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Heterogeneity of the Conserved Ribosomal Ribonucleic Acid Sequences of *Bacillus subtilis*

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ABSTRACT

DOI, ROY H. (University of California, Davis), AND RICHARD T. IGARASHI. Heterogeneity of the conserved ribosomal ribonucleic acid sequences of *Bacillus subtilis*. J. Bacteriol. **92**:88–96. 1966.—Hybrid formation was demonstrated between *Bacillus subtilis* ribosomal ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) from various bacterial species. The high degree of complementarity between *B. subtilis* ribosomal RNA and the DNA from *B. cereus* and *B. stearothermophilus* suggested a method to test whether the same RNA sequences were hybridizing with the DNA from these two species. Saturation studies with 16*S* and 23*S* RNA preparations from *B. subtilis* showed that a definite number of complementary sites was present in each DNA. Base composition analyses of the RNA in the hybrid demonstrated that ribosomal RNA sequences were involved. Hybrid competition studies revealed that *B. stearothermophilus* ribosomal RNA could compete totally against *B. subtilis* ribosomal RNA for *B. stearothermophilus* DNA, although it could compete only partially against the *B. subtilis* ribosomal RNA hybridizing with *B. cereus DNA*. These observations were made independently with both 16*S* and 23*S* ribosomal RNA preparations. These results revealed that different nucleotide sequences of *B. subtilis* ribosomal RNA were hybridizing with the DNA from *B. cereus* and *B. stearothermophilus*. Two possible interpretations of these results are: (i) different nucleotide sequences from a homogeneous ribosomal RNA population are hybridizing with heterologous DNA preparations, and (ii) ribosomal RNA cistrons are heterogeneous.

Ribosomes, which play a key role in protein synthesis, are composed of approximately 60% ribonucleic acid (RNA) and 40% protein in bacteria (5, 23). The 70*S* ribosomes are composed of one 50*S* and one 30*S* subunit, containing RNA with sedimentation coefficients of 23*S* and 16*S*, respectively (16). It has been demonstrated by hybridization techniques that deoxyribonucleic acid (DNA) sequences complementary to ribosomal RNA exist (24, 25). The results indicated that several complementary sites for both the 16*S* and 23*S* RNA preparations were present. Furthermore, evidence for the conservation of ribosomal RNA sequences among various organisms has been presented (3, 8, 9).

Among *Bacillus* species, the ribosomal RNA of *B. subtilis* hybridizes efficiently with the DNA from *B. cereus* and *B. stearothermophilus* (8, 9), which promotes the following question. Is the ribosomal RNA sequence which is hybridizing with the DNA of these two heterologous species identical? Fractionation methods and chemical procedures for determining nucleotide sequences are not available. However, from recent analyses of hybrids between ribosomal RNA and heterologous DNA (8), an indirect procedure for approaching this problem was made apparent.

The results to be presented reveal that a fraction of the *B. subtilis* ribosomal RNA which hybridizes with *B. cereus* DNA cannot be competed against by *B. stearothermophilus* ribosomal RNA, although the latter competes against all the *B. subtilis* RNA which hybridizes with *B. stearothermophilus* DNA. This fact suggests that the fraction of *B. subtilis* ribosomal RNA which hybridizes with *B. cereus* DNA has a sequence of nucleotides different from that fraction involved in hybrid formation with *B. stearothermophilus* DNA.

MATERIALS AND METHODS

Bacterial strains. The following organisms were used: *B. subtilis* W23, *B. cereus* T, *B. stearothermophilus* (ATCC 12016), *Escherichia coli* K-12, *Pseudomonas aeruginosa* (ATCC 10197), and *Proteus vulgaris* (ATCC 2427).

Media. All cells except *B. stearothermophilus* were grown in Penassay medium (Difco). The medium for *B. stearothermophilus* was 2% Tryptone (Difco). All cultures were agitated on a rotary shaker at 37 C, with the exception of *B. stearothermophilus* which was shaken at 55 C.

Preparation, purification, and hybridization of DNA and RNA. DNA was prepared and purified by the method of Marmur (18), with minor modifications (8).

The preparation of uniformly labeled ribosomal RNA (8) and its purification by the phenol method (12) have been described. The separation of 16*S* RNA and 23*S* RNA by methylated albumin kieselguhr (MAK) column chromatography (17) required three to four passages of each RNA species through the MAK column (25). A linear NaCl gradient of 0.55 to 1.2 M was used.

Hybrid formation was analyzed by the methods of Nygaard and Hall (19) and Hall and Spiegelman (13). In some experiments, the ribonuclease-treated annealing mixture was passed through a G-100 Sephadex column before analysis was done by the membrane filter technique (3).

