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Asymmetric Template Function of Microbial Deoxyribonucleic Acids: Transcription of Ribosomal and Soluble Ribonucleic Acids

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In *Bacillus subtilis* and *Escherichia coli*, 16 and 23*S* ribosomal ribonucleic acid (rRNA) hybridize exclusively with the heavy (H) strand of methylated albumin-kieselguhr (MAK)-fractionated complementary deoxyribonucleic acid (DNA) strands. All the soluble RNA (4*S* RNA) in *B. subtilis* and 66 to 75% of the 4*S* RNA in *E. coli* also hybridize with the H strand. Interspecific hybridization shows that *E. coli* 23*S* rRNA also binds selectively to the DNA H strand of *Salmonella typhimurium*. The hybridization peak for all three cellular RNA components is specifically located in the late-eluting region of the absorbance profile of the DNA H strand. The early-eluting region of the light (L) strand preferentially inhibits the hybridization between the peak region of the H strand and 23*S* rRNA. These regions are considered to represent the transcribing sequences and their complements for 23*S* rRNA in the separated H and L strands of DNA, respectively.

As we reported recently, alkali-denatured deoxyribonucleic acid (DNA) preparations from *Bacillus subtilis* (14, 29, 30) and from several microbial species (31) representing DNA varieties of the AT, GC, and equimolar types can be separated by a technique of intermittent gradient elution from a column of methylated albumin-kieselguhr (MAK) into two distinct components, designated, by virtue of their buoyant densities, as light (L) and heavy (H). It was concluded on the basis of standard criteria, such as transforming activity and hypochromicity in annealing experiments and nucleotide composition, that the components isolated from *B. subtillis* DNA represented preparations of complementary strands (14, 29, 30). The direct chemical analysis of the L and H components isolated from DNA specimens of the AT type showed them to exhibit a high degree of complementarity and a bias in the distribution of purines and pyrimidines. In the L and H components derived from DNA of the equimolar and GC types, the bias appears limited to guanine and cytosine (31). These preparations which we believe represent two families of strand fragments, each derived from one of the original DNA chains (31), as well as other strand preparations reported in the literature (4, 5, 8, 19, 25, 32) exhibit one feature in common, namely that the L strand is purine-rich (or guanine-rich) and the H strand is pyrimidine-rich (or cytosine-rich). We were interested in pursuing the biological implications of this chemical asymmetry, especially with respect to the in vivo function of the L and H strands of DNA.

In this paper, we present evidence from hybridization experiments that, in *B. subtilis* and *Escherichia coli*, 16 and 23*S* ribosomal ribonucleic acid (rRNA) species are transcribed exclusively from the pyrimidine-rich (or cytosine-rich) H strand of DNA. All the soluble RNA (4*S* RNA) in *B. subtilis* and 66 to 75% of the 4*S* RNA in *E. coli* are also transcribed from the H strand of DNA. Interspecific hybridization between *E. coli* 23*S* ribosomal RNA and separated DNA strands of *Salmonella typhimurium* further substantiates the finding that ribosomal RNA is copied exclusively from the H strand of DNA. Detailed hybridization tests on the entire MAK elution profile of denatured DNA and competition experiments with individual L fractions demonstrate that ribosomal RNA transcribing sequences (+) and their complements (−) in DNA can be completely separated into two distinct and nonoverlapping fractions by chromatography on

MAK columns. These experiments offer additional and independent evidence that the L and H components, isolated from denatured DNA of *E. coli* and *S. typhimurium* where base complementarity was less obvious (31), do indeed represent preparations of fragmented complementary DNA strands. Similar results for *B. subtilis* have been recently reported from another laboratory (23).

MATERIALS AND METHODS

Bacterial strains. We are grateful to D. Dubnau for *B. subtilis* A26u⁻, a uracil auxotroph of strain 168 (7); to J. Dubnau for *S. typhimurium* pyr401, a pyrimidine and arginine auxotroph of strain LT-2, and *E. coli* K140u⁻, a uracil auxotroph; to W. Maas for *E. coli* 3050 ura7, a uracil auxotroph. The following bacterial strains served as the source of DNA: *B. subtilis* W23 (29), *E. coli* K140 and 3050, *S. typhimurium* pyr401. RNA was prepared from *B. subtilis* A26 and *E. coli* 3050.

Labeling media. Low-uracil medium for *B. subtilis* A26u⁻ consisted of minimal medium (37) supplemented with 0.5% glucose, 1% glutamate, 0.04% vitamin-free Casamino Acids (Difco), 50 μg of L-tryptophan/ml, and 8 μg of uracil/ml. Low-uridine medium for *E. coli* 3050 ura7 consisted of minimal medium (6) supplemented with 0.2% glucose, 0.04% vitamin-free Casamino Acids, 100 μg of methionine/ml, 1 μg of thiamine/ml, and 25 μg of uridine/ml.

