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Interactive Control of *Rhodobacter capsulatus* Redox-Balancing Systems during Phototrophic Metabolism

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In nonsulfur purple bacteria, redox homeostasis is achieved by the coordinate control of various oxidation-reduction balancing mechanisms during phototrophic anaerobic respiration. In this study, the ability of *Rhodobacter capsulatus* to maintain a balanced intracellular oxidation-reduction potential was considered; in addition, interrelationships between the control of known redox-balancing systems, the Calvin-Benson-Bassham, dinitrogenase and dimethyl sulfoxide reductase systems, were probed in strains grown under both photoheterotrophic and photoautotrophic growth conditions. By using cbb_I (*cbb* form I operon)-, cbb_{II}-, *nifH*-, and *dorC*-reporter gene fusions, it was demonstrated that each redox-balancing system responds to specific metabolic circumstances under phototrophic growth conditions. In specific mutant strains of *R. capsulatus*, expression of both the Calvin-Benson-Bassham and dinitrogenase systems was influenced by dimethyl sulfoxide respiration. Under photoheterotrophic growth conditions, coordinate control of redox-balancing systems was further manifested in ribulose 1,5-bisphosphate carboxylase/oxygenase and phosphoribulokinase deletion strains. These findings demonstrated the existence of interactive control mechanisms that govern the diverse means by which *R. capsulatus* maintains redox poise during photoheterotrophic and photoautotrophic growth.

Rhodobacter capsulatus is a nonsulfur purple photosynthetic bacterium that exhibits diverse respiratory abilities, allowing this organism to grow under a variety of environmental conditions. Branched respiratory electron transport pathways allow *R. capsulatus* to grow aerobically in the dark, either chemoautotrophically or chemoheterotrophically, by using O_2 as the terminal electron acceptor. Indeed, its high capacity for aerobic chemoautotrophic growth distinguishes it from other well-studied nonsulfur purple bacteria, such as *R. sphaeroides* and *Rhodospirillum rubrum* (26). Like other organisms of this group, *R. capsulatus* can also grow anaerobically in the light, either photoautotrophically or photoheterotrophically, using cyclic photosynthetic electron transport to generate a proton motive force. These organisms can grow fermentatively as well. Due to such metabolic versatility, *R. capsulatus* provides an excellent system with which to gain insight into the control of redox homeostasis. Yet, compared to the thorough and well-characterized redox control studies of *Escherichia coli* (for a review, see reference 17 or 51 and references therein), knowledge of the control of redox homeostasis in *R. capsulatus* is somewhat limited.

During phototrophic growth, various electron acceptors are employed, in a hierarchical manner, to maintain a balanced redox state in *R. capsulatus* (50). In the presence of organic carbon under light anaerobic growth conditions (photoheterotrophic growth), the redox-balancing mechanism(s) consists primarily of the Calvin-Benson-Bassham (CBB) reductive pentose phosphate pathway (CBB system). Under some growth conditions, the dinitrogenase enzyme complex (dinitrogenase system), the dimethyl sulfoxide (DMSO) reductase (DMSOR) system, or other systems yet to be identified or implicated in redox control are employed. Specific reactions of the CBB pathway allow CO_2 to function as a sink for excess reducing equivalents generated by the metabolism of carbon substrates such as L-malate and succinate. Thus, the predominant role of the CBB pathway during photoheterotrophic growth is to balance the oxidation-reduction potential of the cell (13, 24, 55). The capacity for CO_2-dependent growth under photoautotrophic growth conditions is accomplished primarily by the CBB system, where the chief role of the CBB pathway is to provide the cell with carbon via the assimilation of CO_2. The duality of roles of the CBB system leads to an interplay between the maintenance of redox poise and the control of carbon metabolism under photoheterotrophic and photoautotrophic growth conditions. The dinitrogenase system is synthesized in most phototrophs when the organism is placed in an ammonia-free environment. This system enables *Rhodobacter* to grow under conditions in which dinitrogen is the sole source of nitrogen (N_2-dependent growth); i.e., the cells catalyze the reduction and assimilation of atmospheric dinitrogen to ammonia, accompanied by the reduction of protons to molecular hydrogen. The process of dinitrogen fixation requires much reducing power and is an energy-intensive process (5). Not only does the dinitrogenase system play a primary role in nitrogen metabolism (23), but it has also been shown to be involved in redox homeostasis in *Rhodobacter* and *Rhodospirillum rubrum* (22, 50). Photoheterotrophic growth with a poor nitrogen source such as glutamate signals the cell to synthesize the dinitrogenase system (for a review, see reference 25 and references therein). Under such growth conditions, the excess reducing equivalents generated by the oxidation of carbon substrates, such as malate, are consumed by the reduction of protons and consequent evolution of molecular hydrogen by a hydrogenase-like activity of the dinitrogenase system. This allows the

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TABLE 1. Bacterial strains and plasmids utilized in this study

Strain or plasmid	Genotype and phenotype[a] or relevant characteristic(s)	Reference(s)
R. capsulatus		
SB1003	*cbbLS*$^+$ *cbbM*$^+$ *cbbP*$^+$ PH_a^+ PH_g^+ RubisCO$^+$ PRK$^+$	58
SBI/II	*cbbLS cbbM cbbP*$^+$ PH_a^- PH_g^+ RubisCO$^-$ PRK$^+$	40
RCP	*cbbLS cbbM cbbP*$^+$ PH_a^+ PH_g^+ RubisCO$^-$ PRK$^+$	37, 50
SBP	*cbbLS*$^+$ *cbbM*$^+$ *cbbP* $PH_a^{+/-}$ PH_g^+ RubisCO$^-$ PRK$^-$	40
SBP-PHC	*cbbLS*$^+$ *cbbM*$^+$ *cbbP* PH_a^+ PH_g^+ RubisCO$^-$ PRK$^-$; derepresses nitrogenase system	37, 50
Plasmids		
pRK2013	Helper plasmid for triparental conjugation	14
PXLB	Translational fusion; *cbb*$_I$*::lacZ*; Tet^r	40
PXFB	Translational fusion; *cbb*$_{II}$*::lacZ*; Tet^r	40
pALS53	Translational fusion; *dorC::lacZ*; Tet^r	A. Shaw and A. G. McEwan unpublished data
pHU316	Translational fusion; *nifH::lacZ*; Tet^r	21
pVKD1	*R. sphaeroides cbb*$_I$ translational promoter fusion to *lacZ*; Tet^r	6

[a] PH_a, photoheterotrophic growth with ammonia as the nitrogen source; PH_g, photoheterotrophic growth with glutamate as the nitrogen source; PH_a^-, lack of photoheterotrophic growth with ammonia as the nitrogen source and requirement of an alternative electron acceptor (DMSO); $PH_a^{+/-}$, gradual photoheterotrophic growth (barely grows; slow doubling time compared to wild type).

cell to balance its intracellular redox potential (20). Physiological studies have shown that a link between carbon metabolism and nitrogen metabolism exists that is intimately associated with the control of intracellular redox poise in *R. capsulatus* (50), *R. sphaeroides* (22, 43), and *R. rubrum* (22). Specifically, in the absence of a functional CBB system (achieved through the inactivation of genes encoding key and unique enzymes of the CBB pathway), spontaneous variants of strains with photoheterotrophic competency (PHC) and CBB deficiency dissipate excess reducing equivalents as H_2 gas by derepressing the dinitrogenase system (22, 50). Respiration of the auxiliary oxidant DMSO or trimethylamine-*N*-oxide (TMAO) through the DMSOR system has also been shown to play an important role in the maintenance of redox poise during phototrophic growth of *R. capsulatus* (27, 44). Indeed, DMSO respiration allows growth of CBB-deficient strains of *R. sphaeroides* (11, 18, 19, 55) and *R. capsulatus* (40, 50) under photoheterotrophic growth conditions in the presence of a fixed nitrogen source. Thus, the reduction of DMSO or TMAO serves as an additional mechanism by which to dissipate excess reducing equivalents generated by carbon metabolism.