The competitive hybrid formation experiments were described previously (7, 8). The typical annealing mixtures contained 25 μg of heat-denatured DNA, 1 to 5 μg of radioisotope-labeled ribosomal RNA, an increasing amount of unlabeled RNA (0 to 500 μg), and 0.3 M KCl-0.01 M tris(hydroxymethyl)-aminomethane (Tris) buffer (*p*H 7.0), in a total volume of 0.6 ml. The annealing mixtures were incubated for 3 hr at 70 C, and slowly cooled to room temperature. Previous results (8) had shown that there were no significant changes in the relative amount of hybrid formed at temperatures of incubation from 50 to 75 C. The annealing mixtures were treated with 10 μg of ribonuclease at room temperature for 15 min before being analyzed for hybrids. The methods for base ratio analyses (14) and for radioactive assays (6) have been described.

Materials. The following chemicals were used: uridine-H^3 (4.4 c/mole), Schwarz Bio Research, Inc., Orangeburg, N.Y.; P^{32} phosphoric acid, Oak Ridge National Laboratories, Oak Ridge, Tenn.; crystalline pancreatic ribonuclease, Worthington Biochemical Corp., Freehold, N.J.; type B-6 membrane filter, Schleicher & Schuell Co., Keene, N.H.

RESULTS

Hybrid formation between B. subtilis ribosomal RNA and heterologous DNA. In a previous communication (8), hybrid formation between *B. subtilis* ribosomal RNA and various *Bacillus* DNA preparations, as analyzed by the technique of Nygaard and Hall (19), was reported. These results were confirmed by use of equilibrium sedimentation as the method of analysis. In Fig. 1a to f, the banding profiles of hybrids in CsCl are presented. It is clearly evident from the banding profiles that ribosomal RNA is banded at a position slightly denser than the DNA; this position is indicative of DNA-RNA hybrids (24). *B. subtilis* ribosomal RNA formed hybrids with DNA from the following organisms: *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *E. coli*, and *P. vulgaris*. Very little or no hybrid formation is evident with *P. aeruginosa* DNA. Similar results with *P. vulgaris* and *P. pseudomonas* DNA were obtained when the annealing mixtures were assayed by the membrane filter technique. These results confirmed the earlier findings (8). All subsequent analyses of RNA-DNA hybrid formation were performed with the membrane filter technique (19).

The favorable hybrid formation between *B. subtilis* ribosomal RNA and *B. cereus* and *B. stearothermophilus* DNA suggested the following rationale for analyzing the conserved sequence of the *B. subtilis* ribosomal RNA. The *B. subtilis* ribosomal RNA hybridizing with the two DNA preparations could be either identical or dissimilar in nucleotide sequence. If the identical sequence is hybridizing with both DNA preparations, then ribosomal RNA from *B. cereus* and *B. stearothermophilus* should compete equally well against this RNA during hybrid formation with one of the DNA preparations. If the competition of the heterologous RNA preparations does *not* occur equally well, then it would suggest that different sequences of *B. subtilis* ribosomal RNA were hybridizing with the two DNA preparations.

To perform such an analysis on the two molecular species of ribosomal RNA, the labeled bulk ribosomal RNA of *B. subtilis* was fractionated into its 16*S* and 23*S* components by repeated MAK column chromatography (25). Similar purification procedures were used to obtain 16*S* and 23*S* RNA from *B. cereus* and *B. stearothermophilus* for the hybrid competition studies.

Saturation of various DNA preparations with B. subtilis ribosomal RNA. Saturation experiments were performed to determine the amount of *B. subtilis* 16*S* RNA and 23*S* RNA which would hybridize with *B. cereus* and *B. stearothermophilus* DNA. An increasing amount of labeled *B. subtilis* RNA was hybridized with a constant amount of DNA until no further increase in hybrid formation was observed. Saturation curves were obtained for both the 16*S* RNA and the 23*S* RNA with each DNA species (Fig. 2a to c). From the specific activity of the labeled ribosomal RNA, it was possible to calculate the amount (in micrograms) of RNA which was hybridized with the

various DNA preparations at saturation levels. These results are listed in Table 1. Both 16S RNA and 23S RNA have their own saturation levels for all the DNA preparations. Column D in Table 1 illustrates the level obtained when both 16S RNA and 23S RNA were present in the annealing mixture at the same time. A comparison of column C and column D in Table 1 indicates that the saturation levels for 16S RNA and 23S RNA were additive. The amount of *B. subtilis* ribosomal RNA which hybridized with *B. cereus* and *B. stearothermophilus* DNA was 74 and 42% of the homologous hybridization, respectively.

