Complex I and Its Involvement in Redox Homeostasis and Carbon and Nitrogen Metabolism in *Rhodobacter capsulatus*

MARY A. TICHI, WIM G. MEIJER, AND F. ROBERT TABITA*

Department of Microbiology and the Plant Molecular Biology/Biotechnology Program, The Ohio State University, Columbus, Ohio 43210-1292

Received 24 April 2001/Accepted 21 September 2001

A transposon mutant of *Rhodobacter capsulatus*, strain Mal7, that was incapable of photoautotrophic and chemooautotrophic growth and could not grow photoheterotrophically in the absence of an exogenous electron acceptor was isolated. The phenotype of strain Mal7 suggested that the mutation was in some gene(s) not previously shown to be involved in CO₂ fixation control. The site of transposition in strain Mal7 was identified and shown to be in the gene *nuoF*, which encodes one of the 14 subunits for NADH ubiquinone-oxidoreductase, or complex I. To confirm the role of complex I and *nuoF* for CO₂-dependent growth, a site-directed *nuoF* mutant was constructed (strain SBC1) in wild-type strain SB1003. The complex I-deficient strains Mal7 and SBC1 exhibited identical phenotypes, and the pattern of CO₂ fixation control through the Calvin-Benson-Bassham pathway was the same for both strains. It addition, it was shown that electron transport through complex I led to differential control of the two major *cbb* operons of this organism. Complex I was further shown to be linked to the control of nitrogen metabolism during anaerobic photosynthetic growth of *R. capsulatus*.

*Rhodobacter capsulatus* is a nonsulfur purple phototrophic bacterium that exhibits a wide range of metabolic capabilities, making it and related organisms probably the most versatile of prokaryotes (28). *R. capsulatus* grows under dark, aerobic (chemoautotrophic or chemoheterotrophic) conditions using branched respiratory electron transport pathways; in addition, these organisms can grow under anaerobic (photoautotrophic or photoheterotrophic) conditions in the light via cyclic photosynthetic electron transport to generate a proton motive force. Fermentative growth is also an option.

During phototrophic growth of *Rhodobacter*, redox poised is achieved through the interplay of cyclic photosynthetic electron transport and specific redox-balancing mechanisms of anaerobic metabolism (32). Indeed, it has been suggested that the electron acceptors involved in photosynthetic metabolism function as a sink for excess reducing equivalents to prevent the overreduction of cyclic electron transport; this interaction and control of redox poised and electron transport occur at the level of the ubiquinone pool (12). Under photoheterotrophic growth conditions, the oxidation of C₄-dicarboxylic acids (such as l-malate and succinate) can result in overreduction of the ubiquinone pool (32). The excess reducing equivalents, at the level of the reduced ubiquinone pool, are transferred to NAD⁺ by reverse electron flow mediated by complex I (NADH ubiquinone oxidoreductase) (8). Reducing equivalents stored in the reduced pyridine nucleotide generated by complex I activity may then be dissipated via metabolic systems involved in balancing the intracellular redox state of the organism. These metabolic systems include the Calvin-Benson-Bassham (CBB) reductive pentose phosphate pathway (CBB system) and dinitrogenase system, among others. The dimethyl sulfoxide (DMSO) reductase (DMSOR) system also serves to directly sustain the redox poised of the ubiquinone pool for photosynthetic electron transport (30, 31). Additionally, during photooxygenating metabolism (in which CO₂ serves as the primary carbon source), molecular hydrogen serves as the reductant, and by means of a membrane-bound hydrogenase, electrons are donated directly into the ubiquinone pool (18, 40, 54).

Complex I is the enzyme that mediates reverse electron flow between the reduced quinone pool and NAD⁺ during photooxygenating and photoheterotrophic growth in *R. capsulatus* (7, 20, 29). *R. capsulatus* contains a single complex I enzyme complex (19), and most of the respective *nuo* genes have been cloned and sequenced (5, 6, 19). Bacterial complex I of *Paracoccus denitrificans* (63), *Thermus thermophilus* (65), *Escherichia coli* (59), and *R. capsulatus* (5, 6, 19) contain a minimum of 14 different subunits homologous with mitochondrial complex I (which consists of at least 40 different subunits). Due to its complexity, complex I is the least-understood component of the various known respiratory complexes. Only the *R. capsulatus* complex I was shown to readily reverse electron flow between the quinone pool and NAD⁺ (7, 9).

