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Molecular Cloning of the Ribosomal RNA Genes of the Photosynthetic Bacterium *Rhodopseudomonas capsulata*

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Summary. Chromosomal segments of *Rhodopseudomonas capsulata* carrying the ribosomal operons and cloned with the cosmid vector pHC79 have been identified by cross hybridization with γ^{32}P-ATP labeled rRNAs. At least seven rRNA operons are present in the *R. capsulata* chromosome. By R-loop analyses of DNA-RNA hybrids, two distinct loop structures of sizes 1.50 kb and 2.52 kb corresponding to the 16S and 23S RNA molecules, respectively, were detected. Intact 23S RNA molecules can be isolated from *R. capsulata* ribosomes by sucrose density centrifugation. However, fragmentation of the 23S RNA molecule into a 16S-like molecule was observed during gel electrophoresis. Restriction mapping and hybridization of a 9 kb *Pst*I fragment that contained one copy of the rRNA operon showed the following sequence of the RNA genes in *R. capsulata* 16S, 23S, and 5S. A spacer region of 0.91 kb was found between the 16S and the 23S RNA genes.

Introduction

Members of *Rhodospirillaceae* govern a broad range of metabolic activities. They are able to grow phototrophically under anaerobic conditions or chemotrophically under aerobic conditions. They assimilate nitrogen and CO_2 as well as other carbon and nitrogen sources. Phototrophic bacteria are interesting model systems for cell differentiation (reviewed in Drews and Oelze 1981).

Although the photosynthetic activities, the cell organization and metabolic routes of these bacteria have been studied extensively (Clayton and Sistrom 1978), the organization of the genome remains largely unknown (Saunders 1978). One reason was the failure of an efficient genetic transfer system as it has been described for many other bacteria. Marrs and coworkers, who detected the gene transfer agent in strains of *Rhodopseudomonas capsulata*, have mapped, using this defective phage, genes of *R. capsulata* coding for photopigment markers (Marrs 1974; Yen and Marrs 1976). Various conjugative systems were constructed in *R. capsulata* and the closely related *Rhodopseudomonas sphaeroides* by means of inserting P1-type plasmids from *Escherichia coli* and *Pseudomonas* species (Sistrom 1977; Tucker and Pemberton 1979; Yu et al. 1981; Marrs 1981). Low levels of chromosomal mobilization and gene complementation have been shown.

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To understand the organization and regulation of *R. capsulata* genes, we have initiated a project of cloning and identification of structural and regulatory genes from this bacterium. In the process of screening a cosmid colony bank, we have identified chromosomal segments that contain the rRNA genes of *R. capsulata*. Unusual rRNA processing in *R. sphaeroides* and *R. capsulata* has been proposed (Lessie 1965; Marrs and Kaplan 1970; Mackay et al. 1979). The 23S (1.1×10^6 M_r) RNA molecule appeared to be a precursor of the 0.53×10^6 M_r RNA molecules of the 50S ribosomal subunit. This unusual processing sequence is believed to take place in some other *Rhodopseudomonas* species as well (Gray 1978).

Materials and Methods

Strains. The bacterial strains used are listed in Table 1.

Media. Escherichia coli strains were grown in LB medium (Bacto Tryptone 10 g, NaCl 5 g, 1,000 ml distilled water, pH 7.0). Ampicillin (Ap, Hoechst, Frankfurt, FRG) was added to media at 50 µg/ml final concentration. The wild-type *Rhodopseudomonas capsulata* strain 37b4 (Deutsche Sammlung von Mikroorganismen Göttingen, DSM 938) was cultivated chemotrophically in the dark or phototrophically in the light with RÄH medium (Drews 1965).

Reagents. Restriction enzymes, T4 ligase and polynucleotide kinase were obtained from Boehringer, Mannheim or Bethesda Research Laboratory, Neu-Isenburg, FRG, γ^{32}P-ATP was purchased from Amersham-Buchler, Frankfurt.

