JOURNAL OF BACTERIOLOGY, Oct. 1976, p. 28-34
Vol. 128, No. 1

Printed in U.S.A.

Characterization of the Hybridization Between Purified 16S and 23S Ribosomal Ribonucleic Acid and Ribosomal Deoxyribonucleic Acid from *Escherichia coli*

PATRICK P. DENNIS* AND DOUGLAS H. NORDAN

Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

Received for publication 10 May 1976

An assay to distinguish specifically between 16S and 23S ribosomal ribonucleic acid (rRNA) has been developed. The assay involves hybridization of radioactive rRNA to deoxyribonucleic acid (DNA) from λ*ilv*5 transducing phage, which carries an rRNA transcription unit. Radioactive 16S or 23S rRNA can be specifically and completely competed from hybrids by using highly purified nonradioactive 16S or 23S competitor RNA, respectively. The preparation and purification of 16S rRNA and 23S rRNA are described in detail. The hybridization assay is extremely sensitive and efficient; 65 to 70% or more of the input radioactivity hybridizes to specific DNA in the absence of homologous competitor RNA, and at saturation virtually all of the specific DNA sequences are hybridized to rRNA. The results indicate that: (i) the 16S rRNA and 23S rRNA prepared as described are greater than 99% pure; (ii) 16S rRNA and 23S rRNA hybridize with equal efficiency and in equal molar amounts to λ*ilv*5 DNA; (iii) at saturation, about one molecule of 16S and one molecule of 23S rRNA are hybridized per genome equivalent of λ*ilv*5 DNA; (iv) essentially no cross-hybridization occurs between 16S and 23S rRNA; and (v) the sequence homology between 16S and 23S rRNA is negligible.

Genes coding for the ribonucleic acid (RNA) components of the ribosome in *Escherichia coli* are organized into transcription units (14). The primary RNA transcript from these units contains an adenosine 5'-triphosphate, sediments at 30S, and has a molecular mass of about 2.0×10^6 daltons. Treatment with ribonuclease III in vitro releases species that are slightly larger than, but contain, the entire sequences of 23S ribosomal RNA (rRNA), 16S rRNA, and 5S rRNA (4,7,8,12). In addition to the 5S rRNA fragment, several other low-molecular-weight species are released from the 30S precursor (7). Two of these presumably represent the precursors of glutamic acid-accepting transfer RNA ($tRNA_2^{Glu}$) and isoleucine-accepting transfer RNA ($tRNA_1^{Ile}$), which map in the spacer region between the 16S and 23S rRNA genes on separate rRNA transcriptional units (10, 18). The gene order and the direction of transcription within these units have been demonstrated to be promotor, 16S rRNA, tRNA, 23S rRNA, and 5S rRNA (10).

The cotranscription of 16S and 23S rRNA suggests that the early stages of assembly of the 30S and 50S ribosomal subunits in vivo are closely related. To facilitate the study of the early events relating to ribosome assembly, we have developed specific assays for 16S rRNA and 23S rRNA. In this paper the assays are described in detail. They involve hybridization of radioactive rRNA to specific DNA from λ*ilv*5 transducing phage, which carries a complete copy of an rRNA transcription unit (18). Large amounts of highly purified nonradioactive competitor 16S or 23S rRNA completely and specifically eliminate the hybridization of radioactive 16S or 23S rRNA, respectively, to the λ*ilv*5 DNA. In contrast to earlier observations (1, 11), the results reported here indicate that 16S rRNA and 23S rRNA do not cross-hybridize and that the sequence homology between 16S and 23S rRNA is negligible.

MATERIALS AND METHODS

Bacterial growth. The bacterial strain used for the preparation of 16S and 23S rRNA was NF314, a *leu*$^-$ derivative of rifampin-sensitive *Escherichia coli* B strain AS19. The growth medium was minimal AB (13) supplemented with 0.4% vitamin-free Casamino Acids and 0.2% glucose. Cultures were started by a 10^4-fold dilution of a fresh, stationary-phase culture and grown at 37°C; growth was monitored as absorbance at 460 nm (A_{460}). At an A_{460} of 0.10, a 100-ml portion of a 1-liter culture was removed and labeled with [^{3}H]uracil (specific activity, 25 Ci/mmol; 3 μCi/ml). When the A_{460} of the radioactive culture reached 0.4, nonradioactive uracil (50 μg/ml) was added. When the culture reached an A_{460}

of 0.80, rifampin was added to a final concentration of 20 μg/ml for 30 min. The cultures were cooled to 0°C and harvested by centrifugation. The radioactive and nonradioactive cell pellets were used for preparation of radioactive and nonradioactive 16S and 23S rRNA.

