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Mutation of the Ser2 Codon of the Light-Harvesting B870 α Polypeptide of *Rhodobacter capsulatus* Partially Suppresses the *pufX* Phenotype

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The exact function of the *pufX* gene product of *Rhodobacter capsulatus* is uncertain, but deletion of the *pufX* gene renders cells incapable of phototrophic growth on a minimal medium, and photosynthetic electron transfer is impaired in vitro. However, suppressor mutants that are able to grow phototropically are readily isolated. Two such suppressor mutants were characterized as to their phototrophic growth properties, their fluorescence at different incident light intensities, the integrity of their chromatophores, and their abilities to generate a transmembrane potential. We found that the photosynthetic apparatus in the suppressor mutants was less stable than that of the pseudo-wild-type and primary mutant strains and that the suppressor mutants used light energy less efficiently than the pseudo-wild-type strain. Therefore, the suppressor strains are more precisely designated partial suppressor mutants. The locations and sequences of the suppressor mutations were determined, and both were found to change the second codon of the *pufA* gene. It is hypothesized that the serine residue specified by this codon is important in interactions between the B870 α protein and other membrane-bound polypeptides and that suppressor mutations at this position partially compensate for loss of the PufX protein. A model is proposed for the function of the PufX protein.

The purple nonsulfur bacterium *Rhodobacter capsulatus* is capable of both respiratory and phototrophic growth under appropriate conditions. Since the photosynthetic apparatus can be gratuitously induced during aerobic dark growth simply by lowering the oxygen concentration, *R. capsulatus* is a good model organism for mutational studies of the assembly and function of photosynthetic membrane-bound electron transport complexes.

Energy capture during phototrophic growth is accomplished by what is herein designated the photosynthetic unit. The photosynthetic unit of *R. capsulatus* is composed of three types of integral membrane polypeptide complexes: the light-harvesting antennae (although several designations have been given for light-harvesting complexes in different species of purple bacteria, in this report we use B800-850 for LH2 and B870 for LH1 complexes); the reaction center, which mediates the formation of a transmembrane potential by using the energy captured by the antenna complexes; and the ubiquinol:cytochrome *b*-c_1 complex (*b*-c_1 complex), which converts the transmembrane potential to a proton gradient. A variety of experimental and theoretical data are consistent with the view that in wild-type cells of purple nonsulfur bacteria, these three types of complexes are organized in the membrane in such a way as to enhance functional interactions with each other (16, 34). A structural interaction between the *R. capsulatus* reaction center and B870 antenna complex was indicated by the finding that a single amino acid change of the B870 α polypeptide caused the loss of the B870 complex and resulted in a change in the position of the reaction center. Consequently, the reaction center was capable of charge separation, but it was uncoupled from the *b*-c_1 complex (13, 14) such that the cells were incapable of phototrophic growth.

It was at one time thought that photosynthetic electron transfer required only the reaction center and *b*-c_1 complexes in combination with cytochrome c_2 and ubiquinol (24). However, it is now clear that the situation is more complex. At least in *R. capsulatus*, a membrane-bound cytochrome, cytochrome c_y, operates in parallel with cytochrome c_2 (15). Furthermore, in both *R. capsulatus* and *Rhodobacter sphaeroides*, at least one more protein, the *pufX* gene product, is necessary for normal photosynthetic electron transport (7, 19). The photosynthetically impaired *pufX* deletion strain *R. capsulatus* ΔRC6(pTL2) was found to give rise to photosynthetically competent suppressor mutants at a relatively high frequency. Preliminary studies of these suppressor mutants showed that they had different photosynthetic growth rates at high light intensities relative to the pseudo-wild-type strain ΔRC6(pTB999). Absorption spectra and sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles indicated that the stoichiometries of the components of the photosynthetic unit were also altered in the suppressor mutants (18).

In this report, we evaluate the effects of two of these suppressor mutations on low-light photosynthetic growth, light energy harvesting, formation of a transmembrane potential, and chromatophore structure. We also present the genetic mapping and DNA sequences of the *pufX* suppressor mutations. A summary of the literature on *pufX* and suppressor mutants is used to formulate a model of the function of the PufX protein.

MATERIALS AND METHODS

Bacterial strains and plasmids. *R. capsulatus* ΔRC6 is a *puf* operon deletion derivative of the wild-type strain B10 (4). *R. capsulatus* ΔRC6* is a B800-850$^-$ Crt$^-$ derivative of ΔRC6 that carries a *pufX* suppressor mutation on the chromosome. *R. capsulatus* ΔRC6*crtD* is a mutant of ΔRC6 that accumulates the carotenoid neurosporene; its spectrum in the 400- to 500-nm region facilitates the interpretation of flash spectroscopy experiments. The construction of plas-

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mids pTB999 (which carries the *puf* operon) and pTL2 (which carries a *pufX*-deleted *puf* operon) have been described previously (19, 33). Plasmid pTL30 is identical to pTL2 except that the *Hin*dIII site at the 3′ end of the Ω interposon has been filled in to create an *Nhe*I site. This change was made to facilitate mapping of the suppressor mutations and does not alter the phenotype from that observed for ΔRC6(pTL2). Plasmid pΔ4 contains the *puf* operon promoter and the *pufQ* gene (1), expression of which is necessary for optimal formation of the B800-850 complex in ΔRC6 (9).

Mapping and sequencing of suppressor mutations. The locations of the suppressor mutations on plasmids pTL2-7 and pTL2-9 were mapped by isolating segments from these plasmids and using these segments to replace the corresponding regions of the plasmid pTL30. The DNA sequences of segments from the suppressor plasmids that were found to suppress the *pufX* deletion were obtained by the dideoxy termination method (30).

Growth experiments. Low-oxygen and phototrophic cultures were grown as described previously (19) except that four light intensities were used to assess the photosynthetic growth properties of the strains tested. Specific light intensities were obtained by varying the distance between lamps and culture tubes and were measured with a model LI-185B photometer equipped with an LI-190SB quantum sensor (Li-Cor, Lincoln, Neb.).