Base ratio analysis of RNA hybridized with heterologous DNA. To analyze the base ratio of the *B. subtilis* RNA which was hybridized with the heterologous DNA preparations, the hybrid annealing mixture after proper incubation and ribonuclease treatment was passed through a G-100 Sephadex column. The hybridized RNA-P^{32} was separated from most of the RNA fragments which resulted from ribonuclease treatment. Figure 3 illustrates such a column fractionation of the hybrid material. The hybridized RNA was associated with the DNA as represented by the optical density profile, whereas the unhybridized RNA fragments eluted after the hybrid. The hybrid material was collected on membrane filters, hydrolyzed with alkali, and analyzed for its base composition by the isotope dilution technique (14).

Table 2 illustrates the base composition of the RNA hybridized with the various DNA preparations. It is evident that the RNA has a base composition very similar to the ribosomal RNA of *B. subtilis*. Also, the base composition indicates the absence of *B. subtilis* messenger RNA (6, 7). A slight decrease in the guanylate plus cytidylate composition of the RNA hybridized with *B. cereus* DNA was noted.

Hybrid competition experiments. By use of several criteria it was evident that *B. subtilis* ribosomal RNA was hybridizing with heterologous DNA preparations. A series of hybrid competition experiments was performed to determine whether the *B. subtilis* RNA which was hybridizing with *B. cereus* DNA was identical to that hybridizing with *B. stearothermoiphlus* DNA. The experiments were performed as follows. A constant amount of DNA and a constant amount of labeled ribosomal RNA were placed in a series of tubes. An increasing amount of unlabeled RNA was added to this series of annealing mixtures, and, after proper incubation, the amount of hybrid formed in each tube was analyzed. In the control tube, only DNA and the labeled RNA were present, and the amount of hybrid formed in the control tube was considered as 100% hybrid formation. If the unlabeled RNA competed against the labeled RNA for the DNA, the amount of hybrid formed, as represented by radioactive counts, was decreased. This amount is plotted in Fig. 4 to 6 as the percentage (in counts per minute) remaining in the hybrid.

In Fig. 4, a homologous competition experiment is illustrated with *B. subtilis* RNA and DNA. It is obvious from the results that both 16S RNA and 23S RNA can compete effectively against homologous RNA sequences for DNA. In Fig. 5, the results of hybrid competition experiments with heterologous DNA are illustrated. In these experiments, labeled *B. subtilis* ribosomal RNA was hybridized with *B. stearothermophilus* DNA. Unlabeled 16S RNA from *B. stearothermophilus* and *B. cereus* was able to compete effectively against labeled *B. subtilis* 16S RNA for *B. stearothermophilus* DNA (Fig. 5a). Similar results were obtained with the 23S RNA of *B. subtilis* (Fig. 5b). In both cases, the homologous and heterologous unlabeled RNA competed effectively against the labeled *B. subtilis* RNA for *B. stearothermophilus* DNA. This suggested that the sequence of *B. subtilis* RNA which hybridized with *B. stearothermophilus* DNA was common to the ribosomal RNA populations of both *B. cereus* and *B. stearothermophilus*.

Different results, however, were obtained when labeled *B. subtilis* 16S RNA and 23S RNA were hybridized with *B. cereus* DNA. Unlabeled *B. cereus* 16S RNA competed effectively against labeled *B. subtilis* 16S RNA for *B. cereus* DNA as expected (Fig. 6a). However, unlabeled *B. stearothermophilus* 16S RNA could compete against *only a fraction* of the labeled *B. subtilis* RNA for the *B. cereus* DNA (Fig. 6a).

Also, the unlabeled *B. stearothermophilus* 23S

FIG. 1. *Analysis of hybrid formation between Bacillus subtilis ribosomal RNA-H^3 and various DNA preparations by equilibrium sedimentation in CsCl. The annealing mixtures contained 100 μg of heat-denatured DNA, 6 μg of ribosomal RNA-H^3 (1.1×10^4 counts per min per μg), and 0.3 M KCl-0.01 M Tris (pH 7.2), in a final volume of 1.3 ml. The mixtures were incubated at 70 C for 5 hr and cooled to room temperature. Solid CsCl was added to the annealing mixture to give a density of 1.762 g/cc; to this solution was added enough CsCl solution (density = 1.762) for a final volume of 4.0 ml. The solutions were centrifuged at 33,000 rev/min in a Spinco SW 39 rotor at 25 C for 60 hr. The assay procedures were described previously (13). DNA from the following organisms was used: (a) B. subtilis, (b) B. cereus, (c) B. stearothermophilus, (d) Escherichia coli, (e) Proteus vulgaris, (f) Pseudomonas aeruginosa.*

