R. sphaeroides	
2.4.1	wild type	W. R. Sistrom
CCOP1	2.4.1*ccoP*::ΩTp^r	*14*
PRRA2	Δ*prrA*::ΩSp^r St^r PS$^-$ RC$^-$ B875$^-$ B800−850$^-$ Crt$^-$	*13*
PRRC4	2.4.1*prrC* in-frame deletion from codon 31 to 129, both inclusive; PS$^+$	this study
PRRCA1	2.4.1*prrCA*Δ*Eco*0109I−*Eco*0109I^b::Ω Sm^r Sp^r; PS$^-$	*13*
	Plasmids	
p18Not	pUC18 with *Not*I sites at both ends of the MCS	*53*
pBluescriptII	Ap^r withT3 and T7 promoters	Stratagene
pRK415	Tet^r	*54*
pCF200Km	pCF200 derivative + 1.4-kb *Bam*HI Km^r DNA^b in the *Nru*I site of *tet* from pCF200; Km^r Sm^r Sp^r (*pucB*−*lacZ* transcriptional fusion)	*55*
pCF1010	pLV106 modified to contain unique *Pst*I, *Not*I, *Nsi*I, *Avr*II, *Stu*I, *Bsp*MII, and *Xba*I restriction sites between the 2.0-kb Ω Sm^r/Sp^r and the 5.1-kb *lacZYA′*, IncQ/IncP4 (transcriptional fusion vector)	*56*
pJE1263	pBSII derivative containing an approximately 5.2-kb insert harboring the Prr region; Ap^r	this study
pJE1883	pBSII/*Hinc*II−*Cla*I/∼268-bp *Nru*I−*Cla*I fragment from pUI1643; internal fragment to *prrC*; Ap^r	this study
pJE1884	pBSII/*Pst*I−*Xho*I/∼324-bp *Pst*I−*Xho*I fragment from pJE82; internal fragment to *prrA*; Ap^r	this study
pJE1891	pUI1160*Sma*I/∼0.4-kb *Nci*I from pUI1643	this study
pJE1893	pUI1156*Sma*I/∼0.4-kb *Nru*I-*Eco*0109I from pUI1643	this study
pJE2120	pBSII/*Eco*RV/∼310-bp *Eco*RV−*Aat*II^c; internal fragment to *spb*; Ap^r	this study
pJE2198	pRK415*Kpn*I/*Kpn*I fragment containing *prrC*::*phoA1*	this study
pJE2200	pRK415*Kpn*I/*Kpn*I fragment containing *prrC*::*phoA2*	this study
pJE2864	pSUP203 *Pst*I^c/∼1.8-kb *Eag*I^b fragment with *puc*(263-bp *Eag*I−*Xmn*I)::*sacB* from WS215	this study
pJE3383	pJE1263 derivative containing a *prrC* in-frame deletion from codon 31 to 129, both inclusive	this study
pJE3387	pJE2864 *Sca*IΔ/∼3651-bp^b fragment containing the *prrC* in-frame deletion	this study
pSUP203	pBR325 derivative; Mob$^+$ Ap^r Cm^r Tet^r	*57*
pUI1156	pBSII containing *phoA* gene with MCS in front in a specific reading frame	*d*
pUI1160	pUI1156 *Bam*HI^b containing the *phoA* gene with MCS in front in a different reading frame	this study
pUI1643	pBS/*Bam*HI−*Hin*dIII, 4-kb *Bam*HI−*Hin*dIII fragment cloned from chromosome I of 2.4.1; Ap^r	*13*
pUI1645	pRK415/*Ecl*136II, *Xho*I^b 1.3-kb fragment containing *prrC* from 2.4.1 transcribed opposite to the vector *tet* and *lac* promoters; Tc^r	*13*
pUI1663	pUI1830 (*pufB*−*lacZ* transcriptional fusion)/approximately 1.2-kb *Bam*HI^b fragment from pUC4K at *Nru*I in *tet* conferring Km^r; Sm^r Sp^r (*pufB*−*lacZ* transcriptional fusion)	this study
pUI1664	pCF1010 *Nru*I/approximately 1.2-kb *Bam*HI^b fragment from pUC4K at *Nru*I in *tet* conferring Km^r; Sm^r Sp^r (transcriptional fusion vector)	this study
pUI1830	*pufB*−*lacZ* transcriptional fusion; Sm^r Sp^r Tet^r	*e*
pWS215	p18Not/*puc*(0.8-kb *Pst*I−*Xmn*I)::*sacB*	W. Smith

[a] PS$^-$ indicates cells are photosynthetically incompetent; RC$^-$ indicates cells do not have reaction center pigment−protein complexes. [b] The 5′ overhangs were made blunt with Klenow fragment of DNA polymerase I before cloning. [c] The 3′ overhangs were made blunt with T_4 DNA polymerase before cloning. [d] M. Wood, J. M. Eraso, and S. Kaplan, unpublished data. [e] L. Gong and S. Kaplan, unpublished results.

demonstration of redox regulation of gene expression in *R. sphaeroides* arose from the work of Hallenbeck et al. (*16*, *17*), which showed defined redox effects on the transcriptional regulation of Calvin cycle gene expression following the addition of the electron acceptor, DMSO, to cells growing photoheterotrophically. In consideration of these and other observations, we proposed the presence of a redox signaling pathway in *R. sphaeroides* involving the cbb_3 cytochrome *c* oxidase/Rdx protein(s), which serve as the primary oxygen sensor to generate a signal inhibitory for PS gene expression. This signal is ultimately transmitted to the *prrBA* two-component activation system to regulate PS gene expression. CcoQ, a component of the cbb_3 oxidase, was then shown to be involved in the transduction of the oxygen-sensing inhibitory signal from the cbb_3 oxidase to a downstream regulatory element, proposed to be either PrrC or PrrB, a membrane-localized histidine kinase (*18*, *19*). In this report we present evidence implicating the involvement of PrrC in this signal transduction pathway, and on the basis of our genetic data we propose that PrrC is the link between CcoQ and the PrrBA two-component activation system. Additional evidence reveals that the redox-dependent signaling via the Cco/Rdx proteins is also, in part, involved in the regulation of anaerobic respiration in *R. sphaeroides* 2.4.1. PrrC is a member of a family of proteins that are well conserved throughout evolution. The implications pertaining to its regulatory role in redox regulation of gene expression will be discussed.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Growth Conditions. Bacterial strains and plasmids used in this work are described in Table 1. *Escherichia coli* strains were grown at 37 °C on LB medium (*20*) supplemented, when required, with the following antibiotics: tetracycline (Tet), 20 μg/mL; ampicillin (Ap),[1] 150 μg/mL; kanamycin (Km), 25 μg/mL; streptomycin (Sm) and spectinomycin (Sp), 50 μg/mL. *R.*

[1] Abbreviations: Ap, ampicillin; cbb_3, cbb_3-type cytochrome *c* oxidase; CO_2, carbon dioxide; Crt, carotenoids; CTAB, hexadecyltrimethylammonium bromide; DMSO, dimethyl sulfoxide; DMSOR, dimethyl sulfoxide reductase; ICM, intracytoplasmic membrane; Km, kanamycin; KU, Klett units; LH, light-harvesting; PCR, polymerase chain reaction; Prr, photosynthesis response regulator; PS, photosynthesis; RC, reaction center; SE, spheroidene; SIS, Sistrom's medium; SO, spheroidenone; Sm, streptomycin; Sp, spectinomycin; Tet, tetracycline; TM, transmembrane; Tp, trimethoprim; Ω, omega cassette.

sphaeroides 2.4.1 strains were grown at 30 °C on Sistrom's medium A (SIS) (*15*) containing succinate as the carbon source and supplemented as required with the following antibiotics: tetracycline, 1 μg/mL; kanamycin, 25 μg/mL; streptomycin, spectinomycin, and trimethoprim (Tp), 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 a medium incident light intensity of 10 W/m^2 and sparged with 95% N_2−5% CO_2. Strains grown anaerobically in the dark were cultured in SIS medium supplemented with 0.1% yeast extract in the presence of DMSO as electron acceptor. Potassium tellurite (K_2TeO_3) was used at 10 μg/mL to inhibit *E. coli* growth when necessary.