Preparation of ^{3}H-labeled 4, 16, and 23S RNA species. *B. subtilis* A26u⁻ was grown at 37 C with constant shaking in 250 ml of the low-uracil medium containing 19.6 μCi of uracil-5-^3H (specific activity, 23 Ci/mmole, Schwarz BioResearch Inc.) per ml. When the bacterial growth approached the stationary phase, cells were washed and resuspended in the same medium containing a high concentration of non-radioactive uracil (100 μg/ml) and allowed to incubate for another 40 min. Actinomycin D (5.4 μg/ml; a gift from Merck & Co., Inc.) was then added for 10 min to exhaust messenger RNA (mRNA; reference 16). The cells were chilled and washed, and the pellet was frozen over dry ice-acetone. RNA was extracted by the method of Oishi and Sueoka (24). RNA from *E. coli* was prepared from strain 3050 ura7 grown at 37 C with constant shaking in 140 ml of the low-uridine medium containing 35 μCi of uridine-5-^3H (specific activity, 8.0 Ci/mmole, Schwarz BioResearch) per ml. At the onset of stationary phase, cells were washed twice and resuspended in double the volume of the same medium containing a high concentration of nonradioactive uridine (200 μg/ml) and allowed to incubate for another 60 min to chase the radioactive mRNA. The same extraction procedure was used for the isolation of *E. coli* RNA with the elimination of lysozyme treatment. RNA samples (1 to 2 mg) were equilibrated to 0.3 M NaCl buffered at *p*H 6.7 by 0.05 M sodium phosphate and applied to a MAK column (39) to purify and separate 4, 16, and 23*S* RNA. The column was eluted by means of a linear gradient of either 0.3 M to 1.2 M NaCl or 0.3 M to 1.4 M NaCl; a total of 500 to 600 ml of eluent was used. A superior separation of 16 and 23*S* rRNA is obtained through the application of the intermittent gradient elution technique (29; for details, see legend of Fig. 1). Pooled 4, 16, and 23*S* fractions were dialyzed overnight in the cold against dilute (0.1 or 0.01) standard saline citrate (SSC = 0.15 M NaCl and 0.015 M sodium citrate, *p*H 7). Concentrations of RNA were determined by ultraviolet absorption ($A_{260}^{1\%}$ = 240).

Isolation, denaturation, and strand separation of DNA. The isolation of the bacterial DNA preparations and their separation, after alkali denaturation, into L and H components were recently described in detail (28, 29, 31). Individual or pooled L and H fractions were subjected to extensive dialysis in the cold against 1 liter of 0.1 × SSC. After dialysis, the fractions of any given column were adjusted to a uniform concentration.

Hybridization of isolated L and H strands with ^{3}H-labeled ribosomal and soluble RNA species. Hybridization between 4, 16, or 23*S* RNA species and isolated DNA strands was performed in liquid as described by Nygaard and Hall (22) and modified by Kennell and Kotoulas (15). In general, the hybridization mixture (1 to 5 ml) was composed of individual or pooled L and H DNA fractions (10 to 40 μg), ^{3}H-labeled 23 or 16*S* RNA (0.6 to 2.0 μg) or 4*S* (0.2 to 0.8 μg) RNA in 6 × SSC. Hybridization assays performed on any given MAK column of denatured DNA were always uniform with respect to the volumes of the mixtures and the concentration of nucleic acids (see legends of Fig. 2–4). In addition, competitive hybridization assays were performed between various DNA L fractions and ^{3}H-labeled 23*S* RNA competing for a given H fraction at a ratio of L/H = 2 (see legends of Fig. 5 and 6). Incubation of hybridization mixtures was carried out either at 67 C for 18 hr or at 68 C for 4 to 5 hr. After incubation, the hybrids were collected on filters (type B-6, 24 mm; Schleicher & Schuell Co.), washed, and treated with pancreatic ribonuclease (20 μg/ml) and ribonuclease T1 (20 units/ml) as outlined by Gillespie and Spiegelman (10). The filters were introduced into scintillation vials, and 1 ml of 3% NH_4OH was added, followed by 15 ml of scintillation mixture (2).

