JOURNAL OF BACTERIOLOGY, Jan. 1998, p. 416–421 Vol. 180, No. 2
0021-9193/98/$04.00+0

The ATP Synthase *atpHAGDC* (F_1) Operon from *Rhodobacter capsulatus*

ROBERTO BORGHESE, MASSIMO CRIMI,† LUCA FAVA, AND BRUNO ANDREA MELANDRI*

Laboratory of Biochemistry and Biophysics, Department of Biology, University of Bologna, 40126 Bologna, Italy

Received 2 September 1997/Accepted 6 November 1997

The *atpHAGDC* operon of *Rhodobacter capsulatus*, containing the five genes coding for the F_1 sector of the ATP synthase, has been cloned and sequenced. The promoter region has been defined by primer extension analysis. It was not possible to obtain viable cells carrying *atp* deletions in the *R. capsulatus* chromosome, indicating that genes coding for ATP synthase are essential, at least under the growth conditions tested. We were able to circumvent this problem by combining gene transfer agent transduction with conjugation. This method represents an easy way to construct strains carrying mutations in indispensable genes.

ATP synthase is a multisubunit enzyme that catalyzes the respiratory or photosynthetic synthesis of ATP coupled to the exoergonic flux of protons across the inner membrane of mitochondria, the thylakoid membranes of chloroplasts, or the plasma membrane of bacteria. The enzyme can also promote the active translocation of protons driven by ATP hydrolysis (8, 9, 25). The ATP synthase consists of two parts, an extrinsic sector (F_1) formed by five different subunits (α, β, γ, δ, and ε) with a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ and connected through a thin stalk to the second part, an intrinsic membrane sector (F_0) formed by a minimum of three proteins (a, b, and c) with a stoichiometry of ab_2c_{8-12}. In photosynthetic membranes, F_0 is consistently formed by four different subunits in all cases studied to date (7, 11, 28), the fourth subunit, b′, being a duplication of b. The extrinsic F_1 sector contains three sites in which the synthesis or hydrolysis of ATP is catalyzed and three noncatalytic nucleotide binding sites, whose role is still not understood. The catalytic reaction is coupled to the transmembrane proton flow through the F_0 sector presumably by means of long-range conformational changes propagated along the stalk structure. A rotational catalytic mechanism has been proposed; according to this mechanism, the interaction of the single-copy subunits confers, cyclically in time, different affinities for ATP, ADP, and phosphate to the catalytic site in each αβ pair (reviewed in reference 2). This model has received decisive support from the atomic structure of F_1 from bovine heart mitochondria, recently resolved at 2.8 Å by X-ray crystallography (1). The rotational catalytic model is also in agreement with recent functional experiments with isolated F_1 (5, 21, 22).

The gene structure of the ATP synthases has been studied for a great number of organisms. For the enzymes from photosynthetic organisms, sequences have been elucidated, for at least some subunits of F_1, for about 16 higher plants and 6 algae. Of 23 complete sequences of genes coding for eubacterial F_0F_1 ATPase found in the databases, 16 show a unique *atp* operon with the F_0 genes preceding the genes for F_1. The seven remaining species, all photosynthetic, have the *atp* genes split into two operons (three in *Synechococcus* sp. strain 6716 [28]). The cyanobacteria *Synechococcus* sp. strain 6301 (4), *Anabaena* strain PCC 7120 (18), and *Synechocystis* strain PCC 6803 (14) and probably the green sulfur bacterium *Chlorobium limicola* (34) possess one operon comprising the F_0 genes together with the first three F_1 genes and a second operon with the genes for F_1 β and ε subunits. In the *Rhodospirillaceae* family members *Rhodospirillum rubrum* (6, 7) and, probably, *Rhodopseudomonas blastica*, for which only the F_1 sequence is known (27), the structural genes of F_0 and F_1 are organized into two separate operons. Surprisingly, the gene organization and sequences for the ATP synthase of the two species of *Rhodospirillaceae* most intensively studied from biochemical and genetic standpoints (i.e., *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*) are still unknown.

In this paper, we present the complete gene sequence of the F_1 operon (*atpHAGDC*) of *Rhodobacter capsulatus* B100. We also describe a procedure that makes possible the introduction of chromosomal deletions in essential genes followed by complementation with a new copy of the same genes. This method will allow us to perform easy site-directed mutagenesis studies on *Rhodobacter capsulatus* F_0F_1 ATPase.

Cloning and sequence analysis of the *atpHAGDC* operon. The probe to be used for library screening was made by amplification of a portion of the gene coding for the α subunit (*atpA*). The primers used for PCR (5′ GACCGTCAGACCG GCAAGACCGC 3′ and 5′ AGTCTACAGCGACGACGAC GCGG 3′) were designed based on the sequence of the close relative *Rhodospirillum rubrum* and chosen in highly conserved regions in the middle of the α subunit. The screening of 550 colonies gave six positive clones, one of which was chosen for further study. The sequencing of 8 kb carrying the *atp* operon was completed by use of the dideoxynucleotide chain termination method (23).