Purification of 16S and 23S rRNA. A method similar to that described by Smith et al. (16) was used to purify 16S and 23S rRNA. All operations were performed at 0°C unless specified otherwise. The cell pellets were suspended in 1/20 volume of buffer I [10mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.4); 30 mM NH_4Cl; 10 mM $MgCl_2$; and 6 mM 2-mercaptoethanol] and disrupted with a French pressure cell. After the addition of deoxyribonuclease I (1 μg/ml), the lysates were centrifuged at 15,000 × *g* for 30 min to remove unbroken cells and cellular debris. Ribosomal particles were pelleted from the supernatants by centrifugation (60Ti rotor; 50,000 rpm; 5.0 h; 5°C). The pellets were then dissolved in buffer II (same as buffer I except with 0.3 mM $MgCl_2$) and dialyzed for 2 h against 100 volumes of buffer II to dissociate ribosomes into free 50S and 30S subunits. The 50S and 30S subunits were then separated by zone sedimentation on 35-ml 10 to 30% sucrose density gradients (SW27 rotor; 24,000 rpm; 12 h; 5°C) in buffer II (Fig. 1A). The gradients were collected from the top, and the A_{260} was monitored continuously with a Gilford 2400 recording spectrophotometer. The peak fractions containing the 30S and 50S ribosome subunits were collected separately. The subunits were precipitated by adjusting the Mg^{2+} concentration to 10 mM and adding 0.7 volume of ethanol. After several hours at 0°C, the subunits were pelleted by centrifugation (10,000 rpm; 10 min), redissolved in a small amount of buffer II, and resedimented on buffer II-sucrose gradients as described above (Fig. 1B and C). The peak fractions containing the subunits were again collected and concentrated by ethanol precipitation.

The purified subunits obtained were suspended in TAE buffer (0.1 M Tris-hydrochloride [pH 8.1], 10 mM sodium azide, 1 mM ethylenediaminetetraacetate) and extracted with TAE-saturated phenol at room temperature as described previously (12). The NaCl concentration of the aqueous phases containing the RNA were adjusted to 0.2 M, and the RNA was precipitated with 2 volumes of ethanol at −20°C for 12 h. The RNA precipitates were pelleted by centrifugation (10,000 rpm; 10 min) and suspended in 2× SSC buffer (0.3 M NaCl, 30 mM sodium citrate). The solutions were extracted twice with ether to remove most of the residual phenol and subsequently subjected to zone sedimentation through 35-ml 6 to 30% sucrose density gradients in 2× SSC (SW27 rotor; 24,000 rpm; 18 h; 5°C). The peak fractions containing whole-length RNA molecules from the 16S and 23S regions of the respective gradients were collected (Fig. 1D and E). The peak at the top of the gradients results from the residual phenol in the gradients. In addition, some breakage, especially of the 23S rRNA, which occurs during preparation of the subunits and phenol extraction, is apparent.

The RNA from the peak regions of the gradient was precipitated with ethanol, dissolved in 2× SSC, and stored at −80°C. The concentrations of the RNA solutions were determined spectrophotometrically assuming that an A_{260} of 1.0 was equivalent to 50 μg/ml. The specific activities of the ^3H-labeled 16S and 23S rRNA preparations were 4.8×10^5 cpm/μg and were determined by spotting measured portions on nitrocellulose membrane filters and counting in a liquid scintillation counter.

Preparation of λ*ilv*5 DNA. Bacteriophage λ and λ*ilv*5 were prepared by heat induction of *E. coli* strains NO1374 (F⁻ *trkA401 kdpABC5 lac⁻ gal⁻ spc*ʳ *str*ʳ *fus*ʳ λC1857S7, obtained from M. Nomura) and NF955 (C600 *thi⁻ leuB⁻ ilvC⁻ str*ʳ λC185757 b_{515} λ*xis*6 λC1857S7 *ilv*5, obtained from N. Fiil), respectively. The λ*ilv*5 transducing phage was separated, according to density, from the helper phage by CsCl equilibrium centrifugation (9). The DNA was phenol extracted from the purified phage particles (19).

For the hybridization experiments, the DNA was denatured with alkali and immobilized on nitrocellulose membrane filters as described previously (3). The concentration of the DNA was 5 μg/filter and was determined prior to denaturation in 2× SSC assuming that A_{260} of 1.0 was equal to a concentration of 50 μg/ml.

RNA-DNA hybridization assay. The hybridization assay employed was a modification of the Gillespie-Speigleman method (6) as described previously (12). The DNA filters were incubated in 1.2 or 2.0 ml of 2× SCC containing ^3H-labeled RNA for 18 to 20 h at 67°C. The filters were removed from the hybridization vials, washed extensively with 2× SSC, treated with ribonuclease, washed again, dried, and counted in a liquid scintillation counter.

In competition-hybridization experiments, various amounts of nonradioactive, highly purified 16S or 23S rRNA were added to each assay as competitor. For simplicity, the amount of nonradioactive competitor RNA added to each assay is given in microgram-equivalents of 16S rRNA or 23S rRNA. A microgram-equivalent of 16S rRNA is 0.37 μg and corresponds to about 620 fmol; a microgram-equivalent of 23S rRNA is 0.63 μg and also corresponds to about 620 fmol (16). Thus, these values (i.e., a microgram-equivalent of 16S rRNA and a microgram-equivalent of 23S rRNA) represent equimolar amounts of the two RNA species.