Table 1. Bacterial strains

Strain	Genotype
Escherichia coli K12	
BHB 2600	803 *supE supF* rk^- mk^+ *met*
DH1	*recA*$^-$ rk^- mk^- *nal* A^r
BHB 3000	SF8/pHC 79
BHB 2688	N205 *recA*$^-$ (*λimm 434 cIts b2 red3 Eam4 SAM 7*)/*λ*
BHB 2690	N205 *recA*$^-$ (*λimm 434 cIts b2 red3 Dam15 SAM7*) *λ*
Rhodopseudomonas capsulata	prototrophic wild-type

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Cosmid and Chromosomal DNAs Isolation. Cosmid DNA was isolated as described before using the method for plasmid DNA isolation (Yu et al. 1981). Chromosomal DNA was isolated from anaerobically grown *R. capsulata* cells by the procedure described (Marmur 1961; Klotz and Zimm 1972).

Determination of G+C Molecular % and Chromosome Size Measurements. The molecular percentage of guanine plus cytosine base composition was determined by measuring the middle denaturation temperature, Tm (Marmur and Doty 1962), using a Perkin-Elmer 124 double beam spectrophotometer coupled to a Hewlett-Packard X-Y Recorder 7044A. Alternatively, the G+C molecular % was also determined by the buoyant density of the DNA after isopycnic centrifugation using *E. coli* DNA (50–51 G+C mol %) for comparison (Flamm et al. 1972).

For the chromosome size measurement, total DNA was extracted from exponentially growing *R. capsulata* cells and the corresponding cell count was determined by cell and colony counting.

Cosmid Cloning. Cosmid cloning with the cosmid vector-pHC79 was done essentially as described (Hohn and Collins 1980). The vector DNA was digested with the restriction enzyme *Bam*HI (recognizing sequence G'GATCC) and the wild-type *R. capsulata* chromosomal DNA was digested with the restriction enzyme *Sau*3A (recognizing sequence N'GATCC) which cuts more randomly on the chromosome than *Bam*HI. Ligation of the fragments and the vector was done in the presence of T4 ligase. For the in vitro packaging, extracts of the *E. coli* strains BHB 2688 and BHB 2690 were used (Hohn 1979). The packaged DNA was transduced into *E. coli* strains BHB 2600 or DH1. Positive transductants (Ap^r, Tet^s) were transferred to microtiter plates (holding 98 wells each) by sterile toothpicks. The clones were kept viable in LB medium with 40% glycerol at −70° C.

Isolation of Ribosomal RNAs. Ribosomal RNAs were isolated from chemotrophically grown *R. capsulata* cells at stationary phase. The cells were harvested by centrifugation at 8,500 rpm for 15 min at 4° C (Sorvall centrifuge RC2B, rotor GSA). Digestion buffer (0.2 M Tris-acetate, pH 7.8, 0.15 M magnesium acetate, 0.03 M potassium acetate, 1% Triton X-100, 0.1% iodoacetate or diethylpyrocarbonate, Sigma, 10 ml, was added to cells of 1 l culture. The cells were sonicated three times for 15 s at 0° C. The larger cell debris and membrane-bound chromosomal DNA were centrifuged down at 8,000 rpm (rotor SS34, Sorvall RC2B centrifuge). The supernatant was layered onto 15 ml cushion buffer (50 mM Tris-acetate, pH 7.8, 13 mM magnesium acetate, 20 mM potassium acetate, 1 M sucrose) in polycarbonate centrifuge-tubes. They were centrifuged at 30,000 rpm (rotor Ti60, Beckman ultracentrifuge L2-50) for 2.5 h at 1° C. The sediment was resuspended with 5 ml 50 mM sodium acetate, pH 5.0 and 0.25 ml 20% SDS solution, mixed and stored in an ice bath for 5 min. An equal volume of phenol (freshly distilled and saturated with 50 mM sodium acetate buffer pH 5.0) was added, shaken vigorously and left on ice for 5 min. The tubes (Corex) were then centrifuged at 10,000 rpm (rotor SS34, Sorvall RC2B centrifuge) for 10 min at 4° C. The upper aqueous phase was taken up and extracted with chloroform: isoamyl alcohol (24:1) twice. The final extract was adjusted to 0.3 M sodium acetate with a 3 M stock solution pH 5.0. The RNA was precipitated by adding two volumes of ethanol at −20° C overnight. The RNA was pelleted by centrifugation at 10,000 rpm (rotor SS34, Sorvall RC2B centrifuge) for 10 min and washed twice with 70% ethanol. The final pellet was dissolved in sterile water and stored at −70° C.