Fluorescence measurements. Infrared fluorescence was evaluated at different incident light intensities, using a technique based on that of Youvan et al. (32). Cultures were grown to stationary phase under low aeration; the cells were pelleted and then resuspended to a density of 1.2×10^{10} CFU/ml. Serial twofold dilutions were made from this suspension, and 100 μl of each dilution (equal numbers of cells for each strain) was placed in a microtiter plate with flat-bottom wells. The plate was placed over a 1-cm-thick 1 M cupric sulfate solution illuminated from below with fluorescent lamps and was photographed through a Wratten 87C infrared filter (Eastman Kodak). The intensity of the excitation light was varied by using neutral density filters.

Preparation of chromatophores. Chromatophores were prepared as described previously (33) except that a buffer consisting of 20 mM *N*-morpholinepropanesulfonic acid (MOPS), 100 mM choline chloride, and 1 mM $MgCl_2$ (pH 7.0) was used. These preparations were further purified, when necessary, by sucrose gradient centrifugation. Crude chromatophores were layered on a 10-20-30-40% (wt/wt) sucrose step gradient over a 60% cushion and centrifuged at 97,000 × *g* for 230 min. Chromatophores were collected from the 20/30% sucrose interface, pelleted (149,000 × *g* for 75 min), and resuspended in the chromatophore buffer.

Spectroscopy experiments. Flash spectroscopy experiments were carried out as described previously (5, 26). Crude chromatophore preparations were normalized to a reaction center concentration of 100 nM, and intact cells were normalized to a reaction center concentration of 50 nM, as determined with a reduced-minus-oxidized extinction coefficient of 29.8 $mM^{-1} \cdot cm^{-1}$, using the wavelength pair 605-540 nm (6).

Chemically induced and light-induced carotenoid bandshift experiments were carried out with crude chromatophore suspensions normalized to a reaction center concentration of 100 nM for the light-induced bandshift experiments and to 200 nM for the chemically induced bandshift experiments. In both cases, pairs of identical cuvettes were made up. In the light-induced carotenoid bandshift experiments, a baseline was recorded and light of >800 nm was used to induce the bandshift in the sample cuvette as the difference spectrum was recorded over the wavelength range of 550 to 400 nm at 50 nm/min. In the chemically induced carotenoid bandshift experiments, valinomycin was added to both cuvettes to a final concentration of 2 μM and a baseline was recorded. A pulse of saturated KCl was added to the sample cuvette to induce the bandshift (with an equal volume of the choline chloride buffer added to the reference cuvette), and the difference spectrum was recorded over the 550- to 400-nm wavelength range at 50 nm/min.

Electron microscopy. Electron microscopy was carried out on sucrose gradient-purified chromatophores from low-oxygen-grown cultures. The chromatophores were dialyzed overnight against 2% ammonium acetate; the suspensions were mixed with 3% ammonium molybdate and placed on copper grids coated with collodion and carbon (27). The excess suspension was gently blotted from the grid, and observations were made with a Zeiss EM C10 electron microscope.

RESULTS

Low-light photosynthetic growth. To assess the photosynthetic growth properties of the mutants relative to the pseudo-wild-type strain ΔRC6(pTB999), cultures of the pseudo-wild-type strain, the primary mutant ΔRC6(pTL30), and the two suppressor mutants were grown at light intensities of 15, 30, 60, and 120 microeinsteins · $m^{-2} \cdot s^{-1}$. As shown in Fig. 1, reductions of the light intensity led to decreases in the exponential growth rates of all strains, but the decreases in growth rates of the suppressor strains were larger than the decreases seen in the pseudo-wild type; we therefore conclude that these two suppressor strains use light energy less efficiently than the pseudo-wild-type strain.

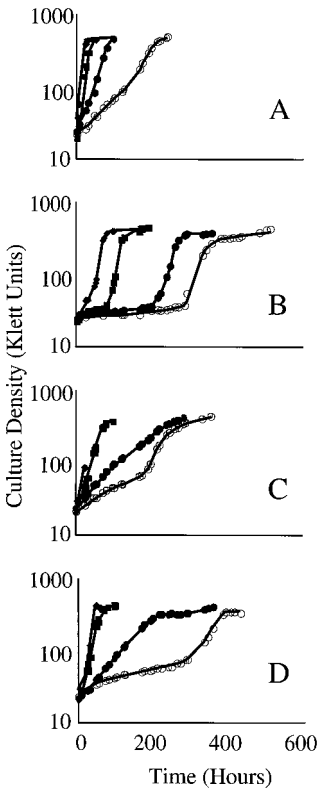

FIG. 1. Phototrophic growth of four strains of *R. capsulatus* at four light intensities. (A) ΔRC6(pTB999), the pseudo-wild type; (B) ΔRC6(pTL30), the primary mutant; (C) ΔRC6(pTL2-7), a plasmid-borne suppressor mutant; (D) ΔRC6(pTL2-9), a plasmid-borne suppressor mutant. Open circles, 15 microeinsteins · $m^{-2} \cdot s^{-1}$; closed circles, 30 microeinsteins · $m^{-2} \cdot s^{-1}$; squares, 60 microeinsteins · $m^{-2} \cdot s^{-1}$; diamonds, 120 microeinsteins · $m^{-2} \cdot s^{-1}$.

Infrared fluorescence. Cells which cannot efficiently use absorbed light emit excess energy in the form of fluorescence. The abilities of the strains used in the growth studies described above to use harvested light were assessed by recording the infrared fluorescence from cells exposed to several light intensities. Strain ΔRC6(pΔ4) was a positive control; it contains the B800-850 complex but no B870 or reaction center complexes. Therefore, this strain is incapable of phototrophic growth, and light energy trapped by the antenna complex is released as fluorescence. Strain ΔRC6*(pTL2) lacks carotenoids and the B800-850 complex and was used as a negative control. As can be seen in Fig. 2, the *pufX* deletion strain ΔRC6(pTL30) released more light as fluorescence than the pseudo-wild-type strain ΔRC6(pTB999). The suppressor strains ΔRC6(pTL2-9) and ΔRC6(pTL2-7) also exhibited enhanced fluorescence relative to ΔRC6(pTB999). The intensity of fluorescence from cells that contained pTL2-7 was similar to that obtained with ΔRC6(pTL30), whereas cells that contained pTL2-9 yielded greater fluorescence. We attribute these differences to the different amino acid substitutions in these two strains (see below). These results corroborate the growth curve data in showing that the suppressor and primary mutant strains are not able to use harvested light as efficiently as the pseudo-wild type.