Previously, it was established that complex I-deficient mutants of *R. capsulatus* could not grow photoautotrophically due to overreduction of the quinone pool. That is, electrons donated to ubiquinone from hydrogen oxidation could not be used for NAD⁺ reduction (20). Moreover, mutants of *R. capsulatus* that lack a functional complex I cannot grow photoheterotrophically with malate as the carbon source; such strains require the addition of an exogenous electron acceptor such as DMSO to overcome this defect (7). Further studies concerning the role of complex I in redox homeostasis under phototrophic growth conditions have not progressed beyond these initial studies (7, 20). During the course of examining the factors...
involved in regulating CO₂ fixation in *R. capsulatus* and *R. sphaeroides*, we isolated mutations that were shown to disrupt complex I. Furthermore, our studies indicated that the control of both carbon and nitrogen metabolism is linked to complex I function in *R. capsulatus*.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** Relevant *R. capsulatus* and *E. coli* strains as well as plasmids used or constructed in this study are listed in Table 1. 

**Transposon mutagenesis.** Transposon mutagenesis was performed on wild-type strain B10 using suicide vector pSUP9011. Mutant selection using *R. capsulatus* was initiated because it, unlike most nonsulfur purple bacteria, grows well under aerobic chemoautotrophic growth conditions. This additional aerobic *R. capsulatus* was initiated because it, unlike most nonsulfur purple bacteria, grows well.

**Plasmids**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. capsulatus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10</td>
<td>Wild type, originally isolated from St. Louis, Mo. (soil)</td>
<td>57</td>
</tr>
<tr>
<td>SB1003</td>
<td>Rif derivative of wild-type strain B10</td>
<td>66</td>
</tr>
<tr>
<td>Mal7</td>
<td>Isolated by random transposon mutagenesis, derivative of B10, Kan'</td>
<td>This work</td>
</tr>
<tr>
<td>SBC1</td>
<td><em>nuoF</em>:Gm' derivative of SB1003</td>
<td>This work</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JM109</td>
<td>recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 (lac-proAB)</td>
<td>64</td>
</tr>
<tr>
<td>SI7-1apir</td>
<td>St' Ty' Spc', λpir and mobilizing factors on chromosome</td>
<td>39</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pACYC184</td>
<td>Cm'Tc', low copy number</td>
<td>4</td>
</tr>
<tr>
<td>pUC19</td>
<td>Ap', high copy number</td>
<td>41</td>
</tr>
<tr>
<td>pSUP9011</td>
<td>Km', suicide vector containing Tn5::mob</td>
<td>47</td>
</tr>
<tr>
<td>pRK2013</td>
<td>Km', helper plasmid for trivalent conjugation</td>
<td>13</td>
</tr>
<tr>
<td>pXLB</td>
<td>Tc', <em>R. capsulatus</em> cbb₃ translational promoter fusion to lacZ</td>
<td>38</td>
</tr>
<tr>
<td>pXFB</td>
<td>Tc', <em>R. capsulatus</em> cbb₃ translational promoter fusion to lacZ</td>
<td>38</td>
</tr>
<tr>
<td>pUC1318-Gm</td>
<td>pUC1318 containing a Gm' cassette cloned as a 1.6-kb <em>HindIII</em>-SpeI fragment from plasmid pFKR-1</td>
<td>S. A. Smith, this laboratory</td>
</tr>
<tr>
<td>pJPTC</td>
<td>Tc', mobilizable suicide vector containing a single <em>EcoRI</em> site located in the multiple cloning site</td>
<td>43</td>
</tr>
<tr>
<td>pMa17-1</td>
<td>pACYC184 containing the Tn5::mob site on a 6.0-kb <em>EcoRI</em> fragment of chromosomal DNA from strain Mal7</td>
<td>This work</td>
</tr>
<tr>
<td>p19M7-4.8EH</td>
<td>pUC19 containing 3.7 kb of <em>R. capsulatus</em> chromosomal DNA and 1.1 kb of Tn5::mob plasmid pMa17-1 cloned as a 4.8-kb <em>EcoRI</em>-HindIII fragment</td>
<td>This work</td>
</tr>
<tr>
<td>p19M7-3.8ESp</td>
<td>pUC19 containing 3.7 kb of <em>R. capsulatus</em> chromosomal DNA and ~0.15 kb of Tn5::mob plasmid p19M7-4.8EH cloned as a 3.8-kb <em>EcoRI</em>-Smal fragment into <em>EcoRI</em> and <em>Sphi</em> sites</td>
<td>This work</td>
</tr>
<tr>
<td>p19-nuoFGM</td>
<td>p19M7-3.8ESp with a Gm' cassette cloned into the blunt-ended <em>NcoI</em> site of <em>nuoF</em> as a 1.6-kb blunt-ended <em>PstI</em> fragment from plasmid pUC1318-Gm</td>
<td>This work</td>
</tr>
<tr>
<td>pJPTC-nuoFGM</td>
<td>p19-nuoFGM cloned into vector pJPTC by linearizing with <em>EcoRI</em></td>
<td>This work</td>
</tr>
</tbody>
</table>