The genes coding for the five subunits of the ATPase F_1 component were identified by their homology to corresponding *atp* genes from both prokaryotes and eukaryotes. The order of the genes in the operon is *atpHAGDC*, corresponding to subunits δ, α, γ, β, and ε, respectively. In *Rhodobacter capsulatus*, no genes coding for F_0 were found upstream of the *atpHAGDC* operon, like the situation found in *Rhodopseudomonas blastica* and *Rhodospirillum rubrum* (6, 27). This organization seems to be unique to the *Rhodospirillaceae.*

All of the reading frames corresponding to the *atp* genes are preceded by a canonical Shine-Dalgarno sequence. The first codon is ATG in four genes, *atpA*, *-G*, *-D*, and *-C*, but is a GTG in the first gene of the operon, *atpH*. Interestingly, the same feature, specific for the δ subunit, has been found in the close relatives *Rhodospirillum rubrum* and *Rhodopseudomonas blas-*

* Corresponding author. Mailing address: Università di Bologna, Dipartimento di Biologia, via Irnerio 42, 40162 Bologna, Italy. Phone: 39-51-351293. Fax: 39-51-242576. E-mail: melandri@alma.unibo.it.

† Present address: Istituto Policattedra, Facoltà di Scienze, Università di Verona, 37134 Verona, Italy.

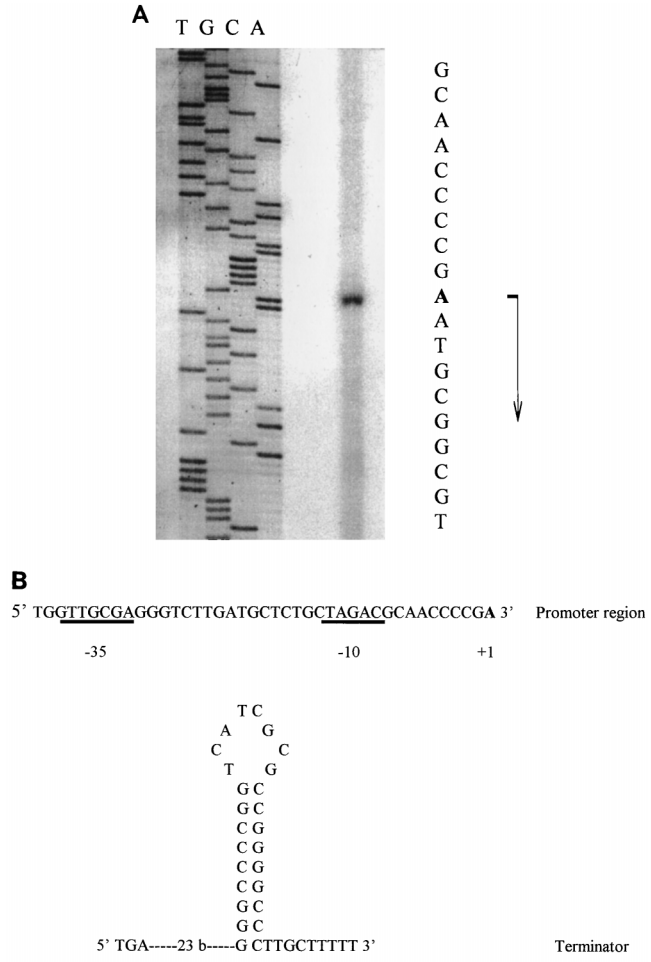

FIG. 1. (A) Determination of transcription initiation. The sequencing ladder, to the left of the lane containing the primer extension product, shows the actual sequence of the template strand; for the sake of clarity, however, the letters at the top of the lanes have been changed to correspond to the sense strand, and the sequence should be read from top to bottom. The corresponding sequence, on the right, should also be read from top to bottom, as indicated by the arrow. The boldface A corresponds to the 5′ end of the mRNA. (B) For the promoter region, the sequence includes 40 bases upstream of the start of transcription, which is marked by +1. The −10 and −35 elements are identified by their distance from the +1 position. For the terminator, the TGA at the 5′ of the sequence corresponds to the stop codon of the last gene of the operon (*atpC*).

tica (6, 27) but not in any other prokaryote checked; in these other prokaryotes, *atpH* begins with a more typical ATG.

The transcription initiation site was determined by primer extension (3). The 5′ end of the message was mapped at an adenine residue, 108 nucleotides upstream of the GTG start codon of the δ subunit (Fig. 1A). A possible promoter sequence was recognized around the −10 and −35 regions. Similar elements have been found in *Rhodobacter capsulatus* in a number of other operons, particularly operons involved in bacteriochlorophyll and carotenoid biosynthesis, in which identification of the putative promoter elements has been substantiated by mutagenesis analysis (15). In particular, the TTG stretch in the −35 element is completely conserved (Fig. 1B).

A terminator sequence is clearly recognizable 23 bp downstream of the *atpC* stop codon (Fig. 1B). This sequence shows the characteristics of a typical rho-independent terminator, including a stem-loop structure, a GC-rich region at the base of the stem, and a stretch of five T's at the end of the structure.