Flash spectroscopy experiments. Chromatophore suspensions from strains ΔRC6*crtD*(pTB999), ΔRC6*crtD*(pTL2), ΔRC6*crtD*(pTL2-7), and ΔRC6*crtD*(pTL2-9) were examined in flash spectroscopy experiments, and their abilities to form a transmembrane potential as a result of photosynthetic electron transfer were monitored at 490 minus 475 nm (the carotenoid

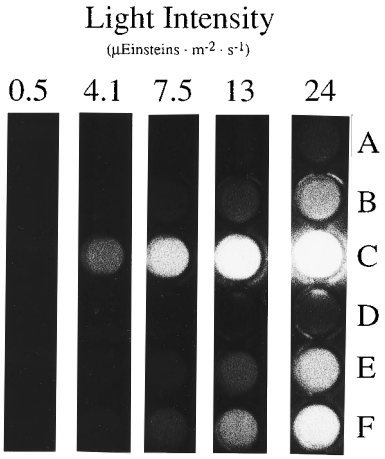

FIG. 2. Relative fluorescence of five strains of *R. capsulatus* at five light intensities. (A) ΔRC6(pTB999), the pseudo-wild-type strain; (B) ΔRC6(pTL30), the primary mutant; (C) ΔRC6(pΔ4), a B870$^-$ RC$^-$ positive control; (D) ΔRC6*(pTL30), a B800-850$^-$ chromosomal suppressor mutant; (E) ΔRC6(pTL2-7), a plasmid-borne suppressor mutant; (F) ΔRC6(pTL2-9), a plasmid-borne suppressor mutant.

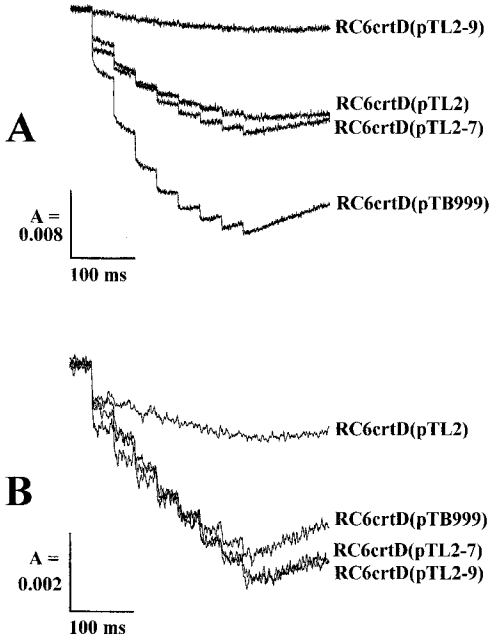

FIG. 3. Responses of the carotenoid bandshift (490 minus 475 nm) in chromatophores (A) and intact cells (B) from strains ΔRC6*crtD*(pTB999), ΔRC6*crtD*(pTL2), ΔRC6*crtD*(pTL2-7), and ΔRC6*crtD*(pTL2-9) to eight actinic flashes. Intact cells were resuspended in 2 M sucrose to a reaction center concentration of 50 nM, and chromatophores were suspended to the same reaction center concentration in 20 mM MOPS–100 mM choline chloride–1 mM $MgCl_2$ (pH 7.0). The E_h was brought to about 150 mV by the addition of a few crystals of sodium ascorbate. Traces were overlaid for ease of comparison.

bandshift). The carotenoid bandshift is due to changes in the absorption maxima of carotenoid pigments that result when an electrochemical potential is established across the membrane of chromatophore vesicles (11). When chromatophores are illuminated, the electron and proton transfers associated with photosynthetic energy transduction cause changes in the membrane potential, which have been designated phases I, II, and III (12). Phases I and II occur within 200 μs after the onset of illumination and are mainly due to primary events within the reaction center. Phase III takes place in about 7 ms and is due to the turnover of the cytochrome b-c_1 complex (10, 12). Results for the pseudo-wild-type strain ΔRC6*crtD*(pTB999) and the primary mutant ΔRC6*crtD*(pTL2) are shown in Fig. 3A, where it can be seen that the primary mutant strain generated reduced phases I and II of the carotenoid bandshift on the first flash (not kinetically resolved on this time scale, but indicated by the sharp decrease in absorbency), and there was very little phase III seen (the slower decrease in absorbency over the time period between flashes). With subsequent flashes, there was a relatively small increase in the magnitude of the total bandshift. These results confirm the impairment in electron and proton transport previously reported in more detail (19). Surprisingly, chromatophores from the two suppressor strains ΔRC6*crtD*(pTL2-7) and ΔRC6*crtD*(pTL2-9) were as poor as or worse than those from the primary mutant strain at forming a transmembrane potential in response to flashes of light (Fig. 3A), despite the fact that they could both grow phototrophically (Fig. 1). However, when these experiments were repeated with intact cells rather than chromatophores, it was evident that suppressor strain cells were capable of generating a transmembrane potential comparable to that observed in the pseudo-wild type (Fig. 3B). These results imply that the process of chromatophore preparation from cells of the suppressor mutants ΔRC6*crtD*(pTL2-7) and ΔRC6*crtD*(pTL2-9) causes a disruption of the in vivo properties of the photosynthetic unit.