RESULTS

Chromatographic separation of RNA on MAK columns by intermittent gradient elution. We found that the intermittent elution technique for the separation of complementary strands of DNA on MAK columns (29, 31) is also applicable to the separation of cellular RNA components. Perfect resolution can be obtained between 16 and 23*S* rRNA and some resolution between 4 and 5*S* RNA species. The separation between 16 and 23*S* rRNA from *B. subtilis* is superior to the conventional MAK profiles obtained when a simple linear salt gradient is employed (Fig. 1). Evidence based on rechromatography of *B. subtilis* L and H DNA fractions has been recently presented (31) which clearly demonstrated that the fractions are

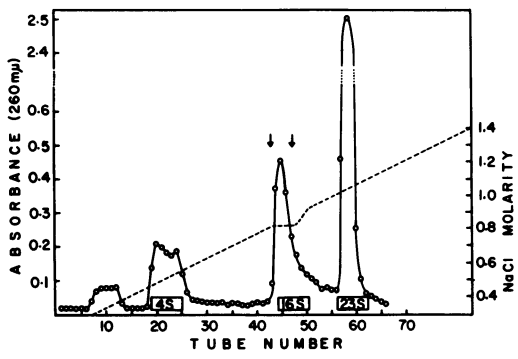

FIG. 1. *Separation of cellular RNA components from B. subtilis RNA by intermittent gradient elution from a MAK column. Isolated RNA (2.3 mg) was applied to a standard MAK column and eluted by means of a linear salt gradient of 0.3 to 1.4 M NaCl (total volume, 600 ml). The gradient was cut at tube 43 and reconnected at tube 47. Recovery (as per cent of input RNA): total, 85; 4S RNA, 17; 16S rRNA, 23; 23S rRNA, 39.*

homogenous and not artifacts produced by the intermittent elution procedure. The ^{3}H-labeled RNA preparations from *B. subtilis* and *E. coli* were subjected to chromatography on MAK columns and eluted similarly by means of an intermittent gradient elution. The separated ^{3}H-labeled 4, 16, and 23*S* RNA species were then used in the hybridization assays. We refer to the soluble RNA fraction as 4*S* even though it includes the 5*S* RNA species. No attempts were made to purify 5*S* RNA for hybridization assays. Fractionated ^{3}H-labeled 23*S* RNA from *E. coli* was also used in interspecific hybridization with *S. typhimurium* DNA fractions.

Hybridizability of total L and H components of B. subtilis DNA with ^{3}H-labeled cellular RNA species: general properties. The ability of the separated L and H components of DNA to form stable complexes with ribosomal and soluble RNA was tested by the liquid hybridization technique (15, 22). Assays of cellular RNA components known to be coded by a small fraction of the genome (9, 11, 44) require high DNA/RNA inputs. Competition from DNA-DNA reannealing which occurs more readily in liquid was completely eliminated by the use of separated complementary strands of DNA (*see below*). *B. subtilis* DNA, denatured by means of alkali, was separated on a MAK column into L and H components (Fig. 2). We first examined the hybridizing capacity of total L and H components saturated with ribosomal and soluble RNA. ^{3}H-labeled purified 16 and 23*S* RNA as well as 4*S* RNA hybridized exclusively with the pooled H component; no radioactivity was bound to the L