**Media and growth conditions.** *E. coli* strains were grown aerobically on Luria-Bertani medium (2) at 37°C with appropriate antibiotic selection. Phototrophic cultures of *R. capsulatus* were grown in front of banks of incandescent lights at 30 to 33°C as previously described (11, 37); phototrophic cultures were grown anaerobically in Ormerod’s medium (36) supplemented with 0.4% (wt/vol) K₂-malate and 1 μg of thiamine/ml. The nitrogen source was provided either

**TABLE 1. Bacterial strains and plasmids used in this study**
as 30 mM ammonia, 6.8 mM L-glutamate, or N₂ gas (95% N₂–5% CO₂). Photosynthetic cultures were sparged continuously with 1.5% CO₂–98.5% H₂. Chemoheterotrophic cultures were grown aerobically in the dark in a defined medium containing 0.4% fructose as the carbon source at 30 to 33°C in PYE medium. The concentrations of antibiotics used for selection of the *R. capsulatus* strains were as follows: rifampin, 50 µg/ml; kanamycin, 5 µg/ml; trimethoprim, 100 µg/ml; gentamicin (Gm), 10 µg/ml; and tetracycline (Tet), 3 µg/ml for stock cultures or 0.5 µg/ml for plasmid maintenance during phototrophic growth conditions. For *E. coli*, the antibiotic concentrations for plasmid maintenance were: ampicillin, 100 µg/ml; chloramphenicol, 100 µg/ml; kanamycin, 10 µg/ml; gentamicin, 15 µg/ml; and tetracycline, 6 µg/ml. DMSO was used at a concentration of 30 mM.

**Cell extracts and enzyme assays.** Culture samples (10 to 20 ml) from 400-ml bottle cultures (37) were harvested in late exponential phase (optical density at 660 nm [OD₆₆₀] = 0.9 to 1.2), washed in buffer (25 mM Tris-HCl, 1 mM EDTA [pH 8.0]), and disrupted by sonication. The resultant cell debris was removed by centrifugation for 15 min at 18,000 × g at 4°C. β-Galactosidase activity was measured by continuously monitoring the increase in absorbance at 405 nm from the substrate-dependent production of *o*-nitrophenol (33). Specific activities were calculated using the change in steady-state *A*₄₀⁵ per min (55).
Ribulose 1,5-bisphosphate carboxylase activity was assayed by measuring ribulose 1,5-bisphosphate-dependent 14CO2 fixation into acid-stable material (60). Phosphoribulokinase (PRK) activity was measured following previously described protocols (50) except that ribulose 5-phosphate was generated from ribose 5-phosphate by the addition of 5 U of phosphoribosyltransferase (Sigma Chemical, St. Louis, Mo.). Protein concentrations were determined using the Bio-Rad protein assay dye-binding reagent (Bio-Rad Laboratories, Hercules, Calif.) using bovine serum albumin as a standard.

RESULTS

Identification of nuoF gene as the site of Tn5::mob transposition. Subclones derived from plasmid pMal7-1 were generated so that DNA sequence analysis could be performed using M13/pUC forward and reverse sequencing primers (Fig. 1). The site of insertion was determined to be in the 5′ part of the nuoF gene. These sequencing results in strain B10 were compatible with the previous gene order and sequences described by Dupuis et al. (8), also using strain B10.