The amino acid sequence derived from the gene structure is confirmed by the amino-terminal sequence of the peptides published by Gabellini et al. (10). The latter study has indicated, however, that all amino-terminal *N*-formylmethionines have been removed in the mature peptides. This holds also for the terminal amino acid of the δ subunit corresponding to the code of valine (probably also an *N*-formylmethionine). With this fact taken into account, the relative molecular weights of the five subunits should be 54,670, 50,097, 31,076, 18,760, and 13,357 for α, β, γ, δ, and ε, respectively, corresponding to an overall molecular mass of 377,494 Da.

Four other open reading frames (ORFs) were found upstream and downstream of the *atpHAGDC* sequence (see Fig. 3A). All of these ORFs have a high degree of sequence similarity with ORFs found around the *atp* operon of the other photosynthetic bacterium, *Rhodopseudomonas blastica* (27). The overall organization is identical in the two species, except that *Rhodopseudomonas blastica* has two extra ORFs. One, ORF5, is found immediately upstream of *atpH* and is apparently transcribed in the opposite direction; the second, ORF6, is placed between *atpG* and *atpD*. *Rhodobacter capsulatus* ORF1, which we have sequenced for only 443 bases, corresponds to ORF2 in *Rhodopseudomonas blastica* with 89% identity. ORF1 also has 50% sequence identity with *Escherichia coli clpA*. *Rhodobacter capsulatus* ORF2, ORF3, and ORF4 correspond to *Rhodopseudomonas blastica* ORF3, ORF4, and ORF7 with identities of 56, 65, and 62%, respectively. Apparently there is no correspondence with sequences upstream of the *atpHAGDC* operon of *Rhodospirillum rubrum*, the other closely related photosynthetic bacterium (6).

We show in Fig. 2 the sequence alignments of β, γ, and ε subunits from *Rhodobacter capsulatus*, *E. coli* (30), and bovine mitochondria (29), together with structural information, where available. The amino acid sequences of the α and β subunits confirm that they are the most conserved ones in the whole ATP synthase complex. The identities with sequences of other photosynthetic bacteria are striking (79 and 89% with *Rhodospirillum rubrum* and *Rhodopseudomonas blastica* β subunits, respectively; 74 and 86% with *Rhodospirillum rubrum* and *Rhodopseudomonas blastica* α subunits, respectively (6, 27); the sequence homology with nonphotosynthetic eubacterial ATP synthases is also very extensive (e.g., 69 and 55% identities with *E. coli* β and α subunits, respectively) (30). The homology is also quite strong with the ATP synthase from eukaryotic organisms (78 and 68% identities with β and α subunits, respectively, in bovine mitochondria) (29). In Fig. 2A, the alignment of β subunit sequences indicates that identity is particularly marked in several key sectors, including the catalytic site (1), where all the amino acid residues are entirely conserved in the three sequences. Subunit γ, only partially resolved by X-ray crystallography of bovine heart mitochondria F_1 (1), constitutes the central axis of the complex in the form of a coiled-coil helical stem in the middle of the αβ hexamer. In ATP synthases of higher-plant chloroplasts, the γ subunit is some 40 residues longer and carries a short 8 amino acid sequence, containing two conserved cysteines, involved in the activation of the catalytic activity of the enzyme by thiol-reducing agents (20). This sequence should be present immediately downstream of the blandly conserved segment of the γ subunit sequence at positions 180 to 200 but is absent in *Rhodobacter capsulatus* (Fig. 2B), in agreement with previous observations for other *Rhodospirillaceae* (6, 27) and cyanobacteria (4, 14, 18, 28).

Construction of a deletion mutant. Gene transfer agent (GTA) particles produced by *Rhodobacter capsulatus* cells pack, randomly, pieces of DNA about 4.6 kb long, either from