For separation of the rRNA species, 0.5 mg total RNA was loaded carefully on top of an 11 ml 10%–30% linear sucrose gradient prepared in 100 mM NaCl, 10 mM Tris-HCl, pH 7.4, 0.5% SDS, 1 mM EDTA and 100 µg/ml heparin (Sigma) in an SW41 nitrocellulose centrifuge-tube. The gradients were centrifuged at 40,000 rpm (rotor SW41, Beckman ultracentrifuge L2-50) for 8 h at 20° C. Fractions of 0.5 ml were collected by downward displacement of the gradient and the elution profile was recorded with a Perkin-Elmer Spectrophotometer and Servogor Recorder RE543. The 23S and 16S rRNAs were fractionated and purified by two cycles of centrifugation on a 5%–20% sucrose gradient in the above buffer. The fractions containing the 5S RNA were collected after the first sucrose gradient centrifugation and then purified on a 10% polyacrylamide gel.

Kinasing RNA. RNA, 3 µg in 10 µl 0.1 M Tris buffer, pH 9.5 was hydrolyzed at 85° C for 5 min. After cooling on ice, kinase buffer was added to give a final concentration of 50 mM Tris, pH 9.5, 10 mM $MgCl_2$, 5 mM dithiothreitol, 4 µM ATP, 50 µCi γ^{32}P-ATP and 10 units of polynucleotide kinase (Boehringer, Mannheim) in a total volume of 25 µl and the sample was incubated at 37° C for 30 min. The reaction was terminated by the addition of 45 µl 2 M ammonium acetate, 20 µl 50% glycerol and 50 µg *E. coli* tRNA (Boehringer, Mannheim). The labeled RNAs were separated from free ATP by filtration through a 10 × 1 cm G-50 Sephadex column eluted with sterile distilled water.

In situ Colony Hybridization. Cosmid clones were transferred from the master microtiter plates to an LB agar plate with the help of a stamp equipped with 48 needles that fit exactly into the wells of the microtiter plates. These colonies were grown up directly on nitrocellulose discs (Schleicher and Schuell, Dassel) which were placed on the LB agar medium plus ampicillin (50 µg/ml). The colonies were denaturated by placing the filters on Whatman 3MM paper that had been soaked with 0.5 M NaOH for 10 min. The filters were blotted dry and treated as above with 0.5 M Tris-HCl, pH 7.5, 1.5 M NaCl for 5 min. They were then air dried and incubated for 1 h with 1 mg/ml proteinase K (Merck, Darmstadt) in 1 × SSC (0.15 M NaCl, 15 mM sodium citrate, pH 7.0). The filters were washed once in 2 × SSC and air dried before treatment in chloroform followed by extensive washing in 0.3 M NaCl. The filters were baked at 80° C for 2 h in a vacuum oven. Hybridization with γ^{32}P-ATP labeled RNA (1×10^6 cpm/filter) was done overnight at 65° C in 3 × SSPE (3 mM EDTA, 30 mM $NaH_2PO_4 \cdot H_2O$, 0.45 M NaCl, pH 7.0), 0.02% Ficoll, 0.02% Polyvinylpyrrolidone and 0.1% sodium dodecyl sulfate. The hybridized filters were washed twice at 65° C with 2 × SSPE and 1% SDS for 45 min each. The final wash was 30 min at 37° C with 0.2 × SSPE. The filters were air dried and autoradiographed for 32 h at −70° C.

Agarose Gel Electrophoresis and Southern Blot. For DNA separation, agarose gel electrophoresis was performed in

horizontal gels (18.5 × 16 × 0.3 cm) with 0.6% agarose (Serva high EEO grade) in 1 × E buffer (50 mM Tris-HCl, pH 7.8, 1 mM EDTA, 20 mM sodium acetate) at 50 mA constant current overnight. The gels were stained with ethidium bromide and photographed under UV light with Polaroid Type 52/PolaPan film.

For RNA separation, formaldehyde agarose gel electrophoresis was performed as described in Yu and Drews (1982) according to the procedure of Rave et al. (1979). Horizontal slab gels (18.5 × 16 × 0.3 cm) of 0.75% agarose and 6% formaldehyde were prepared in N buffer (18 mM Na_2HPO_4, 2 mM NaH_2PO_4). RNA samples (5–10 µg) were incubated in 50% formaldehyde (Merck, 99.7% concentration), 6% formaldehyde in N buffer at 60° C for 5 min, cooled quickly in ice and 0.5 volume of 50% glycerol in 50% formamide containing bromphenol blue dye was added. Electrophoresis was carried out at 1 V/cm overnight at 4° C.