In this study, reporter-gene promoter fusions were employed to examine the expression of the CBB, dinitrogenase, and DMSOR systems in response to different environmental and metabolic signals. CBB-deficient strains and a dinitrogenase-derepressing strain of *R. capsulatus* were used in these studies. Contributions of the different redox systems to photoautotrophic carbon metabolism and the interaction with nitrogen metabolism were explored, and the results of these studies reflect on the overall control of redox homeostasis in *R. capsulatus*.

MATERIALS AND METHODS

Bacterial strains and reporter gene fusion plasmids. The genotypes and phenotypes of the *R. capsulatus* strains and reporter-gene fusion plasmids utilized in this study are summarized in Tables 1 and 2. CBB-deficient strains SBI/II and SBP and subsequent spontaneous variants derived from them, strains RCP and SBP-PHC, respectively, were previously described, and their growth potential in various media has been reported (37, 40, 50). Strain SBI/II lacks form I and form II ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) due to the introduction, respectively, of a spectinomycin resistance cartridge in *cbbL* and a kanamycin resistance cartridge in *cbbM*, while strain SBP lacks phosphoribulokinase due to the introduction of the Ω-spectinomycin cassette into *cbbP*. In order to achieve photoheterotrophic growth comparable to that of wild-type strain SB1003, an exogenous electron acceptor, such as DMSO, must be provided during photoheterotrophic growth with a fixed nitrogen source (ammonia). *R. capsulatus* strains that have acquired PHC in the absence of external electron acceptors like DMSO and can use ammonia as a fixed nitrogen source include strains RCP and SBP-PHC. Strain RCP (*R. capsulatus* photoheterotrophically competent) is a spontaneous variant of strain SBI/II that maintains the *cbbLS cbbM* phenotype, while strain SBP-PHC is a spontaneous variant of strain SBP that maintains the *cbbP* phenotype. The redox-balancing mechanism that allows photoheterotrophic competency in the absence of a functional CBB cycle remains to be established in strain RCP, while derepression of the dinitrogenase enzyme

TABLE 2. Redox-balancing systems for photoheterotrophic growth conditions in strains of *R. capsulatus*

Strain	Redox-balancing system under following growth condition[a]			
	M-A	M-A-D	M-G	M-G-D
SB1003	CBB[b]	CBB; DMSOR[c]	CBB; Nitrogenase[d]	CBB; nitrogenase; DMSOR
SBI/II	No growth[e]	DMSOR	Nitrogenase	Nitrogenase; DMSOR
RCP	Unknown[f]	Unknown; DMSOR	Unknown; Nitrogenase	Unknown; nitrogenase; DMSOR
SBP	Poor growth	DMSOR	Nitrogenase	Nitrogenase; DMSOR
SBP-PHC	Nitrogenase	Nitrogenase; DMSOR	Nitrogenase	Nitrogenase; DMSOR

[a] M-A, malate-ammonia; M-A-D, malate-ammonia-DMSO; M-G, malate-glutamate; M-G-D, malate-glutamate-DMSO.
[b] CBB, CBB system.
[c] DMSOR, DMSOR system.
[d] Nitrogenase, dinitrogenase enzyme complex.
[e] No growth in the absence of an exogenous oxidant, such as DMSO.
[f] Unknown, redox-balancing mechanism remains to be established.

complex has been shown to be essential to allow strain SBP-PHC to maintain photoheterotrophic competency (50).

Media and growth conditions. *E. coli* strain JM109 (57) containing reporter gene fusion plasmids was grown aerobically on LB medium (1) at 37°C with appropriate antibiotic selection. Photoheterotrophic cultures of *R. capsulatus* were grown anaerobically in Ormerod's medium (36) supplemented with 0.4% DL-malate, as required, and 1 μg of thiamine/ml. The nitrogen source was provided as either 30 mM ammonia or 6.8 mM L-glutamate. Photoheterotrophic and photoautotrophic growth of all cultures was monitored by measuring the optical density at 600 nm (OD_{660}) of cultures with a Beckman spectrophotometer. The concentrations of antibiotics used for selection of the *R. capsulatus* strains were as follows: rifampin, 50 μg/ml; kanamycin, 5 μg/ml; spectinomycin, 10 μg/ml; tetracycline, 3 μg/ml for stock cultures or 0.5 μg/ml for plasmid maintenance under phototrophic growth conditions. For *E. coli*, the antibiotic concentrations for plasmid maintenance were as follows: kanamycin, 20 μg/ml; tetracycline, 6 μg/ml. DMSO and TMAO were each utilized at a concentration of 30 mM.

Conjugation techniques. *E. coli* strain JM107 (57), containing mobilizable helper plasmid pRK2013 (14), was used in triparental matings in order to independently conjugate the respective reporter gene fusion plasmids into recipient *R. capsulatus* strains. Donor and recipient cultures were grown to a high turbidity (late log phase) in peptone-yeast extract (PYE) medium (56) containing appropriate antibiotics and washed three times with PYE medium prior to mating in order to eliminate any interference from the presence of antibiotics. Following conjugation, exconjugants were grown at 30°C on PYE agar plates containing rifampin and tetracycline and (if appropriate) spectinomycin and/or kanamycin as counterselective agents against *E. coli*; tetracycline was provided for plasmid maintenance.