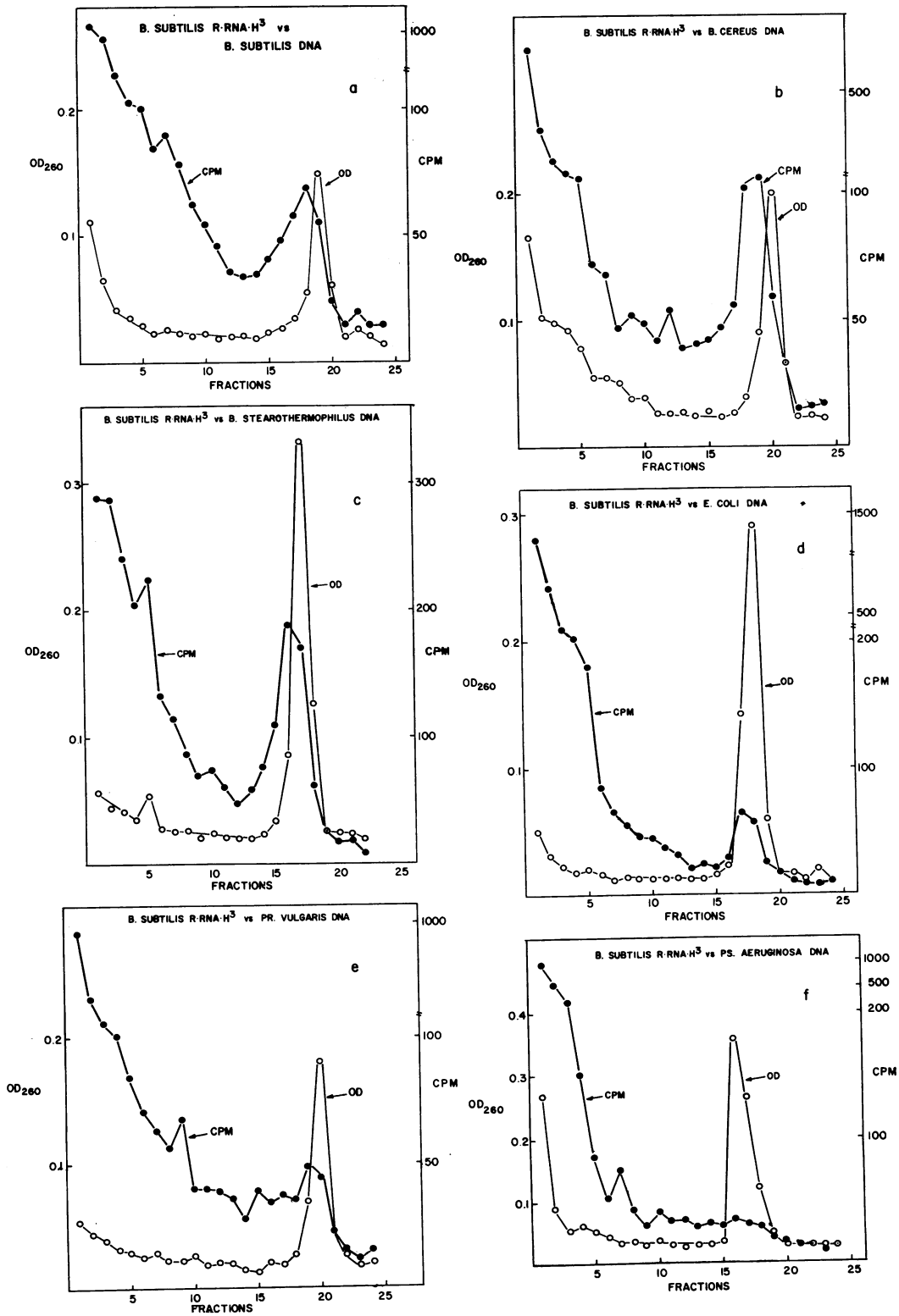
B. SUBTILIS R·RNA·H3 vs
B. SUBTILIS DNA
a
B. SUBTILIS R·RNA·H3 vs B. CEREUS DNA
b
B. SUBTILIS R·RNA·H3 vs B. STEAROTHERMOPHILUS DNA
c
B. SUBTILIS R·RNA·H3 vs E. COLI DNA
d
B. SUBTILIS R·RNA·H3 vs PR. VULGARIS DNA
e
B. SUBTILIS R·RNA·H3 vs PS. AERUGINOSA DNA
f
OD260
CPM
OD
FRACTIONS

