*Proc. Nat. Acad. Sci. USA*
Vol. 68, No. 8, pp. 1786–1790, August 1971

# Transcriptional Organization of the Ribosomal RNA Cistrons in *Escherichia Coli*

W. FORD DOOLITTLE* AND NORMAN R. PACE†

Division of Research, National Jewish Hospital and Research Center; and
Department of Biophysics, University of Colorado School of Medicine, Denver, Colo. 80206

*Communicated by S. Spiegelman, May 17, 1971*

**ABSTRACT** **The data presented support the hypothesis that 16S, 23S, and 5S ribosomal RNAs of *Escherichia coli* are transcribed *in vivo* from transcriptional units consisting of single cistrons for these species arranged in the order 16S–23S–5S, with transcription beginning at the 16S end.**

In eukaryotic cells, linked 18S, 28S, and 7S ribosomal RNA cistrons are transcribed as a unit into a single polynucleotide "precursor" (45S rRNA), which is ultimately cleaved to produce one molecule of each of the three rRNA species (1, 2). The three linked cistrons together comprise a single "transcriptional unit", which we define here as a segment of DNA bounded by a single site for RNA polymerase attachment (promoter) and a single site for polymerase release (terminator). Many such rRNA transcriptional units are confined to a restricted region of the eukaryotic chromosome; adjacent units may be separated by a segment of (nonribosomal) "spacer" DNA (1).

The genetic organization of the cistrons for bacterial rRNA species (16S, 23S, and 5S rRNA) is similar to that of eukaryotes in that: (*a*) the number of gene copies (per genome) for each RNA species is the same (3, 4), (*b*) these cistrons are confined to one (5) or possibly two (6, 7) small regions of the genome, (*c*) 16S, 23S, and 5S rRNA cistrons are tightly linked (8, 9), and (*d*) adjacent 16S, 23S, and 5S gene clusters are separated from each other by segments of spacer DNA (10).

Data presented here support the hypothesis that the linked 16S, 23S, and 5S rRNA cistrons in *Escherichia coli* comprise single transcriptional units arranged in the order 16S–23S–5S, with transcription initiating at the 16S end. The fact that no "precursor" comparable to eukaryotic 45S rRNA has yet been found in bacteria may imply that cleavage into 16S and 23S (and possibly 5S) moieties occurs before transcription of the unit is complete.

## MATERIALS AND METHODS

For the preparation of intact $^3$H-labeled RNA from rifampicin-inhibited cells, a culture of the rifampicin-sensitive, *E. coli* B strain AS19 (11) growing in medium M56 (12), modified to contain 0.5 mM $Na_2HPO_4$ and 50 mM Tris·HCl (pH 7.3), and supplemented with 0.5% tryptone (Difco) and 1 $\mu$Ci/ml [$^{32}$P]orthophosphate (International Chemical and Nuclear Corp.), was chased by the addition of excess unlabeled orthophosphate, and after 30 min of further growth at 20°C, was chilled to 0°C in ice. Rifampicin (Pittman-Moore) was added to 200 $\mu$g/ml at 2 min and [5-$^3$H]uridine (New England Nuclear Corp.) was added to 0.04 mCi/ml (10 $\mu$g/ml) at 4 min after chilling. At 6 min the culture was warmed to 20°C and incubation was continued for an additional 40 min. Cultures were harvested by centrifugation and RNA was extracted and resolved on 2.8% polyacrylamide gels (13, 14).