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, MA), Promega Corp. (Madison, WI), U.S. Biochemical Corp. (Cleveland, OH), Boehringer Mannheim Biochemicals (Indianapolis, IN), and GibcoBRL (Rockville, MD). Chromosomal DNA for sequencing was extracted by the CTAB (hexadecyltrimethylammonium bromide)/NaCl procedure (*21*).

Computer Programs. Sequence analyses were performed with the computer programs DNA Strider (Institute de Recherche Fondamentale, Commissariat a l'Energie Atomique, France) and Oligo 4.0 (National Biosciences Inc., Plymouth, MN). Blast searches and secondary structure predictions were performed with the Expasy molecular biology server (http//www.expasy.ch). ClustalW (http//www.cris.com/~ketchup/genedoc/shtml) and Genedoc (http//www.clustalW.genome.ad.jp) were used for alignments. Image Tool from the University of Texas Health Science Center in San Antonio (UTHCCSA) was used to quantitate protein amounts in Western blots.

Conjugation Techniques. Plasmids were mobilized by triparental matings from *E. coli* DH5αPhe strains into *R. sphaeroides* strains as described elsewhere (*22*).

Construction of the in-Frame Deletion Mutation in prrC. The protocol for the construction of in-frame deletion mutations has been described previously (*23*, *24*). With pUI1643 as the template, two primary PCR reactions were performed with primers PrrC DEL1 FWD (5′-GTCAGCGCGATCGTGTCGGGTTCGGATCTGCCGAGGAAGAC-3′) and PrrC DEL2 REV (5′-GGTCAGGAACAGCAGGAACGACAGCTGCG-3′), and with PrrC DEL1 REV (5′-GTCTTCCTCGGCAGATCCGAACCCGACACGATCGCGCTGAC-3′) and PrrC DEL2 FWD (5′-GGTGCGACTGGTTCAGCGCGGGCTGCG-3′), to generate two 40-bp overlapping DNA fragments (455 and 821 bp in length, respectively), both bearing the same 297-bp in-frame deletion within *prrC* in the region of overlap. Thus, 99 codons were deleted from *prrC*, extending from codon 31 through codon 129, leaving a presumed cryptic *prrC* gene comprising 132 codons. The 41-mer PrrC DEL1 FWD and PrrC DEL1 REV are complementary to each other. The two overlapping primary PCR products were used as templates for the secondary PCR, using the two outside primers, PrrC DEL2 FWD and PrrC DEL2 REV, and recombination within the overlapping sequence allowed for the amplification of a product 1351 bp in length, which is the secondary PCR product and which contains the in-frame deletion within *prrC*, as well as an intact *prrA* gene. This DNA fragment was purified with the QIAquick gel extraction kit (Qiagen Inc., Santa Clarita, CA) and restricted with the restriction enzymes *Pst*I and *Pvu*II to yield a fragment of 1283 bp, which was cloned into pJE1263 previously digested with *Pst*I and *Pvu*II. This plasmid was called pJE3383, and it is 4935 bp in length.

To cross the in-frame deletion into chromosome I at the *prrC* locus, pJE3383 was digested with *Xba*I and *Hin*dIII, to release a 3651-bp fragment containing the *prrC* deletion mutation, an intact *prrA* gene, and flanking sequences on both sides. This fragment was cloned into pJE2864, a newly created suicide vector for *R. sphaeroides*. pJE2864 is a pSUP203 derivative with an ~1.8-kb *Eag*I fragment from WS215 containing a *puc*::*sacB* transcriptional fusion, in which the *Xmn*I site at the proximal end of the *pucB* gene was fused to *sacB*, encoding the levansucrase of *Bacillus subtilis*. This cloning generated a DNA fragment containing the downstream regulatory region (DRS) of *puc* (*25*) starting at the *Eag*I site, fused to *sacB*. This fusion contains only 18 codons of the *pucB* gene and the short *puc* promoter, thus offering a very small target for recombination with the chromosome at the *puc* locus. The expression of *sacB* in the presence of sucrose is lethal for *R. sphaeroides* (*26*).

The resulting plasmid, pJE3387, was conjugated into PRRA2 cells, and cells undergoing single crossover events were selected by plating exconjugants on Tet, using K_2TeO_3 to select against *E. coli* growth. *prrA* is located immediately downstream of *prrC*. Whereas PRRA2 cells are incapable of growth under photosynthetic conditions (*9*), the Tet^r exconjugants were photosynthetically competent, due to the presence of a wild-type *prrA* gene in pJE3387. The streak-purified Tet^r exconjugants were then challenged with sucrose as described (*26*), in the absence of Tet, and the cells were grown under photosynthetic conditions. These conditions were chosen so as to allow for high expression of the anaerobically regulated short *puc* promoter, and therefore increase the levels of levansucrase in the cells. Additionally, it had been previously demonstrated that the absence of PrrC does not prevent *R. sphaeroides* cells from growing photosynthetically (*13*). Isolated genomic DNA from Tet^s, photosynthetically competent recombinants was analyzed by PCR and subsequent sequencing, to show the presence of the *prrC* in-frame deletion and the wild-type nature of *prrA*. The strain was designated PrrC4.

Construction of the prrC::phoA Translational Fusions. Two DNA fragments containing the same *prrC* promoter sequence were used. One extended to the *Nci*I site, prior to the sequence encoding the putative transmembrane domain (TM), and the other extended to the *Eco*0109I site, after the presumed TM sequence. These fragments were made blunt-ended (see Table 1) and cloned into the *Sma*I site of the PhoA fusion vectors pUI1160 and pUI1156, respectively, to create plasmids pJE1891 and pJE1893, which contain in-frame *prrC*::*phoA* translational fusions. Since these plasmids do not replicate in *R. sphaeroides*, they were restricted with *Kpn*I, and the *Kpn*I fragments containing the *prrC*::*phoA* fusions were cloned at the *Kpn*I site of pRK415 in an orientation opposite to that of the resident *tet* promoter. These

were designated pJE2198 (*prrC*::*phoA*1) and pJE2200 (*prrC*::*phoA*2), respectively.

Quantitative Determination of LH Spectral Complexes. Crude cell-free lysates were prepared by passage through a French pressure cell, followed by centrifugation. The Meinhardt equation was used to determine the levels of LH spectral complexes after spectrophotometric analysis of the samples, as described elsewhere (*6*, *27*). All analyses were performed in duplicate at least twice and the data presented are the average of the values obtained.

Carotenoid Analyses. Photopigments were extracted and analyzed as described previously (*15*, *28*).

RNA Isolation and Northern-Blot Hybridization Techniques. RNA was isolated from cells grown anaerobically in the dark and assayed as described previously (*20*, *29*, *30*).