RESULTS

Hybridization of 16S and 23S rRNA to λ*ilv*5 DNA. The λ*ilv*5 transducing phage carries an rRNA transcriptional unit (2). This unit contains 16S, 23S, and 5S rRNA genes and a gene coding for $tRNA_1^{Ile}$ (10; unpublished data). The length of the double-stranded phage DNA is about 46,000 base pairs; the combined length of mature 16S and 23S rRNA is about 4,500 bases. Thus, about 5% of the phage DNA is complementary to the 16S and 23S rRNA sequences.

The hybridization of increasing amounts of ^3H-labeled 16S or 23S rRNA to an excess of λ*ilv*5 DNA is illustrated in Fig. 2. In both instances, the amount of radioactivity associated specifically with λ*ilv*5 DNA increases in propor-

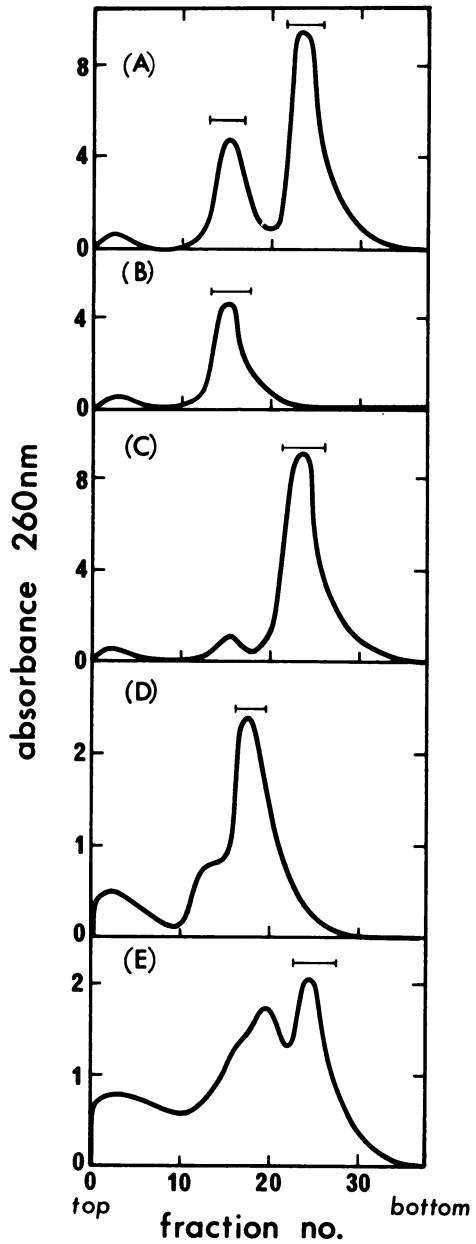

FIG. 1. *Purification of 16S and 23S rRNA. The sucrose density gradient profiles of ribosome subunits and rRNA extracted from purified subunits are illustrated. The bars over the peaks indicate the material recovered from each gradient: (A) initial separation of 30S (peak in fraction 15) and 50S (fraction 23) ribosomal subunits; (B) rerun of the 30S subunits obtained from material collected from gradients identical to those for (A); (30S peak in fraction 15); (C) rerun of 50S subunits obtained from material collected from gradients identical to those for (A) (50S peak in fraction 23); (D) sedimentation profile of 16S rRNA extracted from 30S subunits obtained from gradients identical to those for (B) (16S peak in fraction 17); (E) sedimentation profiles of 23S rRNA extracted from 50S subunits obtained from gradients identical to those for (C) (23S peak in fraction 24). The A_{260}-absorbing material at the top of the RNA gradients (D and E) represents primarily residual phenol. The material sedimenting in the 14S to 18S region of the 23S rRNA gradient is at least 98% partially degraded 23S rRNA and about 1 to 2% 16S rRNA as determined by hybridization competition (data not included).*

tion to the input RNA concentration. Under these assay conditions (i.e., 20 μg of λ*ilv*5 DNA per assay), between 80 and 85% of the ^{3}H-labeled 16S and 23S rRNA enters into RNA-DNA hybrids (Table 1). The hybridization efficiency is related to the concentration of specific DNA such that, at an infinite DNA concentration, virtually all of the radioactivity enters into hybrids (3). Thus, 16S rRNA and 23S rRNA appear to be equally efficient in forming RNA-DNA hybrids.