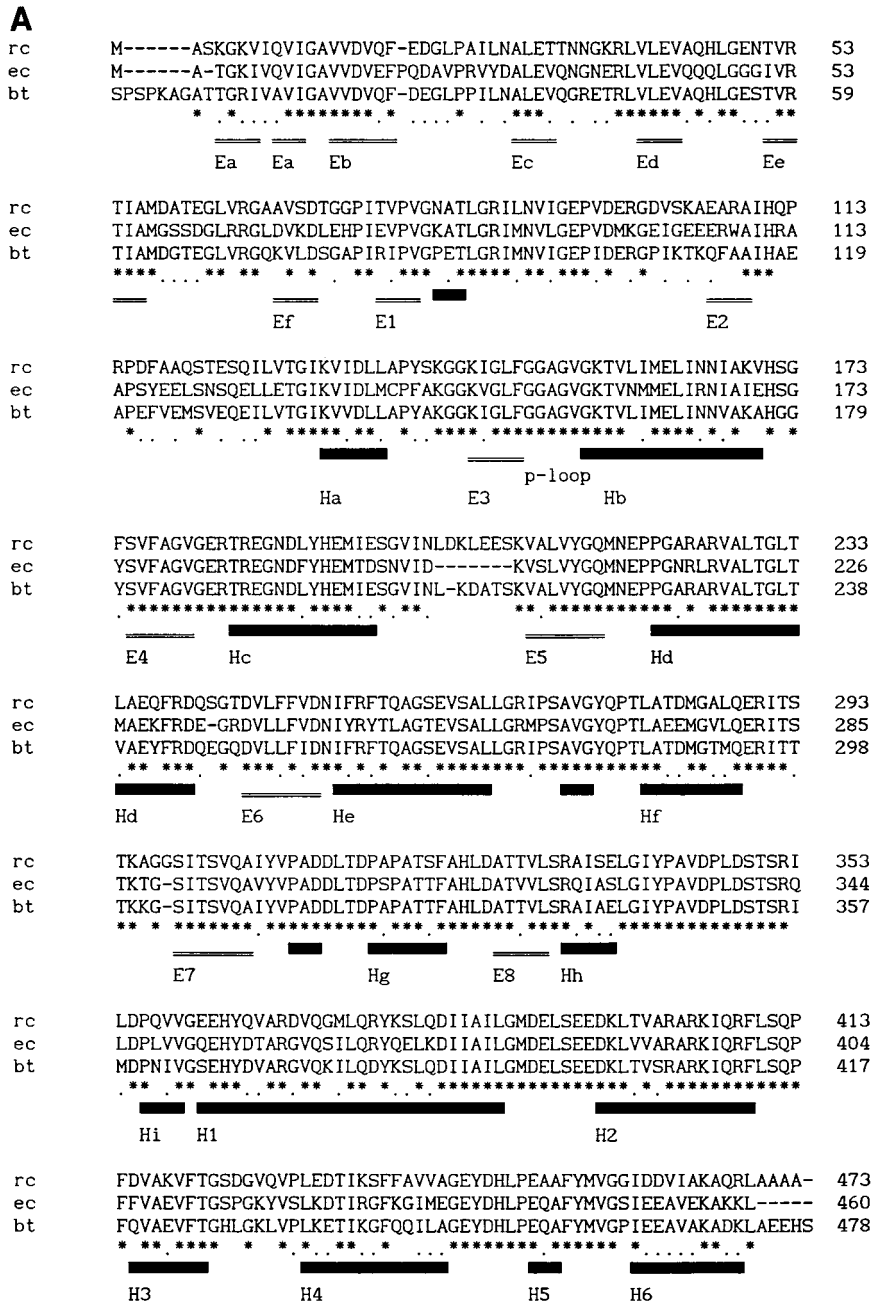

FIG. 2. Alignment of the amino acid sequences of β (A), γ (B), and ε (C) subunits of *Rhodobacter capsulatus* (rc), *E. coli* (ec), and bovine heart mitochondria (bt). Asterisks indicate identical residues, dots indicate conservative substitutions, E's and double underlines indicate β strands, and H's and black boxes indicate α helices. The secondary structures of β and γ subunits have been obtained from the atomic coordinates of bovine heart mitochondria F_1 (1), and the secondary structural elements of the ε subunit have been obtained from nuclear magnetic resonance models of *E. coli* (33).

the chromosome or from resident plasmids, and transfer them to acceptor cells, where they are integrated into the chromosome by homologous recombination (17). This results in an exchange between the incoming DNA and the corresponding chromosomal DNA which is lost in the process. The genetic characteristics of transfer using GTA particles make it a method of choice for the introduction of gene deletions into the chromosome. To this end, two plasmids, pRCA107 and pRCA108 (Fig. 3A), were constructed upon cloning in the broad-host-range plasmid pRK415 (13). pRCA107 carries a complete deletion of the *atpHAGDC* operon, which is replaced by a Km^r cassette; pRCA108 has the cassette inserted at the *Eco*RV site downstream of the operon. In both cases, the resistance cassette is flanked by portions of *Rhodobacter capsulatus* DNA to allow for easy homologous recombination.

Transfer experiments using GTA particles were carried out as described previously (35) with rifampin-resistant strain J1 (31) plasmid-containing cells as the donors and wild-type B100 (12) cells as the acceptors. In each of a number of trials, no *atpHAGDC* deletion mutants were detected despite extensive