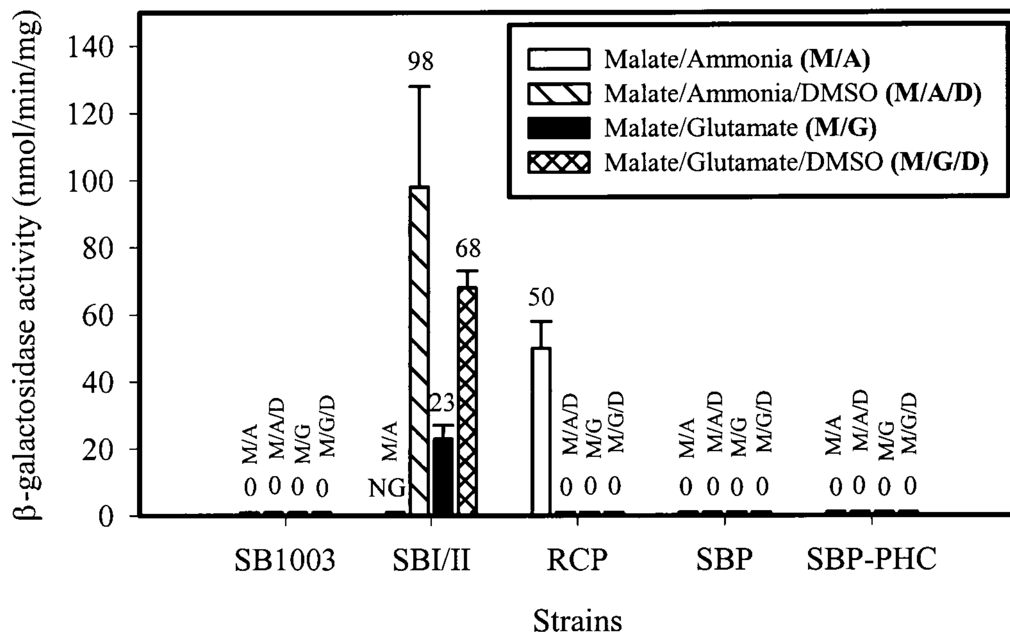
Cell extracts and enzyme assays. Culture samples (10 to 20 ml) were harvested in late log phase (OD_{660} = 0.9 to 1.2), washed in buffer (25 mM Tris-Cl, 1 mM EDTA [pH 8.0]), and disrupted by sonication. The resultant cell debris was removed by centrifugation at 18,000 × *g* for 15 min at 4°C. β-Galactosidase activity was measured as previously reported (40); the production of *o*-nitrophenol (31) was continuously measured over a time period of 10 min by monitoring the increase in A_{405} on a Spectronic GENESYS 2 spectrophotometer. Specific activities were calculated by using the change in steady-state A_{405} per minute and the extinction coefficient for *o*-nitrophenol of 3.1×10^3 cm^2/mmol (54). Levels of activity were checked for cells taken at different stages of growth, with no significant change in the enzyme activity patterns noted. However, to ensure reproducibility of all comparisons, all samples were taken from cultures at close to the same OD_{660} and it was ensured that this never exceeded 1.2. Protein concentrations were determined by the Bio-Rad protein assay dye binding reagent (Bio-Rad Laboratories, Hercules, Calif.) using bovine serum albumin as the standard.

RESULTS

Interplay of the CBB, dinitrogenase, and DMSOR systems under photoheterotrophic growth conditions. Various systems are employed by *R. capsulatus* to balance the intracellular oxidation-reduction potential when the organism is exposed to specific environmental conditions (Table 2). During photoheterotrophic growth on carbon substrates such as L-malate, the CBB system is expressed to allow CO_2, produced as a result of the oxidation of the organic substrate, to function as an electron sink for the excess reducing equivalents generated during metabolism. The dinitrogenase system, in conjunction with the CBB cycle, serves as a redox-balancing tool during photoheterotrophic growth with a poor nitrogen source (such as glutamate); growth with ammonia represses the synthesis of this system. Furthermore, the DMSOR system contributes to redox poise under phototrophic growth conditions in the presence of the auxiliary oxidants DMSO and TMAO. No additional mechanism(s) employed to remove reducing equivalents is known. Specific reporter gene promoter fusions were used to monitor the expression of the CBB, dinitrogenase, and DMSOR systems in CBB-deficient strains and photoheterotrophically competent CBB-deficient strains (which included a dinitrogenase-derepressing strain) of *R. capsulatus* (Table 2). The responses of the selected redox-balancing systems to different environmental and metabolic signals under photoheterotrophic growth conditions were established by using each of these strains.

The CBB system. In *R. capsulatus*, form I RubisCO is not synthesized during photoheterotrophic growth with malate as the carbon source and ammonia as the nitrogen source (39, 48). However, form I RubisCO synthesis is observed during growth with reduced carbon substrates, such as butyrate, or during photoautotrophic (1.5% CO_2–98.5% H_2) growth conditions (16, 39, 48). Consistent with the established regulation of cbb_I (the *cbb* form I operon), wild-type strain SB1003 did not exhibit cbb_I promoter activity under photoheterotrophic growth conditions with ammonia as the fixed nitrogen source in the absence or presence of the exogenous electron acceptor DMSO (Fig. 1A). In addition, no cbb_I promoter activity was detected during growth with glutamate (which also serves as an ancillary carbon source to L-malate) in the absence or presence of DMSO (Fig. 1A). Thus, normal regulation of the cbb_I promoter was maintained in wild-type strain SB1003 regardless of the nitrogen source (ammonia or glutamate) or the presence or absence of an auxiliary oxidant (DMSO). Additionally, strains SBP and SBP-PHC exhibited wild-type regulation of the cbb_I promoter for all of the photoheterotrophic growth conditions tested (Fig. 1A). By contrast, strains SBI/II and RCP showed definitive cbb_I promoter activity during photoheterotrophic growth (Fig. 1A). cbb_I promoter activity was observed in strain SBI/II with either ammonia or glutamate as the nitrogen source in the presence of DMSO or in the absence of DMSO when glutamate was used as the nitrogen source. Strain RCP exhibited cbb_I promoter activity only during growth with ammonia as the nitrogen source in the absence of DMSO (Fig. 1A). Interestingly, strain SBI/II exhibited threefold higher cbb_I promoter activity during photoheterotrophic growth in the presence of glutamate when supplemented with DMSO (Fig. 1A); strain SBI/II does not grow in the absence of DMSO with ammonia as the nitrogen source due to the lack of an expressed redox-balancing mechanism (40, 50).

In contrast to cbb_I, cbb_{II} was expressed under all growth conditions that require the CBB system (39, 40); for photoheterotrophic conditions with L-malate as the carbon source, the primary role of CO_2 fixation via the CBB cycle (using only cbb_{II}) is to maintain the redox poise of the cell. Wild-type strain SB1003 exhibited cbb_{II} promoter activity under photoheterotrophic growth conditions with either ammonia or glutamate as the nitrogen source, in the absence or presence DMSO (Fig. 1B). Strain SBI/II, however, exhibited a 10-fold induction of cbb_{II} promoter activity when cultures were supplemented with DMSO while retaining wild-type control during growth in the absence of DMSO (Fig. 1B). Strain RCP expressed low basal levels of cbb_{II} promoter activity under all of the photoheterotrophic growth conditions tested (Fig. 1B). Similar to strain RCP (Fig. 1B), additional PHC strains that were derived from RubisCO-deficient strain SBI/II (M. A. Tichi and F. R. Tabita, unpublished data) were also shown to exhibit basal, uninduced levels of cbb_{II} expression under the four growth conditions depicted in Fig. 2B. Thus, upon developing photoheterotrophic competency, RubisCO-deficient strains such as strain RCP regained the normal low basal levels



