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Cloning and Characterization of *senC*, a Gene Involved in Both Aerobic Respiration and Photosynthesis Gene Expression in *Rhodobacter capsulatus*

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The purple nonsulfur photosynthetic eubacterium *Rhodobacter capsulatus* is a versatile organism that can obtain cellular energy by several means, including the capture of light energy for photosynthesis as well as the use of light-independent respiration, in which molecular oxygen serves as a terminal electron acceptor. In this study, we have identified and characterized a novel gene, *senC*, mutations in which affect respiration as well as the induction of photosynthesis gene expression. The protein coded by *senC* exhibits 33% sequence identity to the yeast nucleus-encoded protein SCO1, which is thought to be a mitochondrion-associated cytochrome *c* oxidase assembly factor. Like yeast SCO1, SenC is required for optimal cytochrome *c* oxidase activity in aerobically grown *R. capsulatus* cells. We further show that *senC* is required for maximal induction from the *puf* and *puh* operons, which encode the structural polypeptides of the light-harvesting and reaction center complexes.

The purple nonsulfur photosynthetic bacterium *Rhodobacter capsulatus* is a highly versatile organism that is capable of growing under a wide range of nutritional and environmental conditions. When grown anaerobically, these cells synthesize pigments and pigment-binding polypeptides that comprise a photosystem that can efficiently convert light energy into a useful form of cellular energy. In contrast, cells grown in the presence of molecular oxygen do not synthesize a photosystem; instead, the cells utilize a respiratory chain for energy production. Many species of bacteria contain a branched respiratory chain that ends with at least two distinct terminal oxidases (19, 47). In *R. capsulatus*, one branch (Fig. 1) contains a *cb*-type cytochrome *c* oxidase termed C_{ox} (or cytochrome b_{410}) (20, 30, 55) and the other branch contains a bb_3-type quinol oxidase termed Q_{ox} (or cytochrome b_{260}) (56). Electron transfer in the *Rhodobacter* C_{ox} branch is similar to that of the mitochondrial respiratory chain in that they both involve cytochrome bc_1 and a cytochrome *c* (c_2 or c_y) as electron carriers (2, 24, 55, 57). In contrast, quinol oxidase obtains electrons directly from the quinone pool, thus bypassing the requirement for cytochrome bc_1 and a cytochrome *c* (2, 54).

Genetic and biochemical analyses have also indicated that the mitochondrial enzyme is more complex than that of prokaryotic versions in that the isolated mitochondrial enzyme contains up to 10 nucleus-encoded subunits in addition to the three core subunits (COXI, COXII, and COXIII) that are coded by the mitochondrial genome (12, 27, 46, 49). In addition to possible functional roles, some of these nuclear loci appear to be required for translation of the mitochondrion-encoded *COX* loci (13) or in assembly of an active enzyme complex (41). An example of the latter case can be found in the yeast nucleus-encoded mitochondrial membrane protein SCO1 (9, 40, 41). Although its exact function is unknown, SCO1 is required for the accumulation of a functional cytochrome *c* oxidase complex presumably by promoting assembly of the COXI and COXII subunits (28). Alternatively, SCO1 may act to stabilize the cytochrome oxidase oligomer, since in the absence of SCO1, COXI and COXII are rapidly degraded.

In this study, we demonstrate that *R. capsulatus* contains a gene, termed *senC*, that is a homolog of the yeast *SCO1* gene. Like the requirement of SCO1 for cytochrome *c* oxidase activity in mitochondria, mutational analysis demonstrates that SenC is required for optimal *cb*-type cytochrome *c* oxidase activity. Rather unexpectedly, *senC* is shown to be located within a cluster of *trans*-acting regulatory genes that control photosynthesis gene expression in response to alterations in oxygen tension and light intensity (10, 11, 34, 43). We also demonstrate that a strain with a deletion of *senC* also fails to maximally induce photosynthesis gene expression in response to a reduction in oxygen tension, indicating that SenC may also coordinate synthesis of the bacterial photosystem in response to the rate of cellular respiration.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *R. capsulatus* cells were grown at 34°C in the complex medium PYS, in RCV 2/3 PY (53), or in the defined medium RCV, pH 6.8 (51). Spectinomycin and kanamycin concentrations were 10 μg/ml, whereas tetracycline concentrations were 0.5 μg/ml. Dark aerobic growth conditions were achieved by growing 10-ml cultures in 250-ml flasks that were shaken at 300 rpm. Dark semiaerobic growth was achieved by growing 200-ml cultures in 250-ml flasks with a cotton stopper that was shaken at 90 rpm. Light anaerobic (photosynthetic) growth conditions were achieved by completely filling 18-ml screw-cap tubes with growth medium. Illumination was provided by banks of incandescent Lumiline 60-W lamps which were maintained at an intensity of 250 lux as monitored through use of a model 755 Weston Illuminator Meter (Weston Instruments Inc.). To prevent secondary effects such as self-shading, cell turbidity was monitored with a Klett-Summerson spectrophotometer (red filter), and the cells were harvested between 50 and 120 Klett units.