As shown (Fig. 2), the nuo genes are organized as a single 18-kb gene cluster that encodes 14 subunits of complex I (subunits NuoA to NuoN) (9, 19). The 14 nuo genes of R. capsulatus encode proteins homologous to the 14 major subunits of mitochondrial complex I and complex I from P. denitrificans and E. coli (9, 27, 59). In contrast to the compact nqo operon of P. denitrificans (61) and the nuo operon of E. coli (59), the nuo operon of R. capsulatus contains additional reading frames. These potential gene products are not related to any other subunits of complex I in eukaryotes or bacteria and are speculated to function somehow in regulation and protein assembly (19, 61).

The deduced amino acid sequence of the nuoF product, the site of Tn5::mob insertion in strain Mal7, contains a typical nucleotide-binding sequence motif (GXGXXG) (63). These residues are involved in the binding of NADH. Thus, NuoF is an important component for electron transport to and from the enzyme (63).

Construction of nuoF mutant strains. To provide confirmation of the phenotype and results obtained with transposon mutant Mal7, strain SBC1 (nuoF) was constructed as described in Materials and Methods in strain SB1003, with the orientation of the Gm resistance cassette depicted (Fig. 2). The Gm interposon insertion in nuoF was situated in position 5403 of the nuo operon (at an NcoI site), approximately 190 bp downstream from the Tn5::mob transposition site (Fig. 2).

Analysis of complex I-deficient mutants of R. capsulatus. Strain Mal7 could not grow photoautotrophically (1.5% CO2–98.5% H2) or chemoautotrophically (H2–CO2–air) as well as photoheterotrophically on malate in the absence of DMSO (Table 2). The nuoF site-directed mutant of R. capsulatus, strain SBC1 showed a phototrophic growth phenotype comparable to that of strain Mal7 (Table 2). Photoautotrophic and
chemoautotrophic cultures of complex I-deficient strains were supplemented with DMSO, since the addition of DMSO to phototrophic cultures rescued the growth of these strains. Distinct from phototrophic growth conditions, strains Mal7 and SBC1 were not able to achieve growth under autotrophic conditions when DMSO was added. By contrast, wild-type strains B10 and SB1003 grew under all conditions (Table 2), and all strains (mutants and wild type) could grow under dark aerobic conditions with either fructose or malate as the carbon source (Table 2).

Thus, it is apparent that complex I-deficient strains of *R. capsulatus* were not able to grow under conditions in which the CBB pathway is required to maintain the redox poise of the cell (phototrophic growth in the absence of DMSO) or under conditions in which CO₂ assimilation provides all the carbon for subsequent cell metabolism (chemo- or phototrophic growth). This phenotype is comparable to CBB-deficient strains of *R. capsulatus* (38, 50) and ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO)-deficient strains of *Rhodobacter sphaeroides* (11). Unlike *R. sphaeroides*, CBB-deficient strains of *R. capsulatus* do not require an exogenous electron acceptor (DMSO) for phototrophic growth when glutamate is used as the nitrogen source (50). When CBB-deficient strains of *R. capsulatus* are supplemented with glutamate, the dinitrogenase system is induced, presumably providing a sufficient compensatory electron sink to support phototrophic growth in the absence of a functional CBB cycle. Thus, it was of interest to determine if the dinitrogenase system could support growth of complex I-deficient strains in the absence of DMSO (Table 3).

Unlike wild-type strains B10 and SB1003, strains Mal7 and SBC1 could not grow in the absence of DMSO with either glutamate or N₂ as the supplied nitrogen source, whereas growth was rescued when cultures were supplemented with DMSO (Table 3). These findings are in stark contrast to a complex I-deficient strain of *R. capsulatus* generated by Herter et al. (19), which was reported to be able to grow phototrophically in the absence of DMSO (even under nitrogen-fixing growth conditions). Our results do support the findings of Dupuis et al. (7), who suggested that complex I is absolutely essential for the maintenance of redox homeostasis, as previously isolated mutants of complex I (7), as well as the mutants in this study, could not grow under phototrophic conditions in the absence of DMSO.