Southern blots of DNA were done as described (Southern 1975) and hybridized to γ^{32}P-ATP labeled RNA as in the in situ colony hybridization.

R-Loop Analyses. Cosmid DNA was digested to completion with restriction endonuclease. For the preparation of DNA-RNA duplex molecules, DNA (8 µg/ml) and rRNA (13 µg/ml) were incubated in the following mix (Woolford and Rosbash 1979): 10 µl 5 × Pipes buffer (Pipes, Sigma, 0.5 M pH 6.8, 5 mM EDTA, 2.0 M NaCl), 35 µl formamide (Merck, 99% concentration, purified by treatment with Bio Rad mix-bed ionic exchanger AG501-×8), 5 µl DNA and RNA, H_2O was added to a final volume of 50 µl. The above mix was heated 2 min at 75° C and then cooled slowly to 54° C (two step method, cf. Brack 1981) and incubated at this temperature for 4–6 h.

The samples were spread according to the method of Davis et al. (1971) by adding 3 µl of the DNA-RNA hybridized mix to 25 µl formamide, 5 µl Tris-buffer B (1 M Tris, pH 8.5, 0.1 M EDTA), 10 µl H_2O, 2 µl plasmid pBR322 DNA as size marker and 5 µl cytochrome c (Sigma, 1 mg/ml). The hypophase used was 5 ml formamide and 250 µl Tris-buffer B made to a final volume of 25 ml with water. The grids were rotary-shadowed with Pt/Pd (80:20% w/w) using a Balzers evaporation unit Mikro-BA3 (Balzers, Fürstentum Liechtenstein) and the DNA-RNA duplex molecules were observed in a Zeiss EM-10CR electron microscope.

Results

Rhodopseudomonas capsulata Chromosome

The base composition of *R. capsulata* strain 37b4 chromosomal DNA was 65 ± 2 mol% guanine plus cytosine (G+C) (Fig. 1). Members of the genus *Rhodopseudomonas* have % molecular G+C ranges from 64–73 (De Bont et al. 1981). The genome size of *R. capsulata*, as measured by the amount of DNA per cell during exponential growth, is about 2.2×10^9 daltons. In *R. sphaeroides*, the genome size was determined to be 1.6×10^9 daltons (Gibson and Niederman 1970). There was no difference in DNA content between chemotrophically or phototrophically grown cells. Both microorganisms have chromosome sizes comparable to other bacteria and have by rough estimation coding capacities for 2,000–3,000 average size proteins.

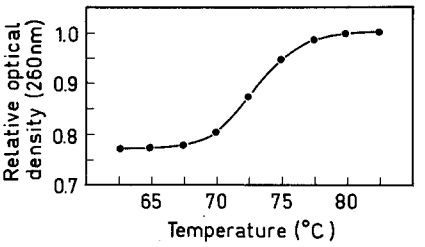

Fig. 1. Spectrophotometric melting curve in 10 mM Tris buffer pH 7.8 of *Rhodopseudomonas capsulata* DNA

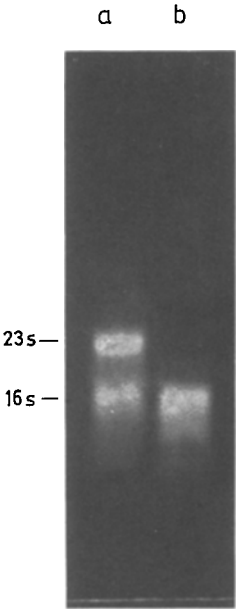

Fig. 2. 0.75% formaldehyde agarose gel electrophoresis of ribosomal RNAs from (a) *Escherichia coli* (b) *Rhodopseudomonas capsulata*

Characteristics of rRNAs from Rhodopseudomonas capsulata

Ribosomal RNAs were isolated from ribosomes of chemotrophically grown *R. capsulata* cells and separated in formaldehyde agarose gel electrophoresis. A broad band of size 16S and smaller, but no 23S rRNA species were detected when compared to the 16S and 23S rRNAs isolated from *E. coli* (Fig. 2). However, if the rRNAs were separated in a 10%–30% linear sucrose gradient, 23S, 16S, 5S, and 4S RNAs comparable to those of *E. coli* could be found (Fig. 3). Apparently, the 23S RNA molecule was fragmented into a 16S-like RNA molecule in *R. capsulata* under the conditions of formaldehyde gel electrophoresis.