Competition-hybridization of 16S and 23S rRNA. A small but constant amount of ^{3}H-labeled 16S or 23S rRNA was incubated with 10 μg of λ*ilv*5 DNA in the presence of 0 to 12 μg-eq of homologous and heterologous 16S or 23S nonradioactive competitor (Fig. 3). Competition with 12 μg-eq of the homologous competitor results in about 95% competition. Double-reciprocal plots of these data extrapolate to values of 1.0 on the ordinate and indicate that, at infinite homologous competitor concentrations, competition is virtually 100% (Fig. 4). From these curves it can also be seen that 5.3 μg-eq of homologous 16S rRNA or 23S rRNA competitor per assay results in 90% competition of the hybridization of ^{3}H-labeled RNA. This means that RNA is present in somewhat less than 10-fold excess over specific DNA sequences and that 0.53 μg-eq of 16S or 23S rRNA are hybridized to the 10 μg of λ*ilv*5 DNA. Further analysis of the competition curves indicates that at saturation the 10 μg of λ*ilv*5 DNA will hybridize 0.575 to 0.580 μg-eq of 16S and 23S rRNA (see Addendum in Proof). This represents equimolar amounts of the two rRNA species and, as expected, accounts for one 16S and one 23S rRNA hybridized per genome-equivalent of λ*ilv*5 DNA or 5 to 6% of the total nucleotide sequences of the transducing phage DNA.

Heterologous competition experiments were also performed. A small amount of ^{3}H-labeled 16S or 23S rRNA was hybridized to λ*ilv*5 DNA in the presence of 0 to 12 μg-eq of heterologous nonradioactive 23S or 16S rRNA, respectively (Fig. 3). No evidence of competition by heterologous RNA was observed even at a 20-fold excess of competitor (i.e., 12 μg-eq) over ribosomal DNA sequences. This observation clearly estab-

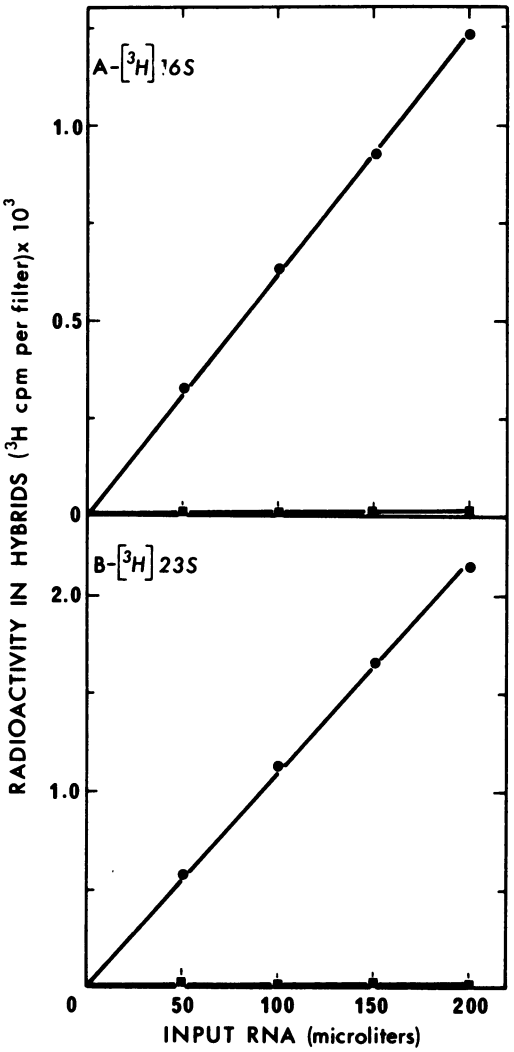

FIG. 2. *Hybridization of ^{3}H-labeled 16S and 23S rRNA to λ and λilv5 DNA. Purified ^{3}H-labeled 16S rRNA and 23S rRNA isolated from sucrose gradients identical to those illustrated in Fig. 1D and E were used for hybridization. The specific activity of the RNA species was 4.8 × 10^5 cpm/μg. (A) Hybridization of ^{3}H-labeled 16S RNA to an excess of λilv5 DNA is illustrated; an input of 50 μl corresponds to 1.46 × 10^3 cpm (5.09 fmol of 16S rRNA). (B) Hybridization of ^{3}H-labeled 23S RNA to an excess of λilv5 DNA is illustrated; an input of 50 μl corresponds to 2.65 × 10^3 cpm (5.43 fmol of 23S rRNA). Each assay was in 2 ml of 2× SSC and consisted of radioactive input RNA and six filters containing DNA; two of the filters contained 5 μg (167 fmol) each of λ DNA and four contained 5 μg (167 fmol) each of λilv5 DNA. The 20 μg of λilv5 DNA per assay (667 fmol) thus represents about a 30-fold excess of specific DNA over specific RNA at the highest input RNA concentration. The average radioactivity per λ DNA filter (■) and per λilv5 DNA filter (●) is illustrated. The radioactivity associated with the four λilv5 filters per assay varied by less than ±5% of the average values shown.*

TABLE 1. *Hybridization efficiency of 16S and 23S RNA to λilv5 DNA*

rRNA	Input radioactivity[a]	Radioactivity[a] in hybrids	Hybridization[b] efficiency (%)
16S	1,460	1,236	85
23S	2,650	2,200	83

[a] The input radioactivity and the radioactivity in hybrids are for an input of 50 μl of ^{3}H-labeled RNA. The radioactivity in hybrids was obtained from the λilv5 hybridization curves corrected for nonspecific λ DNA hybridization. The radioactivity in hybrids is four times that illustrated in Fig. 2 and accounts for the fact that each assay contained four identical λilv5 DNA filters.