### Control of CBB system in complex I-deficient strains Mal7 and SBC1

The levels of activity for the key enzymes of the CBB pathway, RubisCO and PRK, of strain Mal7 were determined, and strain Mal7 exhibited levels of activity similar to the wild-type strain B10 during phototrophic growth with DMSO (data not shown). Likewise, levels of RubisCO and PRK activity for strain SBC1 were comparable to those of wild-type strain SB1003 during phototrophic growth with DMSO, and typical enhanced levels for these enzymes (25, 46) were found in wild-type strains under phototrophic growth conditions (1.5% CO₂–98.5% H₂) (data not shown).

Complex I-deficient strains Mal7 and SBC1 maintained wild-type control of the cbb₁ promoter during phototrophic growth supplemented with DMSO when either ammonia or glutamate was used as the nitrogen source; moreover, levels of cbb₁ promoter activity in strain Mal7 were similar to those in wild-type strain B10, and the levels of cbb₁ promoter activity in strain SBC1 were comparable to those in its wild-type strain (SB1003) during phototrophic growth with DMSO (Table 4). Thus, like the levels of RubisCO and PRK activity, complex I-deficient strains exhibited wild-type control of the expression of the two CBB pathway operon promoters when grown phototrophically in the presence of DMSO.

Consistent with the established regulation of the cbb₁ operon in *R. capsulatus* (38, 53), wild-type strains B10 and SB1003 did not exhibit cbb₁ promoter activity under phototrophic growth conditions in the absence or presence of DMSO, regardless of the nitrogen source; cbb₁ promoter levels were significantly enhanced, as expected, in wild-type strain B10 after photoautotrophic (1.5% CO₂–98.5% H₂) growth (data not shown).

Obviously, the inability to grow photoautotrophically precludes measurements of either RubisCO/PRK or cbb₁ promoter activity under these conditions.

### Table 2. Growth phenotypes of complex I-deficient strains (Ma17 and SBC1) compared to those of wild-type strains (B10 and SB1003) of *R. capsulatus*.

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Growth of strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B10</td>
</tr>
<tr>
<td>Chemoautotrophic</td>
<td></td>
</tr>
<tr>
<td>Fructose/O₂</td>
<td>+</td>
</tr>
<tr>
<td>Malate/O₂</td>
<td>+</td>
</tr>
<tr>
<td>Chemoautotrophic</td>
<td></td>
</tr>
<tr>
<td>CO₂/H₂/O₂</td>
<td>+</td>
</tr>
<tr>
<td>CO₂/H₂/O₂/DMSO</td>
<td>+</td>
</tr>
<tr>
<td>Phototrophic</td>
<td></td>
</tr>
<tr>
<td>Malate</td>
<td>+</td>
</tr>
<tr>
<td>Malate/DMSO</td>
<td>+</td>
</tr>
<tr>
<td>Phototrophic</td>
<td></td>
</tr>
<tr>
<td>CO₂/H₂</td>
<td>+</td>
</tr>
<tr>
<td>CO₂/H₂/DMSO</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 3. Phototrophic growth with various nitrogen sources in the absence and presence of the ancillary electron acceptor DMSO.

<table>
<thead>
<tr>
<th>Carbon/nitrogen source</th>
<th>Redox balancing system(s) used</th>
<th>Growth of strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B10</td>
</tr>
<tr>
<td>Malate/ammonia</td>
<td>CBB</td>
<td>+</td>
</tr>
<tr>
<td>Malate/ammonia/DMSO</td>
<td>CBB, DMSOR</td>
<td>+</td>
</tr>
<tr>
<td>Malate/glutamate</td>
<td>CBB, nitrogenase</td>
<td>+</td>
</tr>
<tr>
<td>Malate/glutamate/DMSO</td>
<td>CBB, nitrogenase, DMSOR</td>
<td>+</td>
</tr>
<tr>
<td>Malate/N₂/DMSO</td>
<td>CBB, nitrogenase</td>
<td>+</td>
</tr>
</tbody>
</table>

Downloaded from http://jb.asm.org/ on September 8, 2017 by guest
activity levels in complex I-deficient mutants. Previous results indicated that mere incubation of phototrophically grown photoautotrophic-inefficient strains in an organic carbon-free, photoautotrophic (1.5% CO\textsubscript{2}-9.5% H\textsubscript{2}) gas environment is sufficient to enable the CBB enzymes and promoters to be induced in the absence of growth (38, 53).