Genomic library construction and cloning. Hybridization analysis (data not shown) indicated that a 15-kbp *Bgl*II DNA restriction fragment contained *regA* and *hvrA* as well as approximately 14 kbp of DNA upstream of this region. Consequently, a library of wild-type *R. capsulatus* genomic DNA was constructed by electroelution from an agarose gel slice that contained 9.5- to 23-kbp *Bgl*II restriction fragments. Purified DNA was then ligated into *Bam*HI-digested λ-Dash vector (Stratagene) and packaged into *Escherichia coli* λ host strain LE392, using a Stratagene Gigapack kit. Bacteriophage λ plaques were subse-

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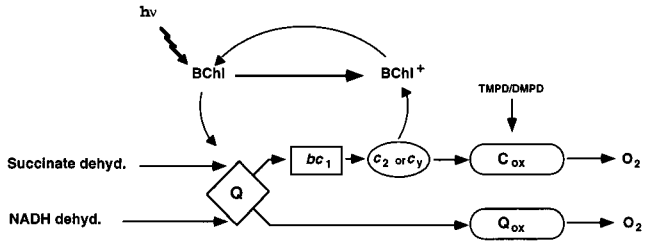

FIG. 1. The branched respiratory chain of *R. capsulatus*. It is believed that the branch point is at the ubiquinone pool (Q), where electrons can either be directly donated to the ubiquinol cytochrome *c* oxidase complex Q_{ox} (cytochrome b_{260}) or diverted to the *cb*-type cytochrome *c* oxidase complex C_{ox} (cytochrome b_{410}). In the latter case, electrons are passed through the cytochrome bc_1 complex (bc_1) and cytochrome c_2 or c_y, both of which are electron transfer components also used by the bacterial photosystem ($Bchl^+ \rightarrow Bchl$) (16, 24). Bchl, bacteriochlorophyll; dehyd., dehydrogenase; DMPD, *N,N'*-dimethyl-*p*-phenylenediamine monohydrochloride.

quently screened for the proper insert by hybridization with a ^{32}P-labeled *regA*-specific oligonucleotide probe. The insert DNA from the λ *Bgl*II clone was then subcloned from flanking *Xba*I sites into similar sites present in the vector pTZ19U to create plasmid pJB101.

Plasmid pMWS3.1, which contains the regulatory gene cluster encoding *senC*, *regA*, and *hvrA*, has been described previously (43). Plasmid p3.1ΔBgl was constructed from pMWS3.1 by replacing a 2.1-kbp *Bgl*II restriction fragment of pMWS3.1 with a *Bam*HI fragment containing an Ω transcription termination sequence (37). Plasmid p3.1ΔBamFS was created by cutting p3.1ΔBgl at the unique *Bam*HI site within *senC*, filling in the overhanging ends by using deoxynucleoside triphosphates and Klenow fragment, and ligating the newly generated blunt ends to create a 4-bp frameshift mutation within the *senC* gene. The reporter plasmids pXCA935 and pCB701Ω, for measuring *puf* and *puh* expression, respectively, have been described previously (5, 52).

Genetic manipulations. The insertion mutation to generate strain JB-1 was constructed by cloning a *Bam*HI restriction fragment containing a kanamycin resistance gene (pKIXX; Pharmacia) into the indicated *Bam*HI site within *senC* on p3.1ΔBgl. The plasmid-borne *senC* insertion mutation was subsequently used to create a chromosomal disruption of *senC* in strain St. Louis, using Gene Transfer Agent-mediated transduction as described previously (53).

Sequence analysis. A 2.1-kbp *Bam*HI restriction fragment from pJB101 which hybridized to a *regA*-specific probe during Southern blot analysis (39) was subcloned into M13 mp18/19 for sequence analysis. Sequence information was obtained from both strands by the dideoxynucleotide chain termination method, using a Sequenase DNA sequencing kit (U.S. Biochemical Corp.). DNA sequence was analyzed on a μVax computer, using programs from the Wisconsin Genetics Computer Group.

Spectral, protein, and enzymatic analyses. In vitro absorption spectroscopy was performed with a Beckman DU-50 recording spectrophotometer. Cultures for spectral analysis were grown semiaerobically as described above, chilled in an ice bath, pelleted at 4°C by centrifugation for 10 min at 5,000 × *g*, and resuspended in 10 mM Tris-HCl (pH 8.0)–1.0 mM EDTA (TE). These cells were then disrupted in a French press at 16,000 lb/in^2. Extracts were centrifuged at 3,500 rpm for 10 min at 4°C. The resulting supernatant was then centrifuged at 47,000 rpm for 90 min at 4°C. The pellet, which was enriched in intracytoplasmic membranes, was resuspended in cold TE (pH 8.0) to a concentration of 2 mg/ml and kept on ice. Protein concentration was determined by the method of Bradford (7) (Bio-Rad kit 500-001).