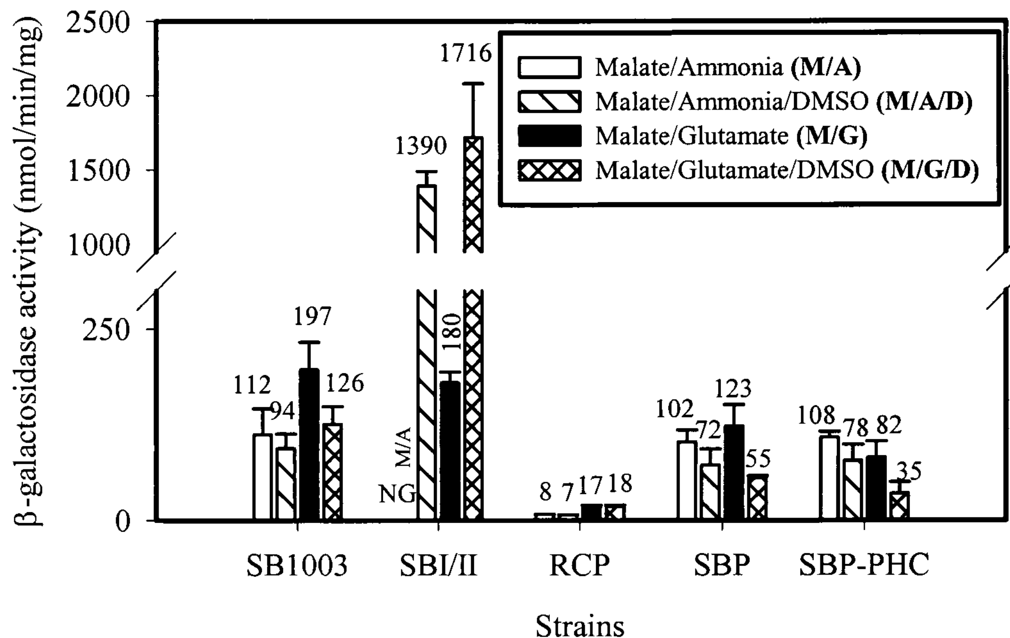


FIG. 1. *cbb*$_{\text{I}}$::*lacZ* (A) and *cbb*$_{\text{II}}$::*lacZ* (B) promoter activities during photoheterotrophic growth of *R. capsulatus* CBB-deficient strains. β-Galactosidase activities were determined in three or four independent cultures assayed in duplicate. NG indicates no growth under photoheterotrophic growth conditions with ammonia as the nitrogen source in the absence of DMSO.

of *cbb*$_{\text{II}}$ promoter activity during photoheterotrophic growth; however, the absolute promoter activity levels were, for unknown reasons, considerably reduced and were similar to those observed with *cbbR*$_{\text{II}}$ strains (53). Strains SBP and SBP-PHC exhibit comparable wild-type (strain SB1003) control of *cbb*$_{\text{II}}$ promoter activity during photoheterotrophic growth in the presence of ammonia, although somewhat less activity was observed with malate-glutamate-DMSO (Fig. 1B). Among all

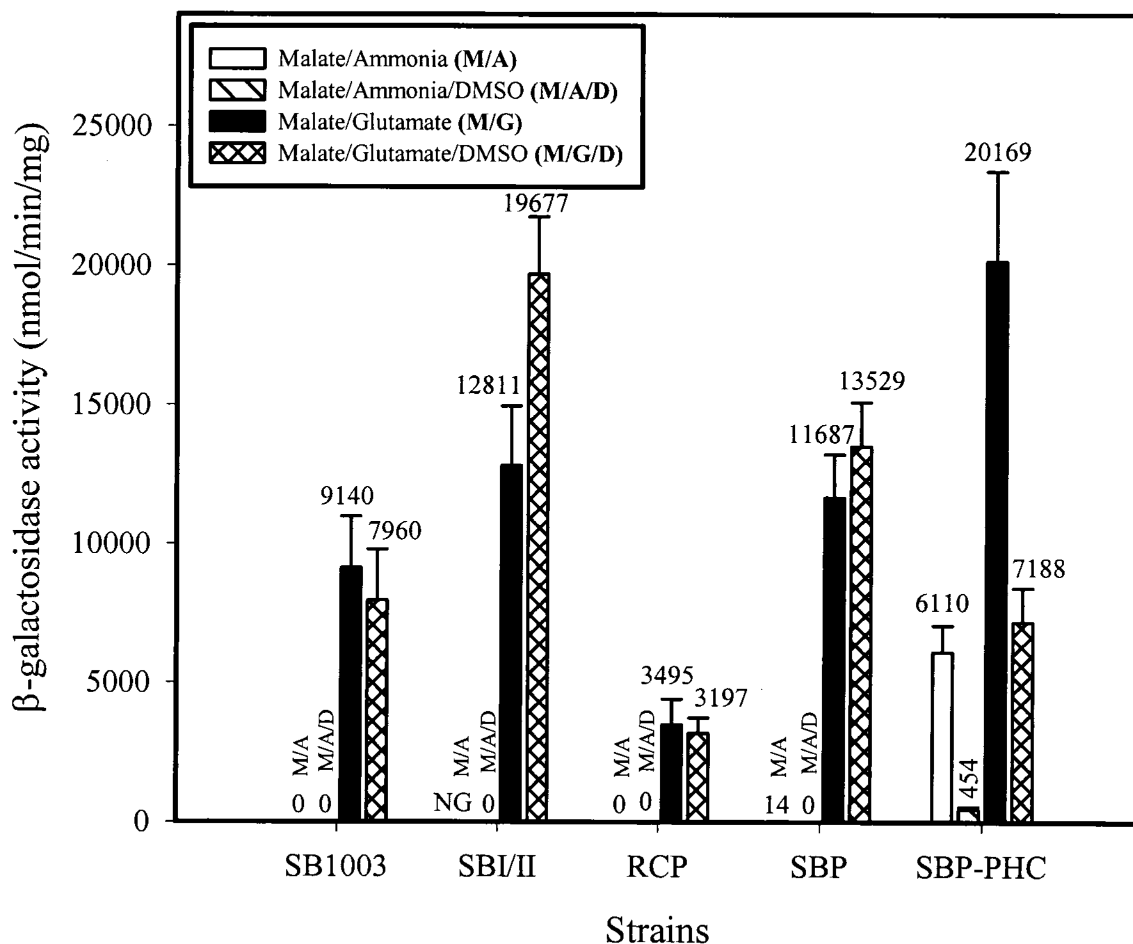


FIG. 2. *nifH*::*lacZ* promoter activity during photoheterotrophic growth of *R. capsulatus* with either ammonia or glutamate as the nitrogen source. β-Galactosidase activities were determined in four or five independent cultures assayed in duplicate. NG indicates no growth under photoheterotrophic growth conditions with ammonia as the nitrogen source in the absence of an ancillary electron acceptor (DMSO).

of the CBB-deficient strains, it appears from these results that only strain SBI/II showed substantial activation of both cbb_I and cbb_{II} promoter activities in the presence of DMSO. Apparently, basic regulatory mechanisms are altered in this strain.

The dinitrogenase system. Previous studies indicated that the conventional molybdenum dinitrogenase system of *R. capsulatus* (encoded by the *nifHDK* genes) provides a sufficient compensatory electron sink in the absence of an operational CBB pathway under photoheterotrophic growth conditions with glutamate as the nitrogen source. Redox poise is also accomplished in the presence of ammonia in PHC strains of *R. capsulatus*. Under these conditions, the organism derepresses the synthesis of an active and unmodified dinitrogenase enzyme complex (50). Consistent with this regulatory scheme, strain SBP-PHC exhibited *nifH* promoter activity when grown photoheterotrophically in the presence of ammonia, while the wild-type strain and additional CBB-deficient strains repressed *nifH* promoter activity (Fig. 2). Also consistent with previous findings (50), the *nifH* promoter activity of strain SBP-PHC was decreased 13.4-fold and 2.8-fold under DMSO-supplemented growth conditions when either ammonia or glutamate, respectively, was used as the nitrogen source; *nifH* promoter activity of parent strain SBP was hardly affected by the addition of DMSO under growth conditions permissive for dinitrogenase expression (Fig. 2). Even though strain RCP exhibited a 2.5-fold decrease in *nifH* promoter activity compared to wild-type strain SB1003, the promoter activities observed during malate-glutamate growth for both strains were not appreciably altered by the addition of DMSO (Fig. 2). *nifH* promoter activity increased 2.5-fold in strain SBI/II during photoheterotrophic growth with glutamate supplemented with DMSO compared to that in wild-type strain SB1003.