To determine if the complex I mutants could induce these enzymes and promoters, the time course for induction of β-galactosidase activity was determined using \( \text{cbb}_{I}:\text{lacZ} \) and \( \text{cbb}_{II}:\text{lacZ} \) promoter fusions in wild-type strain SB1003 and mutant strain SBC1 following transition to a photoautotrophic (1.5% CO\textsubscript{2}-98.5% H\textsubscript{2}-ammonia) environment. The transition to a photoautotrophic growth environment resulted in induction, and a constant level of \( \text{cbb}_{I} \) promoter activity and \( \text{cbb}_{II} \) promoter activity in wild-type strain SB1003, while \( \text{cbb}_{I} \) promoter activity was not induced in strain SBC1 and \( \text{cbb}_{II} \) promoter activity was maintained at levels obtained during photoheterotrophic growth in the presence of DMSO (Fig. 3A). Similar results were obtained when total Rubisco and PRK activity was compared in strains B10 and Mal7 (Fig. 3B). It appears that complex I-deficient strains of \textit{R. capsulatus} were deficient in their ability to transduce the signal that influences the CBB system to be induced under photoautotrophic growth conditions.

### DISCUSSION

During photosynthetic growth of \textit{Rhodobacter}, the interplay between specific metabolic redox-balancing mechanisms (such as the CBB, dinitrogenase, and DMSOR systems, among others) is important to maintain the redox poise of the cyclic electron transport chain (12, 16, 23, 30, 45, 50). This aspect of the physiology of \textit{Rhodobacter} will undoubtedly contribute to an understanding of interactive control of the diverse metabolic capabilities exhibited by these organisms.

The cyclic photosynthetic electron transport chain of \textit{Rhodobacter} begins with absorption of light, leading to the oxidation of P-870 and subsequent reduction of ubiquinone in the photosynthetic reaction center. Following this, electron transport through the ubiquinone pool, cytochrome \( b/c \) complex, and cytochrome \( c_{5} \) completes the cycle (for a review, see reference 52 and citations therein). The actual interaction between cyclic electron transport and redox poise was suggested to occur at the level of the ubiquinone pool, since cyclic photosynthesis requires oxidized ubiquinone as an electron acceptor. An overreduction of the ubiquinone pool has been postulated to potentially saturate the photosynthetic pathway (8, 12). Thus, complex I contributes to maintenance of the redox state of the ubiquinone pool by transferring excess reducing equivalents to NAD\(^{+}\), generating NADH (7, 20). In this way, complex I functions mainly as an electron sink by keeping part of the ubiquinone pool oxidized, allowing cyclic photosynthesis to continue. Excess reducing equivalents are typically dissipated by CO\textsubscript{2} assimilation via the CBB system or, in some instances, by the dinitrogenase system. Indeed, the control of carbon and nitrogen metabolism and redox homeostasis is linked in \textit{Rhodobacter} (23, 50). From the results presented here, it is apparent that complex I plays a major role in balancing reducing equivalents generated through carbon and nitrogen metabolism and redox homeostasis in \textit{R. capsulatus}.

Mutants lacking a functional complex I were not able to grow under photoautotrophic conditions (Table 2) or under photoheterotrophic conditions in the absence of an external electron acceptor (Table 2). This is because electrons transferred to ubiquinone from the oxidation of hydrogen (photoautotrophic metabolism) or from the oxidation of carbon substrates such as L-malate and succinate (photoheterotrophic metabolism) could not be used for NAD\(^{+}\) reduction by complex I (7, 20). When substrates such as L-malate are added to cultures, CO\textsubscript{2} is produced from malate metabolism, and this “metabolic” CO\textsubscript{2} subsequently serves an important function, as it is the preferred electron acceptor for reducing equivalents produced during carbon oxidation. Thus, the CBB system, rather than serving as a major means for generating organic carbon (as in photoautotrophic metabolism), plays more of a role to balance the oxidation-reduction potential of the cell during photoheterotrophic growth (12, 26, 56). Therefore, in the absence of a functional complex I enzyme complex, reducing equivalents cannot be provided for CO\textsubscript{2} fixation through the CBB system.