FIG. 1

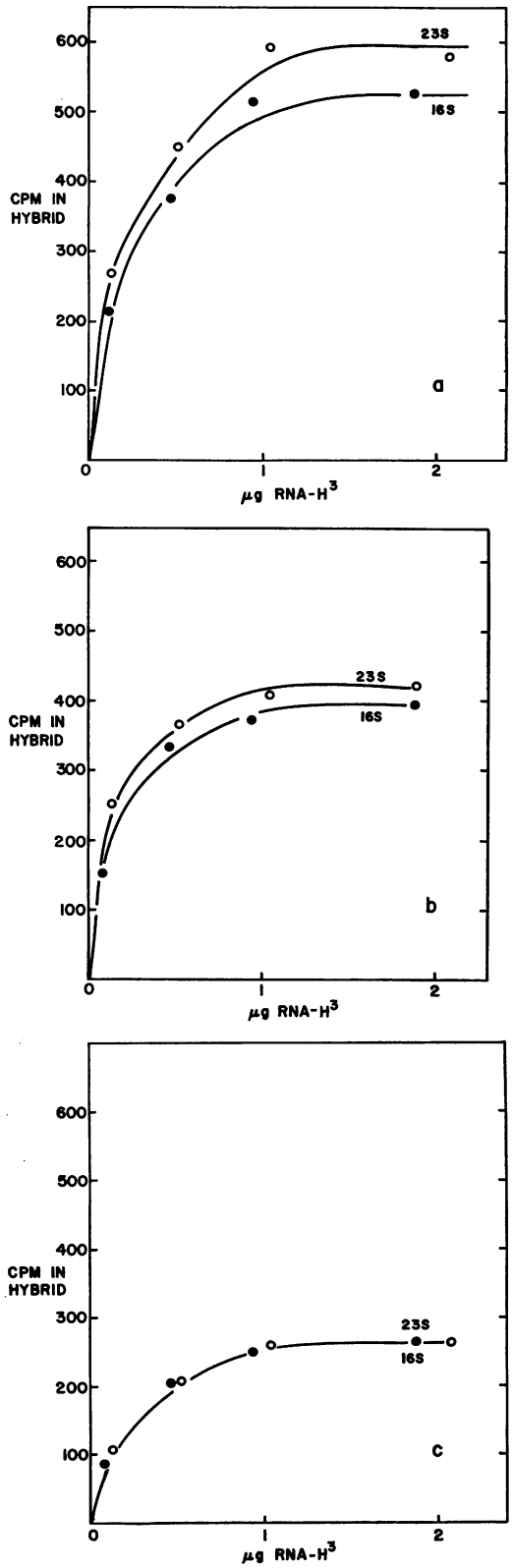

TABLE 1. *Saturation of various DNA preparations with Bacillus subtilis ribosomal RNA*

Source of DNA	(A) 16S RNA (μg per 100 μg of DNA)[a]	(B) 23S RNA (μg per 100 μg of DNA)[a]	(C) A + B	(D) 16S + 23S RNA (μg per 100 μg of DNA)[b]
B. subtilis.....	0.22	0.26	0.48	0.53
B. cereus.....	0.17	0.18	0.35	0.39
B. stearothermophilus.	0.11	0.11	0.22	0.22

[a] These values were obtained from the experiments described in Fig. 2a to c.

[b] The values in column D were obtained from similar experiments in which saturating levels of both 16S RNA and 23S RNA were present in the annealing mixture.

RNA competed against only a fraction of the labeled *B. subtilis* 23S RNA for *B. cereus* DNA (Fig. 6b). With each species of RNA, approximately 10% of the *B. subtilis* RNA could not be competed against effectively. The same situation was obtained when labeled *B. subtilis* 16S RNA and 23S RNA were both present in the annealing mixture, and *B. stearothermophilus* 16S RNA and 23S RNA were used to compete for the *B. cereus* DNA. Again, approximately 10% of the labeled *B. subtilis* RNA was not competed against effectively by the *B. stearothermophilus* RNA. A base ratio analysis of the RNA remaining as hybrid after the competition revealed the following base composition in moles per cent: cytidylate, 20.4; adenylate, 27.4; uridylate, 20.5; guanylate, 31.6. The base composition of the RNA was essentially that of *B. subtilis* ribosomal RNA (Table 2). These results suggested that the *B. subtilis* ribosomal RNA hybridizing with *B. cereus* DNA had some nucleotide sequences different from the *B. subtilis* ribosomal RNA hybridizing with *B. stearothermophilus*.