For the preparation of RNA for oligonucleotide mapping, [$^3$H]guanosine was used for prior labeling, and [$^{32}$P]orthophosphate for labeling in the presence of rifampicin. A culture of AS19 grown at 26°C for several generations in a modified medium M56 [supplemented with phosphate-free acid-hydrolyzed casein (Nutritional Biochemicals) to 0.1% and [8-$^3$H]-guanosine (New England Nuclear Corp.) to 0.15 mCi/ml (10 $\mu$g/ml), and containing 0.5 mM $Na_2HPO_4$,] was centrifuged and resuspended in the same medium containing excess unlabeled guanosine and phosphate at only 0.05 mM. Under these conditions, growth resumes in 3–5 min and continues at the original rate (75-min generation time) until turbidity has increased by 20–30 Klett units. Once growth resumed, carrier-free [$^{32}$P]orthophosphate was added to 5.0 mCi/ml and, 30 sec later, rifampicin was added to 200 $\mu$g/ml to prevent further initiation of transcription. The culture was shaken for an additional 20 min (RNA synthesis ceases within 10 min; ref. 14) and chilled and harvested by centrifugation. RNA was purified by phenol extraction and the rRNA species were resolved by polyacrylamide gel electrophoresis (14). 16S and 23S rRNA were eluted from 1-mm gel slices by shaking overnight with 1 ml of buffer E (13), containing 0.1% sodium dodecylsulfate, and concentrated by ethanol precipitation. The precipitates were redissolved in STE buffer (0.1 M NaCl–0.001 M EDTA–0.05 M Tris·HCl, pH 6.9) and loaded onto 0.7 × 8.0 cm columns of cellulose (Whatman CF-11) as detailed by Franklin (15). All of the rRNA was eluted from such columns in a 13:87 (v/v) mixture of ethanol and STE buffer, while the majority of the contaminating acrylamide remained bound. Eluate fractions were again concentrated by ethanol precipitation and dissolved in 0.003 M EDTA, pH 7.4. The rRNA species were digested with T1 ribonuclease, and the resulting oligonucleotides were resolved by two-dimensional electrophoresis (16). Spots corresponding to $\overline{G}p_{16}$, $pGp_{28}$, and $\overline{G}p_{23}$ derived from 16S and 23S RNA were cut from the electropherograms, and their contents of $^3$H and $^{32}$P were measured by scintillation counting.

The kinetics of $^{32}$P incorporation by AS19 in low-phosphate medium were measured under the same conditions used for

Abbreviation: rRNA, ribosomal RNA.

* Present address: Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia.

† Reprint requests should be addressed to Dr. Pace at the National Jewish Hospital and Research Center.

preparation of RNA for oligonucleotide mapping, except that [$^3$H]guanosine was present at only 0.04 mCi/ml, [$^{32}$P]orthophosphate was added to only 0.1 mCi/ml, and rifampicin was not added. Samples were pipetted into cold 5% trichloroacetic acid at various times after [$^{32}$P]orthophosphate addition. The ratio of $^{32}$P to $^3$H in acid-precipitable material in each sample was determined by liquid scintillation counting.

## RESULTS

### Rationale of approach

Three models for the functional organization of 16S and 23S rRNA genes are shown in Fig. 1. (For simplicity, possible positions of the 5S rRNA gene are not shown, and the 10% size differences between the known immediate precursors of 16S rRNA (p16) and 23S rRNA (p23) and the mature forms are ignored). If the rates of RNA chain elongation are the same for both species, the 23S gene will harbor $2n$ polymerase molecules at any instant, while the 16S gene will carry $n$ polymerase molecules. In model A, the genes are each independent transcriptional units; in models B and C, they share a common transcriptional unit carrying a total of $3n$ polymerase molecules.

The antibiotic rifampicin blocks initiation of transcription, but it does not affect completion of nascent RNA chains (17, 18). If rifampicin and some radioactive isotope that enters RNA, for example [$^{32}$P]orthophosphate, are added simultaneously to a growing *E. coli* culture, the isotope will be incorporated into the nascent chains as they are completed. The amount of $^{32}$P deposited in any given nucleotide sequence in 16S or 23S rRNA will be proportional to the number of RNA polymerase molecules reading across the DNA segment and defining that nucleotide sequence; the amount of $^{32}$P will thus be proportional to the distance of that segment of DNA from the transcription initiation (promoter) site. By examination of the specific radioactivities ($^{32}$P per mole nucleotide) of particular nucleotides derived from 16S and 23S rRNA labeled in the presence of rifampicin, we can distinguish among the alternative models described in Fig. 1.