[b] The hybridization efficiency is dependent upon the amount of specific λilv5 DNA per assay. In this experiment, each assay contained 20 μg of λilv5 DNA. At an infinite DNA concentration, virtually 100% of the specific input RNA will hybridize. At 10 μg of λilv5 DNA per assay, only about 65 to 71% of the specific RNA hybridizes (see the legends of Fig. 3 and 5).

lishes that 16S rRNA and 23S rRNA do not cross-hybridize and strongly argues against extensive sequence homology between the two major species of rRNA.

The purity of the 16S and 23S rRNA preparations used in these experiments was examined by nearly saturating λilv5 DNA with either 16S or 23S rRNA and competing with 0 to 32 μg-eq of heterologous nonradioactive 23S or 16S rRNA, respectively (Fig. 5). The DNA is nearly saturated, as evidenced by the 60% reduction in radioactivity hybridized when a small, but constant, amount (about 1.2 μg-eq) of cold homologous competitor is included in the assay. Inclusion of up to 32 μg-eq of nonradioactive heterologous competitor in the assays results in no or at most a 4% reduction in radioactivity hybridized. In this area of the competition curves, further addition of 0.15 to 0.20 μg-eq of homologous competitor results in about a 5% reduction in radioactivity hybridized (Fig. 3). Thus, it is clearly apparent that cross-contamination of the 16S and 23S rRNA preparation is negligibly small and less than 1%.

DISCUSSION

Purification of 16S and 23S rRNA. For the preparation of uncontaminated 16S and 23S rRNA, precautions must be taken (i) to eliminate messenger RNA (mRNA) from the ribosomes, (ii) to obtain 30S subunits that are free from 50S subunits, and (iii) to obtain 50S subunits that are free from 30S subunits. The

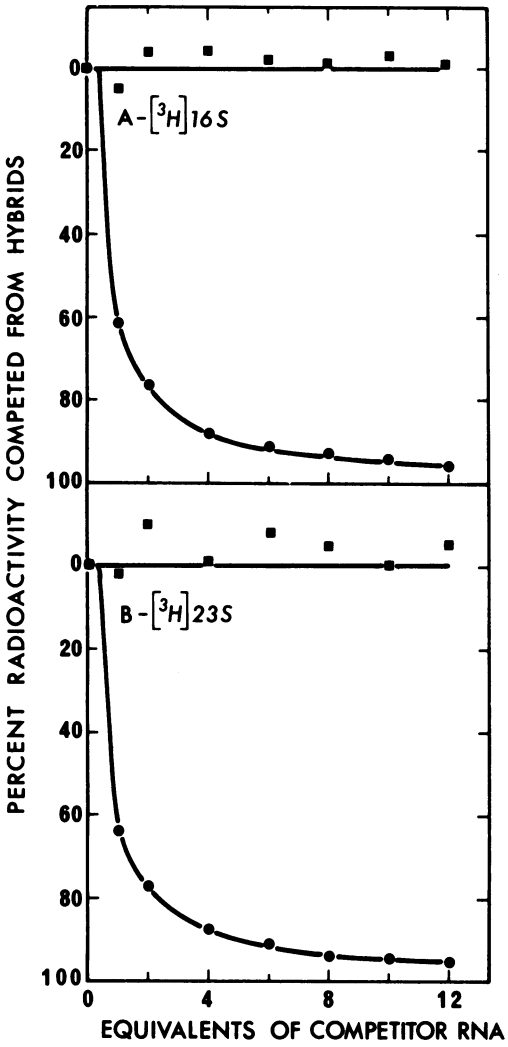

FIG. 3. *Competition-hybridization with 16S and 23S rRNA. (A) Hybridization of ^{3}H-labeled 16S rRNA to λilv5 DNA in the presence of homologous 16S (●) and heterologous 23S (■) competitor RNA. (B) Hybridization of ^{3}H-labeled 23S rRNA to λilv5 DNA in the presence of homologous 23S (●) and heterologous 16S (■) competitor RNA. Hybridizations were done in the presence of 0 to 12 μg-eq of competitor RNA. A microgram-equivalent of 16S rRNA is 0.37 μg (620 fmol) and that of 23S rRNA is 0.63 μg (620 fmol); these values represent equimolar amounts of the two rRNA species and were determined from their respective molecular weights (17). The input radioactivities per assay were 2.7 × 10^3 cpm (9.40 fmol) for ^{3}H-labeled 16S rRNA and 9.9 × 10^3 cpm (20.2 fmol) for ^{3}H-labeled 23S rRNA. Each assay was in 1.2 ml of 2× SSC and consisted of one filter containing 5 μg of λ DNA (167 fmol) and two filters each containing 5 μg of λilv5 DNA (total of 333 fmol). Thus, in the absence of competitor RNA, λilv5 DNA is present in 15- to 30-fold molar excess over specific RNA. Similarly, at the highest competitor concentration, the 12 μg-eq of competitor RNA (7.45 pmol) represents a 20-fold molar excess over λilv5 DNA. Three separate assays, each containing two λilv5 DNA filters, were used to determine the zero competitor points. All of the other values are averages of the data from two filters from a single assay. The total radioactivity hybridized per assay in the absence of competitor rRNA was 1,916 cpm for ^{3}H-labeled 16S rRNA and 7,060 for ^{3}H-labeled 23S rRNA and correspond to a hybridization efficiency of 71%.*