DISCUSSION

The analysis of heterologous DNA-ribosomal RNA hybrid formation by the cesium chloride

FIG. 2. *Hybrid formation of Bacillus subtilis ribosomal RNA-H³ with various DNA preparations as a function of RNA concentration. The annealing mixtures (0.6 ml) contained 25 μg of heat-denatured DNA, various amounts of B. subtilis 16S RNA-H³ (9.2 × 10³ counts per min per μg) or 23S RNA (9.2 × 10³ counts per min per μg), and 0.5 M KCl-0.01 M Tris (pH 7.0). The mixtures were incubated at 70 C for 3 hr and slowly cooled to room temperature before assay. (a) B. subtilis DNA; (b) B. cereus DNA; (c) B. stearothermophilus DNA.*

density gradient technique confirms the earlier results obtained by Doi and Igarashi (8) with the membrane filter method. Furthermore, the results obtained with *E. coli* and *P. vulgaris* DNA illustrate that intergeneric DNA-RNA hybrid formation is possible. Although base ratio analyses were not performed on the RNA-DNA hybrids of *E. coli* and *P. vulgaris*, it is presumed that sufficient complementarity exists between their ribosomal RNA cistrons and *B. subtilis* RNA for the formation of ribonuclease-resistant complexes. Hybrid formation between ribosomal RNA and DNA of many organisms has also

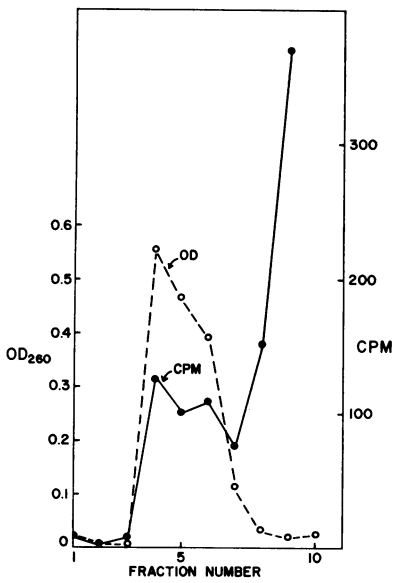

FIG. 3. *Sephadex column separation of DNA-RNA-H^3 hybrid from RNA-H^3 fragments after ribonuclease treatment. The Sephadex G-100 column (1.5 by 16 cm) was equilibrated with 0.5 M KCl-0.01 M Tris, pH 7.0, (KT) buffer. The hybrid annealing mixture (0.6 ml) after ribonuclease treatment was added to the column and eluted with KT buffer. The flow rate was 0.6 ml/min. Fractions (2 ml) were collected and analyzed for radioactivity and optical density at 260 mμ. The optical density peak (fractions 4 to 7) represents the DNA-RNA hybrid region. The radioactive label is in the RNA.*

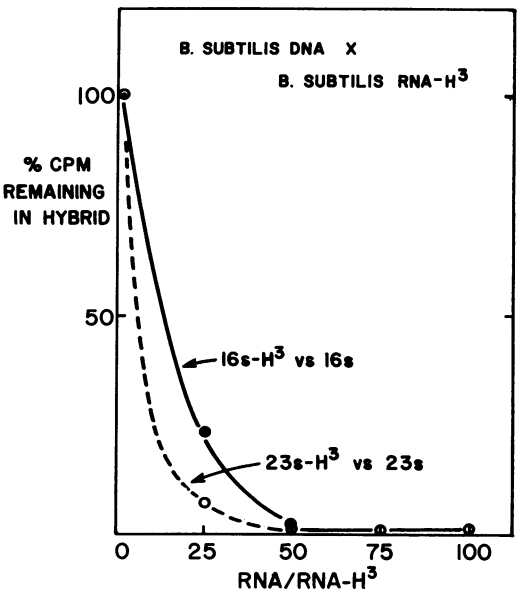

FIG. 4. *Homologous competition of unlabeled Bacillus subtilis RNA against labeled RNA for DNA. Each annealing mixture (0.6 ml) contained 25 μg of heat-denatured B. subtilis DNA, 2 μg of 16S RNA-H^3 (9.2×10^3 counts per min per μg) or 2 μg of 23S RNA-H^3 (9.2×10^3 counts per min per μg), an increasing amount of unlabeled B. subtilis 16S or 23S RNA as indicated in the abscissa, and 0.5 M KCl-0.01 M Tris buffer, pH 7.0. The details of hybridization and assay are given in Materials and Methods. The control contained no unlabeled RNA, and the hybrid formed in the control was considered to be 100%. The ordinate shows the relative amount of hybrid formed in the presence of increasing amounts of unlabeled homologous RNA. Unlabeled 16S RNA and 23S RNA were used to compete only against labeled 16S RNA and 23S RNA, respectively.*

TABLE 2. *Base composition of the Bacillus subtilis ribosomal RNA hybridized with various DNA preparations*

Source of DNA	Moles per cent[a]				GC	G/C	A/U
	Cytidylate (C)	Adenylate (A)	Uridylate (U)	Guanylate (G)			
					%		
B. subtilis	17.3	30.3	18.3	34.1	51.4	1.97	1.66
B. cereus	19.1	29.2	22.2	29.5	48.6	1.54	1.32
B. stearothermophilus	16.0	30.9	17.4	35.7	51.7	2.23	1.78
B. subtilis ribosomal RNA (unhybridized)	22.6	27.0	20.2	30.2	52.8	1.33	1.34

[a] These values are accurate to ±2%.