Radioactive probes were made from highly purified plasmid DNA containing *prrC* (pJE1883), *prrA* (pJE1884), and *spb* (pJE2120) (Table 1). The relative RNA concentrations in the blots were normalized for differences in concentration during loading by use of [α-^{32}P]dCTP-labeled DNA probes encoding *R. sphaeroides* ribosomal RNA (*31*).

Enzyme Assays and Protein Determination: (A) β-Galactosidase Assays. R. sphaeroides cultures used for the determination of β-galactosidase activity were grown as described previously (*9*), and assays were performed as described elsewhere (*32*). The data provided are the averages of at least two separate experiments each performed in duplicate.

(B) Cytochrome c Oxidase Activity. Cytochrome *c* oxidase activity was measured spectrophotometrically by monitoring the oxidation of reduced horse heart cytochrome *c* (Sigma, St Louis, MO) at 550 nm and 30 °C (*33*), and the experiment was performed as described (*19*), but without the addition of myxothiazol. The data provided are the averages of at least two separate experiments. The NADI reaction was also performed as described elsewhere (*34*).

(C) Alkaline Phosphatase Assays. Alkaline phosphatase was assayed as previously described (*35*, *36*). The data provided are the averages of at least two separate experiments, each performed in duplicate.

(D) Immunoblot Analyses. R. sphaeroides cells were grown and assayed as described previously (*9*), and electrotransfer of the proteins and detection with polyclonal antibodies against alkaline phosphatase and DorA and secondary goat anti-rabbit alkaline phosphatase immunoglobulin were performed as described (*36*, *37*). TCA precipitations were performed as described elsewhere (*20*).

RESULTS

Transcriptional Organization of the prr Region. Previous complementation data involving a PrrA mutant strain with the *prrA* gene suggested that there was a promoter for *prrA* immediately upstream of the gene (*9*). This presumptive promoter lies within the 3′ region of the upstream *prrC* gene, which overlaps *prrA* (*13*). In addition, the presence of a second promoter, located upstream of *prrC* was suspected on the basis of the observation that apparent polar mutations of *prrC*, *prrC*1, and *prrC*2 (*13*) expressed phenotypes consistent with a decrease in *prrA* activity, i.e., a reduction in LH spectral complex levels and PS gene expression. It was therefore important to determine whether *prrC* and *prrA* are cotranscribed and to ascertain whether previously constructed *prrC* interruption mutations confer polarity on a *prrC*−*prrA* transcript. In addition, data obtained with the related organism *Rhodobacter capsulatus* showed the existence of an operon encompassing the genes *senC*, *regA* (*prrC* and *prrA* homologues) and *hvrA* (*38*), an *spb* (*39*) homologue.

The transcriptional organization of the *prr* region in *R. sphaeroides* 2.4.1 was determined by use of riboprobes internal to the genes *prrC*, *prrA*, *spb*, and *prrB* (for which the data are not shown) in Northern blot analysis by isolating total RNA from anaerobic dark-grown cells. The results are shown in Figure 1A. Whereas the *spb* probe (lane 3) hybridized to two transcripts of approximately 430 and 1500 bp, corresponding to *spb* and *prrA*−*spb*, respectively, the *prrC* probe (lane 2) hybridized to only one transcript of ∼1400 bp, corresponding to *prrC*−*prrA*. The smaller band within lane 2 corresponds to a degradation product of the above-mentioned transcript. The *prrA* probe (lane 1) hybridized to three transcripts, of which the smallest, ∼700 bp, is the strongest and corresponds to *prrA*. Additionally, the ∼1400- and ∼1500-bp mRNAs that hybridized to the *prrC* and *spb* probes, respectively, were also hybridized by the *prrA* probe. These results are depicted schematically in Figure 1B, in which potential transcription terminators are also denoted. The *prrB* probe hybridized to one transcript of approximately 1550 bp, which corresponds to a monocistronic *prrB* gene (data not shown). In contrast to the transcriptional organization of this region in *R. capsulatus*, we found no evidence for the existence of a transcript encompassing *prrC*−*prrA*−*spb*, which would have been expected to be at least 1900 bp in size.

The above results taken together with those described earlier indicate that *prrC* and *prrA* are transcribed coordinately, and therefore deletion/interruption mutations made in our laboratory with Ω cassettes (*40*) are partially polar on *prrA*, a gene previously shown to be very important in being able to turn on PS gene expression when present in 4−5 copies/cell (*9*).

Construction of the in-Frame Deletion Mutation of prrC. To ascertain the role of the *prrC* gene without the complicating effects on the downstream gene *prrA*, we constructed an in-frame deletion mutation within *prrC*. The construction is described under Experimental Procedures. The extent of the deletion was such that the *prrA* promoter internal within *prrC*, (which is located ∼145 bp upstream of the *prrA* initiation codon), was left intact, as judged by the full and complete levels of *prrA*-dependent downstream PS gene expression, also see below. The strain was designated PrrC4.

When grown on SIS agar plates in the presence of oxygen, mutant PrrC4 gave rise to stable colonies with a darker red pigmentation than the wild type. This darker pigmentation, similar to the pigmentation observed in cells containing multiple copies of *prrA* (*9*), is an indication of the production of the photosystem under aerobic conditions. Similar pigmentation has additionally been observed in mutant strains defective in the cbb_3 cytochrome *c* oxidase and Rdx proteins (*14*, *19*) (Roh and Kaplan, manuscript in preparation), as well as in the in-frame deletion mutation in CcoQ (*19*).

Phenotypic Analysis of PrrC4: Analysis of LH Spectral Complexes in PrrC4. Previous mutants isolated in our laboratory that show a deep red coloration when grown under aerobic conditions have been shown to synthesize the