The *Eco*RI, *Hin*dIII, *Pst*I and *Bam*HI digests of *R. capsulata* chromosomal DNA were fractionated on a 0.6% agarose gel and hybridized to γ^{32}P-ATP labeled total rRNAs of *R. capsulata* (Fig. 4). Multiple bands representing presumably different copies of the rRNA genes were seen to be present in the *R. capsulata* chromosome. At least seven rRNA operons could be detected in the *R. capsulata* genome by hybridization of γ^{32}P-ATP labeled 5S RNA to the *Eco*RI-digested *R. capsulata* chromosomal DNA (data not shown).

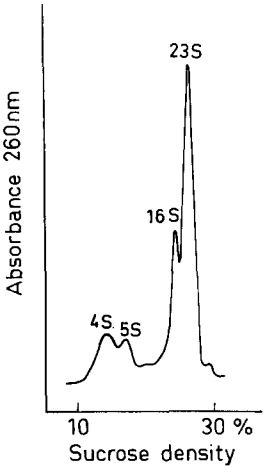

Fig. 3. Linear 10%–30% sucrose density gradient separation of ribosomal RNAs of *Rhodopseudomonas capsulata*

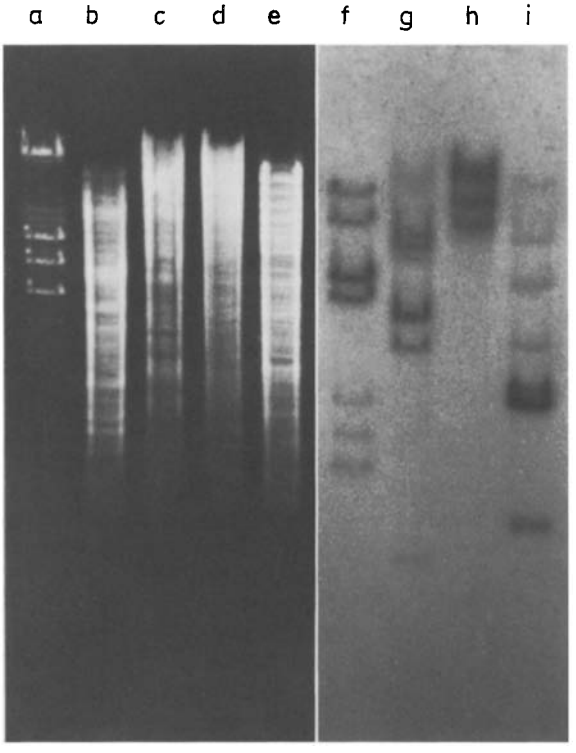

Fig. 4. 0.6% agarose gel electrophoresis of DNA fragments from (a) *Eco*RI fragments of λ phage as size markers (21.8, 7.5, 5.5, 4.8, 3.4 kb). Lanes b, c, d, and e were *Rhodopseudomonas capsulata* DNA digested with restriction enzymes *Eco*RI, *Hin*dIII, *Pst*I and *Bam*HI, respectively. Lanes a, b, c, d, and e were stained with ethidium bromide and visualized under UV light. Lanes f, g, h, and i were Southern blots of lanes b, c, d, and e to nictrocellulose and hybridized to γ^{32}P-ATP labeled ribosomal RNAs isolated from *R. capsulata*. Hybridization and autoradiography were done as described in Materials and Methods

Cloning of rRNA Genes

We have used the large cloning capacity of cosmid vectors in the preparation of a gene bank of *R. capsulata*. A collection of about 900 cosmid clones that have *R. capsulata* DNA fragments of size ranges from 35–45 kb was available. These clones, when statistically distributed, will give a good