The DMSOR system. Excess reductant generated by photosynthetic electron transport is transferred via the ubiquinone pool to the periplasmic terminal electron acceptor DMSOR during phototrophic growth in the presence of DMSO or TMAO (27–29). Thus, DMSO respiration via the DMSOR system (encoded by the genes *dorCDA*) contributes to the maintenance of redox homeostasis. Accordingly, *dorC* promoter activity was examined in wild-type strain SB1003 and the CBB-deficient strains of *R. capsulatus* in order to assess *dor* expression during photoheterotrophic growth. During growth in the presence of DMSO, *dorC* promoter activity was induced in all *R. capsulatus* strains regardless of the supplied nitrogen

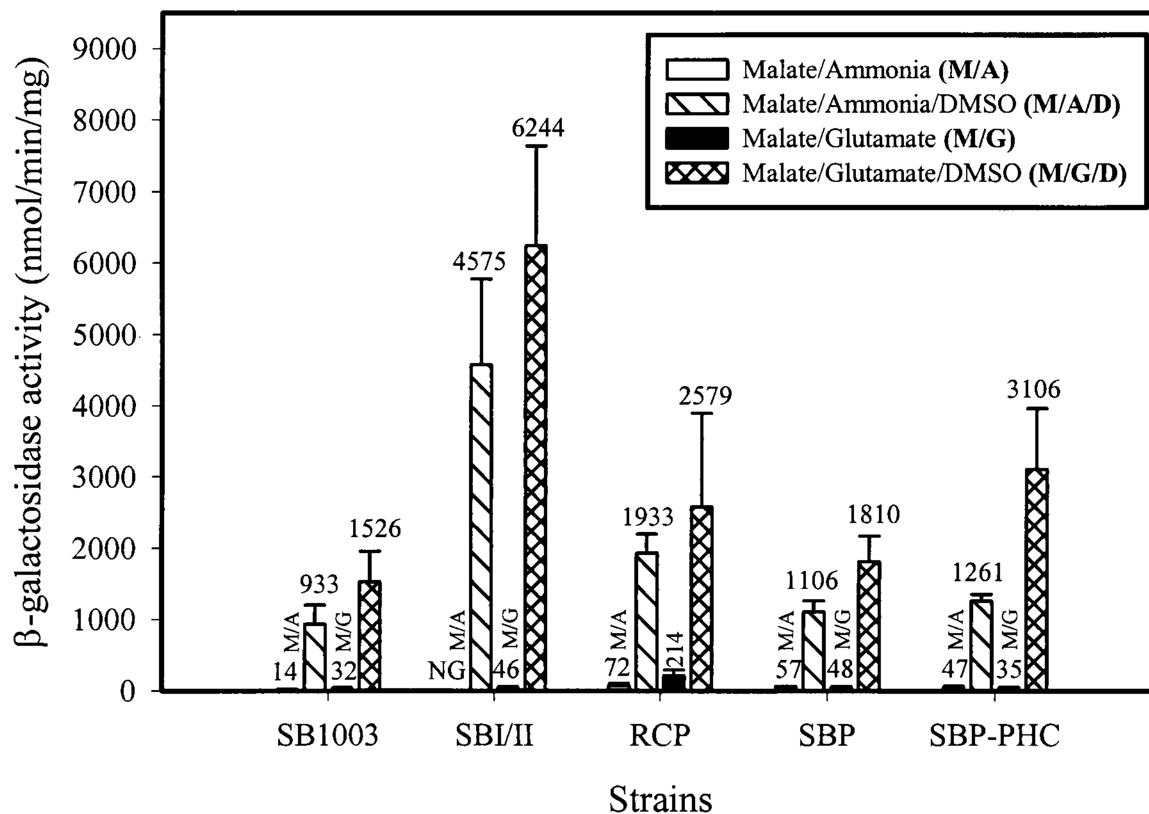


FIG. 3. *dorC*::*lacZ* promoter activity of *R. capsulatus* under photoheterotrophic growth conditions with or without DMSO. β-Galactosidase activities were determined in four or five independent cultures assayed in duplicate. NG indicates no growth in the absence of DMSO under conditions with ammonia as the nitrogen source.

source (Fig. 3). Compared to wild-type strain SB1003, strain SBI/II exhibited enhanced *dorC* promoter activity (4.1 to 4.9-fold) during photoheterotrophic growth with DMSO when either glutamate or ammonia, respectively, was used as the nitrogen source (Fig. 3). These results were consistent with the enhanced cbb_I, cbb_{II}, and *nifH* promoter activities obtained in strain SBI/II under DMSO-supplemented growth conditions.

PHC and coordinate regulation of redox poise and carbon metabolism. The first indication that the PHC phenotype and the control of *cbb* expression might be linked in *R. capsulatus* came from studies with strain RCP, where low basal levels of cbb_{II} promoter activity were obtained under all of the photoheterotrophic growth conditions tested. In addition, unlike all of the other strains, including the wild type, strain RCP expressed cbb_I promoter activity only during growth with ammonia in the absence of DMSO (a condition under which the PHC phenotype is obligatory for growth) (Fig. 1A and B).

The hierarchical use of electron acceptors and control mechanisms involved in phototrophic redox homeostasis in *R. capsulatus* differs from the situation found in *R. sphaeroides* and *R. rubrum* (50). Specifically, coordinate control of the DMSOR and dinitrogenase systems in PHC strains appears to be unique to *R. capsulatus*. The development of a PHC phenotype and the control of *cbb* expression also differed in *R. capsulatus* and *R. sphaeroides*. This was particularly evident when heterologous expression experiments were performed with an *R. sphaeroides* translational cbb_I::*lacZ* promoter fusion vector to examine the effect of the PHC phenotype on *R. sphaeroides cbb* promoter activities in the *R. capsulatus* wild-type and PHC strain backgrounds (Table 3). Plasmid pVKD1 contains, in addition to the cbb_I promoter of *R. sphaeroides*, the upstream and divergently transcribed *R. sphaeroides cbbR* gene (6, 7). Endogenous *R. capsulatus* CbbR proteins do not recognize *R. sphaeroides cbb* promoters; thus, transcription of this cbb_I promoter in the *R. capsulatus* background is dependent on its cognate CbbR protein. It is clear that the *R. capsulatus* cbb_I promoter is not expressed in photoheterotophically grown wild-type *R. capsulatus* (Fig. 1A). However, when an *R. sphaeroides cbbR*-cbb_I promoter plasmid (pVKD1) was used, β-ga-

TABLE 3. Promoter activity using an *R. sphaeroides* cbb_I::*lacZ* promoter fusion in *R. capsulatus* wild-type strain SB1003 and strain RCP under photoheterotrophic growth conditions

Photoheterotrophic growth condition	β-Galactosidase activity[a] of strain:	
	SB1003	RCP
Malate-ammonia	15 ± 6	1,233 ± 166
Malate-ammonia-DMSO	13 ± 6	734 ± 263
Malate-glutamate	20 ± 5	422 ± 157
Malate-glutamate-DMSO	8 ± 2	364 ± 90

[a] β-Galactosidase activities (mean ± standard deviation) are expressed as nanomoles per minute per milligram and were determined in three independent cultures of each strain (assayed in duplicate) containing plasmid pVKD1 from *R. sphaeroides*.