The nucleotides of interest in this analysis are those reflecting (*a*) the $^{32}$P specific activity of the mean nucleotide (midpoint) of the 16S or 23S rRNA molecule and (*b*) the specific activity of the 5′ terminus of the 23S rRNA molecule. T1 ribonuclease releases 160–180 mol of 3′-guanylic acid (Gp) from each mole of 16S rRNA and about twice this number of Gp residues from 23S rRNA. If we assume that these Gp residues are distributed uniformly throughout their parent molecules, the observed $^{32}$P specific activity of the Gp population from 16S or 23S rRNA (labeled in an experiment such as that described above) is that of the midpoint of the RNA molecule, the mean Gp ($\bar{G}p$). T1 ribonuclease also releases from 23S rRNA its 5′-terminal nucleotide, 3′,5′-guanosine diphosphate ($pGp_{23}$) (19). Both Gp and pGp are readily separated from each other and from other T1-generated oligonucleotides by the elegant two-dimensional electrophoretic techniques of Sanger and Brownlee (16). The alternative models described in Fig. 1 offer definite predictions as to the amount of radioactivity deposited in these selected nucleotides after transcription initiation has been halted by rifampicin.

In *model A*, the 16S and 23S rRNA genes are independent transcriptional units. After addition of rifampicin and [$^{32}$P]-orthophosphate, one-half of the $n$ polymerase molecules re-

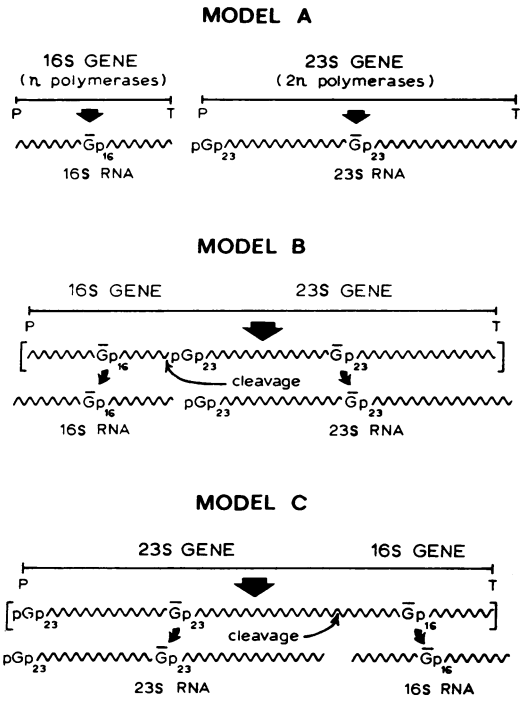


FIG. 1. Three models for the transcriptional organization of 16S and 23S rRNA cistrons. In each model, the 16S rRNA gene carries $n$ polymerases while the 23S rRNA gene carries $2n$ polymerases, distributed randomly across its length. In *model A*, each gene is an independent transcriptional unit with its own promoter (P) and terminator (T) sequence. In *models B* and *C*, both genes are part of the same transcriptional unit, sharing a single promoter and terminator, and are transcribed without interruption by any RNA polymerase that binds at P. The primary transcription products of these units (enclosed in brackets) *may* be cleaved into 16S and 23S rRNA moieties before their synthesis is complete, and thus may not exist independently in the cell. The symbols $\bar{G}p_{16}$, $\bar{G}p_{23}$, and $pGp_{23}$ denote, respectively, the centermost Gp residues of mature 16S rRNA and of 23S rRNA, and the 5′ terminus of 23S rRNA.

siding on the 16S gene deposit $^{32}$P in the $\bar{G}p$ of the 16S rRNA (termed $\bar{G}p_{16}$), while $n$ polymerase molecules transcribe the segment of 23S rDNA defining the midpoint Gp of 23S rRNA ($\bar{G}p_{23}$). Since pGp is the 5′-terminus of mature 23S rRNA, it is derived from a DNA segment at or near the promoter site in the 23S transcriptional unit. Therefore *no*, or very few, polymerase molecules will incorporate radioactivity into $pGp_{23}$. As mentioned above, the $^{32}$P specific radioactivity of any given RNA mononucleotide is proportional to the number of polymerases transcribing the DNA nucleotide complementary to it; thus relative label incorporation into the nucleotides in question is given by the relative number of polymerase molecules depositing $^{32}$P in these nucleotides after rifampicin addition. The expected relative specific radioactivities of $\bar{G}p_{16}$, $pGp_{23}$, and $\bar{G}p_{23}$ for *model A* would be $n/2{:}0{:}n = 1{:}0{:}2$.