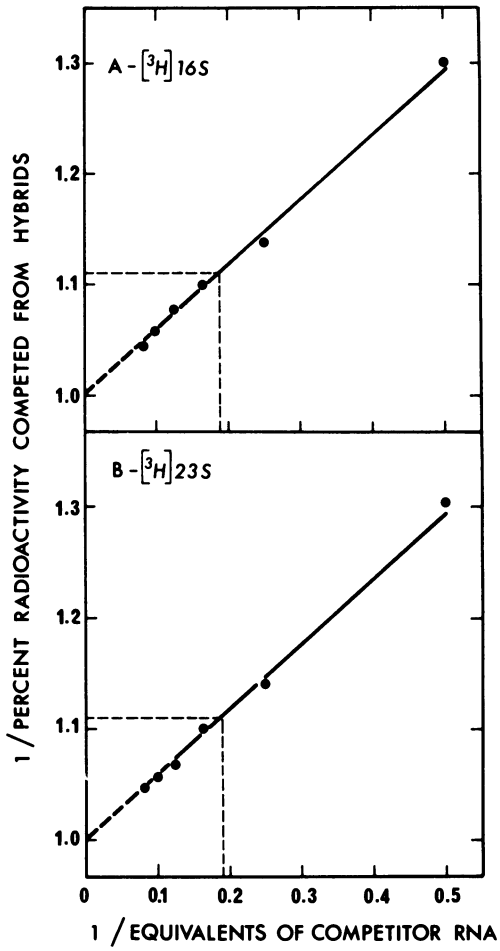

FIG. 4. *Competition of ^{3}H-labeled 16S rRNA and ^{3}H-labeled 23S rRNA from RNA-DNA hybrids by homologous RNA. The homologous competition data from Fig. 3 are replotted as the reciprocal of percent radioactivity competed from hybrids versus the reciprocal of microgram-equivalents of homologous competitor rRNA: (A) competition of ^{3}H-labeled 16S rRNA; (B) competition of ^{3}H-labeled 23S rRNA. The curves extrapolate to a value on the ordinate equivalent to the reciprocal of the percent radioactivity competed from hybrids at an infinite homologous competitor RNA concentration. The light, dashed line indicates the competitor concentration (5.30 μg-eq or 3.28 pmol) required to obtain 90% exclusion of the ^{3}H radioactivity from the RNA-DNA hybrids.*