Since chromatophores from the suppressor mutants appeared to have disruptions of the photosynthetic unit, the orientation of the reaction center cytochrome *c* docking site in chromatophores was evaluated. This was done by monitoring the oxidation and reduction kinetics of the reaction center special pair in the presence and the absence of reduced horse heart cytochrome *c*, which was added as a potential electron donor. In intact, wild-type chromatophores, most of the oxidizing sites of the reaction centers face the interior of the spherical chromatophore vesicles, and exogenous cytochrome *c* is a poor electron donor (25). Typically about 10 to 18% of reaction centers in a chromatophore preparation are able to oxidize exogenous cytochrome *c*, and this is attributed to a small population of broken or sheet-like chromatophores generated during cell disruption (28). As can be seen in Fig. 4, in the presence of exogenous cytochrome *c*, the pseudo-wild-type strain ΔRC6*crtD*(pTB999) and the primary mutant ΔRC6*crtD*(pTL30) both showed only a small increase in reaction center special pair reduction relative to the control after the first flash, indicating that these chromatophore preparations were of the normal topology and that the reaction centers were inserted in the membrane correctly. On the other hand, the suppressor strains ΔRC6*crtD*(pTL2-7) and ΔRC6*crtD*(pTL2-9) both showed greater levels of reduction of the special pair after each flash in the presence of exogenous cytochrome *c* (Fig. 4). This means that the exogenous cytochrome *c* was able to transfer electrons to the reaction center special pair, which in turn implies either that these preparations contained reaction centers oriented such that the cytochrome *c* docking site was on the outside of chromatophore vesicles or that a significant fraction of the chromatophores was present as topologically open sheets.

Electron microscopy of chromatophores. Because the experiments described above indicated that the chromatophores from the suppressor strains might be present as sheets or otherwise grossly disrupted, the gross structure of the chromatophores was assessed. This was done by negatively staining sucrose gradient-purified chromatophores from each strain with ammonium molybdate and examining them by transmission electron microscopy. As the micrographs in Fig. 5 show,

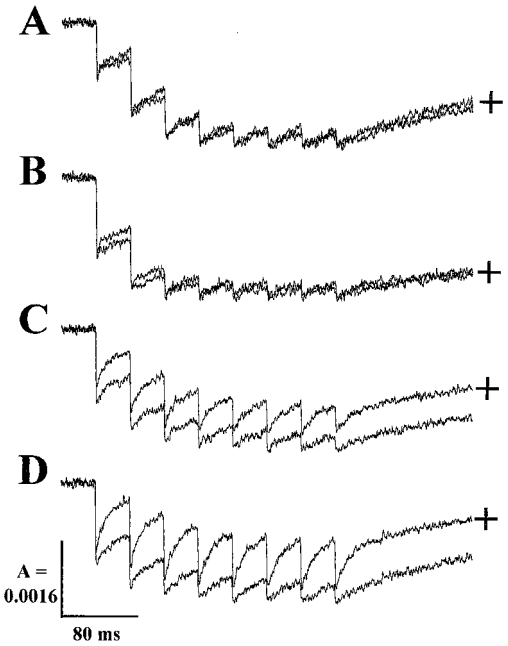

FIG. 4. Oxidation and reduction of the Bchl special pair (605-540 nm) in chromatophores from *R. capsulatus* ΔRC6*crtD* strains carrying pTB999 (A), pTL30 (B), pTL2-7 (C), and pTL2-9 (D) in the presence (+) and absence of horse heart cytochrome *c*. Chromatophores were suspended to a reaction center concentration of 50 nM in 20 mM MOPS–100 mM choline chloride–1 mM $MgCl_2$ (pH 7.0), and the E_h was brought to about 150 mV by the addition of a few crystals of sodium ascorbate. Antimycin A was added to a concentration of 2.5 μM, and valinomycin was added to a concentration of 2 μM. Traces were overlaid for ease of comparison.

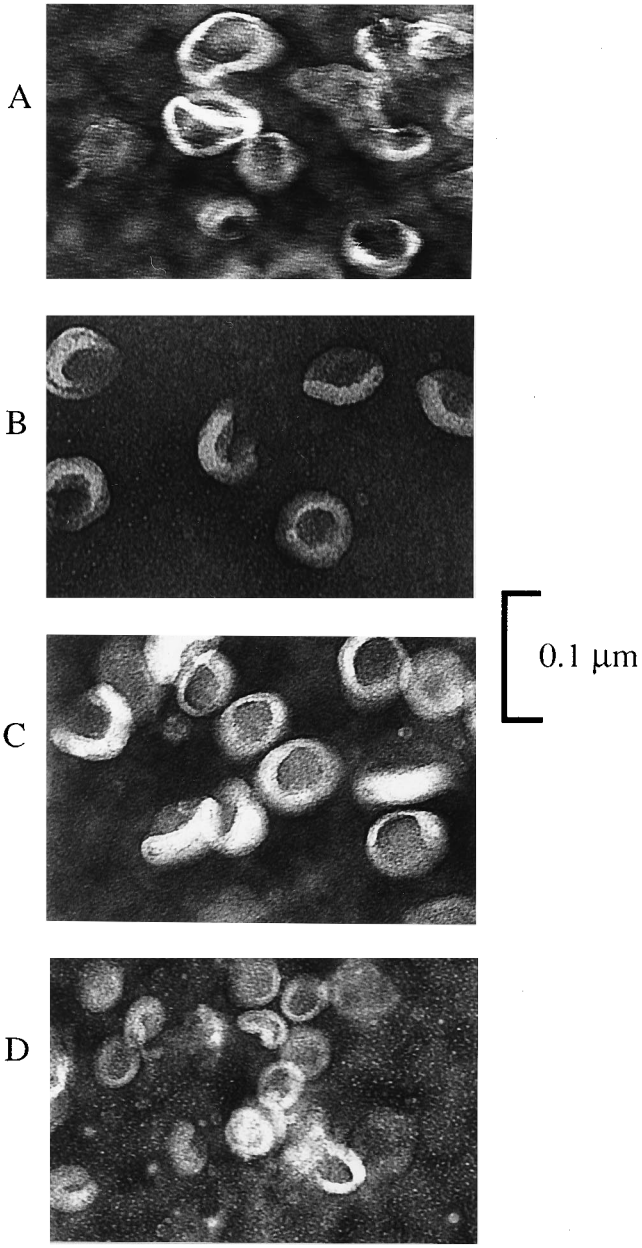

FIG. 5. Electron micrographs of sucrose gradient purified, negatively stained chromatophores from *R. capsulatus* ΔRC6 strains carrying pTB999 (A), pTL30 (B), pTL2-7 (C), and pTL2-9 (D).