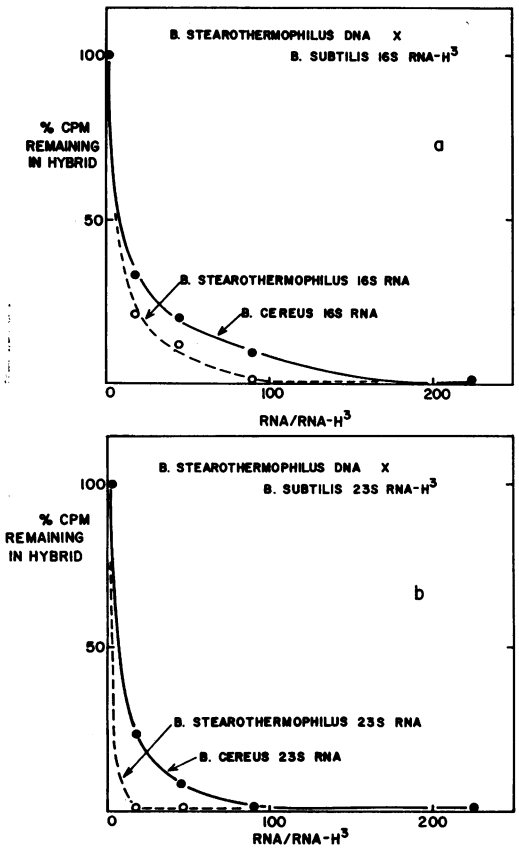

FIG. 5. *Competition of unlabeled Bacillus stearothermophilus and B. cereus RNA against labeled B. subtilis RNA for B. stearothermophilus DNA during hybridization. Each annealing mixture contained 25 μg of heat-denatured B. stearothermophilus DNA, 2 μg of B. subtilis 16S RNA-H³ (9.2 × 10³ counts per min per μg) or 2 μg of B. subtilis 23S RNA-H³ (9.2 × 10³ counts per min per μg), an increasing amount of unlabeled B. stearothermophilus or B. cereus 16S or 23S RNA as indicated in the abscissa, and 0.5 M KCl-0.01 M Tris buffer, pH 7.0. See Fig. 5 for other details. (a) 16S RNA competition; (b) 23S RNA competition.*

been reported by Dubnal et al. (9) and by Attardi et al. (3). Among microorganisms, the sequence of nucleotides in the ribosomal RNA cistrons appears to have been conserved highly.

The base ratio analysis of the *B. subtilis* RNA which hybridized with *B. stearothermophilus* and *B. cereus* DNA demonstrates that ribosomal RNA is indeed hybridizing with heterologous DNA. The composition is very similar to that of the *B. subtilis* RNA hybridized with *B. subtilis* DNA. The hybrids formed under the conditions used in these experiments are different from the RNA-DNA complexes found by Opara-Kubinska et al. (21). In their experiments, the base composition of the RNA complexed with DNA after ribonuclease treatment had a composition similar to RNA core. The results of the present experiments suggest that much greater homology and complementarity were obtained, since the RNA in the ribonuclease-treated hybrid had a composition resembling that of the original RNA.

The saturation curves of *B. subtilis* ribosomal RNA with *B. stearothermophilus* and *B. cereus* DNA illustrate that the sites available for the *B. subtilis* ribosomal RNA are limited to a fixed number. Although *B. cereus* DNA has a base composition more dissimilar from that of *B. subtilis* than *B. stearothermophilus*, it still hybridizes