With *model B*, $n/2$ polymerase molecules will also deposit label in $\bar{G}p_{16}$. However, since the 16S and 23S genes are part of the same transcriptional unit, all the $n$ polymerase molecules originally residing on the 16S gene will read into the 23S gene, depositing $^{32}$P in $pGp_{23}$ and $\bar{G}p_{23}$. Also, $n$ polymerase molecules associated originally with the 23S gene will label

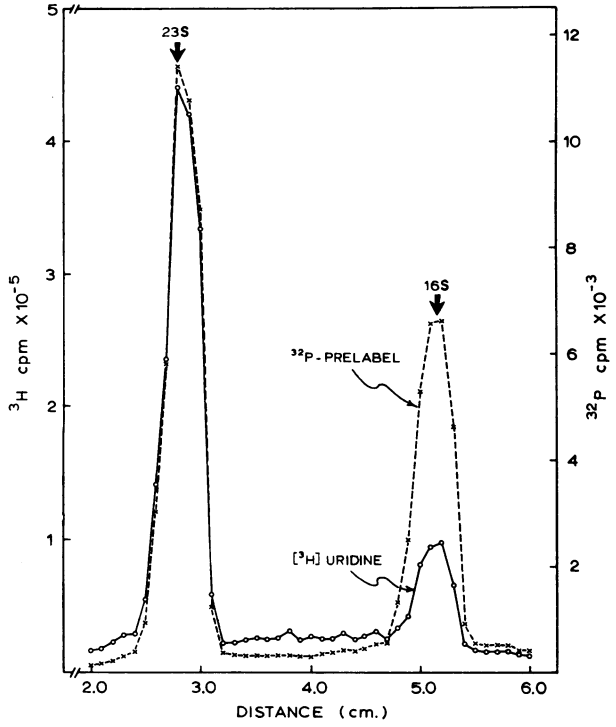


FIG. 2. Labeling of 16S and 23S rRNA after addition of rifampicin. RNA from a culture that had been labeled with [$^{32}$P]-orthophosphate was labeled with [$^{3}$H]uridine in the presence of rifampicin and resolved on 2.8% polyacrylamide gels. Specific activity of 16S ($^{3}$H/$^{32}$P) = 12.6; 23S = 38.0; ratio 1:3.

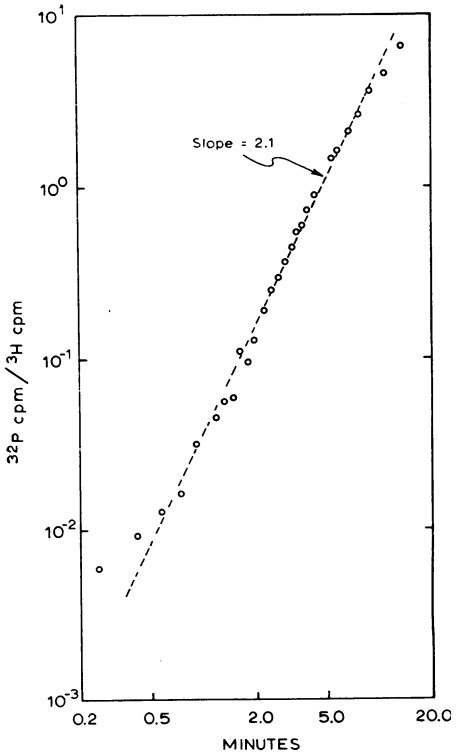


FIG. 3. Kinetics of $^{32}$P incorporation by $^{3}$H-labeled AS19 in low-phosphate medium in the absence of rifampicin. The incorporation of $^{32}$P into trichloroacetic acid-insoluble material was measured.

$\bar{G}p_{23}$. The expected relative specific activities of the nucleotides examined would be $\bar{G}p_{16}$: ($pGp_{23}$)/2: $\bar{G}p_{23}$ = $n$/2: (2$n$)/2: (2$n$) = 1:2:4. (The specific $^{32}$P radioactivity of $pGp_{23}$ is divided by two since it contains 2 mol of phosphate per mole of nucleotide, and hence a $^{32}$P specific radioactivity proportional to 2$n$ for each $n$ polymerase depositing label at the 5′ terminus of 23S rRNA). By similar reasoning for *model C*, where 23S gene precedes the 16S gene in a single transcriptional unit, the expected values would be $\bar{G}p_{16}$: ($pGp_{23}$)/2: $\bar{G}p_{23}$ = $2^{1}/_{2}n$:0:$n$ = 1:0:(0.4).