TABLE 4. Levels of CBB and DMSOR promoter systems in wild-type strain SB1003 during photoautotrophic growth in the presence or absence of DMSO with ammonia G glutamate as the nitrogen source

Phototrophic growth condition	β-Galactosidase activity (nmol/min/mg)[a]		
	cbb$_I$::*lacZ*	*cbb*$_{II}$::*lacZ*	*dorC*::*lacZ*
CO_2-H_2-ammonia	162 ± 8	794 ± 59	9 ± <1
CO_2-H_2-ammonia-DMSO	27 ± 14	1,192 ± 206	795 ± 171
CO_2-H_2-glutamate	0	510 ± 28	12 ± 1
CO_2-H_2-glutamate-DMSO	0	602 ± 108	1,283 ± 184

[a] β-Galactosidase activities were determined in three independent cultures assayed in duplicate.

lactosidase activity was obtained under all of the photoheterotrophic growth conditions tested in an *R. capsulatus* strain RCP background and very low, but demonstrable, basal levels of activity were detected in wild-type *R. capsulatus* (Table 3). *R. sphaeroides cbb*$_I$ promoter activity in *R. capsulatus* strain RCP was highest when the PHC growth phenotype was obligatory (malate-ammonia medium) and decreased up to fourfold as additional redox-balancing systems were used by the organism (Table 3), i.e., when glutamate was used as a nitrogen source (and the dinitrogenase system was synthesized) or when DMSO was added to cultures (when the DMSOR system was synthesized). The high level of *cbb*$_I$ expression obtained in a malate-ammonia medium is very similar to what occurs when *R. capsulatus cbb*$_I$ expression is monitored in strain RCP (Fig. 1A), suggesting that the basic environment in *R. capsulatus* RCP is responsible for regulating CbbR-dependent transcription, no matter whether transcription is directed by the *R. sphaeroides* or *R. capsulatus* CbbR protein and the cognate *cbb*$_I$ promoter. The fact that very low levels of *R. sphaeroides* CbbR-dependent *cbb*$_I$ promoter activity was observed in a wild-type *R. capsulatus* environment is compatible with the finding that *R. capsulatus* CbbR-dependent *cbb*$_I$ promoter activity was not even detected under these growth conditions. Perhaps the low-level expression of the *R. sphaeroides cbb*$_I$ promoter, compared to the *R. capsulatus cbb*$_I$ promoter, was due to the presence of the unique upstream activator region in the promoter-distal region of the *R. sphaeroides cbb*$_I$ promoter (6, 7).

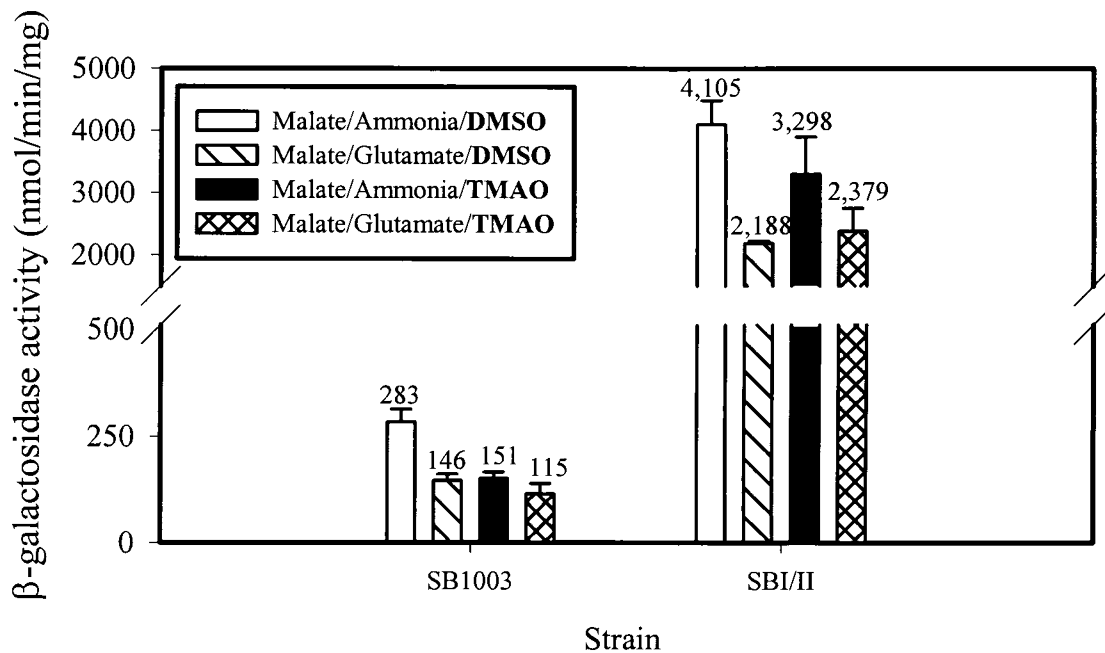
Integrative control of the DMSOR redox-balancing system with photoautotrophic carbon metabolism. To assess the integration of the DMSOR and CBB systems with photoautotrophic metabolism, *dorC* and *cbb* promoter activities were examined in wild-type strain SB1003. *dorC* promoter activity in wild-type strain SB1003 was not significantly altered under photoautotrophic (CO_2-H_2) growth conditions when cultures were supplemented with DMSO in the presence of ammonia or glutamate (Table 4). Likewise, *cbb*$_{II}$ promoter activity in wild-type strain SB1003 was not significantly changed when the exogenous electron acceptor DMSO was added under photoautotrophic growth conditions, no matter whether ammonia or glutamate was used as the nitrogen source (Table 4). These results were analogous to *cbb*$_{II}$ promoter activities obtained for wild-type strain SB1003 during photoheterotrophic growth with malate, where the addition of DMSO did not affect activity (Fig. 1B). In wild-type strain SB1003, *cbb*$_I$ promoter activity, however, decreased sixfold during photoautotrophic growth in the presence of DMSO compared to growth in the absence of DMSO (Table 4). These data suggested that the presence of DMSO has a selective effect on expression of the *cbb*$_I$ promoter in *R. capsulatus* during photoautotrophic growth. No *cbb*$_I$ promoter activity was observed in the wild-type strain under photoautotrophic growth conditions when glutamate was added as a potential carbon and nitrogen source in the absence or presence of DMSO (Table 4). It is apparent from these results that the DMSOR and *cbb*$_I$ systems are reciprocally regulated under photoautotrophic conditions, suggesting that electron flow through the DMSOR system negatively impacts *cbb*$_I$ expression.