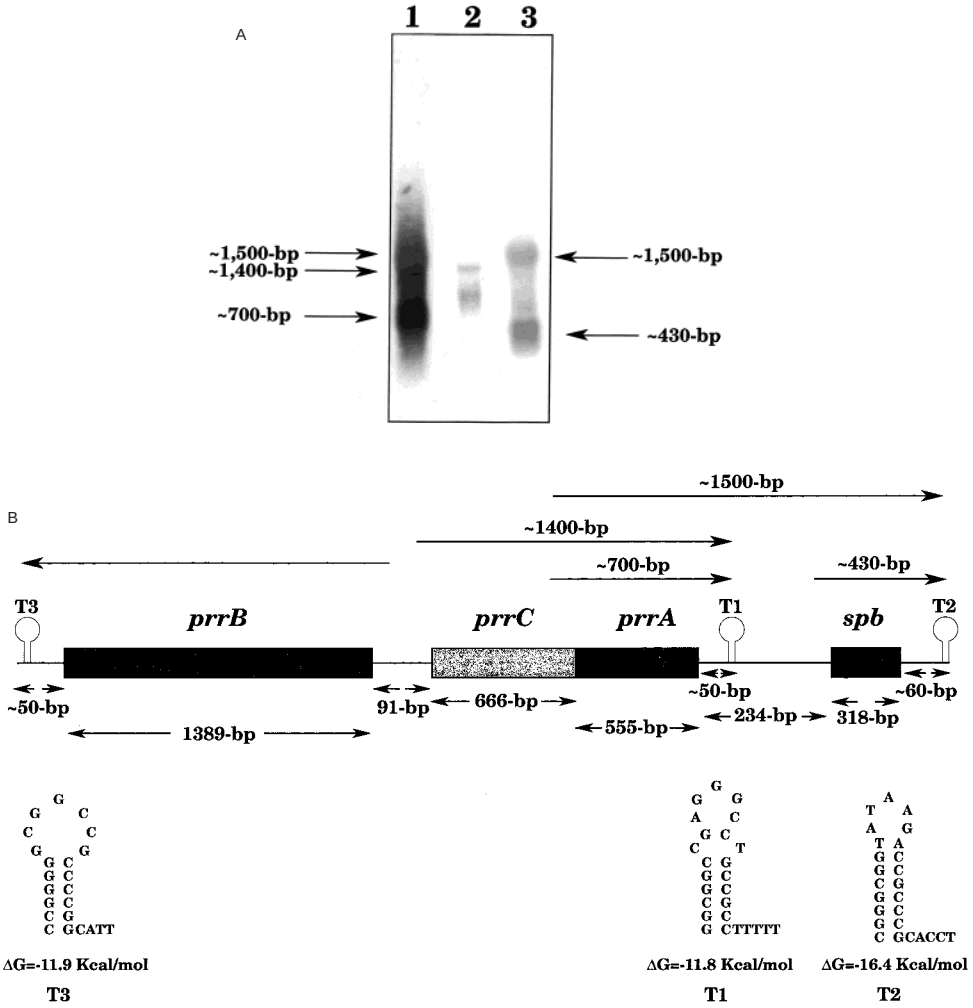

FIGURE 1: Northern hybridization analysis of *prrA*, *prrC*, and *spb*. Cells were grown in Sistrom's medium supplemented with 0.1% yeast extract anaerobically in the dark, in the presence of DMSO. (A) Total RNA was extracted, and after electrophoresis the Northern blots were probed with internal riboprobes to the three genes. Approximately 10 μg of RNA was loaded per lane. Lanes 1, 2, and 3 refer to riboprobes internal to *prrA*, *prrC*, and *spb*, respectively. Numbers refer to the sizes of the different transcripts. (B) Schematic representation of the *prr* region. The location of *prrB*, *prrC*, *prrA*, and *spb* is indicated by the shaded areas. The arrows indicate the direction of transcription and the different transcripts. T1, T2, and T3 refer to putative transcription terminators. ΔGs were calculated with the program Oligo 4.0. Numbers represent lengths of genes and intergenic regions.

photosystem aerobically. To show that this was also true for the deletion mutant PrrC4, crude extracts from aerobically grown cultures of this mutant, as well as wild type, were prepared, and the levels of the LH spectral complexes, B800−850 and B875, were measured spectrophotometrically (Figure 2). Whereas the wild-type produced reproducibly negligible levels of the LH spectral complexes (0.02 ± 0.005 and 0.06 ± 0.001 nmol of Bchl/mg of protein, for the B800−850 and B875, respectively), mutant PrrC4 exhibited 10 and 21-fold, 0.2 ± 0.004 and 1.28 ± 0.1 nmol of Bchl/mg of protein, increased levels of B800−850 and B875, respectively. Higher levels of the B875 with respect to the B800−850 were expected on the basis of similar findings showing that the B800−850 LH spectral complex requires preferentially higher amounts of the yellow carotenoid spheroidene (SE) for assembly (*28*) than does the B875 LH complex. Also bacteriochlorophyll (Bchl) is limiting under aerobic conditions (*19*) and is preferentially used in the assembly of RC and B875 complexes (*41*, *42*). For a direct comparison, a CcoQΔ mutant produced 0.7 ± 0.1 and 5.7 ± 0.9 nmol of Bchl/mg of protein, for the B800−850 and B875, respectively (*19*).

These data suggested that the in-frame deletion mutation in *prrC* led to the oxygen-insensitive formation of the photosystem, which is normally repressed in wild type under aerobic conditions. This is to be contrasted with data previously obtained with the interruption mutations, which had a polar effect on *prrA* (*13*) and thus support the premise that *prrA* is expressed normally in PrrC4. Finally, this phenotype is similar to the phenotypes of the previously constructed *ccoP* and *rdxB* interruption mutations (*14*) and the *ccoN* and *ccoQ* in-frame deletion mutations (*19*). In these mutant strains we know that the *prr* region is intact and functions normally (*14*, *18*).

When the levels of the LH spectral complexes were determined from wild-type and PrrC4 cells grown anaerobically in the dark in the presence of DMSO as electron acceptor (Figure 2), the LH spectral complex levels were virtually identical between the mutant and wild type, although the levels of LH spectral complexes were much higher compared to aerobic conditions, as anticipated. When the LH complexes were examined in the previously described PrrC deletion/interruption mutants (*13*) under these same conditions, significantly lower levels were observed, namely,

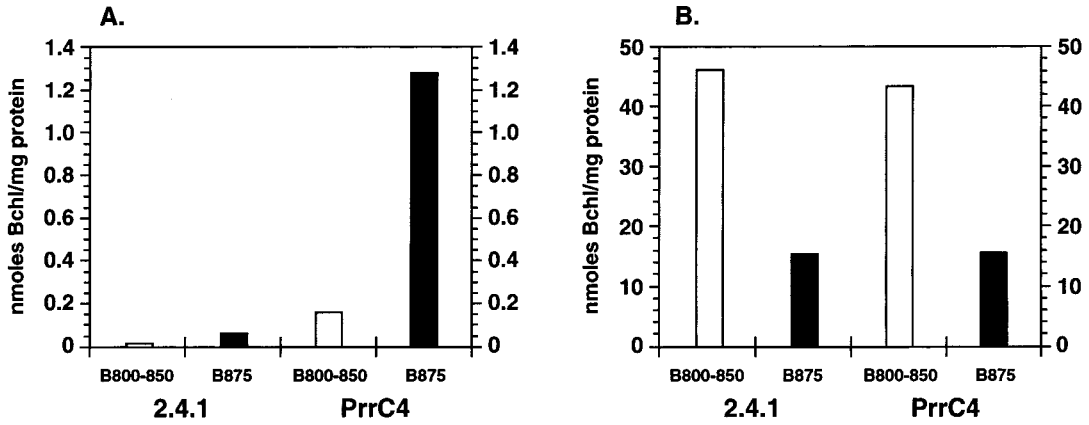

FIGURE 2: Spectral complex formation in wild-type and PrrC4 mutant cells. Cells were grown aerobically (A) sparged with a mixture of 30% O_2, 69% N_2, and 1% CO_2 in Sistrom's medium or in Sistrom's medium supplemented with 0.1% yeast extract anaerobically in the dark (B), in the presence of DMSO. Cells were harvested at a cell density of approximately 20 and 50 KU, respectively, and passed through a French press, and cell-free lysates were prepared for spectral analysis. Samples contained approximately 1−1.5 mg/mL protein. Units represent nanomoles of Bchl per milligram of protein. Light blocks represent the LHII (B800−850 LH complex). Dark blocks represent the LHI (B875 LH complex). Experiments were performed in duplicate. Standard deviations were less than 10%.

Table 2: Light-Harvesting Complexes in Wild Type and PrrC4[a]

	B800−850	B875	SE/SO ratio[b]
2.4.1	38.3	26.4	3:1
PrrC4	41.6	23.3	3:1

[a] Cells were grown photoheterotrophically at 10 W/m^2. Units are nanomoles of Bchl per milligram of protein. Standard deviations were <10%. [b] Spheroidene/Spheroidenone Ratio.

~19% and ~53% for the B800−850 and B875, respectively, compared to the values obtained with PrrC4.