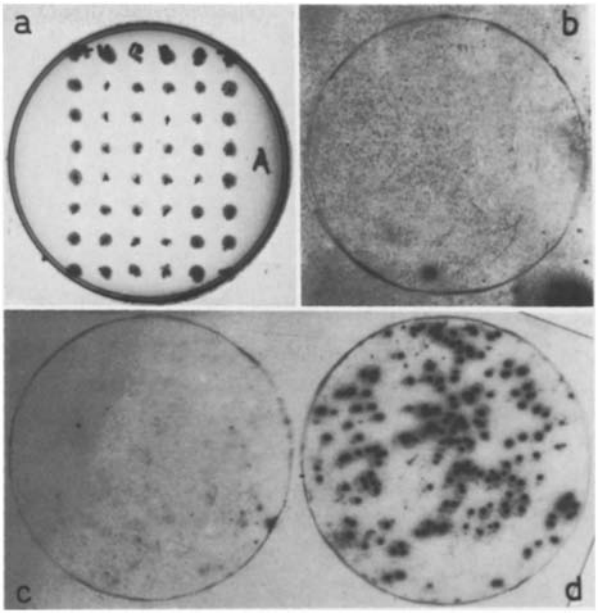

Fig. 5a–d. In situ colony hybridization for the screening of *Rhodopseudomonas capsulata* cosmid gene bank. **a** Colonies containing cosmids were selected on LB medium plus ampicillin (50 µg/ml) after replication from master microtiter plates. **b** Radioautogram of the positive cosmid clone pRC1 after hybridization to γ^{32}P-ATP labeled ribosomal RNAs isolated from *R. capsulata*. **c** and **d** were radioautograms representing a negative (c) and a positive (d) cosmid clone after the second colony screening assay

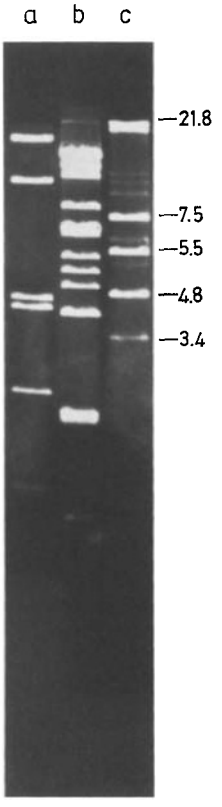

Fig. 6. 0.6% agarose gel electrophoresis of DNA fragments from (a) *Hin*dIII digestion of cosmid clone pRC1 DNA (b) *Hin*dIII digestion of the cosmid clone pRC2 DNA (c) *Eco*RI digestion of λ DNA as size markers

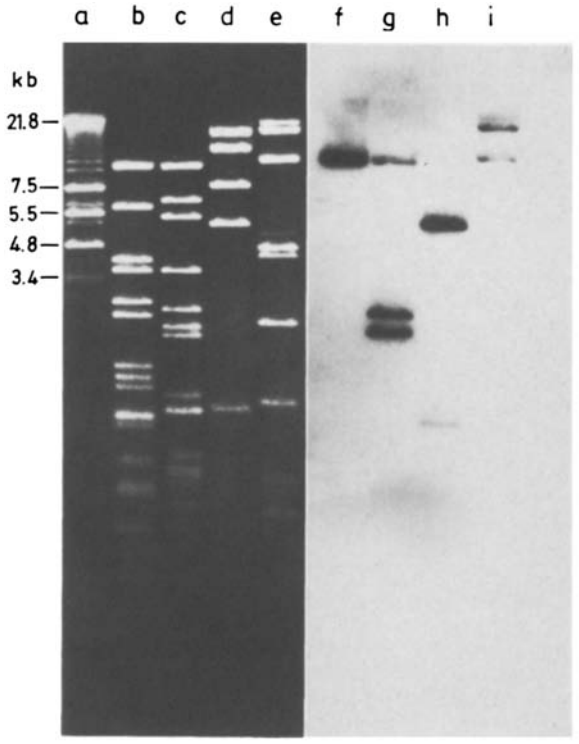

Fig. 7. 0.6% agarose gel electrophoresis of DNA fragments from (a) *Eco*RI digestion of DNA as size markers. Lanes b, c, d, and e were the restriction digestion products of cosmid clone pRC1 DNA with *Pst*I, *Bam*HI, *Eco*RI and *Hin*dIII respectively. Lanes f, g, h, and i were Southern blots of Lanes b, c, d, and e to nitrocellulose and hybridized to γ^{32}P-ATP labeled ribosomal RNAs isolated from *Rhodopseudomonas capsulata*