CBB-deficient strains of \textit{R. sphaeroides} (11, 16, 17) and \textit{R. capsulatus} (38, 50) exhibited a phenotype similar to that of complex I-deficient strains. Indeed, \textit{nuo} (Table 2) and \textit{cbb} (38, 50) disruption mutants were unable to achieve photoheterotrophic growth with a fixed nitrogen source in the absence of an exogenous electron acceptor such as DMSO.

\textit{Rhodobacter} species are especially sensitive to imbalances in the redox state (30). The permissive effect of DMSO respiration through the DMSOR system, to allow both complex I- and CBB-deficient strains to grow photoheterotrophically, suggests that both types of mutants were affected by an excess of re-

### TABLE 4. \( \text{cbb}_{I} \) and \( \text{cbb}_{II} \) promoter activity measured in wild-type and complex I-deficient strains

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>( \text{B10 or SB1003} )</th>
<th>( \text{Ma17 or SBC1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{cbb}_{I}:\text{lacZ} )</td>
<td>( \text{cbb}_{II}:\text{lacZ} )</td>
</tr>
<tr>
<td>Photoheterotrophic (B10, Ma17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malate/ammonia/DMSO</td>
<td>0</td>
<td>297 ± 35</td>
</tr>
<tr>
<td>Malate/glutamate/DMSO</td>
<td>0</td>
<td>342 ± 24</td>
</tr>
<tr>
<td>Photoheterotrophic (SB1003, SBC1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malate/ammonia/DMSO</td>
<td>0</td>
<td>141 ± 24</td>
</tr>
<tr>
<td>Malate/glutamate/DMSO</td>
<td>0</td>
<td>80 ± 7</td>
</tr>
</tbody>
</table>

\(^{a}\) β-Galactosidase activity was determined from three to four independent cultures assayed in duplicate. Strains were grown under photoheterotrophic conditions with either ammonia or glutamate as the nitrogen source in the presence of DMSO.
FIG. 3. Time course of β-galactosidase activity induction using form I promoter fusion (cbb_H::lacZ) and form II promoter fusion (cbb_H::lacZ) (A) and total RubisCO and PRK activity (B) following the transition to a photoautotrophic (1.5% CO₂–98.5% H₂–ammonia) environment. Late-exponential-phase (OD₆₆₀ = 0.9 to 1.2) malate-grown photoheterotrophic cultures of wild-type strain SB1003 and strain SBC1 (A) or wild-type strain B10 and strain Mal7 (B) grown with malate-ammonia-DMSO were washed three times with minimal medium and incubated photoautotrophically (1.5% CO₂–98.5% H₂) in the absence of the exogenous electron acceptor DMSO. At time zero, incubation began under photoautotrophic conditions; wild-type strains grew further under these conditions.
ducing equivalents, most likely generated at the level of the ubiquinone pool. Since the DMSOR system serves to directly sustain the redox poise of the ubiquinone pool for photosynthetic electron transport (30, 31, 45), it is not surprising that the addition of DMSO rescues the growth of both CBB- and complex I-deficient strains. Thus, both the CBB system and complex I are required for redox homeostasis during photoheterotrophic growth conditions in the absence of ancillary electron acceptors.

However, there is a difference in DMSO tolerance between CBB- (50) and complex I-deficient strains of \textit{R. capsulatus} in that complex I-deficient strains were unable to grow during photoheterotrophic conditions permissive for the dinitrogenase system (Table 3). Growth was impossible for complex I-deficient strains when glutamate or \( \text{N}_2 \) was used as the nitrogen source (Table 3). Previous work established that the dinitrogenase system serves as a compensatory electron sink in \textit{R. capsulatus} in the absence of a functional CBB system (50). In fact, dinitrogenase-catalyzed proton reduction by the hydrogenase-like activity of the dinitrogenase system requires a major energy commitment by the cell, and this activity contributes extensively to redox homeostasis (21, 48). The dinitrogenase system is such an efficient reductant of excess reducing equivalents that CBB-deficient strains of \textit{R. sphaeroides} and \textit{Rhodospirillum rubrum} (23), plus \textit{R. capsulatus} (50), derepress synthesis of the dinitrogenase system and abrogate normal control mechanisms in order to achieve photoheterotrophic competency in the absence of exogenous electron acceptors.