When PrrC4 mutant cells were grown under photosynthetic conditions (10 W/m^2), the absence of *prrC* did not impair photosynthetic growth in *R. sphaeroides* cells (*13*), nor was there any difference between the amounts of LH spectral complexes produced by mutant PrrC4 and the wild type (Table 2). Of further interest, the relative composition of the carotenoids SE and SO remained the same as in wild-type cells. The ratio SE/SO was approximately 3:1 for both strains (Table 2). Previously (*14*, *19*), we have observed that when electron flow through the cbb_3 cytochrome *c* oxidase and the Rdx redox proteins is altered, SO comprises greater than 90% of the total carotenoids (Crt) under photosynthetic growth conditions. This dramatic change in Crt composition has led us to suggest that, in wild type, electron flow through the cbb_3 complex can take place anaerobically with the reduced Crt, SE, achieving normally high levels. All of these data indicate that photosynthetic growth in PrrC4 is similar to that of the wild type, and the only obvious defect resulting from the *prrC4* deletion mutation is the absence of the oxygen-generated inhibitory signal that maintains the repression of PS gene expression in the presence of oxygen.

Aerobic Growth. The presence of LH spectral complexes and therefore the induction of the photosystem under aerobic conditions of growth in mutant PrrC4 had absolutely no effect on growth of this strain under these conditions, growth rates for both mutant and wild-type being identical (data not shown).

Analysis of PS Gene Expression in PrrC4 Using puc::lacZ and puf::lacZ Fusions. The production of LH spectral complexes under aerobic growth conditions in the PrrC4 mutant suggested that expression of the operons encoding their cognate apoproteins, namely, *puc* for the B800−850 and *puf* for the B875, might be derepressed. This had been found previously for other mutations (*ccoP*, *rdxB*, and *ccoQ*) leading to a similar phenotype (*14*, *18*, *19*). Thus, the promoter activities of the *puc* and *puf* operons were determined by use of *lacZ* transcriptional fusions, and the results are shown in Figure 3. Expression of *puc* (open bars) and *puf* (solid bars) was activated by 6.6- and 4.2-fold under aerobic conditions of growth, respectively, when compared to wild type. In addition, complementation of the PrrC4 mutant with *prrC* brought expression down to levels observed for the wild type. Under anaerobic conditions in the dark with DMSO, expression of both fusions was similar in the mutant and in wild type. Effects of multiple copies of *prrC* were very subtle. For example, the wild type showed decreased levels of expression of *puf*, but not *puc*, to approximately 62% and 67% under aerobic and anaerobic growth conditions, respectively.

Whereas the expression of *puc* and *puf* in the PrrC4 mutant grown aerobically is similar to levels observed for the wild type grown photosynthetically, the levels of LH spectral complexes were considerably less than would be observed for wild-type cells grown photosynthetically. This phenomenon is routinely observed in those mutant strains showing the phenotype observed here and is readily explained. Even in wild-type cells there is an excess of apoproteins and cognate mRNAs produced relative to the levels of LH spectral complexes, whose abundance is stringently regulated by the levels of Bchl (*7*). In addition, we now know that under aerobic conditions, several critical genes whose gene products are involved in porphyrin biosynthesis as well as, specifically, in Bchl biosynthesis (*bchE*) are subject to regulatory control by FnrL in addition to the PrrBA activation system (Oh, Eraso, and Kaplan and Eraso and Kaplan, manuscripts in preparation).

It was previously determined in this laboratory that oxygen-insensitive synthesis of the LH spectral complexes in the CcoP and RdxB mutants was strictly dependent on PrrA. Therefore these redox protein complexes appear to be in the same signal transduction pathway from oxygen to the response regulator PrrA (*18*). The PrrCA1 deletion mutant, in which both *prrC* and *prrA* are absent, was examined and

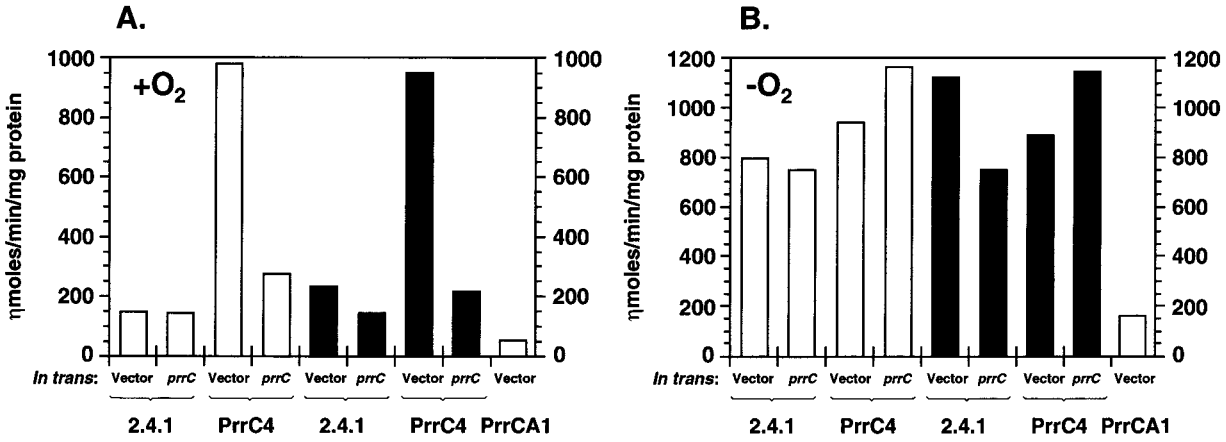

FIGURE 3: PS gene expression in wild type, PrrC4, and PrrCA1. β-Galactosidase values under aerobic (A) and anaerobic (B) conditions of growth are shown. Wild type and PrrC4 contain the *puf*::*lacZ* and *puc*::*lacZ* transcriptional fusions in pUI1663 and pCF200Km, respectively, in trans. They additionally contain either the pRK415 vector alone or pUI1645 containing *prrC*. PrrCA1 contains only the *puc*::*lacZ* fusion. Experiments were performed in duplicate. Standard deviations were less than 15%. Open bars represent the *puc*::*lacZ* fusion. Solid bars represent the *puf*::*lacZ* fusion. β-Galactosidase values are expressed in nanomoles per minute per milligram of protein.

shown to produce only background levels of LH spectral complexes, even under anaerobic growth conditions (*13*). These data indicate that PrrC is also in the same signal transduction pathway as PrrA, although upstream from it. Even though the mutations in *prrC4* and *prrCA1* are different, the final nature of the mutation in *prrCA1* is such that it combines both the inactivation of *prrC* and *prrA*. Thus, the polarity of *prrC* on *prrA* is of no consequence. When gene expression was monitored in the PrrCA1 double mutant strain (*13*) (Figure 3), only background levels of *puc* operon expression were observed. Thus, as shown for *cco* and *rdx* mutations (*18*), the presence of mutations in *prrA* renders the turn-on of PS gene expression in the presence of oxygen null.

DorA Expression in Wild Type and PrrC4. It had previously been shown that mutations in either *ccoP* or *rdxB* resulted in altered expression of the *dorCBA* operon, which encodes the structural components of DMSO reductase (DMSOR) (*37*). Since the PrrC4 in-frame deletion mutant showed altered redox-dependent signaling similar to that of the Cco−Rdx proteins, we suspected there might be additional effects of PrrC4 on *dor* expression in the presence of oxygen.

In Figure 4 we show the results of a Western immunoblot experiment using anti-DorA antibody against crude extracts from cells grown aerobically. As a control, wild-type was also grown anaerobically with DMSO. In contrast to the wild type, which produced barely visible amounts of DorA in the absence of DMSO, mutant CcoP1 produced 2−4-fold more DorA, as had been previously shown (*37*). More importantly, the amount of DorA present in mutant PrrC4 was strikingly similar to that found in CcoP1. Thus, under aerobic growth conditions, the in-frame deletion of *prrC* leads to a derepression of DorA, a protein involved in anaerobic respiration.