**Determination of relative specific activities of intact RNA**

If the T1 ribonuclease-generated Gp residues are distributed uniformly across the mature 16S and 23S rRNA molecules, the specific radioactivity of Gp derived from either species should be the same as that of the intact RNA molecule. In principle, simple comparison of the specific radioactivities of intact, mature 16S and 23S rRNA labeled in the presence of rifampicin should distinguish the alternative models of Fig. 1. Accordingly, cultures of *E. coli* were grown in $^{32}$P-containing medium for several generations to allow uniform labeling of all RNA species. They were washed free of the $^{32}$P and rifampicin and [$^{3}$H]uridine were added. After sufficient time for polymerase read-out and maturation of the $^{3}$H-labeled RNA species, RNA was purified by phenol extraction and resolved on polyacrylamide gels. Fig. 2 shows a typical gel profile obtained in such an experiment. Since very little RNA is made after rifampicin addition, specific radioactivities of 16S and 23S rRNA can be defined by the ratio $^{3}$H/$^{32}$P. In eight such experiments, the average value of this ratio was 1:3. The same value has been obtained by Pato and von Meyenburg in similar experiments (20). As described above, the expected ratios according to models A, B, and C are 1:2, 1:4, and 1:(0.4), respectively. While this sort of experiment rules out model C for the organization of the ribosomal RNA genes, certain sources of error make distinction between models A and B impossible. These potential errors are lag in inhibition by rifampicin, lag in equilibration of exogenous label with internal pools, and preferential breakage of the larger and therefore more labile 23S rRNA during extraction.

**Determination of relative nucleotide specific radioactivities**

Measurements of the specific radioactivities of the T1-released nucleotides $pGp_{23}$, $\bar{G}p_{16}$, and $\bar{G}p_{23}$ are subject to error from the same sources. However, the effect can *only* be to minimize or exaggerate the differences in relative specific radioactivities. Lag in rifampicin action or breakdown of 23S rRNA will decrease these differences, while lag in pool equilibration will increase them, for any model. In all cases, the specific radioactivities of the nucleotides must remain in the order ($pGp_{23}$)/2 < $\bar{G}p_{16}$ < $\bar{G}p_{23}$ for model A, in the order $\bar{G}p_{16}$ < ($pGp_{23}$)/2 < $\bar{G}p_{23}$ for model B, and in the order ($pGp_{23}$)/2 < $\bar{G}p_{23}$ < $\bar{G}p_{16}$ for model C. That is, measurement of the specific radioactivity of $pGp_{23}$, the 5′ terminus of 23S rRNA, allows a confident choice among the models of Fig. 1.

To provide sufficient incorporation of $^{32}$P into RNA for measurement of the specific radioactivity of single nucleotides, [$^{32}$P]orthophosphate labeling was performed at very low phosphate concentration (0.05 mM), and rifampicin was added 30 sec after the isotope. The kinetics of $^{32}$P incorporation into acid-insoluble material under these conditions, but without the addition of rifampicin, are shown in Fig. 3. The

TABLE 1. *Relative specific activities of $\bar{G}p_{16}$, $pGp_{23}$, and $\bar{G}p_{23}$ from 16S and 23S [$^3H$]rRNA labeled with $^{32}P$ in the presence of rifampicin*

| | Radioactivity (cpm above background) | | | | |
|---|---|---|---|---|---|
| | Expt. 1 | Expt. 2 | Expected for model A | B | C |
| $\bar{G}p_{16}$ | | | | | |
| $^{32}P$ | 33,100 | 6,500 | | | |
| $^3H$ | 37,400 | 165,000 | | | |
| $^{32}P/^3H$ | 0.886 | 0.039 | | | |
| (normalized) | (1.0) | (1.0) | 1.0 | 1.0 | 1.0 |
| $pGp_{23}$ | | | | | |
| $^{32}P$ | 434 | 143 | | | |
| $^3H$ | 103 | 993 | | | |
| $(^{32}P/^3H) \div 2$ | 2.10 | 0.072 | | | |
| (normalized) | (2.4) | (1.8) | 0.0 | 2.0 | 0.0 |
| $\bar{G}p_{23}$ | | | | | |
| $^{32}P$ | 97,700 | 44,600 | | | |
| $^3H$ | 38,100 | 332,000 | | | |
| $^{32}P/^3H$ | 2.567 | 0.134 | | | |
| (normalized) | (2.9) | (3.4) | 2.0 | 4.0 | 0.4 |