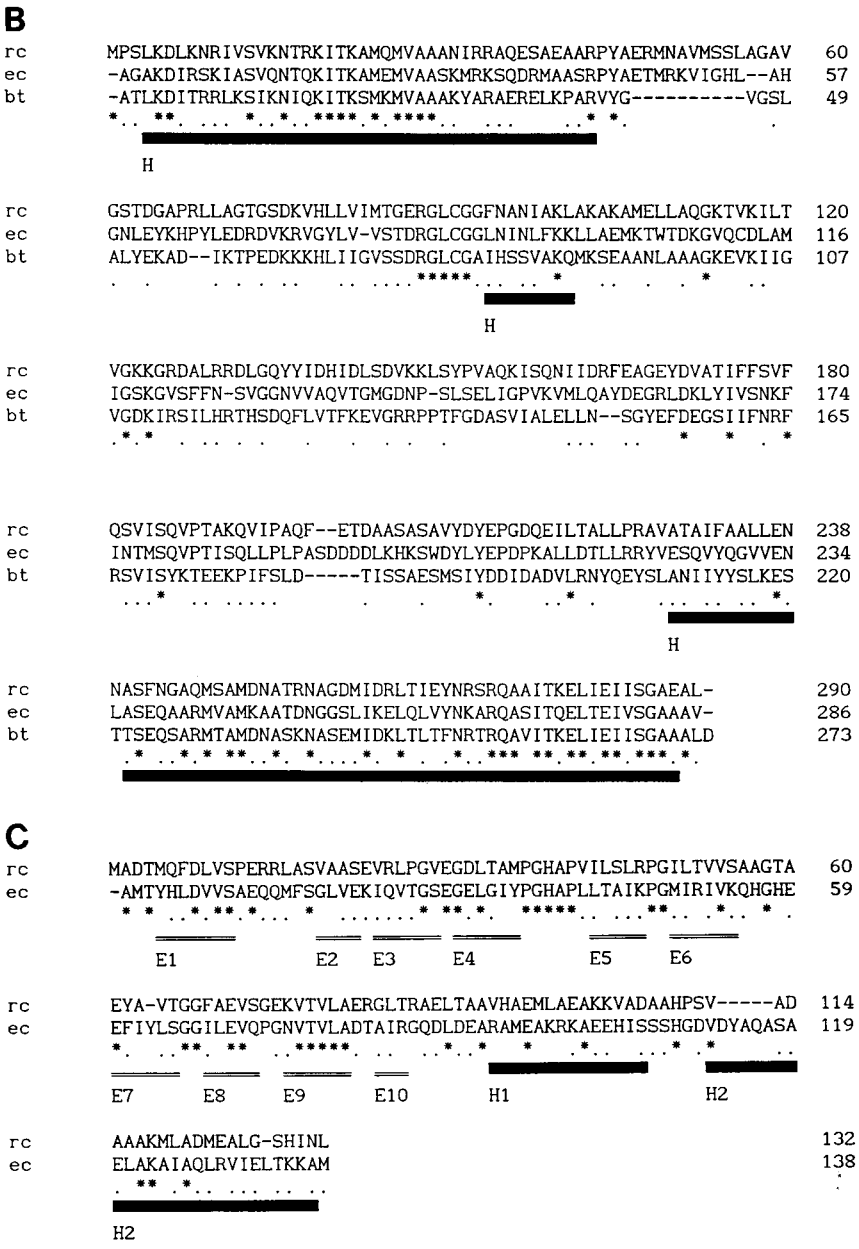

FIG. 2—*Continued.*

efforts. In a typical experiment, slow-growing colonies resistant to kanamycin, putative *atp* mutants, started to appear on RFD2 minimal plates (standard RCV medium [32] modified with fructose as the carbon source and dimethyl sulfoxide [DMSO] as the final electron acceptor and supplemented with 0.05% yeast extract) after 8 to 14 days under aerobic, photosynthetic, or anaerobic growth conditions. Control colonies, with the Km^r cassette insertion downstream of the operon, grew in 2 to 9 days under corresponding conditions. No growth was detected under pure fermentative conditions (24), on RFD2 medium supplemented with sodium bicarbonate but lacking DMSO, even after prolonged incubation. The putative mutants were checked for aerobic growth on RCV standard minimal medium, with malate as the carbon source. Under these conditions, cells missing the ATP synthase complex are not expected to grow. All of the kanamycin-resistant putative *atp* mutants grew on RCV medium, indicating that a functional ATP synthase was still present. In addition, genomic DNA was isolated from five putative mutants and from strain RCAK1 carrying the Km^r cassette inserted downstream of the operon. Total DNA digestions were probed with the γ-subunit gene and with the Km^r gene. All of the putative mutants had conserved the *atpHAGDC* operon and showed no Km^r cassette insertion (data not shown), indicating that the resistance trait was due to spontaneous mutations, whereas the RCAK1 control strain hybridized to both probes, showing that the Km^r cassette had been inserted into the chromosome. The impossibility to isolate *atp* deletion mutants represents a good indication that *Rhodobacter capsulatus* cannot grow without a functional ATP synthase under all growth conditions tested.