the preparations from ΔRC6*crtD*(pTB999) and ΔRC6*crtD*(pTL30) seemed to be largely composed of intact, spherical chromatophores. Thus, the loss of the PufX protein does not cause a change in the gross structure of the chromatophores. Likewise, preparations from the suppressor mutants appeared intact and similar in gross structure to wild-type chromatophores (Fig. 5). Some variation in sizes of chromatophores was seen, and although the gross structures appeared intact, the possibility that chromatophores might have holes in them large enough to allow the passage of ions or cytochrome *c* is not ruled out by these experiments.

Chemical and continuous light induction of a carotenoid bandshift. The permeability of chromatophores to ions was assessed by measuring the membrane potential induced by a potassium ion gradient. The rationale for this experiment was that although the chromatophore vesicles from the suppressor strains did not appear to consist of topologically open sheets in the electron micrographs (Fig. 5), it was possible that they contained relatively small lesions that allowed free passage of ions, thus reducing the formation of an electrochemical gradient across their membranes (Fig. 3A). It was also possible that the access of exogenous cytochrome *c* to the reaction center was by diffusion through membrane holes that were too small to be visible in the electron micrographs. This possibility was addressed by using valinomycin and KCl to generate a membrane potential across the chromatophore membrane, independent of electron transfer (11). Chromatophores were prepared and suspended in a buffer in which the usual KCl was replaced by choline chloride. The addition of valinomycin, which transports potassium ions across membranes, has no effect at this stage, because of the absence of potassium. The addition of a pulse of saturated KCl to the experimental cuvette (balanced by an addition of an equal volume of buffer to the reference cuvette) sets up a substantial potassium gradient across the membrane, and the valinomycin allows potassium ions to move to dissipate this concentration gradient. Since they are transported as cations, however, their movement generates a membrane potential, inside positive (same polarity as the photosynthetically generated gradient of protons). The membrane potential is proportional to the potassium ion gradient and generates the carotenoid bandshift (11). As shown in Fig. 6A, this procedure generated a carotenoid bandshift in all the strains used here, as indicated by the peaks and valleys in the spectra. For direct comparison, continuous, light-induced

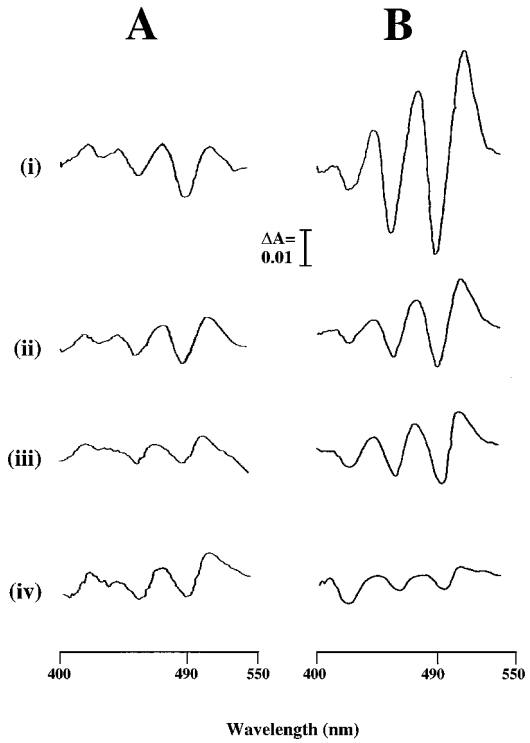

FIG. 6. Chemically induced (A) and light-induced (B) carotenoid bandshifts in chromatophores from *R. capsulatus* ΔRC6*crtD* strains carrying pTB999 (i), pTL30 (ii), pTL2-7 (iii), and pTL2-9 (iv). For the chemically induced bandshift experiments, chromatophores were resuspended to a reaction center concentration of 200 nM in 20 mM MOPS–100 mM choline chloride–1 mM $MgCl_2$ (pH 7.0), and the E_h was brought to about 150 mV by the addition of a few crystals of sodium ascorbate. Valinomycin was added to a final concentration of 2 μM. A chloride ion gradient was imposed by the addition of 50 μl of saturated KCl to the sample cuvette coincident with the addition of 50 μl of buffer to the reference cuvette. For the light-induced bandshift experiments, chromatophores were resuspended to a reaction center concentration of 100 nM in the same buffer and poised at the same E_h, but no valinomycin was added. The sample cuvette was exposed to light of >800-nm wavelength while the reference cuvette was kept dark.

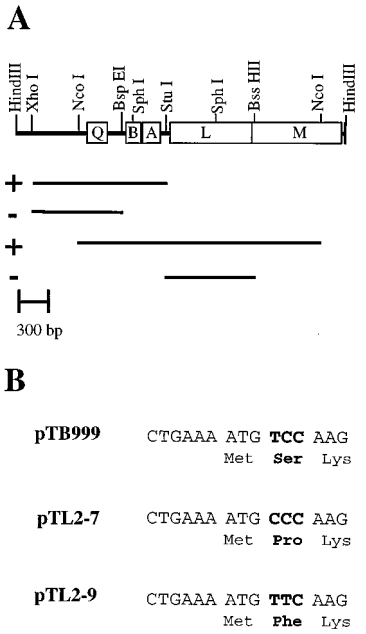

FIG. 7. (A) *puf* operon fragments used to map the suppressor mutations in plasmids pTL2-7 and pTL2-9; (B) nucleotide sequence of the first three codons of the *pufA* gene and upstream sequences from pTB999, pTL2-9, and pTL2-7.

bandshift experiments were done with spectra obtained over the same time scale (Fig. 6B), which show a much greater carotenoid bandshift in ΔRC6(pTB999) than in the mutant strains. Thus, chromatophores from all strains had the ability to maintain a chemically induced membrane potential, but the ability to form a membrane potential in response to continuous (Fig. 6B) or flash (Fig. 3) illumination was severely impaired in chromatophores from the primary mutant and suppressor strains. We interpret these results as showing that the chromatophores from the mutant strains are not significantly more leaky than those from the pseudo-wild type.