Spots corresponding to $\bar{G}p_{16}$, $pGp_{23}$, and $\bar{G}p_{23}$ (see text) were cut from two-dimensional electropherograms of purified 16S and 23S rRNA that had been labeled first with [$^3H$]guanosine and then with $^{32}P$ in the presence of rifampicin. Specific radioactivity ($^{32}P$: $^3H$) is normalized so that the value for $\bar{G}p_{16}$ specific radioactivity is equal to 1.0.

slope, 2.1, of the log–log plot of $^{32}P$ incorporation is considerably greater than unity, which implies that the specific radioactivity of the internal phosphate pool increases throughout the experiment (21). As noted above, neither this lag in label equilibration nor the delay in addition of rifampicin can affect the *order* of specific radioactivities of $\bar{G}p_{16}$, $pGp_{23}$, and $\bar{G}p_{23}$.

For determination of nucleotide specific radioactivity, RNA was uniformly labeled with [$^3H$]guanosine for several generations. The $^3H$ label was then diluted by addition of unlabeled guanosine, and [$^{32}P$]orthophosphate and rifampicin were added. The doubly-labeled 16S and 23S rRNA molecules were isolated by polyacrylamide gel electrophoresis, and oligonucleotides resulting from T1 ribonuclease digestion were resolved by two-dimensional electrophoresis (16). An autoradiogram of a two-dimensional fingerprint of 23S rRNA is shown in Fig. 4. Spots corresponding to $\bar{G}p_{16}$, $pGp_{23}$, and $\bar{G}p_{23}$ derived from 16S and 23S rRNA were cut from the electropherograms, and the content of $^3H$ and $^{32}P$ measured by scintillation counting. $^{32}P$ specific radioactivity was determined as the ratio of $^{32}P$ (incorporated in the presence of rifampicin) to $^3H$ (prior label). Results of two such experiments are shown in Table 1. It should be noted that the molar yield of $pGp_{23}$ is 1/350 that of $\bar{G}p_{23}$, as determined by the relative quantities of $^3H$ (the uniform initial label) in these two nucleotides. This is the ratio usually obtained with T1 digests of intact 23S rRNA; this provides assurance that pGp was derived only from the 5′ end of 23S rRNA.

The *order* of specific radioactivities (Table 1) was $Gp_{16} < (pGp_{23})/2 < \bar{G}p_{23}$, as predicted by model B. There is also reasonable quantitative agreement between the predicted relative specific radioactivities for model B (1:2:4) and the

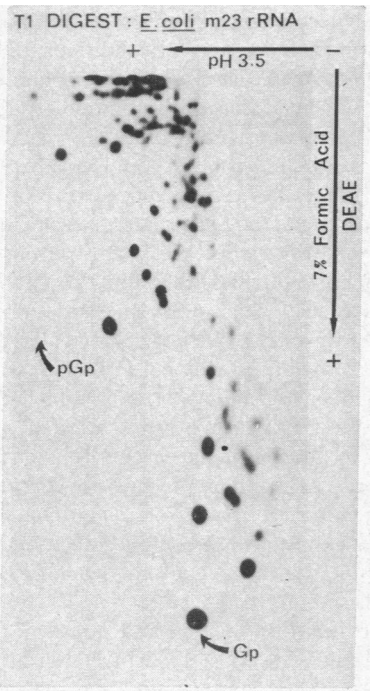


FIG. 4. Two-dimensional electrophoresis of *E. coli* 23S rRNA. Uniformly $^{32}P$-labeled *E. coli* 23S rRNA was completely digested with T1 ribonuclease (Sankyo) and resolved by two-dimensional electrophoresis, all as detailed in ref. 16.

average observed values (1:2.1:3.2). As stressed earlier, quantitative agreement is not required to distinguish between the three models of Fig. 1; the closeness of agreement probably indicates that the errors introduced by lag in label equilibration and delay in rifampicin addition are partially compensatory. In a third experiment, in which the specific radioactivity of $\bar{G}p_{16}$ was not measured, the relative specific radioactivities of $pGp_{23}$ and $Gp_{23}$ were 2.0 and 3.6, respectively.