For β-galactosidase activity assays, cultures were grown either anaerobically, aerobically, or semiaerobically as indicated above. Cultures were subsequently harvested at a cell density of 1.5×10^8 cells per ml, disrupted by sonication, and assayed for β-galactosidase activity as described previously (53). Units of activity refer to amount of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolyzed per minute per milligram of protein.

C_{ox} activity was determined by assaying the *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) oxidation rate for each strain as follows. Cells were grown in triplicate either aerobically or semiaerobically as described above and harvested in log phase at 50 or 100 Klett units, respectively. Intracytoplasmic membrane-enriched fractions were prepared as described above. A 200-μg membrane-enriched preparation was incubated in a 3-ml volume of TE (pH 8.0) containing 10 mM ascorbate and 1.0 mM TMPD. The rate of oxygen uptake upon the addition of TMPD was determined polarographically, using a Clark oxygen electrode (Yellow Springs Instruments). A visual determination of cytochrome *c* oxidase activity in colonies by the addition of a-naphthol and dimethyl-*p*-phenylenediamine (NADI test) was performed as described by Marrs and Gest (32).

Nucleotide sequence accession number. The sequence of *senC* has been deposited in GenBank and assigned accession number L12050.

RESULTS

Cloning and sequence analysis of *senC*. We recently described a cluster of regulatory genes in *R. capsulatus* that control expression of the *puf*, *puh*, and *puc* operons in response to changes in environmental growth conditions. Two of the regulatory loci, *regB* and *regA*, code for a sensor kinase and partner response regulator, respectively, that control *puf*, *puh*, and *puc* operon expression in response to anaerobiosis (4, 26, 34, 43). A third regulatory locus, *hvrA*, codes for a *trans*-acting factor that controls light-mediated regulation of *puh* and *puf* expression (10). Analysis of previously described sequence upstream of the *regA* loci (43) led us to believe that there was an open reading frame that terminates 53 bp upstream from the start of *regA*. To analyze this region further, a region of DNA 5′ of *regA* was cloned from the genome of *R. capsulatus* as described in Materials and Methods and subsequently sequenced (Fig. 2). The results of this analysis indicated the existence of an open reading frame, which we have termed *senC* (sensor of C_{ox}), that is located between the previously described genes *regB* and *regA* (34, 43). The *senC* open reading frame is preceded by the putative ribosome binding sequence GGAG and also contains a codon usage that is biased for transcribed *R. capsulatus* genes (data not shown). Previous work has demonstrated that the region of DNA containing *senC* is cotranscribed with that of *regA* and *hvrA* (10).

The deduced 23.2-kDa polypeptide encoded by *senC* contains 221 amino acids with an average acidic isoelectric point of 4.4. A sequence similarity search through the GenBank data base demonstrated that SenC has 33% sequence identity and 52% sequence similarity to the yeast nucleus-encoded protein SCO1 (Fig. 3A) (41). SCO1 is known to be targeted to the mitochondria by amino-terminal mitochondrial targeting sequences (23) which are subsequently processed by cleavage upon transport (41). It is therefore not surprising that the alignment of SenC begins at amino acid 68 of SCO1. A hydrophobicity profile of SenC (Fig. 3B) demonstrates that its amino terminus contains a 27-amino-acid stretch of hydrophobic residues which are flanked by charged amino acids, a motif typical of membrane-spanning polypeptides (17). SCO1 contains a similar hydrophobic region and is also known to be tightly associated with the inner mitochondrial membrane (9, 41).

There is also a lower level of sequence similarity to an additional yeast protein, YBR0308 (45), as well as two prokaryotic proteins in the database known as Msp5 and orf193 (15, 50), whose functions remain unknown. An area of noted sequence conservation between all of these proteins is the sequence motif CPDVCP present in SenC (amino acids 133 to 138) and similar variant sequences in SCO1, YBR0308, Msp5, and orf193 (Fig. 3). As shown in the alignment in Fig. 3C, this sequence motif is a portion (a half site) of the Cys-rich region of bacterial ferridoxins that is known to form a ligand with iron (8).

***senC* is required for optimal cytochrome *c* oxidase activity.** The high degree of sequence similarity of SenC to SCO1 suggests that SenC may play a role similar to that of SCO1, that is, in the assembly of an active cytochrome *c* oxidase complex. To determine if this is the case, we constructed a chromosomal disruption of the *senC* open reading frame by inserting a kanamycin resistance cassette into codon 179 of *senC* (see Materials and Methods and Fig. 2B for strain construction). The resulting kanamycin-resistant strain, JB-1, was subsequently characterized for C_{ox} activity by subjecting mutant colonies to the NADI reaction (32). *R. capsulatus* colonies containing a functional C_{ox} complex are $NADI^+$, which is indicative of the C_{ox}-catalyzed synthesis of indophenol (a blue compound) from