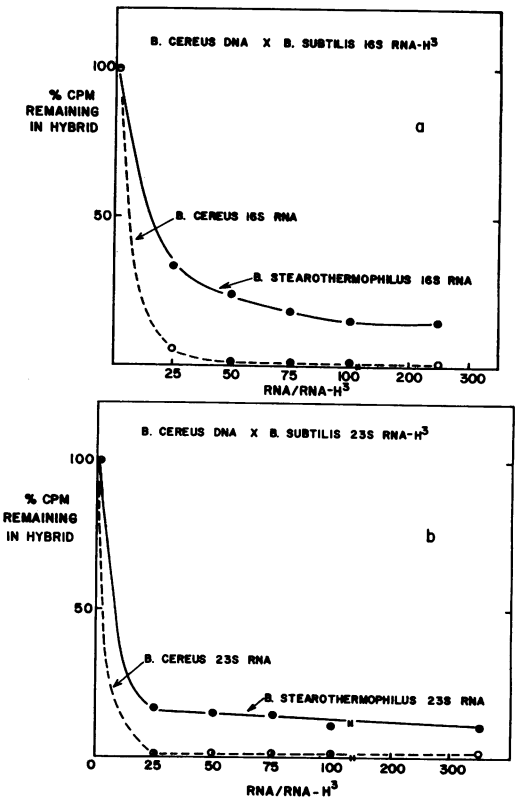

FIG. 6. *Competition of unlabeled Bacillus cereus and B. stearothermophilus RNA against labeled B. subtilis RNA for B. cereus DNA during hybrid formation. Each annealing mixture contained 25 μg of heat-denatured B. cereus DNA, 2 μg of B. subtilis 16S RNA-H³ (9.2 × 10³ counts per min per μg) or 2 μg of B. subtilis 23S RNA-H³ (9.2 × 10³ counts per min per μg), an increasing amount of B. cereus or B. stearothermophilus 16S or 23S RNA as indicated by the abscissa, and 0.5 M KCl-0.01 M Tris buffer, pH 7.0. See Fig. 5 for other details. (a) 16S RNA competition; (b) 23S RNA competition.*

more efficiently with *B. subtilis* ribosomal RNA. The results have been obtained consistently with various preparations of DNA from both species. The absolute amount of RNA hybridizing with DNA is slightly higher than that reported previously (8), but within the range reported by several investigators. Yankofsky and Spiegelman (24) reported that the present methods employed for hybridization yield a minimal amount of RNA-DNA hybrids. The maximal amount of ribosomal RNA which can hybridize with homologous DNA is therefore still unknown. A range of 0.3 to 0.5 μg of ribosomal RNA per 100 μg of DNA has, however, been observed (3, 8, 9, 20, 22, 24). Slight variations have been observed depending on the ribosomal preparations employed.

The fact that *B. cereus* RNA can compete against *B. subtilis* RNA for *B. stearothermophilus* DNA indicates that all three species contain some ribosomal RNA nucleotide sequences in common. This is true, although their DNA preparations range from 33 to 50% in guanylate plus cytidylate composition. The fact that *B. stearothermophilus* RNA cannot compete against all the *B. subtilis* RNA for *B. cereus* DNA suggests that *B. cereus* and *B. subtilis* contain common nucleotide sequences different from that found in *B. stearothermophilus*. In a logical extension of this relationship, these results illustrate that different sequences of *B. subtilis* ribosomal RNA are hybridizing with each DNA.

These results could be interpreted in two other ways. (i) Different nucleotide sequences from one or several identical ribosomal RNA cistrons are hybridizing with the two heterologous DNA preparations; or (ii) cistrons for 16*S* and 23*S* RNA preparations are heterogeneous. In this regard, the *B. subtilis* nucleus contains 2×10^9 (11) to 10^{10} (15) molecular weight equivalents of DNA. From the saturation studies and the molecular weights of the ribosomal RNA (16), there are approximately 2 to 10 cistrons for each species of ribosomal RNA per nucleus of *B. subtilis*. The assumption that the *B. cereus* nucleus contains approximately the same number of cistrons for ribosomal RNA, as well as the fact that only 10 to 15% of the *B. subtilis* RNA is hybridized with *B. cereus* DNA after competition from *B. stearothermophilus* RNA, favors the first interpretation presented. This would also suggest that ribosomal RNA cistrons are identical. However, the possibility of the heterogeneous nature of ribosomal RNA cistrons cannot be disregarded. By use of another method Aronson and Holowczyk (2) concluded that ribosomal RNA fractions of *P. aeruginosa* and *E. coli* were heterogeneous.

Of interest is the fact that heterogeneity of ribosomal RNA would result in a heterogeneous population of ribosomes, since each 70*S* ribosome contains only one 23*S* RNA and only one 16*S* RNA. If the conformation of the ribosome depends on the primary structure of the ribosomal RNA, it is possible that ribosomes may be structurally different depending on the RNA contained, and may have a relative affinity for messenger RNA and transfer RNA during protein synthesis. Although specificity during protein synthesis is believed to reside in the nucleotide sequences of messenger RNA and the anticodon in the transfer RNA preparations, it is possible that some *specificity* and the *rate* of protein synthesis may be affected by the ribosome population which is present. During sporulation of bacilli, there is a rapid turnover or degradation, or both, of ribosomal RNA (4, 10) accompanied by the synthesis of messenger RNA and proteins essential for sporulation (1, 7). It is of interest to know whether ribosomes are heterogeneous, and whether a change in the population of ribosomes may somehow be involved in regulatory processes during differentiation.

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