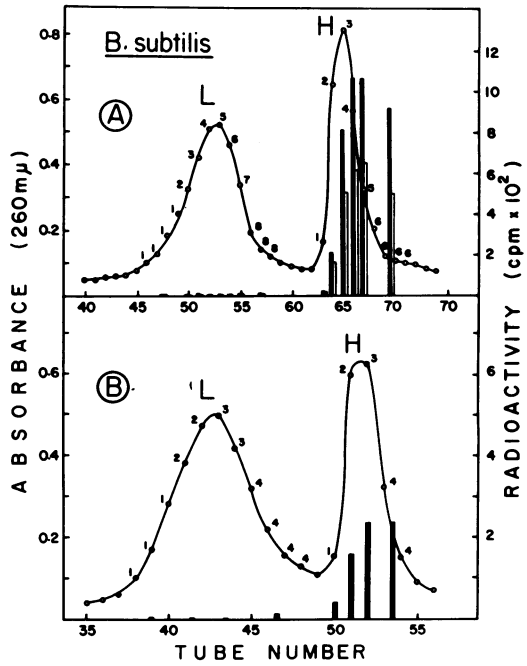

FIG. 2. *Hybridization of separated complementary strands of B. subtilis DNA with ^{3}H-labeled cellular RNA components. A, Hybridization of 16 and 23S RNA with L and H DNA fractions. Alkali-denatured DNA (3.4 mg) was applied to a MAK column and eluted with an intermittent salt gradient between 0.7 and 1.5 M NaCl (total volume, 500 ml). The gradient was cut at tube 45 and reconnected at tube 53. Recovery (as per cent of input DNA): total, 56; fraction L, 30; fraction H, 26. Individual or pooled L and H fractions were dialyzed against 0.1 × SSC. The pooled fractions are designated by the same number on the elution profile. Each reaction mixture (3.6 ml) was composed of 17.3 μg of DNA, 0.61 μg ^{3}H-labeled 16S RNA (specific activity, 14,600 counts per min per μg) or 0.66 μg of ^{3}H-labeled 23S RNA (specific activity, 14,000 counts per min per μg) and incubated for 18 hr at 67 C. The hybridization to 16S rRNA is illustrated by the open bars and to 23S rRNA by the closed bars. B, Hybridization of 4S RNA with L and H DNA fractions. Alkali-denatured DNA (3.4 mg) was applied to a MAK column and eluted with an intermittent salt gradient as in part A. The gradient was cut at tube 38 and reconnected at tube 43. Recovery (as per cent of input DNA): total, 86; fraction L, 33; fraction H, 22; the remaining OD was non-DNA. Individual and pooled L and H fractions were treated as described in part A. Each reaction mixture (3.0 ml) was composed of 13.8 μg of DNA, 0.23 μg of ^{3}H-labeled 4S RNA (specific activity, 16,300 counts per min per μg) and incubated for 18 hr at 67 C.*

component (Table 1). Table 1 further illustrates that pooled L and H fractions, mixed in equal proportions and annealed with any of the ^{3}H-labeled cellular RNA species, resulted in an

TABLE 1. *Hybridizability of total L and H strands of Bacillus subtilis DNA at saturation levels of stable RNA components*

RNA component	DNA strands[a]	Total amt in incubation mixture[b]		Counts/min complexed after ribonuclease	Per cent DNA-H hybridized	Per cent unfractionated DNA hybridized[c]
		DNA	RNA			
		μg	μg			
23S	H	20	2	1,498	0.47	0.26 (36); 0.25 (24)
23S	L	20	2	7		
23S	L + H (1:1)	20	2	236	0.07	
23S	L + H (1:1)	40	4	385	0.06	
16S	H	20	2	630	0.21	0.13 (24, 36)
16S	L	20	2	2		
16S	L + H (1:1)	20	2	105	0.04	
16S	L + H (1:1)	40	4	208	0.04	
4S	H	20	0.8	275	0.08	0.03 (36); 0.04 (21)
4S	L	20	0.8	9		
4S	L + H (1:1)	20	0.8	45	0.01	
4S	L + H (1:1)	40	1.6	89	0.01	

[a] See Table 2 (column 2) for the recoveries of the L and H DNA strands.

[b] Volume of the reaction mixtures was 2 ml for 23 and 4S and 2.4 ml for 16S; incubation was carried out at 67 C for 18 hr. The specific activities (counts per min per μg) for 23, 16, and 4S were: 16,080, 14,680 and 16,870, respectively.

[c] Saturation values for *B. subtilis* RNA were taken from the references given in parentheses.

85% inhibition of complex formation due to L and H interaction. These controls demonstrate that in the liquid hybridization procedure complementary DNA strands (L and H) can reanneal with ease and thereby compete effectively with the RNA-DNA reaction. No interference in the annealing reaction was encountered when fractionated complementary strands were used separately (*see* Table 1).

These findings imply that in *B. subtilis* the three major stable RNA components of the cells are transcribed only from H strands of the DNA which elute as the second peak during fractionation on MAK columns (*see* Fig. 2). If only one strand of DNA is being transcribed in vivo during the synthesis of these RNA components, twice the amount of DNA templates should be available for hybridization when separated DNA strands are used under saturation conditions. This notion is borne out in the comparison shown in Table 1 between published saturation-plateau values (21, 24, 36) of *B. subtilis* RNA components for denatured unfractionated DNA and for the isolated H strands. The proportion of DNA complementary to ribosomal and soluble RNA has doubled at saturation levels when only the H component was available for hybridization. These results provide additional and independent evidence that the L and H components obtained by fractionation on a MAK column of denatured *B. subtilis* DNA represent highly purified preparations of complementary strands. It is appropriate to mention in this connection that any deficits in the recoveries of the H component eluted from the column contribute to variations in the saturation-plateau values. The hybridization properties of three H components derived from separate MAK column fractionations were compared at the same DNA/RNA inputs. The availability of complementary sequences to 23S rRNA diminished as the recoveries of the H component of DNA eluted from MAK columns decreased (Table 2).