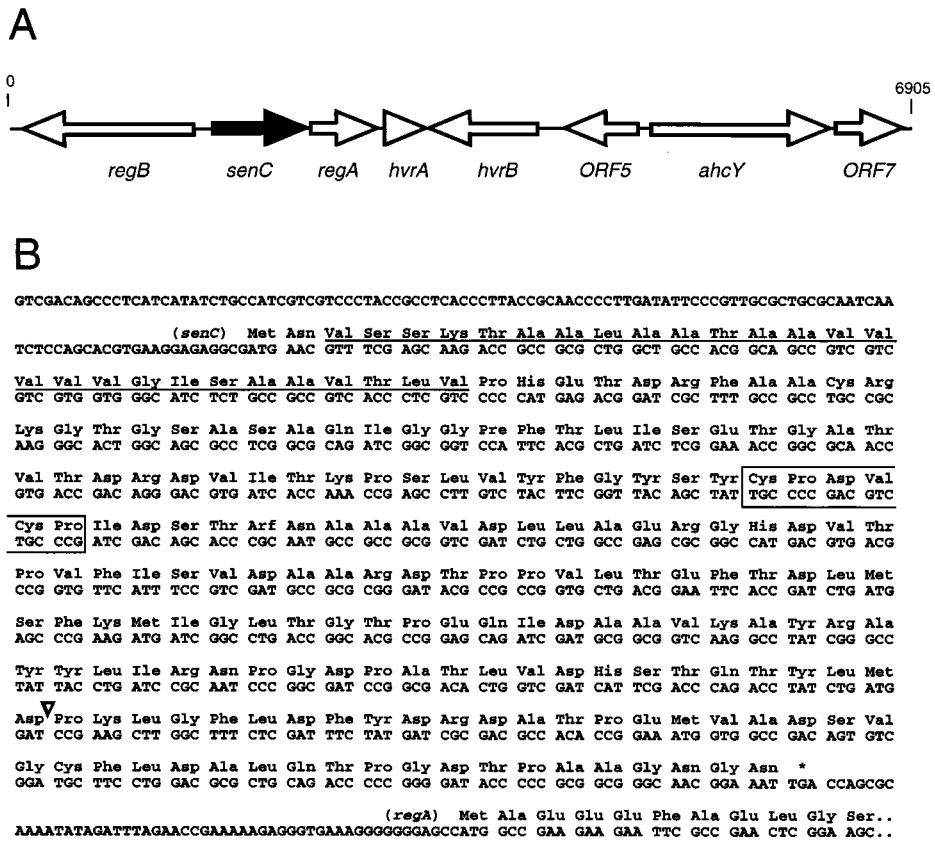

FIG. 2. (A) The photosynthesis regulatory gene cluster. The *senC* open reading frame lies in a cluster of genes, many of which are known to be involved in controlling the synthesis and induction of the bacterial photosystem (3). (B) DNA sequence of the *senC* locus. The predicted amino acid sequence of SenC, as well as the amino-terminal region of RegA, is shown above the nucleotide sequence. A computer-predicted membrane-spanning α helix is underlined. The predicted iron-binding domain (see text) is boxed. The inverted triangle indicates the position of the interposon disruption present in strain JB-1.

α-napthol, using the exogenous electron donor *N*,*N*-dimethyl-*p*-phenylenediamine monohydrochloride. When performing the NADI test (data not shown), we observed that the wild-type parental strain St. Louis rapidly (<5 min) catalyzes the synthesis of indophenol, as indicated by the production of a dark blue product. As a negative control, the *R. capsulatus* C_{ox} mutant strain M7 was also assayed (16, 20, 30, 32). M7 fails to synthesize a 32-kDa subunit of the C_{ox} enzyme (20) and consequently was NADI negative, as indicated by the retention of the red colony color. For the mutant JB-1, we observed that the colonies remain red ($NADI^-$) after a 5-min incubation with the NADI reagents, which indicates that *senC* mutations, like SCO1 mutations, are defective in C_{ox} activity. Interestingly, we also observed that upon further exposure of JB-1 colonies to NADI reagents (ca. 20 min), the center of the colony began to exhibit a noticeable blue color as a consequence of production of indophenol. In contrast, the negative control strain M7 remained red (negative) even after a prolonged incubation (ca. 1 h). From this qualitative analysis, we conclude that *senC* mutations exhibit greatly reduced, but not entirely absent, C_{ox} activity.

To better quantitate cytochrome *c* oxidase activity, we measured the ability of isolated membrane fractions obtained from respiring cells to oxidize the exogenous electron donor TMPD. Previous genetic studies have established that TMPD oxidation occurs via C_{ox}, which utilizes electrons obtained from TMPD to reduce oxygen to water (20). Thus, oxygen consumption, as promoted by the oxidation of TMPD in the presence of membranes, directly reflects C_{ox} activity. As shown in Fig. 4, membrane fractions obtained from an aerobically grown wild-type strain exhibit a level of cytochrome *c* oxidase activity 3.4-fold higher than that observed in membrane fractions obtained from semiaerobically grown wild-type cells. An increase in C_{ox} activity in aerobic compared with photosynthetically grown cells has been observed in previous studies (14, 31). Also consistent with previous studies (30) is our observation that membrane fractions obtained from strain M7, which lacks C_{ox}, exhibit a very low amount of oxygen consumption. In contrast, membrane fractions obtained from the *senC* mutant strain JB-1 have constitutive C_{ox} activity at a level which is similar to that observed with semiaerobically grown wild-type cells. This result indicates that JB-1 does not completely fail to synthesize a functional C_{ox} enzyme complex but instead does not maximally induce C_{ox} activity under aerobic conditions. This conclusion is supported by the results of the NADI reaction, which indicate that JB-1 colonies eventually turn blue upon prolonged incubation (discussed above).