Mapping and sequencing of suppressor mutations. Preliminary genetic mapping of the secondary mutations in the suppressor strains showed that the two used in this study, ΔRC6(pTL2-7) and ΔRC6(pTL2-9), have suppressor mutations located on the complementing plasmid (18). Higher-resolution complementation analyses with *puf* operon segments localized the suppressor mutations on plasmids pTL2-7 and pTL2-9 to a region within the *pufBA* genes. As shown in Fig. 7A, four *puf* operon fragments from the suppressor plasmids were tested for the ability to suppress the *pufX* phenotype. Two of the fragments, the *Xho*I-to-*Bsp*EI and *Stu*I-to-*Bss*HII fragments, did not suppress. The remaining two fragments, the *Xho*I-to-*Stu*I and *Nco*I fragments, which did suppress, overlapped in the *Bsp*EI-to-*Stu*I region. This region, which encodes the *pufBA* genes, was sequenced. Both plasmid-borne suppressor mutations were found to be in the second codon of the *pufA* gene; in plasmid pTL2-7, this mutation changed the normally encoded serine to a proline, whereas in pTL2-9, this serine codon has been changed to a phenylalanine codon (Fig. 7B).

DISCUSSION

It has been known for some time that the *pufX* gene product is necessary for optimal phototrophic growth of *R. capsulatus*. In the absence of the *pufX* gene, the B870/reaction center complex ratio is increased, and the movement of protons and electrons (as quinols) from the reaction center to the b-c_1 complex is impaired (19). Suppressor mutants, which enhance the ability of *pufX* mutants to grow phototrophically, do not restore the wild-type stoichiometry of the photosynthetic unit (18). These facts pointed to a structural role for the *pufX* gene product. The further characterization of suppressor mutants reported in this paper provides additional information about the role of the *pufX* gene product.

As shown in Fig. 1, the ΔRC6(pTL2-7) and ΔRC6(pTL2-9) suppressor strains grew more slowly at low light intensities than ΔRC6(pTB999) (the pseudo-wild-type strain), and the suppressor strains released more of the light energy absorbed by the antenna complexes as fluorescence (Fig. 2). Because the B870 complexes of the suppressor strains still absorb light of the same wavelength as the wild-type strain (18), the bacteriochlorophyll (Bchl) coupling that is believed to be primarily responsible for the absorption properties of this antenna complex must exist (29). We interpret the enhanced fluorescence of the suppressor strains to indicate that they contain disruptions of the B870 complex which reduce the efficiency of energy transfer from pigments in the B870 to the reaction center complexes. The low level of fluorescence of the suppressor strains (relative to the control strain which lacks the B870

complex) indicates that the proximity or orientation of B870 relative to the reaction center is only subtly changed. The enhanced fluorescence seen in the suppressor strains may be due in part to higher than normal B800-850 antenna-to-reaction center ratios and does not quantitatively reflect the ability of these strains to use light energy to create a transmembrane potential (see below). This could account for the observation that the levels of fluorescence in the suppressor strains were not less than in the primary mutant strain, which must transduce a smaller amount of the absorbed light energy to account for its lack of phototrophic growth.

To our surprise, the light-induced carotenoid bandshift in chromatophores from the two suppressor strains ΔRC6*crtD*(pTL2-7) and ΔRC6*crtD*(pTL2-9) were no larger than with chromatophores from the *pufX* primary mutant, when monitored either while flashing with actinic light (Fig. 3A) or by exposure to continuous illumination (Fig. 6B). Similar results were obtained with Oxonol VI (3) as a potential-sensitive probe (data not shown). However, when experiments were repeated with intact cells, we found that the two suppressor strains had bandshifts very similar to that of the pseudo-wild-type strain and much larger than that of the primary mutant (Fig. 3B). These results suggested that either the suppressor strains made leaky chromatophores, so that a transient potential collapsed almost instantaneously, or that chromatophore isolation from cells somehow disrupted the photosynthetic electron transfer machinery so that it was not capable of generating a transmembrane potential. To view the gross structure of chromatophores, electron micrographs were taken (Fig. 5). These showed that the chromatophores were spherical and appeared to be topologically closed. The possibility of membrane defects not visible in the micrographs, but which could still cause the membrane to be permeable to ions, was evaluated by the chemically induced carotenoid bandshift. Membrane potentials generated by valinomycin-KCl pulse (11) showed that a carotenoid bandshift similar to that for the pseudo-wild type was maintained over a relatively long time frame (Fig. 6A), and so membrane permeability to ions does not seem to account for the reduced carotenoid bandshifts seen in the flashing experiments.

As mentioned in the introduction, the photosynthetically deficient *R. capsulatus* strain PBS108 yields chromatophores with reaction centers that are not correctly oriented in the membrane, because of a point mutation in the gene encoding the α subunit of the B870 complex (13, 14). By analogy with strain PBS108, it was possible that reaction centers of strains ΔRC6(pTL2-7) and ΔRC6(pTL2-9) were displaced during disruption of cells to make chromatophores. We therefore assessed the abilities of chromatophores from these strains to oxidize external cytochrome *c*. It is apparent that the suppressor strains possessed a greater number of reaction centers that were accessible to external cytochrome *c* (Fig. 4), indicative of abnormal reaction center orientation.