NADI Test. The NADI reaction (*34*) was used to help determine if cytochrome *c* oxidase was affected in the PrrC4 mutant. Cells containing mutations in *ccoP* remained red after 25 min following treatment with the NADI reagent, indicative of a NADI$^-$ phenotype, as anticipated on the basis of previous results (*14*). PrrC4 cells turned blue within 3−4 min, although not as rapidly (3 min) as either the wild-type

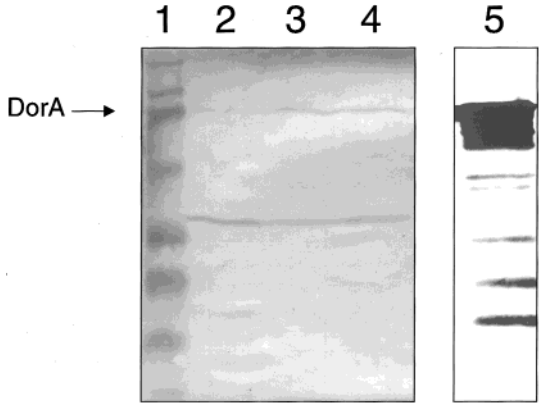

FIGURE 4: Synthesis of DorA. Whole-cell proteins (100 μg) were TCA-precipitated, resuspended in 50 mM Tris (pH 8.0), subjected to SDS−PAGE, and blotted onto nitrocellulose membranes. The presence of DorA was detected with polyclonal antiserum to DorA and visualized by the AP detection system (Promega). Lane 1, molecular weight markers (Bio-Rad); lane 2, 2.4.1 aerobic; lane 3, CCOP1 aerobic; lane 4, PrrC4 aerobic; lane 5, 2.4.1 anaerobic with DMSO (approximately 10 times less protein was loaded). DorA is indicated. Proteins were normalized for recovery.

or PrrA2, which had been included in this experiment as controls. Thus, PrrC4 cells are NADI$^+$; see below.

Cytochrome c Oxidase Activity. To investigate the effect of the *prrC4* deletion mutation specifically on *cbb*$_3$ oxidase activity, cytochrome *c* oxidase activity was determined in crude extracts of wild type and the PrrC4 mutant, from cells grown under anaerobic dark conditions with DMSO as electron acceptor. Under these conditions *cbb*$_3$ is the exclusive cytochrome *c* oxidase present in *R. sphaeroides* (*2, 19, 43*). Whereas the wild type showed 0.176 ± 0.001 μmol of oxidized horse heart cytochrome *c* min^{-1} (mg of protein)$^{-1}$, mutant PrrC4 showed very similar *cbb*$_3$ cytochrome *c* oxidase activity, 0.162 ± 0.01 μmol min^{-1} (mg of protein)$^{-1}$. These data suggested that PrrC4 has no major effect on *cbb*$_3$ oxidase activity under the conditions tested. Thus, as seen earlier, the in-frame deletion mutation of *prrC* phenotypically resembles that of a CcoQ deletion mutant, in that they both lead to the turn-on of PS gene expression aerobically, but neither of them affects *cbb*$_3$ oxidase activity (*19*). Preliminary data from experiments in progress in our laboratory (Eraso and Kaplan, manuscript in preparation)

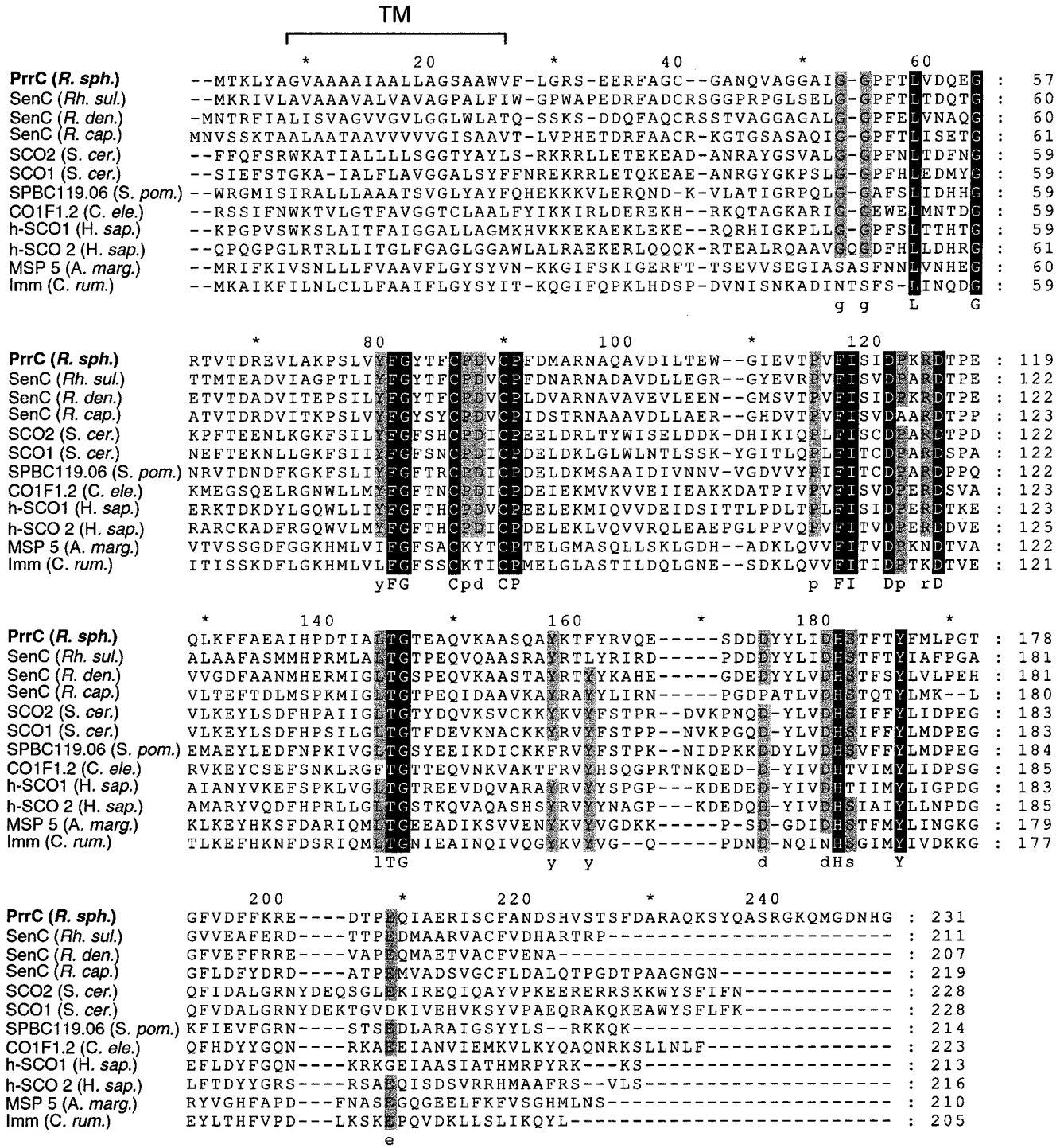

FIGURE 5: Alignment of *prrC* with its homologues. The amino acid sequence encoded by the *prrC* gene (top sequence) was compared with sequences in GenBank. The sequences are the following: PrrC (*R. sphaeroides*), SenC (*Rhodovulum sulfidophilum*), SenC (*Roseobacter denitrificans*), SenC (*Rhodobacter capsulatus*), Sco2 (*Saccharomyces cerevisiae*), Sco1 (*S. cerevisiae*), SPBC119.06 (*Schizosaccharomyces pombe*), CO1F1.2 (*Caenorhabditis elegans*), h-Sco1 (*Homo sapiens*), h-Sco2 (*H. sapiens*), MSP 5 (*Anaplasma marginale*), and Imm (*Cowdria ruminantium*). Totally conserved residues are boxed in black squares. Highly conserved residues are shaded in gray. TM indicates the region where the transmembrane domain is localized in PrrC. For simplicity the residues before the TM domain in all the eukaryotic homologues, as well as in Imm, are not shown.

reveal that the activity of cytochrome *c* oxidase might be affected in cells lacking PrrC when grown aerobically, as opposed to anaerobic dark DMSO. However, under these conditions both the cbb_3 and aa_3 oxidases are functional, and experiments are in progress to discern whether this effect is on the cbb_3 and/or aa_3 oxidases.