## DISCUSSION

The measurements presented are consistent with model B (Fig. 1) for transcriptional organization of the rRNA cistrons, and inconsistent with models A and C. Data supporting model B have also been obtained by Kossman *et al.* (22), who used a different approach. These workers observed that transcription of 16S rRNA sequences (as determined by hybridization competition) began prior to transcription of 23S rRNA sequences, when a required amino acid was restored to a starved $RC^{str}$ amino-acid auxotroph.

Both approaches are subject to the criticism that they demonstrate *only* that the 23S rRNA gene is preceded in its transcriptional unit by a segment of DNA approximately half the length of the 23S rRNA gene itself. If the product of transcription of this DNA segment is *not* 16S rRNA, however, then that product must be very rapidly degraded, since no other RNA species of the expected size and abundance is found in the cell. Consistent with the notion that the DNA segment preceding the 23S rRNA gene *is*, in fact, the 16S rRNA gene are the data of Colli and Oishi (8) and Colli *et al.* (9), with *Bacillus subtilis*, which show that both rRNA species can hybridize with fragments of single-stranded DNA of molecular weight $1.9 \times 10^6$, i.e., close to the expected combined molecular weights of the two genes.

The postulated common precursor of 16S and 23S rRNA cannot be detected under normal conditions, and it is likely that either (*a*) enzymatic cleavage occurs before transcription is complete, or (*b*) liberation of the 16S moiety results from a special type of transcription termination event which leaves RNA polymerase bound to DNA and capable of continued transcription of the 23S rRNA gene in the presence of rifampicin. A precursor of both rRNA species might be isolatable under conditions where cleavage or termination cannot occur. Pettijohn *et al.* (23) have found an RNA species of the appropriate size which was generated during *in vitro* transcription of the rRNA genes of *E. coli*. A large stable RNA species is also formed *in vivo* by cultures of *B. cereus* grown in the presence of 8-azaguanine (24, 25) (which this organism incorporates into RNA). We have detected, by polyacrylamide gel electrophoresis, a species of RNA of the molecular weight expected of a common precursor of 23S and 16S rRNA ($1.7 \times 10^6$) in cultures of *B. megaterium* grown in the presence of this analog. Appropriate hybridization experiments should determine whether it contains sequences characteristic of both species.

We have previously presented an analysis of the incorporation of [$^3$H]uridine into 5S rRNA in *E. coli* after rifampicin addition (14). In these experiments, the specific activity (as $^3H/^{32}P$ initial label) of 5S rRNA was 1.2–1.7 times as great as that of 23S rRNA. An attractive interpretation of this result is that the 5S rRNA gene is part of the same transcriptional unit that encompasses the major rRNA genes, and lies between the 23S rRNA cistron and the termination site. This interpretation is consistent with the recent findings of Colli *et al.* (9) that, in *B. subtilis*, the gene for 5S rRNA is associated with the 16S–23S rRNA gene clusters, and that the 5S rRNA cistron is more closely linked to the 23S rRNA cistron. If model B (Fig. 1) is modified to include the 5S rRNA gene near the terminator, then when $2n$ polymerase molecules incorporate label into $\bar{G}p_{23}$, approximately $3n$ polymerases should deposit label in the Gp residue at the midpoint of 5S rRNA ($\bar{G}p_5$). The specific radioactivity ratio, $\bar{G}p_{23}:\bar{G}p_5$ (which, as explained above, equals the ratio of specific radioactivities of the intact parent molecules) should be $2n:3n$, or 1:1.5, within the range observed.