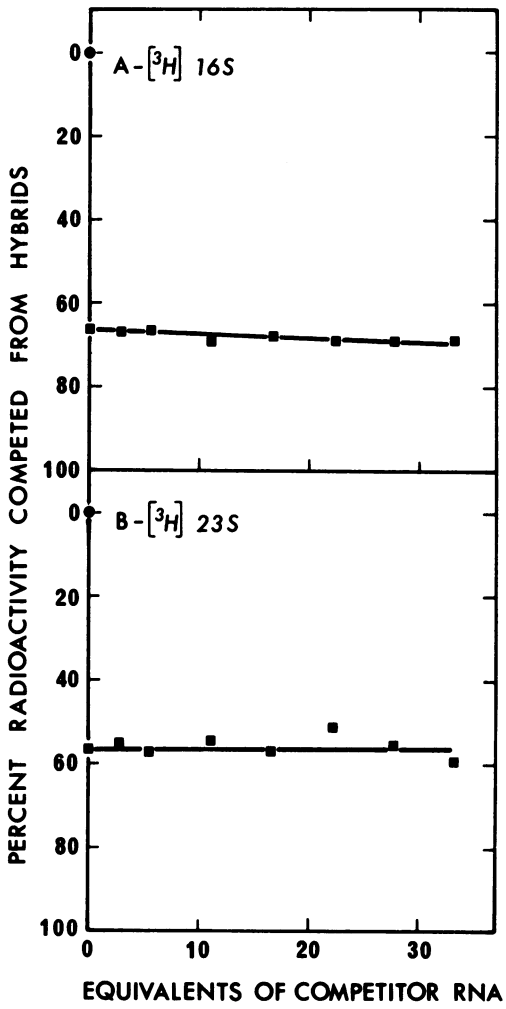

FIG. 5. *Competition hybridization with heterologous rRNA. (A) Hybridization of ^{3}H-labeled 16S rRNA to λilv5 DNA in the presence of a constant amount of homologous 16S competitor RNA and 0 to 32 μg-eq of heterologous 23S competitor RNA (■). (B) Hybridization of ^{3}H-labeled 23S rRNA to λilv5 DNA in the presence of a constant amount of homologous 23S competitor RNA and 0 to 32 μg-eq of heterologous 16S competitor RNA (■). The input radioactivity for the ^{3}H-labeled 16S rRNA was 5.25 × 10^3 cpm/assay (18.3 fmol) and that for the ^{3}H-labeled 23S rRNA was 9.60 × 10^3 cpm/assay (19.6 fmol). Each assay consisted of one filter containing 5 μg of λ DNA (167 fmol) and two filters each containing 5 μg of λilv5 DNA (total of 333 fmol). In the absence of homologous competitor (●), 3,720 cpm of ^{3}H-labeled 16S rRNA hybridized (71% efficiency) and 6,231 cpm of 23S rRNA hybridized (65% efficiency) specifically to λilv5 DNA filters. Addition of a constant amount of homologous competitor (about 1.2 μg-eq or about 800 fmol per assay) nearly saturated the DNA and resulted in a 66% reduction of ^{3}H-labeled 16S rRNA hybridization and a 57% reduction of ^{3}H-labeled 23S rRNA hybridization (defined in the legend of Fig. 3). The abscissa gives the microgram-equivalents of heterologous competitor per assay in the presence of a constant amount of the homologous competitor.*

mRNA contamination can be eliminated by a 30-min treatment with the antibiotic rifampin prior to harvesting of the cells (15). The 23S rRNA extracted from 50S subunits has a tendency to break near the middle to produce two half molecules that sediment between 14S and 18S. Thus, the RNA extracted from 30S subunits contaminated with a small amount of 50S subunits and isolated from the 16S region of a sucrose gradient will almost certainly contain significant 23S rRNA contamination. Likewise, after dissociation of 70S ribosomes into subunits, the 30S subunit may dimerize and cosediment with 50S subunits. The RNA extracted from these 50S subunits and isolated from the 23S region of a sucrose gradient will contain some 16S rRNA. To avoid these difficulties, ribosome subunits were purified by two successive rounds of zone sedimentation through sucrose density gradients. The RNA species were phenol extracted from the purified subunits and reisolated after another zone sedimentation through sucrose. The resulting preparations of 16S and 23S rRNA were judged to be greater than 99% pure. The RNA isolated from the 14 to 18S region of the 23S rRNA gradient was found to be at least 98% partially degraded 23S rRNA.

Hybridization of 16S and 23S rRNA to λ*ilv*5 DNA. In the presence of a constant and excess amount of λ*ilv*5 DNA, the amount of radioactive 16S or 23S rRNA entering into RNA-DNA hybrids is proportional to the amount of input RNA (see reference 3 for a complete discussion). At 10 μg of λ*ilv*5 DNA per assay, 65 to 70% of the input radioactivity hybridizes; at 20 μg per assay, the hybridization efficiency increases to 80 to 85%; and at an infinite DNA concentration, virtually all of the input radioactivity would hybridize. Thus, the efficiency of hybridization of both 16S rRNA and 23S rRNA and also of mRNA (3) to specific DNA carried on λ transducing phages is extremely high according to the assay described. It is also clearly evident that, at saturation of the DNA, equimolar amounts of 16S rRNA and 23S rRNA are hybridized, and virtually all of the specific sites on the DNA are hybridized to rRNA.

The fidelity of 16S and 23S rRNA hybridization is demonstrated by competition with homologous and heterologous rRNA. Homologous competitor RNA is 100% efficient in displacing radioactivity from hybrids, whereas heterologous competitor RNA exhibits no competitive

properties even at a 20-fold molar excess over available DNA sites (Fig. 3). Presumably, these general relationships are valid whenever the specific RNA hybridizing to the denatured DNA lacks sequence homology with other RNA molecules in the hybridization mixture. In the present case, the data clearly illustrate that 16S rRNA and 23S rRNA do not compete for the same binding sites within the rRNA transcriptional unit and therefore do not cross-hybridize. From these experiments, it is concluded that the amount of sequence homology between 16S and 23S rRNA is negligibly small.

These observations are in contrast to earlier reports of cross-hybridization between 16S and 23S rRNA from *E. coli* (1, 11). The simplest explanation for the difference is that the earlier preparations of 16S and 23S rRNA were cross-contaminated for the reasons discussed above.

In this regard, Fellner and Ebel (5) presented a ribonuclease T1 fingerprint of 23S rRNA from *E. coli*, which shows little similarity to the corresponding 16S rRNA fingerprint. In addition, the nucleotide sequences of several of the products representing about 10% of the 23S rRNA sequence were determined and compared with known 16S sequences. Again, no significant homology of the two rRNA species was apparent.

ACKNOWLEDGMENTS

We wish to thank Gordon Tener for his critical evaluation of this manuscript.