PrrC Homologues. A significant number of PrrC homologues have been found in GenBank since the sequence of *prrC* was first reported from our laboratory (*13*). We aligned some of the existing homologues, and the results are shown in Figure 5. These data show that this family of proteins is highly conserved in both prokaryotes and eukaryotes. Furthermore, the putative transmembrane domain, hydrophobic in nature, that had been reported earlier (*13*) is shared by all family members, suggesting that these proteins are localized in the cell membrane. See Discussion.

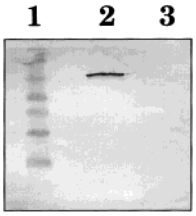

FIGURE 6: Synthesis of PrrC::PhoA. Crude extracts were subjected to SDS−PAGE and blotted onto nitrocellulose membranes. The presence of the PrrC::PhoA fusions was detected with polyclonal antiserum to PhoA and visualized by the AP detection system (Promega). Lane 1, molecular weight markers (Bio-Rad); lane 2, 2.4.1(PrrC::phoA2); lane 3, 2.4.1(PrrC::phoA1).

PrrC Topology. To determine if PrrC is localized to the cell membrane, we made translational fusions to the *phoA* gene, encoding alkaline phosphatase, and studied their activities in *R. sphaeroides*. Whereas no detectable activity was found in the PrrC::PhoA1 fusion, in which PhoA is fused to PrrC before the conserved hydrophobic domain (TM), the activity remained high, 172.4 ± 4.4 in PrrC::PhoA2, where the fusion junction is located after the hydrophobic domain. When *R. sphaeroides* cells were tested for the presence of the hybrid fusion protein by using antibodies against alkaline phosphatase (Figure 6), the fusion protein was only detected in cells containing PrrC::PhoA2. Taken together, these data show that the fusion protein is active and stable only when the PhoA moiety is located in the periplasm (PrrC::PhoA2) and indicate that PrrC is localized to the cell membrane, with its amino-terminal end protruding into the cytoplasm. A more thorough topological analysis of PrrC is currently underway.

DISCUSSION

Our recent studies employing mutations in both *ccoNOQP* and *rdxBHIS*, as well as double mutant strains containing mutations at either of these loci combined with mutations in *prrBA*, are consistent with our model that electron flow through the cbb_3 cytochrome *c* oxidase and the Rdx proteins is responsible for the generation of a redox-dependent signal that is inhibitory to PS gene expression in *R. sphaeroides* when growing under aerobic conditions. This proposed inhibitory signal could be transmitted through the CcoQ protein, which is associated with the cbb_3 oxidase complex and which we believe functions as a "transponder", relaying the signal to the PrrBA two-component gene activation system. This signal relay pathway constitutes a new paradigm for the regulation of gene expression. The redox state of the electron transport chain, as dictated by the availability of reductant and the presence of the electron acceptor(s), which together determine electron flow, is monitored by CcoQ and ultimately regulates PS gene expression.

One prediction of this model is that if additional signaling proteins are involved in this signal transduction pathway, their disruption should lead to a phenotype similar to those already described for other members of the pathway (*14*, *19*). One such protein, PrrC, was found in this study to be specifically involved in the transduction of the inhibitory signal derived from cbb_3 to the downstream PrrBA two-component activation system under aerobic conditions. Because of the intimate structural relationship between *prrC* and *prrA* we constructed an in-frame deletion mutation within *prrC* in which 99 codons were deleted from the gene, leaving a cryptic *prrC* gene comprising 132 codons. The in-frame deletion of *prrC* led to substantial increases in the amount of LH spectral complexes and PS gene expression under aerobic growth conditions, when compared to the wild type. This was consistent with previous results (*14*, *19*) and was in agreement with PrrC being a member of this signal transduction pathway. In addition, similar to the results of Mouncey and Kaplan (*4*) obtained with mutant CcoP1, which lacks a functional cbb_3 oxidase, the level of DorA, a component of the DMSOR complex, was also found to be elevated in mutant PrrC4 under aerobic conditions, compared to the wild type. However, in contrast to mutant CcoP1, the activity of the cbb_3 cytochrome *c* oxidase was found to be comparable to that of the wild type in the PrrC4 mutant, indicating that PrrC is not required for either activity or assembly of the cbb_3 oxidase.

A more direct role for PrrC in some as yet unknown electron transport reaction could not be ruled out, since the -CXXXC- motif (Figure 5) could possibly function as a redox-active metal binding site. In the case of the PrrC homologues found in *Saccharomyces cerevisiae*, such as Sco1 and Sco2, this motif has been proposed to be involved in copper binding for assembly of the terminal cytochrome *c* oxidase in yeast (*44*, *45*). To our knowledge, no data pertaining to the role played by the other homologues of this family are available.

Mutant PrrC4 showed a marginally longer time for the NADI reaction to develop than the wild type but was much more rapid than that in mutant CCOP1, suggesting a slight reduction in the total cytochrome *c* oxidase activity in the PrrC4 mutant, compared to the wild type. When the levels of cytochrome *c* oxidase were measured directly, no significant effect of the *prrC4* mutation on cbb_3 oxidase activity was observed. It is possible that in the PrrC4 mutant the aa_3 cytochrome *c* oxidase might be affected, thus showing an overall lower rate of color development. Alternatively, considering the semiaerobic conditions present in colonies growing on petri dishes in the presence of oxygen and the inherent differences in colony development, slight differences in color development may not be significant. The NADI reaction was substantially slower in SenC mutants, the PrrC homologue in *R. capsulatus*, with respect to the wild type. Compared to PrrC4 mutants, the reaction in SenC mutants was approximately 25% of wild type (*46*). Experiments are in progress to ascertain the significance of this result.

An interruption mutation of the *R. capsulatus* PrrC homologue, SenC, led to a general decrease in PS gene expression in this organism (*46*), and similar to *R. sphaeroides*, *senC* and *regA* are reported to be transcribed in a polycistronic message (*38*). Thus, our earlier results (*13*) and those obtained with *R. capsulatus* are entirely consistent and, as indicated above, mutations in *prrC* can be polar on *prrA* if constructed without the care demonstrated here.