***senC* is required for optimal induction of photosynthesis gene expression.** Upon disruption of *senC*, we observed that JB-1 colonies grown on standard peptone-yeast agar plates appeared to be less pigmented than colonies of the wild-type parent strain St. Louis (data not shown). Spectral analysis of membrane fractions obtained from anaerobically grown cells confirms that JB-1 has reduced levels of light-harvesting bacteriochlorophyll complexes (800- and 850-nm absorbance peaks) relative to the parent (Fig. 5). These observations in-

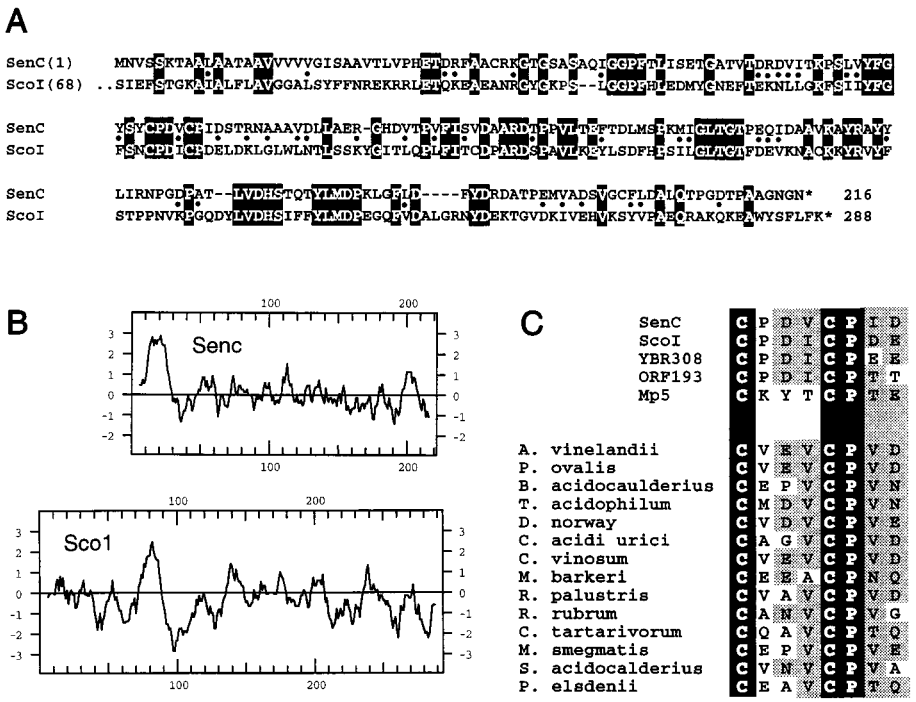

FIG. 3. (A) Sequence alignment of the predicted primary amino acid structures of SenC and SCO1. The sequences have 33% identity (inverse highlighted) and 52% similarity (dots). (B) Alignment of hydropathy profiles of SenC and SCO1. (C) Alignment of conserved segments of SenC and SCO1 to an iron-binding motif found in ferridoxins from a range of species (*Azospirillum vinelandii*, *Pseudomonas ovalis*, *Bacillus acidocaldarius*, *Thermoplasma acidophilum*, *Desulfovibrio norway*, *Clostridium acidiurici*, *Chromatrum vinosum*, *Methanosarcina barkeri*, *Rhodopseudomonas palustris*, *Rhodospirillum rubrum*, *Clostridium tartarivorum*, *Mycobacterium smegmatis*, *Sulfolobus acidocalderius*, and *Peptostreptococcus elsdenii*) (8).

dicate that SenC, or perhaps cytochrome *c* oxidase activity, may affect the synthesis of the photosynthetic apparatus.