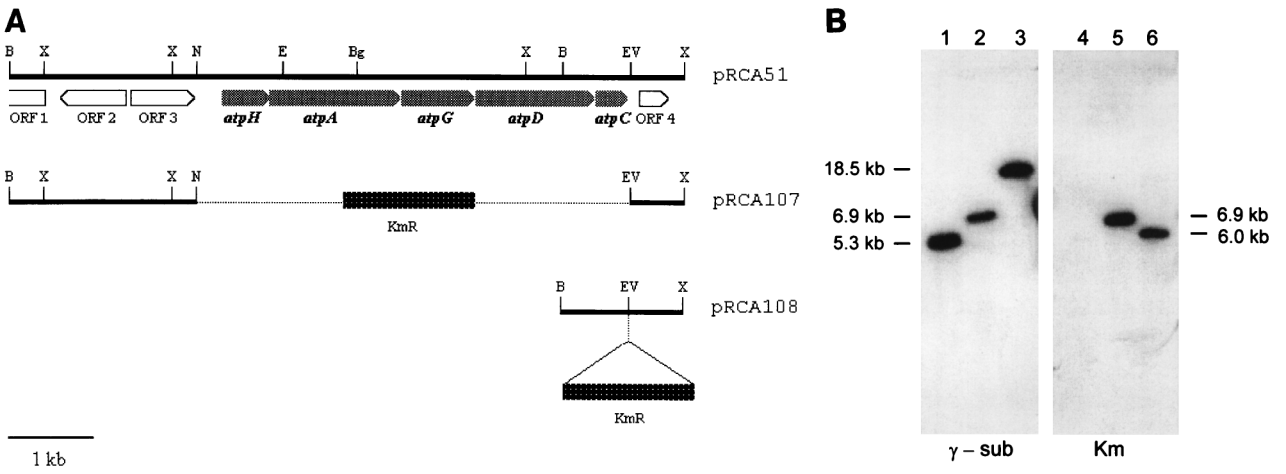

FIG. 3. (A) Restriction maps of plasmids pRCA51, pRCA107, and pRCA108. Thick lines represent *Rhodobacter capsulatus* DNA. Gray bars indicate *atp* genes, and open bars represent ORFs. Solid bars in pRCA107 and pRCA108 are kanamycin resistance cassettes inserted at the positions shown. Restriction site abbreviations: B, *Bam*HI; X, *Xho*I; N, *Not*I; E, *Eco*RI; Bg, *Bgl*II; EV, *Eco*RV. Vectors are not shown. (B) Hybridization pattern of *Rhodobacter capsulatus* total DNA digested with *Eco*RI. Lanes: 1 and 4, wild-type B100; 2 and 5, strain RCAK1; 3 and 6, deletion mutant RCAK4. Hybridization was done with a γ-subunit probe (lanes 1 to 3) and then with a kanamycin resistance probe (lanes 4 to 6). The two panels are pictures of the same filter hybridized with the first probe, stripped, and rehybridized with the second probe.

The problem of introducing a genomic deletion in the *atpHAGDC* operon or, more generally, in an indispensable gene, was addressed in a different way by combining transfer using GTA particles and conjugation procedures. We reasoned that it would be possible, in principle, to insert a genomic deletion via GTA particles followed by complementation with a wild-type copy of the operon present on a plasmid introduced by conjugation. Given the efficiency of transfer using GTA particles for a gene present on a plasmid and the efficiency of conjugation, measured in control experiments, it was calculated that approximately 4×10^9 cells were required for one positive event. The GTA-conjugation experiment was started with 3.5 ml of a *Rhodobacter capsulatus* B100 full-grown culture ($\sim 7 \times 10^9$ cells) and 3.5 ml of GTA containing filtrate from a J1 donor strain culture. This mixture was then centrifuged, resuspended in 3.5 ml of RCV minimal medium, and incubated at 30°C for 1 h. Conjugation of plasmid pRCA51 (pRK415 carrying the *atpHAGDC* operon) (Fig. 3A) was carried out by adding to the *Rhodobacter capsulatus* cell suspension 750 μl each of the two *E. coli* strains used in triparental matings, and the mixture was plated as described previously (26), on RCV minimal medium plus kanamycin. Under these conditions, three colonies that were then found to be resistant to both kanamycin and tetracycline (marker carried by pRCA51) appeared, suggesting the presence of both the Km^r cassette and the complementing plasmid. These colonies were grown as liquid cultures on RCV minimal medium with kanamycin but in the absence of tetracycline. Under these growth conditions, the selective pressure for plasmid maintenance is considered to be the presence of a functional ATP synthase rather than the antibiotic resistance. After repeated subculturing, all of the colonies derived from these cultures tested positive for resistance to both kanamycin and tetracycline. Under the same conditions and without antibiotic selection, the vector, pRK415, was lost in up to 40 to 50% of the cells. Plasmid preparations confirmed that the complementing plasmid was still present. For direct evidence of the genomic insertion of the Km^r cassette, genomic DNA was isolated from wild-type strain B100, strain RCAK1, and one of the strains with double resistance, RCAK4. Total DNA *Eco*RI digestions were checked by hybridization with the γ-subunit gene (*atpG*) and the Km^r gene. The hybridization results are shown in Fig. 3B. Wild-type strain B100 hybridizes to the γ-subunit probe, showing a 5.3-kb restriction fragment (Fig. 3B, lane 1), but not to the Km^r gene (lane 4). Strain RCAK1 (lanes 2 and 5) shows the same hybridization band with both probes (see pRCA108 restriction map in Fig. 3A). These 6.9-kb signals correspond to the 5.3-kb *Eco*RI restriction fragment of the wild type plus the 1.6-kb Km^r gene inserted at the end of the operon. Strain RCAK4 probed with the γ-subunit gene (lane 3) shows a signal corresponding to a fragment of 18.5 kb, which is exactly the size of the plasmid pRCA51 linearized with *Eco*RI. In lane 3, there is no trace of the 5.3-kb hybridization band that represents the wild-type chromosomal copy of *atpHAGDC*, indicating that the operon has been deleted from the chromosome. In lane 6, hybridization of the same RCAK4 DNA with the Km^r gene indicates that the resistance cassette has been inserted into the chromosome in place of the *atpHAGDC* operon. The size of this fragment does not correspond to the 6.9 kb of the hybridization band in lane 5 minus the 5.1 kb of the *atp* operon because the *Eco*RI site present inside the operon has been lost in the process of deletion. From the experiment just described derives the possibility of testing the effect of site-directed mutations in essential genes in *Rhodobacter capsulatus* simply by substituting in the wild-type copy, on the incoming plasmid, a mutated version of the gene under study.