PrrC was found to be highly conserved in both prokaryotes and eukaryotes (Figure 5). The best studied homologues are the *S. cerevisiae* Sco1 and Sco2 proteins. Both proteins are localized to the mitochondrial inner membrane in this organism (*47*, *48*), and the carboxy-terminal end of Sco1 was found to be located in the inner mitochondrial space (*49*). Disruption of *sco1*, unlike that of *sco2*, results in the lack of cytochrome *c* terminal oxidase, and the effect was found to be posttranscriptional (*45*, *47*). Thus, Sco1 has been

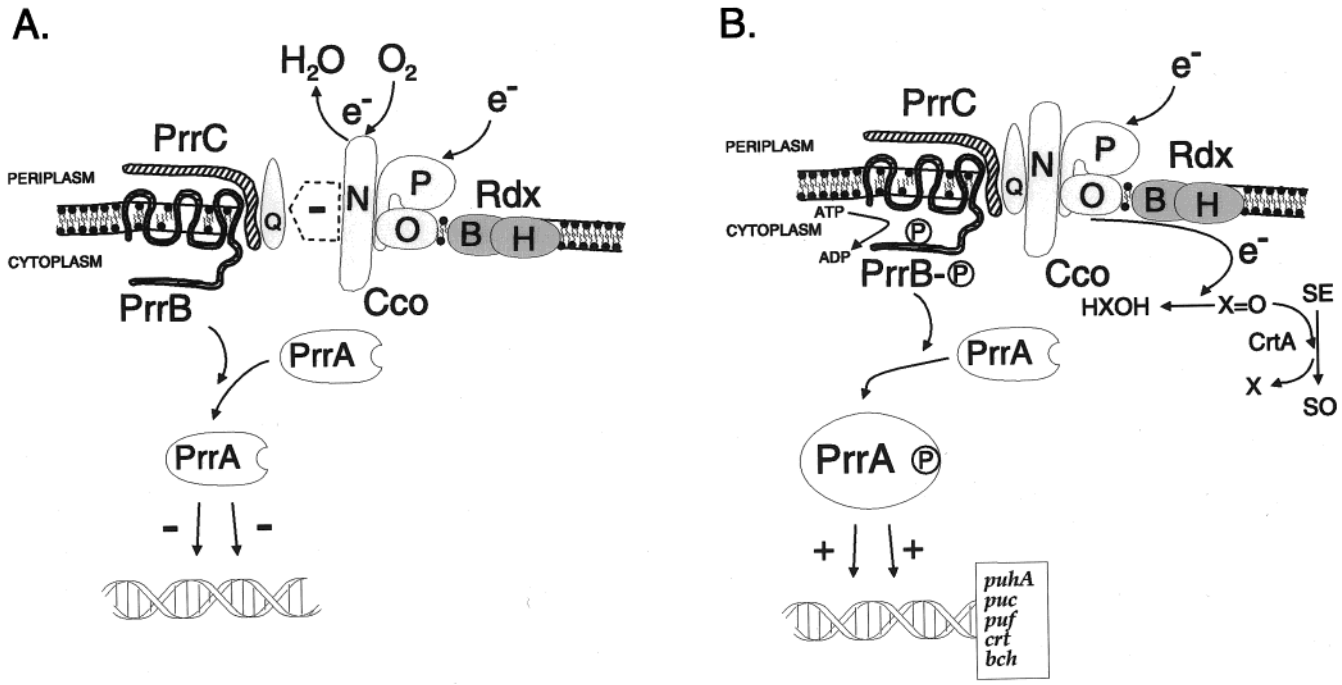

FIGURE 7: Model depicting the role of PrrC, as well as other proteins involved in the signal transduction pathway, generating an inhibitory signal by cbb_3 to the PrrBA two-component activation system involved in PS gene expression in *R. sphaeroides*. (A) Aerobic conditions; (B) anaerobic conditions. The minus sign inside the dashed pentagon indicates an inhibitory signal. + and − indicate activation and lack of activation, respectively, of the genes depicted inside the box, which are regulated by PrrA.

proposed to be involved in cytochrome *c* oxidase assembly (*44*, *45*). In addition, either Sco1 has been proposed to bind copper via the highly conserved (-CXXXC-) domain, and thus make copper available for the assembly of the oxidase (*47*, *50*), or alternatively, the (-CXXXC-) domain in SenC has been proposed to be an iron binding "half-site", similar to the 4-Cys domain present in bacterial ferredoxins (*46*, *51*). No firm conclusions as to the role of Sco2 are available.

Inasmuch as PrrC is concerned, the *R. sphaeroides* protein was found to be localized to the cytoplasmic membrane, as predicted by the existence of a hydrophobic domain of sufficient length to span the membrane (*13*), with its amino terminal end located in the cytoplasm. This is reminiscent of the localization in the inner mitochondrial space of Sco1 in yeast. Thus, with the exception of PrrA, all other proteins presumed to be involved in the redox signal transduction pathway from oxygen to PrrA (Cco, Rdx, etc.) are membrane-associated.

Given these as well as previous results (*14*, *18*, *19*), we propose a model in which PrrC is functionally located between the cbb_3 cytochrome *c* oxidase and the PrrBA two-component activation system (Figure 7), and that it is intimately involved in the transfer of the signal emanating from the electron transport chain via the cbb_3 and Rdx proteins to the response regulator PrrA. Intrinsically, and as previously proposed (*18*, *19*), this signal must be of an inhibitory nature under aerobic growth conditions (Figure 7A). This inhibitory signal, carried via PrrC, might be responsible for either the inhibition of the kinase activity of PrrB or the stimulation of its phosphatase activity or both, as proposed previously and on the basis of genetic evidence (*18*, *40*), ultimately leading to a lack of phosphorylation of the response regulator PrrA, to render it inactive for activation of PS gene expression. In contrast, under anaerobic growth conditions the inhibitory signal is either absent or greatly reduced (Figure 7B). The cbb_3 cytochrome *c* oxidase is still active under these conditions (*18*, *19*), although oxygen is not available, funneling electron flow, as suggested (*18*), to the downstream Rdx components. The absence of the inhibitory signal would result in the subsequent phosphorylation of PrrA, by PrrB, and concomitant activation of PS gene expression. Therefore the state of PrrC, by virtue of the absence of an inhibitory signal from upstream redox components, is responsible for PrrB activation. Whether PrrC and PrrB interact directly with each other remains to be determined. Work in progress in our laboratory toward the purification of the native PrrB, as well as PrrC, will make the pertinent experiments possible.

The involvement of the *rdx* locus in this signal transduction pathway has been shown previously (*14*, *18*), but the nature of this involvement was not clear (*19*). However, experiments in progress in our laboratory (Roh and Kaplan, manuscript in preparation) should more accurately define the role of the *rdx* locus in this signal transduction pathway.

Data obtained with *R. sphaeroides* (Oh and Kaplan, unpublished results) and with *Bradyrhizobium japonicum* (*52*), showed that CcoQ copurifies with the cbb_3 cytochrome *c* oxidase in both organisms, and therefore it may be intimately associated with this oxidase. On the basis of these preliminary observations we favor a model (Figure 7) in which the signal emanating from either redox flux or the oxidized/reduced state of an electron carrier, from the interaction of cbb_3 with oxygen, is monitored by CcoQ, through its interaction with the cbb_3 oxidase, and is subsequently transmitted to PrrC, which we propose is downstream of CcoQ in the signal transduction pathway. Although other functional interactions are not discarded, this is the simplest model, at the present time and with the available data, to explain how redox flux is transmitted to the PrrBA two-component activation system in *R. sphaeroides* 2.4.1. Lack-

ing evidence to the contrary, it appears that we may have now identified all of the components that are involved in this signal transduction pathway from oxygen to the activation of PS gene expression. Experiments are in progress that will further test this hypothesis.

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