Previous studies from our laboratory have indicated that synthesis of the *R. capsulatus* photosynthetic apparatus is regulated, in part, by controlling expression of the strongly inducible *puf* and *puh* operons, which code for structural proteins of the light-harvesting and reaction center complexes, as well as for an essential protein for bacteriochlorophyll biosynthesis (3–6, 34, 43). To address whether reduced pigmentation in JB-1 cells is a result of alterations in photosynthesis gene expression, we assayed the levels of *puf* and *puh* expression by using reporter plasmids that contain translational fusions to *lacZ*. As shown in Fig. 6A, analysis of β-galactosidase activity expressed from cells that harbor the *puf*::*lacZ* reporter plasmid pXCA935 indicates that the *senC* mutant strain JB-1 exhibits a 40 or 57% reduction in *puf* operon expression when grown

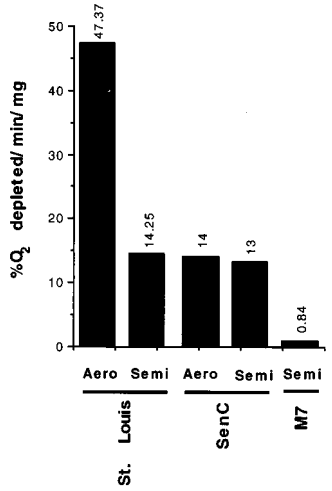

FIG. 4. Cytochrome *c* oxidase activity in membranes isolated from wild-type and JB-1 cells grown under either fully aerobic or semiaerobic conditions. The assay measures the rate of oxygen consumption in a closed reactor vesicle containing membrane fractions as well as the exogenous cytochrome *c* oxidase electron donor TMPD.

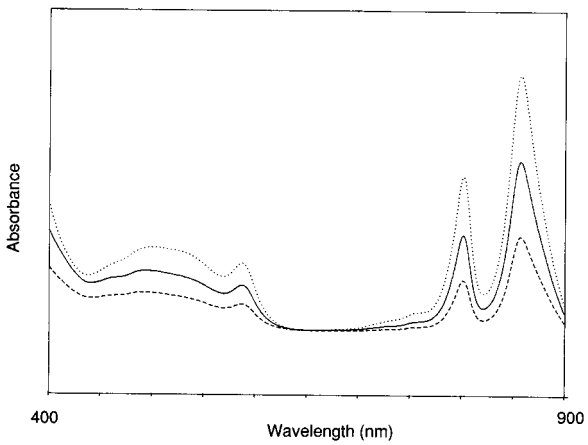

FIG. 5. In vivo absorption spectrum of membrane fractions isolated from strains St. Louis (solid line), JB-1 (dashed line), and M7 (dotted line). Each cell line was grown semiaerobically at 34°C and harvested at 100 Klett units. The absorbance peaks at 800 and 850 nm correspond to absorption by bacteriochlorophyll associated with light-harvesting and reaction center polypeptides.

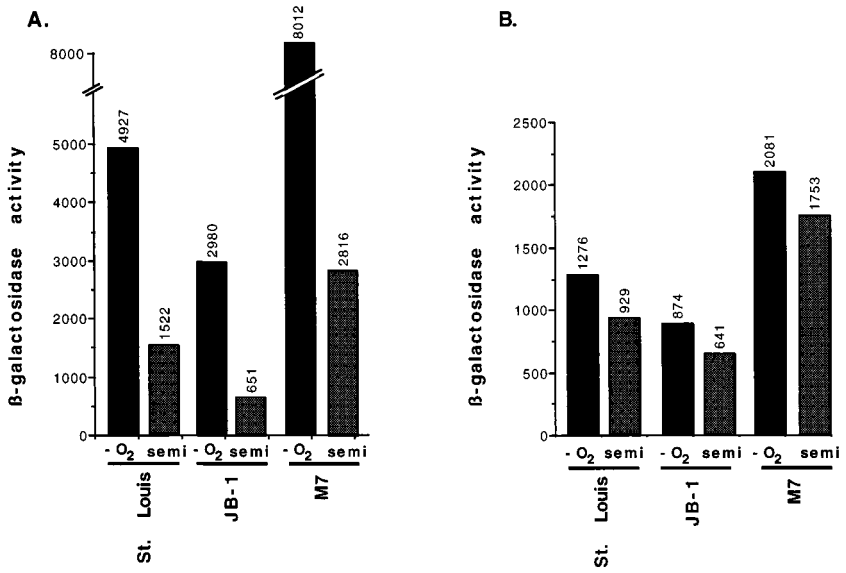

FIG. 6. Effect of the *senC* mutation on photosynthesis gene expression. (A) β-Galactosidase activity in cells containing plasmid-borne fusions of *lacZ* to the *puf* operon promoter. Cells were grown either anaerobically (black bars) or semiaerobically (grey bars). (B) β-Galactosidase activity in cells harboring a *puh*::*lacZ* fusion construct. Values shown are averages of three experiments, with an average standard deviation of 5%. Units refer to the amount of ONPG cleaved per minute per milligram.

under light anaerobic (photosynthetic) or dark semiaerobic conditions, respectively. Similar results were obtained with the *puh*::*lacZ* reporter plasmid pCB701Ω (Fig. 6B).