Hybridization pattern of individual L and H fractions of B. subtilis DNA with ^{3}H-labeled ribosomal and soluble RNA species. The hybridization pattern of the entire MAK column

TABLE 2. *Availability of the H strand of Bacillus subtilis DNA for hybridization with 23S RNA as a function of its recovery from methylated albumin-kieselguhr columns*

Column no.	Recovery—per cent of input DNA			Per cent of DNA-H hybridized[a]
	Total	Fraction L	Fraction H	
1	88	44	44	0.55
2	75	42	33	0.49
3	60	32	28	0.41

[a] Reaction mixtures had an input ratio of DNA/RNA = 10.

elution profile of *B. subtilis* denatured DNA was assayed by annealing individual L and H fractions, after adjusting to the same DNA concentration, with ^{3}H-labeled cellular RNA species. As illustrated in Fig. 2 and already described for total DNA H component (Table 1), ^{3}H-labeled 16 and 23*S* RNA and 4*S* RNA hybridized exclusively with the H strand of DNA. It can be seen that the preferential annealing of cellular RNA species with the H strand is quite specific, being mainly restricted to the late-eluting portion of the H component (Fig. 2A and B). The shift in the hybridization peak was more pronounced in the case of 16 and 23*S* RNA and less in the case of 4*S* RNA. Since the individual L and H fractions were normalized to the same DNA concentration before annealing, the degree of localization of sequences coding for stable RNA components can best be demonstrated from the increase in the per cent of DNA-H hybridizable of the individual H fractions along the absorbance profile. The ratios of per cent of DNA-H hybridizable to 16 and 23*S* RNA molecules, namely of H_5/H_1 (Fig. 2A) were 51 and 28, respectively. The ratio of H_3/H_1 or H_4/H_1 for 4*S* RNA (Fig. 2B) was 6. The data suggest (*see below*) that MAK is capable of recognizing finer compositional differences within fragments of complementary strands.

Hybridization of separated L and H components of E. coli DNA with ^{3}H-labeled ribosomal and soluble RNA. We were naturally interested in the generality of this observation, especially in the case of DNA specimens belonging to either the equimolar or GC varieties in which the distribution bias between the L and H fractions was limited to guanine and cytosine (31). The hybridizability of L and H fractions derived from denatured *E. coli* DNA and normalized to the same concentration was therefore assayed. Figure 3A and B illustrates very clearly that in *E. coli*, as in *B. subtilis*, ^{3}H-labeled 16 and 23*S* RNA hybridized exclusively with the H fraction of denatured DNA. As can be seen in Fig. 3A and especially in Fig. 3B, there is a remarkable shift in the hybridization peak to the extreme right-hand region of the absorbance profile of the DNA H fraction. The hybridization peaks seen in the *E. coli* DNA elution profiles are by far more displaced from the absorbance peaks than the observed shifts in the elution diagram of *B. subtilis* DNA (*compare* Fig. 3A and B with Fig. 2A). This displacement again demonstrates that DNA sequences which code for rRNA can be resolved by chromatography on MAK into relatively clean fractions. The ratios of per cent of DNA-H hybridizable to 16 and 23*S* RNA of the two extreme sides of the absorbance profile of the H component, namely