Conclusions. We have cloned and sequenced the *atpHAGDC* operon coding for ATP synthase from *Rhodobacter capsulatus*. The operon contains only the five genes of the extrinsic sector (F_1), while the genes of the transmembrane subunits (F_0) are in a different region of the chromosome, resembling the situation found in the close relatives *Rhodospirillum rubrum* and *Rhodopseudomonas blastica*. Cloning and sequencing of the F_0 operon are in progress.

Rhodobacter capsulatus was reported to grow quite poorly under pure fermentative conditions, with fructose as the carbon source (24). Substantial growth was observed when DMSO or trimethylamine-*N*-oxide was used as an exogenous electron sink (16). We tried to isolate F_1 deletion mutants under both growth conditions. Cells were incubated either anaerobically in the dark or in the light (as additional energy source) or aerobically with the respiratory chain used as an electron sink. No

such deletion mutants were obtained under any of these growth conditions. It was possible, however, to isolate strains with a chromosomal deletion in the *atpHAGDC* operon after conjugation with an F_1-containing plasmid. Our data can be considered in the light of previous conflicting results on the requirement of oxidative phosphorylation for growth under conditions of anaerobic respiration with DMSO or trimethylamine-*N*-oxide (16, 19, 24). Regarding the question of whether anaerobic electron flow is required just to dissipate the reducing power generated during fermentation or is necessarily associated with oxidative phosphorylation, our results clearly favor the second hypothesis.

This work represents the first step in the genetic characterization of the ATP synthase system in this photosynthetic bacterium. The well-developed genetics of this species and the good biochemical characterization of its energy-producing components make it a preferred system for genetic and structural analysis of photosynthetic ATP synthesis in prokaryotes. We have described here a genetic procedure that will make possible the easy introduction of mutated copies of the *atpHAGDC* operon for site-directed functional and structural analyses.

Nucleotide sequence accession number. The DNA sequence presented has been assigned the EMBL accession no. X99599.

This work was supported by the European Community under contract BIO2-CT93 0078.

We are grateful to A. G. W. Leslie and J. E. Walker, MRC Laboratory, Cambridge, United Kingdom, who allowed access to the atomic coordinates of bovine heart mitochondrial F_1. We also thank Paola Turina for helpful discussions and for preparation of Fig. 3. The work of our students, Daniela Costa, Andrea Romano, and Luigi Altomare, is also acknowledged.