The high-resolution mapping and DNA sequencing of the suppressor mutations in plasmids pTL2-7 and pTL2-9 showed that they are both located in the second codon of the *pufA* gene, which normally encodes a serine in the α polypeptide of the B870 antenna complex (Fig. 7). Suppressor mutations for *pufX* have also been mapped and sequenced in *R. sphaeroides*, and although none of the suppressor mutations in *R. sphaeroides* occurred in this αSer2 codon, 90% of the 48 independent mutations mapped were located in the *pufB* and *pufA* genes encoding the B870 complex polypeptides (2). Of these, 91% were located in sequences encoding residues on the periplasmic side of the transmembrane segment of the α or β polypeptide. The isolation of *R. sphaeroides pufX* suppressors

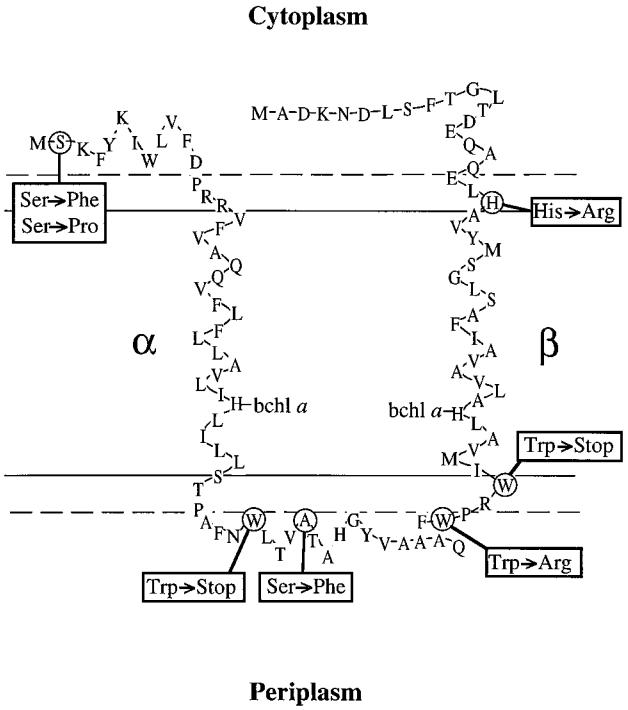

FIG. 8. Schematic diagram of the *R. capsulatus* B870 α and β polypeptides showing the locations and nature of the suppressor mutations found in the B870 complexes of *R. capsulatus* and *R. sphaeroides*. Note that the αAla47 residue of *R. capsulatus* is a Ser residue in *R. sphaeroides*. Mutated residues are circled and linked to boxes indicating the nature of the mutation. The solid lines represent the proposed boundaries of the hydrophobic portion of the membrane. The dashed lines represent the proposed boundaries of the polar head groups of the phospholipid molecules. After Jackson and Prince (13) and Zuber and Brunisholz (35).

was carried out with strains that were merodiploid for the *pufB* and *pufA* genes; that is, there were copies of the *pufB* and *pufA* genes on the chromosome and on the complementing plasmid. Subsequently, suppressor mutant plasmids were transferred into a host that lacked the *pufB* and *pufA* genes for further evaluation (2).

A summary of the locations and nature of the suppressor mutations in the *pufB* and *pufA* genes of *R. capsulatus* and *R. sphaeroides* is shown in Fig. 8. We group the suppressor mutations into three broad categories. In the first category are suppressor mutations that restore the PS^+ phenotype and produce a B870 complex in the absence of additional, wild-type copies of the *pufB* and *pufA* genes. This category contains the αSer2→Pro and αSer2→Phe mutations in *R. capsulatus* and the βHis20→Arg and αSer47→Phe changes in *R. sphaeroides* (2). Neither of these Ser residues is highly conserved within the α subunit of 25 antenna complexes that have been sequenced, nor has it been suggested that they are involved in pigment binding (35). In contrast, the βHis20 is found in 23 of the 25 β polypeptide sequences compared by Zuber and Brunisholz, but this residue is not thought to be involved in pigment binding (35); instead, it has been reported that in *Rhodospirillum rubrum*, this histidine is required for in vitro antenna complex assembly (23).

The second category of suppressor mutations consists of *R. sphaeroides* mutations (αTrp43→stop and βTrp44→stop) that restore the PS^+ phenotype only when a wild-type copy of each of the *pufB* and *pufA* genes is present (2). Neither of the category 2 suppressor mutant proteins formed the B870 com-

plex in the absence of the wild-type proteins, and they reduced the level of B870 absorption in a *pufBA*$^+$ background (2). As shown in Fig. 8, these mutations result in changes to highly conserved Trp residues in the periplasmic domains of the αβ dimer. The residue βTrp44, for example, is conserved in the B870 β apoproteins of 11 different species of bacteria and has been proposed to interact with the Bchl molecule that is liganded to the His residue six positions N terminal (Fig. 8) (23, 35). Loss of this residue in the βTrp44→stop mutant simultaneously results in the absence of the βTrp47 residue, also highly conserved in the β apoprotein from both B870 and B800-850 complexes (34, 35). The crystal structure of the *Rhodopseudomonas acidophila* antenna complex shows that the equivalent Trp residue is in contact with the B850 Bchl pigment (21).

The third category of suppressor mutants contains the βTrp47→Arg suppressor of *R. sphaeroides*, which was photosynthetically competent in the absence of additional, wild-type *pufBA* alleles, but the B870 complex in this strain was spectroscopically undetectable and levels of the α and β apoproteins (as measured in Western blots [immunoblots] with anti-B870 antibodies) were very low (2). This category also includes the strain described by McGlynn et al., which completely lacked the B870 light-harvesting complex as well as the PufX protein and was capable of phototrophic growth equal to that of the isogenic *pufX*$^+$ strain (22).

To understand how the three categories of suppressor mutations might overcome the effects of the loss of the PufX protein and, by extension, what the role of the PufX protein might be, it is helpful to consider the structures of light-harvesting antenna complexes. In this regard, the recent publications of the 2.5-Å (1 Å = 0.1 nm) resolution crystal structure of the B800-850 complex from *Rhodopseudomonas acidophila* (21) and the 8.5-Å resolution electron density map of the B870 complex of *Rhodospirillum rubrum* (17) are of great value. Since the amino acid sequences of light-harvesting antenna complex proteins are so similar (35), we use these structural data to help extrapolate to a general model.