The transcriptional unit for the rRNAs of mammalian cells and that proposed here for bacterial cells are similar in that both include cistrons for the two major rRNA species, with the gene for the smaller of these (16S or 18S rRNA) closest to the initiation site (26). They are apparently dissimilar in that the bacterial 5S rRNA cistron is included in the transcriptional unit, while the mammalian 5S rRNA gene is excluded. However, a fourth mammalian rRNA, 7S rRNA, *is* transcribed from the same unit as 18S and 28S rRNA, and remains covalently bonded to the latter species during early stages of ribosome maturation (2). It is possible that mammalian 7S rRNA, and not 5S rRNA, is the evolutionary homolog of bacterial 5S rRNA.

We thank Profs. C. R. Woese, Howard V. Rickenberg, and Sol Spiegelman for their suggestions concerning, and encouragement of, this study. This work was supported by USPHS grants GM 16569 from the National Institute of General Medical Sciences and FR 05474 from the National Institutes of Health. This paper is contribution No. 446 from the Department of Biophysics, University of Colorado School of Medicine.

1. Birnstiel, M. L., M. Chipchase, and J. Speirs, in *Progr. Nucl. Acid Res. Mol. Biol.*, ed. J. N. Davidson and W. E. Cohn (Acadademic Press, New York, N.Y., 1971), Vol 11, pp. 351–390.
2. Pene, J. J., E. Knight, Jr., and J. E. Darnell, *J. Mol. Biol.* **33**, 609 (1968).
3. Pace, B., and N. R. Pace, *J. Bacteriol.*, **105**, 142 (1971).
4. Pace, B., Ph.D. thesis, Department of Microbiology, University of Illinois (1969).
5. Yu, M. T., C. W. Vermeulen, and K. C. Atwood, *Proc. Nat. Acad. Sci. USA*, **67**, 26 (1970).
6. Cutler, R. G., and J. T. Evans, *J. Mol. Biol.*, **26**, 91 (1967).
7. Smith, I., D. Dubnau, P. Morrell, and J. Marmur, *J. Mol. Biol.*, **33**, 123 (1968).
8. Colli, W., and M. Oishi, *Proc. Nat. Acad. Sci. USA*, **64**, 642 (1969).
9. Colli, W., I. Smith, and M. Oishi, *J. Mol. Biol.*, **56**, 117 (1971).
10. Purdom, I., J. O. Bishop, and M. L. Birnstiel, *Nature*, **227**, 239 (1970).
11. Sekiguchi, M., and S. Ida, *Proc. Nat. Acad. Sci. USA*, **58**, 2315 (1967).
12. Monod, J., G. Cohen-Bazire, and M. Cohen, *Biochim. Biophys. Acta*, **7**, 585 (1951).
13. Pace, N. R., D. Bishop, and S. Spiegelman, *Proc. Nat. Acad. Sci. USA*, **58**, 711 (1967).
14. Doolittle, W. F., and N. R. Pace, *Nature*, **228**, 125 (1970).
15. Franklin, R. M., *Proc. Nat. Acad. Sci. USA*, **55**, 1504 (1966).
16. Sanger, F., and G. G. Brownlee, in *Methods in Enzymology*, ed. L. Grossman and K. Moldave (Acadademic Press, New York, N.Y., 1968), Vol. 12, pp. 360–381.
17. Wehrli, W., J. Nüesch, F. Knüsel, and M. Staehlin, *Biochim. Biophys. Acta*, **157**, 215 (1968).
18. Sippel, A., and G. Hartmann, *Biochim. Biophys. Acta*, **157**, 218 (1968).
19. Takanami, M., *J. Mol. Biol.*, **29**, 323 (1967).
20. Pato, M. L., and K. von Meyenburg, *Cold Spring Harbor Symp. Quant. Biol.*, **35**, 497 (1970).
21. Britten, R. J., and B. J. McCarthy, *Biophys. J.*, **2**, 49 (1962).
22. Kossman, C. R., T. D. Stomato, and D. E. Pettijohn, submitted to *Nature* (1971).
23. Pettijohn, D. E., G. O. Stonington, and C. R. Kossman, *Nature*, **228**, 235 (1970).
24. Chantrenne, H., *J. Cell. Comp. Physiol.*, **65**, *Suppl 1*, 149 (1964).
25. Grünberger, D., R. N. Maslova, and F. L. Sorm, *Collect. Czech. Chem. Commun.*, **29**, 152 (1964).
26. Reeder, R. H., and D. D. Brown, *J. Mol. Biol.*, **51**, 361 (1970).