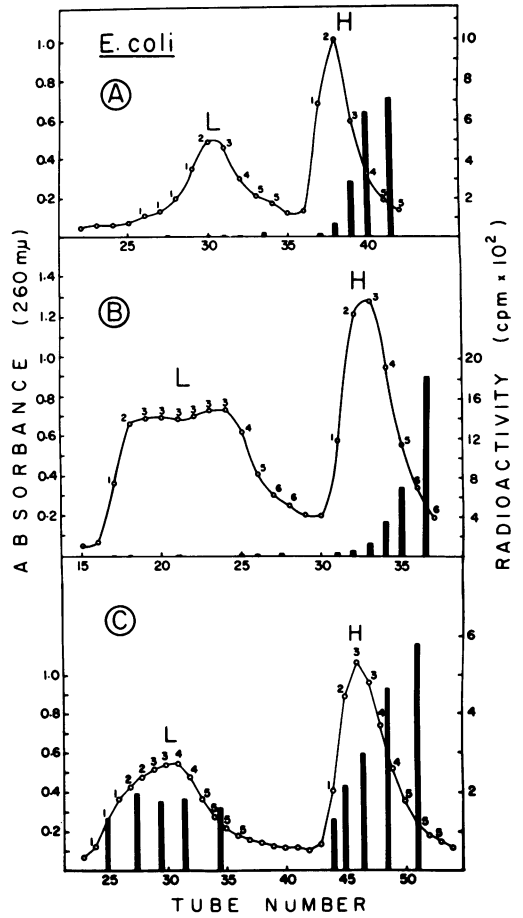

FIG. 3. *Hybridization of separated complementary strands of E. coli DNA with ^{3}H-labeled cellular RNA components. A, Hybridization of 16S RNA with L and H DNA fractions. Alkali-denatured E. coli DNA (2.9 mg) was applied to a MAK column and eluted with an intermittent salt gradient between 0.6 and 1.5 M NaCl (total volume, 500 ml). The grad'ent was cut at tube 25 and reconnected at tube 31. Recovery (as per cent of input DNA): total, 89; fraction L, 40; fraction H, 47. Each reaction mixture (5 ml) was composed of 26.8 μg of DNA, 0.77 μg of ^{3}H-labeled 16S RNA (specific activity, 13,600 counts per minute per μg) and incubated for 18 hr at 67 C. B, Hybridization of 23S rRNA with L and H DNA fractions. Alkali-denatured DNA (4.3 mg) was applied to a MAK column and eluted with an intermittent salt gradient. The gradient was cut at tube 16 and reconnected at tube 23. Recovery (as per cent of input DNA): total, 77; fraction L, 45; fraction H, 32. Each reaction mixture (3.2 ml) was composed of 27.1 μg of DNA, 0.71 μg of ^{3}H-labeled 23S RNA (specific activity, 9,300 counts per min per μg) and incubated for 18.5 hr at 67 C. C, Hybridization of 4S RNA with L and H DNA fractions. Alkali-denatured DNA (3.4 mg) was applied to a MAK column and eluted with an intermittent salt gradient 0.55 and*

H_5/H_1 (Fig. 3A) and H_6/H_1 (Fig. 3B), were 50 and 52, respectively.

The hybridizability of *E. coli* L and H DNA fractions with ^{3}H-labeled 4*S* RNA is illustrated in Fig. 3C. It was found that *E. coli* soluble RNA hybridizes predominantly with the H fraction and to a lesser extent with the L fraction. The annealing pattern of *E. coli* 4*S* RNA to the DNA H component is similar to the pattern for *B. subtilis* 4*S* RNA. The hybridization peak is not as displaced as in the case of rRNA, and the ratio of per cent of DNA-H hybridizable to 4*S* RNA of fraction H_5 to fraction H_1 (Fig. 3C) was 5. The proportion of soluble RNA which hybridized with the H and L components varied slightly depending on the source of *E. coli* DNA which was employed. For example, when ^{3}H-labeled 4*S* RNA from *E. coli* strain 3050 was annealed to its homologous DNA, the proportion hybridizable to H and L was 66 and 34%, respectively, as compared to 75 and 25% when DNA from *E. coli* strain K140 was used. Similar variations in the extent of hybrid formation by *E. coli* 4*S* RNA with unfractionated denatured DNA of other strains of *E. coli* have been reported in the literature (11). The proportion of 4*S* RNA hybridizable to the L strand did not diminish upon the use of an L component which was subjected to rechromatography on a small MAK column (31). One may conclude that this minor class of hybrids was specific to the L component and was not due to artifacts produced by the presence of some contaminating fragments of the H component. Competitive hybridization with cold homologous (4*S*) and heterologous (16 and 23*S*) RNA species revealed that the hybridizability of ^{3}H-labeled 4*S* RNA to either the L or the H component was affected to the same extent only by competition from cold soluble RNA and not from cold rRNA (for details, see legend of Fig. 3C). One may therefore conclude that the minority class of hybrids formed between the DNA L component and *E. coli* 4*S* RNA represents the same specific interactions as the majority class of hybrids formed with the DNA H component. Although great care was taken during the preparation of the RNA components, it cannot be ruled out entirely that some degradation products of mRNA were present in the ^{3}H-labeled soluble RNA fraction.

FIG. 3—*continued*
1.4 M NaCl (total volume, 500 ml). The gradient was cut at tube 24 and reconnected at tube 32. Recovery (as per cent of input DNA): total 96; fraction L, 43; fraction H, 49. Each reaction mixture (5.2 ml) was composed of 40 μg of DNA, 0.5 μg of ^{3}H-labeled 4S RNA (specific activity, 14,800 counts per min per μg) and incubated for 19 hr at 67 C. Pooled L and H fractions normalized to 10 μg of DNA/ml were annealed separately to ^{3}H-labeled 4S RNA at an input ratio DNA/RNA = 50 in the presence of competing cold 4 or 23S RNA. The ratio of cold to hot RNA was 6:1. The reaction mixtures were incubated for 7 hr at 68 C. Competition from cold 4S RNA reduced the counts hybridizable to L and H components by 56 and 59%, respectively. No competition was observed from 23S RNA.

Interspecific hybridization of separated strands of S. typhimurium DNA with E. coli 23S RNA. The hybridization pattern of L and H DNA fractions from a genetically related species such as *S. typhimurium* was tested with *E. coli* ^{3}H-labeled 23*S* RNA. The interspecific hybridization results again in the exclusive binding of 23*S* RNA to the H component of *S. typhimurium* DNA (Fig. 4). The hybridization peak is shifted to the extreme right-hand region of the absorbance profile of the DNA H fraction as was observed for *E. coli* (*compare* Fig. 4 with Fig. 3B). Since the assays included the tail end of the H component, the peak region of hybridization to 23*S* RNA was higher and more displaced than in the *E. coli* pattern. In conclusion, the results clearly demonstrate the striking homology between *E. coli* and *S. typhimurium* DNA H strands for 23*S* RNA coding sequences. This homology is not unexpected in view of the other similarities between the two species previously reported (27, 34, 35, 42).