The B870 antenna complex is thought to surround the reaction center, with a stoichiometry of 16 αβ dimers per reaction center (17). The antenna structures appear to be closed rings (17, 21) in that there is no obvious pathway for quinones, which are membrane soluble, and cytochrome c_y, which is membrane bound, to move through these complexes. Data from Karrasch et al. and McDermott et al. indicate that the transmembrane α helices in the αβ dimers are not in contact with each other, but that there are interactions between the extramembrane segments of α and β proteins (17, 21). The two types of antenna apoproteins seem to form the inner (α) and outer (β) walls of transmembrane cylinders, with the Bchl and carotenoid pigments located between these two walls (17, 21). Therefore, the overall structures would form a barrier to the lateral diffusion of quinols within the membrane, since noncovalently bound carotenoid and Bchl molecules tightly fill the space between the α and β proteins (shown most clearly in the B800-850 structure). As noted above, the primary structures of α and β light-harvesting proteins are highly conserved among the purple photosynthetic bacteria. They consist of central hydrophobic, membrane-spanning domains flanked by hydrophilic N- and C-terminal domains, which are found in the cytoplasm and periplasm, respectively (35). The PufX proteins from *R. capsulatus* and *R. sphaeroides* share this three-domain structure and model as integral membrane proteins, although they do not contain the amino acid residues associated with pigment binding (8, 21, 35). The PufX protein of *R. sphaeroides* was localized to chromatophore membranes and found to copurify with the B870-reaction center core complex (7).

We use the information summarized above and the results of a number of independent studies (2, 7, 17, 18, 19, 21, 22) to propose a speculative model in which the PufX protein creates a void in the structure of the B870 antenna complex, where a local absence of pigments allows passage of quinones between the reaction center and the b-c_1 complex.

In this model, the category 1 suppressor mutations (see above) would compensate for the loss of the PufX protein by disrupting interactions between αβ dimers that comprise the B870 antenna complex, which interacts with the reaction center. This suggestion is based in part on the structure of the B800-850 complex from *Rhodopseudomonas acidophila*, in which the only direct contacts between the two polypeptides in the αβ dimer are found in the cytoplasm or in the periplasm (21). Thus, the *R. capsulatus* cytoplasmic αSer2→Phe and αSer2→Pro suppressor mutations might reduce the stability of the B870-reaction center core complex and allow more facile quinone-quinol exchange between the reaction center and b-c_1 complexes in the absence of the PufX protein. Additional consequences of this reduced stability would be that the reaction center is liable to displacement during cell breakage and that these suppressor mutants are not able to harvest light energy as efficiently as the pseudo-wild-type strain.

The category 2 mutations are proposed to overcome the loss of the PufX protein by changing the pigment-binding capability of a B870 apoprotein, such that the mutant apoprotein effectively assumes the role of PufX in the presence of wild-type B870 proteins; that is, it would compete with wild-type proteins to form an αβ dimer with reduced affinity for pigment, and B870 complexes that contain this dimer would allow the passage of quinones and quinols, through the B870 structure, between the reaction center and b-c_1 complexes.

Category 3 mutants lack the B870 complex (see above) and thus would allow relatively unrestricted movement of quinones between the reaction center and b-c_1 complexes.

Mathai et al. have presented relevant evidence that physical restriction of the diffusion of quinones and quinols in membranes impairs electron transfer (20). These authors restricted void spaces in chloroplast and mitochondrial membranes by decreasing the osmolality of the medium surrounding vesicles that had been prepared in moderately high osmolality buffers. Their results are consistent with the idea that optimal movement of quinones and quinols depends on intramembrane voids, such as might be formed in our model of PufX-B870 interaction.

In our model, the absence of the PufX protein would result in an increase in the number of B870 antenna Bchl molecules, and this could account for the increased absorption at 870 nm seen in the primary mutant strains (7, 19). The B870 Bchl content of *R. sphaeroides pufX*$^+$ and *pufX* deletion strains has been carefully measured and found to increase in *pufX* deletion strains by 1.16 to 1.49 B870 per reaction center (22). These values are what would result from the displacement by PufX of pigment from one to four α/β dimers per B870 complex. Additionally, it has been reported that the loss of the PufX protein results in changes in B870 complex fluorescence polarization, which were interpreted as showing that in a *pufX* deletion mutant, the number of interacting Bchl pigment molecules in the B870 antenna complex was greater than in the pseudo-wild-type strain (31).

In summary, we conclude that the primary effect of the loss of PufX (the deficiency in phototrophic growth) is suppressed in the secondary mutants described as a result of changes in the B870 proteins that allow quinone transfer between the reaction

center and b-c_1 complexes. However, the structural role of PufX in maintaining B870-reaction center interaction is not restored to the wild type in the suppressor mutants. The reduced photosynthetic growth rates at low light intensities and increased fluorescence seen in the *R. capsulatus* suppressor mutants indicate that these strains do not use light energy as efficiently as the pseudo-wild-type strain. Thus, these suppressor strains are more precisely designated partial suppressors of the PS^- phenotype exhibited by *pufX* gene deletion mutants. We propose that our data and those of Barz and Oesterhelt (2) on the locations and characteristics of *pufX* suppressor mutations are consistent with a model for the role of the PufX protein in which the PufX protein interacts with a B870 antenna surrounding the reaction center in such a way as to facilitate passage of quinones and quinols between the reaction center and b-c_1 complexes. In this model, the suppressor mutants alter the structure of the B870 complex such that quinone transfer between the reaction center and b-c_1 complexes is possible in vivo, to allow phototrophic growth. Our proposed role for the PufX protein is consistent with all properties of *pufX* and suppressor mutants that have been published. High-resolution structural studies of B870-reaction center core complex crystals might reveal if our speculations about the general interactions of the PufX protein are correct and provide specific details about which proteins participate in these proposed interactions.

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