The addition of DMSO to cultures of complex I-deficient strains incubated in an ammonia-free environment rescued growth under nitrogen-fixing conditions (Table 3). Whether the permissive effect of DMSO respiration on the ability of complex I-deficient strains to grow under nitrogen-fixing conditions was due to balancing of the oxidation-reduction state of the ubiquinone pool or to sufficient electron flow to ferredoxin I (electron donor to nitrogenase) (24) remains to be determined. In any event, complex I function appeared to be linked to nitrogen metabolism in \textit{R. capsulatus}, unlike the findings in a previous report (20).

Control mechanisms involved in mediating the function of the \textit{nuo} operon of \textit{R. capsulatus} remain to be established. Transcriptional regulation of the \textit{nuoA} gene locus of \textit{E. coli} involves regulation by the global two-component regulatory system ArcB/A as well as by FNR (references 3 and 51 and citations therein). In \textit{R. capsulatus}, a global two-component signal transduction system, RegB/A (PrrB/A), has been shown to play a role in the regulation of key operons involved in photosynthesis, including operons that encode structural genes for the light-harvesting complexes (\textit{puf} and \textit{puc}) and the photosynthetic reaction center (\textit{puf}) involved in photosynthetic gene expression (22, 34). In addition, the Reg/Prr system has been implicated in controlling \( \text{CO}_2 \) fixation (43, 53) as well as nitrogen fixation and \( \text{H}_2 \) oxidation (10, 23, 44). Thus, during photosynthetic conditions, the Reg/Prr system regulates components of the cyclic electron transport chain as well as systems involved in redox homeostasis.

Complex I appears to provide linkage between the poise of the photosynthetic electron transport chain while balancing the means by which reducing equivalents are generated during carbon metabolism. Conceivably, the Reg/Prr system might also play a role in regulating the \textit{nuo} operon of \textit{R. capsulatus} and \textit{R. sphaeroides}. Additionally, the Reg/Prr system has been shown to be involved in the transcription of \textit{cbb} \( \text{II} \) promoter expression as well as maximal expression of the \textit{cbb} \( \text{II} \) promoter under photoautotrophic growth conditions in \textit{R. capsulatus} (53). Complex I-deficient strains appeared to be blocked in a necessary signal for induction of \textit{cbb} expression (Fig. 3). Perhaps a redox signal (such as the flow of reductant through the ubiquinone pool) is transduced through a pathway that involves complex I and is then conveyed to the Reg/Prr signal transduction pathway. Possibly the absence of a functional complex I enzyme complex disrupted a necessary redox signal required by the Reg/Prr system, which in turn affected \textit{cbb} induction.

In \textit{R. sphaeroides}, the \textit{cbb} \( \text{III} \) cytochrome \( c \) oxidase senses the redox state of the quinone pool and transduces a signal to the Reg/Prr system, which in turn regulates expression of photosynthesis genes in response to \( \text{O}_2 \) (35). Obviously, further studies are required to elucidate a potential linkage between the Reg/Prr signal transduction regulatory system and complex I in \textit{R. capsulatus} during photoheterotrophic and photoautotrophic growth conditions. Additional control mechanisms could also be involved in mediating the function of the \textit{nuo} operon of \textit{R. capsulatus}. Indeed, a characteristic LysR-type consensus DNA-binding motif (\( \text{T-N}_{11}-\text{A} \)) (14) was localized as an inverted repeat separated by a 21-bp spacer in the promoter region of the \textit{nuo} operon of \textit{R. capsulatus} (data not shown), raising the possibility that LysR-type transcriptional regulators could also be involved in controlling \textit{nuo} expression in \textit{R. capsulatus}. More detailed studies need to be done to address these possibilities.

**ACKNOWLEDGMENTS**

We thank A. G. McEwan for helpful discussions concerning this work. This work was supported by Public Health Service grant GM 45404 from the National Institutes of Health.

**REFERENCES**


units are all essential for the biogenesis of the respiratory NADH-ubiquinone oxidoreductase. Mol. Microbiol. 28:531–541.


38. Whitman, W., and F. R. Tabita. 1976. Inhibition of ribulose 1,5-bisphosphate carboxylase/oxygenase activity in vivo in mutants of Rhodobacter sphaeroides carrying the gene cluster encoding the energy-trans-