Localization of the nontranscribing regions for 23S RNA in the L strand: competition experiments. Since it is now obvious that the transcribing templates for ribosomal RNA, which we de-

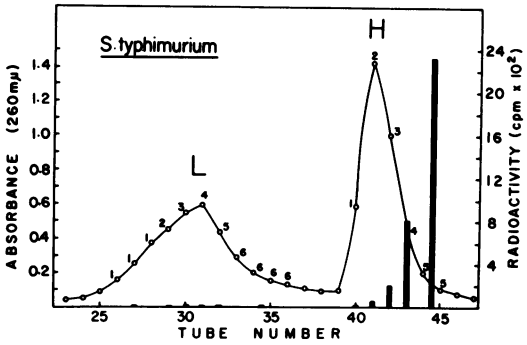

FIG. 4. *Interspecific hybridization of separated complementary strands of S. typhimurium DNA with E. coli ^{3}H-labeled 23S rRNA. Alkali-denatured DNA (2.9 mg) was applied to a MAK column and eluted as described in Fig. 3A. The gradient was cut at tube 25 and reconnected at tube 31. Recovery (as per cent of input DNA): total, 77; fraction L, 48; fraction H, 29. Each reaction mixture (5.0 ml) was composed of 20.4 μg of DNA, and 0.59 μg of ^{3}H-labeled 23S rRNA (specific activity, 9,300 counts per min per μg) and was incubated for 18 hr at 67 C.*

fine as plus (+), are specifically located in the late-eluting portion of the absorbance profile of the DNA H strand, one may inquire whether their complements, namely the minus (−) templates, can be localized in the early-eluting portion of the L strand. This could be ascertained by competitive hybridization which would demonstrate whether the early-eluting L fractions can preferentially inhibit the hybridization between the peak region of the H fraction and rRNA. The results of such competitive hybridization in *B. subtilis* are illustrated in Fig. 5. Fraction H_4 which comprised the hybridization peak when annealed with 23*S* RNA (for comparison, see Fig. 2A) was tested individually with the competing L fractions at a ratio of $L_{1\text{-}5}/H_4 = 2$. Maximal inhibition (74 and 69%) of hybrid formation between fraction H_4 and 23*S* RNA resulted from competition by fraction L_1 and L_2 located at the earliest eluting portion of the L component. This region of the absorbance profile can be considered to represent the complementary portion of fraction H_4. Almost no inhibition (10%) was encountered with fraction L_5 located at the tail end of the L component. Similar results were obtained for *E. coli* as shown in Fig. 6. Fraction H_7 which represents the hybridization peak (for comparison, see Fig. 3B) was annealed to 23*S* RNA in the presence of competing L fractions. Maximal inhibition (69 and 76%) resulted from competition with fractions L_1 and L_2, whereas L_7 had no effect on hybrid formation. Despite the fact that fraction L_2 was slightly more effective as a competitor than L_1, the sharp decrease in the per cent of inhibition with fraction L_3 to L_7 is more impressive in *E. coli* than in *B. subtilis* (*compare* Fig. 5 and 6). In *E. coli* fractions H_7 and L_1, L_2 represent a more precise localization of the plus and the minus templates for 23*S* RNA, respectively. On a later occasion, we shall report observations on the restoration of transforming activities and hypochromicity in annealing experiments of individual L and H fractions of *B. subtilis* DNA which further demonstrate that various complementary sequences in DNA can be completely separated into two distinct and nonoverlapping fractions by chromatography on a MAK column.

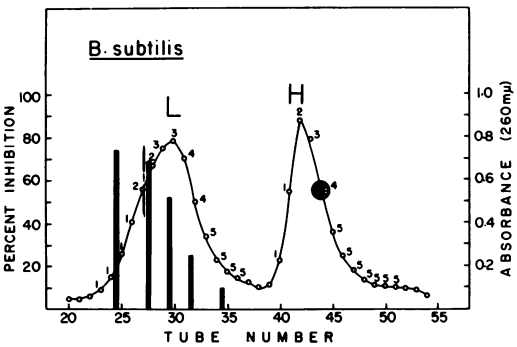

FIG. 5. *Inhibition, by various competing L fractions, of hybrid formation between the H4 DNA fraction and ^{3}H-labeled 23S rRNA in B. subtilis. Alkali-denatured DNA (4.1 mg) was applied to a MAK column and eluted as described in Fig. 1A. The gradient was cut at tube 22 and reconnected at tube 30. Recovery (as per cent of input DNA): total, 83; fraction L, 41; fraction H, 30. Each reaction mixture (4 ml) was composed of 8.8 μg of H4 DNA (shown encircled), 15 μg of the competing L DNA fraction (as numbered on the elution profile) and 0.55 μg of ^{3}H-labeled 23S rRNA (specific activity, 14,000 counts per min per μg) and incubated for 4.5 hr at 68 C. The counts bound to fraction H4 alone were 619 counts/min which constituted 0.5% DNA-H hybridized to 23S RNA.*