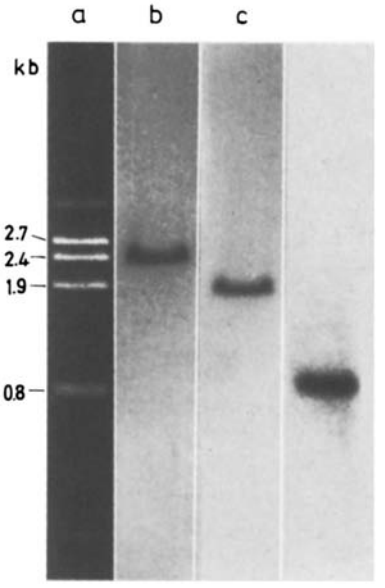

Fig. 9. Hybridization of γ^{32}P-ATP labeled 23S, 16S, and 5S RNA to the cloned *Pst*I fragment of the cosmid clone pRC1 DNA. (a) *Bam*HI digestion of the *Pst*I fragment. (b) hybridization of γ^{32}P-ATP labeled 23S RNA to (a). (c) hybridization of γ^{32}P-ATP labeled 16S RNA to (a). (d) hybridization of γ^{32}P-ATP labeled 5S RNA to (a)

chance of having all unique sequences of *R. capsulata* DNA (Clarke and Carbon 1976).

Positive cosmid clones were identified by hybridization with γ^{32}P-ATP labeled total RNAs (Fig. 5). Two cosmid clones pRC1 and pRC2 were purified and their DNA isolated. *Hin*dIII restriction digestion of the pRC1 and pRC2 DNAs showed that they originated from different segments of the *R. capsulata* genome (Fig. 6). Nick-translated pRC1 and pRC2 DNAs hybridized to digested *R. capsulata* chromosomal DNA showed that the two cosmid clones have not undergone genetic rearrangement (data not shown).

Arrangement of rRNA Genes

The DNA from the cosmid clone pRC1 was digested with *Bam*HI, *Eco*RI, *Pst*I and *Hin*dIII restriction endonucleases analyzed on 0.6% agarose gel and hybridized to γ^{32}P-ATP labeled total rRNAs of *R. capsulata* (Fig. 7). A single 9 kb *Pst*I fragment was found to hybridize to the total rRNAs (Fig. 7). This *Pst*I fragment was isolated and characterized further by restriction digestion. The restriction mapping data were summarized in Fig. 8. The orientation of the RNA genes in this *Pst*I fragment was determined by selective hybridization of γ^{32}P-ATP labeled 23S, 16S, and 5S RNA to *Bam*HI-digested fragments (Fig. 9). The orientation of the RNA genes was 16S, 23S, and 5S in the 9 kb *Pst*I fragment (Fig. 8). The exact location of the RNA genes was determined by R-loop analyses. Figure 10 shows the rRNA-rDNA duplexes found within the cloned *Pst*I fragment of pRC1 DNA. We found one small and one large loop inside this 9 kb *Pst*I fragment. Their size distribution is summarized in Table 2. The location of these loops is shown together with the restriction digestion data in Fig. 8.

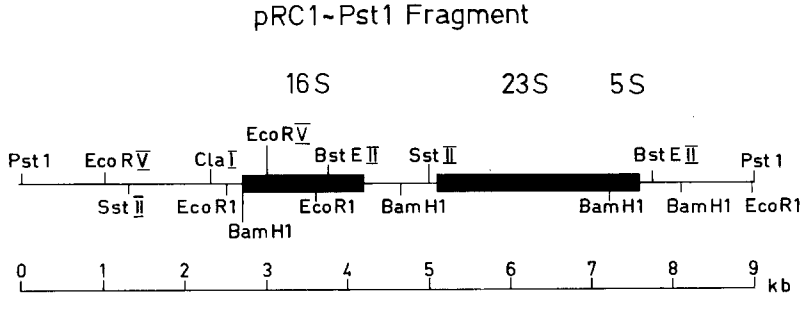

Fig. 8. Restriction endonuclease map of the cloned *Pst*I fragment of the cosmid clone pRC1 DNA. The coding regions for the ribosomal RNA genes are shown by blackened regions