As mentioned above, *senC* is located just upstream of the regulatory genes *regA* and *hvrA*, which have been shown in a previous study to be involved in the *trans* activation of *puf*, *puh*, and *puc* (Fig. 2A) (10, 43). Since Northern (RNA) blot analysis has indicated that *senC*, *regA*, and *hvrA* are cotranscribed (10), it is possible that the observed effect of the *senC* insertion mutation on photosynthesis gene expression is not a result of disruption of SenC synthesis per se but is instead a consequence of polarity on downstream *regA* expression. To test this possibility, we used complementation analysis to further investigate the operon organization of *senC* and *regA* as well as to test for polarity of the mutation. As diagrammed in Fig. 7, three plasmids were constructed for this analysis. The control plasmid p3.1ΔBgl, which contains both *senC* and *regA*, fully complemented JB-I with respect to the NADI test, pigment formation, and *puf* operon expression. In addition, p3.1ΔBgl also fully complemented the two *regA*-deficient strains REG1 and MS01 with regard to pigment formation and *puf* expression. p3.1ΔBgl therefore fully expresses both *senC* and *regA*.

To confirm the results of Northern blot analysis, which indicate that *regA* is cotranscribed with *senC*, plasmid p3.1ΔBamΩ was constructed from p3.1ΔBgl by cloning a transcriptional termination element (Ω) (37) into a *Bam*HI site present at codon 179 of *senC* (Fig. 7). As expected, this plasmid fails to complement the NADI and photosynthesis gene expression defects exhibited by the *senC* mutant strain JB-1. In addition, this plasmid does not complement the photopigment and gene expression defects exhibited by the *regA*-disrupted strains REG1 and MS01, which confirms that *regA* is indeed transcribed in an operon with *senC*.

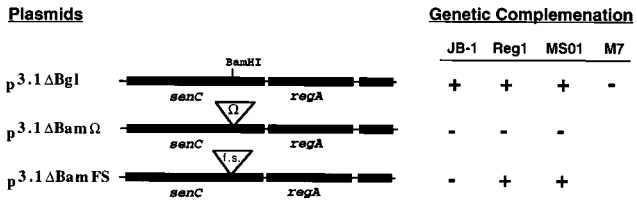

FIG. 7. Genetic complementation analysis of the *senC* and *regA* mutant phenotypes. +, restoration of both the NADI-deficient (where applicable) and photosynthesis-reduced phenotypes in the presence of the complementing plasmids. Complementing plasmids are described in detail in the text.

To test for polarity of the chromosomal *senC* disruption harbored by JB-1, we constructed plasmid p3.1ΔBamFS by cutting p3.1ΔBgl with *Bam*HI, filling in the ends with Klenow enzyme, and religating the newly generated blunt ends to create a four-base frameshift disruption within codon 179 of the plasmid-encoded copy of *senC* (the frameshift introduces a new in-frame stop codon which results in the synthesis of a truncated SenC lacking 32 amino acid residues at the carboxyl terminus). Importantly, this plasmid *trans* complements the *regA* mutant strains REG1 and MS01, which indicates that p3.1ΔBamFS expresses RegA (Fig. 7). More importantly, plasmid p3.1ΔBamFS does not complement the NADI$^-$, reduced pigmentation, and reduced photosynthesis gene expression phenotypes exhibited by strain JB-1. In conclusion, the finding that p3.1ΔBamFS, which supplies only RegA in *trans*, complements the phenotype exhibited by *regA* mutants but does not complement that of the *senC*-disrupted strain JB-1 indicates that the reduction in photosynthesis gene expression observed for JB-1 is not a result of a decrease in *regA* expression.

Cytochrome *c* oxidase activity also affects photosynthesis gene expression. We next addressed whether the observed reduction in photosynthesis gene expression in JB-1 cells was a result of pleiotropic effects caused by a SenC-mediated reduction in cytochrome *c* oxidase activity or was instead a consequence of a more direct role of SenC in regulating photosynthesis gene expression. To test these possibilities, we assayed photopigment production and *puf* expression in strain M7, which lacks a functional cytochrome *c* oxidase (20, 32). If the reduction in *puf* and *puh* expression observed upon disruption in SenC is the result of a pleiotropic effect, then a mutation in

cytochrome *c* oxidase should yield a similar reduction in photosynthesis gene expression. As shown in Fig. 5, spectral analysis of strain M7 demonstrates that this strain actually exhibits an elevation in photopigment production relative to that observed for the parent stain, a phenotype that is distinctly different from that observed with JB-1. Measurement of *puf* expression in M7 is in agreement with the spectral analysis in that strain M7 exhibits a 1.6-fold increase in *puf* expression when grown under anaerobic conditions and a 1.9-fold increase when grown under semiaerobic conditions relative to that observed with the parent (Fig. 6).