To confirm that the control of the *cbb*$_{II}$ system is independent of controls on the DMSOR system, an experiment was designed to take advantage of the fact that the DMSOR system catalyzes both DMSO and TMAO respiration; despite this, *dorC* promoter activity decreases during photoheterotrophic growth when TMAO is used as an exogenous electron acceptor instead of DMSO (47). Using either DMSO or TMAO as the electron acceptor for the DMSOR system did not significantly affect *cbb*$_{II}$ promoter expression in either wild-type strain SB1003 or strain SBI/II under photoheterotrophic growth conditions (Fig. 4A and B). In addition, strain SBI/II maintained up-regulated expression of the *cbb*$_{II}$ system in the presence of either TMAO or DMSO. By contrast, however, the addition of TMAO to photoheterotrophic cultures of strains SB1003 and SBI/II resulted in a three- to fivefold decrease in *dorC* promoter activity compared to the activities obtained in the presence of DMSO (Fig. 4B).

DISCUSSION

Nonsulfur purple bacteria couple their ability to assimilate carbon dioxide and dinitrogen to photosynthetic energy generation and the production of required reducing equivalents (15). However, knowledge of how various redox-balancing systems interact and contribute to successful photoheterotrophic or photoautotrophic metabolism is limited. In the present study, the expression of three important redox-balancing mechanisms, the CBB, dinitrogenase, and DMSOR systems, was shown to be either coordinately regulated or influenced by the presence of one system or the other. This is necessary to ensure balance in the use of reducing equivalents generated by phototrophic metabolism (Fig. 5). The control of anaerobic respiratory pathway gene expression in *R. capsulatus* is comparable to the situation in *E. coli*, where there is also coordinate and integrative control over the redox-balancing systems (for a review, see reference 17 and references therein). However, in the present study, evidence for linkage in the control of key redox-balancing systems (i.e., those important for CO_2 fixation, nitrogen fixation, and DMSO respiration) is presented for the first time in both the photoheterotrophic and photoautotrophic growth modes.

The interplay between the dual roles (maintenance of redox poise and carbon metabolism) of the CBB system has been shown to correlate with the expression of the DMSOR system. The integration of the CBB system and the DMSOR system in phototrophic metabolism is not unprecedented. Phototrophic growth on highly reduced substrates such as butyrate and propionate is known to depend upon the addition of exogenous CO_2 as an electron acceptor (52). Under these conditions, the



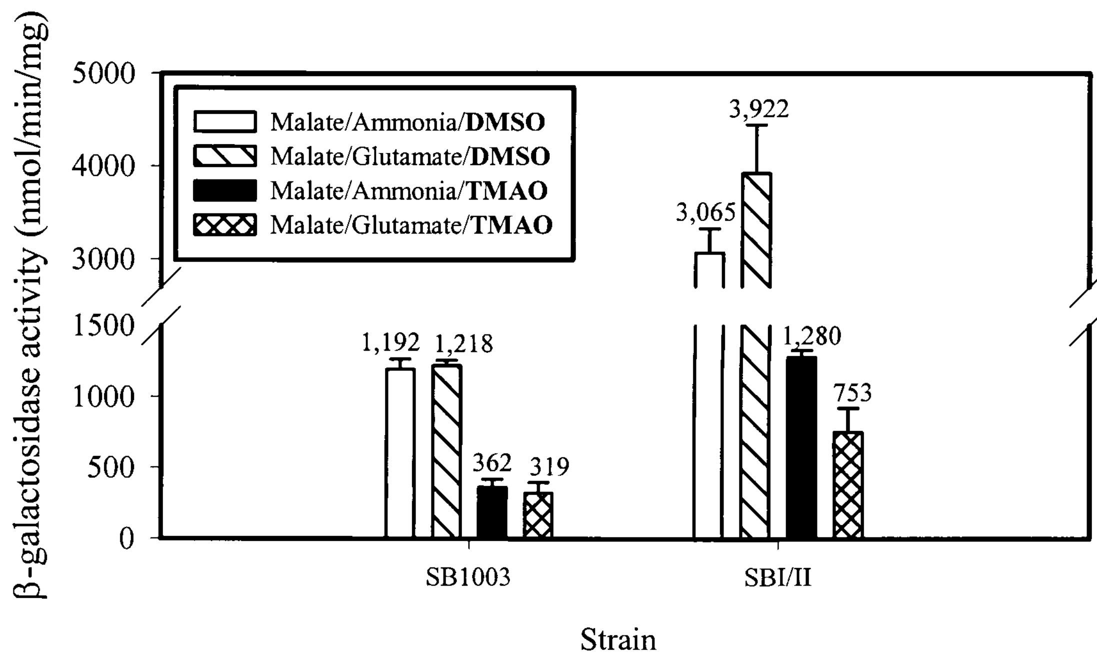


FIG. 4. *cbb*$_{II}$::*lacZ* (A) and *dorC*::*lacZ* (B) promoter activities in wild-type *R. capsulatus* strain SB1003 and strain SBI/II during photoheterotrophic growth with either DMSO or TMAO as the supplied exogenous electron acceptor in the presence of either ammonia or glutamate as the nitrogen source.

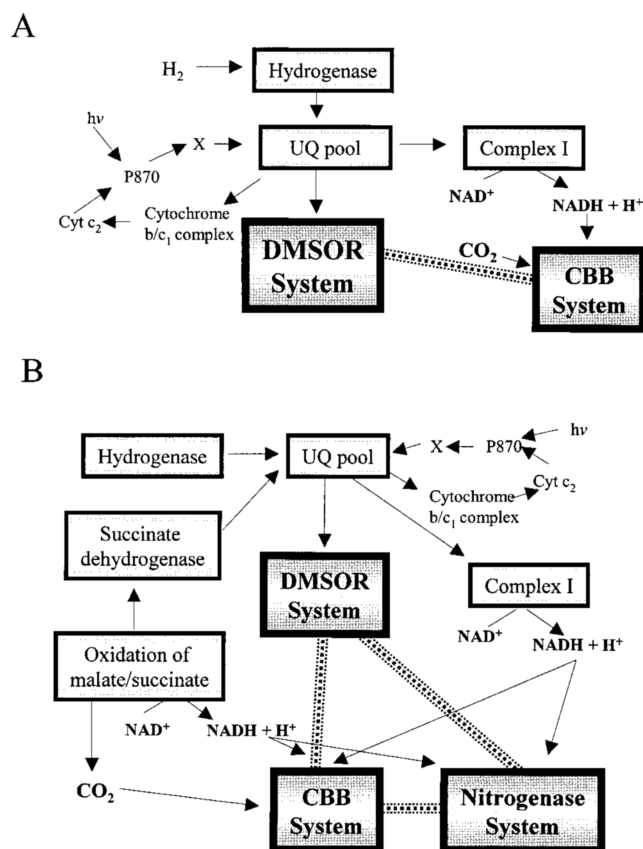


FIG. 5. Mechanisms involved in adaptation of the efficiency of energy conservation to intracellular requirements under photoautotrophic (A) and photoheterotrophic (B) growth conditions. Cyclic photosynthetic electron transport and the specific redox-balancing mechanisms of the CBB, DMSOR, and nitrogenase systems contribute to redox homeostasis in *R. capsulatus*. NADH generated by the oxidation of carbon substrates or reverse electron flow via complex I is dissipated by the CBB system (A and B) and perhaps the nitrogenase system (B). Flux of reductant from the ubiquinone pool is transduced to the DMSOR system under phototrophic growth conditions (A and B). The dotted line indicates the influence of DMSO reduction on specific redox-balancing mechanisms under phototrophic environmental conditions (A and B).