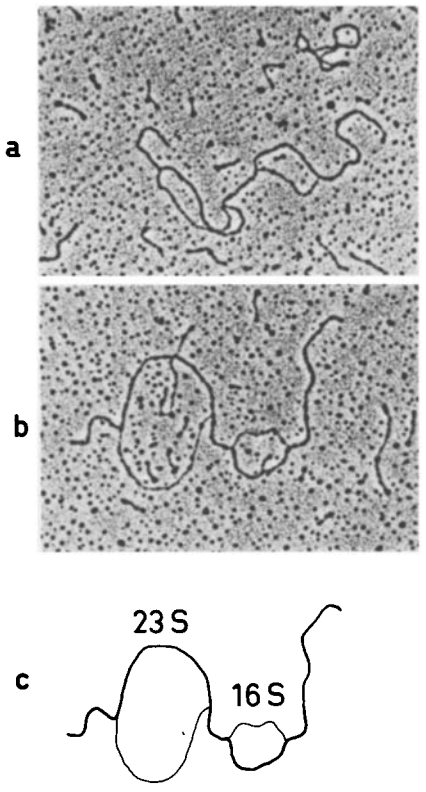

Fig. 10. R-loop analyses of rRNA-rDNA duplexes formed within the cloned *Pst*I fragments of the cosmid clone pRC1 (a) and (b). Double-stranded plasmid pBR322 DNA was used as internal size marker. The orientation of the loops as well as double and single strands are shown in the corresponding line tracing (c)

Table 2. Organization of the rRNA genes in the *Pst*I fragment of pRC1

Length (kb)[a]					
Total	Long end	16S loop	Spacer	23S loop	Short end
9.07 ±0.62[b]	2.83 ±0.31	1.50 ±0.18	0.91 ±0.08	2.52 ±0.22	1.39 ±0.13

[a] Sizes were determined by using plasmid DNA pBR322 (4,362 bp) as internal size marker

[b] The mean of 14 molecules ± S.D.

Discussion

Two unique chromosomal DNA segments of *R. capsulata* carrying the rRNA operon have been cloned in the cosmid vector pHC79. By R-loop analyses, we have found two loop structures corresponding roughly to those of 16S and 23S rRNA coding regions of *E. coli* rDNAs (rrnB operon: 1,542 bp and 2,904 bp respectively, Brosius et al. 1981). The 23S coding region in *R. capsulata* was 2,523 bp, which was shorter than that of *E. coli* and *Anacystis nidulans* (2,904 bp, Tomioka et al. 1981). The spacer region between the 16S and the 23S rRNA genes was estimated to be 0.91 kb in *R. capsulata*. This spacer region was longer than that of *E. coli* (440 bp spacer region in rrnB operon, Brosius et al. 1981) and *A. nidulans* (540 bp, Tomioka et al. 1981). Longer spacer regions have been reported in *Chlamydomonas* chloroplast (1.68 kb, Rochaix and Malnoe 1978), in maize and tobacco chloroplasts (about 2.0 kb, Bedbrook et al. 1977; Kusuda et al. 1980) and in spinach chloroplast (about 1.4 kb, Whitfeld et al. 1978).

The studies of Marrs and Kaplan (1970) and Mackay et al. (1979) showed clearly that the 0.53 and 0.42×10^6 M_r rRNAs of mature *R. sphaeroides* 50S ribosomal subunit resulted from the cleavage of a 1.1×10^6 M_r (23S) RNA precursor. In *R. capsulata* an intact 23S RNA molecule could be readily isolated by sucrose density gradient centrifugation. This 23S RNA molecule was fragmented into a 16S-like RNA molecule under the conditions of formaldehyde agarose gel electrophoresis. This fragmentation process occurred presumably under destabilizing conditions by means of specific endonucleolytic cleavage on particular region(s) of the 23S RNA molecule. Heating and the absence of divalent ions such as Mg^{++} and Ca^{++} during the RNA extraction have also been shown to induce this fragmentation process (Leaver and Ingle 1971); although the isolation of intact 23S RNA molecules has been reported in the absence of Mg^{++} from *R. sphaeroides* (Borda et al. 1969). Besides *Rhodopseudomonas* species, 23S RNA fragmentations have been reported in *Anacystis nidulans, Agrobacterium tumefaciens, Bdellovibrio bacteriovorus* and in some plant chloroplasts (Doolittle 1973; Schuch and Loening 1975; Meier and Brownstein 1976; Leaver and Ingle 1971).

The *R. capsulata* rRNA genes cross-hybridized to those of rRNAs isolated from *E. coli* (data not shown). Comparative analyses of the 16S ribosomal RNA sequences have been used to explore prokaryotic phylogeny (Fox et al. 1980). The cloning of the rRNA genes of *R. capsulata* completes genetic analysis of this ancient group of bacteria.

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