DISCUSSION

Cytochrome *c* oxidase accessory factors are conserved among kingdoms. The results of our sequence and mutational analysis indicate that SenC is a bacterial homolog of the yeast nucleus-encoded protein SCO1. This conclusion is based on the observations that these proteins exhibit a high degree of sequence identity (33%) and that a disruption of *senC*, like that observed for *SCO1* (41), affects the synthesis of a fully active cytochrome *c* oxidase complex. Biochemical and mutational analysis indicates that SCO1 forms a precomplex with COXI and COXII in the inner mitochondrial membrane and that this precomplex is subsequently converted to a functional cytochrome *c* oxidase complex (41). SCO1 is therefore proposed to have a role as an assembly factor in cytochrome *c* oxidase synthesis. As noted in previous studies, *R. capsulatus* does not synthesize a mitochondrial-type (aa_3) cytochrome *c* oxidase complex; instead, this species synthesizes a *cb*-type enzyme complex. The *cb*-type enzyme complex is distinctly different in that it contains a COXI homolog that is associated with two *c*-type cytochromes rather than with COXII and COXIII homologs. SenC and SCO1 exhibit a high degree of sequence identity and exhibit similar phenotypes, which indicates that they have similar roles involving a possible interaction with the COXI subunit, which is the only subunit that is conserved among these enzymes.

One question raised by this study is whether there are as yet undiscovered additional eubacterial homologs of the numerous nucleus-encoded accessory polypeptides that have been shown to be required for synthesis and assembly of cytochrome *c* oxidase in yeast and mammalian systems (41, 48). We feel that such a possibility exists and that there are several reasons why additional eubacterial accessory polypeptides have not been discovered. One of the foremost arguments against the existence of eubacterial accessory factors is the observation that biochemical purification of the eubacterial enzyme complex does not show the presence of the numerous accessory polypeptides that are observed in similar enzyme preparations obtained from yeast and mammalian mitochondria (27, 29, 33, 46). This discrepancy, however, can be rationalized by assuming either that eubacterial accessory polypeptides are not associated with the mature holoenzyme complex or that they are loosely associated and subsequently stripped off during detergent-mediated solubilization and purification of the enzyme complex. It is also likely that there are additional accessory polypeptides that have not yet been genetically characterized since only a few genetic studies have been undertaken on eubacterial cytochrome oxidases (1, 21, 32). An additional problem, which is highlighted by this study, is the possibility that mutations of eubacterial accessory polypeptides may not exhibit tight phenotypes. This is clearly the case of disruption of *senC*, which results in a phenotype that is not as severe as those produced by mutations in the yeast *SCO1* gene.

SenC has an additional role in regulating photosynthesis gene expression. There are many lines of evidence which suggest that the processes of photosynthesis and respiration are closely interrelated (reviewed in reference 42). For example, biochemical and mutational evidence suggests that both photosynthetic and respiratory processes have in common electron transport components, i.e., ubiquinones, cytochrome c_2, and the cytochrome bc_1 complex (2, 16, 24, 38, 54, 57). It has also been shown that photosynthetically grown cells contain cytochrome oxidase activity that is localized within an intracytoplasmic membrane complex that also houses pigment and cytochrome components of the bacterial photosystem (31). Oxygen uptake experiments with cells that contain photopigments have demonstrated that illumination causes a rapid and reversible inhibition of respiration (38, 44). This light-induced inhibition of oxygen uptake is apparently a result of soluble cytochrome c_2, as well as other, secondary cytochromes such as c_y, bypassing cytochrome oxidases in favor of donating electrons directly to the photooxidized reaction center (16, 24, 25, 57). Even though it is clear that the levels of photosynthesis and respiration are coordinately regulated, the exact nature of the mechanism involved remains unknown. Molecular oxygen, however, appears to be a key modulator in controlling these alternative growth modes.

Since electron transport components of respiration and photosynthesis are shared, it is interesting that the disruption of SenC also leads to a reduction in photosynthesis gene expression. Several of our observations indicate that SenC may have a direct rather than an indirect effect on photosynthesis. This conclusion is supported by our observation that the isogenic strain M7, which lacks C_{ox} activity, exhibits an increase rather than a decrease in photosystem gene expression as was observed for disruption of *senC*. This finding demonstrates that the reduction of *puf* and *puh* expression in JB-1 is not a pleiotropic effect of reduced C_{ox} activity. The results of our complementation analysis also indicate that the reduction of *puf* and *puh* expression in JB-1 is not a consequence of polarity of the *senC* mutation on *regA* expression. Our conclusions are further strengthened by a recent study of Eraso and Kaplan (18) which describes the sequence and mutational analysis of a SenC homolog from *Rhodobacter sphaeroides*. Although their study did not address the effect of disruption of *senC* on C_{ox} activity, they did see a similar phenotype with respect to photosynthesis gene expression as described in our study.

The mechanism whereby a disruption of SenC leads to an alteration in photosynthesis gene expression is unclear; however, we are pursuing the possibility that SenC has an active (rather than passive) role in governing the level of photosynthesis gene expression. One mechanism by which SenC could affect *puf* and *puh* expression is by affecting the RegB-RegA phosphorelay circuit in a manner analogous to that observed for accessory polypeptides that control the flow of phosphate in the sporulation phosphorelay circuit (22, 35, 36). This model will have to be confirmed by additional analysis of the RegB-RegA phosphorylation cascade that controls *puf* and *puh* expression. The recent isolation of biochemically active RegB and RegA components should facilitate such studies (26).

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