CBB system is obligately required for growth (11, 12, 39). An auxiliary oxidant, DMSO or TMAO, can substitute for CO_2 under these phototrophic growth conditions (44). Moreover, during phototrophic growth on less-reduced carbon substrates (e.g., L-malate), the DMSOR system can also replace the need for a functional CBB system in *R. capsulatus* (40, 50). The results of the present investigation indicated that cbb_I of the CBB system of *R. capsulatus* was responsive to activation of the DMSOR system under photoautotrophic growth conditions, while cbb_{II} was unaffected by the DMSOR system under either photoheterotrophic or photoautotrophic growth conditions. By contrast, RubisCO-deficient strain SBI/II exhibited a different response in that both cbb_I and cbb_{II} promoter activities were raised to photoautotrophic (1.5% CO_2–98.5% H_2) wild-type levels under photoheterotrophic growth conditions in the presence of DMSO. In fact, all of the redox systems, as exemplified by the respective promoter fusions, were up-regulated in strain SBI/II.

In *Rhodobacter*, redox homeostasis is achieved through the interplay of cyclic photosynthetic electron transport and specific redox-balancing mechanisms of anaerobic metabolism during phototrophic growth (30). It has been suggested that the electron acceptors involved in photosynthetic metabolism function as a sink for excess reducing equivalents or prevent the overreduction of the cyclic electron transport system. This interaction between redox poise and electron transport occurs at the level of the ubiquinone pool (13; Fig. 5). Respiratory electron flow to the DMSOR system has been shown to branch from cyclic electron transport at the level of the ubiquinone pool (27, 28); thus, activation of the DMSOR system under phototrophic growth conditions may siphon reductant from the ubiquinone pool. Studies of the related organism *R. sphaeroides* indicated that flux from the ubiquinone pool is transduced through a pathway involving cbb_3-type cytochrome *c* oxidase, while a signal involved in the flow of reductant is conveyed to the PrrBA (RegBA) signal transduction pathway (33–35). In *R. capsulatus*, the two-component signal transduction system RegBA (PrrBA) has been shown to be involved in the regulation of operons important for photosynthetic gene expression (46), CO_2 fixation (53), and nitrogen fixation and H_2 oxidation (8, 50). Indeed, the current model suggests that the RegBA system responds to the overall intracellular redox state (22, 49), although more-detailed studies are required to elucidate the specific redox-sensing mechanisms that influence the Reg system of *R. capsulatus* (4, 8). Under photoautotrophic growth conditions in *R. capsulatus*, the RegBA global regulatory system was shown to be involved in activation of cbb_I promoter expression, as well as maximal expression of the cbb_{II} promoter (53). It is possible that by activating the DMSOR system, which alters the oxidation-reduction potential of the ubiquinone pool, a redox signal is transmitted to a regulatory system that, in turn, controls the expression of key operons involved in phototrophic metabolism. In *R. capsulatus*, the RegBA system plays more of a critical role in regulating cbb_I since this operon is up-regulated only during photoautotrophic metabolism while cbb_{II} is expressed under a variety of conditions. This could explain the sensitivity of cbb_I to activation of the DMSOR system under photoautotrophic growth conditions. Alternatively, additional, unknown factors that have been postulated to be involved in expression of the CBB system in *R. sphaeroides* (6, 7) and *R. capsulatus* (53) could play a critical role in transmitting a redox signal to control key operons involved in redox homeostasis.

During photoheterotrophic metabolism, redox poise is also achieved by the coordinate integration of the DMSOR and CBB systems, as well as the dinitrogenase system. Indeed, in the absence of an operational CBB system, spontaneous variants of *R. capsulatus* derepress the dinitrogenase system, resulting in photoheterotrophic competency (50). Dinitrogenase-catalyzed proton reduction and the consequent evolution of H_2 gas are important for maintenance of redox poise in *R. capsulatus* (20). The current study monitored the interplay between the CBB and dinitrogenase systems, as well as the DMSOR system, in CBB-deficient and PHC strains of *R. capsulatus*. Although the specific regulatory mechanism(s) involved in the derepression of dinitrogenase in PHC mutant strains of *R. capsulatus* remains to be established, it should be noted that the PrrBA (RegBA) two-component regulatory system is in-

volved in the maintenance of the PHC phenotype of an *R. sphaeroides* dinitrogenase-derepressing strain (22). Additionally, the Reg system was shown to be involved in the control of nitrogen fixation in wild-type *R. capsulatus* (8) and *Bradyrhizobium japonicum* (3).

The specific integration of redox mechanisms with the derepression of the dinitrogenase system in *R. capsulatus* differs from the situation in *R. sphaeroides* (50). For example, activation of the DMSOR system under photoheterotrophic growth conditions diminishes *nif* expression in a dinitrogenase-derepressing strain of *R. capsulatus* while exhibiting no effect in *R. sphaeroides* (22, 42). We have also observed differences in *R. sphaeroides* and *R. capsulatus* cbb_I promoter expression in an *R. capsulatus* PHC strain background. This could be due to differences in general redox response between cbb_Is of the two organisms, the differential effects of specific metabolic signals on the cognate CbbR proteins, or a combination of both possibilities. With different ecological niches in aquatic ecosystems (41), it is not unexpected that *R. capsulatus* and *R. sphaeroides* differentially regulate processes involved in the control of redox homeostasis in response to the environmental milieu. An indication of this possibility was previously suggested by the demonstration of differences in the roles of the global regulatory systems of FnrL (47, 59, 60) and RegBA (PrrBA) (2, 9, 10, 32, 46) during phototrophic growth in *R. sphaeroides* and *R. capsulatus*.

A question that must be addressed concerns the potential role and coordinate control of specific metabolic signals with redox homeostasis in response to environmental factors. In *R. sphaeroides* (7) and *Rhodopseudomonas palustris*, whose genomic sequence was recently completed (http://www.jgi.doe.gov/tempweb/JGI microbial/html/index.html), it is apparent that a single *cbbR* gene controls the transcription of the two major *cbb* operons. A separate upstream and divergently transcribed *cbbR* gene, however, controls each *cbb* operon in *R. capsulatus* (38, 39, 53). LysR-type transcriptional regulators, such as CbbR, generally utilize a metabolite or coinducer produced by the pathway they regulate (45). Clearly, the current study has demonstrated that a complex interrelationship of specific redox-balancing systems exists in *R. capsulatus* and probably other nonsulfur purple bacteria. Since activation of the DMSOR system affects control of the CBB system under photoautotrophic environmental conditions and in some instances may also cause up-regulation of promoter sequences important for redox balancing under photoheterotrophic growth conditions, it is important to determine if these observed regulatory events are coordinated with the appearance of and subsequent interaction with a specific metabolic signal metabolite(s). Perhaps strain SBI/II can be effectively used in such investigations since there is dramatic up-regulation of operons important for redox control in this strain. Continued studies of the nature of the signal(s) that influences both CbbR and the more global redox-sensing pathways required for photoheterotrophic and photoautotrophic growth are warranted.

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