This work was supported by funds from the Department of Microbiology at The University of British Columbia and the National Research Council of Canada, grant A-0394.

ADDENDUM IN PROOF

A figure illustrating the saturation of λ*ilv*5 DNA with 16S or 23S rRNA sequences has not been included. However, these saturation profiles can be obtained directly from the competition data illustrated in Fig. 3 as follows. The amount of 16S or 23S rRNA hybridized to λ*ilv*5 DNA at a given homologous competitor concentration is equal to the product: [homologous competitor concentration] × [1 − (percentage radioactivity computed from hybrids)]. In this experiment, the concentration of radioactive rRNA was negligibly small and has consequently been ignored in the calculation.

This method of obtaining a saturation profile is more sensitive than the usual method of adding increasing amounts of radioactive RNA to a constant amount of DNA, because the amount of input radioactivity in each assay remains small and constant; consequently, the nonspecific binding of radioactivity to the DNA filters remains reproducibly low.

LITERATURE CITED

1. **Attardi, G., P. Huang, and S. Kabat.** 1965. Recognition of ribosomal RNA sites in DNA. I. Analysis of the *E. coli* system. Proc. Natl. Acad. Sci. U.S.A. 53:1490–1498.
2. **Collins, J., N. Fiil, P. Jørgensen, and J. Friesen.** 1976. Gene cloning of *Escherichia coli* chromosomal genes important in regulation of ribosomal RNA synthesis. *In* O. Maaløe and N. O. Kjeldgaard (ed.), Alfred Benzon Symposium IX. Control of ribosome biosynthesis. Munksgaard, Copenhagen.
3. **Dennis, P. P., and M. Nomura.** 1975. Regulation of the expression of ribosomal protein genes in *Escherichia coli*. J. Mol. Biol. 97:61–76.
4. **Dunn, J., and F. W. Studier.** 1973. T_7 early RNAs and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. Proc. Natl. Acad. Sci. U.S.A. 70:3296–3300.
5. **Fellner, P., and J. P. Ebel.** 1970. Observations on the primary structure of the 23S ribosomal RNA from *E. coli*. Nature (London) 325:1131–1132.
6. **Gillespie, D., and S. Spiegelman.** 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. 12:829–832.
7. **Ginsberg, D., and J. A. Steitz.** 1975. The 30S ribosomal precursor RNA from *Escherichia coli*. J. Biol. Chem. 250:5647–5654.
8. **Hayes, F., M. Vasseur, N. Nikolaev, D. Schlessinger, J. S. Widada, A. Krol, and C. Branlant.** 1975. Structure of a 30S pre-ribosomal RNA of *E. coli*. FEBS Lett. 56:85–91.
9. **Jaskunas, S. R., L. Lindahl, and M. Nomura.** 1975. Specialized transducing phages for ribosomal protein genes of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 72:6–10.
10. **Lund, E., J. Dahlberg, L. Lindahl, S. R. Jaskunas, P. P. Dennis, and M. Nomura.** 1976. Transfer RNA genes between 16S and 23S rRNA genes in rRNA transcription units of *E. coli*. The Cell 7:165–177.
11. **Maugiorotti, G., D. Apirion, D. Schlessinger, and L. Silengo.** 1968. Biosynthetic precursors of 30S and 50S ribosomal particles in *Escherichia coli*. Biochemistry 7:456–472.
12. **Nikolaev, N., L. Silengo, and D. Schlessinger.** 1973. Synthesis of a large precursor to ribosomal RNA in a mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 70:3361–3365.
13. **Nomura, M., and F. Engbaek.** 1972. Expression of ribosomal protein genes as analyzed by bacteriophage Mu-induced mutations. Proc. Natl. Acad. Sci. U.S.A. 69:1526–1530.
14. **Pace, N.** 1973. Structure and synthesis of the ribosomal ribonucleic acid of prokaryotes. Bacteriol. Rev. 37:562–603.
15. **Pato, M., and K. von Meyenburg.** 1970. Residual RNA synthesis in *Escherichia coli* after inhibition of initiation of transcription by rifampicin. Cold Spring Harbor Symp. Quant. Biol. 35:497–504.
16. **Smith, I., D. Dubnau, P. Morell, and J. Marmur.** 1968. Chromosomal location of DNA base sequences complementary to transfer RNA and to 5S, 16S and 23S ribosomal RNA in *Bacillus subtilis*. J. Mol. Biol. 33:123–140.
17. **Van Holde, K., and W. Hill.** 1974. *In* M. Nomura, A. Tissieres, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Wu, M., and N. Davidson.** 1975. Use of gene 32 protein staining of single strand polynucleotides for gene mapping by electron microscopy: application to the $\phi 80 d_3 ilvsu^+ 7$ system. Proc. Natl. Acad. Sci. U.S.A. 72:4506–4510.
19. **Zubay, G., D. Chambers, and L. Cheong.** 1970. Cell-free studies on the regulation of the lac operon. *In* J. Beckwith and O. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.