REFERENCES

1. **Abrahams, J. P., A. G. W. Leslie, R. Lutter, and J. E. Walker.** 1994. Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. Nature **370:**621–628.
2. **Boyer, P. D.** 1993. The binding change mechanism for ATP synthase. Some probabilities and possibilities. Biochim. Biophys. Acta **1140:**215–250.
3. **Buggy, J. J., M. W. Sganga, and C. E. Bauer.** 1994. Nucleotide sequence and characterization of the *Rhodobacter capsulatus hvrB* gene: HvrB is an activator of *S*-adenosyl-L-homocysteine hydrolase expression and is a member of the LysR family. J. Bacteriol. **176:**61–69.
4. **Cozen, A. L., and J. E. Walker.** 1987. The organization and sequence of the genes for ATP synthase subunits in the cyanobacterium *Synechococcus* 6301. J. Mol. Biol. **194:**359–383.
5. **Duncan, T. M., V. V. Bulygin, Y. Zhou, M. L. Hutcheon, and R. L. Cross.** 1995. Rotation of subunits during catalysis by *Escherichia coli* F_1-ATPase. Proc. Natl. Acad. Sci. USA **92:**10964–10968.
6. **Falk, G., A. Hampe, and J. E. Walker.** 1985. Nucleotide sequence of the *Rhodospirillum rubrum atp* operon. Biochem. J. **228:**391–407.
7. **Falk, G., and J. E. Walker.** 1988. DNA sequence of a gene cluster coding for subunits of the F_0 membrane sector of ATP synthase in *Rhodospirillum rubrum*. Biochem. J. **254:**109–122.
8. **Fillingame, R. H.** 1990. Molecular mechanics of ATP synthesis by F_1F_0-type H^+-transporting ATP synthases, p. 354–391. *In* T. A. Krulwich (ed.), The bacteria. Academic Press, Inc., New York, N.Y.
9. **Futai, M., T. Noumi, and M. Maeda.** 1989. ATP synthase (H^+-ATPase): results by combined biochemical and molecular biological approaches. Annu. Rev. Biochem. **58:**111–136.
10. **Gabellini, N., Z. Gao, C. Eckerskorn, F. Lottspeich, and D. Oesterhelt.** 1988. Purification of the H^+-ATPase from *Rhodobacter capsulatus*, identification of the F_1F_0 components and reconstitution of the active enzyme. Biochim. Biophys. Acta **934:**227–234.
11. **Herrmann, R. G., J. Steppuhn, G. S. Herrmann, and N. Nelson.** 1993. The nuclear-encoded polypeptide Cfo-II from spinach is a real, ninth subunit of chloroplast ATP synthase. FEBS Lett. **326:**192–198.
12. **Hillmer, P., and H. Gest.** 1977. H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*: H_2 production by growing cultures. J. Bacteriol. **129:**724–731.
13. **Keen, N. T., S. Tamaki, D. Kobayashi, and D. Trollinger.** 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene **70:**191–197.
14. **Lill, H., and N. Nelson.** 1991. The *atp*1 and *atp*2 operons of the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Mol. Biol. **17:**641–652.
15. **Ma, D., D. N. Cook, D. A. O'Brien, and J. E. Hearst.** 1993. Analysis of the promoter and regulatory sequences of an oxygen-regulated *bch* operon in *Rhobobacter capsulatus* by site-directed mutagenesis. J. Bacteriol. **175**:2037–2045.
16. **Madigan, M. T., J. C. Cox, and H. Gest.** 1980. Physiology of dark fermentative growth of *Rhodopseudomonas capsulata*. J. Bacteriol. **142:**908–915.
17. **Marrs, B.** 1974. Genetic recombination in *Rhodopseudomonas capsulata*. Proc. Natl. Acad. Sci. USA **71:**971–973.
18. **McCarn, D. F., R. A. Whitaker, J. Alam, J. M. Vrba, and S. E. Curtis.** 1988. Genes encoding the α, γ, δ, and four F_0 subunits of ATP synthase constitute an operon in the cyanobacterium *Anabaena* sp. strain PCC 7120. J. Bacteriol. **170:**3448–3458.
19. **McEwan, A. G., S. J. Ferguson, and J. B. Jackson.** 1983. Electron flow to dimethylsulphoxide or trimethylamine-N-oxide generates a membrane potential in *Rhodopseudomonas capsulata*. Arch. Microbiol. **136:**300–305.
20. **Miki, J., M. Maeda, Y. Mukohata, and M. Futai.** 1988. The γ-subunit of ATP synthase from spinach chloroplasts. Primary structure deduced from the cloned cDNA sequence. FEBS Lett. **232:**221–226.
21. **Noji, H., R. Yasuda, M. Yosida, and K. Kinosita.** 1997. Direct observation of the rotation of F_1-ATPase. Nature **386:**299–302.
22. **Sabbert, D., S. Engelbrecht, and W. Junge.** 1996. Intersubunit rotation in active F-ATPase. Nature **381:**623–625.
23. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
24. **Schultz, J. E., and P. F. Weaver.** 1982. Fermentation and anaerobic respiration by *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata*. J. Bacteriol. **149:**181–190.
25. **Senior, A. E.** 1990. The proton translocating ATPase of *Escherichia coli*. Annu. Rev. Biophys. Biophys. Chem. **19:**7–41.
26. **Sharak Genthner, B. R., and J. D. Wall.** 1985. Ammonium uptake in *Rhodopseudomonas capsulata*. Arch. Microbiol. **141:**219–224.
27. **Tybulewicz, V. L. J., G. Falk, and J. E. Walker.** 1984. *Rhodopseudomonas blastica atp* operon. Nucleotide sequence and transcription. J. Mol. Biol. **179:**185–214.
28. **Van Walraven, H. S., R. Lutter, and J. E. Walker.** 1993. Organization and sequences of genes for the subunits of ATP synthase in the thermophilic cyanobacterium *Synechococcus* 6716. Biochem. J. **294:**239–251.
29. **Walker, J. E., I. M. Fearnley, N. J. Gay, B. W. Gibson, F. D. Northrop, S. J. Powell, M. J. Rusnwick, M. Saraste, and V. L. J. Tybulewicz.** 1985. Primary structure and subunit stoichiometry of F_1 ATPase from bovine mitochondria. J. Mol. Biol. **184:**677–701.
30. **Walker, J. E., M. Saraste, and N. J. Gay.** 1984. Nucleotide sequence, regulation and structure of ATP-synthase. Biochim. Biophys. Acta **768:**164–200.
31. **Wall, J. D., and K. Braddock.** 1984. Mapping of *Rhodopseudomonas capsulata nif* genes. J. Bacteriol. **158:**404–410.
32. **Weaver, P. F., J. D. Wall, and H. Gest.** 1975. Characterization of *Rhodopseudomonas capsulata*. Arch. Microbiol. **105:**207–216.
33. **Wilkens, S., F. W. Dahlquist, L. P. McIntosh, L. W. Donaldson, and R. A. Capaldi.** 1995. Structural features of the ε subunit of the *Escherichia coli* ATP synthase determined by NMR spectroscopy. Nat. Struct. Biol. **2:**961–967.
34. **Xie, D. L., H. Lill, G. Hauska, M. Maeda, M. Futai, and N. Nelson.** 1993. The *atp*2 operon of the green bacterium *Chlorobium limicola*. Biochim. Biophys. Acta **1172:**267–273.
35. **Yen, H.-C., and B. Marrs.** 1976. Map of genes for carotenoid and bacteriochlorophyll biosynthesis in *Rhodopseudomonas capsulata*. J. Bacteriol. **126:**619–629.