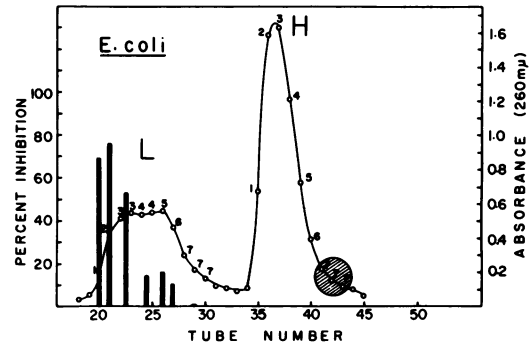

FIG. 6. *Inhibition, by various competing L fractions, of hybrid formation between the H7 DNA fraction and ^{3}H-labeled 23S rRNA in E. coli. Alkali-denatured DNA (4 mg) was applied to a MAK column and eluted as described in Fig. 3A. The gradient was cut at tube 19 and reconnected at tube 26. Recovery (as per cent of input DNA): total, 93; fraction L, 40; fraction H, 53. Each reaction mixture (5 ml) was composed of 10.8 μg of H7 DNA (shown encircled), 21.6 μg of competing L DNA fractions (as numbered on the elution profile) and 0.59 μg of ^{3}H-labeled 23S rRNA (specific activity, 9,300 counts per min per μg) and incubated for 5 hr at 68 C. The counts bound to fraction H7 alone were 1,230 counts/min which constituted 1.26% DNA-H hybridized to 23S RNA.*

DISCUSSION

The L and H components obtained from denatured microbial DNA molecules after MAK chromatography should be regarded as families of strand fragments of a molecular weight ranging from 10×10^6 to 20×10^6 (23, 31). The fragmentation of the bacterial chromosome which occurs during the initial isolation of DNA probably increases the compositional heterogeneity of our

strand preparations. In all the DNA preparations examined previously (31), the H components were richer in cytosine than the L components, and in the AT types they were also richer in thymine. Hybridization with rRNA provides another criterion for assessing the distribution of cytosine-rich sequences in DNA by virtue of the high content of guanylic acid in rRNA. The experiments described here suggest that along the MAK elution profile of fragmented denatured DNA there exists a gradient in the distribution of cytosine-rich sequences. The L strands eluted first are relatively poor in cytosine and rich in guanine, whereas the H strands eluted last are the richest in cytosine. The underlying principle in the separation of complementary strands of DNA by our fractionation technique may very well be due to the sensitive recognition by methylated albumin of any variation in the cytosine content among the various families of L and H strands.

The experiments described here are in agreement with previous findings which show the selective copying of the pyrimidine-rich H strand of DNA in vivo (1, 20, 23, 40, 43) as well as in vitro (3, 14, 26). There are instances, however, which show that the purine-rich L strand of DNA is also involved in the transcription process. Unpublished data revealed that of the hybridizable mRNA synthesized during a nutritional shift down growth of *B. subtilis*, 10 to 15% was complementary to the L strand and the remaining bulk of the transcripts (85 to 90%) was complementary to the H strand. Additional evidence for the involvement of the purine-rich L strand in the transcription process was implicated in this report for soluble RNA in *E. coli* and was demonstrated elsewhere for messenger synthesis after phage infection (12, 13, 38). Furthermore, the existence of operons with opposite polarities in bacteria (18, 33, 34) provides indirect evidence for a switch in transcription from one strand to the other.

The correlation between the relatively higher content of pyrimidine or cytosine in the H strand and its preferential transcribing function is compatible with the concept (41) that pyrimidine-rich sequences are involved in the regulation of transcription. At present, no direct evidence exists which demonstrates that pyrimidine or cytosine clusters are the signals for the initiation of transcription. There is good indirect evidence, however, from in vitro studies (17) which demonstrates that purine nucleoside triphosphates are preferentially found at the triphosphate end of newly synthesized RNA chains. Experiments are now in progress in our laboratory designed to correlate the transcribing DNA strand with various genetic markers and to specifically isolate those rare mRNA species which are transcribed from the L strand during synchronous growth of germinating *B. subtilis* spores.

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